













THE DIFFERENTIATION AND SPECIFICITY OF STARCHES IN RELATION  
TO GENERA, SPECIES, ETC.

STEREOCHEMISTRY APPLIED TO PROTOPLASMIC PROCESSES AND PRODUCTS, AND AS A STRICTLY  
SCIENTIFIC BASIS FOR THE CLASSIFICATION OF PLANTS AND ANIMALS

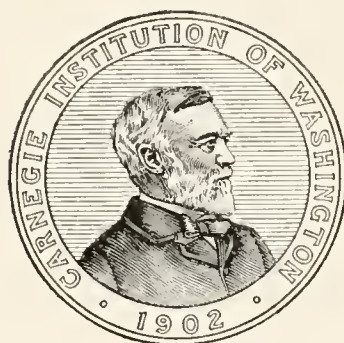
BY

EDWARD TYSON REICHERT, M.D.

*Professor of Physiology in the University of Pennsylvania  
Research Associate of the Carnegie Institution of Washington*

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## PREFACE.

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The present memoir, which is purely in the nature of a report of a preliminary investigation, is complementary and supplementary to Publication No. 116 of this Institution, entitled "The Differentiation and Specificity of Corresponding Proteins and other Vital Substances in Relation to Biological Classification and Organic Evolution: The Crystallography of Hemoglobins," in the preface of which the following statement was made of the hypothesis upon which the research was founded, and of the support of the hypothesis by the results of the inquiry:

"The trend of modern biological science seems to be irresistibly toward the explanation of all vital phenomena on a physico-chemical basis, and this movement has already brought about the development of a physico-chemical physiology, a physico-chemical pathology, and a physico-chemical therapeutics. The striking parallelisms that have been shown to exist in the properties and reactions of colloidal and crystalloidal matter *in vitro* and in the living organism lead to the assumption that protoplasm may be looked upon as consisting essentially of an extremely complex solution of interacting and interdependent colloids and crystalloids, and therefore that the phenomena of life are manifestations of colloidal and crystalloidal interactions in a peculiarly organized solution. We imagine this solution to consist mainly of proteins with various organic and inorganic substances. The constant presence of protein, fat, carbohydrate, and inorganic salts, together with the existence of protein-fat, protein-carbohydrate, and protein-inorganic salt combinations justifies the belief that not only such substances, but also such combinations, are absolutely essential to the existence of life.

"The very important fact that the physical, nutritive, or toxic properties of given substances may be greatly altered by a very slight change in the arrangement of the atoms or groups of molecules may be assumed to be conclusive evidence that a trifling modification in the chemical constitution of a vital substance may give rise to even a profound alteration in its physiological properties. This, coupled with the fact that differences in centesimal composition have proved very inadequate to explain the differences in the phenomena of living matter, implies that a much greater degree of importance is to be attached to peculiarities of chemical constitution than is universally recognized.

"The possibilities of an inconceivable number of constitutional differences in any given protein are instanced in the fact that the serum albumin molecule may, as has been estimated, have as many as 1,000 million stereoisomers. If we assume that serum globulin, myoalbumin, and other of the highest proteins may have a similar number, and that the simpler proteins and the fats and carbohydrate, and perhaps other complex organic substances, may each have only a fraction of this number, it can readily be conceived how, primarily by differences in chemical constitution of vital substances, and secondarily by differences in chemical composition there might be brought about all of those differences which serve to characterize genera, species, and individuals. Furthermore, since the factors which give rise to constitutional changes in one vital substance would probably operate at the same time to cause related changes in certain others, the alterations in one may logically be assumed to serve as a common index of all.

“In accordance with the foregoing statement, it can readily be understood how environment, for instance, might so affect the individual’s metabolic processes as to give rise to modifications of the constitutions of certain corresponding proteins and other vital molecules which, even though they be of too subtle a character for the chemist to detect by his present methods, may nevertheless be sufficient to cause not only physiological and morphological differentiations in the individual, but also become manifested physiologically and morphologically in the offspring.

“Furthermore, if the corresponding proteins and other complex organic structural units of the different forms of protoplasm are not identical in chemical constitution, it would seem to follow, as a corollary, that the homologous organic metabolites should have specific dependent differences. If this be so, it is obvious that such differences should constitute a preëminently important means of determining the structural and physiological peculiarities of protoplasm.

“It was such germinal thoughts that led to the present research, which I began upon the hypothesis that if it should be found that corresponding vital substances are not identical, the alterations in one would doubtless be associated with related changes in others, and that if definite relationships could be shown to exist between these differences and peculiarities of the living organism, a fundamental principle of the utmost importance would be established in the explanation of heredity, mutations, the influences of food and environment, the differentiation of sex, and other great problems of biology, normal and pathological.

“To what extent this hypothesis is well founded may be judged from this partial report of the results of our investigations: It has been conclusively shown not only that corresponding hemoglobins are not identical, but also that their peculiarities are of positive generic specificity, and even much more sensitive in their differentiations than the ‘zoöprecipitin test.’ Moreover, it has been found that one can with some certainty predict by these peculiarities, without previous knowledge of the species from which the hemoglobins were derived, whether or not interbreeding is probable or possible, and also certain characteristics of habit, etc., as will be seen by the context. The question of interbreeding has, for instance, seemed perfectly clear in the case of *Canida* and *Murida*, and no difficulty was experienced in forecasting similarities and dissimilarities of habit in *Sciurida*, *Murida*, *Felida*, etc., not because hemoglobin is *per se* the determining factor, but because, according to this hypothesis, it serves as an index (gross though it be, with our present very limited knowledge) of those physico-chemical properties which serve directly or indirectly to differentiate genera, species, and individuals. In other words, vital peculiarities may be resolved to a physico-chemical basis.”

Before and since the inception of the foregoing research, data have been slowly accumulating which point more and more strongly to the extremely important interrelationships that exist between the intramolecular configurations of various substances that play active rôles in life’s processes and the configurations of protoplasm. Hence, any progress in the application of stereochemistry to metabolic processes brings us closer to an understanding of those peculiar mechanisms of protoplasm which give rise to the phenomena which in the aggregate constitute life in its normal and abnormal manifestations.

Hemoglobin, next to protoplasm, is unquestionably the most important organic substance of vertebrate life, and in conjunction with the stroma with which it is associated is an active functioning protein, the main function of which is the conveyance of oxygen from the external organs of respiration to the internal organs of respiration or the tissues generally. Starch is similarly an extremely important

constituent of a vast number of forms of plant life, but its rôle in vital processes, while, on the whole, as essential to the continuance of life, is of an entirely different character. Moreover, the general and special characters of these substances in relation to those of the bodies which originate them, and the mechanisms of their formation, are likewise strikingly different. Hemoglobin constitutes nearly the whole of the erythrocyte or red-blood corpuscle, and that portion of the erythrocyte which is not this substance may properly be regarded as being in the nature of an adjunct, but nevertheless essential. In early embryonic life the erythrocytes are nucleated and probably derived directly from the mesoblastic elements, and they increase in number by mitosis. Later, proliferation occurs in all parts of the circulation, in certain capillary areas more than others, especially in those of the liver, spleen, and bone-marrow. During the progress of fetal development the erythrocytes, primarily spherical and nucleated, in time lose their nuclei, and become smaller, and take on the peculiar disk or cup-shaped form of postnatal life. After birth the red bone-marrow is the chief or sole seat of formation of erythrocytes. It is the common conception that in this structure these corpuscles arise from nucleated red cells which exist at first as colorless, nucleated erythroblasts, and subsequently as smaller, denser, colored, nucleated normoblasts. The former, which are looked upon as the hereditary representatives of the embryonal erythrocytes, are generally conceived to be converted into normoblasts by mitosis, and the latter in turn to become ordinary erythrocytes upon the disappearance of the nuclei by solution or extrusion. It is, however, more likely, as suggested in 1882 by Malassez, and very recently (1912) by the investigations of Emmel by means of plasma cultures, that the erythrocyte of late fetal and post fetal life is formed from the *cytoplasm* of the erythroblast by a simple process of budding and detachment. According to either conception the erythrocyte is a separated portion of the mother substance that has been set free in a highly specialized life-sustaining medium, but in a distinctly modified form, inasmuch as it has a much higher hemoglobin content and is lacking in the amœboid activities and power of reproduction of the parent substance, the latter differences being readily accounted for in the absence of nuclear matter. Starch, on the other hand, is a synthetic product of metabolic activity which bears no resemblance to the protoplasm that gave rise to it, and which is destined to serve an entirely different purpose from that of hemoglobin in the life-history of the organism. With *hemoglobin* as it exists associated with the stroma in the erythrocytes we are dealing with an *active, living, functioning, highly specialized form of protoplasm*; with *starch*, we deal with an *absolutely inert, non-living, non-functionating, extremely complex carbohydrate in the nature of a stored-up pabulum, and a synthetic product of plastids which are specialized forms of protoplasm*. In the hemoglobin research it was shown that the hemoglobin molecule is modified in specific relationship to genus, species, etc., which may be taken to mean that the form of protoplasm that is expressed by the term erythrocyte is correspondingly stereochemically modified; with starch it has been found, as will be seen by the context, that the molecule is likewise changed in specific relationship to genera, species, etc., which accordingly may also be

taken to mean that during synthesis the products of activity are altered in their molecular peculiarities in specific relationship to the stereochemic modifications of the forms of protoplasm which produce them. In other words, one may lay down the dictum *that each and every form of protoplasm existent in any organism is stereochemically peculiarly modified in specific relationship to that organism, and that, as a corollary, the products of synthesis will be modified in conformity with the molecular peculiarities of the protoplasm giving rise to them.* It follows, therefore, that if the plastids of any given plant be of different stereochemic structure from those of others, *the starch produced will show corresponding stereochemic variations, and hence be absolutely diagnostic in relation to the plant.* Abundant evidence will be found in the pages which follow in justification of this statement. Moreover, if such differences are diagnostic, it is evident that they constitute *a strictly scientific basis for the classification of plants.*

The author takes advantage of this opportunity to record his heartfelt obligation to the Carnegie Institution of Washington for the grants which made this investigation possible; and also to President R. S. Woodward and Dr. S. Weir Mitchell for invaluable assistance—assistance easy of mention, but difficult of adequate expression.

EDWARD TYSON REICHERT.

FROM THE S. WEIR MITCHELL  
LABORATORY OF PHYSIOLOGY,  
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# PART I.

## THE STARCH-SUBSTANCE AND STARCH-GRAIN.

THEIR FORMS, STRUCTURE, MECHANISMS OF FORMATION, CLASSIFICATION, PROPERTIES, COMPOSITION, DECOMPOSITION PRODUCTS, SYNTHESIS, RELATED BODIES, METHODS OF DIFFERENTIATION, AND DIFFERENTIATION AND SPECIFICITY IN RELATION TO GENERA, SPECIES, ETC.

GENERAL APPLICATIONS OF THE RESULTS OF THIS RESEARCH.

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# CHAPTER I.

## INTRODUCTION.

### OBJECTS OF THE RESEARCH.

This research was undertaken with three primary objects in view: First, to determine if the hypothesis underlying the hemoglobin investigation would be supported by the stereochemic peculiarities of other complex synthetic metabolites; second, to add materially to our knowledge of one of the most important substances in the life-history of both plant and animal kingdoms; and third, to throw open fields of investigation which offer extraordinary promise, particularly in adding to our knowledge of the all-important properties of protoplasm. In the previous research it was clearly demonstrated that hemoglobin exists in isomeric forms which are specifically modified in relation to genera and species, and differing to so marked a degree that by means of such variations species and genera could be recognized. Starch was selected as the second subject of investigation, primarily, as stated in the Preface, because of its being so different from hemoglobin in its relations to the parent substance, in its chemical composition and constitution, and in its rôle in life's processes; and, moreover, because of its especial value in such an inquiry owing to its extremely large and complex molecule and high carbon content.

### THE STARCH-GRAIN.

Starch exists *in situ* both as "soluble starch" and starch-grains or granules, almost solely as the latter. In the soluble form it has been found in the cell-sap of the epidermal cells of *Saponaria officinalis* and *Arum italicum*, and in a number of other plants. Starch-grains have long been recognized as occurring in a great variety of forms. Fritzsche, in 1834, recorded that not only are the starches from different plants not alike, but also that *often* the form was so characteristic as to determine the plant, or at least indicate the genus and family to which the plant belonged. This observation was confirmed by Schleiden and other investigators. Nägeli, the most noted authority on starches, stated in his elaborate memoir published in 1858, after examining over 1,200 different kinds of starches, that all genera of a natural order frequently contain starch-grains which are closely related, and that *sometimes* a distinction is shown between genera, and then usually also between species, so that plants may be classified into natural groups according to the structure of their starches. Nägeli's records also show that starches of like gross histological characters may be found in entirely different genera, families, and orders; and, moreover, that even in the same family the starches of different genera may differ more or less markedly, or, indeed, be entirely unlike. Even though such differences in form exist, it is by no means necessarily implied that such variations are an expression of different kinds of starch, because they may be entirely accounted for upon the basis of attendant conditions.

The starch-grain, excepting perhaps at its earliest stage of formation, is a spherocrystal, and is produced by specialized protoplasmic bodies which are designated plastids. The forms of the starch-grains are dependent in part upon the molecular peculiarities of the plastids, the form of the plastid, and the position of the plastid in relation to the surface of the grain; and in part upon a number of incidental conditions, such as variations in the composition of the cell-sap, the presence of foreign bodies in the form of crystals of protein or other solid masses, mutual pressure of starch-grains, etc. Were the plastid

free to operate in the absence of disturbing external conditions, the histological characteristics of the grain would doubtless be specific in relation to the peculiarities of the plastid; but the conditions in the plant are ever-changing; and, as is well known, a given substance being deposited in a crystalline or a non-crystalline state under varying attendant conditions may take on different forms.

The cell-sap is not only of varying composition in different plants, but also changing from time to time in the same plant or cell, which factor of itself may materially modify the shape of the grain. Such an influence is illustrated in the crystallization of calcium oxalate, which substance may be found in a variety of forms in different plants and different parts of the same plant, and modifiable by changes in conditions in the given part of a plant in which the crystals arise. It may be seen as needles, rods, cryptocrystalline forms, rosette aggregates, tetragonal pyramids, rhombic plates, combination of pyramid and prism, combination of rhombohedron and hemipyramid, and in various other forms which are stated to belong to the tetragonal or monoclinic systems. In many plants the form of these crystals is quite as distinctive of the plant as the form of the starch-grain, yet the differences are attributed to extraprotoplasmic conditions, and not to any inherent molecular peculiarities of the substance itself. There are many instances on record to show that such variations may be dependent solely upon the presence of certain substances in the mother-liquor, or upon other incidental conditions: Thus, for instance, in the case of sodium chloride, which under ordinary conditions crystallizes in cubes, but which in the urine or in a solution of urea appears as octohedrons; or in ammonium fluosilicate which crystallizes from an aqueous solution in hexagonal forms at 6° C., in cubes at 13°, and in both forms at intermediate temperatures.

Not only are starch-grains during their growth subjected to the influences of the peculiarities of composition of the cell-sap, but also to changes in temperature which may amount to many degrees, and also to alterations in the plastids themselves, which are ever influenced in their operations by variations in both internal and external conditions. It seems, therefore, obvious that the histological peculiarities of different starches may be expressive merely of variations in the conditions attending starch formation, without in the least indicating differences in the starch molecules themselves any more than differences in the forms of geometric models indicate differences in the wood of which they are made. It follows, as a consequence, that if there are inherent differences in the starches of different plants, as was found in hemoglobin in relation to animals, such variations must be determined by methods that are available for the differentiation of isomers.

#### STEREOCHEMISTRY AND SOME OF ITS APPLICATIONS.

Modern science has brought to light extraordinarily important disclosures in explanation of the differences in the properties of isomerides, and this development has resulted in the formation of a new department of chemistry that is known as *stereochemistry*, but which as yet seems to be but little understood among the rank and file of students. As the laws and principles of this new science underlie the present series of investigations, it seems desirable to set forth certain basic facts which will serve particularly to show: (1) that it is theoretically possible for a complex carbon compound, such as starch or hemoglobin, to exist in a countless number of stereoisomeric forms; (2) that the slightest alteration in the configuration or arrangement of the component units of a molecule may give rise to a change of properties that may be profound, and sometimes of a predictable specific character; (3) that stereochemistry is inseparably associated with protoplasmic processes, and hence with the problems of nutrition, species, disease, heredity and the innumerable manifestations of protoplasmic activity which in the aggregate constitute life.

For many years the chemical composition of substances was expressed by their *molecular formulæ*, but in the course of time this was found to be inadequate to designate

certain peculiarities of substances which experience determined to be owing to differences in chemical constitution, or the manner of linkage of the molecular components, and hence should be expressed by *structural formulæ*. Still later it was recognized that even the structural formulæ, which obviously indicate the relations of the atoms and groups of the molecule to one another in only *two* dimensions of space, are insufficient, and that a full conception of the causes underlying the differences in isomers rests in the recognition of the arrangements of the molecular components or units in the *three* dimensions of space or, in other words, to be recorded by *space formulæ*.

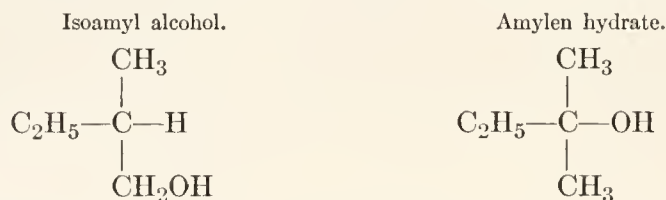
Stereochemistry, therefore, treats of the physical and chemical properties of atoms or structural units in space of three dimensions, the arrangement of the component units of the molecule being expressed by the term *configuration of the molecule*. This branch of chemistry seems to have had its inception in an article by Wollaston in 1808, in which he states that in order to understand the mutual relations of atoms we must have a geometrical conception of their arrangements in all dimensions of space. But like many a gem, it lay by the wayside unnoticed. Many years later (1848) Gmelin wrote that no controversies as to the mode of writing formulæ could be settled without the support of a recognized conception of the arrangements of the atoms of the molecule. After another such long period (1872), Wislicenus forcefully pointed out the insufficiency of structural formulæ, and suggested that the deficiencies in our conceptions of the relations of atoms could be accounted for in "the different positions of atoms in space."

About a decade previous to this (1861), Pasteur made the first substantial step in laying the foundation of stereochemistry by his investigations with tartaric acids, in which he found that under proper conditions there could be obtained three kinds of tartaric acid differing in crystalline form, and in optical, chemical, and physiological properties. Two forms of these crystals he determined to be enantiomorphous, that is, related to one another as an object is to its mirror-image, the hemihedral faces which appear on the right side of one of the forms appearing on the left side of the other. Crystals that were composed of equal quantities of these substances showed an absence of hemihedrism. When the enantiomorphous forms were examined in polarized light it was found that they were oppositely but equally optically active, one form rotating the ray of polarized light to the right (dextro-rotatory), and the other to the left (lævo-rotatory); and that when they were present in equal quantity the acid was inactive (racemic). When solutions of ordinary tartaric acid (dextro-tartaric acid) and of racemic tartaric acid (in the form of tartrates, with a little albumin), were subjected to the influence of *Penicillium glaucum*, it was found, upon testing with the polarimeter, that as fermentation proceeded, in the first solution optical activity decreased, and that the second or optically inactive solution became active, but herein the plane of polarization was rotated to the left, or in the opposite direction, and hence was lævo-rotatory. As fermentation progressed, optical activity was correspondingly increased, and reached its highest limit when fermentation ceased. Hence, in the racemic solution the acid was split, the dextro-tartaric acid being consumed by the *Penicillium* as in the first solution, leaving the lævo-acid. All three acids are in chemical composition and structural formula identical, yet, as will be observed, they differ markedly, not only in their crystalline forms and optical properties, but also to an extraordinary degree in their physiological peculiarities. This latter phenomenon, as will be pointed out, is in its applications one of the most important in the whole domain of protoplasmic processes.

The principles underlying the work of Pasteur were developed in 1874 by van't Hoff and Le Bel, working independently, who found that every optically active carbon compound contained at least one *asymmetric* carbon atom, that is, an atom which has attached to it four *dissimilar* elements, groups, or masses; and, moreover, that in order to explain why this asymmetry should cause crystalline and optical asymmetry and the accompany-

ing differences in chemical properties, it is necessary to represent the arrangement of the atoms in the three dimensions of space. When substances have the same kinds of atoms and the same number of each of the kinds of atoms, they are *isomers* and have the same *molecular formula*. If they differ in any of their properties there is a difference in the linkage of their components which is expressed in differences in their *structural formula*; or, if they have the same structural formula but differ in their properties, while the components have the same linkage the units differ in their positions in the three dimensions of space, and are then distinguished as *stereoisomers*.

Now, it is an extremely important fact, as pointed out by Fischer, that stereoisomers may show far greater differences in their properties than may be observed among related isomers. As shown by van't Hoff, substances which contain one or more asymmetric carbon atoms are optically active, and it has since been found that every optically active carbon compound contains at least three carbon atoms, one of which is asymmetric. If a compound containing one asymmetric carbon atom be so modified that one of its attached atoms, groups, or masses is substituted by another so as to give rise to a plane of symmetry, as in the case of isoamyl alcohol to form amylen hydrate, when the hydroxyl group is replaced by hydrogen, and the hydrogen by a hydroxyl group, optical activity disappears for the obvious reason that asymmetry no longer exists in the molecule:



Each stereoisomer, whether it contain one or more asymmetric carbon atoms, is enantiomorphous, or, in other words, there are two complementary forms which are characterized by having opposite but equal effects on polarized light, and also by other differences, all of which are due to modifications in the space relations of the components of the molecules. There are therefore two types of optically active stereoisomers which are designated the dextro and lævo forms in respect to their optical effects. Moreover, inactive isomers may exist if the two enantiomorphous forms be mixed in equal proportion to form a mechanical mixture, as in racemic tartaric acid; and also when the units of the molecules of the two forms combine in the molecule in equal quantity to form a true compound, as in meso-tartaric acid. In the first instance, the substance is inactive because of *external* compensation, the effect of the dextro-molecule being compensated for by that of the lævo-molecule; in the second instance the compensation is *internal* and due to the units which constitute one half of the molecule neutralizing those of the other half. Obviously, those of the first kind can be separated into the two enantiomorphous forms, while those of the second can not. The first are known as *racemic* substances, and the second as *meso*-substances. There are, therefore, in the case of tartaric acid, four distinct stereoisomeric forms: dextro-rotatory, lævo-rotatory, racemic, and meso-tartaric acids, respectively. The dextro-active, the inactive, and the lævo-active forms may be likened physically to the differences in the three images seen when the head is viewed in the three-sided mirror.

When a substance contains a number of asymmetric carbon atoms, the number of possible stereoisomers increases with each addition, and soon becomes considerable, so that there may be many of each of the dextro, lævo, and inactive forms. When the number is relatively small, as in the members of the aldohexose group which have the formula  $\text{CH}_2\text{OH}(\text{CHOH})_4\text{COH}$ , in which there are only four such atoms, the theoretical

number is sixteen. These are in pairs, twelve of the sixteen are known, and three occur in nature. The *relative* configurations may be expressed by the following formulæ:

Unknown.		Unknown.		Glucoses.		Mannoses.	
				Dextro.	Lævo.	Dextro.	Lævo.
CH <sub>2</sub> OH H.OH H.OH H.OH H.OH H.OH COH	CH <sub>2</sub> OH HO.H HO.H HO.H HO.H HO.H COH	CH <sub>2</sub> OH H.OH H.OH H.OH HO.H HO.H COH	CH <sub>2</sub> OH HO.H HO.H HO.H H.OH H.OH COH	CH <sub>2</sub> OH H.OH H.OH HO.H H.OH H.OH COH	CH <sub>2</sub> OH HO.H HO.H H.OH HO.H HO.H COH	CH <sub>2</sub> OH H.OH H.OH HO.H HO.H HO.H COH	CH <sub>2</sub> OH HO.H HO.H H.OH H.OH H.OH COH
Guloses.		Idoses.		Galactoses.		Taloses.	
Dextro.	Lævo.	Dextro.	Lævo.	Dextro.	Lævo.	Dextro.	Lævo.
CH <sub>2</sub> OH HO.H H.OH HO.H HO.H COH	CH <sub>2</sub> OH H.OH HO.H H.OH H.OH COH	CH <sub>2</sub> OH HO.H H.OH HO.H H.OH COH	CH <sub>2</sub> OH H.OH HO.H HO.H HO.H COH	CH <sub>2</sub> OH H.OH HO.H HO.H H.OH COH	CH <sub>2</sub> OH HO.H H.OH H.OH HO.H COH	CH <sub>2</sub> OH H.OH HO.H HO.H HO.H COH	CH <sub>2</sub> OH HO.H H.OH HO.H H.OH COH

The *relative* arrangements of the component units of molecules are determined by an arbitrary standard, because there is no known way of ascertaining the *absolute* configuration. If, therefore, we adopt in any pair a standard formula for the dextro form, the formula of the lævo form will, of course, be the mirror-image of that of the first; and the standards for the several pairs will, in their relations to one another, be established upon the same basis. In addition to this differentiation in accordance with the *qualitative* effects upon polarized light, the various dextro and lævo forms differ in their *quantitative* effects; and, moreover, each form may exist in more than one form. Inasmuch as each asymmetric group has a rotatory value of its own, dextro or lævo, it seems obvious that when a compound contains a number of such groups the rotatory power must correspondingly be influenced. This conception led van't Hoff to the assumption that when a compound contains a number of asymmetric groups the rotatory power is the algebraic sum of the rotational values of the several groups. Both Guye and Walden have confirmed, but Rosanoff has disputed, this assumption; but the whole subject of the causes of the quantitative differences in optical activity is under discussion.

For many years it was believed that the asymmetric carbon atom is specific to living matter, just as was held in respect to organic substances until Wöhler in 1828 synthesized urea. He in common with Darwin, van't Hoff, and almost every discoverer or expositor of some great and revolutionary truth or doctrine was subjected to intemperate criticism, and so insistent were his critics that when all attempts failed to locate the mysterious vital entity *in vitro*, the proposition that the necessary living factor lay in the presence or manipulations of the chemist certainly carried destructive criticism to its ultra-microscopic limit. Since Wöhler's time, hundreds and thousands of organic substances have been synthesized *in vitro*; and it has likewise been found that the asymmetric carbon atom is an atom not merely of life, but of nature. In fact, so impressed were chemists with the specificity of the carbon atom in respect to living matter that its very presence was regarded as sufficient of itself to determine whether or not a given substance was of vital origin. This is well-illustrated in the instances of petroleum, bitumen, and asphaltum, whose origin had been a matter of speculation for generations; but which, since Ragusin has shown that petroleum is optically active, has been considered settled.

Starch is given the molecular formula  $n(C_6H_{10}O_5)_n$ , and were it known, or had we good reason for believing, that the molecule is a complex polymeride of preformed groups such as  $C_6H_{10}O_5$ , or of larger or smaller groups, as is commonly stated, the number of possible stereoisomers could readily be determined were the molecular weight known. Assuming that the latter is as high as 25,000, as set forth upon the basis of apparent osmotic pressure, the number of stereoisomers would be a matter of hundreds rather than of thousands. But, as stated in the preface, there is abundant evidence to be convincing that starch is not a uniform substance and that it may exist in countless different forms, and hence that the word starch should be used to express not only an individual, but also a group of substances which have certain fundamental typical characteristics in common, but which differ from each other in individual ways.

The existence of starch in a vast number of different chemical forms can be accounted for: (1) in forms of isomers having structural formulæ which differ so little that all have the typical characteristics of a given prototype; (2) in forms of stereoisomers; and (3) in mechanical mixtures of two or more isomers or stereoisomers. Starch, like cellulose, is undoubtedly a complex or aggregate of molecular units of great molecular weight, high carbon content, and extreme complexity of molecular configuration. According to the very recent experiments of Fouard (page 83) with a *non-colloidal* solution of starch, the lowest possible molecular weight is 15,000. Figures by former observers ranged usually between 20,000 and 30,000. Knaffe prepared a body from glycogen ("animal starch") by the agency of chlorine which had a molecular weight of 23,630, from which glycogen was regenerated having a molecular weight of 15,350. Geinsbergen recorded for cellulose 5,508, Bumeke and Wolfenstein 1,944, and Nastukoff 6,480.

The very striking relationships between starch, cellulose, and glycogen in their elementary composition, molecular formulæ, color reactions with iodine, decomposition products in the form of dextrans and sugars, and in a number of other ways that need not be specifically stated, are very familiar to every biologist. Hence, a knowledge of the chemical peculiarities of one substance becomes of direct value in explaining or indicating peculiarities of another. Our information of the chemistry of the starch molecule is very limited, but the common conception that it is a complex polymeride of molecular units which are in the nature of preformed hexose or other similar groups is not only apparently without justification, but is opposed by the logical conception, and by modern literature, that it is an extremely complex *labile* aggregate of unknown groups of *ionic* units. The investigations of Cross and Bevan with cellulose, extending during a period of over ten years, have brought to light many important facts from which deductions may be made in respect to starch. For instance, these authors have shown that there is not a single cellulose, but a group of celluloses that is divisible into subgroups in accordance with their chemical peculiarities. Their classification is based on: (1) the degrees of resistance to hydrolytic and oxidative agents; (2) the per cent yield of furfural when decomposed by boiling HCl.Aq; and (3) the elementary composition in respect to the ratio C : O. The most important findings of a fundamental character may be tabulated as follows:

	Cotton. Subgroup A. Bleached cotton.	Wood cellulose. Subgroup B. Jute cellulose.	Cereal cellulose. Subgroup C. Straw cellulose.
Elementary composition { C.... O.... ratio.	44.0 to 41.4 50 1 : 1.13	43.0 to 43.5 51 1 : 1.18	41.5 to 42.5 53 1 : 1.26
Furfural.....	0.1 to 0.4	3.0 to 6.0	12.0 to 15.0
Other characters:.....	No active CO groups. Pass through the reactions involved in their solutions as xanthate without hydrolysis to soluble derivatives.	Some free CO groups. In xanthate reactions they are partially resolved to alkali-soluble derivatives.	Considerable activity of CO groups. Still less resistant than the preceding subgroup, and more especially the furfural yielding components which are selectively attacked under certain conditions.

These groups were found by Cross and Bevan to "pass by imperceptible gradations into a heterogeneous class of natural products which, while possessing some of the characteristics of the celluloses proper, are so readily resolved by hydrolytic treatment that they must represent a very different constitutional type or types. To this group of complex carbohydrates the name hemicellulose has been assigned," which group is stated to be readily resolved into crystalline monoses. From the foregoing data it is obvious that there are certain forms of cellulose that are not strictly speaking isomeric, and that there are also forms that are isomerides. Cellulose is optically active, and therefore contains one or more asymmetric carbon atoms, and, as a corollary, each isomer may have a number of stereoisomeric forms, the number varying with the number of asymmetric carbon atoms. Reasoning from this, starch may likewise exist as groups which are not strictly speaking isomeric, yet in which the molecules differ so little in their molecular and structural formulæ as to have the essential characteristics of a given prototype; secondly, each group may be made up of a number of stereoisomers; and thirdly, the starches of nature, as observed in the starch-grains of a given plant, may, as it seems certain, be variable mechanical mixtures of two or more different chemical forms.

The number of possible starch stereoisomers is entirely problematical. Miescher has estimated that the serum albumin molecule having 40 carbon atoms may have as many as a thousand million stereoisomeric forms. If we assume that the molecular weight of starch is as low as 15,000, and that the molecular formula is  $n(\text{C}_6\text{H}_{10}\text{O}_5)_n$ , the total number of carbon atoms in the molecule is at least 550. What proportion are asymmetric is unknown, but judging from the relatively high percentage in such comparatively simple substances as the aldohexoses, and the striking tendency for protoplasm during the synthesis of organic substances to form bodies with asymmetric carbon and asymmetric nitrogen atoms, it is probable that nearly all are asymmetric. Moreover, if we conceive, as we should upon the present basis of our knowledge, that the molecule is not a polymeride of preformed atomic groups, but an aggregate of labile groups of ionic units, the possible number of stereoisomers is absolutely inconceivable.

It has already been pointed out that a trifling transposition of elements, groups, or masses attached to an asymmetric carbon atom may cause a marked change in crystalline form, and in optical, chemical, and physiological properties; and also that in stereoisomers changes in the configuration of the molecule, however slight, may give rise to greater differences than may be shown by isomerides which have entirely different structural formulæ. This is a matter of the most profound fundamental importance in connection with protoplasmic processes, and in it we seem to have the key to unlocking many baffling problems of physiology, toxicology, and pathology—not to speak of those of general biology; nor is it necessary to enter into speculation for illustrations of such applications, because many instances in literature, dating from Pasteur's experiments, are at one's disposal.

In earlier pages, experiments of Pasteur were referred to which showed the marked differences in the dextro-, lævo-, and racemic tartaric acids in relation to *Penicillium glaucum*, the dextro form being consumed, the racemic form being split into the dextro and lævo forms, and the dextro form being used, but the lævo form discarded and remaining in solution. It has since been shown that the dextro forms of glucose, mannose, galactose, and fructose are fermentable, or, in other words, consumed by various kinds of micro-organisms as foodstuffs; while the lævo forms are not at all or but inappreciably affected. Again, when mandelic acid (racemic) is subjected to the action of *Penicillium glaucum* the compound is split, the lævo form is consumed, but not the dextro form; whereas, in the presence of *Saccharomyces ellipsoideus*, the dextro form but not the lævo form disappears. With glyceric acid treated with *Penicillium* and *Bacillus ethaceticus*, respectively, the primary splitting process is the same as with mandelic acid, but the *Penicillium* uses the dextro form and the *Bacillus* the lævo form.

These astonishing manifestations of selectivity, which of course mean important differences in the configurations of the molecules and specific relationships of these differences to the protoplasmic structures and nutritive mechanisms of the organism, must be of the deepest fundamental significance. Not only do these organisms thus distinguish, as it were, between the dextro and lævo isomers, but also between different dextro forms. Thus, while *only* the dextro varieties of hexoses are fermentable, *not all* of them are fermentable; and those which are exhibit different degrees of fermentability that vary in specific relationship to their molecular configurations. As has been shown, dextro-glucose, dextro-mannose, and dextro-fructose are closely alike in their configurations, and all three are attacked with readiness; dextro-galactose and dextro-talose are likewise closely related, but the former is fermentable by means only of a specially prepared yeast-juice, while the latter is, so far as known, unfermentable. All of these differences are readily explainable upon the basis of the existence of slight differences in the relative positions of OH groups. The enzyme emulsin is without action on both  $\alpha$ -methyl dextro-glucoside and  $\alpha$ -methyl dextro-galactoside, but it breaks down  $\beta$ -methyl dextro-glucoside and  $\beta$ -methyl dextro-galactoside; whereas, the destruction by yeast is the reverse, the former substance being metabolized but not the latter.

Studies of the properties of stereoisomers from the aspects of toxicity and general physiological actions have afforded interesting and even startling examples of the as yet little appreciated importance of molecular configuration of substances in their relations to protoplasmic processes. A few instances hastily gathered from the researches of various investigators are all that are required at this juncture. Brion, in experiments on dogs with the dextro-, lævo-, meso-, and racemic tartaric acids, found marked differences in the extent to which they were oxidized. He recorded that the lævo and meso forms are destroyed in equal degree, the dextro form to a less degree, and the racemic form to a very slight degree. Pohl, two years before, had also found, in experiments on rabbits and dogs, that tartaric acid (dextro form) is only to a slight extent destroyed in the system. Neuberger and Wohlgenuth, in investigations on rabbits with dextro-, lævo-, and racemic arabinose, recorded that when these substances were given by the mouth the percentages which disappeared were in order, lævo-, racemic, and dextro-arabinose; and they also noted that only the lævo form gave rise to glycogen formation. Beyerinck recorded that the nutritive values of the stereoisomeric tartaric, fumaric, and maleic acids may be markedly different. Nagano noted differences in the absorbability of certain kinds of sugars: Thus, a 2.5 per cent solution of dextro-mannose was distinctly less absorbable than the same concentration of dextro-galactose, dextro-glucose, and dextro-fructose, while lævo-xylose was decidedly more absorbable than lævo-arabinose. With stronger solutions differences were noted in the first three. Mayer found that dextro-mannose is more readily oxidized than lævo-mannose, but that, unlike the difference noted in the enantiomorphous forms of arabinose, both (instead of only one) led to the formation of glycogen. Neuberger and Mayer, in experiments on rabbits with the three mannose stereoisomers, recorded not only that the dextro form is the best in respect to nutritive value, but also that both the dextro and racemic forms are glycogen-builders, the latter being partially split, and all three yielding a derivative of the glucose series. McKenzie found that the dextro- $\beta$ -oxybutyric acid is more readily broken down than the lævo form, and that after giving racemic salts, racemic and lævo acids were excreted in the urine, the dextro acid disappearing in the body.

Instances to show that the configuration of molecules is of great importance in relation to the degree of toxicity and general physiological actions are numerous. Maleic and fumaric acids are stereoisomers, and in the experiments of Ishizuka on dogs it was found that the former is distinctly the more toxic, and confirmatory results are recorded by Kahlenberg and True in experiments with *Penicillium glaucum* and *Lupinus albus*.

Mayer noted, in investigations with dextro and lævo forms of nicotine on guinea-pigs, that the former is only half as poisonous as the latter, and that the two differ markedly in certain respects in their physiological actions. Poulsson, in studies of the actions of the cocaine group, found that the application of a 5 per cent solution of racemic cocaine gives rise to a quicker and more intense but less lasting action than the same strength of solution of the lævo form. Albertoni, in his investigations of the actions of certain drugs on the brain, recorded differences in cinchonine and cinchonidine, which are stereoisomers, the former being the dextro form and the latter the lævo form. The latter is the less active, and its actions differed somewhat from those of the other. Ehrlich and Einborn found that the physiological actions of lævo-hyoscyne (scopolamine) and racemic hyoscyne (atropine) differ. Piutti records that dextro-asparagine is sweet and lævo-asparagine tasteless; and Menozzi and Appiani discovered the same peculiarity in glutamic acid. Werner and Conrad, in examinations of odors, noted differences in the esters of the dextro- and lævo-trans-hexohydro-terephthalic acids; and Schmidt and Tiemann state that the optically active terpenes are more odorous than the racemic forms. Cushny, in experiments with atropine (racemic hyoscyamine) and its optical isomers dextro-hyoscyamine and lævo-hyoscyamine, found "that the two hyoscyamines differ to a marked extent in their pharmacological actions, the lævo-rotatory natural base possessing a very powerful action on the terminations of the nerves of the salivary glands, heart, and iris, while the dextro-rotatory artificial base is almost devoid of effect on these organs, but exercises a stronger stimulating action on the central nervous system of the frog. The action of atropine (racemic hyoscyamine) is the resultant of the actions of its components, lævo- and dextro-hyoscyamine, and it thus affects the nerve terminations about half as strongly as lævo-hyoscyamine, while possessing a more distinct stimulant action on the central nervous system." In another study in which the dextro-, lævo-, and racemic hyoscines were the subjects, it was recorded that lævo-hyoscyne acts twice as strongly as the racemic form on the secretory nerves of the salivary glands and on the inhibitory nerves of the heart; and that lævo- and racemic hyoscines have the same effect on the central nervous system in man and animals and on the terminals of the motor nerves of the frog, in which animal, however, they seem to be without effect on the central nervous system.

Such facts might be materially amplified, but anyone who knows them and has any real appreciation of the meanings of the differences in physiological, pharmacological, and toxicological actions, and of the relations of these differences to the configurations of the molecules, must, if he does not conceive of the stupendous importance of stereochemistry to protoplasmic processes, be absolutely devoid of imagination.

Moreover, the fact that *a given isomeride may be transmuted into another form* under conditions which are identical with or similar to those which exist in plant and animal life is one of apparently the greatest and most widespread fundamental biological importance. It has already been found that many stereoisomers show marked evidences of unstability, tending under certain conditions to be changed with the greatest readiness from one form into another; that the degree of unstability varies, the direction of the change usually being from the more unstable to the less unstable configuration; and that in some, and perhaps in all, instances, the transmutation may be more or less markedly accelerated or inhibited by very simple conditions, as has been shown in the case of certain sugars and related bodies, ethylenes, cobalt-amines, diazo-compounds, chromium and silicon and platinum compounds, and other bodies. Such transformations may be brought about *directly*, whereby one form is changed into another by a simple transposition of molecular components; or *indirectly*, by the formation of an intermediate compound. Direct transmutation may be caused by *spontaneous* change, in accordance with the laws governing the establishment and maintenance of equilibrium of solutions;

by *mere solution* in water; by a *slight increase of temperature*; by the action of *sunlight*; by *crystallization*; by *repeated recrystallization*; by the actions of certain *chemical agents*, such as traces of halogens, alkalies, certain acids, etc.

It has been found in recent investigations that the transmutation of stereoisomers can be accelerated by bases, by salts generally, and by certain acids, etc.; and inhibited by alcohol, phenol, certain acids, and a number of other bodies. The readiness with which such transformations may occur is instanced in the following cases: When  $\alpha$ -glucose in aqueous solution is converted into  $\beta$ -glucose; when maleic acid is heated slightly above the melting-point and the stereoisomeric fumaric acid appears; when the benzyl- $\beta$ -amidocrotonic ester is converted by sunlight into the  $\alpha$  form; when the solution of  $\alpha$ -methylcinnamic acid is crystallized, yielding the  $\beta$  form; when the aceto-acetic ester phenylhydrazone is by simple repeated recrystallization transformed into a stereoisomer having a much higher melting-point and other differences in properties; when in the reversal of the enzymic maltose-dextrose reaction isomaltose appears instead of the initial maltose; when maleic acid is converted into fumaric acid by the agency of the combined catalytic actions of sulphur dioxide and sulphureted hydrogen; and when  $\alpha$ -glucose in solution is transmuted with great rapidity into  $\beta$ -glucose by the agency of a mere trace of alkali, etc.

Perhaps among all of the instances of the transmutation of stereoisomers that might be quoted, none is of broader and more fundamental importance in its bearings in biology than when under certain conditions there occurs a spontaneous conversion of one form of glucose into another when in aqueous solution. The solution of natural glucose is that of an equilibrated mixture of two stereoisomerides,  $\alpha$ -glucose and  $\beta$ -glucose, the former having the lower solubility, but the higher crystallizability and rotatory power. The rotatory power of the pure solution, as found by Dubrunfaut in 1846, lessens with marked slowness, which change has been shown in recent years to be due to the transformation of some of the  $\alpha$  form into the  $\beta$  form. If such a solution be concentrated until crystallization occurs, some of the  $\alpha$  form crystallizes out, thus causing the solution to be thrown out of equilibrium, which disturbance immediately tends to be compensated for by a conversion of some of the  $\beta$  form into the  $\alpha$  form, to replace the moiety of the latter which has passed into solid form. If, now, the preparation be warmed so as to cause a re-solution of the crystalline  $\alpha$  form there will be a conversion of some of the  $\alpha$  form that is in solution into the  $\beta$  form until equilibrium of solution is again established. If, now, we suppose that only one or the other of these forms is especially adapted to the requirements of protoplasm, as is to be inferred from the illustrations previously given, it is clear how through such transformations there may be maintained not only an equilibrium of solution, which often is of such vast importance in vital processes, but also a continuous supply of pabulum in a form that is especially adapted to nutritive exactions.

Such instances indicate not only how readily a given form of stereoisomer may, in order to meet nutritional needs, or because of casual conditions, be transmuted into another form which may have markedly different properties, but also that inasmuch as the conditions existent in living matter are of a highly favorable character to stereoisomeric transformations, that such changes are continually going on, and inferentially of the greatest importance. Coupling the foregoing statements with the recognized extreme lability or instability of protoplasm, it requires no effort of thought to conceive of how the more or less marked and continual changes in conditions, internal and external, to which organisms are subjected may bring about with equal or greater facility transformations in the configurations of the *stereochemic units of protoplasm*, thus altering to a greater or less extent the physico-chemical mechanism, and in turn giving rise to physiological and morphological changes which may be temporary or permanent, and even heritable.

Anyone who is familiar with modern biology, general and medical, need not give more than a few moments' thought to the foregoing facts and deductions without being led irresistibly to the application of stereochemistry to the explanation of the infinitude of enzymic actions which constitute so essential a part of life's processes; of the mechanisms that are concerned in the determination of sex, species, and genera; of the mechanisms of fecundation and heredity; of the changes produced in the labile protoplasm of organisms by the alterations of environment, and their effects on structure and function; of the mechanisms which underlie tumor formation, malformations, and reversions; of the specificities of toxins, hemolysins, and agglutinins; of anaphylaxis; of symbiosis; of bodily idiosyncrasies; of asepsis, infection, and immunity; of the selectivities of different cells and tissues for different substances; of the restrictive activities of hormones in relation to the specific reactive organs; of the specificities of the actions of medicinal substances; of the oft predetermined physiological or pharmacological properties of substances synthesized *in vitro*; of the extraordinary tolerance of pigeons and dogs to morphine; of the differences in the susceptibility of different species and races of animals to disease; of the seemingly chemical identity of the component parts of different forms of lichens which arise from the same algæ; and of a host of other commonplace and special vital phenomena.

These statements, as barren as they are of detail and accompanying facts and observations which would add greatly to their interest, applications, and value, must be adequate to subserve the purposes for which they are intended: that is, (1) to show by the results of laboratory experience that even trivial changes in the configurations of the molecules of stereoisomers may cause more or less marked, or even profound, alterations in the properties of substances in their relations to protoplasm, and of protoplasm itself; (2) to show that different forms of organisms exhibit specific selectivities for substances in accordance with the configuration of the molecules, some organisms utilizing the racemic form and others the *lævo* or *dextro* form, or both, but with different avidities, and also that in a given organism one form is complementary, as it were, to one kind of protoplasm, but not to another; and (3) to give evidence which leads to the inevitable deduction that if there be, as justifiably held, countless stereoisomeric forms of starch, each form differing, however little, in its configuration from that of the others, each must have specific individual properties by which it can, by appropriate means, be absolutely distinguished from the others, whatsoever the number.

Pasteur, in explanation of the selectivity of *Penicillium glaucum* for dextro-tartaric acid, likens it to the mechanical relations of "male and female screws." Fischer, to whom we owe more than to any other individual for our conceptions of the actions of enzymes, states that the explanation of the selectivities of these mysterious entities probably rests in the complementary configurations of the enzymes and the bodies acted upon, an enzyme not affecting any substance that has not a molecular configuration complementary to its own, or, figuratively speaking, when the adjustments are not like those of "lock and key." Ehrlich would conceive of a similar complementary relationship between the configuration of the toxine and that of the protoplasm. And thus one might go on, but this would take us far beyond the necessary restrictions of this memoir.

Yet a final word: The history of starch from the moment of the utilization of carbon dioxide and water to form an aldehyde, through the various steps of synthesis of mono-saccharoses, disaccharoses, and polysaccharoses to the ultimate appearance of starch, and the reversal of these steps when the starch is consumed as food, is upon logical grounds conceived to be one continuous and consecutive enzyme action. The enzymes synthesize starch and its precursors, and no other substances, because they can build up only such chemical structures as have configurations complementary to themselves, each tending naturally to build those forms which have the closest configuration; and likewise each analyzes only such substances as have the same stereochemic relationships. If, as stated, protoplasm uses

only such organic substances as have a complementary stereochemic form, it follows, as a corollary, that starch has a corresponding configuration, and that if starch has such a conformable structure so also must have the enzymes that produce it. In other words, every enzyme formed by any given kind of protoplasm is specifically produced to carry out operations which are directly or indirectly essential to the existence of the protoplasm itself, and must *ipso facto* bear a stereochemic relationship to its mother substance; therefore, protoplasm, enzyme, and product have in common the same fundamental stereochemic peculiarities. In fact, as the results of these researches go to show, every synthetic organic substance produced by any given kind of protoplasm through the agency of its enzymes has a configuration in agreement with that of the protoplasm. If, as must be admitted, corresponding kinds of protoplasm in different organisms differ, the corresponding synthetic metabolites will differ; and, conversely, if the latter differ, so must the former. Hemoglobins, which are corresponding substances, have been shown to differ in specific relationship to genera and species; and the same extraordinary phenomenon has been brought to light in respect to starches.

Therefore, from specific stereochemic differences in corresponding substances we are led to corresponding specific differences in protoplasm; from these to differences in vital processes in general; and from these in turn to those which characterize life's processes in all of their enigmatic phases. Indeed, it is far from visionary to conceive that through the advances of science along the lines indicated it will be found that inasmuch as any given organism, for instance, gives rise to a number of enzymes which are the essential instruments by which the necessary vital processes are directly or indirectly carried out, the sum of the configurations of the enzymes represents a corresponding sum or composite of the configurations of the component units of protoplasm. Therefore, each configuration formula would be as specific of the enzyme and the form of protoplasm as is that of glucose; and the sum or composite of these formulæ would be as specific of the organism as the aldohexose group of formulæ is specific of the aldehydoses. *Hence, we may logically assume that the time may come when any given form of protoplasm or any given organism may be expressed by the physiological chemist as specifically in terms of configuration formulæ as it now is described by the biologist in terms of morphology.*

#### DIFFERENTIATION OF STEREOISOMERS.

Our methods for the differentiation of stereoisomers are, on the whole, inexact or even absolutely crude; and in some instances, as in the preparation, separation, and identification by Fischer and his pupils of members of a single group, such as the aldohexoses, the work is tedious and difficult. Obviously no aid is to be expected by centesimal analysis and, as a consequence, dependence rests upon such procedures as will elicit differences in optical reactions, crystalline form and crystallizability, solubility, melting or gelation point, color, color reactions, digestibility or fermentability, decomposability, toxicity, physiological and pharmacological actions, etc. Optically active substances owe their effects on light to either *inter-molecular* or *intra-molecular* arrangements; that is, in the first place, to the arrangements of the molecules in relation to each other, and, in the second place, to the arrangements of the component molecular units within the molecules themselves. Sodium chlorate, for instance, is optically active when in crystalline form, but inactive when in solution; saccharose is active when in crystalline form, and also when in solution. In the first instance, optical activity is attributable to intermolecular arrangement, the molecules being so disposed in relation to one another as to cause asymmetry; in the second instance, activity is due primarily to the configuration or asymmetry of the units of the molecule itself; and activity becomes more marked in both instances as the number of asymmetric molecules of the substance present is greater.

Starch, like saccharose, is optically active in both solid state and solution. Starch grains are, as a rule, markedly optically active, which activity is due in part to the asymmetric molecules, and in part to the intermolecular arrangements of the starch in the solid form. If grains of potato starch, for instance, are examined in water with the polarizing microscope, and the slide be gradually heated to the minimal temperature of gelatinization, it will be found that gelatinization and swelling will begin at a given point or points, and that the progress of the process can accurately be traced by the corresponding disappearance of optical activity. This alteration is obviously due to a breaking down of the intermolecular structure of the grain; but the gelatinized starch is nevertheless still optically active. Brown, Morris, and Millar found the specific rotatory power of 2.5 to 4.5 per cent concentrations of soluble starch at 15.5 C. to be  $(\alpha)_D = +202$ . In differentiating isomerides which are in crystalline form, the melting-point, or, in other words, the temperature at which the intermolecular organization is broken down, is one of the most important means of distinction. Hence, as the starch-grain is a spherocrystal, the temperature of gelatinization must be regarded as being equally important in the differentiation of starch stereoisomers. In various parts of this memoir, especially in Chapters IV and VI, the various methods employed in the differentiation of starches are given adequate consideration to meet the conditions of the investigation.

#### CONCEPTIONS, METHODS, PLAN, AND CONDUCT OF THIS RESEARCH.

Opinions may differ widely in regard to various features pertaining to the conceptions, methods, plan, and conduct of this research. Thus, it might be held that such differences as have been noted in different starches might be attributable to mixtures of a unit substance which may exist in multiple forms having variable physical but not chemical properties, but such a view finds insignificant support in the literature of starch, or in the results of this research; while, on the other hand, the evidence points positively to the existence of a vast number of stereoisomers, which, however, may be multiple in the starch of any species or even in any given starch-grain. As regards the methods selected, choice was based in part upon the results of recorded work, and in part upon deductions upon general principles. The histological method, in use for two centuries, has shown that starch-grains from different plants appear in a great variety of forms, and that peculiarities of form may be definitely related to the genus or species, etc.; the polariscope has demonstrated differences in the degrees of polarization, differences in the interference figure or "cross," and differences in reaction in the presence of the selenite plate; iodine and anilines have been found to vary in their reactions with different starches; temperatures of gelatinization have been recorded by Lippmann and others as ranging within wide limits; the degrees of digestibility of both raw and boiled starches are stated by different authors to vary markedly; and a number of "swelling reagents" have been found to elicit differences in minute histological structure, as well also in the intensity of action.

In the preliminary work of this research it was found that digestion experiments, whether upon raw or boiled starch, were of no value in detecting differences in the starch-substance *per se*. Apart from this method, all of the others enumerated were used. The selection of the particular anilines and swelling reagents was based to only a minor degree upon the results of experience. It is quite probable, indeed almost certain, that better results are to be obtained by other reagents; but this must be shown by experience. It has been taken for granted that the phenomenon of swelling, whether brought about by heat or chemical reagents, is a manifestation of adsorption affinity that is a specific property of the starch, and variable in relation to the molecular configuration and intermolecular structure of the different hypothetical stereoisomers; and, conversely, that quantitative and qualitative differences in the reactions to these agents are indicative of corre-

spondingly different stereoisomers, which is entirely consistent with the facts embodied in the literature of adsorption-affinities—or whatever term one may use to express the very conspicuous and important *residual-affinities* that exist in substances in which, according to the laws of stoichiometry, the affinities are satisfied; or, in other words, in which there exists *chemical saturation*.

Neither heat nor any of the other gelatinizing and swelling agents used (chloral hydrate, chromic acid, pyrogallic acid, ferric chloride, and Purdy's solution), except probably chromic acid, causes any apparent notable degree of decomposition of the starch molecule during the periods of observation; but they do change the raw starch into a distinctly different constitutional form by altering the intermolecular arrangements. The individuality in the behavior of the chromic acid, coupled with its peculiarities, leads to the belief that the first effect of this reagent is a constitutional transmutation, as above stated, which is probably followed by a series of oxidation processes. No doubt more or less decomposition is brought about sooner or later by pyrogallic acid and ferric chloride, both of which substances are in concentrated solution; but with Purdy's solution the decomposition, if any, that may be caused by the small amount of alkaline hydrate present need not be considered. It is manifest from this that these methods of gelatinization represent essentially a group of a number of different groups of chemical methods which are available for the study of the changes in the starch molecule and its derivatives through all of their ramifications of changes of constitution and decomposition to the ultimate  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , etc.

Doubt may exist as to whether or not the primary objects of the research would not have been better accomplished by a study of a less number of specimens by a larger number of methods, especially by methods akin to those employed by Fischer and his pupils in the separation and identification of stereoisomers, and by Cross and Bevan in the study of cellulose. But this alternative plan, for certain reasons, was practically impossible; and, as regards the one adopted, it was believed that a few methods would be sufficient to elicit such fundamental differences as might be necessary in a preliminary investigation, and that the larger the number and variety of starches the better the idea of what is to be expected from the really serious research that must follow. Then again, it might seem that a study of a large number of members of a single genus, or of representatives of quite a number of genera of only a single family, might be sufficient as an index of what is to be anticipated in other corresponding groups; but the wisdom of the plan adopted of examining in a number of instances, as in *Liliaceæ*, *Amaryllidaceæ*, and *Iridaceæ*, representatives of a dozen or more genera, and as in *Lilium*, *Narcissus*, and *Iris* quite a number of species and varieties, is clearly shown by the records. And it is also evident that the examination of representatives of scattered families and genera has brought fruitful results.

#### ASSISTANTS AND SOURCES OF SUPPLY OF MATERIAL.

The very exacting demands upon the head of one of the most important departments of a first-class medical school, and the enormous amount of labor required in an investigation of this character, made it necessary for the author to assign certain laborious and routine parts of the work to assistants. At the inception of the research it was believed by the writer, who is not a botanist, that the best results were to be obtained by the constant assistance of an expert botanist. To this end an arrangement was made with the late Dr. Louis Krautter, instructor in physiological botany in the University of Pennsylvania, but whose tragic and lamentable death occurred before the investigation had been started. The author secured the material, laid down the methods and lines of investigation, directed the work, and made the photomicrographs which accompany the histological and polariscopical descriptions, but the routine laboratory studies of the properties of the starches were of necessity but regretfully assigned to assistants. Nearly all of this

laboratory work was done by Dr. Elizabeth E. Clark, B. A. (Bryn Mawr), M.D. (Women's Medical College of Philadelphia), who was well fitted for such an investigation by her training in the laboratories of these colleges, and who devoted two years to the investigation. Dr. Clark made all of the studies recorded in Part II, with the exception of a group including the studies of *Vicia*, *Lathyrus*, *Quercus*, *Castanea*, *Lilium*, *Tulipa*, *Convallaria*, *Amaryllis*, *Crinum*, *Sprekelia*, *Hæmanthus*, *Hymenocallis*, *Leucoium*, *Crocus*, *Sparaxis*, *Curcuma*, *Maranta*, and *Zamia*, and some incidental investigation here and there. Practically all of the studies included in the latter group, and also the determinations of the *temperatures of gelatinization*, were made by Miss Martha Bunting, B. L. (Swarthmore), Ph.D. (Bryn Mawr), whose painstaking work in various laboratories is so well known to biologists as to render an introduction needless. The author has also received help from Dr. Clark and Miss Bunting and other assistants in getting together the literature quoted, and in other ways; and for three and a half years he has devoted to this research all of the hours that could possibly be taken from the very exacting requirements of professorial work.

It goes without saying that, inasmuch as the author is not a botanist, the botanical data given are based upon other and recognized authorities. Free use has been made of the voluminous work of Engler and Prantl, *Die Natürlichen Pflanzenfamilien*, begun in 1889, but unfortunately still incomplete; of the *Index Kewensis*; of Warming's *Systematic Botany*; and of many other standard authorities, especially of the admirable four-volume *Cyclopedia of American Horticulture* by Bailey, which often has been followed quite closely. The classification and nomenclature of plants are undergoing continual change, and it has been puzzling at times how best to identify plants and to state relationships, but the data of this character found at the heads of the various chapters have been based on the highest authorities. The brief descriptive introductory notes on the different genera, species, etc., will doubtless prove of value to many readers in refreshing their memories, as well as in other ways. Altogether over 300 starches were studied, a goodly number, but ridiculously small when one recalls to mind that the total known number of plant species is over 230,000 (over half of which are *Monocotyledones* and *Dicotyledones*)—not to speak of the thousands of varieties, hybrids, etc. Most of the material from which the specimens were prepared was obtained from E. H. Krelage & Son, Haarlem, Holland; James Veitch & Sons, London; Henry A. Dreer and Henry F. Michell & Co., Philadelphia; J. M. Thorburn & Co. and Peter Henderson & Co., New York; Bobbink & Atkins, Rutherford, New Jersey; and Reasoner Brothers, Oneco, Florida. Some were secured from or through the Botanical Department of the University of Pennsylvania by the courtesy of Prof. J. M. Macfarlane (to whom the author is indebted for help in many ways), and quite a number from dealers and growers in various parts of the world.

#### GENERAL CHARACTERS OF THE INVESTIGATION AND RECORDS.

A glance through the pages of this memoir will be all that is necessary to convince the reader of the superficiality and general crudeness of the investigation; and that the records of the laboratory studies of the properties and differentiation of the various starches are of a *purely tentative character* and therefore, for comparative purposes, in the nature of merely *temporary standards*. It is obvious that if data are to be had that are to be accepted as *constants*, certain absolutely essential conditions which have not been sufficiently recognized in this work must be satisfied. As, for instance, every plant from which starch is obtained must positively be identified botanically; the starch must be so prepared as to eliminate, as far as possible, without injury to the molecule, all contaminating substances; examinations should in every instance be made with two or more specimens obtained from different plants of the same species; the influences of age, environment, and

other conditions must be determined; the time-reactions of the effects of each reagent should be repeatedly observed, as often as occasion demands, in the case of each specimen under given conditions of experiment in order to establish mean standards, and so on. However, if the three fundamental objects stated in the beginning of this chapter have been fulfilled, and if by throwing open the shutters investigators may perceive roads and pathways leading to the elucidation of the extraordinarily important relations that exist between stereochemistry and protoplasmic processes, the labor has not been in vain.

In the final arrangement of this report it has been found to be a mechanical necessity to carry the detailed laboratory records into a separate volume, and these accounts now compose Part II of this publication. This arrangement, it is hoped, will make this matter easy of reference, although the first impression may be that they have been taken out of their more logical position.

## CHAPTER II.

### THE STARCH-SUBSTANCE, AND THE STRUCTURE, FORM, AND MECHANISM OF FORMATION OF THE STARCH-GRAIN.

The wide distribution of starch in plant-life, its great food-value to both plants and animals, and its extensive field of usefulness in domestic life and in many of the arts, sciences, and trades, have made this substance a subject of study for generations. In 1836, Poggendorff, in reviewing the literature of starch, wrote:

No substance has been more investigated and yet less known. It affords a striking proof of the diffuse manner in which a subject may be treated if it fall into improper hands. After ten years of investigation, in which the most various views have been set up on the nature of starch, during which all its characteristics as a proximate vegetable substance have been discussed, we are little or nothing in advance of the old point of view; and, although perhaps we may not be wholly without some extension of our knowledge on secondary points, we are still entirely without fundamental grounds in proof of our having arrived at the truth.

Even as late as 1885 Brown and Heron wrote:

There is probably no one subject in the whole range of chemistry which has attracted more workers during the last 60 years than the transformation which starch undergoes when submitted to the action of diastase or dilute acids; and in no respect are the opinions of chemists, even at the present time, more at variance.

As recently as 1895, Meyer, in his elaborate memoir on starch, states that our knowledge of the chemical substances which compose the starch-grain and the products of decomposition is very meager.

Notwithstanding the accumulation of an exceedingly voluminous literature and the many advances in our knowledge of the chemistry of starch since the time of Poggendorff, there are doubtless not a few biochemists who will hold that the statement of this investigator and critic is applicable in a very large measure to our information at the present day.

The synthesis, proximate constituents, microscopical structure, and molecular constitution of starch, and the exact processes and products of the disintegration of starch through the actions of dilute acids, alkalis, heat, oxidizing agents, enzymes, etc., are but few of the very many instances of important problems which remain partially or wholly unsolved. However, there are sufficient data pertaining to the subject-matter of this chapter to show that starch is produced only in certain plants and in certain parts of plants, it being substituted by some analogous substance or substances in non-starch-producing plants or plant parts; that the starch-substance from different sources is not identical, but exists in a number of forms; that the starch-grain is produced by specific starch-forming structures which are more or less markedly differentiated, not only in different plants but also in different parts of the same plant; that the forms and other structural characteristics of the starch-grain differ in certain ways in specific relationship to the peculiarities of the starch-producing structures and other specific intracellular conditions; that the starch-grains produced by any given part of the plant may be very variable in size, form, and other structural features, yet as a whole exhibit a certain type; that the starch-grain is a spherocrystal, and therefore has properties which render it available for study by certain crystallo-

graphic methods; and that the chief or essential primary proximate decomposition products, and conversely probably the essential final synthetic substances, are erythrodextrin, achroodextrin, maltose, and dextrose.

VARIOUS VIEWS OF THE NATURE OF THE STARCH-SUBSTANCE, AND OF THE STRUCTURE, FORM, AND MECHANISM OF FORMATION OF THE STARCH-GRAIN.

Although starch was known to the ancients, as shown by the writings of Dioscorides, our knowledge of this substance had its origin practically in the microscopical examination by Leeuwenhoek in 1716, by which he differentiated two fundamental structural components. He found that when the grains were heated in water the inner part disappeared, leaving nothing but the integuments (Hüllen); and that in the excrement of birds that had fed on grain the same integuments could be found. From these observations he concluded that starch consists of a kernel or nucleus which is fit for nourishment (nährenden Substanz), and an outer, insoluble non-nutritive envelope. From this time until the early part of the last century no material addition was made to the literature of starch, at which period an era of research was initiated by Vaquelin (1811) and Kirchoff (1811), the former finding that when starch is subjected to torrefaction it is converted into a gummy substance soluble in water; and the latter discovering that weak acid changes starch into gum and sugar. The results of these investigations were confirmed by a number of contemporaneous experimenters.

During the following twenty-five years, however, very little was added to our knowledge apart from certain discoveries relating to the decomposition products and to the agents which give rise to them. In 1819, De Saussure (Ann. de chim., 1819, XI, 379) found that after setting raw starch aside for two years there were present a sort of paste and sugar; a substance insoluble in cold water, but soluble in hot water, and giving a blue reaction with iodine; and a body that was insoluble even in hot water, and which closely resembled cellulose. To the substance soluble in hot water he gave the name *amidine*, and to the cellulose-like body the name *ligneux amylicée*.

Some years later Raspail (Ann. de sciences natur., Oct. et Nov. 1825, et Mars, 1826; quoted by W. Nägeli, Der Stärkegruppe, Leipzig, 1874) reported that starch when heated on a plate is converted in part into a sort of gum that is soluble in cold water, leaving a residual insoluble sheath. The former stained blue with iodine, but the latter not. Raspail's statement was at once disputed by Caventou (Ann. de chim., 1826, XXXI, 358), who recorded that all parts of the grains stain blue with iodine; that raw starch does not contain a substance that is soluble in cold water; and that when starch is subjected to dry heat at 100° it is changed into a soluble substance resembling the amidine of De Saussure, which substance he looked upon as a modified starch (*amidon modifié*).

It was then shown by Guibourt (Ann. de chim. et phys., 1829, XL, 183) that raw starch does contain a substance that is soluble in cold water. He found that when starch-grains have been comminuted in a mortar they are rendered partially soluble in cold water; that this solution yields a blue reaction with iodine, and that upon drying it yields a residue that is insoluble in cold water. The soluble substance he identified with the amidine of De Saussure and the gum of Raspail, which latter, however, he holds is not a gum, as stated by Raspail. He records that there is still another component (the integuments) which stains blue with iodine, but is insoluble in cold water.

Following Guibourt's article there appeared four contributions by Guérin-Varry (Ann. de chim. et phys., 1834, LVI, 225, and LVII, 108; 1835, LX, 32; 1836, LXI, 66). In the first of these Guérin-Varry states that starch consists of three substances: One (*amidine*) that is soluble in cold water; another (*amidin soluble*) that is insoluble in cold water by itself, but which is held in solution by amidine; and an insoluble substance (*amidine tégumentaire*), the integuments or capsules of the grains. The amidin tégumentaire was

found to constitute 2.96 per cent of the starch, while the remaining 97.04 per cent was recorded as being made up of 60.45 parts of amidine and 39.55 parts of amidin soluble.

Payen and Persoz (Ann. de chim. et phys., 1834, LVI, 337) regarded the starch-substance as a single body, which they refer to as *amidone*.

Guérin-Varry in his second article states that the amidone of Payen and Persoz is a mixture of two substances, one soluble and the other insoluble in cold water; and in his third article he studies the actions of diastase on starch, and the characteristics of starch-paste and the sugar formed, and he refers to the great resistance of raw starch to the action of malt extract. He placed a preparation of raw potato starch and malt extract in a sealed tube, keeping it at a temperature of 20° to 26° C. for 63 days, and found upon microscopic examination at the end of this period that the grains showed no alteration. In his fourth article he further considers the three components of starch, as held by him (see also Chapter III, pages 86 and 177).

Fritzsche (Ann. d. Physik u. Chemie, 1834, XXII, 129) seems to have been the first to study the form and structure, and the mechanism of formation of the starch-grain. He, in the first place, takes exception to the assertion of Raspail that every grain is composed of two substances, one an enveloping membrane or integument (*Hülle*) which is insoluble in water, and another part which is intractantegumentary and soluble in water. The author's studies were mostly made with potato starch, but confirmed by observations on other starches. He states that the grains of potato starch have a variety of forms and sizes, and that the normal shape is that of a somewhat compressed, oval structure. A universal and constant characteristic, he records, is the presence of concentric rings which vary in distinctness, regularity, arrangement, and number. These rings proceed from a spherical point which, on account of its peculiar chemical relation, he terms the kernel or nucleus (*Kern*) of the grain (in later years and at present known as the *hilum*). The hilum was not always found to be located at the center, but might be anywhere in the long axis of the grain. From the transparency of the hilum he was led to believe that it is a funnel-like hole, which he thought proved by the fact that when a grain is compressed between glass plates the hilum retains its position and canals are never observed to run from this point peripherally. About this spherical hilum rings are arranged, the inner ones usually uniformly, and the outer ones spread out more on one side of the grain than the other, producing an oval shape. Every grain he states is composed of as many concentric layers as there are rings.

Fritzsche then considers the question as to whether the outermost layer is produced first in the form of a skin (*Haut*), and then the inner part by infiltration; or whether the hilum is the first part formed and the layers deposited on it. This he found most easily answered by examining compound grains which are found among the simple grains, and which he states may be regarded as deviations or monstrosities. Observations of these grains led him to conclude that all grains are formed by the deposition of the outer layers upon the inner. The compound grains he believes have arisen through the union of two grains in apposition by means of the deposition of a common combining layer, or by a smaller grain being inclosed by a layer of a larger grain during the process of growth of the latter, such grains always having a small, distinct space present between the fused grains. The outermost layer of the starch-grain was found to have a special density, by which it can resist external influences better than the inner layers and he gives this as the reason why unbroken grains are insoluble in water, and why crushed grains are soluble. The reason for the greater density of the outermost layer he attributes to its being in contact for a long time with the cell-sap which contains a large amount of vegetable albumin; or that the grains may be covered with a precipitate that could easily be formed from an albuminous body. He believes that differences in the densities of the layers account for their visibility, and that the variations in density are perhaps owing to the influence of



if starch be rubbed for a period of half an hour in a mortar with double the volume of water, there is formed a viscid stiff "salve," capable of being drawn into threads. A large number of the granules when seen under the microscope appeared to be crushed in various ways, and partly ground into small flakes. The inner layers are combined with more water by friction, exhibiting a fine floecular or granular but connected mass, which is colored blue with iodine, while all the water surrounding the mass remains wholly uncolored. On heating the grains upon a small plate he observed that one can trace gradual changes brought about by heating, and that thus may be found the best explanation of the structure of the grains. The first action is one of drying, by which the so-called nucleus or hilum is converted into an air-bubble. The several layers separate simultaneously, and in consequence of inspissation the lines of separation become sharper, darker, and broader, and also even recognizable broader and narrower layers of air, the layers hanging closer together at some places than at others. By degrees the separate layers peel away from each other like the scales of a bulb. If the grains are boiled in water their outlines grow more and more distinct, but the particles cling together in the form of a paste-like mass. Under the microscope, by means of iodine, he found that we may recognize the separate and swollen granules, while the water added is never colored blue. He believes from these phenomena that while starch may take up a large quantity of water, it can never be properly dissolved. The layers of the starch-grain he regards as being more aqueous as they lie further inward. By testing with iodine he noted that all parts of the grain are stained equally, and that while there may be slight differences in the external layers in relation to solvents, differences which he believes arise from the adhesion or infiltration of traces of albumin, fat, or wax, such differences merely delay the action of the solvents.

Schleiden studied the phenomena of erosion and formation of the grains in the potato. During erosion the grains retained their solidity to the last moment, and were only gradually attacked from the exterior inward, the extremities of the longitudinal sections offering the greatest resistance, on which account the grains after a time resembled knotty twigs. In young growing potatoes he found exceedingly minute granules and large grains, the former being the more numerous. He states that if we regard the minute granules as the rudiments of structure, and take the different sizes as indexes of their age, the younger the granules the more truly spherical they appear, the ovoid or irregular outline being subsequently acquired, and the deviations from the original form being caused by the unequal thickness of the outer layers. The innermost layers continue to exhibit the original spherical form which the youngest granules present. Starch therefore grows, he concludes, by the deposition of outer new layers upon the inner older layers. This he found confirmed when starch from the other plants were compared with grains from the potato, as, for instance, grain from *Dieffenbachia seguine*. Schleiden also noted that the forms of starch-grains are exceedingly various, and he made a tabular list of grains from various sources based upon histological peculiarities (see page 64).

The view that starch-grains grow by the external apposition of layers was asserted by Walpers (*Botanische Zeitung*, 1851, ix, 329) to be disproved by the fact that in twin grains, which occur so abundantly, the hila are not located near the line of fusion of the component grains, but always near the circumference; and that the formation of "between-layers" which takes place at the line of contact is not explained by deposition of layers from without. The theory of external apposition he states is contradicted by the fact or assumption that the most outer layer is the oldest, and the layers within the youngest, in succession from periphery to center. The outer layers he states are usually the densest, yet they must be able to stretch to attain the maximum size of the grain. Walpers holds that to adjust such an indisputable contradiction, two possible answers present themselves: 1) the outer older layers grow simultaneously with the growth of the interior, which can not be the case, because the lamellation indicates an

intermittent growth; or (2) in many kinds of starch both external and internal lamellation go on independently. The latter assumption he holds explains the peculiar structure of starch-grains often found in Chile arrowroot (*Alstrameria*), but rarely found in potato starch, in which several common dense layers surround a fused group of starch-granules.

Maschke (Jour. f. prakt. Chemie, 1854, LVI, 400), from studies of the structure of wheat starch, concluded that the grains have the form of membranous vesicles, the peripheral layer consisting of cellulose, which in starch-paste does not stain blue with iodine. He heated starch in water to various temperatures. At 40° C. the grains or vesicles showed large numbers of well-defined rings which are alternately light and dark; at 60°, instead of intact rings, outlines of smaller grains were noticed in the center of the large grain; at 70° breaks and cracks were found, due to the swelling of the vesicles; and at 100° the grains were rather irregular in shape, resembling collapsed bags. On the addition of iodine he noted in the blue mass, granular brown-colored lumps. These phenomena can be explained, he states, if one assumes that every grain is composed of from 3 to 5 vesicles placed concentrically one within the other, and between them the granular amylopectin or starch. The appearance and disappearance of so many rings he attributes to the swelling and the separation of the granular amylopectin, ultimately leaving the 3 to 5 circles which are the envelopes of as many concentrically arranged vesicles that compose the grain. He believes that he has proved the existence of such an arrangement by means of the action of iodine and sulphuric acid. The internal part of the starch he conceives to consist of a soluble and an insoluble substance, and that by evaporation the former is converted into the latter, whereas by solvents, such as lye or hot water, the reverse occurs. These two modifications of starch, therefore, are believed by him to preëxist in the grains. The explanation of the light and dark rings, he writes, is to be found in these two modifications of starch, the light rings representing the insoluble modification and the dark rings the soluble modification. The hilum he describes as a central hollow of the innermost vesicle, which hollow may, owing to drying, be without contents, or it may be filled with amylopectin in solution. The insoluble starch, he assumes, is present in granules around which the soluble form of starch is present in liquid form.

The view that starch-grains grow by external accretion, and that the outer layers are the youngest and last formed, received further support in the investigations of Crüger (Botanische Zeitung, 1854, XII, 41) of the starch-grains in the plant-cells of *Philodendron grandifolium*, *Dieffenbachia seguine*, *Batatas edulis*, and other plants, the starch of *Batatas edulis* being especially suitable for the study of compound grains. He endeavored particularly to determine where and how the outer layers are formed. In a number of the plants examined the younger cells contained only round grains, whereas in older plants there were large grains of various irregular forms, from which facts he believes that the forms found in the older cells were round originally and developed into larger grains which depart during growth from the spherical form because of the deposition of layers on the outside. He makes the observation that all starch-grains are formed upon the protoplasmic layer which lines the inner surface of the cell. The starch-grain is formed here, he holds, as long as it is capable of further growth and as long as protoplasm exists in the cell. In all kinds of starches with definite layers and definite eccentric hila it was seen that the hilum is always located in the part of the grain farthest removed from the point of attachment of the grain. When the part of the starch resting upon the protoplasm or chlorophyll was examined it was found that the outside layer at this point behaved differently with iodine from other parts of the grain, for while the mass became *blue* this outer layer turned *yellow* or *dark brown*, the same color as the protoplasm or chlorophyll assumes, but it did not take the stain readily. This outer layer is described as being of varying thickness in different starches, in some hardly perceptible; and he regards it as a substance which is in the process of becoming starch, and that it probably contains nitrogen or albuminous material.

Small grains treated with iodine were colored *yellow* or gave no color reaction; and young grains reacted very slowly, and the blue color was not so pronounced as in the older grains. The parenchyma cells of *Batatas edulis* were found to contain large numbers of single starch-grains. In later stages of development the grains by enlargement approach each other, and ultimately form groups with the flattened surfaces of the grains in contact with each other. At this stage the transition substance was still perceptible, but in older cells it was absent. A compound grain he therefore regards as being nothing more than two or more single grains which develop singly, and that later, owing to the disappearance of the transition substance between them and by the development of a common outer envelope, become one compound grain.

The divergent views as to whether the starch-grain grows by a stalactite-like deposition upon the outer surface or whether the grain, like the wood- or bast-fibers, increases in size by a deposition within, led Harting (*Botanische Zeit.*, 1855, XIII, 905) to an investigation, from which he found that in the early stages of development of the starch-grain an integument is present, this being especially prominent in the starch of the potato tuber. This was colored *brown* with iodine, not blue like the starch-layers beneath it. According to his view the integument belongs to the "epigon cell" which produces the starch-grain. In the mature grain an integument was no longer discernible, all layers now being colored blue with iodine. He believes that it must be this integument, or the integument of the epigonen or mother cell, which holds the groups of grains together in such plants as *Smilax sypilitica*. The similarity after absorption of water of the "cambial layer," or outer layer of the starch-grain, to the cross-section of the cambial layer which produces wood- and bast-fibers, is so striking that he believes one is warranted in the supposition that it is the outermost layer of the starch which is the cambial wall of the cell, and that the deeper layers are formed later, and that while new layers are being formed on the inner surface of the previously formed structure, the latter and the cambial wall increase in size through intussusception.

Reinsch (*Neue Jahrbuch. f. Pharm.*, 1855, III, 65) states that the granules of potato starch contain dextrin and sugar already formed, which can be dissolved in water from the pulverized grains.

Melsens (*Institut, Ire sect.*, 1857, xxv, 161; quoted by W. Nägeli, *Der Stärkegruppe*, Leipzig, 1874) found that when starch-grains were treated with weak acids, pepsin, or diastase they become so changed that they no longer yield a blue reaction with iodine, yet retain their form, therefore having a non-starch skeleton or framework.

In 1858 there appeared the elaborate monograph of Carl Nägeli (*Die Stärkekörner. Morphologische, physiologische, chemisch-physicalische und systematisch-botanische Monographie*, Zürich, 1858, 25 Tafellen, 625 S.) which covers a wide field of inquiry. To this author chiefly is due the conception, which even to the present day receives almost universal acceptance, that the starch-grain consists fundamentally not only of two substances, *granulose* and *cellulose*, but also that they have markedly different properties. The differentiation of these components was brought about by subjecting raw starch to the action of saliva for several months at 45° to 55° C. The larger portion of the grains was slowly dissolved, while the remaining part was found to retain the form and structure of the original grains. To the former he gave the name *granulose* and to the latter *cellulose*, which latter he thought identical with the substance of the same name of plant structures, and which he regarded, when obtained in this way, as being in its purest form. He looked upon the grains as being mixtures of *granulose* and *cellulose* in which these substances are combined in the form of a sort of a diffusion. The proportions of the two substances, he records, differed in different kinds of starch and in the different layers of the same grain, but the quantity of *granulose* was considerably greater than that of *cellulose*, the latter being present often in very small quantities, probably representing only one-eighth

of the entire mass. The quantity of cellulose was found to be in direct proportion to the resistance of the grains to swelling and solvent media. For this reason, he concludes, the dense layers contain relatively more cellulose than the soft layers, and the external more than the internal parts. The cellulose, he states, is not insoluble, but difficultly soluble. Besides granulose and cellulose the grains were found to contain water, both in the fresh and air-dried condition, and also "condensed gases;" but usually no other substances were present in perceptible quantities, although sugar, dextrin, and soluble starch may exist in small amount. He thought that large quantities of gases are condensed in the grains, which idea was doubtless suggested by the evolution of gas during decomposition processes. Differences in the reactions of starch granulose and cellulose with iodine were recorded: The granulose was found to take up iodine from a weak solution and to become blue before the cellulose becomes colored, and this occurred even though the iodine solution had to penetrate the cellulose to reach the amylose. While the amylose was colored a bright red to a blue or a black, according to the quantity of starch present, pure cellulose was colored a dull-red, or a brownish-red, which differences formed a means of distinguishing one from the other.

Studies of the mechanism of development of starch-grains led Nägeli to the view of growth by intussusception, and hence to oppose the theory of growth by external accretion that was held by many of his predecessors. It must be remembered, he writes, that all grains in every stage of development are solid, and that only in abnormal cases, owing to solution, are they hollow. If growth takes place by deposition on the outside, then the young grain and the inner layers of the large growing grain must be identical in form, structure, and substance. While the forms of the two are very similar they nevertheless differ very markedly, there being present in the interior of large growing grains "layer complexes" which are never found in the small mature grains. In all kinds of starch, without exception, the substances of the young and of the large growing grains are different. In the large grains there are present, from the periphery to the center, alternate dense and less dense layers. During no stage of the development is there a soft layer on the outer surface, but always in the growing grain a dense peripheral layer. Swelling solvent media, such as hot water, acids, and alkalis, act on the inner substance of large grains, as well as on the "part-grains" of "half-compound grains" (see page 66), and dissolve this substance, while the small mature grains are not acted upon. The external layer in all stages of development of the grain is the same, so that solvents disorganize and dissolve the entire inner substance in both large and small grains in a similar manner, but leave the outer layer in the form of a membrane. Young grains up to a certain stage are entirely homogeneous, but after a time layers become evident in the interior of the grains. In other grains, which in earlier stages show no structural differentiation, inclosed part-grains appear whereby such grains become half-compound grains.

The phenomena of form and structure of compound grains, Nägeli holds, are also in opposition to the apposition theory of growth. The part-grains increase in size by growth along the inner and not the outer radius. If growth occurred from outside there must be pressure exerted on the outside of the grains, but the compound grains show no trace of such external pressure, since the part-grains have sharp edges and corners and flat surfaces. Not only, he states, is growth not by apposition, but the half-compound and many of the compound grains do not originate by the fusion of simple grains, but both are produced by internal processes, the originally homogeneous substance becoming lamellated and divided into part-grains. He regards it unlikely that there may also be a deposition on the exterior of the grain, because the peripheral layer of large and small and young and old grains is identical in its resistance to the action of solvents, and because one would have to assume that the external layer, as it is covered by the newly deposited substance, changes its nature, and that as the layers are deposited each in turn takes on

the properties of the external layer. He believes that the conditions which regulate the growth of the grain by intussusception bear a very close relationship to the physical and chemical properties of the grains. This he sums up in the following law: The more rapid the growth in any part of the grain, the softer the substance at that point, and the more readily it is acted upon by swelling solvents, and since the chemical composition runs parallel with growth, the larger will be the amount of granulose in relation to cellulose. The least growth, Nägeli holds, takes place in the exterior part of the grain, as can be seen in both simple and half-compound grains, which grains from the earliest stages do not increase in thickness but grow in the same plane. While the outer layer under all circumstances is the densest and richest in cellulose content, the small young grains, unlike the mature grains, consist of a dense substance throughout. In these grains there appears at the center a soft hilum which is a very small point, and which increases in size and density, while the surrounding substance does not change much in density.

In a later contribution (*Botanische Zeit.*, 1881, XL, 633) Nägeli again discusses the mechanism of formation of starch-grains, and adheres to the theory of growth by intussusception. He assumes that the differentiation of the substance into lamellæ is present in all starch-grains, and that the homogeneous appearance of some grains is due to the optical appliances not being sufficiently powerful to perceive a lamellation which may be exceedingly indefinite and indistinct. That the grains are solid can be demonstrated, he states, in the sprouting of starch-containing plant parts, in which the grains, like inorganic crystals, dissolve from the surface inward until they disappear completely, during all stages of which solution the grains are solid. The variations in the densities of the lamellæ he attributes to differences in the water-content. The lamellæ are not of uniform thickness throughout, and the inequalities he states may be distributed regularly or irregularly over the entire layer. Very often two or more layers fuse into one, or one layer may split into two or more. Such splitting takes place principally in the dense layers, but it may occur in the more watery loose layers. A thick lamella which on one side is simple may on the other side be split into several parts of equal density, between which there may appear parts of less density. Complete and incomplete lamellæ are arranged about a common central point which sometimes is the mathematical center of the grain, but usually eccentric for the entire grain and concentric only for the inner layers. The form of the individual layers and the arrangement of the lamellæ from the center to the periphery, in other words, the structure of the grain, he states are due to the form of the hilum. In grains with a lenticular hilum the short axis of the grain is in a line with the shortest diameter of the hilum, which is centrally located, and the layers are usually of uniform thickness. In grains with an elongated hilum, the axis of the greatest diameter of the grain is in line with the greatest diameter of the hilum, which is central, and the layers are rather uniform. In spherical grains the hilum lies at the center of the grain, and the layers are always circular, and have a uniform thickness. All radii of such grains are equal. Another type of grain is one in which the hilum is spherical and eccentrically located, and in which the axis passes through the center, on one side of which it extends through the longer radius and on the other the shortest radius of the grain. Such a type is the potato starch.

The principal forms of grains with eccentric nuclei are: (1) spherical with the hilum between the center and the periphery; (2) elongated (lanceolate to conical), with uniform ends or one end more attenuated, the hilum being located at either the narrower or wider end; (3) wedge-shaped, elongated (1 to 3 times as long as wide), compressed on one side, narrow and not compressed on the other side, where the hilum is located; (4) expanded (0.66 to 2.5 as wide as long), compressed, the hilum located near the narrow end; (5) irregular forms with projecting edges and angles.

Nägeli notes that in some instances lamellated grains have several hila, with as many systems of lamellæ. Such a grain appears as an aggregate of several lamellated part-

grains which are surrounded by a common lamellated envelope. A grain of this type may be said to be "half-compound." These part-grains number 2 to 10, rarely 25 to 40, in one grain. If they become relatively large, and the common enveloping layers are thin, a transition type between the simple and the compound grain is formed. If the lamellation becomes rather indistinct, the half-compound grain resembles a single grain, but it can easily be distinguished from the latter by the presence of several hila. Half-compound grains occur usually in grains with eccentric hila. The structure of the part-grains is similar to that of the simple grains. The part-grains of a half-compound grain are usually simple, but rarely are half-compound, each with several hila (see Chapter V).

In another part of the memoir Nägeli studies the formation of starch-grains in "chlorophyl vesicles." Starch-grains, he writes, occur not infrequently in the granular or vesicular protoplasmic structure of the cell-contents, but they are constant constituents of the chlorophyl vesicles. The formation of the grains within the chlorophyl, he found, can often be traced. The grain is described as appearing in a homogeneous green mucus in the form of small points that increase in size and often attain sufficient dimensions to be recognized as starch-grains. In some instances these particles remain rather small, and during their entire development are inclosed entirely by the chlorophyl; in other cases they steadily increase in size, gradually push out through the surrounding chlorophyl, and finally lie free. Grains lying together in the same vesicle may become flattened on their contact surfaces and be united as a compound grain. Such stages of development were observed in the leaf of *Begonia dichotoma*. In the oval chlorophyl vesicles of *Nitella synearpa* 3 to 10 white points were seen and recognized as white starch-granules, very minute in size and not becoming larger. In young cells of *Chara hispida* the chlorophyl vesicles are arranged in series and are polygonal in shape. They contained 1, 2, or 3, rarely 4, starch-grains, which color blue with iodine. In older cells the chlorophyl grains are considerably larger, with their margins more rounded. In such cells the chlorophyl vesicles are entirely filled with starch-grains, containing 1 to 4, rarely 7, starch-grains, which are in contact with each other, and at the surface still covered with a thin layer of chlorophyl. In still older cells the chlorophyl had disappeared entirely. The chlorophyl vesicles are arranged along the cell-wall, and they are compressed, with the flat surfaces in contact with the cell-wall.

The starch-grains at first, without coming in contact with each other, lie side by side in the same plane, and they grow chiefly in the plane parallel with the cell-wall, and become more or less tabular. Later they touch each other, and their ends become superimposed upon each other. The young grains are perfectly homogeneous, but later one or more points are noticed which are probably areas where solution has taken place. Chlorophyl vesicles in the leaf parenchyma of *Begonia* sp. are perfectly homogeneous at first, and consist only of green-colored protoplasm. After they have increased in size one observes from 2 to 7 shining dots. In still larger chlorophyl vesicles there are 1 to 3, very rarely 6, starch-grains which are recognized by means of iodine. With the growth of the starch-grains the green protoplasm associated with the formation of the grains becomes more and more replaced by starch, and finally instead of protoplasm one observes only starch-grains. In the younger stages the chlorophyl vesicles have 3 to 7 small dots, while among the mature grains none consists of more than 3 part-grains. The explanation of this phenomenon is that either an unequal number of starch-grains originates in a chlorophyl vesicle at different periods of growth, or, as in *Phipsalis*, some of the points which are visible in the early stages are oil droplets; or it may be that the chlorophyl vesicles divide, as in *Chara* and *Nitella*.

Studies of the solubility of raw starch, and also of the structure of the starch-grain, were made by Jessen (Ann. d. Physik u. Chemie, 1859, cvi, 497), who states that one can easily convince himself of the solubility of raw starch by crushing the grains of potato

starch, maranta starch, etc., in a little water, when the beginning of solution is soon noted, for the entire mass becomes viscous and mucilaginous. On the addition of more water a clear solution is obtained, on whose surface float torn and broken "integuments" of the starch-grains, while the unbroken grains sink to the bottom. The filtrate shows no precipitate, but becomes blue with iodine. Jessen agrees with Harting (page 23) that the starch-grain has a structure like that of cells, and that the concentric lines on the grains are cell-walls or condensed layers between which the soluble starch is deposited. In a later research (page 29) Jessen records that starch consists of three substances: the integuments, starch soluble in cold water, and starch insoluble in cold water.

Wicke (Ann. d. Physik u. Chem. 1859, cviii, 359) holds, in opposition to many previous observers, that pulverized starch-granules are almost entirely insoluble in cold water, only very small portions going into solution.

The statement of Nägeli that the cellulose of the starch-grain remains as a residue after the solution of the granulose seemed to von Mohl (Botanische Zeit., 1859, xvii, 225) to be not well-founded. Von Mohl therefore undertook a series of experiments to prove or disprove this point. He used chiefly the starch from the rhizome of *Canna indica*, and for the extraction of the substance (granulose) which was colored blue with iodine, he utilized saliva. At a temperature of 35° to 40° C. the extraction of the soluble substance began slowly, and proceeded regularly from the periphery of the grain to the center. At a temperature of 50° to 55° the extraction was completed in several hours. An examination of the grains from which the soluble constituents had been thus removed showed that they had lost in weight; that they float more readily in water; that they are less refractive to light; and that they are smaller, but just how much was difficult to determine. The lamellation underwent no change, except that in many cases, as in sprouting wheat, it became more evident during the process of solution. The grains from which the soluble matter had been extracted by saliva behaved exactly towards polarized light as do unchanged grains.

Von Mohl opposes Nägeli's statement that granulose and cellulose can be differentiated by iodine. He states that whether iodine produces a red or a blue color depends neither on the fact that the object stained may be starch (granulose) or cellulose, nor on the amount of iodine present, but essentially on the behavior of the organic substance toward water. If a small amount of water is absorbed a red coloration ensues; if a larger amount, there is a blue coloration. One can produce, he states, a beautiful blue in cellulose, and a red or violet in starch (granulose) without bringing about a chemical change in the objects colored, the coloring being regulated by the amount of water absorbed. It is therefore clear, he holds, that the blue coloration of cellulose with iodine is in no wise proof that the cellulose is changed wholly or partly into starch; and, moreover, that iodine furnishes no means whatever of differentiating granulose from cellulose.

Furthermore, von Mohl holds it can be demonstrated, from both physical and chemical standpoints, that the substance (cellulose) remaining in starch-grains after treating the grains with saliva is *not* identical with the cellulose of plants. The so-called cellulose of the starch-grain, he states, is very brittle, while plant cellulose is tenacious to a remarkable degree. The effects of polarized light on the two are opposite. Caustic potash dissolves starch cellulose instantly, but swells up plant cellulose, and dissolves it only after a number of hours. In nitric acid and muriatic acid starch-grains are dissolved at once, but plant cellulose is dissolved only by boiling. Other chemical reagents showed similar contrasts in their effects on the two substances. Von Mohl proposes the name *farinose* as a substitute for the term cellulose of Nägeli.

The nature of the substance of raw starch that is soluble in cold water was also examined by Delffs (Ann. d. Physik u. Chemie, 1860, civ, 648), who macerated comminuted starch-grains in water for 24 hours, after which a clear liquid was filtered off which gave a blue reaction with iodine. The soluble constituent he regards as being a

form of dextrin, which he thinks differs from the three forms derived by the action of malt extract, sulphuric acid, and torrefaction, respectively. He views this substance as being in the nature of a starch-building material, and he discusses in this connection the mechanism of the formation of the starch-grain. He writes that if one assumes the starch-grain to be an organized body which grows by intussusception there must be present a substance which is soluble and which can enter the grains by osmosis. To serve the function of starch-building no substance is more suitable for the purpose than the various kinds of gums and dextrans, because they on the one hand have the proper solubility, and on the other belong to a group that is isomeric with the insoluble starch-substance, to which group belongs the soluble constituent of starch. This substance which may be built up into starch he states may be termed *amylogen*, and he holds that, if these views are correct, the question as to the age of the various layers of the starch-grain is solved, for the outer layers must be regarded as the oldest, the greater age and density of the outer layers protecting the soluble contents of the grain.

Objection was made by Knop (Chem. Centrablatt, 1860, v, 367) to the statement of Jessen and of Delffs that by crushing the starch-grain a gelatinous substance is dissolved out by cold water. Knop believes that the heat generated while rubbing the grain is sufficient to cause gelatinization; but Jessen (Ann. d. Physik u. Chemie, 1864, cxxii, 482) showed later that the ground pulp has a temperature of only 22°, and therefore, in opposition to Knop, not sufficiently high to cause gelatinization.

Shortly after the investigations of Jessen, Delffs, and Knop, Flückiger (Zeit. f. Chemie, 1861, iv, 104), in a brief article, showed that pulverized starches from various sources are partially dissolved, especially in the presence of calcium chloride.

According to Dragendorff (Jour. f. Landwirthsch, 1862, vii, 211), starch consists of 3 or 4 components: (1) a base which remains after starch has been heated to 60° in a concentrated solution of sodium chloride in a 1 per cent muriatic acid; (2) true starch which is insoluble in cold water; (3) Schultze's amidulin (Delff's amylogen); and (4) occasionally dextrin.

The influence of light on the formation of starch in chlorophyll granules was studied by Sachs (Botanische Zeit., 1862, xx, 365), who concludes that starch is to be regarded as a product of the assimilative activity of the chlorophyll substance, this activity, being due to the agency of strong light, the starch being built up from CO<sub>2</sub> and water in the presence of mineral salts, and being distributed to growing buds and storage centers. He believes that starch formation occurs through a series of transformations in the chlorophyll bodies, and that the starch of non-green parts of plants has wandered from the green parts.

In a later article (*ibid.*, 1864, xxii, 289) Sachs reports the results of experiments in connection with the formation and disappearance of starch in a number of plants. He found that starch which originates in chlorophyll granules under the influence of light disappears when the plant is withdrawn from the light; that starch formed during the day is partly dissolved during the night; and that the entire starch-content disappears in the dark in 48 hours. In accordance with his results, he assumes that during a summer night of 8 hours one-sixth of the starch is dissolved. It would seem, he states, that these reversed processes should throw light on the mechanism of the formation of starch-grains.

From a study of the chemical properties of starch in relation to various solvents, especially haloid salts, glycerol, and saliva, Kabsch (Zeit. f. analyt. Chemie, 1863, ii, 216) held that the assumption is not justified that starch consists of two different substances, one of which is real starch (granulose) and soluble in saliva (Nägeli and von Mohl), in dilute acids (Melsens), and in cold water after crushing (Reinsch, Jessen, Delffs, and others); and the other substance (cellulose according to Nägeli, and farinose according to von Mohl) insoluble in these media. The grains he found appear at first very small, solid, and granular, and during growth become more or less condensed, and arranged in layers which

vary in density, the outermost and densest part resisting all action of chemical substances longer than the less dense layers by which the latter are surrounded and impregnated.

In further investigations of the constituents and decomposition products of starch, Jessen (*Jour. f. prakt. Chemie*, 1868, cv, 65) concludes that starch-grains are composed mainly of three constituents: (1) the envelopes, or cell-membranes, or integuments, which are insoluble in hot or cold water (the amidine tégumentaire of Guérin-Varry); (2) the starch-substance, or *amylogen*, which is soluble in cold water (the amidone of Payen and Persoz; the amylogen of Delffs; and the amidine of Guérin-Varry etc.); (3) starch which is insoluble in cold water, but soluble in water at 55° to 80° C., which may be called *amylin* (the amidin-soluble of Guérin-Varry, and the amylin of Maschke, Delffs, Melsens, and Fr. Schultze). Other constituents were found to be present in very small quantities, and in ordinary analyses some of them may be entirely overlooked. Some of the minor constituents, he states, are dextrin (in wheat, pointed out by Maschke); chlorophyl and wax (in potato, according to Guérin-Varry); nitrogen (0.1 to 0.25 per cent); fat, soluble in alcohol (0.001 per cent in potato and 0.0005 to 0.006 per cent in wheat, according to Rousseau).

The envelope, Jessen states, forms only a small part of the grain (2.96 per cent according to Guérin-Varry; 5.7 per cent in potato, 3.1 per cent in maranta, and 2.3 per cent in wheat according to Fr. Schultze; and 4.8 per cent according to Payen and Persoz). By continued boiling the envelopes pass more or less into solution, and they behave generally like other cell membranes and give the reactions for cellulose. Often they became blue in the presence of iodine, owing, he assumes, to adherent particles of starch. He states that when these are removed the envelopes are colored with iodine only upon the addition of chloride of zinc, dilute sulphuric acid, etc.

The proportions of amylogen and amylin Jessen gives as 58.68 per cent and 38.38 per cent respectively. He accepts the figures of Guérin-Varry (60.45 and 39.55 respectively), although he asserts that the proportion of amylogen is probably less. Both substances yield a blue reaction with iodine. The amylogen exists chiefly in the inner parts of the grains, and it can be obtained by placing the crushed grains in cold water. The inner layers he believes to be the youngest. Starch-paste he holds is neither a simple substance nor a chemical compound, but a mechanical mixture of all of the constituents of the grains. Amylogen, he found, passes into dextrin very readily, far more readily than amylin; and the transformation occurs in a pure solution at room temperatures after 2 to 3 days. Whether or not amylogen and amylin of different kinds of starches are different, he does not know; and he states that very little is known about the composition and properties of amylogen, and that in chemical composition amylogen and amylin may be the same.

Sachs (*Text-Book of Botany*, 1875) adopts in modified form Nägeli's view of the two proximate constituents of starch. He states that every grain of starch consists of starch, water, and inorganic substances. The starch has the same percentage composition as cellulose, to which it bears the greatest similarity of all known substances in both chemical and morphological properties. It occurs in the grain in two forms: one, granulose, which is the more easily soluble and which gives a blue reaction with iodine; and the other, starch-cellulose, which shows less solubility and which comes nearer to cellulose. Both occur in every part of the grain. If the granulose is extracted, the cellulose remains behind as a skeleton which shows the internal organization of the whole grain, but is less dense, and its weight represents only about 2 to 6 per cent of the weight of the whole grain. The internal organization of the grain he holds is not homogeneous, and that it differs in relation to the varying proportions of water in the several parts. Every part of the grain contains water, the amount usually increasing from without inwards, and attaining a maximum at a fixed point in the interior. With the increase in the proportion of water there is a decrease in cohesion and density, and also in refractivity, on which partly the

power of perceiving these differences depends. The outermost, most dense, and least watery layer is succeeded by a more watery layer beneath, and this in turn is followed by a still more watery layer, and so on until the innermost and least dense and most watery layer surrounds a very watery part, the nucleus.

Sachs believes that the hypothesis of the growth of the starch-grain by intussusception alone affords an explanation of all of the phenomena arising during the growth of the grain. New particles of formative material become, he thinks, intercalated between those already existing, both in radial and tangential directions, by which means the proportion of water at the points of deposition is lessened. Did the formation of layers occur by external deposition, grains would be formed in which the outermost layer is the most watery; but this never occurs, because the outermost layer is always the least watery and the most dense. According to the view of growth by apposition, he contends that the nucleus would possess the properties of young grains, which are dense, whereas in mature grains the nucleus is always soft. The theory of growth by apposition he believes could be accepted to explain only the formation of partially compound grains if we were to assume that the common layers which inclose the single simple grains had been deposited about them, in which case the common layers would have a different form and the fissures in the interior of such grains would remain unexplained.

In 1874 a monograph appeared by W. Nägeli (*Beiträge zur näheren Kenntniss der Stärkegruppe in chemischer und physiologischer Beziehung*, Leipzig, 1874, S. 106) in which he treats especially of the effects of dilute acids on starch, and of the means of preparation and the properties of *amylodextrin*. (See Chapter III, p. 114.) He notes that while starch in a natural condition is insoluble in water, it becomes soluble after long soaking, or when the grains are broken; and that there are several modified forms of starch which are characterized by their different powers of resisting solution and by their reactions with iodine. In the solid state these forms are said to take color in the order of blue, violet, red, orange, and yellow, as their power of resistance to solution and their affinity for iodine increase. Iodine added to a starch solution will always turn it blue, because, he states, on boiling with water the other modifications gradually go over into the blue-reacting form. This latter form, being the more readily soluble, possesses the power of absorbing the less soluble modifications; the disappearance of the former precipitates the latter. The various kinds of starches, he states, may be distinguished by the different proportions of these modified forms. The difference in the substances composing the starch groups may, he believes, be a chemical one, but that it more likely rests on the physical condition of greater or less distribution.

Musculus (*Botanische Zeit.*, 1879, xxxvii, 345) accepts the view of C. Nägeli that starch consists fundamentally of two substances (granulose and cellulose), but he goes on to show that the cellulose (referred to by him as amylo-cellulose) is nothing else than an insoluble modification of granulose. Granulose can by drying, he states, be transformed into cellulose, and cellulose can be transformed by sodium hydroxide into granulose. If alcohol be added to the sodium-hydroxide solution of cellulose, a gelatinous precipitate results which, after repeated washing, shows all of the properties of granulose. This precipitate is completely soluble in hot water, and the solution is colored blue with iodine. If the precipitate is dried, it undergoes a partial transformation into cellulose.

The first of an important series of contributions which have had a marked influence on our views, even up to the present, was published by Schimper (*Botanische Zeit.*, 1880, xxxviii, 881; 1881, xxxix, 185, 201; 1883, xli, 121), in which he studies the structure and mechanism of formation and other properties of the starch-grains, and also the specific starch-forming structures of various plants. He records in the first article that if the chlorophyl-grains are spherical, the starch-grains may appear at all points of the chlorophyl-grains; but if the form is plate-like, the starch-building is limited to the equatorial zone,

and that such chlorophyl-grains may produce six or more starch-grains. Starch-grains which are produced within the chlorophyl-grain, and remain surrounded by it, attain a concentric structure, and most grains originating in this way remain very small and without definite structure. In *Vanilla planifolia* the mature starch-grains consist of hundreds of colorless, polyhedral granules of equal size, which originate as very small points in the chlorophyl, and become larger, and at the same time polyhedral through mutual pressure. Starch-grains which develop at the periphery of the chlorophyl-grain show a differentiation into hila and lamellæ. Such grains are always eccentric, and the growing side of each grain is that to which the chlorophyl-grain is attached.

It is obvious, Schimper states, that the unequal growth on opposite sides of the hilum is the result of unequal "nourishment." Such a conclusion, he finds, is supported by the fact that where starch-grains are partly in contact with chlorophyl-grains they become gibbous at the points of contact. Starch-grains developing in flat chlorophyl-grains are wedge-shaped at first, being flattened in the same manner as the chlorophyl-grains. Where starch is produced very actively, the chlorophyl-grain assumes an isodiametric form, decreases in density and later in size, until finally it is present as a mere remnant, while at the same time the starch-grain becomes denser and assumes an oval shape. With the disappearance of the chlorophyl the growth of the starch-grain ceases. In chlorophyl-grains capable of producing starch-grains within their entire mass, the starch-grains may appear near the surface of the chlorophyl, and later on break through. Such grains are eccentric.

Schimper's examination of non-green starch-bearing plants showed that starch-grains are not surrounded by ordinary protoplasm, but are inclosed by or attached to refractive, spherical, or spindle-shaped bodies which are but little affected by alcohol and are rather unstable. A study of the youngest stages of these bodies (later designated *leucoplasts*) demonstrated their presence before starch-grains are formed and that the starch-grains, at their appearance and during their development, indicate similar relations to these bodies as are borne by other starch-grains to chlorophyl granules. The starch formed in these albuminous-like bodies has an eccentric structure like the eccentric grains produced by chlorophyl-grains, and the entire behavior of these bodies is like that of chlorophyl-grains, and therefore they are starch-builders. Schimper then gives the mechanisms of formation of starch by starch-builders in non-chlorophyllous plants and refers to the formation of starch in roots as an instance of starch-building by these colorless bodies, or leucoplasts. He also noted that in most cases the colorless starch-builders may, under the influence of light, be transformed into chlorophyllous bodies.

In the second and third communications Schimper (*Botanische Zeit.*, 1881, xxxix, 185, 201) goes more in detail into the processes and stages of development of starch-grains in different plants. He found that the grains growing in many chlorophyllous plants show certain constant peculiarities. Tabular grains have irregular lobes and are laterally depressed, and at times are porous; their broad surfaces are uneven and present a spotted appearance, owing in part to surface structure and in part, in many cases, to internal vacuoles. These phenomena he ascribes to partial solution, which occurs when starch is used for the growth of the organ in which the starch-grains are formed, as in germinating seeds of *Zea mays*. Even after the starch-bearing organs cease to grow, or slacken growth, starch is formed. There may originate spherical structureless grains; or corroded grains already present in the cells may take on new growth, which occurs in the form of a layer around the corroded grain, thin at first, but gradually becoming thicker and more refractive. This layer shows the projections and depressions of the corroded grain. Layers subsequently formed go through the same process, but the roughness of the surface becomes less pronounced as new layers are deposited, so that the mature grain appears smooth; but by suitable illumination the original corroded grain may be seen in the center of the mature grain.

Schimper states that the processes of formation of starch-grains were very similar in the several plants studied. The most important stages in the development he gives as follows: (1) the appearance of starch-grains in the form of strongly refractive bodies; (2) the differentiation of the originally homogeneous grain into a central watery hilum and a dense peripheral layer; (3) in later stages the surrounding of the hilum by three layers, the middle always being watery (a watery layer never occurs at the outside, and such a layer probably originates by an alteration of an original dense layer); (4) the number of layers increases, and the outer is always the most dense; (5) the watery content of the inner parts of the grain increases with the increase in the volume of the grain.

Schimper found that pressure on starch-grains causes the formation of numerous cracks, which in simple grains are perpendicular, but never parallel to the lamellæ. The cohesion of the starch-grains varies in a very striking manner with the line of direction in which the pressure or strain is exerted—in a tangential direction it is very small, but in a radial direction it is great. In the tangential direction he holds that there is no elasticity of the substance, whereas in the radial direction it is marked.

Schimper refers to the observation of previous investigators that in the swelling of starch-grains in water the water is stored much more abundantly in a direction parallel than perpendicular to the lamellæ, and he states that one reason for this is that the cracks, originating during the drying of starch, always run at right angles to the lamellæ, and that cracks would also appear in other directions if the water were distributed equally in the grains. The fact that the water is stored in larger quantities in a tangential than in a radial direction causes tension in the grains. The grain is assumed to consist at first of a homogeneous substance of uniform density. If the ever-increasing tension resulting from the unequal storing of water reaches a point where the elasticity can no longer withstand the tension, the substance of the center of the grain must expand and assume a condition of greater swelling, and become less refractive. Observation shows, he states, that when the grain has attained a certain size during swelling, the feebly refractive but much swollen hilum appears at the center. The formation of the hilum is therefore brought about by the action of tension, this tension being caused by unequal deposition of water molecules, and not (as was assumed by Nägeli) by the deposition of starch molecules. The formation of the hilum decreases the tension of the grain, but with the storing up of new substance in the dense layer around the hilum the tension increases until it finally is sufficient to overcome the elasticity. The result is that the dense layer is differentiated into three layers—a soft middle layer and dense inner and outer layers. The dense outer layer behaves just as the first layer, and when its tension reaches a certain intensity it also undergoes division into a soft inner layer and a dense outer layer. As a result of the storing of new substance in the grain the inner parts are expanded, the soft layers increase in density, and the dense layers increase their water-content as they become softened, and, consequently the inner parts of the grain are less resistant to swelling and solvent media than the outer parts.

Schimper holds that the form of the starch-grain is determined by the manner of "nourishing." Concentric grains result, he states, when they are completely surrounded by the starch-producing substance or chloroplast, while eccentric grains are found when the plastid is in contact with a part of the surface of the growing grain, the most rapid growth taking place at the point or points of contact of the starch-forming substance. Flat grains with a central hilum originate in lens-shaped chlorophyll-grains, and their flat sides are parallel to the flat sides of the chlorophyll-grains. Elongated starch-grains are formed in spindle-shaped chlorophyll-grains. The different shapes of the starch-grains can be explained only by unequal "nourishment." If we try, he states, to conceive the manner in which the grain is nourished by its mother substance, we imagine the latter to be a form of solution which impregnates the chlorophyll-grain. The starch-grain and the chlorophyll-grain which forms it do not lie in the cell-sap, but are embedded in the protoplasm.

In later communications by Schimper (Botanische Zeit., 1883, xli, 121; Jahrbücher f. wiss. Bot. 1885, xvi, 7) studies are made especially of the origin and functions of chlorophyll-grains and homologous structures. Leucoplasts, he writes, originate in the vegetating cells of the plant, and but rarely from chloroplasts. Certain fruits which are green in the young stages and white in the mature condition contain leucoplasts which develop from the chloroplasts, as, for instance, in the white fruit of the snow-berry, *Symphoricarpus racemosus*. Leucoplasts are widely distributed and serve as starch-builders; rarely are they functionless, as in the roots of *Dahlia*. Many leucoplasts may be transformed into chloroplasts, especially when they are exposed to light of sufficient intensity, but some leucoplasts are incapable of this change. In non-chlorophyllous starch-producing plants, or parts of plants, the starch is formed by leucoplasts.

Referring to chromatophores, Schimper records that they assume very different forms, and that it is difficult to discover an external relation between them. The green tabular chloroplasts, the colorless (usually very delicate) spherical leucoplasts, and the chromoplasts with manifold forms differ widely from each other. Nowhere is the capacity shown by living substance to assume such diverse forms and properties as is exhibited by these simple protoplasmic structures. One and the same chromatophore, as a leucoplast, can form and store up starch from assimilated materials, or as a chloroplast can decompose carbonic acid and produce organic substance from carbon and water, or as a yellow or red chromoplast can fill the passive rôle of attraction for animals. Chromatophores originate exclusively from preëxisting chromatophores. In the simpler plants, as in *Chlorophyceæ* and *Diatomacææ*, leucoplast formation is a subsequent process, that is, chromatophores which contain pigment are transformed into colorless chromatophores, or leucoplasts; but in higher plants the opposite process takes place. This condition in the simplest plants leads to the belief that leucoplasts are to be regarded as metamorphic forms of pigmented, assimilating chromatophores. The chromatophores of a large number of *Angiosperms* form protein crystals, and the shape of the chromatophore is often due to the presence of these crystals, and thus the shape and structure of the starch-grain are correspondingly influenced. In *Phaius* (orchid), starch-grains are produced which have an eccentric structure and some are triangular in shape. These are developed on an elongated chromatophore, the elongation being due to the presence of protein crystals.

That these crystals thus indirectly influence the shape of starch-grains is also shown in the rhizomes of some *Zingiberacææ*. In such plants the chromatophores of the rhizomes always produce elongated, spindle-shaped, or needle-like crystals, and these cause the odd structure of the starch-grains. In the rhizomes of *Canna*, large, flattened, triangular, markedly eccentric starch-grains develop. These grow on leucoplasts which form two kinds of crystals—flattened octohedra and needle-like. The grains lie both on and within the chromatophores, and in the latter location attain an eccentric structure which is influenced by the presence of crystals. Colorless bodies, *pyrenoides*, are found in the chromatophores of many *Algæ*, and appear to act as nuclei of the chromatophores. They bear a close relation to starch-formation. In these plants, the starch-grains which originate within the chromatophores appear first and in the largest numbers about the pyrenoides, which they surround in the form of a shell. Observations indicate that the pyrenoid substance is in the nature of a reserve material that is used by the chromatophore in starch-building, and that they are probably crystals, since they behave like protein crystals of the higher plants.

Schimper also believes that in young cells of certain of the lower plants, cell nuclei furnish material for starch-formation before the chromatophores are developed; but starch subsequently appears everywhere in the cells about the chromatophores, by which time the nuclei apparently have ceased to yield starch-forming material.

Chloroplasts and pyrenoids in *Algæ*, chloroplasts and paramylum bodies of *Euglenæ*, and pyrenoids in bacilli were reported by Schmitz (Die Chromatophoren der Algen, 1882; Jahr. f. wiss. Bot. 1884, xv, 1).

Vines (*The Physiology of Plants*, 1886) assumes a difference in the functions of chlorophyll corpuscles and leucoplasts in that in the former the synthetic processes begin with such simple substances as CO<sub>2</sub>, water, and salts, and are effected under the influence of light, whereas in the latter they begin with tolerably complex substances, such as asparagin and glucose, light not being essential.

The contention of Schimper that starch-grains are formed by apposition brought forth another contribution by C. Nägeli (*Botanische Zeit.*, 1882, XL, 633) in defense of the intussusception theory. Nägeli writes that he was led to the adoption of the intussusception theory because in the originally dense grain there appears a soft hilum, and that later, when the dense layers have attained a certain thickness, there is inserted a soft layer. He assumes that the starch-grain is composed of very small invisible particles (which he here refers to as *micellæ*) which have a crystalline character and grow as crystals do, and which attract water to their surfaces. If unilateral pressure is applied to the grains, tangential as well as radial cracks form. The cohesion of the grain he holds is less in the radial than in the tangential direction, on account of the alternate dense and soft layers; and the cohesion of the single layers is also greater in the radial direction. For various reasons it seemed to Nägeli that the soft layers possess a gelatinous, brittle consistency, and are not semifluid, and therefore that cracks might occur in these layers, which would not be the case were they semifluid. Stains and solvents in small quantities, he states, penetrate the starch-substance and are deposited in varying proportions. Such deposition is dependent on two causes: on the relation of the stain and the solvent to the starch and the peculiar micellar constitution of the starch-substance, and on the dynamic action which is conditioned by the constitution of the starch-substance. The molecules of the stain he conceives to remain dissolved in the imbibitional liquid, or extracted from it and deposited in the starch micellæ. That different stains behave differently is shown by the dissimilar relations of starch-grains toward the same stain in different solvents, and by their relation towards different stains in the same solvent. Starch-grains in their natural condition, he found, may not take stain, while the swollen grains take an intense stain. This he explains upon the assumption that unchanged starch substance offers a greater resistance to the deposition of stains because of the regular and compact arrangement of the micellæ. In the swollen, disorganized starch substance the micellæ are disarranged, and therefore lack the power of resistance against foreign molecules.

Nägeli believes that the softer parts of starch can not be regarded as a paste-like substance disorganized by swelling, because the cohesiveness of the grain prevents such a disorganization. Tension in the starch-grain which surpasses the limit of elasticity brings about a rendering of the substance, but not a swelling. In grains dried and again moistened, cracks were found evident only in the unchanged starch-substance. By slow action of artificial swelling agents, cracks appear, and disorganization takes place in proportion to the strength of the disorganizing medium. He holds that if the negative tensions which result from the growth-processes within the grains are to be explained satisfactorily by the storing of water (as Schimper believes), and not by a storing of starch-substance according to the intussusception theory, the formation of the soft center or hilum and the softer layers can not be accounted for, and by such an explanation the interior of the grain must be disrupted by cracks.

Nägeli also attacks Schimper's view that water is stored more abundantly parallel than perpendicular to the lamellæ, and that on this account different conditions of tension are caused. He contends that the starch-grain at every stage of its growth is surrounded by a watery liquid, by an intermicellar water-containing system, whose tensions are always at an equilibrium. When the grain is dried cracks are formed, which shows that the equilibrium is disturbed by drying; and the cracks have a radial direction, crossing the layers at right angles, which is proof that more water is lost in a tangential rather than in the

radial direction. He found that when artificial swelling media act upon the starch-grain slowly, the volume of the grain is increased and radial cracks are formed which indicate that during this process more water is stored radially than tangentially.

The existence of tensions Nägeli deduced from the theory of growth by intussusception. Starch-grains in which radial cracks appear on drying do not lose the cracks on moistening, but on the other hand the cracks become larger and hence more evident. The lamellæ of the grain in its natural state have a tendency to store more water tangentially, and therefore grow chiefly in length and breadth, and this tendency is more pronounced in the external layers, so that these in contrast with the inner lamellæ have a positive tension. He states that if this were not the case, on moistening the dried grains the cracks would become imperceptible. Tensions could not occur, he holds, in growth by apposition. If we imagine a starch-grain to lie in the cell-protoplasm or in the cell-liquid, it is in contact with water, and it has absorbed as much water as it can, because the micellæ attract as much water as their molecular force can hold. The particles of micellæ are formed in such a manner that there is an equilibrium between the particles of starch and water. The tensions determine the separation of the soft center or hilum and the softer layers of the grain, and this is brought about by the simultaneous growth by intussusception.

Another fact that Nägeli assumes aids in disproving the apposition theory is the presence of a thin peripheral layer which possesses different properties from the rest of the grain. He states that when this outer layer is very thin it does not react with iodine, and that when somewhat thicker it is colored a reddish-violet, while the part inclosed by this layer always becomes a deep blue. This peripheral layer likewise resists the action of solvents (acids, etc.), while the entire inner mass is dissolved. Since both large and small grains have this peripheral layer or "membrane," growth by intussusception, according to Nägeli, is the only possible explanation of the mechanism of formation of the starch-grain.

The hypothesis proposed by Schimper that the starch-grain is a spherocrystal of a carbohydrate was supported by Meyer (*Botanische Zeit.*, 1881, xxxix, 841), who states that it furnishes the simplest explanation for the lamellation, the apposition-growth of these lamellæ, the later deposition of the outer layers by external accretion, and the relatively low density of the inner parts of the older starch-grains. He compares the growth, the formation of lamellæ, and the variations in density of the different parts of the grain with similar phenomena of spherocrystals generally, and shows analogies in causes and explanations. He reasons that if starch-grains are spherocrystals, it may be assumed with certainty that they grow in a manner analogous to that of other spherocrystals. If spherocrystals of a carbohydrate, such as sugar, are caused to form, and if conditions attending crystallization are altered periodically, as when crystallization takes place at a window where the sun warms the preparation periodically, spherocrystals are deposited in the form of a concentric lamellated structure; but if crystallization occurs under constant conditions of temperature, the crystals show no lamellæ. It is therefore to be inferred, Meyer holds, that the formation of the layers of the starch-grain is due to fluctuations in external conditions. The hila of the spherocrystals of sugar are usually less dense than other parts of the crystals, and they inclose a mother substance, rarely air. Very seldom is a dense hilum observed.

Applying these facts to the growth of the starch spherocrystal, Meyer states that they show that most starch-grains, even in young stages, must have a small, relatively soft center; and that the starch-grains, since the assimilation of the plants is subject to periodic fluctuations, must be built up of layers of alternating density. The youngest, outermost layer is always the most dense, and the successive, deeper-lying layers become less dense according to their age. The varying density, he assumes, is due to the action of ferments. Thus, if one assumes that a layer just formed is exposed to ferment action

for  $n$  hours, it will decrease in density, and it will lose, for example,  $m$  grams of starch-substance which is dissolved by the ferment. If a new layer is now formed, it will be the most dense. If the starch-grain is again exposed  $n$  hours to ferment action, the first layer loses  $2m$  grams of substance, and the last-formed layer, that is, the second layer, loses  $m$  grams by solution.

Meyer states that there is present a second kind of solution, a corrosion of starch, which occurs in the living cell. In and on the chlorophyl-grains solution of the starch-grains takes place if the assimilation of the plants becomes sufficiently low. Starch-grains in non-green cells are also dissolved on the exterior while they are still attached to the starch-builder or leucoplast. Grains which are slowly dissolved from without take on a corroded appearance, owing to a loss of some of their substance, and during the action of the ferment they are surrounded by a layer of less dense substance. The thickness of this layer depends upon the length of time the ferment acts and upon the degree of activity of the ferment. This outer corroded layer remains visible even after new starch is deposited. Such a deposition on corroded grains takes place in both chloroplasts and leucoplasts, and relatively thick layers originate which may be designated secondary layers in contrast with the primary layers produced by the previously described change of conditions of crystallization.

In another article which appeared at about the same time (*Botanische Zeit.*, 1881, xxxix, 856) Meyer reports the results of studies of starch-formation in the rhizomes of *Iris germanica* and *Iris pallida*. The mature grains are described as oval, cylindrical, or spherical, and as showing great diversity of forms and with an eccentric hilum. Usually the grains in the cells nearest the tip of the rhizome are faintly lamellated, but very often closed layers are entirely wanting. In parts of the one-year-old rhizome back from the tips, grains are found, of approximately equal size, in which the closed layers are more prominent than in the tips. The increase in the distinctness of lamellation he explains upon the assumption that there is a solvent action on the starch in the cells. The grains show conical lamellation, and the outlines of the layers which become visible through ferment action are similar to those of the young stages of the grains of the rhizome tips.

Meyer confesses his inability to determine whether the relatively low density of the bases of the successive closed layers has arisen by the contact with the starch-builder and the rapid supply of material, or whether it is a form of lamellation determined by the ferment, or whether it is due to both. It seems certain, however, he states, that the ferment action emphasizes conical lamellation. In older parts of the rhizomes cells are sometimes found filled with starch, but in others they are entirely starch-free. In going from such old (mostly non-starch-bearing) parts toward the tip of the rhizome, starch-builders are found which bear starch-grains; but these grains are mostly attached obliquely to the builders, the grains having moved somewhat, so that now the original bases do not come in contact with the builders. The grains are usually more or less corroded, and commonly the side of the grain in contact with the builder is "eaten out." In parts of the rhizome where the most active solution of the starch takes place, it is found in the autumn that there are grains which have secondary lamellation. The presence of such lamellæ in grains that are usually attached diagonally to the builder supports the theory that in such parts of the rhizome periodic solution and formation of starch-substance take place in the same starch-builder. Since starch-builders have until their death the capacity to produce starch, new grains may be formed or, if corroded grains are present, such grains may take on secondary lamellation; but the new grains are distinguished by their almost regular round form and by their prominent radial striations, which are developed when the grains are swollen in potassium hydroxide. The theory of the growth of starch-grains by apposition furnishes, he states, a simple explanation of all of the phenomena observed in the development of the starch-grains of the *Iris* rhizome.

Meyer, in an elaborate research on starch-grains published in 1895, again takes up the study of the formation and structure of the starch-grain, reference to which will be found in later pages (page 47).

Our understanding of the structure and mechanism of formation of the starch-grain was added to materially by the careful observations of Strasburger (Ueber den Bau und das Wachstum der Zellhäute, Jena, 1882). He states that since there is a great analogy of structure between cell-membranes and starch-grains, it is natural to go from the former to the latter in the study of the structure and growth, and that the large, flat, eccentric starch-grains of *Phaius grandiflorus* (*P. wallichii*) are very suitable for such investigations. The structure of the grains is such that the layers, except the innermost, are not complete, that is, they end blindly on the side of the grain (see plate 102, fig. 611). In general, all starch-grains from the same bulb agree fully in structure and in other properties. When magnified, these grains show definite lamellations which are indicated by broader light and narrower dark lines. The lines or lamellæ may vary greatly in width. Upon this alternation of light and dark lines is based the view of alternation of dense and less dense layers, and of layers that are poor or rich in water. As a matter of fact, he states, there are in the starch-grain, as in the cell-membrane, consecutive lamellæ which resemble each other rather closely. The darker lines are the adhesion surfaces of the consecutive lamellæ. Where these dark lines follow each other very closely they indicate directly the boundaries of the lamellæ; usually, however, they indicate only single adhesion surfaces, and thus separate layer-complexes from each other. The more sharply marked the separation-surfaces appear, the longer are the intervals between the periods of formation of the lamellæ. The dividing lines become more pronounced by individual layers becoming more dense on their outer surfaces. This accentuation, he states, may also be brought about by an interruption in the growth. Small variations in the constitution of the protoplasm which produce the lamellæ, as a difference in the water-content, may also possibly cause the variation.

Strasburger found that by a slow action of potassium hydroxide on starch-grains the grains swell. The surface was found to be the most resistant, and the outermost layer was seen to be not continuous around the entire grain. The anterior and the acute end of the grain was the most resistant and is the oldest part of the grain. With the beginning of the action numerous fine cracks appeared on the surface, and ran with approximate regularity perpendicularly to the direction of the lamellæ; immediately the substance between the cracks was dissolved at a number of points. The entire surface of the grain was now dotted regularly, and the dots were very small and not continuous. At the same time there was seen a rather large crack, which, passing out from the hilum, mantle-like, inclosed the inner part of the anterior half of the grain; it separated the denser outer part from the less dense inner part; the latter escaped from the grain through openings made by the rapidly dissolving posterior lamellæ; the inner mass separated into two parts. The dense layer and the layer-complexes do not swell as much as the less compact layers and radial cracks form in them. These cracks are usually very irregular and anastomose with each other. During the swelling the lines of separation between the layers and the layer-complexes do not become wider, yet they become more evident. The formation of cracks and their direction in the swelling process indicate undoubtedly that a radial structure is present in the grains, and leads to the conclusion that the elements of the grain are minute rods arranged radially. The regularity of the cracks, Strasburger holds, opposes the view that the cracks have arisen through tension in the grains. In the fresh starch-grains the hilum was recognized with difficulty, but in the dried grains it was quite evident. In the latter case it appeared usually to be hollow, and the fissures that crossed the lamellæ at right angles were directed toward the hilum. Such fissures in the dried grains extended only to the concentric lamellæ, and they usually terminated where the lamellæ became incomplete.

Studies were also made by Strasburger of the starch of *Cycas circinalis*. In the relatively large part-grains, each grain consisting of 2 to 8 parts, which constitute a single grain, a radial structure was also observed. Likewise in the centric, oval grains of *Phaseolus vulgaris* a radial structure of the lamellæ was observed. In *Phaius* the starch-builders are rod-like and easily seen, and from them the starch-grains project. The entire starch-grain or only the base was observed to be covered by a delicate membrane separate from the main mass of the starch-builder, and the lamellæ extended only to this membrane. Between this membrane and the mass of the starch-builder was a less dense substance, described by Schimper as being a delicate and more or less swollen layer of the starch-builder which borders on the grain.

Some of the grains, Strasburger found, do not originate in the chlorophyll bodies and starch-builders directly, but in the cell-plasma. Such grains were observed in the macrospores of *Marsilia* (water fern), and the grains were remarkable for the fact that each was covered with a network. The formation of the starch in these macrospores only begins after the macrospore has been developed. The grains are embedded in the protoplasm, and no specialized starch-builders are present. These grains, he states, grow by apposition. Starch-grains in the cells of the medullary rays of *Conifers* are also produced without the agency of starch-builders. In *Pinus sylvestris* the formation of starch begins 4 or 5 cell-lengths from the cambium. In the cells of the rays at this point very small starch-grains appear in the strands of the plasma-network, and appear to come directly from microsomes. When the grains become larger they lie in the meshes of the network, and have microsomes attached to their surfaces. In principle, the process of starch-formation in *Marsilia*, *Pinus*, etc., was not found to differ essentially from the formation in starch-builders, since in both cases protoplasm and microsomes are utilized in the process. Where starch-builders are present there is merely a more extended division of labor in the protoplasmic cell-body.

Strasburger states that it is evident that all eccentric grains originate from differentiated starch-builders, and that the eccentric structure is a result of unilateral deposition on the starch-grain, which explains at once the presence of the starch-builder at only one end of the grain. Not all centric grains, however, originate without the agency of starch-builders, because a uniform growth on the entire outside of the grain is possible only so long as the grain is entirely surrounded by the substance of the starch-builder. Finally, that compound starch-grains originate by the union or fusion of grains originally isolated can be established, Strasburger states, in grains of *Marsilia diffusa*.

In further studies of the structure of the starch-grain, Strasburger records that up to that time (1882) the lesser density of the interior of the starch-grain had not been satisfactorily explained. He compares the lamellæ of cell-walls and starch-grain, and notes that in cell-walls the inner constantly growing lamellæ which border the cell-contents behave differently from the lamellæ of the membranes which are removed from the cell-contents. Every lamella, as it becomes covered by later-formed lamellæ, decreases in its refractive capacity, this decrease being due to an increase in the water-content. The same relation holds good for the starch-grain. The lamellæ deposited on the outside of the grains becomes less refractive but richer in water as they become farther separated from the outer surface. The parts of the grain which cease to increase, on which no new starch is deposited, retain their original density, and their density even increases with age. The anterior or acute end of the very pronounced eccentric *Phaius* grains is distinguished by its density, and it represents the oldest part of the grain. On account of the increased water-content as the lamellæ lie farther from the surface layer, a pulling or state of tension is created in every lamella, and the tendency is to distribute this strain and thus increase the size of the entire starch-grain, and hence on account of the strain in the inner lamellæ, the storage of water in them is favored. This water-storage he con-

ceives takes place in a tangential direction only. The tendency of the grain is to increase constantly on its periphery, but this is hindered by the inner expanding lamellæ. Thus there is a positive tension between every lamella and the next inner lamella, and a negative tension between it and the next outer lamella.

Strictly speaking, Strasburger states, only in centric starch-grains does the water-content of the lamellæ increase from without inward. In eccentric grains the increasing water-content of the lamellæ applies only to the oldest central parts of the grain. The greater density of the surface is due to the lower water-content. The influence of the surrounding medium lowers the swelling capacity of the surface, and it also gives rise to the following phenomenon noted by Harting: By contact with water there very often form denticular projections which are caused by a more rapid swelling of the layers closely packed below the surface, whereby the surface-membrane becomes turned over at some places.

Strasburger notes that by cutting or crushing starch-grains swelling is increased because of the greater or less disorganization. Cohesion in starch-grains is feeblest in a tangential direction, and tension relations are therefore equalized by means of radial cracks. In concentric grains the innermost and most watery layer and the hilum lose most water in drying, and hence the cracks center at the hilum. The adhesion of the lamellæ is stated to be very great, so that the layers can not be separated by means of pressure, while the formation of radial cracks is easily accomplished by pressure. Strasburger writes that the radial structure of starch-grains suggests the conception that the rod-like elements of the grains are crystal needles arranged radially.

The component of raw starch that is soluble in cold water was studied by Bruckner (Monatshefte f. Chemie, 1883, iv, 889) and identified with the *amidulin* described by Nasse (Inaug. Dissert. Halle, 1866) and with the *granulose* of C. Nägeli. Bruckner dried the grains and crushed them between glass plates, causing many cracks. After maceration in water, and repeated filtering, a filtrate was obtained which became blue upon the addition of iodine. In another experiment, the whole grains (*Zea*) were placed with three volumes of water in a cylinder and set aside for three weeks, during which period the mixture was shaken daily. At the end of this time the preparation was filtered, and the filtrate was evaporated to one-fifth of its original volume. No trace of a blue reaction with iodine was obtained. From these results, Bruckner concludes that the outer firm layers or coats serve as a protective membrane to the inner layers, and that the soluble starch can be extracted by cold water only after cutting, breaking, or otherwise injuring the outer layers, and thus exposing the inner soluble portion.

Shubert (Monatshefte f. Chemie, 1884, 472; Jour. Soc. Chem. Ind., 1885, iv, 236) investigated the behavior of starch-granules on being heated, and concluded that changes in form and structure, especially the lamination, are not solely determined by the amount of moisture in the air-dried granules, but depend also on the different physical and chemical properties of each layer. The effect of heat was found sufficient to make these differences more prominent. The starch-granule, under the influence of high temperature, is altered in such a manner that the layers that are rich in granulose are at once converted into soluble starch and dextrin, while the principal portion of the layers that are rich in cellulose undergoes this transformation only after a time. When starch which has been heated is treated with water of the ordinary temperature, the soluble starch and dextrin are removed and an organized residuum is left, which resembles the form and structure of the original granule and contains small quantities of unchanged granulose. This granulose can be further removed by extracting with water, and it appears to be in such a state as to be readily changed in its chemical properties. The grains give up the greater portion of the granulose to the water, thereby losing in mass but not in volume, retaining their structure, the residue consisting chiefly of cellulose. Grains extracted in this way become colored blue, or, at least, bluish-violet, on the addition of sulphuric acid and iodine, and the individual

layers swell up and separate from one another. When the granules are heated more strongly with a drop of water on the object glass, a deep-blue coloration ensues. The residue is not a uniform body, but contains, in addition to cellulose and granulose, a transformation product of starch, similar to dextrin, which reduces Fehling's solution, is colored red by iodine, and undergoes decomposition on treatment with water. It could not be decided whether or not this body was erythro-dextrin. If a large quantity of the grains is triturated with powdered glass, which presumably would not produce decomposition or alteration, the substance yielding the red color can not be removed by repeated treatment with cold water; on the contrary, by the addition of iodine the residue becomes more intensely colored blue, while the rotatory power remains practically unaltered.

The development of the starch-grain in the lactiferous cells of *Euphorbiaceæ*, first observed by Meyer in 1836, was studied by Potter (Jour. Linnean Society, 1884, xx, 446), who describes phenomena of much interest. These grains are formed in the interior of rod- or spindle-shaped corpuscles which lie in the parietal protoplasm of the cell. The starch-grain is at first visible as a thin streak in the interior of the corpuscle. This streak, through the deposition of starch, assumes a rod- or spindle-shape, and both grain and corpuscle increase in size. When the grain has attained nearly its maximum dimensions in length and breadth, the starch-forming corpuscle collects at both ends of the rod-shaped grain and forms masses at the ends which cause the former to assume a remarkable shape, resembling a long bone, such as the tibia. The lactiferous cells are polynuclear, and since when very young their diameter does not much exceed that of the nuclei, it follows that the starch-forming corpuscles which are always formed near the nucleus must be developed at the sides of it. The smallness of the diameter of the lactiferous cell necessitates the starch-forming cell being much longer than broad, and hence it comes about that the primitive shape of the grain should be that of a rod. Later, however, when the cell has increased in diameter, the rod can also increase in diameter. The hilum of these grains is seen in the form of a line in the middle of the grain in the direction of its long axis. The lines of stratification inclose the hilum and are roughly parallel to the outline of the grain. The grains are doubly refractive, and in all respects agree with starch-grains from other sources, since they are developed in the interior of starch-forming corpuscles and are stratified and rendered doubly refractive through the agency of the lamellæ.

The view that the starch-grain is composed, according to C. Nägeli, of granulose and cellulose was opposed by De Vries (Botanische Jahresberichte, 1885, 1, 122), who holds that the so-called starch-skeleton does not consist of cellulose, because most or all of such skeletons are colored blue by boiling in Lugol's solution. He believes that only one carbohydrate (amylum or granulose) is present in the grains, that it may exist in different degrees of density, and hence that the starch cellulose is merely a dense form of granulose.

In the same year Mikosch (Botanische Jahresberichte, 1885, 1, 122) reported the results of his inquiries into the seat of origin of starch-grains. He placed leaves on a sugar solution, and observed that starch-grains arise not only in chlorophyl grains, but also in any part of the protoplasm. He found the same to take place in the potato tuber. In the cotyledonary leaves of *Zea mays* and in the young tissue of *Elodea canadensis* the grains were observed to originate in the plasma of leucoplasts.

At the same time an article by Belzung (Botanische Jahresberichte, 1885, 1, 122) appeared on the mechanism of the development of the starch-grain. He found in sprouts developing in the dark that leucoplasts arise which soon after their appearance form several small starch-grains. After a short period of growth the grains fill the leucoplasts, and finally the grains in one leucoplast may fuse into a single grain, or by atrophy of the leucoplast they may lie free as small grains in the protoplasm. In general, he states, there are three methods of development of starch-grains: (1) The formation of grains within the chromatophore by "resorption," but without marked subsequent growth (seen in sprouts, leaves); (2) the for-

mation in chromatophores, but with marked subsequent growth (in many Cotyledones); (3) the formation at the surface of the chromatophores (*Phaius*). The first method of growth gives rise to a type of small, lamellated starch-grains; the second and third methods to types of large lamellated grains. In the development of types of grains 1 and 2, the growth of the grains takes place presumably by a chemical metamorphosis of the chromatophore-substance, while in the growth of type 3 the rôle of the chromatophore is problematical.

The so-called starch cellulose or skeletons prepared by C. Nägeli by subjecting starch to the prolonged action of saliva, were obtained by Meyer (*Botanische Zeit.*, 1886, XLIX, 697, 713) by the agencies of dilute acids, pepsin, diastase, and saliva. Meyer found that after digestion had proceeded sufficiently long the skeletons are colored yellowish or reddish-brown with iodine, and that they consist of pure amyloextrin. This substance, he notes, crystallizes very readily, usually in the form of spherical spherocrystals (rarely plate-like), resembling starch-grains which have a concentric structure. These spherocrystals, he states, behave similarly to starch-grains in polarized light, except that the dark cross is not orthogonal but diagonal, a phenomenon which evidently is dependent, he states, upon the orientation of the needle-crystals. The saliva-skeletons, as well as the acid-skeletons, behave exactly like the starch-grains towards polarized light. The microchemical similarity of the spherocrystals of amyloextrin and the saliva and acid skeletons proves, he contends, the identity of the substance of the three structures. This statement he supported by the results of experiments with acids, alkali, and chloride of zinc and iodine, by which it was found that all were affected in the same way.

The starch cellulose or skeleton-like substance described by Nägeli and others was prepared by Griessmayer (*Allgem. Bauer- u. Hopfenzeit*, 1887, xxvi, 147) by subjecting 1000 grams of potato starch to the action of 6 liters of a 12 per cent hydrochloric acid for 100 days in the cold. From this preparation, after washing free of acid, sugar, etc., and drying, a quantity of starch cellulose was obtained that weighed 300 grams. This substance was almost completely soluble in boiling water; and by freezing such a solution amyloextrin crystallized out in the form of spherocrystals composed of minute radial needles. Brown and Heron (*Ann. d. Chem. u. Pharm.* 1879, cxcix, 189) question whether cellulose is an original constituent of the grain or an after-formation.

The conception of the rod-like crystalline arrangement of the particles of the starch-grain received further support in the investigations of Buscalioni (*Botanische Jahresberichte*, 1891, xix, 489) with *Zea* starch. The seeds of corn, not quite ripe, were broken up and boiled for 30 seconds in 1 c.c. of chloroform containing several drops of a solution of chromic acid. The pieces of the seeds were spread out and examined under the microscope, and numerous grains of starch swollen to various degrees were noted. In the slightly swollen grains radial streaks were seen which passed from the center of the grain to the periphery, and which were placed in two directions so as to cross each other at right angles, thus giving the grain the appearance of being composed of numerous rhombic pieces regularly arranged. In the more swollen grains the lines were replaced by small points arranged concentrically and radially. If the action of the chromic acid goes on for a longer time every trace of a definite structure vanishes and the whole grain appears to be a hyaline mass.

In a later article (*ibid.*, 1899, xxvii, 282), Buscalioni found in the cortex of the root of *Juncus tenuis*, especially within the endoderm, an accumulation of simple and compound starch-grains. The grains which appear in cells poor in starch were sometimes inclosed in a space of varying form and consistency, and in the nature of a dense mucus substance, so as to constitute a capsule. These capsules lie in a corner of the cell or on the cell-wall, while others are attached to the wall by a little stalk. Aggregations of grains are sometimes inclosed in a common capsule. Capsules were also observed which did not contain a trace of starch. Sometimes pectin, and also cellulose, were present. The author

states that the envelope of the starch-grain undoubtedly originates in the cytoplasm, as is shown in the different stages of development observed in the various preparations. The inclosed grains are stated to have an extraordinary similarity to the grains of the seeds of *Vicia narbonensis*, with the difference that in *Juncus* the capsules usually have stalks, while those of *Vicia* never have.

The cause of the lamellation of the starch-grain is due, according to Zimmermann (Beiträge z. Morph. u. Physiol. d. Pflanzenzelle, 1890; Botanical Microtechnique, Trans. by Humphrey, 1893, 226), to varying water-content, which, according to the author, may be shown by examining moist and dry starch-grains in Canada balsam. He notes that the complete removal of water can only be accomplished by drying at a temperature of 50° to 100° C., and that the use of dehydrating media, such as absolute alcohol, does not give demonstrative results.

In an investigation of the mechanism of the growth of starch-grains, Eberdt (Jahrbücher f. wissensch. Botanik, 1891, xxiii, 293) attempts the support of the then practically abandoned theory of growth by intussusception, and among other things he holds very different views from Schimper regarding the rôle of the colorless starch-builders, or leucoplasts. Eberdt contends that the starch-grains which originate in the interior of chlorophyll granules become larger after they are freed from the granules, which to him indicates a formation by concentric lamellation, a process that can only be explained by growth by intussusception. The appearance of lamellation in starch-grains, he states, be it concentric or eccentric, might be explained by a leaching-out process brought about by plant acids, but the tensions present in the starch-grains would remain to be explained, and hence one must at the same time assume that simultaneously with this process swelling takes place.

Eberdt agrees with Schimper that in non-assimilating plant parts the formation of starch is due to albuminous bodies. Schimper ascribed an active rôle to these bodies, looking upon them as starch-builders, but Eberdt believes that they are passive, and that the plasma is the active agent in the transformation of assimilation products into starch. Eberdt holds that during the life of the plant, when no reserve material is produced, these albumin bodies perform no apparent function, and that one might in many cases regard them as waste products; but when reserve material is being formed the rôle played by them is passive, and they become transformed into starch. This transformation may be brought about, he states, in two ways, either from within outward, as when the form of the starch grain is analogous to the form of the body; or, the transformation takes place gradually from without inward, in which case the body is completely dissolved, and the form of the resulting starch-grain shows no resemblance to its form. In every case the transformation is assumed to take place only in the presence and by the agency of protoplasmic substance, which is the active factor, and which may become green under the influence of light.

Eberdt gives a very lengthy discussion of the subject, but his chief conclusions may be summarized as follows: (1) The origin of the small albuminous bodies (Stärkekörner of Schimper), which on account of their behavior he terms the "Stärkegrundsubstanz," result from a differentiation of the plasma of the cell. (2) These bodies may be attracted to the cell nucleus, and later be arranged in groups or deposited singly about the nucleus. In every instance they are surrounded by a layer of protoplasm which is connected with the peripheral plasma of the cell by plasmic threads. (3) After the individual grains of the groups have been transformed into starch, the plasmic layer completely surrounds every group, or the layer breaks and the groups separate. In the former case the groups are surrounded by the plasma until the starch-grains are mature, and the individual starch-grains show no lamellation, or the grains separate before maturity, and in such cases concentric lamellæ appear, and in some instances eccentric layers. Grains of the last two types may increase in size after lying free in the cell-space. (4) In instances where the bodies of the Stärkegrundsubstanz are not grouped around the cell-nucleus, the plasmic

layer separates, and a part of it is in contact with and completely surrounds every one of these bodies. This plasmic layer brings about the transformation of the body into starch in such a manner that the albumin molecule is split by the action of the plasma. After the solution of the entire body, and after the newly formed starch-grain has broken through the plasmic layer, a part of this plasmic layer remains attached to the grain in the form of a hood, which usually has attachment to the rest of plasma. (5) Such grains always show eccentric lamellation which appears after the grains have broken through the plasmic layer. (6) No growth occurs after the disappearance of the plasmic hoods. (7) The plasmic layer or plasmic hoods might with reason be called starch-builders.

The starch-building processes of non-chlorophyllous structures were also studied by Königsberger (*Botanische Centralblatt*, 1892, XLIV, 47). This author gives the results of his investigations of starch-building in Angiosperms. He does not agree with Schimper that in many plants the starch-grain originates only in the peripheral parts of the chlorophyll-grains and soon breaks through the chloroplast, and on account of unilateral accretion becomes eccentric. He observed starch-grains in the parenchyma cells of *Pelargonium* which were eccentric although entirely inclosed by the chlorophyll.

The following is a very brief summary of Königsberger's results: (1) The formation of reserve starch in Angiosperms takes place through the agency of the leucoplasts and also by the direct activity of the protoplasm itself. The first process, which is observed in many *Monocotyledones* and in only a few *Dicotyledones*, must be viewed as the earlier, from which the second has been developed and predominates in many *Dicotyledones*. (2) In the *Dicotyledones* the leucoplasts, having performed their function, have in many plants entirely disappeared. (3) The beginning of the starch-grain is probably in the form of a deposition of amyloextrin. (4) The capacity to polymerize carbohydrates of less molecular weight into those of greater molecular weight, which latter are in the definite form of reserve material, was originally peculiar to the leucoplasts, but in many of the higher plants was later transferred to the protoplasm. (5) The starch-grains probably originate from amyloextrin, and when the starch is rendered into a soluble form for purposes of transportation it is reverted into amyloextrin.

The form and method of formation of starch-grains were studied coincidentally by Dodel (*Flora oder Allgemeine Botanische Zeitung*, 1892, LXXV, 266) and Binz (*ibidum*, 1892, LXXVI, 34). Both made their studies mostly with the starch from *Pellionia daveauana*. Dodel makes rather general statements, while Binz goes somewhat into details.

Dodel writes that after careful study he reached the conclusion that this plant furnishes the most suitable material for the study of both the morphology of starch-grains and of the starch-builders; that both grains and builders of *Pellionia* are well adapted to answer the question as to whether or not the grains grow by intussusception or apposition; and that he is convinced that the growth is exclusively by apposition. He records that a section of the stem at a young internode shows in the parenchyma region many starch-grains of various sizes and forms, most of which have chloroplasts attached to them. In the region of the fibrovascular bundles, and in the pith, the starch-grains have attained most perfect and uniform development, while towards the periphery of the section the grains and the chloroplasts decrease in size. The form of the grains was found to vary considerably. All of the grains are stated to originate as small spheres which are found in the middle or toward the periphery of the spherical or ovoid chloroplast. As soon as the grains have attained sufficient size to protrude through the green starch-builders, the spherical form is changed, and the grains begin to grow unilaterally, that is, the main point of growth is where they are in contact with the chloroplasts, while the part of the grain that is not in contact with the chloroplast not only grows slowly but finally ceases to grow. The grains, at first spherical, soon become oval, wedge-shaped, reniform, cylindrical, or irregular in shape. All of this diversity of form, he states, is due to growth by apposition.

The form and size of the grain are determined by the form and number of the chloroplasts attached to the grain. Frequently two or more grains were noted to appear simultaneously in different parts of the chloroplast, when a compound grain results.

Dodel then takes up the question as to whether or not the grains are formed by the attached chloroplast, and to answer this he studied the half-grown grains in a section of a stem of *Pellionia* that had been in absolute alcohol for several months, and in which the starch-builders had become decolorized. A section was washed in distilled water, and stained with a weak solution of methyl-violet, when it was found that the half-grown grains, besides being partially surrounded with the hood-shaped chloroplast, were inclosed by a very thin, colorless plasmic layer. In the mature grains this layer was no longer evident. Frequently several chloroplasts were in contact with one starch-grain, this being due to the division of the original chloroplast. The starch-builders increased in mass as long as the starch-grains continued to grow. The lamellæ of the grains of *Pellionia* were not perceptible until a comparatively late stage of development. The young grains, as long as they were spherical, appeared to be homogeneous. It is very striking, he states, that the part of the grain about the hilum, which is the oldest, is not lamellated. The solution of the starch-grains, he found, takes place on the entire surface of the grain, even on the part covered with the chloroplast. During the process of solution the chloroplasts modify their form as the shape of the grains is changed. After a considerable part of the starch-grain has been dissolved the chloroplast may resume its activity, and thus there may be an intermittent activity of the starch-builders. The formation of irregular grains is probably due, he holds, to such secondary activity of the chloroplasts.

According to Binz, the majority of the starch-grains of *Pellionia* are simple, and there are both half-compound and compound grains; but the compound grains do not originate by division of the hilum of the simple grain, as Nägeli assumed. The simple grains, he states, fall into two sharply defined categories: In the young state the grains are regular (spherical) in form, and inclosed by the green starch-builders. After they break through the chloroplasts they assume an oval form and become eccentric. Binz assumed that it is not the inner constitution of the starch-grain which determines the form of the grain, but the position of the grain in relation to the starch-builder. After the grains protrude from the chloroplasts, the latter are in contact with the grain in the form of a hood, and the growth of the grain takes place at the point of contact. Such grains take on a more or less regular form. The second category of simple grains includes grains of irregular form, representing advanced stages of the regular forms. When the grains are subjected to a 4 per cent solution of potassium hydroxide the lamellæ become evident. Two kinds of lamellæ are noted, one kind being complete, and the other being incomplete and wedge-shaped. The complete or closed layers are few in number and pass entirely around the hilum. These layers are evidently formed when the grain is entirely inclosed by the chloroplast. In every grain the innermost part is stated to be soft and watery, then follows a complete dense layer, then a watery layer, and so on, until the outermost or last complete layer is very dense, and the boundaries between the complete and incomplete layers are very marked. The inner complete layers are not complete in the early stages of the growth of the grain, and are only perceptible after the starch-builder has become ruptured by the extrusion of the grain and has assumed a hood-like form, and perhaps not visible before the first of the incomplete layers is deposited. This is a point, states Binz, that has always been used to oppose the apposition theory of growth, although nothing can be proved by it in such application. It is possible that the layers as such are deposited as a homogeneous mass from the very beginning, and that by subsequent changes, as by the varying absorption of water, are rendered apparent.

Binz agrees entirely with Schimper in that the growth of starch-grains occurs by apposition, yet he admits that there are no direct unimpeachable proofs in support of the theory.

The softer layers, he observes, are present in much larger numbers than the denser layers. Very often a grain shows only 4 or 5 dense layers, while between them are numerous soft layers. The lamellæ are separated from each other by dark lines which Binz holds must be regarded as very thin layers, for it is evident, he states, that when two substances of different refractivity are in contact a boundary line must result.

Binz holds that Nägeli's view that layers arise by the splitting of layers already present, is not justified, and that the correct view is that formulated by Schimper, which is that the starch-builder deposits the layers. Whether the lamellæ are deposited directly or whether they are secondary phenomena Binz could not determine; nor was lamellation found to be attributable to the action of ferments, as Meyer assumed.

Binz discusses various other topics, such as the relations between the structure of the chloroplast and the form of the starch-grain; irregular starch-grains, compound and half-compound grains; solution phenomena of starch-grains; and the structure of chloroplasts.

The main conclusions of Binz's researches, briefly stated, are: (1) Nägeli's theory that layers arise by the splitting of other layers does not hold good for the starch-grains of *Pellionia*. (2) The outer layer of the starch-grain is the youngest, and the innermost the oldest. (3) The spherical part of the grain forms when the grain is within the chloroplast, complete layers being formed so long as the grain is entirely within the chloroplast and the wedge-shaped incomplete layers being formed after the grain has broken through the builder, so that the form of the starch-grain is directly related to the form of the chloroplast. (4) The extruded grain grows at the point at which it is attached to the chloroplast. (5) Outgrowths and secondary growths are sharply defined from the original starch-grain. (6) If the chloroplast becomes detached from a certain part of the grain, the growth of the grain ceases at that part. (7) Compound and half-compound grains do not originate by division of the original simple grain, but by the formation of several starch-forming centers in a chloroplast. The compound grains may also originate by the grouping together of several starch-builders, as seen in the pith of *Philodendron* and *Convallaria stanhopea*. All these points, he writes, favor the apposition theory of growth, but the theory can not be proved definitely until the origin of the lamellæ is better understood. (8) The structure of the starch-grain has no influence upon the manner of corrosion, but upon the intensity of it, for the softer parts are more easily corroded than the denser, and the several layers are more easily corroded in a radial than in a tangential direction. (9) The chloroplast consists of a homogeneous ground-mass, or stroma, with embedded pigment spheres, the *grana*. (10) Starch-builders in the form of leucoplasts are present in the growing tips and they are structures homologous to the chloroplasts, since under the influence of light they are transformed into chloroplasts.

The microscopic structure of the starch-grain was studied by Bütsehli (Botanisches Centralblatt, 1893, LVI, 150), who found that starch-paste of medium density, on drying, first assumed a honey-comb structure and subsequently a fibrous form. In gelatinous paste, and in a dilute solution of starch that is frozen, the same characteristic honey-combed appearance was noted. From these results he concluded that the lamellation of starch-grains bears a close relationship to a honey-combed structure.

Examinations of starch-grains in water were found by Bütsehli to indicate that there are traces of a honey-comb structure in the inner layers and also in the hilum, but that such observations did not furnish definite results. When grains of a form of commercial arrowroot (canna starch) were heated in water at 60° to 70° C. until they began to form a paste, it was seen that every layer of the grains is radially striated, and that every layer is composed of "honey-combs" whose walls consist of firm starch-substance, and whose contents are water or dissolved starch-substance. These radial structures, Bütsehli states, do not extend through the entire grain, but alternate with each other in successive layers. This structure was seen in slightly swollen starch, but not when much swollen, because in

the latter there is brought about an increase in volume in the outer layers, and this causes a disintegration of the internal organization. The honey-combed structure was seen even more clearly in the so-called artificial starch-grains which were prepared by condensation of a starch-solution, when under the skin that covers the solution a layer of grains consisting of starch was found. These grains, Bütschli recorded, show a characteristic honey-combed structure, and in polarized light behave exactly like natural starch-grains. Such artificial starch-grains, having a beautiful finely honey-combed appearance, are produced by freezing a dilute starch solution. Bütschli holds that there can be no doubt about the chemical nature of these artificially produced grains, and he also states that the structure of the starch-grain seems incompatible with the theory of growth by intussusception, but entirely in harmony with the theory of growth by apposition. On evaporation of a thin starch-paste there arose at the outer edge of a droplet layers having lamellated structural characteristics bearing a marked resemblance to the lamellæ of the starch-grain. The origin of the honey-combed structure Bütschli conceives to be due to a separation of water during the concentration of the starch-solution, and to further loss of water by freezing.

This investigation was supplemented by Bütschli (*Botanisches Centralblatt*, 1896, LXVIII, 213) with the conclusion that the structure of the spherocrystal of inulin and that of the starch-grain is identical, and that both kinds of spherocrystals have arisen through a honey-combed method of building. He obtained spherocrystals of starch in this way: A watery starch solution was prepared by boiling starch in water for 3 to 4 hours, when the solution filtered until entirely clear. To the filtrate was added an equal volume of a 5 per cent solution of gelatine, and the preparation was then evaporated almost to dryness, when spherocrystals appeared. These crystals had a diameter of 0.05 mm. In polarized light such crystals behaved exactly like the natural starch-grains, and the behavior of the two towards iodine was similar, but solutions of chloride of calcium and of chloral hydrate acted differently on the natural and artificial starches.

Bütschli agrees with Meyer (see page 47) as to the presence of  $\alpha$ -amylose and  $\beta$ -amylose in natural starch, but he obtained only one of them in the form of artificial starch-grains.

Artificial starch-grains have also been prepared by Rodenwald and Kattein (*Sitzungsber. Kgl. pr. Akad. Wiss.*, 1899, XXIV, 628), Roux (*Compt. rend.*, 1905, CXL, 440, 943, 1259), Maquenne and Roux (*ibid.*, 1303), and St. Jentys (page 58). The former heated starch in a solution of iodine and iodide of potassium in a sealed tube for 15 minutes at 130°. A greenish liquid was formed which, they state, consisted essentially of a solution of starch-iodide and some sugar. The clear filtrate, they record, became decolorized upon heating, owing to the liberation of the vaporous iodine, which could be removed by a current of steam. Upon slowly cooling the solution, a white substance separated in the form of starch granules. These granules gave the characteristic blue reaction with iodine; they were insoluble in water; they formed a thick paste on boiling; and they swelled and formed a paste with potassium hydroxide. Roux found that by an incomplete degradation of amylocellulose an artificial starch can be produced which has a cellular structure similar to that of natural starch. These granules, while giving the blue reaction with iodine, do not gelatinize in hot water, and they therefore differ somewhat from both natural starch and the artificial starch-grains of Rodenwald and Kattein (see pages 59 and 111).

The honey-comb theory of Bütschli was opposed by Puriewitsch (*Berichte d. deutsch. Botan. Gesellsch.*, 1897, xv, 246). This experimenter used arrowroot, potato, wheat, and canna starches, whereas Bütschli used only canna starch. Puriewitsch records that starch-grains of arrowroot in the fresh condition show no honey-comb structure, nor is such a structure apparent in starch-grains that have been in water for 24 hours. On slightly swelling the grains, either by heating or by reagents, there is developed a quite definite honey-comb appearance, but this Puriewitsch does not regard as an actual honey-comb

structure. The lines of lamellation of such swollen grains are not regular but zig-zag, and the presence of these small projections gives rise to the illusory honey-combed appearance. Starch-grains from the potato likewise did not show a honey-comb structure in the fresh condition, and in only very few of the grains could such a formation be seen after swelling. Here also the appearance of such a texture is illusory, and merely an optical effect due to the zig-zag lamellæ. In a few instances traces of the honey-comb structure could be seen in *Canna indica*. This form of starch is very suitable for such an investigation on account of the large and prominent lamellæ. Grains from wheat did not show a honey-comb peculiarity, either in the fresh or swollen condition. Puriewitsch therefore declines to accept Bütschli's view of the peculiar honey-combed structure of starch.

An important contribution to the literature of starch, covering quite a broad field of investigation, was published in 1895 by Meyer (*Untersuchungen über die Stärkekörner. Wesen und Lebensgeschichte der Stärkekörner der höheren Pflanzen*, Jena, 1895, mit 9 Tafeln und 99 in den Text gedruckten Abbildungen, S. 318). Meyer notes that even at this time our knowledge of the chemical substances which compose the starch-grain, and of the products of decomposition, is very meager in spite of the enormous amount of work that has been done. Meyer concluded from his investigations that in ordinary starch-grains only amylose and small amounts of amylopectin are present. The former he states is present in two forms, one of which dissolves in water at 100°, but the other not. The difference, he assumes, is due to the existence in starch-grains of anhydrous crystals, of crystals which contain water, of crystals that are soluble with difficulty in water, and of crystals easily soluble in water. The easily soluble modification of amylose he terms  $\beta$ -amylose, and the difficultly soluble he terms  $\alpha$ -amylose.

*$\alpha$ -Amylose.*—Just as there is a dextrose anhydride, Meyer writes, which in cold water does not take up water of crystallization directly, there also appears to be amylose anhydride which upon boiling in water passes into a hydrate very slowly, and this fact has partly given rise to the conception of the so-called starch-cellulose. The term starch-cellulose, he notes, has been applied to very different substances—to mixtures of amylopectin with  $\alpha$ -amylose, of dissolved  $\beta$ -amylose and  $\alpha$ -amylose, of dissolved  $\beta$ -amylose and nitrogenous and fatty impurities, and to amylopectin in an almost pure state. Meyer states that he concluded in 1886 that the skeletons of starch formed during acid and salivary digestion do not consist of a substance that is contained originally in the starch-grain, but of amylopectin, a transformation product of amylose. Later researches, however, showed him that this conclusion was not strictly correct, and that acid skeletons are of variable composition, and consist, depending upon the length of time the acid acts, of a mixture of  $\beta$ -amylose,  $\alpha$ -amylose, and amylopectin; or of  $\alpha$ -amylose and amylopectin; or of amylopectin alone. That amylopectin alone is left after acid action for a sufficient time was proved, he records, by an experiment in which a 12 per cent hydrochloric acid solution was allowed to act on potato starch for eight and one-half years, at the end of which time the skeletons contained only amylopectin. It is different, he records, with the salivary skeletons, which, if the amylose hydrate has been dissolved out of the grains, consist of a mixture of amylopectin and  $\alpha$ -amylose.  $\alpha$ -amylose he obtained by treating starch-paste with malt extract, or by the action of hot dilute hydrochloric acid on whole starch-grains. He found that when starch-grains are treated for a short time with saliva or with a cold dilute acid, and the skeletons extracted with hot water, there remained a residue which differed in its properties from other substances of the grains in that it was insoluble in hot water, and was colored red instead of blue with Lugol's solution.

Meyer believes it probable that  $\alpha$ -amylose is to some extent present in starch-grains from the beginning in crystallized forms which offer great resistance to boiling water, and to the penetration of iodine into the small crystals. That  $\alpha$ -amylose is already contained in the intact grains is shown, he states, in the following experiment: If arrowroot

starch is allowed to soak at 70°, and the soaked grains are treated for several minutes with cold saliva, skeletons are obtained which no longer color blue with iodine, and which, at least in part, must consist of  $\alpha$ -amylose, because the  $\alpha$ -amylose could hardly be formed in so short a time from the solid crystals by the action of the saliva. He believes that it must therefore be assumed that  $\alpha$ -amylose is present from the beginning in varying quantities in many grains. Meyer goes on to state that the results of the experiments made up to that time do not show clearly the relationship of  $\alpha$ -amylose to  $\beta$ -amylose; that it seems to him that the differences between the substances are insignificant; and that the future may show that they are merely hydrous and anhydrous forms of the same body.

*$\beta$ -Amylose.*—Meyer found that when starch-grains are subjected in water to a temperature of 138° there is obtained an apparently uniform solution of amylose, since at this temperature  $\alpha$ -amylose goes into solution as  $\beta$ -amylose. On cooling this solution, amylose separates from its solution in very small microscopic, viscous droplets, which do not mix with water below 138°, and which can only be regarded as a solution of water in amylose. This water-amylose solution can be produced at the swelling temperature of starch-grains, that is, at 60° to 70°, at which temperature the trichites of  $\beta$ -amylose are changed into droplets of viscous solution, while  $\alpha$ -amylose apparently remains unchanged. The trichites of  $\alpha$ -amylose are so minute, he states, that they can not be seen singly, but they may become visible by fusion of a number of individuals in the form of a droplet.  $\beta$ -amylose is insoluble in water at a temperature of 30° or below. At 60° the crystallized  $\beta$ -amylose in small quantity forms a thick solution. At 100° it requires more than half its weight of water to pass from the crystallized to the liquid state.

*Amylodextrin.*—Meyer states that this substance is of great interest because it is present in grains of starch which are colored red with iodine. Grains which color blue with iodine can readily be transformed into pseudomorphs consisting of amylodextrin. Amylodextrin was prepared by Meyer by the action of acids upon starch, 200 grams of starch in 2 liters of water and 8 grams of oxalic acid being heated to 100°. The unchanged amylose is allowed to settle by setting the preparation aside for 12 hours. The filtrate which is rich in amylodextrin is then concentrated and frozen to remove the amylose, and finally the amylodextrin solution is greatly concentrated, from which, upon standing, spherocrystals of amylodextrin separate. By recrystallization and washing with alcohol, a preparation is obtained which colors a pure red with iodine. The quantity of amylodextrin produced by this method was not very great, being only about 2 per cent of the amount of starch. Amylodextrin he found to be difficultly soluble in water (at 8°, 0.13 per cent; at 30°, 1.58; at 6°, 3.98; at 70°, 4.66; at 80°, 9.33; at 90° a dense solution that could not be filtered). It is soluble in 50 per cent boiling alcohol, and more soluble in acid and saline solution than in water.

Meyer also studied the growth of the starch-grains and the relations of the grain to the chromatophore. He states from the results of his researches that he is convinced that the substance of the chromatophore, whether it be chloroplast, chromoplast, or leucoplast, incloses the starch-grain as long as it lies within the lining cell, and that during no period of its existence does the starch-grain come in direct contact with the cytoplasm. The complete inclosure of the starch-grain by the substance of the starch-builder permits a direct influence of the latter on the grain, because every part of the grain is in contact with the mother substance. The chloroplast appeared to be a more or less viscous droplet of a colorless or light-yellow substance (*stroma*), in which was a viscous droplet (*grana*) of a substance colored dark-green with chlorophyl. In the stroma there may also be present well-formed crystals of protein and also starch-grains. In his earlier experiments, Meyer found difficulty in determining whether the stroma is colorless or a very light green, but he now expresses his conviction that the stroma contains no chlorophyl, that it is usually colorless, and seldom yellowish. In the leucoplasts of *Pellionia*, and in the “turned-

green" leucoplasts of potatoes, he observed colorless grana lying in the stroma, and these he designated *albigrana*, but since that time he has not studied them. Inasmuch as only the chromatophores which contain grana separate oxygen, it seemed to him likely that the grana constitute the apparatus of assimilation. The colorless as well as the green parts of the chloroplasts have, he states, the capacity of forming starch-substance.

The starch-grain, Meyer holds, grows entirely within the chloroplast, and the approximate proportion between the thickness of the chromatophore layer and the starch-layers growing below it makes it probable that the chromatophore controls the production of starch-substance. The stroma probably produces the starch, because the grain is most actively added to where the layer of stroma is thickest. Chromatophores embedded in a relatively fine-grained emulsion of cytoplasm show the simplest form—a rather viscous drop. But the form varies and is alterable in relation to contents which originate in the chromatophore. The various forms of these bodies have a close relationship to the forms of the starch-grains growing in them, provided that the shape of the chromatophore is retained for a sufficient length of time.

Protein crystals growing in the chromatophore produce a change in form which may be constant for a long time, and they are very important in relation to the growth of the starch-grain. Pigment crystals act in a similar manner, and occasionally influence the form of the starch-grains. But the most far-reaching changes in the forms of the chromatophore are due to the starch-grains themselves. Not only does the growing starch-grain act upon the shape of the chromatophore, but the varying form of the chromatophore reacts upon the growing grain. There are cases where there are simultaneous growths of eccentric starch-grains and chromatophores, when after a certain stage in development both grain and chromatophore increase in size yet constantly retain the same form. The young chloroplasts of *Dieffenbachia* are mostly spherical and contain mostly centric starch-grains, a few eccentric. On further growth all of these grains were found to become elongated and eccentrically layered, so that the chloroplast bulges out on one side into a thin membrane. A grain which is centric at first may develop into an eccentric or concentric grain, according to the nature of the chromatophore, which is influenced by the surrounding cytoplasm. The pressure of a relatively thin but viscid layer of cytoplasm upon the chromatophore exerts a marked influence upon the shape of the chromatophores, and also therefore upon the starch-grains growing within them. The pressure of the cytoplasm, together with the viscosity of the chloroplast and the pressure of the growing starch-grain, gives rise to an accumulation of chloroplast substance at two diametrically opposite points of the starch-grain, so that the chloroplast assumes the form of a thin-walled sac with thick-walled ends. Several grains may grow in one chromatophore.

*Pellionia* serves as a good example, Meyer states, of the change of form of such chloroplasts as contain two starch-grains. In well-nourished types of this plant the growth of the starch-grains normally exceeds that of the chloroplasts. The margins of the grains push diagonally through the chloroplast, and as the grains grow the tendency is to push the chloroplast out into a layer over the contact surfaces of two grains. In *Dieffenbachia* the chloroplasts are of rather thick consistency, so that two growing starch-grains press out the layer of the chloroplast substance between them more slowly, and owing to the greater mutual pressure of the grains the grains assume an irregular shape. A change in the form of the chromatophore that is far-reaching and very significant in its influence on the ultimate form of the starch-grain is brought about by an active solution of the contained starch-grains. Meyer states that if one observes in *Dieffenbachia* the chloroplasts which surround the normal starch-grains during the various stages of solution, it is seen that the main mass of the chromatophore becomes rounded and that the thin layer of the chromatoplast which covers the largest part of the grain is closely joined to all of the transverse furrows that originate in the grain. At times he observed that the thin

stroma layer is decidedly denser in grains that are undergoing solution than in grains in process of growth, and here and there was noticed a peculiar thickening of the layers in the furrows which results from the flow of the stroma layer to the furrows.

In further studies of form and lamellation, Meyer confirms the observation of Schimper that the unequal growth of the starch-grain on different sides of the hilum is owing to the unequal deposition due to the relations of the grain with the starch-builder. Meyer points out that the starch-grains of *Iris germanica* grow most rapidly upon the side in closest contact with the mass of the leucoplasts, and also that the closed layers are widest on the side lying in closest proximity to the leucoplasts. The form of the last starch-layer deposited is approximately similar to the form of the chromatophore if the chromatophore does not undergo a change of form during the deposition.

The final thickness of the layers, Meyer states, is dependent upon two factors: first, the thickness of the first layers of the grain, and second, the solution *in situ* which removes some of the starch before the occurrence of the following period of starch-growth. Like many spherocrystals of other carbohydrates, starch-grains are composed of alternate loose and rather compact layers, but entirely apart from this difference the layers can be differentiated from each other by the varying proportions of  $\alpha$ -amylose,  $\beta$ -amylose, and amylo-dextrin. In spherocrystals forming from a single substance in a homogeneous mother substance the deposition of the layers occurs in such a manner that the saturation relations or other relations of the mother substance change periodically, so that a complete cessation of growth, or a partial solution, does not necessarily take place. If partial solution occurs, the lamellations become very prominent. Similarly, in the formation of starch-grains there are fluctuations in the state of the mother substance which affect the formation of the lamellæ.

In Meyer's experiments with *Pellionia*, twigs were starved so that the starch-grains were dissolved to such a degree as to leave only open lamellæ. The twigs were now nourished until the deposition of starch began, when it was found that for every day of growth there was formed a thick, dense layer, and for every night a thin, soft layer. During the day the twigs were assimilating actively and the growth of the layer proceeded steadily, but at night there is a small supply of material, so that there may be deposited a thin, loosely compact layer, or there may occur a partial solution of the outer layer.

Experiments were also made by Meyer with the starch of *Adoxa moschatellina* and *Hyacinthus*. The results in general showed that a relatively dense layer is deposited on the grain when the chromatophore produces starch-substance actively and steadily, and that when there is a slow and irregular production of starch-substance a thin, incompact layer is formed. The thickness of the layer is always proportional to the length of the period during which starch is continually produced. An examination of the periphery of a grain that had been exposed in the chromatophore for a long time to the action of diastase showed, Meyer states, that this part of the grain is never surrounded by a loose layer, as some have supposed. The diastase penetrates every part of the grain and brings about internal solution, but forms no sharply separated incompact peripheral layer. Even if the internal solution plays no part in the formation of lamellæ it brings about a porous structure of the layers of the grain exposed to prolonged solvent action. This is demonstrated, he states, by the fact that potato starch-grains, which lie for some time in the sprouting tubers, are less refractive than the grains of the tubers which have sprouted. This factor is, he states, hardly sufficient to account for the presence of loose, porous layers in the central parts of many grains. Such a phenomenon is, he admits, explained by the fact that many starch-grains in their earlier stages of growth are subjected to conditions which cause the formation of open, porous layers. In *Adoxa moschatellina* the lamellation was studied by following the stages of development. In the growing grains of young shoots eccentric layers are deposited corresponding to the form of the chromatophore. As long as the layers are not affected by solvent action, all of the layers, both

dense and porous, are entirely closed. At the apex of the grains the layers are very soft, but entirely closed, and the layers widen toward the base of the grain. Later, during short periods of solution, approximately uniform layers of substance are dissolved from the periphery of the grain, but the solvent action is more marked at the base, with the result of opening some of the lamellae laterally. If such open layers are later surrounded by newly formed closed layers, it is seen that in the periphery single open layers are located between numerous closed layers. When the grain has attained its normal mature size, its base is often subjected to active solution, whereby the grain becomes attenuated and the layers of the base loosened.

Meyer suggests that the dense layers are relatively rich in  $\alpha$ -amylose, and that amylo-dextrin occurs in largest quantity in the less dense, porous layers. It is, he states, possible that there are starch-grains which contain only amylo-dextrin, or amylo-dextrin and  $\beta$ -amylose, since not every chromatophore possesses an equally high capacity of condensation. The less dense layers may be distinguished, according to Meyer, by placing the grains in a solution of methyl violet and adding calcium nitrate, which causes a granular precipitation of these layers, which becomes stained. Such precipitation-staining experiments were carried out *in extenso* by Fischer (page 55).

Referring to the causes of the variations in the forms of starch-grains in the same plant, Meyer writes that the differences are due not only to the fact that the grains were not formed at the same time, but also to the fact that every cell has its own biology, and that even every chromatophore has its own individual properties. In different organs of the same plant starch-grains can take on a great variety of forms. In the tubers of potato, for example, the leucoplasts form mostly solitary, monarch, eccentric, conical, or oval grains, with a length of  $200\mu$ , and having definite irregular layers. The polytone starch-grains of a given plant part differ more from one another than the monotone grains, because in them the original differences are magnified. (Further reference to Meyer's investigations and his descriptions of starch-grains will be found on page 67.)

In opposition to the view that the alternation of light and darkness is a cause, or the cause, of the lamellation of the starch-grain, Fischer (Beihefte z. Botan. Centralbl., 1902, XII, 227) records that when cuttings of *Pellionia daveauana* were kept in the dark for two weeks, and then put for one week where the light of an incandescent lamp would fall on them continuously, a few lamellated grains were found like those described by Meyer as owing their lamellation to alterations of illumination. (See also St. Jentys, page 58.)

Some of Meyer's conceptions and conclusions were criticized by Robert (Ber. d. deutsch. botan. Gesellsch., 1897, xv, 231). According to Meyer,  $\beta$ -amylose remains unchanged during the conversion of raw starch into starch-paste, but Robert states that, if this were true, when the temperature falls below the minimum required for forming paste, the form of amylose that is insoluble in water would again assume its original condition, which is not the case. At the temperature of gelatinization, Robert states, amylose undergoes a permanent change, being converted into a substance capable of swelling to a much greater degree, the nature of the change being apparently that of a hydrolytic splitting of the molecule of  $\beta$ -amylose into smaller molecules of similar composition, and probably a further splitting into still smaller molecules at  $138^\circ$ , at which temperature the paste is soluble in water. Robert contends that the terms  $\alpha$ -amylose and  $\beta$ -amylose are not acceptable, and he proposes as substitutes farinose and granulose, respectively, and he suggests that the term amylose be retained for the substance which results from the gelatinization of granulose ( $\beta$ -amylose). He disagrees with Meyer's statement that every grain is completely and constantly surrounded by the substance of the chromatophore, without, however, mentioning the nature of his observations.

Salter (Jahrbücher f. wissenschaft. Botanik, 1898, xxxii, 117) also followed up Meyer's work, and some of Meyer's statements he confirms, but others he opposes. The following

is a brief summary of Salter's results: (1) The starch-grain, in all stages of its development, is sharply differentiated from the plastid in which it originates. In no instance are transition lamellæ noted. The substance of the grain is separated out by protoplasm, but not produced by the gradual transformation of the successive layers of protoplasm. (2) Meyer's observation that the chromatophore forms a complete and constant but very delicate integument of the starch-grain was confirmed in many instances. (3) The staining reactions prove the accuracy of the view that the lamellated appearance of starch-grains is primarily to be ascribed to the differences in density and also to the varying absorption capacity of the different layers; and that the coloration by stains is due merely to the imbibition of coloring matter between particles, the layers which stain dark being comparatively loose and watery, the darker the staining the less the density. (4) The conclusion reached by Nägeli regarding the uniform density of the young grains, and the manner of formation of the hilum and the first soft lamellæ, was confirmed. (5) All growing grains appear to possess a dense peripheral layer, which gives no indication of a lamellated structure. The lamellæ attain their distinguishing characteristics when covered by the lamellæ formed subsequently. (6) A progressive but not uniform decrease in density is noted in passing from the periphery toward the hilum, or structural center of the grain. (7) Lamellation bears a close relationship to the diverse conditions which arise during the formation of the layers. It is noted that layers soft at first become dense after a time. (8) Changes of the surface of the grains may be owing to ferment action. (9) Every lamella consists of structural elements arranged radially.

The substances designated  $\alpha$ -amylose and  $\beta$ -amylose by Meyer were studied by Syniewski (Annal. d. Chem. u. Phar., 1899, cccix, 282), who found that when starch-paste is treated for several minutes with malt extract all of the paste is dissolved except a small portion that remains as a flocculent mass, which is the amylocellulose of Meyer. According to Meyer this substance, when boiled in water and again treated with malt extract, is for the most part dissolved, leaving a residue that is  $\alpha$ -amylose. Syniewski ascertained in these experiments that from starch-paste amylocellulose could be obtained in quantities ranging from 0.7, 2.4, 3.6, and even to 13 per cent, according to the concentration of the paste; and also under different conditions variable percentages from paste of equal concentration. These facts led to the belief that amylocellulose is formed from the starch-substance that originally is dissolved or swollen. He notes that when a 5 per cent starch-paste is heated in a closed vessel under a pressure of 3 to 4 atmospheres, the starch-substance is entirely dissolved. From this solution on cooling there separated a gelatinous mass which is insoluble in cold water and not acted upon by diastase. On heating this mass it went into solution, and upon cooling it again became gelatinous, but upon frequent repetition a stage was reached when not all of the gelatinous matter was dissolved upon heating. This residue is not soluble in hot water, and is, he states, the  $\alpha$ -amylose of Meyer.

Syniewski found that, from a solution which according to Meyer contained only  $\beta$ -amylose he could obtain amylocellulose, and also that by continued boiling under pressure he could obtain the  $\alpha$ -amylose. Amylocellulose and  $\alpha$ -amylose therefore, he writes, originate subsequently from the substance of the starch-grains that first went into solution. When malt extract was added to starch-grains suspended in water, and the mixture heated to 70°, the starch was found to be dissolved completely without leaving any  $\alpha$ -amylose, which would necessarily remain if it preëxisted in the starch-grain. There is, therefore, states Syniewski, no evidence to uphold the theory that starch is a compound of two substances. To the contrary, he holds, since the starch-substance is homogeneous at 138°, a temperature not favorable for the origin of derived products, even if these by transformation go into solution, that it is safe to assume that the starch-substance is a single body. By the action of sodium peroxide on starch a form of soluble starch occurs which Syniewski regards as the simplest structural element of the complex starch-molecule, and which he terms

*amylogen*. He believes that starch (as well as the products obtained from it, such as Meyer's  $\alpha$ -amylose, Lintner and Düll's amyloextrin, the granulose of various investigators, etc.) consists of many amylogen molecules joined together in the form of carbinol-anhydrid combinations. (See Chapter III, p. 136.)

Strasburger (Text-Book of Botany, 1903) supports the view of the existence of a single fundamental substance, which, however, he admits exists in two forms in the grains, one of which (the inner part) is soluble in water at 100° C., and the other (the outer part) not soluble. He also notes that amyloextrin may be present, and that grains of certain plants, such as *Oryza sativa* var. and *Glutinosa* may consist principally of amyloextrin, and therefore color red with iodine. (See *Alocasia*, *Chelidonium*, and *Macis*.)

Bloemendal (Woch. f. Brau., 1909, xxxiii, 436, 449) is also among the opponents of Meyer's conception of the nature of the substances constituting the starch-grain. Bloemendal studied the chemical composition of various starches, and came to the conclusion that the  $\alpha$ -amylose and  $\beta$ -amylose of Meyer correspond to the cellulose and amylose of Nägeli, and if not identical differ only in water-content; and that the one form can be readily transformed into the other. He states that no amyloextrin is found in normal starch if sufficient care has been exercised in the course of preparation. (See page 170.)

Pfeffer (The Physiology of Plants, 1900-6, 2d edition, 3 vols., Oxford, Trans. by Ewart) records that starch-grains remain within the chloroplastids or leucoplastids in which they were produced until they are dissolved or removed, so that under normal conditions they are never found lying free in the protoplasm or cell-sap. The grains are described as being composed mainly or entirely of amylose, usually turning blue with iodine; but in certain cases, as in the seed-coat of *Chelidonium*, *Oryza*, etc., they are recorded as being mainly composed of amyloextrin, and other dextrins as well, so that a red coloration is produced with iodine. These dextrinous substances are stated to be formed as intermediate products of diastatic action, so that the starch-grains which redden with iodine may be regarded as having undergone partial conversion into sugar. Starch-grains, whether formed by chloroplastids or leucoplastids, are able, he writes, by virtue of their power of imbibition and swelling, to take up dissolved substances, and hence to interpolate new particles between the older ones. They might therefore, states Pfeffer, grow by intussusception, although the researches of Schimper and Meyer have shown that starch-grains usually grow by apposition. The structure and lamellation of the starch-grain, he holds, are mainly the result of its growth by the apposition of successive layers, but, as in the cell, secondary modification is possible by means of solvent and other agencies acting on the surface of the starch-grain. Starch, like reserve cellulose, may be partially or entirely dissolved when required for food, and hence at any time there may occur a solution or a renewed deposition. A starch-grain does not dissolve only from the outer surface, but also from within, so that frequently a skeleton of the grain is produced. Typical spherocrystals often dissolve in an equally peculiar manner, and changes in the condition during their formation may result in the production of denser layers than those first formed.

Pfeffer records that the shape and growth of the starch-grain depend upon a variety of factors, such as the specific character and activity of the amyloplastid (leucoplastid or chloroplastid), the position of the starch-grain in it, and also upon a number of conditions which influence these and other relationships. Hence, starch-grains in the same cell are not always precisely similar, while in diversely differentiated cells of the same plant they may assume widely different shapes, as, for example, those in the lactiferous cells of *Euphorbia* when compared with those in other cells of the same plant. Usually the grain continues to grow only so long as it is in contact with the plastid; and when the latter is attached to one side only, growth takes place in this direction, and as a consequence an eccentric lamellation results. The enlarging starch-grain, he states, not only regulates its own growth by causing the distension and shifting of the plastid, but also, as in case

of a growing crystal, the part already deposited influences the shape of the subsequent additions. The internal strains observed in starch-grains, he holds, could easily be produced by apposition combined with subsequent internal changes, and no arguments as to the mode of growth can be deduced from the supposed molecular structure, which is itself a mere hypothetical abstraction. The power of forming starch is, according to Pfeffer, possessed by etiolated chloroplasts as well as by many non-chlorophyllous chromatophores, but all chromatophores have not this power, and certain chloroplastids never contain starch, perhaps because solvent enzymes may be present which dissolve the starch as fast as it is formed.

The sequence of events which occur in the formation of the starch-grain was studied by Timberlake (Annals of Botany, 1901, xv, 619). His inquiries were made with *Hydrodictyon*. He notes that the pyrenoid (first described by Schmitz) is a spherical protein body that forms a part of the chromatophore, and that it bears a morphological relation to it similar to that of the nucleolus to the nucleus. In cells of this plant chlorophyll is distributed in the whole peripheral protoplasmic layer of the cell. No distinct chromatophores are observable in the cells. In the cells that contain an abundance of starch, practically the whole layer of the protoplasm, from the plasma membrane on the outside to the vacuolar membrane on the inside, is filled with starch-grains, all of which originate in pyrenoids and later are transferred bodily to other parts of the cell. The whole process of starch formation could be traced from certain structural changes occurring in the body of the pyrenoid.

The first indication of the changes leading to the formation of starch consists, Timberlake found, in a differentiation of the body of the pyrenoid into two portions, one of which is destined to become transformed into a starch-grain and the other to remain unchanged. The part that is to form the starch-grain stains less densely, and instead of red becomes a neutral gray or a faint orange, in safranin-gentian-orange stain. The dense homogeneous structure becomes spongy, with regions of varying density. Very often the dense regions are so distributed as to give an alveolar appearance. The denser regions become more prominent and take up the blue stain. Between the fully formed starch-grains and the unchanged remainder of the pyrenoid a thin zone of slightly stained material appears.

When the grain is fully formed it is seen to lie in a vesicle or vacuole in the cytoplasm, but without being surrounded by a differentiated membrane. The mature grain was observed to have practically the shape of the pyrenoid from which it was formed. This point, states Timberlake, aids in establishing that all starch is formed from the pyrenoid. When starch is produced rapidly a second grain will be built at once before the pyrenoid regains its original form. The long axis of the second grain is at right angles to that of the first. As the process proceeds rapidly, the grains as they are formed are continually crowded outward by the last-formed grains, until finally they are densely packed throughout nearly the whole of the protoplast. He therefore looks upon the pyrenoid as being directly the seat of a process which results in the formation of starch, which process, he states, is an exceedingly complicated one, as is shown by the structural and microchemical changes elicited by differences in staining.

Since the pyrenoid seems to be of a protein nature, and since a part of it (at least in *Hydrodictyon*) seems to be converted into starch, Timberlake suggests that the process involves the breaking down of a protein into carbohydrate. He notes that Boubier suggested the hypothesis that the pyrenoid is comparable to the leucoplast of the higher plants, and that the method of starch formation in it is similar to that in the leucoplast. The most serious objection to the comparison suggested by Boubier seems to lie, Timberlake believes, in the fact of the difference in structure between the two. Timberlake states that it is difficult to differentiate the leucoplast from the rest of the protoplasm, and that when it is differentiated it has a granular or reticulate appearance; but the pyrenoid

appears homogeneous, dense, and sharply bounded. Hence the relations of the methods of starch formation in *Hydrodictyon* and in chromatophores without pyrenoids must be regarded at present as uncertain.

In a series of precipitation-staining experiments, Fischer (Beihefte z. bot. Centralbl. 1902, XII, 226) tried various aniline and other dyes, using picric acid as the precipitant instead of calcium nitrate, which was employed by Meyer (page 51). Such grains were found (1) to be unaffected by nigrosin, Hessian purple, diamond red, Kongo red, carmine, aniline blue, and cyanin; (2) to be uniformly colored by acid fuchsin, corralin, eosin, crocein, tropæolin, Martin's yellow, and hemotoxylin; (3) to be in the form of fine precipitates by fuchsin, safranin, indigo carmine, methyl blue, methylen blue, and indulin; (4) to give large crystalline grains by methyl violet and gentian violet; (5) and exhibit radial needles by Bismarck brown, chrysodin, malachite green, brilliant green and thionin. These precipitation-staining reactions were confined to the less dense layers. The outer surface or layer was unaffected.

Kraemer (Botanical Gazette, 1902, XXXIV, 341) conceives the starch-grain to be a direct product of the polymerization of soluble carbohydrates of either the glucose or cane-sugar group, and that during the processes of formation the products consist of three substances, two crystalloidal in the form of starch-cellulose and granulose, and one colloidal. These are assumed to occur as follows: (1) In the point of origin of growth (the hilum) the colloidal substance is associated with a small proportion of cellulose, as also in the alternate lamellæ. (2) In the other layers occurs the granulose associated with a small amount of colloidal substance, and possibly also some cellulose. (3) The peripheral layer of the grain is not readily acted upon by reagents, and is quite elastic and more or less porous, probably consisting of an anhydride of cellulose. (4) In some cases some of the dextrans, or some of the non-colloidal or crystalline carbohydrates, such as maltose, dextrose, levulose, etc., may be present, probably formed as results of alterations taking place in the grain.

Certain aniline dyes were used by Kraemer to differentiate the lamellæ of the starch-grain. Freshly prepared starch-grains, or commercial starches, were treated with weak aniline solutions of safranin and gentian violet and allowed to dry at ordinary temperature, when it was observed that certain parts of the grain took up the stains more readily than others. He also used iodine, water, and various other reagents for the same purpose, and he makes the important observation that the grains from different plant sources do not react the same. The gentian-violet stain was found to be more pronounced in its effect upon potato starch than upon the starch of wheat and corn, the stain being held by the point of origin of growth (the hilum) and by the lamellæ alternating with it. On the other hand, he ascertained that safranin is a better differential stain for wheat starch, being held in certain of the lamellæ (usually not more than three or four of them being affected) and also in numerous radial clefts and channels. Corn starch did not appear to take up these stains as readily as either wheat starch or potato starch, and there was no differentiation of lamellæ, which Kraemer thinks as being probably due to the peripheral layers being denser and less permeable.

In referring to the previous work of Salter (Jahrbücher f. wissensch. Bot., 1898, XXXII, 117), Kraemer takes exception to his statement that the dye has not a selective or specific action on the layers, and he states that Salter's figures show that certain parts of the grain stain more than others, and that he believes Salter's work, as well as that of Meyer (*loc. cit.*), with methyl violet, correspond with his own in that the layers that are colloidal in character take up the stain. He notes that the lamellæ which are not affected by the aniline stains become blue with iodine, the alternate layers and the point of origin of growth remaining unaffected. The layers thus affected by iodine, he states, are the ones that are rich in granulose and more clearly defined in the grains of potato and wheat starch

than in corn starch. These lamellæ, Kraemer found, become crystalloidal in character on treatment with water at 60° or 65° for about an hour, and also with chromic acid, calcium nitrate, saliva, and other reagents.

The effects of these reagents upon different starches were found to be not identical. Upon potato starch the first effect is to make the lamellæ more distinct; this is followed by the development of the crystalloidal character of the lamellæ, which is most pronounced in those lamellæ which are colored blue with iodine; this in turn is followed by the production of small tracts or channels which connect contiguous lamellæ; then larger channels form which are plume-like in appearance, the grain in the meanwhile swelling quite perceptibly, the middle portion becoming clearer and assuming a zig-zag outline, between which and the periphery a number of crystalloidal lamellæ arise; the grain now becomes spherical and marked by a number of concentric lamellæ near the periphery, and the lamellæ finally rupture, followed by a gradual solution of the grain (see page 172).

In wheat starch the development of the crystalloidal character of the lamellæ is followed by the formation of narrow, interrupted or continuous, radial channels near the periphery of the grain, which are sometimes connected with lamellæ located near the middle of the grain; the grain meanwhile swells perceptibly, the center becomes clearer, and the contents are crowded into crescent-shaped halves which are still connected at the poles; the contents of each of the halves consist of crystalloidal lamellæ in which are then produced small tracts or channels connecting the contiguous lamellæ, the halves in some instances finally separating and slowly dissolving. The first effect of reagents upon corn starch is to bring out the point of origin of growth, which becomes larger and in some cases more or less zig-zag in outline; between this and the periphery of the grain there arise more or less interrupted or continuous radial channels, usually the latter; the crystalloidal structure of the grain develops slowly and is most pronounced when the grain has swollen to two or three times its normal size. At this stage the center of the grain has become clear and the point of origin of growth has become obliterated in some cases, and between it and the periphery occur numerous crystalloidal lamellæ similar to those observed in potato starch. Finally, the peripheral layer ruptures and there is a gradual disintegration of the grain. Sometimes it was noted by Kraemer that the grain appears to separate into as many parts as there were arms to the point of origin of growth, particularly when acted upon by saliva or diastase.

Kraemer, in summing up his observations, concludes: "The starch-grain consists of colloidal and crystalloidal substances, these being arranged for the most part in distinct and separate lamellæ, that is, at the point of origin of growth, and in the alternate lamellæ the colloidal substance preponderates, associated with the crystalloid cellulose; whereas, in the other layers the crystalloid substance, consisting for the most part of granulose, occurs in greater proportion. As further evidence of the presence of these crystalloidal and colloidal areas we may say that the peculiar behavior of the colloidal layers toward aniline stains is analogous to the behavior of a section containing mucilage cells towards these dyes, the latter being taken up by the mucilage cells alone." Kraemer holds that differences in the starch-grains show that starch, instead of being a uniform substance, is in fact composed of several substances in varying proportions, but more or less definitely arranged.

Denniston (Trans. Wisconsin Acad. Science, Arts and Letters, 1904, xv, 664), in his studies of the growth and organization of the starch-grain, also made use of aniline dyes to differentiate the different layers, and he furthermore noted that the differences of the various layers of the same grain vary when the grain is mounted in water, iodine solution, and a solution of gentian violet and orange G, respectively. Layers which, for instance, appear single in water may appear double or multiple in a color reagent, and a single layer brought out by one stain may appear as two by means of another. The results of the

staining reactions with canna starch are summarized by Denniston in tables 1 and 2, the order of the parts being from the margin inward.

TABLE 1.

A (in water).	B (in iodine solution).	C (in gentian violet and orange G).
a. Refractive layer.	{ <i>a'</i> . Light blue. <i>a''</i> . Dark blue.	<i>a'</i> . Orange. <i>a''</i> . Light blue.
1. Crevice.		
b. Refractive layer.	b. Pale-blue layer.	{ <i>b'</i> . Dark-blue layer. <i>b''</i> . Light-blue layer.
2. Dark line.	2. Dark line.	
c. Dark, slightly refractive layer.	c. Dark-blue layer.	c. Dark-blue layer.

TABLE 2.

A (in water).	B (in iodine).	C (in alcohol).	D (in gentian violet and orange G).
a. Highly refractive region.	<i>a'</i> . Layer which has not so fully taken on the nature of starch, hence is faintly blue in color. <i>a''</i> . A blue starch layer.	This layer is now pale blue in color, the color becoming lighter from inside toward periphery. There is no sharp line separating two parts.	The layer <i>a'</i> is of different composition and takes orange; <i>a''</i> is starch and takes gentian violet like rest of grain.
1. A dark line, probably a crack filled with watery colloidal mass.	This layer is broader and paler in color.	In alcohol this layer is still broader.	This layer is about the same width as in alcohol. It stains pale blue.
b. Highly refractive layer.	Contracted slightly and stains blue.	Contracted a little more and blue partly removed.	Characteristic blue with gentian violet.
2. A dark line similar to 1.	<i>c</i> and layers anterior to <i>c</i> have contracted, leaving space at 2.	Contraction goes on with consequent broadening of 2.	Stains pale blue, contains relatively small amount of starch.
c. Slightly refractive layer.	In iodine this layer stains uniformly with those next to it on inside. It is pale blue in color.	This iodine is easily removed, leaving layer pale blue in color.	This layer stains less deeply than <i>a</i> or <i>b</i> .

The layers which take the deepest color with iodine and gentian violet Denniston regards as being the more dense, but, in case of such precipitants as were used by Meyer (*loc. cit.*, p. 51) and Fischer (*loc. cit.*, p. 55), the less dense. The limitation of the orange layer, he holds, can not be due to hindrance to the penetration of the dye, because the layer does not become thicker in time and because in the case of crushed grains in which the dye has access at once to all of the layers the parts adjacent to the outer layer do not become yellow. He also noted that the *central* part of *Canna* grains stain yellow, and that frequently *young* grains stain entirely orange with the exception of one or two dots, thus in agreement with the view expressed by others that the young grain is of different composition from the later superposed starch. As further evidence of a differentiation of the outer layer, he found that weak iodine may penetrate to the inner part of the grain, coloring it blue without in the least coloring the outer part (as had been found by Nägeli). The outer layer he believes is in the nature of a transition substance undergoing erosion or deposit.

Further evidence that starch is not a uniform substance was found by Härz (Beihft. z. botan. Centralbl., 1905; Woch. f. Brau., 1905, xxii, 721) in experiments with solutions of chromic acid, and chromic and sulphuric acids, in which the starch-grains were macerated for 24 hours and then washed with cold water. Not only did the various kinds of starch, but also different grains of the same starch, differ widely in their behavior; from which Härz asserts that starch can not be a physically uniform substance which consists of granules differing merely from each other according to a denser or looser constitution of their ultimate complexes. He states that amyloextrin also did not behave like a uniform substance, but seemed to be made up of a number of molecular groups which differ in com-

plexity and density of internal structure, and that in examining the decomposition products of starch it was not until the achroodextrin stage of the degradation of the starch molecule was reached that the products exhibited an apparently uniform molecular condition.

Aniline dyes were used by Gastine (*Compt. rend.*, 1906, *CXLII*, 1207; *Jour. Soc. Chem. Ind.*, 1906, *xxv*, 655) for the detection of rice flour in wheat flour. A sample is treated on an object glass with a solution of a suitable dyestuff, drying the preparation at about 30° C., and then completing the desiccation by heating for a few minutes at 110° to 130° C. The preparation, he states, should be mounted in cedar-wood oil or Canada balsam and examined under the microscope. For staining, a solution of aniline blue or green may be employed of a strength of 0.05 grm. in 100 c.c. of 33 per cent alcohol.

This treatment has the effect of showing the hilum of the minute rice starch-grains, while wheat starch rarely exhibits a visible hilum. In rice flour isolated starch-grains are rare, the grains generally occurring in clusters in starch-bearing cells. These clusters, according to the above method, have a very characteristic appearance, since the hilum of each starch-granule appears as a reddish-colored point, these red points being grouped quite uniformly in symmetrical arrangement resembling a mulberry under a high magnification. Wheat and rice starches do not take up the dyestuff; only the nitrogenous matters are dyed. The fragments of rice flour therefore appear colored; the medium-size and large granules of wheat are practically uncolored, but the small granules of the wheat, in which the interstitial nitrogenous matter is more abundant, are distinctly colored. The grains of corn and buckwheat starches behave like rice. Potato, arrowroot, and sweet potato starches, unlike the cereal and leguminous starches, absorb the dyestuff directly.

According to Maquenne and Roux (*Compt. rend.*, 1905, *CXL*, 1303; 1906, *CXLIII*, 124) starch consists of about 90 per cent of amylocellulose and about 10 per cent of amylopectin. The former they describe as being devoid of gelatinizing power, but the latter as gelatinizable. (See Chapter III, page 112.) Day (*U. S. Dept. Agriculture, Office Expt. Sta. Bull.* 202, 1908) records three substances in starch-grains, which are designated blue amylose, red amylose, and rose amylose, in accordance with the color reaction with iodine. (See Chapter IV, page 166.)

Some extremely interesting and original views of the chemical nature, and also of the cause of the peculiar structure, of the starch-grain were published by St. Jentys (*Bull. d. l'academie d. sc. d. Cracovie*, 1907; *Jahr. ü. d. Fort. d. Theirehemie*, 1907, *xxxvii*, 99), who records that the presence of tannin in the cell-sap, and the fact that a solution of starch yields with this substance a preparation which is insoluble in cold water, led him to the supposition that tannins enter into the composition of the starch-grain. Doubts as to the components of the starch-grain were first aroused in St. Jentys by the results of a series of experiments in connection primarily with the peculiar reactions of the starch-grain and starch-paste with iodine. St. Jentys observes that starch-grains, as is well known, are rarely colored a pure blue with iodine, but usually violet, and sometimes even black. A still greater variety in color may be observed in dried starch-grains and starch-paste which have been treated with iodine, the violet parts gradually going over into a cherry-red, copper-red, or orange, and finally into a brownish-yellow. These various colorings (which Nägeli had already noticed) are due, St. Jentys states, to the presence in the starch-grain of substances which differ in their behavior toward iodine. For example, the starch-grains of potato pulp, from which the tannin had been removed by means of a methyl-blue solution instead of water, were colored an indigo-blue with iodine after the removal of the methyl-blue; the outer surface of the intact grains was colored violet, while the inner part became more of a blue color. Other reagents also acted differently on the outer and the inner parts of the grains.

St. Jentys could extract nothing by cold water from intact grains, but some particles passed into solution from the pulverized grains. Boiling with water, he states, evidently

causes the outer layer to swell or to dissolve, the whole grain producing starch-paste; but the starch does not go into complete solution, because undissolved flakes always remain behind and take on a violet-color with iodine, while the solution turns blue. The process of gelatinization also varied in starches of different origin. For instance, in acorns the starch gelatinized only after being heated in water to 77.5° to 87.5° C., while the other starches did so at a much lower temperature, the difference being due, he states, to the richness of the starch in tannin. The presence of tannins also explains, he believes, the failure to obtain starch-paste from raw boiled potatoes and from acorns. In fact, he found that starch-paste will not be formed if the grains are boiled in a weak solution of tannic acid. The starch in such a mixture is in flakes and the preparation can not be used as a paste. The presence of tannin as a constituent, especially of the outer layer of the starch-grain, is further indicated by the behavior of starch to iodine in solutions of calcium, magnesium, and zinc chloride, of potassium bromide and potassium iodide, and of concentrated lye. The action of these reagents depends, as revealed by the microscope, upon the solution of the outer layer of the grain by these compounds. A certain power of resistance to solutions of diastase is one of the peculiar properties of the outer layer. Starch-grains were found to be affected by diastase only after the outer layer had here and there been eroded by enzymes.

St. Jentys attempted to prove by direct experiments the hypothesis that tannins enter into the composition of the starch-grain. Potato starch was digested with concentrated sodium hydroxide, and from the solution substances were extracted by means of concentrated alcohol which separated spontaneously from the solution, one of which was obtained in crystalline form. These substances gave either a yellow or a copper-red reaction with iodine, and also the characteristic color reaction for tannin with ferric chloride. The alcoholic mother-liquor was colored greenish-yellow, and it also gave the tannin color reaction with ferric chloride. The mass which was insoluble in concentrated lye and alcohol, and which may be compared with Nägeli's granulose, is dissolved readily in water, and it is the substance in which a blue color with iodine predominated. Nor was this part of the starch found to be of uniform composition, as was proved by the addition of a surplus of iodine. The solution which at first was blue turned black, and after successive shakings with chloroform and ether this black solution became violet and then blue, while bodies staining a dark-red or yellow went into solutions in the chloroform and ether. Now that the part which tannin plays in the iodine reaction, as well as in other reactions of starch, was recognized, it was natural, St. Jentys writes, to suppose that the lamellated structure of the grain is due to the presence of tannin. He states that when changes in concentration occur, the formation of concentric layers must be caused by crystallized bodies like tannins. In fact, from solutions of granulose, to which tannin had been added, and allowed to evaporate, there separated lamellated structures resembling the most beautiful starch-grains, such as are found in only a few plants like *Dioscorea* or *Canna*. Adding gallic acid instead of tannin to a granulose solution caused a radial structure to appear with concentric lamellæ, and the grains which separated from the solution resembled the starch-grains of wheat, buckwheat, or Chinese sokes, which is only the more remarkable inasmuch as the grains with a more radially striated structure gave a more violet color, sometimes a red color, with iodine, also a color similar to that of gallic acid. The lamellated grains, as St. Jentys found, could now be studied not only in granulose, but also with methyl-blue solutions, these solutions being allowed to evaporate after the addition of tannic acid.

St. Jentys states that in view of these phenomena it is clear that the starch-grain in the plant is formed neither by apposition nor by intussusception, but by solidification of concentrated solutions; and, furthermore, since tannins also possess the property of being colored with iodine, not every part of the grain which shows a lamellated structure and which is colored by iodine need necessarily be considered a carbohydrate. Since in the

process of the saccharification of starches with mineral acids or with diastase the iodine reaction shows all the changes of color which may be observed in the intact grain, as well as of starch-paste under certain conditions, it may be assumed that this process (contrary to the universally accepted view of the progressive formation of the dextrins) depends upon the splitting up of the compounds of sugar and various tannins in the form of glucosides, and to the successive decomposition of separated or free tannins. St. Jentys believes that numerous observations seem to support this theory. For instance, starch-paste which had been colored blue with iodine on being treated with powdered leather, first turned violet and then became colorless, and it had the property of reducing Fehling's solution. On distilling acidified starch-paste with sulphuric acid in order to test for sugar, an aromatic volatile body with a peculiar, unpleasant odor went into the distillate, which combined with iodine without a color reaction.

#### OCCURRENCE OF THE STARCH-GRAIN IN PLANT LIFE.

C. Nägeli (*loc. cit.*) gave careful study to this subject, and this section is a free translation from his memoir. He states that starch has a very general distribution throughout the vegetable kingdom, but that it is absent from the *Fungi*, *Diatomaceæ*, *Chroococcaceæ*, *Nostocaceæ*, and many other cellular plants, and also apparently from some vascular *Pteridophyta*. Little or no starch is found in colorless parts of plants of one year's growth from which no new structures arise, but on the other hand more or less starch will always be found in tissue containing chlorophyl. The vegetative parts which develop organs often store up considerable amounts of starch in their colorless portions when they are not too near the surface and are of the right age, for example, the underground parts of perennial herbaceous plants, all of which contain a great deal of nutritive material, and from which starch is seldom wholly absent. Furthermore, the stems and branches, also portions of the roots of trees and shrubs, contain starch, which is present in the pith, in the medullary rays, and in the wood-cells up to a certain age of these organs, generally in small quantities, or occasionally in considerable quantities, in the region near the leaves. Finally, it is observed in the pith of this year's sprouts, in the receptacle, and even in the placenta.

In seeds the presence or the absence of starch-grains is more sharply defined than in other portions of the plant which contain nutrient material. Generally all of the genera of one order correspond in this respect, this holding good for eleven-twelfths of the natural families; and the genera of the same order are seldom dissimilar in this particular, and still more rarely the species of a genus. No starch-grains occur in the seeds of about four-fifths of the natural families and about nine-tenths of all the genera of Phanerogams. Starch is present in the seeds of about half of the families and genera of *Gymnospermæ* and *Mono-cotyledones*, while it is absent from the other half. Starch is found in the seeds of about one-sixth of the *Dicotyledones*, and in only one-fourteenth of the families and in a still smaller fraction of the genera of *Gamopetalæ*.

When starch-grains are present in seeds, other reserve foods are almost entirely excluded; if seeds which are rich in starch have perisperm, then the embryo with few exceptions contains oil and no starch-grains; but if perisperm is absent from the seeds, the starch is found in the cotyledons, while only oil is usually present in the cells of the canicle and plumules. Whenever the various genera of an order differ in this respect, it will occur usually among those which have seeds of different sizes, starch being found in the large seeds and lacking in the small seeds. The resting spores of Cryptogams usually have no starch-grains, but if two kinds of resting spores are present they usually agree in that starch is in both either present or entirely absent. Starch is absent in the majority of the pollen grains of Phanerogams.

If we consider the development of the entire plant, the formation of starch always occurs in the tissues at certain stages of their growth and disappears from them at a definite

age. This agrees in general with similar transitions found in vegetable life. Before the decay of the lateral parts the starch is again dissolved and conducted to the main organs, for example, from the periphery and the growing vegetative ends.

Sometimes starch formation is found in all of the plant parts in consecutive order (organs, tissues, cells), while now and then it is omitted from one of them. A noteworthy suspension in starch formation occurs during the transition from the active to the latent period of vegetation, and this may be due to the disappearance of the starch and its replacement by another reserve material, such as fatty oil, or it may be that starch formation has ceased before the end of the period of rest. Thus, in the development of Phanerogams the formation of starch may extend to the ovary, the placenta, the testa, the outer and inner coats of the ovule, and to the embryo; and the starch may remain stored up where it is formed in the embryo, in the endosperm, etc., or it may disappear from these parts.

The underground parts of plants usually contain both simple and compound starch-grains, and usually in equal proportions, although it may appear that there are more of one kind than of the other. The compound grains consist of a larger or smaller number of components, depending on whether there are the same or a greater number of these grains present; if they are in the majority they are composed of from 10 to 12 components; when they are almost exclusively present 20 components; and in exceptional cases as many as 200 to 500 components may enter into the composition of the grain. The components of the compound grains are sometimes of the same size, but more frequently differ in size. The simple grains probably never belong to the centric-lamellated type, but an eccentric structure can in most cases readily be distinguished. In closely allied species, or in the same species, the simple grains are larger in proportion as they outnumber the compound grains, while they become smaller and show a less distinctly developed structure as they decrease in number.

Although the parts of the plant above the ground (bark, pith, wood, leaves, receptacle, and pericarp) are essentially similar in structure to the underground parts, they contain starch-grains which are smaller and less well developed.

Starch formation is less general in the vegetative organs, among which Nägeli includes all organs except the seeds, and often shows considerable variations, even in different parts of the same plant. In this respect nearly related plants may differ so completely that they can easily be distinguished by the place of formation of the starch-grains, while usually no characteristic difference is found in this particular in an entire order of plants.

All forms of starch-grains may occur in seeds, but generally the simple grains with centric lamellæ and the compound grains of many equal components considerably exceed all others in number. The compound grains with many components (usually many thousand) are more likely to be found in the perisperm, and the simple grains with centric lamellæ are less numerous, while those of an eccentric structure very rarely occur. On the other hand, in the cotyledons the simple grains are more numerous, sometimes with centric, sometimes with eccentric structure, the former usually predominating, while the compound grains usually consist of 2 to 8 or in exceptional cases of 24 to 40 components. If starch is found in both the perisperm and the cotyledons the grains show a similar structure.

The starch in seeds, especially in the spores and endosperm, show much likeness in form, variations occurring only within narrow limits. Very often only simple or only compound grains will be found in these parts, although it is not unusual to find both kinds in the cotyledons. All genera of one natural order frequently contain closely related starch-grains, but sometimes a distinction is shown between genera, and usually then between species, so that these may be classified into natural groups according to the structure of their starch.

The starch-grain is found almost exclusively in the plant cells. It is absent from the vascular bundles and also from the latex, except in *Euphorbiaceæ*. It is found in the cells in large number, even to 100, and then of small size; or a few or even one single grain of

considerable size. In the cells they sometimes constitute the only non-nitrogenous nutrient reserve substance; sometimes other equally important compounds, such as fat, cellulose, pectin bodies, etc., are present. In most cases the grains lie immediately within the cell-cavity, and when present in large numbers they fill up the whole space and are sometimes so crowded that they become flattened by mutual pressure into polyhedrous forms. They may be in direct contact with each other, or a thin layer of protoplasm may intervene, so that each grain appears to be lodged in its own compartment. When they are present in smaller masses they frequently form a lining to the wall of the cell, and may if they lie in a single layer become polygonal, owing to their crowded position; if still less numerous they sometimes cover merely the cell nucleus or circumscribed places of the cell.

The starch-grains during their entire existence, or at least in earlier stages, are inclosed within plastids. In the nuclei and in the mucilage cells there may also occur oil-drops, mucus granules, and chlorophyl granules. Starch usually occurs alone in the chlorophyl bodies, but occasionally oil-drops are present. Owing to the crowded position of the grains they are sometimes flattened by mutual pressure and may remain coalesced as a pseudo-compound grain if set free by the disappearance of the surrounding protoplasm.

In the chlorophyl of the *Desmidiaceæ* and the *Zygnemaceæ*, as well as in several other lower forms of *Alga*, the starch appears first in the form of homogeneous rings (globular shells) inclosed in protoplasm, and which later, through radial division, are converted into a compound, spherical, hollow grain.

#### PECULIAR KINDS OF STARCH, AND STARCH-LIKE BODIES.

A substance termed soluble starch was found by Dufour (Bull. de la Soc. vaud. de Sci. nat., XXI, Nr. 93; Zimmermann, Botanical Microtechnique, 1893, 229) in solution in the cell-sap of the epidermal cells of a few plants, notably *Saponaria officinalis*. The chemical composition was not determined, but it agrees with the soluble starch made *in vitro* from starch-grains in forming a solution in water, and in yielding a blue, violet, or red reaction with iodine. Ewart (Pfeffer's Physiology of Plants, trans. by Ewart, 1900-6, I, 473), in referring to the soluble starch in *Saponaria*, etc., states that—

Similarly, the cell-sap in the epidermal cells of *Arum italicum* turns violet when treated with iodine, the color disappearing on heating and returning on cooling. The substance giving this reaction escapes from the cells as soon as they are killed, and the watery extract yields on evaporation a transparent, slightly gummy residue, which turns violet or blue with a watery solution of iodine, but reddish-brown when alcoholic iodine is added, turning blue in the presence of water. After prolonged boiling a more reddish reaction is given, and also partial digestion with diastase or ptyalin, while ultimately the color reaction disappears, a reducing sugar being formed. This "soluble starch" has a very much feebler osmotic value than cane sugar or dextrose, and its molecule is presumably large and complex. Its peculiar distribution points rather to its possessing some biological function (hindrance to transpiration, protection, etc.) than of its having any special value in nutritive metabolism. It may occur in small quantity in the cell-sap of the guard-cells of the stomata, though it seems always to be more abundant in the surrounding epidermal cells, and it may be still present in almost undiminished abundance after a prolonged sojourn in darkness (ten days), although no starch is then present in the mesophyll. The soluble starch soon escapes from the epidermal cells when placed in 50 per cent alcohol, and the same also occurs in absolute alcohol, though more slowly.

Incidentally in this connection might properly be mentioned the discovery of Wederhake (Centralbl. f. allg. Path. u. path. Anat., 1905, xvi, 517) of the occurrence of what he terms genuine starch-grains in the human secretions and excretions. The so-called starch was found in the fresh spermatic juice and in the testis, and also in a gonorrhœal discharge, sputum, tuberculous sputum, pus, and both normal and abnormal urine. The grains yielded a deep-blue reaction with the tincture of iodine, and lost their color upon heating and recovered

it on cooling. But they did not give the starch reactions with iodine-sulphuric acid, methyl violet, and gentian violet. (This might be a form of glycogen, since Claude Bernard found a glycogen in paralyzed muscles that gave a blue reaction with iodine, and because glycogen is an important constituent of the human organism, while starch heretofore has been found only as a transient food-stuff that is confined to the alimentary tract.)

A starch-like substance known as "Floridean or Rhodophycean starch" has been observed in a number of *Florideæ*. This body, which seemingly is not identical in different plants, has been examined by Van Tieghem (Compt. rend., 1865, LXI, 804), Belzung (Ann. d. Sci. nat. Bot., Ser. VII, v, 179; quoted by Zimmermann, *loc. cit.*), Hansen (Mitth. a. d. Zool. Station. z. Neapel, 1893, XI, 276, 283), Golenkin (Alogologische Notizen, 1894, 4) and Burns (Flora, 1894, Erg. bd., 159). Van Tieghem found that the grains of this body agree in most of their chemical properties with ordinary starch, and that in the polarizing microscope they showed a similar cross or interference figure. With iodine, however, a yellow-brown or brownish-red reaction was obtained. Belzung states that the starch of many *Florideæ*, especially the young grains, yields a blue reaction.

The "Phærophycean starch" described by Schmitz (Die Chromatophoren der Algen, Bonn, 1882, 154; Jahrbücher f. wissenschaft. Botanik, xv, 1) is in the form of colorless bodies that are found in the cytoplasm. They do not yield a color reaction with iodine, and it is held by Berthold (Jahrbücher f. wissenschaft. Botanik, XIII, 569) that such bodies do not exist.

Paramylon or paramylum grains have been examined by Klebs (Untersuch. a. d. bot. Institut z. Tübingen, 1883, I, 233), Schmitz (Jahrbuch. f. wissenschaft. Botanik, 1884, xv, 111), Schimper (*ibidem*, 1885, xvi, 199), and Zopf (Schenk's Handbuch, Bd. III, Hefte 2, 1). These grains have been observed in several *Euglenæ* and other low organisms, but there is doubt as to their actual character. They have been seen as disk-shaped, rod-shaped, and ring-shaped forms, and in some instances they have been found to be lamellated after being subjected to the action of certain swelling media, but the lamellation differed from that of ordinary starch-grains, inasmuch as it was in the form of complete concentric rings or plates without a common center or hilum. They do not yield a color reaction with iodine, and they differ in their behavior towards certain swelling and solvent reagents, and certain stains.

Glycogen, which occurs in both plant and animal life, chiefly in the latter, is so closely related to starch as to be called "animal starch." In the saccharification of glycogen, dextrins appear as intermediary bodies, as in the saccharification of starch (see Tebb, page 153). With iodine it yields an orange to a reddish-brown or wine-color reaction, according to the strength of solution and form of the glycogen, the color disappearing on heating and reappearing on cooling, as with starch. It is soluble in water, forming an opalescent solution, and it exists in the living tissues usually as colorless, refractive bodies. Errera (Bot. Zeit., 1886, 316; Zeit. f. w. Mikrosk., III, 277) found that glycogen is widely distributed in fungi, in which it seemingly replaces starch, inasmuch as chromatophores are absent from these organisms and therefore probably no starch formed, and since it seems to serve the purpose of a reserve food. It is also formed, sometimes very abundantly, in yeast cells, as shown by Errera (Recueil de l'Institut botanique, Bruxelles, 1906; Compt. rend., 1885, CI, 253), Laurent (Jahresbr. ü. Gärungorg., 1900, I, 54), Meissner (Centralbl. f. Bakt., 1900, II, 6), Cremer (Ber. d. d. chem. Gesellsch., 1899, XXXII, 2062) and Pavy and Bywaters (Jour. Physiology, 1907, XXXVI, 149). In large fungi, as in *Phallus*, Clautriau (Jahresb. ü. Gärungorg., 1895, VI, 51) has shown that glycogen disappears rapidly during growth, it being altered in the same manner as starch under similar conditions in phanerogams.

Other substances that bear a more or less close relationship to starch are cellulin bodies discovered by Pringsheim (Ber. d. d. botan. Gesellsch., 1883, 288) in the hyphæ of *Saprolegniaceæ*. These bodies occur in the form of spherical, circular, or polyhedral granules which are occasionally lamellated. They do not yield a color reaction with iodine. Noll

found cellulin bodies in *Siphonæ*, and Denniston (Trans. Wisconsin Acad. Sciences, Arts and Letters, 1904, xv, 664) studied them in *Saprolegnia*. Denniston found that they, like the outer layer of starch-grains, show a strong affinity for orange-G aniline, in contradistinction to the inner part of the grain, or the granulose, which shows a correspondingly strong affinity for methyl violet. He regards these bodies as being made up of a substance that is intermediate in character in the synthesis and analysis of starch. (See Denniston, pages 56 and 57.) Related to these bodies are spherical granules found by Weber van Bosse (Ann. Jard. bot. de. Buitenzorg., 1892, viii, 165) in *Phytophysa treubii*. These granules do not yield a reaction with the iodine-potassium iodide solution, but they become blue in the presence of iodine-zinc chloride, and violet on the addition of iodine-sulphuric acid. Bodies in the form of U or V shaped cups, hollow cones, and hollow cylinders, known as fibrosin bodies, were discovered by Zopf (Ber. d. d. botan. Gesellsch., 1887, 275) in several *Erysiphææ*. They do not yield a color reaction with either the iodine-potassium iodide or iodine-zinc chloride solutions.

Some forms of cellulose are in the nature of a food reserve, and bear a close relationship to starch in this respect, and also chemically. Like starch, they yield sugars upon decomposition in the presence of dilute acid or appropriate enzymes, the cellulose-reducing enzymes being designated cytases. (See Brown and Morris (Jour. Chem. Soc. Trans., 1890, lvii, 458), Schulze (Zeit. f. physiol. Chemie, 1890, xiv, 227, and 1892, xvi, 387), Newcombe (Annals of Botany, 1899, xiii, 49), and Heruuaey (Revue d. bot., 1903, xv, 345).) Phætophycean starch, paramylum bodies, cellulin bodies, and fibrosin bodies are doubtless closer relatives of cellulose than of starch. Some forms of plant mucus and cellulose give a blue reaction with iodine.

#### THE CHIEF FORMS AND CLASSIFICATIONS OF STARCH-GRAINS.

It must have been recognized by Leeuwenhoek, and by many of the investigators of the earliest part of the last century, that starches from different sources are not morphologically identical, but if so it does not seem to have attracted any particular attention until the investigations of Fritzsche (Ann. d. Physik. u. Chemie, 1834, xxxii, 129), although Payen and Meyen and others examined a number of different starches. Fritzsche described the starches obtained from a variety of plants, including *Solanum tuberosum*, *Costus speciosus*, *Tulipa gesneriana*, *Fritillaria meleagris*, *Lilium bulbiferum*, *Amaryllis formosissima*, *Bromelia* sp., *Hyacinthus orientalis*, *Iris florentina*, *Ixia crocata*, *Narcissus poeticus*, *Crocus vernus*, *Colchicum autumnale*, *Bulbocodium vernum*, *Gladiolus communis*, *Arum dracuncululus*, *Pisum sativum*, *Canna edulis*, *Hedychium flavescens*, and *H. hirsutum*. He noted not only that the starches from different sources were different, but also that often the form was so characteristic as to determine the plant, or, at least, indicate the genus and family from which the specimen was obtained. This statement was confirmed some years later by Schleiden (Principles of Botany, 1849, 14), who examined a number of starches, mostly not described by Fritzsche. From the differences observed he was enabled to tabulate the various forms, and he published a classification that has continued to be quoted in various standard works, even at the present time.

#### SCHLEIDEN'S CLASSIFICATION OF STARCH-GRAINS.

- I. *Amorphous Starch.* Amorphous starch was found in only two phanerogamous plants, it occurring paste-like in the cells, as in the seeds of *Cardamomum minus* and in the bark of *Smilax ornata* (Jamaica sarsaparilla). In the case of the latter it is not improbable that the method of drying by the fire, common in the preparation of sarsaparilla, may change the character of the starch. The paste is most frequently found in abnormally red roots, and less frequently in the yellow roots, neither of which have hitherto been esteemed in commerce as varieties of the Jamaica sarsaparilla.

II. *Simple Grains.* The majority of plants exhibit perfectly simple individual grains, among which doublets and triplets only occur as exceptions. The following groups may be distinguished:

1. *Roundish Bodies.*

A. *With the central cavity or hilum apparently absent.*

1. Quite small, almost spherical granules, occurring almost everywhere from time to time in the vegetable kingdom as cellular contents, as for instance, in carrots, in the cambium in the winter; in leaves as the bearers of chlorophyl, etc.
2. Large, irregular, knobby, often truncated multiangular grains, as, for instance, in the bulbous buds of *Saxifraga granulata* and in the pseudo-tubers of *Ficaria verna*.

B. *With small roundish central cavities or hila.*

(a) *With a perceptible laminated formation.*

3. Very large, rough grains, deformed as it were. Found in the pith of the *Cycadaceæ*. There are somewhat similar grains in the underground leaves of *Lathræa squamaria*, in which the inner layers form an ovoid grain almost similar to those of potato starch; the few grains formed in external layers, on the contrary, are so irregular, and generally so disproportionately thickened at one or two sides, that the whole grain assumes a broadish triangular figure.
4. Ovoid granules. In the potato.
5. Mussel-like granules. In the bulbs of the larger *Liliaceæ*, as in *Fritillaria* and *Lilium*.
6. Almost triangular. In *Tulipa*.

(b) *With an indistinct or deficient lamellated formation.*

7. Rounded-off polyhedric grains. In the albumen (perisperm) of *Zea mays*.
8. Sharp-edged, polyhedric, very small grains. In the albumen of *Oryza sativa*.

(c) *With an elongated central cavity.*

9. Roundish or oval grains, in a dry condition, generally showing a star-like cleft in the inner layers. In the *Leguminosæ*, as in the seeds of *Pisum* and *Phaseolus*.

(d) *Perfectly hollow, apparently cup-like grains.*

10. Very marked in the rhizome of *Iris florentina* and in kindred species.

2. *Flatly compressed lenticular granules.*

11. Sometimes with, sometimes without, a decided lamellated formation; sometimes with a central, or eccentric, or less rounded, or more elongated, or radiated torn-up cavity or hilum. In the albumen of *Triticum*, *Hordeum*, and *Secale*.

3. *Perfectly flat discs.*

12. With more distinct lamellæ, in which it is, however, at times doubtful whether they pass entirely around or are only menisci laid over one another. The former appeared probable owing to analogy and the phenomena presented in roasting and on dissolving in sulphuric acid. We do not find it in the rhizomes of all the *Scitamineæ*, as Meyer asserts, but exclusively in the *Zingiberaceæ* Lindl; and neither in the *Cannaceæ*, nor in the *Marantaceæ*.

4. *Elongated grains.*

13. With an elongated central cavity in the milk-juice of the indigenous and a few of the tropical *Euphorbiaceæ*.

5. *Very irregular grains.*

14. In the milky juice of many tropical *Euphorbiaceæ*.

III. *Compound Granules.* Here we find a simple grain as an exception in the plant or part of the plant.

1. *The separate grains in the compound grains without evident central cavity or hilum.*
  15. Compounded according to the simplest types in 2, 3, or 4, as in the rhizomes of *Marantaceæ* (West Indian arrowroot). Also in tubers of *Aponogeton*, in the thickened vagination of the leaves of *Marattia*, and in roots of *Bryonia*.
  16. Generally arranged regularly, seldom irregularly, and composed of from 2 to 6. In the bark of the roots of the various sorts of sarsaparilla.
2. *The separate grains in the compound grain having a distinct central cavity or hilum.*
  - (a) *All the parts of the grains of nearly the same size.*
    17. United according to simple types from 2 to 4. The central cavity or hilum small and roundish, as in the tubers of *Jatropha manihot*.
    18. Combined according to simple types from 2 to 4. The central cavity or hilum large and very beautiful, opened in a star-like form, as in the corms of *Colchicum autumnale*.
    19. Combined according to simple types from 2 to 4. The separate grains quite hollow, apparently cup-shaped. A marked form occurs in *Radix ivarancusæ* (*Anatherum ivarancusæ*).
    20. Firmly combined, from 2 to 12 in number, in very irregular groups, as in the rhizomes of *Arum maculatum*.
    21. A large number, often as many as 30, of small roundish grains, very loosely grouped. Frequent, for instance in the stem of the *Bernhardia dichotoma*.
  - (b) *Many smaller grains grown together upon one larger one.*
    22. In the pith of *Sagua rumphii*, etc., and generally in sago.

#### NÄGELI'S CLASSIFICATION OF STARCHES FROM DIFFERENT SOURCES.

##### A. *Grains Simple.*

- I. *Centric.* Hilum in the mathematical center; lamellæ always equal at two corresponding diametrically opposite points.
    - Type 1. Spherical.* When the grain is free both hilum and grain are spherical.
    - Type 2. Lenticular.* When the grain is free both hilum and grain are rounded; grains compressed; sometimes circular or ovoid; sometimes triangular or quadrangular.
    - Type 3. Oval.* When the grain is free both hilum and grain are oval to lanceolate-oval; occasionally kidney-shaped or somewhat curved; when on end they appear circular or somewhat compressed.
    - Type 4. Spindle-shaped.* Grain linear or lanceolate, tapering towards the pointed ends, or of equal width with blunt ends; when on end they appear almost circular.
    - Type 5. Bone-shaped.* Grain elongated and compressed from the narrow aspect, but linear spindle-shaped from the broad aspect, with enlarged laminated ends.
  - II. *Eccentric.* Hilum usually more or less removed from the mathematical center of the grain; lamellæ coarsest and finest at opposite ends of the grain, respectively.
    - Type 6. Inverted cone-shaped.* Grain on end almost circular; slender at the hilum end.
    - Type 7. Cone-shaped.* Grains on end almost circular; decidedly thicker and broader at the hilum end.
    - Type 8. Wedge-shaped or compressed.* Grains flattened, of equal thickness throughout, or thicker but narrower at the hilum end than at the distal end.
    - Type 9. Rod-shaped.*
  - III. *Grains simple and structure obscure.*
    - Type 10. Structure not fully developed, or not identified owing to diminutive size of the grains.* Lamellæ, hila, cavities, fissures, and clefts seldom observed.
- ##### B. *Grains semi-compound.*
- Type 11. Grains semi-compound.* The component part-grains are enveloped partly or wholly by a common substance.

C. *Grains Compound.* The component part-grains not enveloped by a common substance.

I. *Composed of fused part-grains.*

*Type 12. Composed of fused part-grains.* The part-grains are not separated by fissures, and even different grains may be fused with one another.

II. *Composed of separated part-grains.* The part-grains separated by fissures.

*Type 13. Grains in 1 or 2 rows.* From 3 to 11 components arranged in 1 or 2 rows.

*Type 14. Equally divided grains of few components.* From 2 to 10 or more almost equal-size part-grains which when separated have 1 curved surface and 1 or more pressure facets.

*Type 15. Unequally divided grains of few components.* From 2 to 10 or more unequal sized firmly united part-grains, which when separated have 1 curved surface and several flattened pressure facets.

*Type 16. Multiple grains.* From 20 to many thousand firmly united part-grains which when separated are covered with pressure facets.

*Type 17. Hollow spherical grains.* The part-grains are arranged in a spherical layer, as if a globular shell had been divided radially.

For further details of the characteristics of the various types see Chapter V, page 197.

#### MEYER'S CLASSIFICATION OF STARCH-GRAINS.

Meyer, in his memoir *Die Stärkekörner (loc. cit.)*, criticizes Nägeli's conceptions of the "pseudo-compound" and "true-compound" starch-grains (Chapter V), and holds that we must dismiss the idea of a "true-compound" grain in the sense held by Nägeli because there are no starch-grains that have been formed by the separation of an originally single grain. The expression "pseudo-compound starch-grains" may also be rejected because it is now unsuitable, since if there are no true-compound grains there can be no false ones; furthermore, the grains under consideration are not individual starch-grains, but only simple starch-grains held together by a chromatophore substance. The "semi-compound" grains which according to Nägeli arise through the division of the nuclei and the formation of systems of lamellæ without any lines of separation between the cells is also incorrect because such starch-grains arise from several simple starch-grains being inclosed within a common starch-layer. Instead of the old name of compound grains, to avoid confusion, it is better to substitute the expression "complex" starch-grains; but Nägeli's conception and term of "simple grain" may be retained.

Meyer proposes some substitutes and some new terms in place of Nägeli's, as follows:

(a) *Simple or monarch starch-grains.* Grains which have but one hilum.

(b) *Complex starch-grains.* Grains that are formed from several starch-grains which are so crowded in one chromatophore that they become enveloped within common starchy layers, and thus bound into a single individual. Complex starch-grains may be diarch to polyarch.

The approximately monotone starch-grains possess simple symmetrical forms; frequently they are spherical bodies, the shape depending upon the influence which the fluid chromatophore exerted upon the form and lamellæ of growing starch-grains. Grains of a relative monotone type are found chiefly in the nutritive tissues of seeds, in which during the active growth of their cells and chromatophores an energetic and periodic solution of the starch-grains takes place, by means of which the central mass of the grain becomes relatively less dense, which in turn is followed by a uniform growth of the starch which ceases when the seed matures. Similar processes take place in many cotyledons which contain reserve material; also in numerous typical, colorless storage roots and bulbs which are filled with starch during their growth, and contain, if they vegetate normally, relative monotone starch-grains. Such organs during their development, and especially at the period when the reserve material is admitted, depend chiefly upon their neighboring structures for nourishment, and only in extreme cases of need can they draw upon the stored starch-grains. Examples of monotone starch-grains are found in the fleshy scale leaves of *Adoxa*, in the rhizome of *Iris germanica*, and in the mature tubers of *Solanum tuberosum*. In the latter monotone starch-grains may develop within the parenchyma cells in 14 days.

Polytone starch-grains, which are distinguished especially by the periodic inequality of their lamellæ, are found most abundantly and constantly in storage organs which live for several years, in consequence of which the carbohydrate passes through several periods of storage and disappearance. They may be found in the rhizome tips of *Adoxa* in the spring of their second calendar year, and in 3-year-old bulbs of *Hyacinthus*. These grains are also most readily formed in perennial plants, as observations in *Pellionia* have shown.

- (c) *Solitary starch-grains*. Grains which grow singly in one chromatophore.
- (d) *Adelphous starch-grains*. Those which grow along with other grains in one chromatophore. They may be di- to poly-adelphous; up to 6-adelphous would be designated as oligo-adelphous.
- (e) *Monotone starch-grains*. Grains which during their entire life have undergone periods of solution, leaving coherent traces of each of the lamellæ formed during the period of storage. Perfect monotone starch-grains in which there still exists a trace of every lamella which has been formed and in which, even if eccentric, the lamellæ are closed are exceedingly rare. This term is also to be used in all cases in which no distinct characters of a polytone type are present.
- (f) *Polytone starch-grains*. Grains that have during their development undergone two or more periods of solution in which numerous lamellæ completely disappear or decrease in width, interspersed with other periods during which they developed as the relative monotone type. Polytone grains if they have an eccentric structure show a series of lamellæ which are open laterally.

The similarity of the monotone starch-grains at one period in the same plant part and of the various individuals of a definite plant part is a recognized phenomenon. This is the consequence of the specific nature of the chromatophore and cytoplasm and of the approximately similar biology of the latter. Monotone starch-grains which grow at different periods in one cell of a plant may, however, assume very divergent forms, since in the course of the life of the cell changes occur in the size, consistency, and chemistry of the chromatophores and cytoplasm. Thus, in the chromatophores of one cell of the scale leaves of *Adoxa moschatellina* of the first year's growth only monarch eccentric, conical starch-grains are found, while in the same cells of the second year usually only polyarch, almost centric, starch-grains are observed. The small variations in the form of the starch-grains found in the same plant part are not due alone to the fact that they were not formed at the same time, but also because every cell has its own biology, even every chromatophore has its individual properties. In different organs of the same plant grains may assume a great variety of form. Thus, the leucoplast of the tubers of *Solanum tuberosum* form, besides complex and oligo-adelphous grains, mostly solitary, monarch, eccentric, conical, or oval grains, with a length of  $200\mu$ , and which have definite, rather delicate, irregular lamellæ. In the chloroplasts of the potato, large, solitary, inverted conical-shaped starch-grains occur which have at regular intervals refractive lamellæ of equal width.

The polytone starch-grains of one plant part, even in one plant cell, are in general less similar than the monotone grains, since in them the distinctions which originally existed are intensified through the variations caused by the marked solution alterations of the monotone grain.

#### A. *Monarch Starch-Grains*.

##### (a) *Solitary starch-grains*.

- I. *Centric starch-grains*. Hilum in the mathematical center; lamellæ always equal at two diametrically opposite points (Nägeli, Type 1). Such centric grains, as already noted by Nægeli (see Types 1, 2, and 3), occur only in seeds. It is therefore evident that, just as in the seeds the cytoplasm is abundant and dilute, the chromatophores lack density and have a tendency to expansion.

A. *Monarch Starch-Grains*.—*Continued*.

- (1) *Monarch solitary, spherical*. (Nägeli, Type 1.) Hilum spherical in the center of the spherical grain; lamellæ of uniform thickness, forming complete circles. Examples of such grains are found in *Sorghum vulgare* and in *Zea mays*. In the latter, when the grains have reached two-thirds of their growth, they are spherical with centric lamellæ. Grains which have just been removed from immature endosperm cells show the distinct striations of the lamellæ; later, by pressure due to the growth of neighboring grains, the solitary grains become angular; the lamellæ of these angular grains are closed, but are finer at the flattened surfaces.
- (2) *Monarch solitary, centric, lens-shaped*. Hilum and grains rounded and compressed (also rounded-reniform or rounded-oval, compressed). (Nägeli, Type 10.) Examples: Starch-grains from the seeds of *Triticum*, *Secale cereale*, *Hordeum vulgare*. In the chromatophores of the endosperm of *Hordeum* small grains form early, and by the rapid growth of several grains in a relatively small chromatophore become mutually flattened. During the development of the endosperm cells the growth of the grain within the chromatophore is hindered, partly due to new formations, partly through the solution of small grains so that starch-grains of various shapes arise, such as laterally flattened, crescent-shaped, bean-shaped, etc.
- (3) *Monarch solitary, centric, oval grains*. Hilum and grains oval and lanceolate-oval; circular in cross-section; lamellæ equal at two diametrically opposite points, being coarsest at two poles. (Nägeli, Type 3.) Examples: Many grains from the cotyledons of *Vicia faba* and other *Papilionaceæ*. The starch-grains in the cotyledons of *Cicer arictinum* are instances of this form of grain. All the chromatophores lie in the primordial utricle and usually contain 1 starch-grain, or rarely 2, which are flattened upon the sides in contact. Most of the starch-grains correspond in form to that of the chromatophores, which are disk-shaped, although of different densities. From time to time energetic solution takes place as a consequence of rapid growth, so that most of the grains are irregularly outlined and tuberculated in the early stages; later, the irregularities become less pronounced because there is less active solution. The chromatophore surrounds the starch-grain almost uniformly as a green layer, the surface of the grain becomes more and more smooth and increases relatively more in thickness than in width and breadth, since by the deposition of lamellæ of uniform thickness the grain grows more rapidly in the thick diameter than in the longitudinal. In the mature grain a feebly refractive diffuse hilum is observed, the lamellæ are strongly refractive, and the grains have become rounded and almost oval.
- (4) *Monarch solitary, centric, rod-shaped, and cone-shaped grains*. (Nägeli, Types 4 and 5.) Examples: Starch-grains from the lactiferous vessels of the *Euphorbiaceæ*. Observations upon *Euphorbia myrsinites* show that the young starch-grains from the latex are rod-shaped; most of them are irregularly corroded and have a feebly refractive line in the axis, which probably may be due to active fermentation and swelling. Very slightly corroded grains are also rod-shaped with bilateral eccentric lamellæ. Since the younger lamellæ are laid down directly after the growth of the latex vessels terminates, they will be the most dense and hence probably withstand solution better than those lying nearer the middle of the rod-shaped grain, and thus the explanation of the origin of the thicker ends may be similar to that observed in the case of *Oxalis ortgiesi*. The starch-grains from older internodes are bone-shaped and somewhat flattened. The investigation of chromatophores from material which had been carefully hardened and stained with fuchsin showed the grain completely enveloped in the substance of the chromatophore, which, however, was more markedly massed at the two ends of the grain.

II. *Eccentric starch-grains*.

- (5) *Monarch solitary, eccentric, rod-shaped*. Lamellæ on one side the heaviest, and at the diametrically opposite side the finest. Grains are circular in transverse section; both ends of almost equal width and thickness. (Nägeli, Type 9.) Examples: In *Dieffenbachia* and *Iris germanica*. In *Dieffenbachia* beautiful mono-

A. *Monarch Starch-Grains*.—*Continued*.

tone rod-shaped starch-grains arise by starch-grains growing simultaneously with the viscous chloroplast, and becoming unilaterally extended. The thinness of the stretched sides of the chloroplast results in the formation of eccentric lamellæ. When the chromatophore is first mature and a strong polytone growth of the starch-grains prevails, the chromatophore is expanded so that the starch-grain is somewhat broader at the base.

- (6) *Monarch solitary, eccentric, conical*. Lamellæ are the densest at one side and the most delicate at the diametrically opposite side. Grains are conical; circular in transverse sections. Hilum located at the narrower, less dense end. (Nägeli, Type 6.) Examples: Starch-grains in the tuber of *Solanum tuberosum* and rhizomes of *Adoxa*. Starch-grains of Type 6 are found in chromatophores, which show a tendency to growth equally in all directions. Pure forms usually only occur when the monotone grain is half-grown.
- (7) *Monarch solitary, eccentric, inverted-conical*. Grains similar to those in Type 6, but with the hilum at the more dense end. (Nägeli, Type 7.) Examples: Tuber of *Solanum tuberosum*, rhizomes of *Adoxa* and *Iris germanica*, etc. By a flattening and then a sharpening to a point at the base of a grain of Type 6 with closed lamellæ, such type may develop into one of Type 7 with lamellæ open at the base. Grains of Type 7 are usually found in company with those of Types 5 and 6.
- (8) *Monarch solitary, eccentric, flattened*. Examples: Starch-grains in rhizomes of *Zingiber officinale*, *Curcuma zedaria*, *Maranta*, pseudo-tubers of *Phaius grandiflorus*, etc. The starch-grains of Type 8 are formed in the same way as those of Types 6 and 7, only they are flattened.

(b) *Adelphous starch-grains*.

I. *Oligoadelphous starch-grains*. If several starch-grains grow simultaneously in a chromatophore, they behave just as spherocrystals do when growing in an inexhaustible mother liquor. Starch-grains from *Pellionia* are an example. Only in the very earliest stage are the two starch-grains spherical, and, as is apparent, the spherical shape is the more pronounced the larger the chloroplast when the first grains start to form. If the chloroplast is still small when the starch-grains begin to grow, so that the grains develop along with the chloroplast and exceed it in growth, they are very soon prevented from increasing in size on the inner side, and both become flattened. The lamellæ are heaviest within and below, and in purely monotone grains they are always closed, since the crystallization substance between the grains is furnished in the greatest quantity. Flattening of the grains results, as is readily seen if one considers that when two spherical grains grow side by side in a chromatophore, the chromatophore layer being thinnest where the two spheres come in closest contact with their surfaces. The entire process of growth of the diadelphous starch-grains is similar to that of monarch, solitary grains of *Pellionia*.

II. *Polyadelphous starch-grains*. The polyadelphous starch-grains of a chromatophore, which are not easily distinguished from the diadelphous forms, are approximately similar in form and size, though the proportion of the diameter of the smallest to that of the largest is usually as 1 to 4. The greatest diameter of a chromatophore filled with starch-grains which was measured by Nägeli is 106 $\mu$ . Such a chromatophore may, according to Nägeli, contain between 10 and 30,000 growing starch-grains. The form of the polyadelphous grains is mostly polyangular or rounded with centric structure. Some exceptions are found in the flattened forms of *Arenaria* and *A. graminifolia* and *Drymaria cordata* described by Nägeli. The polyadelphous grains are found relatively seldom in rhizomes and roots, although there are some exceptions, but occur most abundantly in the reserved food of seeds. The development of starch-granules in the chromatophore of the endosperm of *Oryza sativa* served as a good example of the formation of polyadelphous starch-grains. In every young seed of *Oryza* the nucleus lies in the abundant cytoplasm in the center of the cell, and is surrounded by small, scarcely

A. *Monarch Starch-Grains.*—*Continued.*

discernible leucoplasts. In certain of the leucoplasts several starch-granules arise almost simultaneously, the number increases although it varies until cell division is complete. The chromatophores are rounded at first, and remain so as long as there is no pressure on them. When the starch-bearing chromatophores have attained about half of their ultimate diameter they begin to flatten against one another, since they are rather crowded in the cytoplasm. The starch-granules, which are angular and much crowded when first recognized, gradually become more and more regular, since they check each other equally in growth. Distinct lamellæ are not present in the developing grain, since at first they are angular and corroded; and only a feebly refractive hilum, which corresponds to the earliest corroded stage of the grain, can be observed in the mature grain.

B. *Complex (Di- to Poly-arch) Starch-Grains.*

It can not be readily demonstrated that complex grains, like certain grains, for example, of *Pellionia*, are descended from adelphous grains. It has, however, been proven that all plant parts in which complex grains are present also at times develop adelphous starch-grains which correspond entirely with the central lamellæ of the complex grains. Furthermore, gradations between the adelphous and the similar complex forms can be found. Additional proof of the connection between adelphous and complex starch-grains is the fact that plant parts which produce few and irregular adelphous grains likewise have relatively few and irregular complex grains in their cells. In the storage scale leaves of *Adoxa* it is noticed that numerous adelphous grains form at first, but only complex grains are present later, and the layers of the latter resemble those of the former. After the two diadelphous starch-grains found in *Pellionia* have attained a certain size in the chloroplast, the chloroplast substance is entirely forced out between the contact surfaces of the grains, or is cut off from the remaining mass of the chromatophore by the growing trichites, so that no more starch-substance is stored in the region of the contact surfaces. If the chromatophore mass between the flattened surfaces becomes inactive or disappears, the two starch-grains again grow into diarch form.

## MUTER'S CLASSIFICATION OF STARCHES.\*

This classification, which is based upon histological and polariscopic peculiarities, is characterized by the designation of each group by some important type of starch:

*Potato group.* Oval or ovoid granules, showing hilum and concentric rings clearly; cross and colors with selenite usually distinct.

*Legume group.* Round or oval granules, hilum marked, rings faint, but rendered visible in cases by chromic-acid solution; cross and colors feeble.

*Wheat group.* Round and oval granules, hilum and rings generally invisible; feebly marked cross and colors.

*Sago group.* Truncated granules, hilum distinct, rings faint; cross and colors usually faint.

*Rice group.* Polygonal granules, hilum distinct, rings faint; cross and colors usually faint.

## KRAEMER'S CLASSIFICATION OF STARCH-GRAINS.†

This classification includes the starches of some of the more important vegetable drugs, together with a few commercial starches, and is based upon morphological and other characters.

A. *Simple spherical grains.*

- (a) *Not more than 5 $\mu$  in diameter:* Cimicifuga, Cypripedium, Frangula, Hydrastis, Leptandra, Piper, Prunus virginiana, Quassia, Quercus alba, Rhamnus purshiana, Spigelia, Viburnum opulus, and Viburnum prunifolium.

\* Organic Materia Medica; quoted by Leffmann and Beam, Food Analysis, 1906.

† Botany and Pharmacognosy, Philadelphia, 1907, 698.

A. *Simple spherical grains.—Continued.*

- (b) *Not more than 10 $\mu$  in diameter:* Calamus, Eunonymus, Gelsemium, Granatum, Quillaja, Sanguinaria, Serpentaria, Tonka, Ulmus, Xanthoxylum.  
 (c) *Not more than 15 $\mu$  in diameter:* Apocynum, Cinchona, Colehici semen (in caruncle only), Convallaria, Sumbul, Valeriana.  
 (d) *Not more than 20 $\mu$  in diameter:* Glycyrrhiza, Phytolacca.  
 (e) *Not more than 30 $\mu$  in diameter:* Rumex, Stillingia.

B. *Compound spherical or polygonal grains.*

- (a) *2 to 3 compound:* Belladonna radix (5 to 15 $\mu$ ), Sassafras (7 to 20 $\mu$ ), Veratrum viride (7 to 20 $\mu$ ).  
 (b) *2 to 4 compound:* Aconitum (4 to 12 $\mu$ ), Cinnamomum (7 to 15 $\mu$ ), Colehici cornus (7 to 20 $\mu$ ), Ipecacuanha (4 to 14 $\mu$ , those of Carthagenia ipecae being uniformly larger), Krameria (20 to 30 $\mu$ ), Rheum (5 to 20 $\mu$ ), Sarsaparilla (7 to 20 $\mu$ ).  
 (c) *2 to 6 compound:* Podophyllum (5 to 12 $\mu$ ).  
 (d) *More than 6 compound:* Capsicum (3 to 7 $\mu$ ), Cardamomum (1 to 4 $\mu$ ), Cubeba (1 to 4 $\mu$ ), Gossypii cortex (5 to 20 $\mu$ ), Mezereum (10 to 15 $\mu$ ), Myristica (5 to 7 $\mu$ ), Pimenta (7 to 10 $\mu$ ), Rubus (3 to 7 $\mu$ ).

C. *Ellipsoidal or ovoid grains.*

Althaea (10 to 20 $\mu$ ), Geranium (10 to 15 $\mu$ ), Glycyrrhiza (5 to 10 $\mu$ ), Pareira (7 to 15 $\mu$ ), Physostigma (25 to 40 $\mu$ ), Rumex (10 to 20 $\mu$ ), Stillingia (15 to 30 $\mu$ ), Strophanthus (2 to 4 $\mu$ ), Zingiber (15 to 30 $\mu$ ).

D. *Grains of characteristic shape.*

Calumba (25 to 35 $\mu$ ), Iris florentina (15 to 30 $\mu$ ), and potato and other starches, such as arrowroot, wheat, corn, yam, canna, bean, pea, cassava.

E. *Altered grains.* Guarana (10 $\mu$ ), Jalapa (15 to 35 $\mu$ ; also 2 to 3 compound grains), Tragacantha (2 to 10 $\mu$ ), turmeric in masses (70 to 140 $\mu$ ).F. *Amylodextrin grains.* Maec contains starch-grains which give a reddish color with iodine. Kramer notes that leaves, herbs, and flowers do not as, a rule, contain starch.

## WINTON'S CLASSIFICATION OF STARCHES.\*

Winton records that the forms of the grains are so numerous, even in the same variety, as to forbid accurate classification, but that the following are the more striking:

1. *Globular.* The starch of the peanut and some grains of maize.
2. *Lenticular.* The large grains of wheat, rye, and barley.
3. *Ellipsoidal.* The starch of legumes.
4. *Ovoid or pear-shaped.* The starch of potato, canna, Bermuda arrowroot, yam, and banana.
5. *Truncated.* Most of the grains of cassava, batata, and sago.
6. *Polygonal.* The starch of maize, rice, oats, and buckwheat.

Winton also gives an analytical key by Moellar to commercial starches that is based upon general histological characteristics.

Many other classifications might be quoted, without, however, any material advantage.

## PROPERTIES OF THE STARCH-GRAIN IN RELATION TO MENDELISM.

Gregory (The New Phytologist, 1903, II, 226) found that the starches of round and wrinkled peas occur in two very different types. (See Plates 8, 9, and 10, figs. 47 to 56.) In the *round* seeds the peripheral cell-layers of the cotyledons contained a few oval starch-grains which did not exceed 0.06 mm. in the greatest diameter. In the third layer the grains reached 0.2 mm. in length, while the more deeply situated cells were crowded with oval grains measuring as much as 0.34 mm. in the greatest dimension. The grains were regular in shape, with a definite center surrounded by well-marked lines of stratification. In the *wrinkled* peas the grains of the peripheral layers were of about the same size as those of the round peas, but were of a different type, occurring in irregular spheres with several

\* The Microscopy of Vegetable Foods, New York, 1906, 645.

centers, thus forming a compound grain which has a strong tendency to break up into smaller parts. In the cells which lie deeply these compound grains never attain a greater length than 0.1 mm. in the greatest dimension. Table 3 gives a list of the seeds examined.

TABLE 3.

Race.	Seed character.	Form of starch-grain.	Race.	Seed character.	Form of starch-grain.
Express.....	Round.	Large.	William the First.....	See below.	Large.
Fillbasket.....	Do.	Do.	Telephone.....	Wrinkled.	Small.
Très nain de Bretagne...	Do.	Do.	Laxton's Alpha.....	Do.	Do.
Maple (purple-flowered) .	Do.	Do.	Serpette nain blanc.....	Do.	Do.
Carter's Telegraph.....	Do.	Do.	Dark Jubilee.....	Do.	Do.
Victoria Marrow.....	Do.	Do.	Early Giant.....	Do.	Do.
Field pea (purple flower).	Indent.	Do.	British Queen.....	Do.	Do.
Purple Sugar.....	Do.	Do.	Windsor Castle.....	Do.	Do.

Gregory notes that seeds of intermediate and dubious shapes were not uncommon in certain of the races. The depressions in these seeds were sometimes mere pitting, as in Victoria Marrow; or they may be so marked that the seed would be described as wrinkled. The latter were especially common in William the First, but microscopic examination showed at once that these seeds are really of the round type. There are, therefore, states Gregory, two entirely different types of wrinkling, and while it is clear that the process by which wrinkling is produced is connected with shrinkage on drying, the regularity of the shrinking of the round type and its irregularity in the two other types can not at present be explained. There occasionally occur among the offspring of hybrids between round and wrinkled types seeds of dubious shape which it is difficult on superficial examination to classify as round or wrinkled. The existence of such seeds and types of doubtful shape was taken by Weldon (*Biometrika*, 1902, 1, 246) to indicate irregularities of Mendelian segregation and dominance, but Gregory states that no seed has been found which upon histological examination allowed of any doubt as to its true character, and consequently that occasionally pitting and spurious wrinkling must be distinguished from the true wrinkling of the wrinkled types.

The nature of the starch-grain in the hybrid, and how the characters of the starch-grains segregate, if they do so at all, in subsequent generations, are points which suggested themselves to Darbishire (*Proc. Roy. Soc., B.* 1908, LXXX, 122), who states that they are matters on which we are ignorant. He found that the starch-grains of the round pea, such as of the "Eclipse," appear as single potato-shaped grains, with an average length of 0.0322 mm. and an average breadth of 0.0213 mm. The length-breadth-index (*i.e.*,  $100 \times \text{breadth} \div \text{length}$ ) is 66.14. Besides these potato-shaped grains, there are extremely few very much smaller grains which are round. The grains of wrinkled peas like the "British Queen" are compound, each consisting of a number of pieces which vary between 2 and 8. These pieces are held together by a refragent yellow substance which does not color blue with iodine, and they are likely to break apart. The commonest types are those with 4, 5, or 6 components; grains with 7 or 8 are rarer; grains with 2 or 3 are intermediate in frequency between those with 4, 5, or 6 on the one hand and 7 or 8 on the other. While the grains with 7 to 8 pieces are not much larger than those with 4, 5, or 6, grains with 2 or 3 are always conspicuously smaller than those with 4, 5, or 6. The average length is 0.0269 mm., the average breadth 0.0248 mm., and the length-breadth-index is 92.19. In these peas are a number of very small single grains which can be distinguished from the pieces of the compound grains by the fact of their being circular and always smaller than the grains consisting of two pieces. Very rarely will be found isolated potato-shaped grains.

The grains of the F<sub>1</sub> cotyledons produced by crossing the round with the wrinkled pea are nearly round; the majority of the grains are single and the remainder compound; the compoundness exhibited by the compound grains in F<sub>1</sub> seeds is intermediate between

singleness and the degree of compoundness in the grains of wrinkled peas; for while in the latter the number of pieces varies between 2 and 8 and the commonest is 6, in the  $F_1$  grain it varies between 2 and 4 and the commonest is 3. The differences in the measurements of the three starches are shown in table 4, by which it will be seen that in shape the  $F_1$  grain is intermediate between the potato-shaped grain and the compound grain, but nearer the latter.

TABLE 4.

	Round. Potato- shaped grain.	$F_1$ . Round grain.	Wrinkled. Compound grain.
	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>
Average length.....	0.0322	0.0276	0.0269
Average breadth.....	0.0213	0.0236	0.0248
Length-breadth-index...	66.14	85.5	92.19

Darbshire also examined the grains of  $F_5$ . These he did not measure, but he states that no differences could be seen between the potato-shaped, compound and round grains from the three types already described. He notes that the evidence points to the fact that the heterozygote round peas in generations subsequent to  $F_1$  are characterized by the possession of irregular round or round grains, and homozygote round peas by potato-shaped grains. Darbshire records that if the association of round grains with heterozygote round and of potato-shaped grains with homozygote round holds good for the  $F_2$  generation, we have a means of distinguishing between DD round and DR round in  $F_3$ , instead of, as at present, having to wait until their progeny are mature in the following year. Another point demonstrated by the nature of grains in  $F_1$ , and borne out by those of  $F_5$ , is that the shape of the grain is inherited separately from its composition—if we may use this term to cover the singleness or compoundness of the grain. In the round pea the grains are single and long; in the wrinkled peas they are compound and round; in the hybrid they may be either single or compound, but are more round than long. In  $F_5$  there are round grains exhibiting much compoundness and others exhibiting little. Possibly there are potato-shaped grains either with no compounds or with few, and intermediate grains either with few compounds or with many. The wrinkled peas of this generation contained, as was to be expected, compound grains, but some of them had in addition, very sparingly, potato-shaped grains. Darbshire also studied the absorptive capacities of the peas in relation to water. The following facts are summed up from the results of his investigations:

1. Although roundness is dominant over wrinkledness in peas, the round starch-grain of the  $F_1$  generation is a blend between the type of grain of the round pea (the potato-shaped) and the type of grain of the wrinkled pea (the compound) in respect of three characters: (a) it is intermediate in shape as measured by its length-breadth-index, that of the potato-shaped grain being 66.14, that of the compound grains 92.19, and that of the round grain 85.5; (b) it is intermediate in the distribution of compoundness, inasmuch as some of the round grains are compound and some single; (c) it is intermediate in the degree of compoundness, inasmuch as amongst those round grains which are compound the most common number of constituent pieces is 3, whereas in compound grains it is 6.
2. In a subsequent generation ( $F_5$ ) the homozygote round peas contain potato-shaped grains and the heterozygote round peas contain round or intermediate grains. But both round and intermediate grains may be associated either with a high or a low degree of compoundness.
3. Potato-shaped grains occasionally occur in wrinkled peas in  $F_5$ , and the evidence suggests that the existence of these grains in wrinkled peas tends to make them less wrinkled.
4. A wrinkled pea takes up more water when it germinates than a round one. The hybrid between a round and a wrinkled pea is intermediate in respect to this character between its two parents.
5. But the intermediateness of the hybrid in absorption capacity is not occasioned by the intermediateness of the starch-grain of the hybrid, because both  $F_2$  peas containing round grains and peas containing potato-shaped grains have the same absorption capacity as the  $F_1$  pea.

6. When, therefore, a round pea is crossed with a wrinkled pea, four separately heritable characters are dealt with: (a) the shape of the pea, whether round or wrinkled; (b) the absorption capacity of the pea as regards water, whether low or high; (c) the shape of the starch-grain, whether long or round; (d) the constitution of the starch-grain, whether single or compound.

(A study of the effects of hybridization, etc., upon the properties of starch and other corresponding substances is now being carried on by the author of the present memoir.)

#### STARCH-GRAIN AS A SPHEROCRYSTAL.

The hypothesis of the crystalline structure of starch was suggested by C. Nägeli (Die Stärkekörner, etc., *loc. cit.*; Botanische Zeitung, 1881, XXXIX, 633), who conceived the grain to be composed of minute crystalline structural elements, which in his earlier writings he designates atoms, later molecules, and finally *micellæ*, and he attempted upon this hypothesis the support of the theory of growth of the starch-grain by intussusception, the explanation of the phenomena of swelling, and optical and certain other properties of the starch-grain. Nägeli writes that the form of the starch-grain or molecule in its earliest stages is unknown, and that while the grains or molecules within their watery envelopes behave as regards their impermeability and growth by apposition in a similar manner to crystals, it does not necessarily follow that they must agree with crystals in respect to form; but to the contrary that it is possible that just those properties which differentiate them from crystals, that is, the chemical changes at the moment of solidification and the attraction of the watery envelopes, may prevent the development of a characteristic crystalline shape. The analogy of the crystals, he also states, does not necessarily lead to a similarity between the form of the starch-molecule and that of the crystal. For other reasons than those given it is probable, he states, that the starch-molecule is originally spherical, which view he holds receives support in the spherical form of all starch-grains in the earliest stages of development. The characters of the starch-grain demonstrate that the molecules that were closely arranged in concentric lamellæ and in radial rows had evidently, by a primary attraction (cohesion), been distributed equally over the entire surface of the grain, thus giving rise to the spherical form. The spherical molecules always maintain this form when they develop singly, and are free in a fluid medium, so that growth may occur equally at all points of the surface; but when they undergo unequal growth upon different sides they soon deviate from their original spherical shape. If the molecules in one part of the grain are still spherical and of equal size it indicates that they have a favorable environment.

The movement of the particles of the solution he conceives to pass preferably in a radial direction, and even if all of the particles in the solution do not closely follow this course, yet it still is the prevailing one. The outer and inner faces of each molecule become much more perpendicular than the lateral face, on account of the movements of the particles, and also increase more rapidly at the poles than at the equatorial zone, and thus become ellipsoidal. As they deviate from the spherical form the water envelopes of the molecules also show an unequal density, since the greater diameter corresponds to the less dense envelope. This is a cause for the molecules at the poles now developing a greater mass than at the lateral surfaces, and this theory also agrees with the facts already given that there is less water lodged between the molecules in the radial than in the tangential direction. In the transverse (tangential) section of the molecule, the interstices between the 3 or 4 molecules which lie beside one another are triangular or quadrangular. Now only these sides of the molecule and the angles which are turned towards the interstices have a chance of having particles from the solution deposited upon them with considerable force and thus growing by the deposit of material. The transverse section of the molecule has become angular, with the angles turned towards the interstices, which have become narrowed. This alteration from the circular cross-section under similar conditions continues and even increases.

As the molecule grows, even if the form were originally spherical, it becomes elongated, polyhedral, or prismatic. The peculiarity of form depends upon the position of the molecule in relation to other molecules; thus the outer and inner sides, if turned towards another molecule, become flattened and pointed if directed towards the interstices. The larger the molecule grows the greater the deviation from the original form, both because the movement of the fluid encourages the deposition of new material at the sides turned towards the interstices and because here the resistance to the water envelopes is diminished.

Nägeli gives in line drawings schematic representations of the form and arrangement of the molecules. In one is shown a hypothetical section of a superficial molecular layer showing among developed molecules or micellæ young and as yet spherical molecules, and the molecules with their envelopes and interstitial canals shown in a tangential direction. In the second figure he represents a part of a hypothetical radial section showing the molecules lying beside one another in horizontal rows belonging to the same molecular layer.

The structural likeness of the starch-grain to spherocrystals of inorganic substances was first strikingly pointed out by Famintzin (Heidelberger Jahrbücher der Literatur, 1869, LXII, 226), who found starch-like forms of crystals of calcium carbonate. He records the parallelisms in the manner of growth, in the lamellation, in the differences in solubility of the inner and outer parts, and in the formation of compound grains and crystals. Erosion phenomena which bear a striking resemblance to those occurring in starch-grains have been observed by Goldschmidt (Zeit. f. Krystallographie u. Mineral., 1904, XXXVIII, 1) in spherocrystals of calcium carbonate.

Schimper (*loc. cit.*) supported the hypothesis of the starch-grain being a crystalline body upon the basis of the cohesive and optical properties which distinguish amorphous from crystalline structures, and he studied with some care the analogies between the starch-grain and ordinary spherocrystals. He observed that the grains break more easily transversely than parallel to the lamellæ, and that such a peculiarity has not been noted in amorphous bodies, because the absence of regularity of arrangement of the molecules makes them split irregularly when crushed. A filamentous crystalline aggregate when crushed, he observes, separates in lines parallel to the bundles, because the force which binds the latter is more easily overcome than the cohesion of the molecules of the individual crystals. The striated nature of the broken faces shows, he states, how the fibers are separated, and that the starch-grains exhibit this same phenomenon, thus resembling radio-fibrous crystalline aggregates and differing from amorphous bodies. The polariscopic characteristics he found to agree entirely with the cohesive properties, and like them to be owing to the crystalline nature of the starch-grain. Viewed in polarized light the "interference figure" is such as starch-grains should exhibit if they are composed of bundles of crystalline uniaxial or rhomboidal elements, and would split up transversely to the lamellæ.

Schimper states that Baily (Philosophical Magazine, 1876) and also von Lang (Ann. d. Physik. u. Chemie, cxxiii; Carl's Reportorium, III) reached this conclusion; and that Mohr's (Botanische Zeitung, 1858) assertion that the arms of the interference cross always run perpendicular to the lamellæ holds good only for a regular centric or spherical structure, as they often intersect eccentric grains at a very sharp angle. In regular centric spherical structures, as well as in the axes of eccentric ones, the doubly refractive elements are straight and extinguish the light in its entire length; the lateral parts of eccentric grains behave differently, as is shown by the bundles and the fissures, which described a curve, and which according to this satisfy the conditions for extinguishing the light in more or less of their length. These characteristics, he writes, like those of cohesion, can be attributed only to the fact that starch-grains consist of crystalline bundles which run parallel to the lamellæ. Starch-grains, he notes, differ from ordinary spherical crystals in their property of swelling, and he proposes to distinguish spherocrystals and all crystalline bodies which possess this property as *crystalloids*. Schimper states that one may conclude that starch-granules

consist of radially arranged crystalloids; that the crystallized starch-substance has the formula  $C_6H_{10}O_5$ ; that there are probably several isomers; that various conditions can be cited to prove that starch-crystals always appear in aggregate bundles and never singly; and that the conditions which account for the occurrence of aggregates are difficult solubility, with low power of crystallization, and the viscosity of the solution in which crystallization takes place, and that any one of these factors may in some cases suffice.

The study of the starch-grain as a spherocrystal was taken up by Meyer in 1881 (*loc. cit.*) and later in 1895 (*loc. cit.*), and he gives the name *trichites* to the hypothetical crystal elements named by Nägeli *micellæ*. Meyer states that the hypothesis that starch-grains are spherocrystals of a carbohydrate furnishes the simplest explanation of the lamellated structure; of the growth of the grain by external accretion; of the later origin of the outer layers; and of the relatively low density of the innermost parts of the mature grains. If the grains are spherocrystals it must be assumed, he states, that they grow like other crystals and show phenomena which characterize spherocrystals. If spherocrystals of a carbohydrate, such as sugar, be caused to form, and if conditions attending crystallization are altered periodically by placing the preparation at a window where the sun warms the solution periodically, concentric, lamellated spherocrystals are formed; but if crystallization take place under constant conditions the crystals show an absence of lamellæ. The hilum of the artificial spherocrystals of sugar was found to be usually less dense than the rest of the crystals, and that it incloses "mother substance," rarely air. Application of these facts to the starch-grain shows, he states, that most starch-grains, even in young stages, have a small, relatively soft center, and that since the plant is subjected to periodic changes in external conditions the grains must be built up of layers of alternating density. In examining crystals of carbohydrates he notes the occurrence of all transitions between spherical-crystal groups and typical spherocrystals. If in the production of crystals they are produced from impure solutions of carbohydrates, they have an apparently homogeneous appearance, in which, as in most intact starch-grains, no radial striations can be perceived. Such spherocrystals are formed from carbohydrates having a high molecular weight, such as amyloextrin and inulin. In the production of crystals of amyloextrin, as long as the solutions contain 20 per cent of amylose and dextrin, spherocrystals form very readily on evaporation or congelation, or by slow cooling of a concentrated solution.

Typical spherocrystals are, writes Meyer, composed of very thin elongated needle-like or thread-like crystal units, which, as before stated, he terms *trichites*. In all spherocrystals the trichites are assumed to be joined in tufts. Concentric lamellation is stated to be a very constant phenomenon in typical spherocrystals. This structural peculiarity, he notes, is observed in spherocrystals of minerals and in many artificial crystals of inorganic substances, and that it has long been observed in the spherocrystals of carbohydrates, in which the lamellæ may appear in the apparently structureless forms and in the coarsely radial thread-like forms. The lamellæ of spherites of amyloextrin are stated to be produced by alternate layers of trichite tufts which vary in their crystalline structure. Such spherites are described as consisting of a hilum or center with radial trichites, and around the hilum layers of trichite tufts. The layers are differentiated by the varying thickness of the trichites; by the varying lengths of the trichites; by the compact or open arrangement of the trichites; and by the extent of branching of the trichite tufts. The trichites are always most easily separated in a radial direction, and spherocrystals are always porous. Starch-grains behave so similarly to spherocrystals of other substances that Meyer holds that one is justified in designating them spherocrystals of amylose; and likewise he would speak of spherocrystals of  $\alpha$ -amylose, of  $\beta$ -amylose, and of amyloextrin. Whether or not amyloextrin crystallizes with amylose into a mixed crystal, or whether three kinds of trichites mix, he postpones for further investigation.

The porosity possessed by starch-grains in common with spherocrystals of other carbohydrates was especially studied. Meyer states that starch-grains are porous like the spherocrystals of inulin and amyloextrin whose individual trichites are not recognizable, and that they contract when water is withdrawn and expand upon the addition of water. The pores are exceedingly small, and can scarcely be seen with the highest power of the microscope. They absorb approximately as much glycerine as water, and also take up alcohol, certain dye solutions, etc. Corn and potato starch placed in a concentrated solution of methyl violet is stained completely. If the stained grains, after being washed, are placed in glycerine, they lose the stain, the coloration disappearing first in the peripheral layers. If the intensely stained grains are put into a dilute solution of calcium nitrate, it can be seen that the methyl violet is deposited in the largest quantities in those layers which are relatively porous and slightly refractive. In Berlin blue the less refractive layers also stained the more deeply.

As in other crystals of carbohydrates, Meyer observes that the trichites of the starch-grain are arranged in a radial manner and placed at right angles to the main mass. In spherical starch-grains the easiest separation was found to be in the direction of the radii, and in eccentric-layered grains along the lines which join the center to the periphery, or perpendicular to the layers. The radial trichite structure is rarely seen very distinctly in starch-grains, but not infrequently such a structure could with definiteness be detected in potato starch. The spherical, concentric-layered grains behave optically exactly as the spherocrystals of amyloextrin, and as though they were composed of trichites arranged radially, and whose small optic axis of elasticity runs lengthwise.

Meyer contradicts the statement of Mikosch that isolated trichites are not doubly refractive, and holds (to the contrary) that every undissolved trichite of a starch-grain, whether it consists of amyloextrin or amylose, behaves just like the isolated trichites of amyloextrin crystals. When spherical starch-grains are allowed to soak slowly, the optical properties of the trichites persist as long as the minutest trichitic radial structure of the swollen grain can be seen. Starch-grains, he notes, are lamellated as are the spherocrystals of inulin and amyloextrin, and the lamellæ become visible because in equal volumes of different layers, the volumes of the pores standing in varying relation to the volumes of the trichites.

Meyer quotes Nägeli's statement that the lamellar lines in starch-grains are either the boundaries between two substances unequally refractive, or the entire space is filled with a substance of varying density—at times watery lamellæ in a denser substance and at other times denser lamellæ in a watery substance.

Meyer states that since starch-grains are composed of various kinds of  $\alpha$ -amylose,  $\beta$ -amylose, and amyloextrin trichites, and even probably of a mixture of crystals of these substances, it is evident that the various layers consist of varying mixtures of these trichites. A direct proof that such a relation exists, he holds, lies in the fact that when sorghum starch is colored with iodine, many layers become blue and others red because some lamellæ contain more amylose and others more amyloextrin, etc. By swelling the blue-colored sorghum starch, or by treating the grains with saliva, it can be further shown that in different layers the number of  $\alpha$ -amylose and  $\beta$ -amylose trichites varies.

Referring to comparisons of the starch spherocrystal with other spherocrystals, Meyer notes that the multiplicity of form observed is in striking contrast with the uniformity of shape of artificial spherocrystals, and that it must be remembered that the relations under which artificial spherocrystals ordinarily grow are similar, while those under which starch-grains grow are very varied. Starch-grains develop in chromatophores which are in the form of viscous droplets, and whose substance completely surrounds the growing grain. These viscous droplets produce the crystallization material, and every layer of each droplet, however thin, may furnish crystallization substance. The amount of crystalline matter formed, he states, is dependent upon the quantity of active mass of the chromato-

phore; therefore the starch-grain will grow most rapidly where the layer of chromatophore substance is greatest. As a consequence, the shape of the starch spherocrystal is dependent upon the shape assumed by the chromatophore.

Bütschli (*loc. cit.*) also noted the correspondence of the structure of the starch-grain and crystals of inulin, and he also prepared artificial starch-grains in the form of spherocrystals which had the crystalline properties of normal starch, and which had a lamellated structure that bore a marked resemblance to the lamellæ of the normal grain. The correspondence between typical spherocrystals and starch-grains in regard to the peculiarities of solution and reformation, and to the mechanism of the formation of layers of varying density may also be found in the studies of Hansen (*Arbeit. d. Botan. Instituts in Würzburg*, 1884, III, 110), Rodenwald and Kattein (*Zeitschr. f. physikal. Chem.*, 1900, XXXIII, 579), and Ostwald (*Lehrb. d. allgem. Chem.*, 1891, I, 1041).

Various statements or claims in relation to Bütschli's honey-comb theory, Nägeli's micellar theory, and Meyer's trichite theory were strongly attacked by Fischer (*Beit. z. biol. d. Pflanzen*, 1898, I, 53; *Beihefte z. bot. Centralbl.*, 1902, XII, 226), who showed in all instances that most of the claims of what these theories or hypotheses explain are inconsistent with facts. Neither Bütschli's nor Meyer's theory, Fischer states, is compatible with the physical properties of inulin crystals, which, as has been shown, are essentially like the starch spherocrystals. Fischer regards Nägeli's hypothesis as being well-conceived and systematically elaborated, but based upon fanciful invention and unsupported by facts, and he states that Nägeli, without producing proof, advances the theory that the crystals, or micellæ, and their imbibed water are disposed in distinct layers and held together by some mysterious force. Fischer compares the particles of water-free starch with other crystals, lime-spar for instance, in respect to their properties of cohesion and refraction, and he goes on to show that the presence of water in the starch-grain is not a physical phenomenon, or one of capillarity, but one of chemical affinity. In regard to Nägeli's hypothesis of growth by intussusception, Fischer states that to accept it we must admit the existence of quite a number of special properties which are inconsistent with our knowledge.

Fischer looks upon Meyer's trichite theory, in the explanation of the cause of the lamellation of the starch-grain, as being untenable, and he disproves Meyer's statement of the influences of alternation of light and darkness, and of the changes in concentration of the mother-liquor, on the formation of the lamellæ. Cuttings exactly like those described by Meyer were kept in the dark for two weeks and then in the condensed light of an incandescent lamp for one week. In one of four experiments stratified starch-grains like those described by Meyer as being caused by the alternating influences of light and darkness were observed. Fischer notes the fact that results confirmatory of his own of the negative effect of light on lamellation were recorded by Salter by treating leaves of *Pellionia* with sugar solutions. (See also *St. Jentys*, page 58.) Fischer's article is quite long and full of detail.

CONCLUSIONS RELATING TO THE STARCH-SUBSTANCE AND THE STRUCTURE AND MECHANISM OF FORMATION OF THE STARCH-GRAIN, BASED CHIEFLY UPON THE FOREGOING LITERATURE AND IN PART UPON OBSERVATIONS RECORDED IN SUBSEQUENT CHAPTERS.

(1) The starch-substance is not a unit body, but exists in a number of stereoisomeric forms. It probably differs specifically in the grains of different plants, in the grains of the same plant, and in the individual grains, and is indicated by differences in the behavior towards iodine, aniline dyes, and various other agents. In any given mature grain it may be found that certain parts behave differently from other parts to reagents, indicating, for instance, not only specific differences in the nature of the starch-substance of the capsular or outer layer and the inclosed part, but also of the different lamellæ and even in different parts of a given lamella. The different lamellæ vary in density and solubility, increasing in density and decreasing in solubility from within outward,

which may be due to molecular differences in the component starch-substances. The outermost or capsular layer is the least soluble in both hot and cold water; it is less digestible in weak acids and enzymes; it gives different color reactions upon certain conditions; and it shows a different degree of resistance to the actions of chloral hydrate and other reagents. Grains of some plants may yield a purple, violet, or red reaction with iodine, owing, it may be, to the presence of erythro-dextrin, but grains which at first become blue may exhibit a range of colors from a purple to a violet and red upon the exposure to the air. When starches have been subjected to swelling agents, and the capsular layer is thus disorganized, the inner part may stain an intense indigo blue, while the capsular portion may be colored blue, purple, violet, red, etc. The grains of certain plants apparently do not contain ordinary starch, but in its place a modified form or a mixture of ordinary starch and erythro-dextrin, or erythro-dextrin, and therefore stain a red-violet or a red with iodine, as reported in the case of the grains of *Oryza sativa* (red violet) and *Glutinosa*, of the seed-coats of *Chelidonium*, and of the arillode of the seed of *Myristica*, etc.

The use of specific terms to signify that the outer or capsular part of the grain is a non-starch substance, as, for instance, the term "cellulose" of Nägeli, and "amylocellulose," is misleading and unwarranted, as there is no good reason for believing that this part is other than a modified form of a typical starch-substance. The term "amylo-dextrin" is as objectionable as amylocellulose, etc., as it infers a form of dextrin entirely apart from the true dextrans, or a mixture of starch and dextrin; and, moreover, it is used by different authors to signify very different bodies. The assumption that starch-substance exists in modified forms in the grains of different starches and in grains of the same starch and in any given grain, and that erythro-dextrin may be present especially in the capsular parts, and that erythro-dextrin may replace starch in the grains of certain species, are sufficient, upon the basis of the literature quoted, to satisfactorily account for the essential differences in the behavior of different grains and of different parts of a given grain towards iodine and other agents.

(2) The cause of the insolubility of uninjured raw starch-grains in cold water seems to be attributable to some form of protective covering which is deposited by the starch-forming structure or by the cell-sap. That such a protective exists is indicated by the fact that any condition which destroys the continuity of this coating, such as the rupture of the grain by grinding with sand or glass, or the erosion of the grains by bacteria or diastase, or the gelatinization or solution of the coats by potassium hydrate and various other agents, renders the grains soluble in cold water and is equivalent to gelatinization proportional in degree to the extent of exposure of the intra-integumentary portion. It would seem that such a protective covering might be for the purpose of preventing the solution of the starch-grain in the cell-sap, yet not interfere with the action of diastase, which is rendered effective by changed conditions in the composition of the cell contents when a consumption of starch for pabulum is rendered necessary. Assuming that starch is produced through the agency of enzymes, it follows, as a corollary, that by virtue of the reversibility of the actions of these substances it is broken down when needed for pabulum into the bodies identical with or similar to those from which it was formed.

(3) The starch-grain at its earliest period of formation is spherical and homogeneous, and during growth tends to develop a hilum and lamellæ and undergo changes in form which, sometimes at least, are more or less characteristic of the species, genus, and family. Both hilum and lamellæ are more or less variable in their properties in different starches, and the lamellæ of any given grain may differ from one another in density and coarseness and other features. The mechanism of the formation of the hilum can probably be accounted for by the factors which give rise to a similar phenomenon in the formation of other spherocrystals, and in the apparently similar conditions which attend the formation of a core and radial fissures during the solidification of molten metals. The starch-grain

in its early stage of development is, as has been shown, a spherical homogeneous mass, but there comes a time when (owing to changes in internal or external conditions) crystallization occurs, during which there arise those conditions of tension which cause contraction changes of such a character as to form the hole or funnel-like cavity, etc., that we understand as the hilum. In grains of many starches after the formation of the hilum fissures form which tend to become more numerous and deeper as the grain approaches full growth, particularly when it is subjected to drying. The grains of some species show no evidence of fissuration upon drying, or even upon dehydration at 120° C., yet those of other species, even while in the cell-fluid, are more or less markedly fissured. This shows, of course, differences in the degrees of molecular tension, and it is not unlikely that these differences are in close relationship to stereochemic differences in the starch-substance. Since the young amorphous grains are, in comparison with the portions of the grains subsequently deposited, rich in phosphorus, we may have here an explanation, or at least an expression, of conditions which may inhibit crystallization in the young grain but permit of crystallization at a certain stage of growth after the deposition of a less rich phosphorus-bearing starch-substance upon this spherical nucleus which may lose some of its phosphorus.

The cause or causes of the lamellation are yet obscure, but there are no reasons for believing that they are essentially different from those giving rise to the same phenomenon in spherocrystals of other substances. The assumption that they are a result of an alternation of light and darkness, or of variations in the concentration of solution, etc., has been clearly disproved, for instance, by Fischer and St. Jentys.

(4) The hypothesis of growth of the starch-grain by intussusception is, as Fischer and others have shown, absolutely untenable; whereas the hypothesis of growth by external accretion is entirely consistent with our knowledge of the structure and growth of other crystalline structures and with the peculiarities of the starch-grain and the phenomena observed in the starch-producing structures.

(5) The form or shape of the starch-grain is not fortuitous, but definitely determined primarily by specific characters of the starch-producing structures, which are differentiated not only in different plants in ways more or less peculiar to the plant, but also even in different parts of the same plant, or even in the same organ; and secondarily by various incidental conditions, such, for instance, as the presence of protein and other crystalline structures within the starch-formers, and to some extent, in certain plants, by the mutual pressure of grains in contact.

(6) The production or synthesis of starch, excepting possibly in the lowest forms of starch-producing plants, is due probably essentially or even solely to specialized starch-producing protoplasmic structures, which may or may not contain chlorophyl; but the presence of chlorophyl, even in the case in chlorophyllous plastids, is not essential to starch formation by these plastids, as is shown in the fact that etiolated plastids may produce starch. Chlorophyl may be and usually is entirely absent from the leucoplasts of bulbs, tubers, corms, rhizomes, etc., which are very active starch-producers. In fact, chlorophyl may be merely in the nature of an energizer. (See page 22 of the Hemoglobin memoir referred to in the Preface.)

(7) The starch-grain is a spherocrystal having properties in common with spherocrystals of other substances, and hence having molecular properties in accord with those of rectilinear crystals. There are not any facts to justify a belief of a special microscopic form of structure of the starch-crystal, such as held by Nägeli's micellar theory or Meyer's trichite theory, which distinguishes the starch-crystal from other spherocrystals. Starch-crystals must have characteristics dependent upon intramolecular structure and intermolecular arrangement which distinguish them from the crystals of other substances, and if the starch-substance exists in a number of stereoisomeric forms, the properties of the crystals will vary accordingly, because of molecular differences.

(8) The forms or shapes of the starch-grain are variable in plants of different species, and in the same plant, and in different parts and even in the same part of a given plant, and are affected to some extent by the age of the plant and by changes in nutritive conditions; but the starch-grains of any given plant have characteristics in common which are sometimes more or less distinctive of the family, genus, species, etc. Greater departures from a given external form should be expected in spherocrystals produced under the peculiar, complex, and varying conditions of starch formation than under the conditions under which rectilinear crystals and spherocrystals generally are formed. In the production of the starch-crystal the growth of the crystal in all organisms, except in the lowest that produce starch, is, it seems, by means of specialized protoplasmic structures acting in conjunction with a cell-sap of peculiar, complex, and variable composition, while in the formation of crystalline substances generally the growth is ordinarily from molecules in a solution of simple composition and of virtually unchanging composition, except in so far as is concerned the loss of the mother-substance from the solution, and attended by comparatively unimportant changes in external conditions. Even though there be variant forms of the mature starch-grains or starch-spherocrystals in a given plant or given part of a plant, the molecular constitution of the starch-substance or the mean molecular constitution of the combination of starch-substances constituting the individual grains may be precisely the same, the vicarious forms representing distortions such as occur in the formation of rectilinear crystals when subjected to mechanical pressure and other conditions which give rise to malformations. It follows from this that while there may be a specific stereoisomeric form or combination of forms of starch in relation to each species or each genus, etc., conditions incidental to crystallization might so affect the form of the grain as to seemingly produce a shape that is foreign to that which might be regarded as belonging to the type of the species or genus. It would, therefore, be as hazardous to conclude that because starch-grains from any two sources differ in form they must necessarily differ in chemical constitution or stereochemic form; or because they are of like shape they must necessarily be of the same chemical constitution or stereochemic structure. The fallaciousness of such conclusions will be clearly shown in subsequent pages. Having a given substance, variations in crystalline form must be expected when certain attendant conditions are variable.

In the formation of starch-spherocrystals four coöperative factors must be conceded as being continually operative: One, *non-biological*, which is common in the formation of spherocrystals of sugar, unilin, leucin, etc., *in vitro*, tends specifically to the production of circumlinear crystals, which consist of radial aggregates; another, *biological*, which operates in the living organism to maintain a non-crystalline or amorphous condition; and another, the presence of foreign bodies, such as crystals of proteins or oxalate, etc., or of multiple starch-grains which by mechanical pressure or otherwise influence the forms of the grains; and finally, various conditions, such as changes of temperature, composition of the cell-sap, etc., which cause a given substance to crystallize in different forms. In other words, the tendency of the starch-substance is to crystallize, which is opposed by the tendency of bodies associated with protoplasmic process to remain in solution or in pseudo-solution, or to be deposited in a colloidal or amorphous solid form, as in the soft and the osseous tissues. Rectilinear crystals of entirely different substances may have precisely the same external features; and, on the other hand, crystals of precisely the same substance may have different external features, in other words, appear in different phases or forms. This doubtless applies to the different starch substances and to the starch spherocrystals. Therefore, the peculiarities of molecular structure, as determined by various reagents and by the polarizing microscope, enable us to determine the presence of different stereoisomeric forms—or, in other words, to show differences in chemical constitution, whereas differences in histology may be mere expressions of variations due to physical or physico-mechanical conditions.

## CHAPTER III.

### PRIMARY AND REVERTED DECOMPOSITION PRODUCTS OF STARCH.

When proteins are subjected to the actions of various hydrolytic agents, such as dilute acids, alkalies, and proteoclastic enzymes, a number of complex non-protein, non-colloidal derivatives or unit structural substances or decomposition products are formed which, because of their close relationship to the parent substances, have been termed the "primary dissociation products." These have been found to differ quantitatively and qualitatively in different proteins; and in certain instances, as in the protamines, differences have been noted which lead to the belief that with better methods of technique the constitutions of corresponding proteins will be found by chemical methods to differ, as has been shown by the crystallographic method in the case of the hemoglobins (see Preface, page vi); and, moreover, that such differences will be found to be specific in relationship to genera, species, etc. The starch-molecule likewise yields non-colloidal derivatives, which, as in the case of the proteins, are closely related to the parent substance and which also, with better laboratory methods, will in all likelihood be found to possess constitutional characteristics peculiar to the kind of starch. In case of both protein and starch, in the decomposition processes giving rise to the non-colloidal derivatives, there are intermediate colloidal products formed which as yet are but little understood as individuals or in their relations, or in the exact processes that occur during their formation.

The starch-molecule is conceived to be a complex polymer. According to Findlay (Physical Chemistry and its Applications in Medicine and Biological Science, London, 1905, 63) the apparent osmotic pressure of boiled starch leads to the value of 25,000 for the molecular weight. Skraup (Monatsb. f. Chemie, 1905, xxvi, 1415), by means of an acetylchlor compound, estimated the molecular weight of soluble starch to be 7,440. Fouard (Compt. rend., 1908, cXLVI, 285, 978) prepared a *non-colloidal* solution of starch, having the mobility of pure water, by filtering a pseudo-solution of partially demineralized hydrated starch at 80° through a collodion membrane. He found that cryoscopic examinations showed no falling of the freezing-point, and that the lowest possible molecular weight must be at least 15,000. This solution, he records, showed by ultra-microscopic examination no diffracting particles, and that it stands between the mineral colloids of insoluble elements and fully dissociated salt solutions. Assuming that the molecular weight is 15,000, and that the molecular formula is  $n(\text{C}_6\text{H}_{10}\text{O}_5)_n$ , the molecule would, according to almost universal conceptions, be regarded as being composed of over 90 single groups of  $\text{C}_6\text{H}_{10}\text{O}_5$ , the groups probably existing in the molecule in multiples of 3 or more; but the assumed existence of such preformed groups can not be admitted, as has been pointed out in Chapter I, page 6.

When comminuted raw starch or boiled starch is subjected to the actions of weak acids, hot glycerol, amyloelastic enzymes, etc., the starch-molecules are broken down into a number of complex primary decomposition products which, as far as our limited knowledge goes, are in the form, chiefly or entirely, of dextrans and sugars. Since a number of these products have been described, and as some differences have been noted in them when obtained from different starches under both like and unlike conditions of experiment, the probability is suggested that starches from different sources of origin, if they are not identical in chemical constitution, might exhibit specific differences in their derivatives, and thus afford a method of specific differentiation of starch in relation to genera, species,

etc. That the starches of different plants are not chemically identical is not only suggested by the marked differences in histological peculiarities, polariscopic properties, behavior to heat, reactions with stains and with various reagents, etc., but also by the differences in the conditions, protoplasmic and otherwise, under which the starch is formed.

The literature bearing upon the subject of the primary decomposition products contains many inconsistencies, contradictions, errors of observation and deduction, and baseless conclusions, and we are as yet very far from being satisfactorily informed as to the exact processes involved, or the exact products formed, or of the various conditions under which the products may be modified. The earliest literature of the products of the decomposition of starch, up to 1836, was reviewed by Poggendorff (*Ann. d. Physik u. Chemie*, 1836, xxxvii, 115). Subsequent publications, up to 1874, were very briefly abstracted by W. Nägeli (*Beiträge zur näheren Kenntniss der Stärkegruppe in chemischer und physiologischer Beziehung*, Leipzig, 1874, 106). Since this time a voluminous literature has accumulated. In 1872 a new era in these investigations was initiated by the discovery of Griessmayer and Brücke, working independently, of the formation of two kinds of dextrin, one giving a red reaction with iodine (*erythrodextrin*) but the other no coloration (*achroodextrin*); and by the rediscovery by O'Sullivan of the sugar described by Dubrunfaut in 1823, and named by O'Sullivan *maltose*.

SYNOPSIS OF THE MORE IMPORTANT LITERATURE UP TO THE INVESTIGATIONS OF  
GRIESSMAYER, BRÜCKE, AND O'SULLIVAN IN 1872.

The observation of Leeuwenhoek in 1716, that the starch-grain consists of a non-nutritive outer coat or integument and an inner nutritive substance, seems to have been the earliest step in the study of the structural units and derivatives of starch, which body, as Poggendorff pointed out in 1836, was up to that time one of the most studied and least understood of all substances. Poggendorff's statement was virtually repeated, ten years later, by Schleiden (*Principles of Botany*, 1849), and twenty years later by von Mohl (*Botanische Zeitung*, 1859, xvii, 225), the latter writing that more microscopical researches with starch-grains have been made, and in the course of time a greater number of contradictory teachings concerning the structure and chemical composition of starch-grains have been uttered than of any other plant structure. In fact, von Mohl's statement might with justification have been written at the present day.

Our knowledge of the primary decomposition products had its origin in the discovery of Vaquelin (*Bull. d. pharm.*, 1811, iii, 54) that by torrefaction starch is converted into a gummy substance (*Stärkemehlgummi*) which resembles gum arabic; and in the discoveries of Kirchoff (*Schweigger's Journal*, 1815, xiv, 389) that dilute acids change starch into a gum and grape-sugar, and that gluten, and also a substance of germinating barley that can be extracted by water, and which acts like weak acid, act likewise. The substance from barley was thought by Kirchoff to be the albuminous matter, or the gluten, of the grain. The records of these investigators received full confirmation by contemporaneous workers. Several years later De Saussure (*Ann. de chim.*, 1819, xi, 379) found that when starch-paste was set aside for two years, not only were gum and sugar formed, both of which could be extracted by cold water, but also two other bodies were present, one of which he named *amidine* and the other *ligneux amylicée*. Amidine was obtained from the residue by solution in hot water; the *ligneux amylicée*, so-called because of its resemblance to cellulose, was found to be insoluble in both hot and cold water. Amidine was also distinguished by its giving a blue reaction with iodine. In 1825 Raspail (*Ann. d. sciences naturelles*, Oct., Nov., 1825; *Mars*, 1826; quoted by W. Nägeli, *loc. cit.*) carried out experiments in which he heated dry starch on an iron plate, and then added water and tartaric acid to the baked grains that had been placed on the stage of the microscope. He found, as had a number of the earlier investigators, that the grains consist of an inner part and an integu-

ment. He believed that these two parts differ in chemical composition, one being soluble and staining blue with iodine and the other being insoluble and not yielding the blue reaction with iodine; which difference he thought due to the presence of an unstable inner substance, or gum, which is expelled on heating and which is soluble in cold water.

The following year Raspail's assertions were disputed by Caventou (Ann. de chim., 1826, xxxi, 358), who states that the starch-grain as a whole is stained with iodine, and that raw starch does not contain any part that is soluble in cold water. He also found that the amidine of De Saussure could be extracted from fresh starch-paste by cold water, and that it was merely a modified starch (amidon modifié).

Several years later Guibourt (Ann. de chim. et phys., 1826, xl, 183), in support of Caventou, ascertained that both the outer coat and the internal substance stain with iodine, and therefore that they are not of different chemical composition, as held by Raspail. He also recorded that when broken-up starch-grains are placed in cold water the inner substance of the grains is dissolved, leaving the integuments. This soluble substance he states is not a gum, and he notes that it gives a blue reaction with iodine. He identified it with De Saussure's amidine, and also with the soluble part of the starch, or the gum, referred to by Raspail. Guibourt's results were confirmed by Berzelius the next year. In 1831 the very important discovery was made by Leuchs (Ann. de chim. et phys., 1831, xxii, 105, 623) that saliva, like the aqueous extract of germinating barley, contains a substance that saccharifies starch-paste. This was confirmed by Schwann (Ann. de chim. et phys., 1837, xxxviii, 358) and other observers, and although it was conceded by the authorities of the day that saliva had this property, it was believed that because of the watery character of the secretion the functions of saliva must be in connection with mastication, gustation, and deglutition, and that its saccharifying property could not be of importance in the living organism (Wagner's Handwörterbuch d. Physiologie, Verdauung, iii, Hfte. 1, 768).

The nature of the gum produced when raw starch is subjected to dry heat and when boiled starch is subjected to weak acids or to an aqueous extract of barley, etc., was made a special study by Biot and Persoz (Ann. d chim. et phys., 1833, lxi, 72), who prepared this substance by means of dilute sulphuric acid. They believed that it is not a product of decomposition, but the inner substance of the starch-grain, that is set free by the bursting of the outer coat. Polariscopic examination showed it to be dextro-rotatory, on account of which they gave it the name of *dextrin*, which name has continued in use to the present time.

During the next year Raspail (*Nouveau système de chimie organique*) recorded his earlier views on the structure and reactions of starch and reports the results of further studies, which led him to the conclusion that the substance contained within the integument is dextrin, and that the body which stains blue with iodine is an unknown, unstable principle. This conclusion was based on the fact that the part of the grain that is dissolved in boiling water is no longer stained blue with iodine, as is also the case with the integuments.

An investigation of fundamental importance was carried out at this time by Payen and Persoz (Ann. de chim. et phys., 1834, lxi, 730), who prepared from germinating barley and other grains, and also from potatoes, a substance which converts starch into dextrin and sugar. They believed that dextrin constitutes the inner substance of the starch-grain and that their prepared substance has the remarkable property of separating the integument of the grain from the inclosed dextrin. Because of this property they named it *diastase*, from the Greek διάσπασις, meaning separation. They differentiated the processes of dextrinization and saccharification, and they believed that the formation of sugar is a subsequent and not a coincident process in the production of dextrin. It is recorded that when 100 parts of starch, 400 parts of water, and 5 to 10 parts of dried malt are heated to 75° for 3 hours, the dextrin liberated from its integuments is transformed into a sugary substance, and when the preparation was heated to boiling-point the formation of sugar was prevented, because at this temperature, they state, the diastase is destroyed.

They prepared diastase by two methods: According to the first method the sprouted grains are crushed and macerated in water. The preparation is strained, and then filtered and heated to 75°, causing a cloudiness due to the precipitation of albuminous substances. This is filtered and sufficient alcohol is added to the filtrate to cause a white flocculent precipitate, which when dried is in the form of a firm, white, uncrystallized mass, without taste, neutral, and soluble in water and in weak alcohol, but not in strong alcohol. By the second method the sprouted grains are crushed and the grains moistened with half their weight of water, the liquid is filtered off, and sufficient alcohol is added to remove its mucilaginous consistency and to precipitate the albuminous substances. The preparation is filtered and the diastase is separated from the filtrate by the addition of sufficient alcohol. They purified the diastase by repeated solution in water and reprecipitation with alcohol. They note that one part of diastase is sufficient to saccharify 2,000 parts of starch, and that reaction takes place in several minutes with a quantity of water less than four times the weight of starch. They also recorded that diastase does not pre-exist in the ungerminating grains, but is formed as needed for the transformation of starch into sugar during germination.

During this and the following years, four articles appeared by Guérin-Varry (Ann. de chim. et phys., 1834, LVI, 225; 1834, LVII, 108; 1835, LX, 32; 1836, LXI, 66), in which he states the following conclusions: The starch-grain consists of three substances—*amidine*, a substance soluble in cold water; an integument, *amidine tégumentaire*, which is insoluble in cold water; and *amidin soluble*, which is insoluble in cold water by itself, but is rendered soluble by the contained amidine. The amidin tégumentaire was recorded as forming 2.96 per cent of the starch. Of the remaining 97.04 per cent, 60.45 parts per hundred were amidine, and 39.55 parts were amidin soluble. He made note of the fact that while whole starch-grains are entirely insoluble in cold water crushed grains are partially dissolved; that broken grains as well as boiled grains are dissolved all but the integuments; and that if the solution of starch be evaporated a residue will be obtained, a part of which is soluble in cold water (amidine) and a part insoluble in cold water (amidin soluble). He gives the methods for preparing these substances and their properties, and he also records the results of the elementary analyses of starch, and gives the molecular formula as  $C_6H_{10}O_5$ .

Guérin-Varry's conclusions regarding the existence of three constituents of starch were criticized by Payen (Ann. de chim. et phys., 1836, LXI, 335), who states that the amidine which is soluble before the evaporation of the solution is "swollen up" amidone distributed through the water, but after evaporation it is amidone with greater cohesiveness than the starch; that the amidine tégumentaire is nothing else than amidone which, by the method of treatment and by the presence of the integuments, has attained greater cohesiveness; and that amidine is finely divided amidone.

The literature of starch was added to this year by two contributions by Fritzsche (Ann. d. Phys. u. Chem., 1834, xxxii, 129, 143), in which he reports studies on the morphology, method of formation and dissolution of starch-grains, and also the microscopic changes which occur in starch when heated in water on the stage of the microscope. He disproves the statement of Raspail that the grains consist of an enveloping membrane that is insoluble in water and an inclosed substance that is soluble in water. Raspail states that when the grains are ruptured the contents are released from the integuments, but Fritzsche found that when the grains were placed in water and pressed between glass plates, by which some of the grains were broken, the inner mass was not dissolved, nor were empty integuments observed. He studied the grains in the resting-stage of the potato and during the period of erosion. From these studies he believed that the grains are formed by the desposition of outer layers upon the inner and that erosion takes place layer by layer from without inward, from which it follows, he states, that the grains do not consist of a membrane and an inner substance that is soluble in water, or of membranous layers with a soluble substance between them, but of a homogeneous mass arranged in concentric layers.

Fritzsche seems to have been the first to observe under the microscope the changes which occur in starch when it is heated in water, and he states that remarkable changes are seen when a thin layer of starch is placed between two thin glass plates and one end of the plates gradually heated until the water boils. The first change observed in potato starch is in the region of the hilum (referred to by Fritzsche as the *Kern*), where cracks are formed, and at the same time the hilum begins to expand in the direction of the part of the grain where the layers are thinnest and where the least resistance is offered. The cracks often spread out irregularly through the grain. Then the water in which the starch is placed passes into the layers of the grains and the grains spread out until all traces of lamellation disappear. While these changes are going on some of the inner, now porous, layers are partly dissolved and the parts undissolved float about as small flakes. If iodine is added to such a preparation the flakes are colored blue, but the swollen grains take on a reddish hue. He also made observations of the effects of acids and alkalies and of alcohol and water. He noted the interesting phenomenon that if small quantities of water are added to alcohol and the starch-grains are boiled in it, the larger the quantity of water the more does the hilum enlarge, from which he concludes that the hilum is composed of a peculiar substance which expands at high temperature.

Various investigators had made elementary analyses of starch antedating this period, and their figures were collected by Brunner (*Ann. d. Phys. u. Chem.*, 1835, xxxiv, 319), to which was added the results of his own labors. The following year Payen (*Ann. de chim. et phys.* 1836, lxi, 355; lxxv, 225) concluded, from analyses of different kinds of starch, that all starches consist essentially of amidone (starch) and dextrin, and that all have the same elementary composition ( $C_6H_{10}O_5$ ), differing merely in their molecular constitution.

Poggendorff (*loc. cit.*) at this time, as previously stated, reviewed the most important parts of the literature of the starches up to this date, and in summing up the results of the several researches makes the following statement: Starch, as present in granular form in the cereals, potatoes, etc., is undoubtedly an originated substance in the general sense that it is a simple nutritive constituent of the plant. Raspail's view that the starch-grain is a sort of a sac filled with a gum-like substance is held to be untenable, whereas Fritzsche's view is deemed in better accord with the facts. Poggendorff writes that he can not tell of what the point or *Kern*, as named by Fritzsche, is composed, or what causes the layers to be separated during the breaking down of the grain, but that we do know with a degree of certainty that the layers consist only of material which we call starch (*Starkemehl*), and we know that the insolubility of the intact grains in cold water is caused either by the fact that the outermost layer has a special cohesion or that it is saturated with an albuminous substance by which the grains are surrounded in the cells. The former view, he states, is shared by Payen, but he believes that the outer layer is the oldest, and that the grain grows by deposition within. He doubts whether starch is dissolved in either cold or hot water; he considers the name amidone superfluous because it represents nothing more than pure starch; and he believes it a question as to whether the substances named amidine and amidin soluble are constituents or products of decomposition of the grains.

From this time (1837) until 1845 very little was recorded in the advancement of our knowledge of starches in regard to their chemistry, or to the agents which break them down, or to their derivatives. Mulder (*Jour. f. prakt. Chemie*, 1838, xv, 299) analyzed potato starch, and from his results he concluded that no water is chemically combined with the starch. In this year Payen published his *Mémoire sur l'amidon* (*Ann. des sciences naturelles*), in which he describes the properties of starch as determined by himself and others.

In 1840 Jacquelain (*Ann. de chim. et phys.*, 1840, lxxiii, 167) found that on heating starch-paste to  $150^\circ$  a substance was found which is very insoluble in cold water, but readily soluble in water at  $70^\circ$ ; and he notes that when it is subjected to a higher temperature dextrin and glucose are formed. The reactions of starch, dextrin, and sugar

with oxide of copper were reported by Trommer (Ann. d. Chem. u. Pharm., 1841, xxxix, 360). During the same year Liebig (Ann. d. Chem. u. Pharm., 1841, xlii, 306) stated that the so-called iodide-starch is not a true chemical compound, the starch being merely impregnated with the iodine; and also that wood fiber is not actually changed into starch, as believed by some, by the action of potassium hydrate or sulphuric acid.

Blondeau de Carolles (Jour. f. prakt. Chemie, 1844, xxxiii, 439) recorded in examinations of potato starch that the grains are composed of a number of layers which have the same nature and composition, but differ from each other by varying density. He notes that starch changes readily at 60° by rubbing the grains; and he also made elementary analyses and prepared several sulphuric-acid products.

The optical reactions of the starch-grains and of their solution were reported by Bioz (Ann. de chim. et phys., 1844, xi, 100). The grains were found to be doubly refractive, and the solution (solutions of dextrin) to be dextro-rotatory. Fürstenberg (Ann. d. Chem. u. Pharm., 1844, 52, 417), in experiments with cereals, recorded the presence of a dextrin similar to that obtained by the action of dilute sulphuric acid or diastase on starch, and that it does not reduce the oxide of copper.

Fehling (Ann. d. Chem. u. Pharm., 1845, lv, 13) experimented on starch with dilute sulphuric acid and found what he described as 9 different products in different proportions according to the strength of the acid and the length of time of the reaction. Fehling's work received support in the investigations of Kalinowsky (Jour. f. prakt. Chemie, 1845, xxxv, 193), who also later reported (*ibid.*, 201) that tannic acid precipitates a solution of starch without forming a compound with the starch.

The pancreatic juice was found by Bouchardt and Dandras (Compt. rend. acad. sci., 1845, xx, 1085) to have the property of saccharifying boiled starch. At the same time Miahle (Compt. rend. acad. sci., 1845, xx, 954, 1485) reported that the methods of Payen and Persoz for obtaining diastase were applicable to the saliva. He filtered the saliva and added to the filtrate from 5 to 6 volumes of absolute alcohol, which caused a small amount of white flocculent precipitate, which was collected and dried at room temperature. This preparation he found to be very energetic, 1 part having the power of converting 2,000 times its weight of starch into sugar. Owing to the apparent identity of its properties with those of diastase, and in order to distinguish it from the diastase obtained from plants, he termed it *salivary diastase*, a term that has fallen into disuse for the preferable name *ptyalin*. Since that time it has been shown, from the investigations of a large number of observers, that amylolytic enzymes are very widely distributed throughout both the animal and plant kingdoms and in the various bodily solids and fluids. The preparations of these earlier investigators were, notwithstanding their energy, very impure, and consisted very largely of inert albuminous matter.

Reissek (Flora, oder allgemeine Botanische Zeitung, 1847, 13), after a careful study of the normal starch-grain and of the metamorphoses which take place when the grains are set aside in water for some time, was led to the conclusion that starch-grains must be viewed as special undeveloped cells, and that in the entire series of starch-grains from the various plants known to us there can be found transition forms from the simple homogeneous, dense grains to the grains whose outer substance has been differentiated into a membrane, and thus formed into definite cell.

Schulze (Jour. f. prakt. Chemie, 1848, xlv, 178) reported a substance which he thought stood between starch and dextrin, and which was found to be insoluble in cold water but readily dissolved in hot water. He gave to it the name *amidulin*, which substance is probably identical with one obtained by Jacquelin by subjecting starch-paste to a temperature of 150°.

The nature of potato starch was studied by Schleiden (Principles of Botany, 1849, 11). He found in tests with iodine and solvents that all parts of the starch-grain are affected

practically equally; and that while there may be slight differences in the external layers arising from the adhesion or infiltration of some traces of albumin, fat, or wax, such differences merely cause a longer or shorter delay of the action of the iodine or solvent. During this year Schwarz (*Ann. d. Chem. u. Pharm.*, 1849, LXX, 54) and Fehling (*Ann. d. Chem. u. Pharm.*, 1849, LXXII, 106) reported their determinations of the quantity of starch present by reducing the starch to sugar by dilute sulphuric acid and finding the amount of sugar by means of the copper test.

Soluble and insoluble forms of starch were examined by Maschke (*Jour. f. prakt. Chemie*, 1852, LVI, 400; 1854, LXI, 1). In studying the structures of starches by the effects of heating (wheat starch was taken as a type), he records that the peripheral layer consists of cellulose which is colored red or brown by iodine, while the inner part is colored blue. Wheat starch was heated to 40°, 50°, 60°, 70° and 100°, and at each temperature examined. At 40° the grains show rings in large numbers, alternating light and dark; at 60° outlines of small grains appear in the center of the large grains; at 70° cracks or breaks are formed by swelling; at 100° the grains are irregular, each resembling a collapsed bag. All of these phenomena, Maschke states, can be explained upon the assumption that every starch-grain is composed of 3 to 5 vesicles of different size placed one within the other, the starch ("amylon") being present between these in granular form. The appearance and disappearance of so many rings he attributes to the swelling up of the ring-like, separated, granular amylon; and he regards the 3 to 5 circles which finally remain as the boundary envelopes of the vesicles which compose the grain. He believes he has proved the existence of such a structure by means of the action of iodine and sulphuric acid. The internal part of the grain, he states, consists of a soluble and an insoluble substance; and by evaporation the former is converted into the latter, but by the use of solvents, such as potassium hydrate, or hot water, or alcohol and sulphuric acid, or when subjected to dry temperature of 150°, the insoluble substance is converted into the soluble modification. The terms soluble starch and insoluble starch are used by him only with reference to the behavior of the two modifications of starch towards cold water. According to Maschke, the numerous light and dark rings which lie between the concentric vesicles are probably due to the presence of different modifications of the starch-substance, and the light rings are the insoluble modification of the starch which is present in granules lying side by side, while the dark rings are of the soluble modification in liquid form in which the light rings are embedded.

Soluble starch was prepared by Béchamp (*Compt. rend.*, 1854, XXXIX, 653) by various means, such as nitric, sulphuric, and acetic acids, and solutions of chloride of zinc and potassium hydrate. The preparation thus obtained was found by Béchamp to differ from dextrin in several characteristics, and also from natural starch, inasmuch as when the solution is boiled to a syrup no cloudiness appeared. In a later article (*Compt. rend.*, 1856, XLII, 1210) he discusses in detail the process of changing natural starch into soluble starch, and he looks upon the latter as a special substance which stands between starch and dextrin.

It was noticed by Crüger (*Botanische Zeitung*, 1854, XII, 41) that all parts of the starch-grain during growth do not react in the same way with iodine. The outer layer of the grain resting upon the plastid behaves, he states, differently from the mass of the grain, this layer staining a yellow or dark brown (the same coloration assumed by the protoplasm and chlorophyll), while the rest of the grain stains blue and also more readily than the outer layer. He regards this outer layer as a substance which is in the process of becoming starch, but which as yet does not stain blue with iodine. Young grains stained very slowly with iodine, and the blue color was comparatively less pronounced than in mature grains. Small grains treated with iodine stained yellow with iodine or showed no color reaction. Crüger does not undertake the determination of the nature of this

transition substance. From the similarities, dissimilarities, and transitions which exist between starch and cellulose, he believes that starch and cellulose are not homogeneous substances, but compound bodies having a common organic element.

Reinsch (*Neue Jahrbücher f. Pharm.*, 1855, III, 65) records that potato starch contains dextrin and sugar which are set free in water when the grains are pulverized. Nägeli (*Tageblatt d. 32, Versammlung deutscher Naturforscher u. Aerzte in Wein*, 1856) found that a substance giving a blue reaction with iodine could be derived from starch without changing the structure. This work is reviewed in his elaborate monograph which was published in 1858 (*loc. cit.*). Payr (*Jour. f. prakt. Chemie*, 1856, LXIX, 425) studied the action of chloride of zinc on the starch of the horse chestnut. He noted that the grains are dissolved, leaving a very small residue. He made preparations in the cold and at 100°, which were filtered, precipitated with alcohol, and dried. From both he obtained a carbohydrate in combination with zinc, but the preparation made at high temperature contained only a twelfth part of the zinc salt that was contained in former. He determined the elementary composition, and concluded that the carbohydrate separated is not starch, because it dissolves easily in water and does not give a blue reaction with iodine; nor could it be dextrin or sugar, he concludes, yet it goes easily into sugar on warming with dilute acid.

In 1857 Wolff (*Jour. f. prakt. Chemie*, 1857, LXXI, 91) examined the proportions of water and the ash constituents of a number of starches. Melsens (*Institut*, 1857, XXV, 161; quoted by W. Nägeli, *loc. cit.*) found that the starch-grains when treated with dilute acids, pepsin, or diastase may be so altered in composition, without changing their form, that they then no longer stain blue with iodine. During the same year Fresenius (*Ann. d. Chem. u. Pharm.*, 1857, 102, 184) reported his observations that as the temperature was lower the intensity of the color reaction of the starch solution to iodine was greater.

Mulder (*Chemie des Bieres*, Leipzig, 1858, 166) found, after subjecting starch to extract of malt, dilute sulphuric acid, and roasting, respectively, that the dextrans formed differ from each other, as was shown by their behavior in relation to certain precipitants.

In 1858 Carl Nägeli published his elaborate monograph (*Die Stärkekörner, etc., loc. cit.*), from which quotations have been made in the preceding chapter, and which will be referred to in subsequent pages. Von Mohl (*Botanische Zeitung*, 1859, XVII, 225) took exception to the statement of Nägeli that when starch-grains are subjected to the action of saliva the granulose is dissolved out while the cellulose remains, and that the reaction of starch and cellulose with iodine furnish a means of differentiation, etc. (See Chapter II, page 27.)

Payen (*Compt. rend.*, 1859, XLVIII, 67) found that the reaction of starch and cellulose with iodine is almost the same, but that it differs under the influence of diastase, and also towards an ammoniacal solution of copper oxide, cellulose being soluble in the latter, but not so with amylose. Niépee de Saint-Victor and Convisart (*Compt. rend.*, 1859, XLIX, 368) ascertained that sugar and dextrin are formed from starch-paste that had been subjected to the action of the sun. Wicke (*Ann. d. Physik. u. Chemie*, 1859, CVIII, 359) states that pulverized starch-granules do not dissolve in water, but this statement is in contradiction to the records of many previous observers and is fully disproved by the investigations of Jessen (*Ann. d. Phys. u. Chemie*, 1859, CVI, 497; 1860, CIX, 361), and later experimenters. Jessen writes that one can easily convince himself of the solubility of raw starch by crushing the starch-grains in a little water, when the beginning of solution is at once noted, the entire mass becoming viscous and mucilaginous. On the addition of more water a clear solution is obtained on whose surface float torn and broken coats of the starch-grains, while the unbroken grains sink to the bottom. The filtered liquid became blue on the addition of iodine, and the iodine-starch is, he states, contrary to C. Nägeli's statement, completely soluble in pure water.

Delffs (Ann. d. Physik. u. Chemie, 1860, cix, 648) confirmed Jessen's statement of the solubility of crushed starch-grains. He also notes that while the soluble constituent of starch has been looked upon as a form of dextrin, its various reactions show that it does not correspond with the three dextrans described by Mulder (*loc. cit.*), and therefore that it must be another form of dextrin if it is to be classed as a body of this kind. He associated this substance with a hypothetical isomer from which the starch-substance is supposed to be formed, and to which he gave the name *amylogen*.

Knop (Chem. Centralblatt, 1860 v, 367) also found that crushed raw starch is soluble in water, but he thought that sufficient heat was formed during the pulverization to gelatinize the grains, and thus cause solution. This assumption, however, was subsequently disproved by Jessen (see page 92).

Museulus (Ann. de chim. et phys., 1860, lx, 203; Compt. rend., 1861, liv, 194) reported that diastase, and also dilute sulphuric acid, changes starch into 2 parts of dextrin and 1 part of sugar, and that the acid may exert a slow but continuous action on the dextrin, so that ultimately the preparation does not yield a color reaction with iodine. When fresh starch-paste was added the reaction was renewed, and it continued until there occurred a loss of color response to iodine; but even at the end of the reaction there was much dextrin remaining, there being present always at this time 2 parts of dextrin to 1 part of sugar. In his later contributions (page 94) he gives equal proportions of dextrin and sugar when the reaction becomes stationary. He thought that the accumulation of sugar prevented further changes of dextrin, but not of starch; and he believed the starch is not converted into dextrin, and dextrin into sugar, but that by a process closely associated with an absorption of water the molecules of starch were split into dextrin and sugar. The theory of this splitting was founded on the fact of the production of these two products in definite ratio.

The work of Musculus received support in the investigations of Payen (Compt. rend., 1861, liii, 217), who recorded that the resulting sugar prevents a further production of sugar, and that by removing the sugar starch may be converted into dextrin and sugar in almost any quantity, but never only into dextrin. Payen, however, opposed the view of Musculus of the splitting of starch into dextrin and sugar.

The disappearance upon heating of the blue coloration of a starch-solution treated with iodine was reported by Duroy (Compt. rend., 1860, li, 1031). Baudrimont (Compt. rend., 1860, li, 825), in seeking the cause of the loss of the color of the iodide of starch on heating, concluded that it is owing to the evaporation of the iodine, the vapor resting on the liquid, and the color returning upon cooling owing to accompanying resolution of the iodine. Pohl (Jour. f. prakt. Chemie, 1861, lxxxiii, 35) offered a different explanation, and holds that hot water has a greater affinity than starch for iodine, whereas with cold water the reverse is true. Schönbein (Jour. f. prakt. Chemie, 1861, lxxxiv, 402) states that Baudrimont's explanation is untenable, and that Pohl's is correct; that iodide of starch is a chemical compound; and that there is no such thing as a colorless iodide of starch. Schönbein's view was supported by Fresenius (Zeit. f. analyt. Chemie, 1862, i, 84), Kraut (Gmelin's Lehrbuch d. organ. Chemie, 1862, iv, 554), and Kemper (Archiv d. Pharm., 1863, cxv, 252). Pellet (Zeit. f. Chem., 1866, x, 352), however, asserted that starch-iodide is insoluble in cold water and that it loses its blue color upon heating because it is soluble in hot water and loses its color when it goes into solution.

The oft-repeated observation of the solubility of pulverized raw starch in cold water was confirmed by Flückiger (Zeit. f. Chemie, 1861, iv, 104), who also noted that the solubility was increased in the presence of potassium chloride. During the same year, Nossian (Jour. f. prakt. Chemie, 1861, lxxxiii, 41) examined the hygroscopic properties of starch (Chapter IV, page 167); and Lippmann (Jour. f. prakt. Chemie, 1861, lxxxiii, 51) reported the results of his experiments on the temperature of gelatinization of different starches (Chapter IV, page 175).

The actions of an ammoniacal solution of oxide of copper on various starches were studied by Weiss and Weisner (Sitzungsber. d. k. k. Acad. d. Wissenschaft z. Wein, 1862, XLVI, 311).

The essential components of starch were recorded by Dragendorff (Jour. f. Landwirthsch., 1862, VII, 211) as follows: (1) True starch; (2) a radical base which remains after starch has been heated to 60° in a concentrated solution of chloride of sodium containing 1 per cent of hydrochloric acid; (3) Schulze's amidulin (Delff's amylogen); and (4) occasionally some dextrin.

Kabsch (Zeit. f. analyt. Chemie, 1863, II, 216) studied the properties of starches in relation to the polariscope, to gelatinization, and to solubility. He noted that the polariscopic appearances undergo change by the action of heat; that the grains, when examined in the polariscope with a quartz plate ground perpendicular to the axis, by turning analyzer they behave like cellulose, being dextrogyrate or lævogyrate, according to conditions; that the doubly refractive properties of animal and vegetable bodies are determined by a fixed, definite arrangement of the molecules, but that the arrangement is not sufficiently uniform, as in crystals, to speak of definite optic axes; that there are a large number of substances which gelatinize or swell starch without dissolving it, the chief of these agents being the easily soluble haloid salts (but some salts prevent swelling, depending upon the concentration of the solution); that starch is completely soluble in glycerine when heated for a sufficient length of time, and that the dissolved starch can be precipitated from solution by alcohol, although the precipitate does not have the properties of the original starch, the properties indicating a modification identical with Delff's amylogen; that starch is completely dissolved by the action of saliva and other ferments; that we are justified in assuming that starch consists of two different substances, one of which, the real starch-substance, is soluble in saliva (Nägeli and von Mohl), in dilute acids (Melsens), and in cold water after crushing (Reinsch, Jessen, Delffs, etc.); the other substance (cellulose according to Nägeli, and farinose according to von Mohl) is insoluble in the above-mentioned media; that the outermost or densest layers, and especially the outermost, resist all action of chemical agents longer than the less dense substances by which the lamellae are surrounded and impregnated; and that the heat generated during the pulverization of starch is a coagent in solution.

Kemper (Archiv d. Pharm., 1863, CXV, 250) found that a weak solution of sugar-free dextrin does not reduce Fehling's solution, but that a concentrated solution does.

Nägeli (Botanische Mittheil, 1863, 251; Sitzungsberichte d. k. k. Acad. d. Wissensch. in München, 1862-1863) studied the reactions of iodine with starch-grains and cell-membranes, the chemical composition of starch-granules and cell-membranes, and the chemical differences of starch-grains.

Jessen (Ann. d. Physik. u. Chemie, 1864, CXXII, 482) opposed the statement of Knop and of Kabsch that heat has anything to do with the solubility of the comminuted starch-grains. Jessen found that the pulp showed a temperature of 20°, which is too low to have any influence.

The following year Payen (Ann. de chim. et phys., 1865, IV, 286) asserted that some of the statements of Musculus published in 1860 are not correct. Payen found that diastase as well as dilute acid will convert dextrin into sugar, and that of the entire product obtained by the action of diastase on starch as much as from 17 to 50 per cent may consist of sugar.

During the same year, Musculus (Ann. de chim. et phys., 1865, VI, 177) studied the properties of what he terms *true dextrin* (achroodextrin), and showed that this substance does not stain blue with iodine.

A year later, Payen (Ann. de chim. et phys., 1866, VII, 382) repeated his experiments, and he again gave evidence to disprove views of Musculus. He found that diastase as well as sulphuric acid will produce varying proportions of sugar, and that sulphuric acid when properly used produces 80 per cent or more of sugar.

Schützenberger (Compt. rend., 1866, 61, 485) recorded that anhydrous acetic acid produces from starch two compounds, one soluble and the other insoluble, both of which yield dextrin. Philip (Zeit. f. Chemie, 1867, x, 400) noted that by boiling starch in dilute acid there was not produced a constant proportion of sugar, as Musculus supposed, but variable proportions according to the quantity of acid used. Löw (Zeit. f. Chemie, 1867, x, 510) broke starch-paste down into formic acid, carbonic acid, and carbon by keeping it at a temperature of 170°.

Jessen (Jour. f. prakt. Chemie, 1868, cv, 65) resolved starch into three essential components: (1) Envelopes or cell-coats which are insoluble in hot or cold water (the amidine tégumentaire of Guérin); (2) starch which is soluble in cold water (the amylogen of Delffs, the amidine of Guérin-Varry); (3) starch which is soluble in hot water (55° to 80°), and which may be called amylin (the amidin soluble of Guérin-Varry and the amylin of Maschke, Delffs, Melsens, and Schulze).

Musculus (Compt. rend., 1869, LXVIII, 1267; 1870, LXX, 857) ascertained that in the breaking down of starch by diastase or acid into *colorless* or *true dextrin* (achroodextrin) there is formed a modification which he terms an *insoluble dextrin*. This dextrin was found to be insoluble in cold water, but soluble in water at 50° to 60°. An aqueous solution yielded a wine color with iodine, and when in an air-dried condition it became colored a violet, yellow, or brown with iodine, and it was found to yield less sugar than starch.

Schwarzer (Jour. f. prakt. Chemie, 1870, cix, 212), in common with Payen, Philip, and others, looked upon Musculus's theory of the splitting of the starch molecule directly into dextrin and sugar as being incorrect, even though the formation of dextrin and sugar occurs in definite ratio. Schwarzer studied the actions of diastase with reference to the quantities and characters of the products under various conditions. He observed that after the reaction to iodine ceased, the formation of sugar was about completed, so that a continuance of the action resulted in the formation of only very small quantities of sugar; that at all temperatures from 60° down to 0°, using varying amounts of diastase, there is formed from 50 to 53 per cent of sugar; that at temperatures above 60° less sugar is formed than at lower temperatures; that if malt extract acts for some time at 70°, it becomes so changed that it forms very little sugar if the temperature be lowered; that if a starch-solution prepared at 70°, which contains 27 per cent of sugar, is lowered in temperature, and diastase is added, the sugar-content can be increased to 52 per cent; that the appearances of the starch preparation when transformed at different temperatures are very varied—at 70° the undissolved starch capsules are grouped into brown flakes, while at lower temperatures white flakes appear which form a thin layer at the bottom of a clear liquid; and that a definite amount of diastase is capable of transforming only a limited amount of starch, since there is a gradual transformation of the diastase into an inactive form.

Schulze and Märker (Jour. f. Landwirtsch., 1872, xx, 56) confirmed the records of several previous investigators that through the action of diastase starch is almost completely converted into definite proportions of dextrin and sugar.

#### THE BASIC INVESTIGATIONS OF GRIESSMAYER, BRÜCKE, O'SULLIVAN, AND MUSCULUS AND GRUBER.

The present day conceptions of the primary decomposition products of starch that are formed through the actions of enzymes, weak acids, etc., may be credited essentially to Kirchoff (1811), Biot and Persoz (1833), Payen and Persoz (1834), Musculus (1860–1870), Griessmayer (1871), Brücke (1872), and O'Sullivan (1872). Kirchoff was the first to announce the formation of sugar; Biot and Persoz prepared and studied the properties of dextrin and gave this substance its name; Payen and Persoz believed that dextrin is the inner substance of the starch-grain and that sugar is formed from it; and Musculus

recorded that definite proportions of dextrin and sugar (2 : 1 or 1 : 1) are formed, the starch molecule being split by the agency of water into dextrin and sugar, and also that two forms of dextrin occur, one of which is not colored with iodine.

Griessmayer (Ann. d. Chem. u. Pharm., 1871, CLX, 40) and Brücke (Sitzungsber. d. k. Akad. d. Wissensch. z. Wien, 1872, LXV, 3 Abth., 200), in independent investigations, ascertained that two dextrins are produced which are readily distinguishable from one another by their behavior with iodine. Griessmayer states that when the solution of starch is set aside there will be formed at first a dextrin that is colored red with iodine, and then a dextrin that gives no color reaction with iodine but which nevertheless has the greater affinity for this reagent. He designated these substances dextrin I and dextrin II, respectively.

Brücke gave to the dextrin that becomes red in the presence of iodine the name *erythro-dextrin*, and to the one which does not become colored, the name *achroodextrin*, both of which terms have had almost universal use up to the present. He also noted that during the conversion of starch into dextrin by the extract of malt a portion of the starch remains after the disappearance of the granulo-se, which portion, like erythro-dextrin, is colored with iodine, but which shows a greater affinity for iodine than granulo-se. This substance he termed *erythramylum*, which he regarded as consisting of a mixture of Nägeli's "cel-lulose" and a substance which is colored red with iodine that is present in dry starch but covered by the granulo-se, so that, as a consequence, its reaction is masked by the blue reaction of the granulo-se. These dextrins, he found, did not reduce copper solutions.

O'Sullivan (Jour. Chem. Soc. Trans., 1872, x, 579) at this time thought that the first two dextrins are identical, and states that neither reduces a copper solution, and that they possess a rotatory power  $(\alpha)_D = +213^\circ$ . O'Sullivan, in referring to investigations of Musculus, Payen, and Schwarzer, in which it was found that dextrin and sugar are formed in about equal proportions, or larger proportions of dextrin than sugar, showed by the copper test for sugar that the proportion of non-reducing to reducing substance is 1 : 2. He, however, did not regard the solution as being a solution of both dextrin and glucose, but as a solution of a peculiar form of a sugar which he designated *maltose*. He showed that maltose is not identical with glucose, because its reducing power corresponds to only 60 per cent of that of the latter, and he identified it with the sugar described by Dubrunfaut in 1823 (Ann. de chim. et phys., 1823, XXI, 178), who at that time stated that it was not glucose, as was then believed.

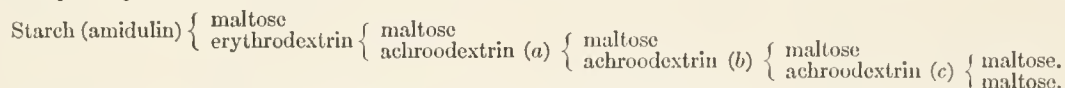
Several years later an important contribution by Musculus and Gruber appeared (Zeit. f. physiolog. Chemie, 1878, II, 177) which did more to mould our conceptions of the processes and products of the saccharification of starch than any other single contribution among the entire starch literature. They extended the investigation of Musculus of 1860, working with starch in the presence of diastase, or dilute sulphuric acid under varying conditions of time, temperature, etc., and recorded the formation of soluble starch, 1 erythro-dextrin, 3 achroodextrins, 1 dextrin in small amount which is unconvertible into sugar, maltose, and dextrose. The achroodextrins were found to differ in their rotatory powers and reducing coefficients. These authors advanced the theory that the starch-molecule is broken down through a number of well-defined stages by repeated hydrolysis; that at each stage there is produced coincidentally a form of dextrin together with maltose; that with each succeeding stage there is formed a dextrin of lower molecular weight than the preceding, and that ultimately there is present a small amount of unconvertible dextrin together with maltose and glucose (see page 122). These authors failed to give evidence that they had studied pure substances, and it was shortly afterward demonstrated by Brown and Morris (Ann. d. Chem. u. Pharm., 1885, XXI, 72) that the distinctive properties of the different achroodextrins, as stated by Musculus and Gruber, could be accounted for upon the assumption of variable mixtures of maltose and a single achroodextrin that had not reducing power, although they also held that a specific body exists in the form of maltodextrin (see page 124).

Since the investigations of Musculus, Griessmayer, Brücke, O'Sullivan, and Musculus and Gruber, a very voluminous literature has accumulated which treats, in a large measure, of soluble starch, amyloextrins, erythroextrins, achroodextrins, and the saccharine products; and also much bearing directly upon the constitution or configuration of the starch-molecule, together with sporadic and unimportant inquiries as to the differentiation of starches from different sources based upon differences in the products of digestion, etc.

NATURE OF THE CHEMICAL PROCESSES INVOLVED IN THE DEXTRINIZATION AND SACCHARIFICATION OF THE STARCH-MOLECULE.

From the time of the publication of the article by Musculus in 1860, in which it was held that the conversion of starch into dextrin and sugar is a process of hydrolysis, it seems to have been almost universally accepted that the mechanisms throughout the breaking down of raw starch into its final sugars and intermediate derivatives are those of repeated simple hydrolysis. This, however, is a convenient if not a justifiable assumption, as will be apparent by the context. There are a number of facts which seem to be opposed to it as an entirety, and which are to be harmonized before it can be accepted, as, for instance, the formation of dextrin by torrefaction, certain marked differences exhibited in the behavior of enzymes and inorganic decomposing agents, both as regards the initial substances acted upon and the final products of activity, the probable importance or necessity of oxygen, as is indicated by the investigations of Sacharow (Russki Wvratsch, 1904, No. 17; Biochem. Centralbl., 1904-5, III, 115) and Grüss (page 146) in enzymic action and by Wortmann (page 186) in bacterial digestion, and the practical identity of the behavior of raw starch and gelatine and other of the so-called organic colloids when placed in water.

Based upon the investigations of Musculus and Gruber, Neumeister (Lehrbuch d. physiologischen Chemie, 1893, Teil I, 32) proposed the following scheme, each step being one of hydrolysis:



Whether or not there may be as many, or more or less, intermediate and final steps and products is yet an open question, but in very recent years the tendency has been toward the acceptance of a simpler scheme, such as the following:



THE PROCESSES IN THE CONVERSION OF RAW STARCH INTO STARCH-PASTE (HYDRATED STARCH), AND INTO PSEUDO-SOLUTION AND TRUE SOLUTION.

While there can be no doubt of the essential part played by water in the swelling, gelatinization, pseudo-solution, and solution of starch, it seems clear that none of these phenomena is due either to hydrolysis (decomposition in which molecules of water are taken up and become an integral part of the molecules), or hydration in the strictly chemical sense (the formation of derivatives in which basic matter is substituted by hydrogen atoms of water, or the actual combination of water so that the molecules of water constitute intramolecular components of the derivatives). The terms hydrolysis and hydration are often used synonymously, but at times incorrectly, because while hydration may mean hydrolysis, it may on the other hand signify a combination with or impregnation of water which is an extramolecular and not an intramolecular phenomenon. According to the recent developments of physical chemistry, none of the processes concerned in the

conversion of raw starch into the so-called soluble starch, of which starch-paste and pseudo-solutions and true solutions are simple modifications, is one of hydrolysis or hydration in the strictly chemical sense, but of adsorption, that is, an extramolecular union with water that is of a physico-chemical character, such, for instance, as is observed in the deposition of moisture on glass and in the taking up of water by hygroscopic substances, in which there may be no true chemical union in the conventional meaning, but a mere surface combination—certainly no hydrolysis or splitting-up of molecules.

Starch-grains do not either gelatinize or pass into solution in their normal state, because apparently of the existence of some form of protective covering which prevents the water from reaching the starch-substance, but when the grains are crushed, or if the coating is otherwise injured, they take up water rapidly and become gelatinous; but if such grains are placed in water containing a sufficient quantity of some agent which hinders or prevents the surface-combination with water, such as alcohol, acetone, alcohol and ether, brine, etc., little or absolutely no swelling or solution occurs. Analogous phenomena have been observed with other so-called colloids, where it has been shown that swelling may be augmented or inhibited by the presence of various substances. Starch in common with organic colloidal substances is hygroscopic, and the so-called process of hydration or hydrolysis to form soluble starch or "hydrate of starch" is explicable on the basis of adsorption, that is, a physico-chemical affinity that is specific and selective, and supplemental to satisfied affinities according to the laws of stoichiometry. The water is conceived to enter into a physico-chemical combination, the energy of this combination or adsorption becoming more marked with an increase of temperature, and observed at its maximum when starch is heated in the autoclave to form a solution so perfect as to be separable by methods of filtration which prevent the passage of molecules in other than in true solution.

Raw starch gradually heated in water slowly takes up water until at a certain level of temperature adsorption occurs very actively, and the grains swell rapidly, the gelatinized capsules burst, and the whole disappears. Before any marked swelling occurs the starch-grain loses its anisotropy, thus showing an intermolecular disorganization, but this can not be regarded as a manifestation of hydrolysis or of the formation of a hydrate in the chemical sense any more than in the case of particles of gelatin, which also when dry are anisotropic or doubly refractive, but not after swelling. The combination is, of course, actually chemical, because all of the so-called physico-chemical reactions are technically chemical, but it is not chemical in the conventional sense any more than the solution of sugar in water is chemical and thus a hydrate is formed.

The striking analogies that have been shown in the behavior of starch, dextrin, gum, cellulose, gelatin, glue, and proteins by the results of the investigations of Hofmeister (*Archiv f. exp. Path. u. Pharm.*, 1890, xxvii, 395; 1891, xxviii, 210), Pauli (*Archiv f. ges. Physiologie*, 1897, lxxvii, 219; 1898, lxxi, 1; *Physical Chemistry in the Service of Medicine*, trans. by Fischer, 1907), Spiro (*Beit. z. chem. Physiologie*, 1904, v, 276), Ostwald (*Archiv f. ges. Physiologie*, 1905, cviii, 563), Fischer (*Œdema*, 1910), and others, render it clear that the explanation of the phenomena of swelling, gelatinization, pseudo-solution, and true solution, as determined by one of these substances, is in all essential respects applicable to all. If, therefore, the phenomena in the case of gelatin are explicable on a physico-chemical basis, the same will hold good for starch, and, therefore, the phenomena attending the so-called hydration of starch are those of adsorption. Moreover, not only are these parallelisms manifested in the behavior towards water and various aqueous solutions and in the reversibility of the taking up of water, but also in changes brought about in colloids by alterations in external conditions, every change leaving its effects temporarily or permanently on the colloid.

As regards the latter, reference may be made to the investigations of Syniewski (*Ann. d. Chem. u. Pharm.*, 1899, cccix, 282), who found that when a 5 per cent starch-paste

was heated in a closed vessel under a pressure of 3 or 4 atmospheres, the starch-substance is dissolved entirely. From the solution on cooling there separated a gelatinous mass that is insoluble in cold water and not acted upon by diastase. On heating, this mass is again dissolved. If this heating and cooling process be repeated a number of times, a stage is finally reached when not all of the gelatinous mass is redissolved on heating. Repeated heating and cooling has therefore altered the constitution of that portion of the starch which has thus been rendered insoluble.

The results of various experiments show that the molecular condition of colloids, such as gelatin, is very sensitive. For instance, Moore and Roaf (*Biochem. Jour.*, 1906, II, 34) found that a solution of gelatin kept for some time at 70° to 80°, and cooled to room temperature has its osmotic pressure considerably increased, which increase gradually disappears upon keeping the solution for some days. The explanation for these changes may lie in changes in the sizes of the molecular aggregates—heat reducing them and cold favoring their rebuilding. Pauli (*Physical Chemistry in the Service of Medicine*, trans. by Fischer, New York, 1907, 61) states that if a gelatin having a temperature which lies between the melting-point and gelation-point be carefully warmed back to the melting-point previously recorded, the gelatin remains *solid*. If, moreover, the gelatin is heated beyond the previously recorded melting-point, and is then carefully cooled to the starting temperature, it remains *liquid*. By repeated heating and cooling both gelatin and melting temperatures are therefore altered, showing that the gelatin has undergone changes in constitution. This is paralleled in starch. Furthermore, granules of dry gelatin behave towards water at different temperatures in all essential respects like crushed starch-grains, undergoing swelling, gradually liquefying, becoming limpid, becoming insoluble, etc. The conversion of raw starch into soluble starch can therefore be accounted for on physico-chemical grounds, while there is neither satisfactory evidence nor good reason for the assumption that there occurs a hydration in a strictly chemical sense.

#### THE PROCESS IN THE CONVERSION OF STARCH INTO DEXTRIN.

The conversion of starch into dextrin is likewise regarded as being due to hydrolysis, but this rests merely upon assumption. This conception had its origin in the early work of Musculus, in which it was recorded that dextrin and sugar are formed in definite ratio, and that the reaction could be worked out theoretically on the basis of the taking up of molecules of water which constitute an integral part of the product; but it has since been shown that such ratios are determined by the laws governing the equilibrium of solution, and not by the nature of the process, and that the chemical processes involved are merely incidental in determining the direction of the reaction, synthetic or analytic, in order to bring about this equilibrium. Dextrin is classed as a polysaccharose but of lower molecular weight than starch, and while there is no clear evidence whatsoever from the chemical point of view of its being a derivative that is formed through the agency of extra-molecular water, it is logical to assume that it may arise through a process of atomic rearrangement and splitting. If dextrin is a derivative of starch in the form of a hydrate, the molecules of water concerned in the reaction must, it seems, have an intramolecular source. Thus, in the formation of torrefaction dextrin it is justifiable to assume that starch must be absolutely dehydrated before the temperature is reached at which the conversion occurs; hence, the water taking part in the hydration must have been derived from intramolecular disorganization.

Furthermore, that dextrin is not formed conjointly in association with sugar by a process of hydrolysis by which water becomes a part of the product, and that it is formed entirely independently, has been rendered obvious by the results recorded by a number of investigators in studies of the products of the actions of enzymes, dilute acid, and other agents. As previously stated, Payen and Persoz (*Ann. de chim. et phys.*, 1834, LIII, 73)

believed that dextrin existed in the starch-grain and that it is converted into sugar. This view was accepted until Musculus (1860), as before noted, stated the existence of a definite ratio between the quantities of dextrin and sugar formed, and formulated the theory of the hydrolysis of starch directly into conjoint molecules of dextrin and sugar. This theory was opposed by a number of contemporaneous workers, but the later work of Musculus and his co-workers, and of Brown and Heron (Ann. d. Chem. u. Phar., 1879, cxcix, 241), and of a number of subsequent experimenters, set the seal of approval, so that it seems to have been accepted without much question, even up to the present, although the investigations of Lintner and Düll (Ber. d. d. chem. Gesellsch., 1893, xxvi, 2533) and others pointed strongly to the contrary, as will be referred to later. That dextrin may be formed without an attendant production of sugar has been shown by subjecting starch to torrefaction, to modified enzymes, and to the restricted actions of high moist heat, glycerine, sulphurous anhydride, etc. Such being the case, the assumption that it is a conjoint product with sugar by a process of hydrolysis is needless and untenable.

The conception of the disaggregation of starch into sugar by consecutive stages of hydrolysis had its origin, as stated, in misconceptions of Musculus, and it received support in the fact that in a related reaction the conversion of dextrin into sugar is owing to hydrolysis, and in the assumption that existed up to a very recent period that diastase, for instance, is an individual or unit substance having properties which are manifested serially in the liquefaction of starch, in the conversion of starch into dextrin, in the conversion of dextrin into maltose, and in the conversion of maltose into glucose, respectively, and, as a consequence, that if one of the processes is hydrolytic, the natural inference is that all must be. It has, however, been clearly demonstrated by the investigations of recent years that enzymes as ordinarily prepared are very commonly composites, each component enzyme exercising its own special properties, so that a product of one enzyme which may not be further altered by that enzyme may become an initial substance in relation to another.\*

The properties of diastase were shown by Bourquelot (Compt. rend., 1887, civ, 71, 177, 576) to be capable of modification in such a manner by the action of moist heat as to materially alter the character of the products of digestion, from which he was led to assume that diastase contains several enzymes which present unequal resistance to the injurious influence to heat. When saliva was heated to 70° its power of forming sugar fell about 90 per cent, and when heated to 71° dextrinization continued, but no sugar was formed. Experiments of later investigators also show that even though sugar formation ceases at the above temperature, dextrin formation continues. Emulsin after being heated, was shown by Jacobsen (Zeit. f. physiolog. Chem., 1892, xvi, 340) to be active in relation to amygdaline, but no longer in relation to hydrogen peroxide.

Duclaux (Traité de microbiologie generale, 11, Diastases, Toxines et Venins, 1899), from the results of his experiments, concluded that diastase consists of two enzymes, one of which (amylase) converts starch into dextrin, and the other (dextrinase) converts dextrin into sugar. Since that time much evidence has accumulated to show not only that enzymes as ordinarily prepared are generally composed of two or more distinct enzymes,

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\* Fischer and Lindner (Ber. d. d. chem. Gesellsch., 1895, xxviii, 984) showed that three enzymes are consecutively active in the conversion of raffinose (a trihexose) into glucose, fructose, and galactose. Buchner and Meisenheimer (Ber. d. d. chem. Gesellsch., 1904, xxxvii, 417) found that two enzymes are active in the alcoholic fermentation of sugar, one (zymase) converting sugar into lactic acid, and the other (lactacidase) converting lactic acid into alcohol and carbon dioxide. Bourquelet (Compt. rend., soc., biol., 1902, liv, 1140) and Bourquelot and Hérrisey (Compt. rend., 1902, liv, 399) in experiments with gentianose (a trihexose which consists of two molecules of glucose and one of levulose) found that two enzymes, invertase and emulsin, which exist in the juice of *Aspergillus*, split it into simple hexoses, one splitting off levulose and the other splitting the glucose. Armstrong and Horton (Proc. Roy. Soc., 1908, lxxx, 312) proved that emulsin of almond contains three enzymes: lactase,  $\beta$ -glucase, and a third enzyme. The enzymic conversion of maltose into glucose has been shown to be due to a special enzyme (glucase) found in variable quantities in the preparations of diastase, ptyalin, amylopsin, etc. (For the sources, actions, etc., of glucase, and for other references, see page 141.)

but also that serial actions may be or are due to independent actions of the several component enzymes. Duclaux's conclusions received support in the investigations of Pottevin (Compt. rend., 1898, cxxvi, 1218), who treated starch-paste with a malt extract that had been heated to 79° to 80° for 20 minutes, and found that the greater part of the starch goes over into dextrin without there being formed any reducing sugars. The starch-paste he prepared by heating 10 grams of starch in 1 liter of water heated to 90° for 30 minutes, and subsequently heated for the same length of time in the autoclave at 120°. The paste thus obtained was sufficiently transparent to permit of the determination of its rotatory power,  $(\alpha)_D = +197.6$ . (See under Dextrins, page 120.) Pottevin's results received confirmation in the investigations of Maquenne and Roux (Compt. rend., 1905, cxi, 1303), who converted amylopectin (see p. 112) by malt extract heated to 80° into dextrins, one of which was non-saccharifiable. The investigations of O'Sullivan (Jour. Chem. Soc. Trans., 1872, LVIII, 579), Petit (La bière et l'industrie de la brasserie, 1896, 179), and Windisch and Hasse (Woch. f. Brau., 1892, xix, 192) also give confirmation to Pottevin's results. Their studies show that heating diastase so alters its properties that as the temperature is higher the proportion of sugar formed falls rapidly, and that a point is soon reached at which dextrin continues to be formed, yet little or absolutely no sugar. When the enzyme is heated to 71° to 75°, saccharification ceases, but not dextrinization.

Schumann (Jour. Soc. Chem. Industry, 1888, vii, 335) and Berge (Jour. Soc. Chem. Industry, 1892, ii, 448; 1897, xvi, 548) prepared dextrin from starch by sulphur dioxide. Raw starch was set aside for 24 hours in 1 per cent acid. It was then collected and washed with fresh water until free from acid. This washed modified starch was reduced by water to a milk at 15° Baumé, and boiled under a pressure of 3 to 4 atmospheres with a 0.5 per cent of saturated sulphurous-acid solution until the first trace of glucose could be detected. The reaction was then stopped, the slight trace of sulphuric acid formed was fixed, and the syrup filtered through animal charcoal and evaporated. Berge made use of either gaseous or liquid sulphur dioxide. With the former a closed vessel is half filled with starch and gaseous sulphurous anhydride passed in until the air is displaced, when the exit-tube is closed and the temperature raised to 120° to 190°. When the conversion is complete the gas is allowed to escape. He notes that below 80° the action on starch is almost *nil*, but between that temperature and 115° soluble starch is formed. At 115° the soluble starch, in the absence of moisture, is converted into a very pure dextrin. Berge obtained a dextrin containing less than 1 per cent of sugar by heating dry potato starch with sulphur dioxide at 140° for 7 hours in a revolving cylinder. With sulphurous acid in solution the action is extremely slow below 45°, but at 100° saccharification to glucose begins and is complete between 135° and 140°. In the case of potato starch granule is rapidly converted, but the starch cellulose quite slowly. Schumann (Jour. Soc. Chem. Industry, 1889, viii, 295) prepared dextrin free from sugar by mixing starch to a thick cream with cold water and treating with 1 per cent of its weight of sulphuric, hydrochloric, or nitric acid. After 24 hours the starch is washed free from acid. This prepared starch is either dried or mixed with water to a cream, and heated to 160° or 170° on an oil-bath or by means of superheated steam.

Glycerine may be used to prepare sugar-free dextrins, as was done by Zulkowski (Ber. d. d. chem. Gesellsch., 1891, xxiii, 3295). Starch dissolved in hot glycerol is converted at first into soluble starch, but by continued heating it is completely broken down into erythro-dextrin, achroo-dextrin and a series of related bodies having increasingly greater solubility in alcohol as the decomposition proceeds.

It is obvious from the foregoing that dextrin and sugar formation are independent processes; that dextrin may be formed without an attendant production of sugar; that dextrin may be produced under conditions unfavorable to, or impossible for, the occurrence of a

process of hydration (at least such as is conceived to occur through enzymatic action), as, for instance, in the dextrinization of soluble starch at 115° in the apparent absolute absence of moisture, and in the production of dextrin by torrefaction. It will be shown in later pages that starch-paste may be liquefied, *i.e.*, converted into "soluble starch," without the formation of either dextrin or sugar, and that the paste may be converted into dextrin and sugar without liquefaction. In other words, the weight of evidence points unquestionably to the view of Lintner and Düll of a series of breaking-down processes, in which one substance is formed from another, in contradiction to the theory of Musculus, which holds that the starch-molecule is decomposed into dextrin-maltose molecules, each of which in turn is subsequently hydrated and split.

THE PROCESS IN THE CONVERSION OF ONE FORM OF DEXTRIN INTO ANOTHER AND INTO SUGAR.

The higher dextrans are supposed during these decomposition processes to be converted into lower dextrans, *i.e.*, dextrans of less molecular weight, of higher solubility in water, and of less solubility in alcohol. According to Lintner and Düll (Ber. d. d. chem. Gesellsch., 1893, xxvi, 2533), starch is a union of high molecular complexes which, under the influence of diastase, dilute acids, and certain other agents, is split up into high molecular components. The first to be formed is *amylo-dextrin* ("soluble starch"), and then in turn *erythro-dextrin*, *achroo-dextrin*, *maltose*, and *isomaltose*, all of the stages going on consecutively and together. Amylo-dextrin ( $C_{12}H_{20}O_{10}$ )<sub>54</sub> gives a dark-blue reaction with iodine. Each molecule of amylo-dextrin, according to them, is broken into 3 molecules of erythro-dextrin,  $(C_{12}H_{20}O_{10})_{18} + H_2O = (C_{12}H_{20}O_{10})_{17} \cdot C_{12}H_{22}O_{11}$ , which gives a reddish-brown reaction with iodine. Each molecule of erythro-dextrin is in turn hydrated into 3 molecules of achroo-dextrin  $(C_{12}H_{20}O_{10})_6 + H_2O = (C_{12}H_{20}O_{10})_5 \cdot C_{12}H_{22}O_{11}$ , which gives no iodine reaction. The molecules of achroo-dextrin are converted into isomaltose according to the following formula:  $9 [(C_{12}H_{22}O_{10})_5 \cdot C_{12}H_{22}O_{11}] + 45 H_2O = 54 C_{12}H_{22}O_{11}$  (isomaltose) = 54 maltose. They believe that both dextrin and starch consist of isomaltose groups. These authors isolated the several non-saccharine bodies by stopping diastatic activity at the necessary stage by boiling, and then repeatedly fractionating the product with alcohol. The progress of the reactions could be followed by the changes in the color-reactions with iodine. (For sugar products, see pages 129, 143, 144, 151, and 152.)

The stages of digestion and the chief products are presented in the following schema, which is closer in accord with the better investigations and most recent developments than one based upon the assumption of a coincident hydrolysis into dextrin-maltose and the splitting of these, and so on:

Stages.	Raw starch or starch-paste.	Reactions with iodine.
1. Starch-erythro-dextrin - achroo-dextrin - maltose-glucose	Soluble starch → Erythro-dextrin → Achroo-dextrin → Maltose → Glucose	Purple to a bluish violet.
2. Erythro-dextrin-achroo-dextrin-maltose-glucose	Erythro-dextrin → Achroo-dextrin → Maltose → Glucose	Reddish-violet, red, reddish-brown, or reddish-yellow.
3. Achroo-dextrin-maltose-glucose	Achroo-dextrin → Maltose → Glucose	No color reaction.
4. Maltose-glucose	Maltose → Glucose	No color reaction.

In this schema it will be observed that a serial action is assumed by which one product is formed after another, in contradistinction to the theory of the coincident formation of dextrin and maltose and the splitting of these molecules. The different stages are char-

acterized by the chief decomposition products present and by the reactions with iodine. No account has been taken of residue. The scheme is, of course, modifiable under different conditions of experiment. Thus, the final product may be maltose and not maltose and glucose, or glucose; and instead of maltose there may be isomaltose; and in some reactions saccharose and other sugars would be present, etc.; according to some investigators either or both amyloextrin and maltodextrin would be included, etc. The color reactions of the second stage will vary in accordance with the nature of the decomposing agent and other conditions.

SOLUBLE STARCH, REVERTED STARCH, COAGULATED STARCH, "ARTIFICIAL STARCH," AND  
"ARTIFICIAL STARCH-GRAINS."

SOLUBLE STARCH.

The term soluble starch has been used so indiscriminately that it not only does not designate any specific body, but it may also apply to a substance so close to raw starch as to give the typical *blue* reaction with iodine, or to one that is a mixture of starch and erythroextrin, or to a specific body that has properties of both starch and erythroextrin, or to solutions of dextrans or dextrin and sugar, etc., which are so far removed from starch as to give no color reaction with iodine. In other words, it has been used to signify the products of decomposition as they exist at any given stage of the process of saccharification from the first modification product to a mixture of achroodextrin, or to "malto-dextrin," in which there may not be a particle of starch, soluble or insoluble, present. Obviously the term soluble starch should be restricted to the first product which yields a blue reaction with iodine. Boiled starches are essentially pseudo-solutions which contain small and variable quantities of dissolved particles of starch, together with a relatively great mass of suspended particles which represent starch in many degrees of molecular condensation. Such pseudo-solutions may be rendered into *perfect solutions* in proportion to the extent of solution of the suspended particles, so that by appropriate procedures a *non-colloidal solution* of starch can be obtained which can be filtered through a colloidal membrane or a Chamberland filter. Pseudo-solutions, such as starch-paste or less dense preparations, can be converted into a clear limpid solution, or true soluble starch, by minute quantities of amylolytic enzymes, by small or large quantities of amylolytic enzymes that have been modified by subjection to a temperature of 75° to 80° for 10 to 15 minutes, by the actions of dilute acids and other chemical agents, and by heating in the autoclave at about 140°.

Starch-paste, starch pseudo-solutions, and starch non-colloidal solutions undergo, upon standing, or upon repeated heating and cooling, more or less *reversion* to insoluble forms of starch. This change becomes apparent at first as a turbidity, which grows more marked as condensation proceeds, and which ultimately may be observed in the form of granules which have the appearance and general properties of starch-grains. This reversion product has been termed *amylocellulose*, or *coagulated starch*, or *artificial starch*, or *artificial starch-grains*. There seem to be many forms of this reverted substance which differ in solubility, digestibility, reaction with iodine, etc. A similar reversion may be caused by a specific enzyme that has been named *coagulase*, but the reversion products are not absolutely identical with those formed spontaneously (see page 111).

Probably the earliest investigator to prepare soluble starch was Guibourt (Ann. de chim. et phys., 1828, XL, 183). This he did by pounding starch-grains in a mortar and placing in water. The rupture of the grains renders them partially soluble in cold water, giving rise to a gelatinous solution that yields a blue reaction with iodine. This observation has been repeated by many investigators, and while it has been held by some that the gelatinization is due to heat formed during the pulverization, this assumption has been totally disproved (page 28), and it has, moreover, been clearly shown by the records

of a number of observers, as already stated, that the degree of solubility is proportional to the degree of comminution of the grains, and that complete comminution is equivalent to complete gelatinization by heat in so far as the actions of digestive agents are concerned. Fritzsche (Ann. d. Physik. u. Chemie, 1834, xxxii, 129), in his study of the lamellæ of the starch-grain, associates the greater density of the outer layer with the insolubility of the raw grains in cold water, as he found that when the grains are crushed some of the inner starch is dissolved in cold water. The solubility of a part of the raw grain in cold water has been confirmed by Jessen, Delffs, Knop, and others of the earlier investigators (p. 28), and later by the investigations of Brown and Heron, Meyer, and Maquenne. Uninjured raw starch-grains are as insoluble *in vitro* in solutions of amylolytic enzymes as they are in cold water, provided the actions of bacteria are prevented. Brown and Heron (Ann. d. Chemie u. Phys., 1878, cxcix, 206) found that starch-grains which showed no change after a considerable time in the presence of malt extract were readily digested after they were crushed. Meyer (Die Stärkekörner, etc., *loc. cit.*) noted that perfect grains of the starch of the potato and of *Dieffenbachia seguina* are dissolved after a time from *within outwards* in successive layers, but grains having clefts or fissures were eroded with channels, pits, cavities, etc. Maquenne (Compt. rend., 1904, cxxxviii, 375) compared the intensities of the digestive actions of diastase and dilute acids on raw starch, crushed starch, and starch-paste. By means, for instance, of malt extract at 55° he found the following percentages of soluble matter produced: Raw starch-grains, 2.8; raw starch broken by trituration, 94.8; and starch-paste, 102.2, the percentages being calculated on the quantity of original starch.

The form of soluble starch that is obtained by simple solution of the comminuted raw grains is doubtless the nearest derivative of natural starch, but a similar or identical form may be prepared by the subjection of the grain to the temperature of complete gelatinization; or by a *slow* solution of raw starch by amylolytic enzymes, dilute acids, etc., avoiding the formation of various decomposition products that are formed during *rapid* action; or by the liquefaction of starch-paste by amylolytic enzymes, dilute acids, high temperatures, etc. The various products thus obtained are not to be regarded as being of uniform composition, as will be evident by the context, and they may be further individualized by modifications of certain of the methods of preparation.

The form of so-called soluble starch that is made by boiling starch in sufficient water to make a liquid preparation is so universally known as not to require special notice. Starch-paste can be converted into liquid soluble starch without the formation of dextrin by heating in the autoclave or by careful restriction of the actions of amylolytic enzymes, dilute acids, alkalies, etc., or by the use of modified enzymes. Syniewski (Ann. d. Chem. u. Phar., 1899, cccix, 282; 1902, cccxxiv, 212) placed a 5 per cent starch-paste made of potato starch in the autoclave, at a temperature of 140°, and obtained a clear solution which gave a blue reaction with iodine. This form of soluble starch is soluble in cold water and is without reducing action on copper solutions. He also prepared soluble starch by treating starch-paste for several minutes with malt extract. Lintner and Düll (Ber. d. d. chem. Gesellsch., 1892, xxvi, 2533) prepared soluble starch ("amylodextrin") by subjecting potato starch to the action of air-dried malt at a temperature of 70°, and arresting the action while iodine still yielded a blue coloration. Hot 40 per cent alcohol was added, and the soluble starch that separated upon cooling was finally purified by repeated fractionation with 30 to 40 per cent alcohol. Upon drying, a light white powder was obtained which was only slightly soluble in cold water, but very soluble in hot water. It yielded a blue reaction with iodine, but it did not reduce Fehling's solution.

Pottevin (Compt. rend., 1898, cxxvi, 1218) found that when malt extract is kept at 79° to 80° for 15 to 20 minutes it loses its power of converting starch into sugar, but retains its power of liquefying starch-paste and of forming dextrin. Petit (Compt. rend., 1905,

CXLI, 1247) discovered that malt infusions react toward tincture of guaiacum like solutions of compounds of iron or manganese. If the malt infusion yields only a faint guaiacum reaction, the addition of traces of alkali, or aeration and acidifying with lactic acid, causes it to give a full reaction. The preparation after this treatment has lost its power of saccharifying, but not its power of liquefying starch-paste. Solutions having the power of liquefying starch-paste at 20° were made by treating a solution of commercial albumin with ferrous and ferric oxides. It was also found that the starch-liquefying action could be considerably intensified by the addition of lactic acid or asparagin.

The liquefaction of starch-paste under pressure may be hindered by the presence of certain substances. Thus, Fernbach and Wolff (Compt. rend., 1906, cxliii, 380) found that while salts which are neutral to methyl orange are without effect, salts that are alkaline are inhibitory, and that traces of alkali may be absolutely preventive. Green (The Soluble Ferments, London, 1901, 31) records that the "diastase of secretion" corrodes starch-grains and disintegrates them before solution, and rapidly liquefies starch-paste, most advantageously at a temperature of 50° to 55°, and that it will withstand heating to 70° without destruction.

Ford (Jour. Soc. Chem. Industry, 1904, xxiii, 414) prepared soluble starch by previous treatment of the raw starch with dilute alkali and acid, repeatedly washing, and then drying in the air. This purified starch was gelatinized, then liquefied at 79° to 80° by means of a trace of diastase, and then the limpid solution boiled to destroy further diastatic action. Ford also prepared a soluble starch that was practically ash-free by repeated precipitation of an acidified solution of starch with alcohol, with the addition of a very small quantity of potassium or sodium chloride to prevent the starch from becoming milky, which results from the entire removal of acid or salts. Soluble starch prepared in this way is described as fairly pure and nearly neutral; that is, it does not give a reaction with rosolic acid or methyl orange, although somewhat acid to phenolphthaleine.

O'Sullivan, Brown and Heron, and many other investigators have prepared soluble starch by arresting diastatic action at the proper time.

A *non-colloidal* or *perfect solution of starch* was made by Fouard (Compt. rend., 1908, cxlvi, 285) by demineralizing the starch, and partial hydration at 80°. The preparation thus obtained can be filtered through a collodion membrane, a 5 per cent solution of colloidal starch yielding in this way a 2.74 per cent non-colloidal solution. Fouard states that this non-colloidal solution shows not a trace of polarization; that it gives an intensely blue reaction with iodine, appearing as a solution rather than as a suspended precipitate; that its viscosity is about that of a 1 per cent solution of sugar, and, therefore, nearly that of water; that it is unstable, becoming cloudy and forming a granular deposit; and that it is probably a heterogeneous system containing dissolved molecules together with starch-molecules in all degrees of condensation. In a subsequent study, Fouard (Compt. rend., 1908, cxlvi, 978) found by ultra-microscopic examination that this non-colloidal solution stands intermediate between mineral colloids of insoluble elements and a perfectly dissociated salt-solution. Freezing causes a slight opalescence preceding the formation of granules. When a 5 per cent pseudo-solution is filtered through several collodion membranes which differ in their density, by varying the proportions of alcohol the filtrate contains increasing proportions of colloidal starch, ranging from 1.518 to 2.365 per cent, and having an increasing rotatory power  $(\alpha)_D =$  from 183° 15' to 191° 50', showing that the membrane acts as an analyzer. The state of perfect solution is changed by dilution or evaporation *in vacuo*. The water therefore not only acts as a solvent, but also is a necessary factor in modifying the state of the starch. During the granular formation the solution shows a very slight acidity, such as might be caused by completely dissociated acid phosphate; and the electric conductivity slowly increases, corresponding to the formation of the granules and the liberation of mineral ions.

In another inquiry, Fouard (Compt. rend., 1908, CXLVII, 931) investigated the alterations which occur in the pseudo-solution upon standing. A neutral pseudo-solution containing 54.3 grams of starch per liter at 15° was studied by removing portions at definite intervals ranging from a few hours to many days, filtering through collodion membranes, and determining the amounts of solid matter and the specific rotatory powers. The preparations, he found, undergo spontaneous gelatinization, and the process goes on during a period of several months. The results of these experiments, Fouard records, confirms his former view of starch existing in different states of molecular aggregation, each state having a definite and specific rotatory power. During the process of gelatinization the collodion membrane transmits these different aggregates in definite order, the first to be checked being the most active polariscopically. The results of this series of experiments are shown in table 5.

The rotatory power of the filtrates remained constant. In another series of experiments a similar pseudo-solution kept at 20° was filtered during a period of 126 hours, and samples taken after the lapse of 6, 30, 78, and 126 hours, respectively, with the results shown in table 6.

The residual colloidal starch gelatinized on the filter during the sixth day, owing to concentration of the solution, causing the amount of starch in the filtrate to fall rapidly. In another inquiry, Fouard (Compt. rend., 1909, CXLVII, 502) found that the specific rotatory power of true solution of starch was reduced by the addition of small quantities of potassium hydrate, the larger the quantity of alkali the greater the effect, the rotatory power falling as low as  $(a)_D = 141$ . Neutralization instantly reverses the modification. He made further observations on the fractions obtained from pseudo-solutions at different stages of spontaneous gelatinization, and noted that as gelatinization advances the specific rotatory power of the dissolved starch falls towards the level of the rotatory power of maltose. He believes that the solution of starch, whether by water or by the action of potassium hydrate, depends upon a reversible hydrolysis, with maltose as its ultimate term. Therefore, starch is simply a molecular aggregate of maltose in variable and unknown form. He concludes that no chemical combination takes place between the starch and alkali, that starch has not acid functions, and that the action is essentially ionic. The presence of perfectly soluble starch in solution lowered the conductivity of the solution, but to a much less extent in the presence of ammonia than potassium. Before coagulation occurs, association of the starch with metallic ions is believed by him to take place.

The use of dilute acids to cause a reduction of starch-grain or starch-paste to soluble starch, or to render raw starch more soluble in cold water, was adopted by a number of the earlier investigators, but it is probable that Béchamp (Compt. rend., 1854, XXXIX, 653; 1856, XLII, 1210) was the first to use this method to obtain soluble starch, which substance he regarded as a special body occurring between common starch and dextrin. Since that time this method, and also various modifications of it, have been in continuous use, and no one, it seems, has so popularized it as Lintner (Jour. f. prakt. Chemie, 1886, XXXIV, 378), as is indicated by the comparatively very frequent references to "Lintner's soluble starch." His method is given as follows: Mix some good potato starch with enough 7.5 per cent hydrochloric acid to cover the starch. Allow this to stand at ordinary room temperature for 7 days, or for 3 days at 40°, when the starch will have lost its gelatinizing property. Decant with cold water until sensitive litmus paper shows no acid reaction,

TABLE 5.

Duration of change in days.	Grams of extract per 1000.	Specific rotatory power.
6	27.44	188°
2.9	21.65	184.1
7.7	17.72	182.45
16.9	14.8	181.3
29.0	13.48	180.5
48.7	12.25	180.24
98	11.43	178.2
174	11.04	178

TABLE 6.

Number of hours of filtering.	Grams of extract per 1000.	Specific rotatory power.
6	27.48	188°
30	29.61	186.3
78	32.13	184.15
126	35.5	184

and dry the starch in the air. This gives a preparation that is readily soluble in hot water, yielding a clear solution. A 2 per cent solution will remain clear or slightly opalescent for two days, after which it will become cloudy, but if no further change takes place this will not interfere with its use for diastatic experiments. A concentrated (10 per cent) solution on cooling becomes salve-like. The solution will reduce only a minute quantity of Fehling's solution, so that this need not enter into consideration in using the starch for experimental purposes. Ten per cent hydrochloric acid will immediately turn starch into soluble starch, differing in this respect from sulphuric acid, for if the latter be employed it will be necessary to use at least a 15 per cent solution and to conduct the experiment at 40°.

Brown and Morris (Jour. Chem. Soc. Trans., 1889, LV, 449) subjected potato starch to the action of 7.5 to 12 per cent hydrochloric acid. With the former, the power of gelatinization was lost in 10 days; with the latter within 24 hours. This alteration is not accompanied by the least change of structure of the starch-grain or in the behavior of the starch-grain in polarized light. In hot water (60° to 70°) the grains form a perfectly limpid solution, and from the solution there separates, on cooling, a substance in the form of a white, flocculent, amorphous precipitate which has all of the properties of soluble starch prepared by the limited action of diastase or dilute acids on starch-paste at elevated temperatures. Both in solution and in the solid state this soluble starch is colored intensely blue in the presence of iodine. In solution it is without reducing power on copper solutions, and it has a specific rotatory power ( $\alpha$ )<sub>D</sub> 3.86 = +216. Even after 20 days the microscopic form of the granules is retained, notwithstanding the profound modification in the starch.

Lintner's method was modified by Lake (Jour. Soc. Chem. Industry, 1894, XIII, 264) by centrifugalization of the starch-hydrochloric acid preparation. The starch is placed in an equal volume of 5 to 10 per cent hydrochloric acid and centrifugalized, the action of the centrifuge causing a very intimate penetration of the starch by the acid. The acid is removed by washing with cold water and with dilute solutions of alkaline carbonates, after which the preparation is centrifugalized, and the starch collected and dried at a temperature of 20° to 40°. Starch prepared in this way dissolves in hot water without gelatinization. The process may also be carried out with nitric, sulphuric, phosphoric, oxalic, lactic, acetic, and other liquid or volatile acids.

Virneisel (Jour. Soc. Chem. Industry, 1899, XVIII, 697) digested starch in a 1 to 2 per cent solution of mineral or organic acid, preferably sulphuric acid, at a temperature of 50° to 55° for about 12 to 15 hours. Dextrin and sugar were not formed.

Blumer (Jour. Soc. Chem. Industry, 1903, XXII, 310) slowly heated starch in a 1 per cent solution of volatile acid for 5 to 6 hours at 115°, the acid afterwards being removed by distillation.

The foregoing processes were modified by Cross (Jour. Soc. Chem. Industry, 1903, XXII, 1008) by heating the starch in a monocarboxylic acid with or without a dehydrating agent, such as alcohol or a concentrated solution of neutral salt. For instance, starch dried at 100° is intimately mixed with one-third to one-half of its weight of glacial acetic acid, heated in a steam-jacketed vessel for 1 to 2 hours, and the starch then freed from acid and dried. Formic or lactic acid may be employed, but preferably in the presence of a dehydrating agent to prevent gelatinization.

Similar procedures were adopted by Wotherspoon (Jour. Soc. Chem. Industry, 1904, XXIII, 29). For instance, dried starch is treated with glacial acetic acid to the extent of 10 to 50 per cent of its weight, and the preparation heated in a closed steam-jacketed converter until the starch is soluble in hot water. If an aqueous acid be employed, an inert dehydrating agent may be used, such as alcohol or brine, to prevent gelatinization. The acid may be distilled off.

Tanret (*Compt. rend.*, 1909, cXLVII, 1775) treated raw starch for 30 minutes with a cold 1 : 1000 hydrochloric acid, washing well with water, drying at 30° until the per cent of water was reduced to 17, then heating at 100° to 110° for 1 hour in a closed vessel. This solution was extracted with 25 per cent alcohol, which dissolved out about half of the starch. About one-fourth of the residue was soluble, and it had a rotatory power  $(a)_D = +188.6^\circ$ . Upon the addition of 95 per cent alcohol to an alcoholic solution, precipitation occurred. The precipitate was washed with absolute alcohol, dried first over sulphuric acid, and then at 100°. The product was slightly soluble in boiling water, and had the following rotatory power:  $(a)_D = +208^\circ$  to  $210^\circ$ . The insoluble portion gave an insoluble blue compound with iodine and resembled the granular amylose of Maquenne and Roux. The residue obtained by evaporating the alcoholic solution gave reddish-violet and red reactions with iodine. (See Dextrins, page 120.)

Welwart (*Chem. Zeit.*, 1907, xxxi, 126) states that the easiest means of making soluble starch is to boil a thin paste in a chamber with formic acid, which produces a more limpid preparation than acetic acid. The acid is subsequently expelled by boiling. Soluble starch may also be prepared by the action of sulphur dioxide at a temperature of 80° to 115°, as was done, for instance, by Berge (*Bull. Assoc. Belge d. Chimistes*, 1897, x, 444). He found that in the presence of either gaseous or liquid sulphur dioxide soluble starch is produced at the above temperature, while at higher temperatures dextrin and sugar may be formed (see pages 128 and 131).

Skraup (*Ber. d. d. chem. Gesellsch.*, 1899, xxxii, 2413) obtained by gentle acetylation of starch a derivative which on saponification yielded a substance which resembled soluble starch, giving a blue reaction with iodine. Chlorine or chlorine-water were used by Siemens and Halske (*Jour. Soc. Chem. Industry*, 1898, xvii, 257). By this method the starch is made into a sludge and introduced into a vessel provided with stirring machinery. After raising the temperature to 45° the starch is treated with chlorine or chlorine-water, the product is then washed with water, and dried in the usual manner. The action of chlorine leads to a partial rupture of the coats of the starch-grain, and the halogen enters the grain and attacks bodies which give to raw and bleached commercial starches an ethereal odor, which are subsequently removed by washing with water.

The chlorine process was modified by Hartwig (*Jour. Soc. Chem. Industry*, 1905, xxiv, 1024), who subjected raw starch, especially corn starch, at a temperature of 50° to 86°, to the action of an excess of chlorine for 4 to 8 days, or as long as necessary to render the starch perfectly soluble in hot water.

Gatin-Gruzewska and Maquenne (*Compt. rend.*, 1908, cXLVI, 540) prepared soluble starch by adding to a 3 per cent starch-paste one-fourth its volume of a hot 40 per cent solution of potassium or sodium carbonate, and treating with one-third its volume of alcohol. A precipitate of "amylopectin" (see page 113) is thrown down. The filtrate contains a quantity of starch in solution. Or, 10 grams of potato starch are placed in 500 c.c. of a 1 per cent solution of sodium carbonate, sufficient water is added to make 1 liter, the preparation is neutralized with acetic acid, an equal volume of water added, and the whole set aside for 24 hours. The filtrate contains starch in solution and stains blue with iodine. Or, if starch be boiled with a concentrated solution of sodium citrate or sulphate, and the mixture be filtered, the filtrate will be found to be rich in soluble starch.

Wolff (*Jour. Soc. Chem. Industry*, 1906, xxv, 437) treated starch at the ordinary temperature for an hour and a half in double its weight of a solution consisting of 2 to 4 parts per 1000 of potassium bichromate and about 15 per cent of sulphuric acid. The starch is then washed until the excess of acid is removed, and then dried at a temperature of about 30°. The bichromate can be replaced by half its weight of permanganate. The starch thus prepared forms a paste which, when heated in the presence of traces of basic

matter, as, for instance, tap-water, becomes fluid and transparent. The liquefied paste gradually gelatinizes when cooled, but is readily liquefied by heating. If the prepared starch be washed in distilled water instead of tap-water containing traces of basic substances, and dried at 30°, it may be converted into soluble starch by heating in the dry state at 80° to 100° for a few hours.

Starch may be liquefied by boiling in a weak solution of aluminum chloride (Jour. Soc. Chem. Industry, 1901, xx, 492). The starch is boiled in the solution of aluminum chloride in a similar manner to that followed when dilute acids are employed, and the aluminum may be separated by the subsequent addition of sodium silicate. The use of aluminum chloride prevents the extraction of objectionable nitrogenous matter from the grains. Hydrogen peroxide and ammonia were used by von Asboth (Chem. Zeit., 1892, xvi, 725) to prepare a soluble starch, which he called amyloextrin. This could be precipitated by alcohol, and it was found to represent about 80 per cent of the original starch. Various other familiar oxidizing agents have been employed by different investigators, especially permanganate of potassium.

Permanganate of potassium was used by von Siemens and Witt (Jour. Soc. Chem. Industry, 1896, xv, 366). The starch is stirred in water and a saturated solution of permanganate is added until the pink color of the latter in the liquid persists. Manganese peroxide is deposited in state of extreme division on the grains, which assume a brown color. The starch is well washed, treated with a dilute solution of hydrochloric acid of a 0.5 per cent strength upward, according to the nature of the starch, then treated with alkali, and as soon as it gives a perfectly clear solution it is filtered off and freed from the manganese salt by sulphurous acid or bisulphite, then washed and dried.

A number of oxidizing agents, including bleaching powder, chloric acid, magnesium dioxide, chromic acid, and potassium permanganate were used by Schmerber (Bull. Soc. Ind. Mulhouse, 1896, 238) to liquefy starch, but the last substance was found to give the best results. Equal quantities of starch and water are mixed thoroughly until the starch is in suspension, to which is then added one-fiftieth of the weight of starch of a warm 40 per cent solution of permanganate. The mixture becomes at first a reddish violet, turning to a dark brown. After 24 hours, to a solution consisting of about one-twentieth the weight of starch there is added hydrochloric acid in 5 volumes of water at intervals during constant stirring until the starch is decolorized, after which the starch is washed repeatedly to remove the acid and manganous chloride, and dried. The starch thus prepared yields a solution that is limpid and transparent. On standing the solution becomes cloudy, which cloudiness disappears upon heating. Schmerber's results were confirmed by Dollfus and Scheurer (Bull. Soc. Ind. Mulhouse, 1896, 241).

Fernbaeh and Wolff (Seventh Internat. Congress Appl. Chem., London, 1909) liquefied starch to the limpidity of water in 15 minutes by subjecting a 5 per cent starch-paste with a few drops of hydrogen peroxide and ammonia at a temperature of 70° to 75°. To each 5 c.c. of paste was added an amount of hydrogen peroxide equal to 0.005 gram of oxygen, and 0.004 gram of ammonia, but an excess of alkali was not found to be harmful, and it noted that other alkalis may be substituted. Weak acidity retarded liquefaction. Other liquefying substances were studied, especially the sulphates of iron and copper. In the liquefaction in the presence of ammonia some product in very small quantity is formed which partially neutralizes the ammonia. With a relatively large amount of hydrogen peroxide together with ferrous sulphate and sodium hydroxide the quantity of unreduced starch was very small, while with hydrogen peroxide alone a large quantity of unreduced starch remains. By subjecting 25 c.c. of the paste with 0.001 gram of ferrous sulphate, 0.042 gram of oxygen in the form of hydrogen peroxide, and 0.0006 gram of sodium hydroxide for 2 hours at a temperature of 75° to 78°, the preparation no longer gave a color reaction with iodine, and it had acquired an acid reaction and a high reducing power. The product

did not form an osazone, and therefore it was not regarded as being saccharine. The reducing substance differed essentially from dextrin because of its being precipitated from concentrated solution by copper sulphate, and therefore more like a gum.

Thompson (*Jour. Soc. Chem. Industry*, 1904, xxiii, 29) treated starch in a neutral, alkaline or acid medium, preferably at high temperature, with more permanganate than necessary to oxidize all of the extractive impurities, the treatment being continued until all the starch was converted into a soluble form.

Wolff and Roux (*Compt. rend.*, 1905, cxli, 1046) modified the permanganate process; they also used bichromate and chlorine instead of permanganate, and they extended our knowledge of the properties of soluble starch. Starch was mixed with twice its weight of a 1 per cent solution of potassium permanganate containing 10 to 15 per cent of sulphuric acid, or 6 to 7 per cent of hydrochloric acid. The liquid becomes decolorized in about 2 hours, when the starch is filtered off, washed, and dried at 30°. A 5 per cent paste made with distilled water is little less viscid than ordinary paste, and generally behaves like ordinary starch-paste, but if treated at 70° in the presence of traces of basic substances such as ammonia, or hydroxides, or carbonates of alkalies or alkaline earths, etc., there occurs immediate liquefaction. This liquefying action takes place very slowly at ordinary temperatures, it is at its maximum at 70° to 75°, and it is somewhat different from the liquefying action of malt because of its persisting above a temperature of 80°. The hot solutions of this liquefied starch gelatinize as the temperature falls, and become a jelly at ordinary temperatures, and again pass into a limpid solution upon heating.

Unless the action of the permanganate is checked the starch is gradually broken down. Leitner (*Zeitsch. f. angew. Chemie*, 1890, 546) followed the progress of the actions by testing the preparation from time to time with iodine in the manner pursued when diastase is used. He recorded that the color reaction, which at first is blue, changes to violet, red-violet, red, and reddish-brown, which becomes weakened, until finally there is an absence of color response. The products, he states, are different from ordinary dextrans, as is shown by their forming precipitates with basic lead acetate and barium hydroxide, and by their acid reaction. They have a slight reducing power, and upon boiling expel carbon dioxide from carbonates.

Glycerol breaks down starch into soluble starch and, if the action is continued or is carried on at a higher temperature, dextrans and related bodies are formed. Zulkowsky (*Ber. d. d. chem. Gesellsch.*, 1891, xxiii, 3295) found that starch heated in glycerine is first changed to soluble starch, and that as the temperature rises erythrodextrin and then achroodextrin are formed, together with a series of bodies which are increasingly soluble in alcohol and which could be separated by means of the differences in solubility. Difficulty was met with in removing the last traces of glycerine, owing probably to the formation of glycerides. This research was supplemented by another by the same author in conjunction with Franz (*Chem. Centralbl.*, 1894, ii, 918; 1895, iii, 557), who studied somewhat in detail the varying products formed at different temperatures. At 190° soluble starch is produced which gives a deep-blue reaction with iodine and has a specific rotation of +188.3°. This starch is precipitated by alcohol, lime-water, or baryta-water. The alcohol precipitate, after drying and upon keeping, is gradually converted into the insoluble form. When kept in strong solution it gradually gelatinizes and becomes insoluble. At 200° erythrodextrin is formed, and at 210° achroodextrin. When the preparation is subjected to prolonged heating related carbohydrates are produced, one resembling gum arabic. Pregl (*Monatsch. f. Chem.*, 1901, xxii, 1049) used 10 times the volume of glycerine and purified the product by filtering the solution into 60 per cent alcohol, collecting and drying the precipitate. The specific rotatory power of the aqueous solution was found to be  $(\alpha)_D = +191.26^\circ$ . From this preparation he made and studied acetyl derivatives.

Iodine was employed by Rodenwald and Kattein (*Sitzungsber. d. Berliner Akad.*, 1899, xxxiii-xxxiv, 628), who heated starch in Lugol's solution in the autoclave for 15 minutes at 130°. A greenish-brown liquid is formed, which as stated consists essentially of iodide of starch and some sugar. The starch iodide, they found, can be obtained pure by dialysis and filtration. The quantity of iodine in combination was ascertained to be constant at 14.3 to 14.8 per cent. The starch iodide is decomposed by heating, and as the iodine is freed it is removed by a current of steam, leaving a clear solution. On cooling, granules separate which give a blue reaction with iodine, and when dried the granules are insoluble in cold water, but form a paste on boiling, and swell in potassium hydrate. Some of the grains measured 0.02 mm. in diameter.

Formaldehyde has a marked decomposing action on starch. Syniewski (*Ann. d. Chem. u. Pharm.* 1902, cccxxiv, 201; *Jahr. ü. d. Fort. d. Tierchemie*, 1902, xxxii, 100) put potato starch in 5 times its weight of 40 per cent formaldehyde, and at the end of two months the starch was found to be completely dissolved. The solution was opalescent, but showed no iodine reaction. Upon evaporation he obtained a homogeneous mass consisting of trioxymethylen and a crystalline condensation product. The addition of water caused a gradual disintegration of the latter, as was indicated by changes through a scale of colors from brown to red and violet and blue, owing to the separation of the formaldehyde. This cleavage could be accelerated by the presence of acid. The product present which gives the blue reaction with iodine he regards as amyloextrin or a body closely related to it. He notes that amyloextrin otherwise prepared is dissolved at once in formaldehyde, with a transition of colors the reverse of that which is observed when the formaldehyde combination is broken up; and the reverse play of colors is also found to occur when the solution is diluted. The amyloextrin thus prepared undergoes crystallization upon evaporation of the solution. Under the microscope, stars composed of needles were first seen, and these were surrounded by a substance arranged in radial concentric layers. These bodies were deceptively like starch-grains, and under the polariscope the similarity was further shown by the double refraction and the interference figure. This amyloextrin was found to form a definite dark-blue iodine compound.

Reichard (*Zeit. ges. Brauw.*, 1908, xxxi, 161) confirmed Syniewski's views regarding the formation of a formaldehyde-amyloextrin compound by the action of 40 per cent formaldehyde at ordinary temperature. He showed that at higher temperatures the process goes on very rapidly, and that the temperature of gelatinization is reduced with an increase in the concentration of the formaldehyde. At 15° to 16° a 38 per cent solution of formaldehyde gelatinizes the starch completely in two days. At 25° 1 gram of starch in 10 c.c. of the same solution of formaldehyde was completely gelatinized in 7 to 8 hours. The action is less rapid if the formaldehyde is neutralized.

Potassium hydrate was used by Béchamp (*Compt. rend.*, 1854, xxxix, 653) to dissolve starch, and it has since been used by a large number of investigators and is one of the most valued agents when pure soluble starch is desired, because it, unlike dilute acids, diastase, glycerol, permanganate, and most other agents, does not give rise to dextrin or to any reducing substance; in other words, with a weak solution of alkali the decomposition of starch ceases at the stage of liquefaction. Tollens (*Nachricht d. k. Gesellsch. d. Wissensch.*, etc., Göttingen, 1873, 762) also used potassium as well as sodium hydroxide to prepare soluble starch.

Wróblewski (*Ber. d. d. chem. Gesellsch.*, 1897, xxx, 218) prepared soluble starch in the following way: 100 grams of rice starch are rubbed with a small quantity of a 1 per cent solution of potassium hydrate, and set aside for 2 to 4 hours, then more of the solution is added until the whole has attained a volume of 600 to 800 c.c. This jelly-mass is heated on a water-bath, stirred until fluid, and then boiled for 20 to 30 minutes, filtered, acetic acid added to slight acidity, precipitated with alcohol, dissolved and precipitated, redis-

solved in a small quantity of water, poured into a large quantity of alcohol under constant stirring, the precipitate washed in absolute alcohol and ether, and finally dried *in vacuo*. This method yields a snow-white preparation which contains little ash. It gives an intense blue reaction with iodine; it does not reduce Fehling's solution; and it is soluble in water to the extent of about 4 per cent. By the prolonged boiling of a dilute solution a small amount of reducing substance is formed. If a strong solution of alkali is used, as by Bülow (Archiv f. d. ges. Physiologie, 1895, LXII, 131), decomposition continues beyond the starch stage.

Wróblewski (Chem. Zeit., 1898, XXII, 375) reported a process for preparing a soluble starch by means of caustic potash that can be filtered through porcelain. On a small scale, the soluble starch can be prepared by rubbing 20 grams of starch in a mortar with 100 c.c. of cold water, pouring this into a 2-liter flask, adding about 1 liter of 0.5 per cent potassium hydrate, and boiling under an inverted condenser for 1½ to 2 hours until it becomes limpid and of a pale-yellow color. The preparation is neutralized with acetic acid, precipitated with an equal volume of alcohol, filtered, and filtrate washed successively with 50 per cent, 95 per cent, and absolute alcohol, and finally with ether, and then dried *in vacuo*. The snow-white powder thus obtained is soluble to the extent of about 3 per cent in water; and it contains about 0.4 to 0.6 per cent of ash. The less the ash the stronger the alcohol necessary to cause precipitation.

The caustic-alkali processes for producing soluble starch were modified by Kantorwicz (Jour. Soc. Chem. Industry, 1904, XXII, 1038; 1905, XXIV, 144) by the introduction of alcohol, acetone, mixtures of alcohol and ether, etc., into the process to prevent the gelatinization of the starch while it is being rendered soluble by the action of the alkali. The starch is mixed with 50 to 90 per cent alcohol, and to this is added two-fifths the weight of the starch of a solution of caustic soda at 30° Baumé. The mass thickens, and is set aside for an hour, then neutralized with acetic acid, and the resulting precipitate filtered off and dried. One part of the dried product with 10 parts of water yields a viscous, liquid-like paste.

Both oxidizing agents and caustic alkalis have, as shown, been found valuable in rendering starch into a soluble form. Syniewski (Ber. d. d. chem. Gesellsch., 1897, xxx, 2415; 1898, xxxi, 1791) combined both by using sodium peroxide. 50 grams of sodium peroxide were dissolved in 500 grams of water and rubbed up with 50 grams of starch in 500 grams of water, and set aside for an hour, after which the preparation was precipitated with 95 per cent alcohol, the precipitate dissolved in water, neutralized with acetic acid, and again precipitated with alcohol. By repeated solution and precipitation a snow-white powder was obtained which is very soluble in hot water, and soluble to the extent of 12.5 per cent in water at ordinary temperature. It is colored blue with iodine, does not reduce Fehling's solution, and has a rotatory power at 20° of +182.66° for a 2.5 per cent solution and +189.5° for a solution of 12.5 per cent. In his second article he gives the rotatory power of a 10 per cent solution as  $(\alpha)_{D_{120}} = +195.3^\circ$ . Solutions containing more than 12.5 per cent could not be read in the polarimeter because of the deposition of a white precipitate, which precipitate, by analysis, he found to be formed from the soluble starch, presumably by dehydration, and therefore a reversion product. This substance was also formed in solutions which gelatinized upon cooling, thus giving rise to a gelatinous mass identical with ordinary starch-paste. He describes the reactions with baryta-water, acetylchloride, and benzolchloride, and also the invertive action of water under pressure, and of alcohol, and diastase.

A form of starch which possesses all of the properties of starch gelatinized at high temperatures may be prepared by treatment with ammonium thiocyanate, according to the process of the Arabel Manufacturing Co. (Jour. Soc. Chem. Ind., 1909, xxviii, 213). 100 parts of dry starch are mixed with 80 parts of a 50 per cent aqueous solution of this salt, to which 40 parts of alcohol are added. The product swells in cold water to form a paste. The thiocyanate can be removed by washing with alcohol, acetone, etc.

Soluble starch has also been prepared by the agency of sodium perborate (Stolle and Kopke, and Fritsche, Chem. Abstracts, Amer. Chem. Soc., 1909, III, 1105), by using 1 to 2 parts to 100 of starch and heating at 30° to 40° for 5 hours. It may also be made in like manner by using a mixture of acetic and nitric acids instead of perborate.

THE REVERSION OF STARCH-PASTE, SOLUBLE STARCH, AND AMYLODEXTRIN INTO COAGULATED AND INSOLUBLE FORMS OF STARCH. "ARTIFICIAL STARCH" AND "ARTIFICIAL STARCH-GRAINS."

The reversion of soluble starch to an insoluble condition was noted by Brown and Heron (Ann. d. Chem. u. Phar., 1879, CLXXXIX, 266), who found that starch-paste upon standing becomes opaque, owing to the formation of an insoluble form of starch. Salomon (Jour. f. prakt. Chemie, 1883, XXVIII, 82) observed the same phenomenon in preparations of what he terms soluble starch (page 115). After precipitating and purifying, dissolving, and reducing to the consistence of a syrup, and setting aside over night, there was deposited a white powder consisting of fine grains almost insoluble in cold water. A large part of the so-called soluble starch separated from a concentrated solution as a white powder. (See page 192.)

The phenomena of the reversion of dissolved starch into an insoluble form were investigated particularly by Rodenwald and Kattein, Wolff and Fernbach, Maquenne, Roux, and Coombes. Rodenwald and Kattein (Sitzungsber. Kgl., pr. Akad. Wiss., Berlin, 1899, XXIV, 628) prepared a form of soluble starch by means of iodine-potassium iodide in conjunction with the autoclave, etc. (see page 109), which when hot is clear, or at most faintly turbid, and which upon cooling deposits a white substance having the form of "artificial starch-grains." Both the filtrate and these artificial grains give the blue reaction with iodine. The grains when dried are insoluble in cold water, but are gelatinized upon boiling, and swell and form a paste in the presence of potassium hydrate.

Wolff and Fernbach (Compt. rend., 1903, CXXXVII, 718) coagulated solutions of starch by means of *amylocoagulase*, an enzyme which they discovered in the green seeds of cereals and associated with amylase in a large number of ripe seeds, germinating seeds, leaves, etc. They found that 5 c.c. of a 10 per cent infusion of air-dried malt is sufficiently strong to coagulate 100 c.c. of a 4 to 4.5 per cent solution of potato starch (prepared in the autoclave at 130° for 2 hours) in 20 to 30 minutes at 15° to 25°. The stronger the solution of starch the quicker the coagulation and the denser and more coherent the mass. With weak solutions the action of the amylocoagulase is hindered or prevented by the antagonistic action of diastase. The coagulum never exceeds 30 per cent of the starch present, and it is readily soluble in hot water. Malt extract loses its coagulating property if subjected to a temperature of 65° for five minutes. A solution of starch prepared by the restricted action of diastase was found to be not so suitable for coagulation as that prepared in the autoclave. Moreover, the reverted starches formed from the soluble starches prepared in these two ways are not identical.

The coagulum, or reversion product, or *amylocellulose*, as it has been termed, was further studied by Maquenne, Fernbach, and Wolff (Compt. Rend., 1904, CXXXVIII, 49), who found that the formation of amylocellulose in the coagulum is more rapid when prepared by the action of amylocoagulase on starch-paste heated at 120° for 15 minutes, or at 100° for 30 minutes, or liquefied by heating in the autoclave at 130° for 2 hours, than when amylocellulose is formed during the spontaneous reversion of starch-paste. The coagulum immediately after its formation contains very little amylocellulose, but the latter increases and may reach 50 per cent of the total coagulum. The amylocellulose obtained by the reversion of starch-paste is saccharifiable by diastase, but not so with that formed by the action of amylocoagulase.

Additional observations were recorded by Fernbach and Wolff (Jour. Fed. Inst. Brewing, 1904, x, 216) regarding the properties of amylocoagulase. They note again the antagonistic actions of diastase and amylocoagulase, and that if the action of the former

predominates coagulation will not occur. Relatively high temperatures, they ascertained, are favorable to the action of diastase, while low temperatures are favorable to amylo-coagulase, and coagulation is favored as the temperature is lower and the duration of heating shorter during the preparation of the solution of starch. With a 2 per cent solution of starch, coagulation may occur at 8° but not at 20°; but with a 4 per cent solution coagulation takes place readily at 15° to 25°. The proportion of coagulum formed may be increased by various agents, and it was raised from 14.4 to 24.1 per cent with sodium hydrate at 15°. They note here that only a part of the amylocellulose is resistive to malt extract at 67°, and also that while the activity of amylocoagulase is destroyed by heating in the moist condition for 5 minutes at 63°, it is not destroyed in the dry state, and that it is present in kilned malts as well as in cured malts.

During the same year two articles were published by Maquenne (*Compt. rend.*, 1904, cxxxviii, 213 and 375), in which he recorded the results of his investigations on the proportions of amylocellulose formed from starch-solutions of different strengths. Such solutions were prepared by gelatinization of starch in a bath of boiling water, and afterwards by heating the preparation in the autoclave for 15 minutes at 120°. These solutions were set aside for 4 days at 9° to gelatinize, and then were saccharified by malt extract, and the results compared with those of similar solutions that were saccharified immediately after being taken from the autoclave. With the reverted solutions the amount of soluble matter formed was found not to increase in proportion to the amount of starch, contrary to what was found in the check solutions. Maquenne regards both raw starch and reverted starch as containing amylocellulose in different stages of condensation, that amylocelluloses are obtained from comminuted grains by solution in cold water, that they are therefore present in reverted starch-paste and in raw starch-paste, and that they are dissolved at high temperatures and therefore not apparent in freshly prepared starch-paste.

Further studies of the reversion phenomena of soluble starch were made by Roux (*Compt. rend.*, 1905, cxl, 440, 943, 1259). In the first of these articles he shows that the reversed action may proceed at any temperature between 0° and 150°, but that at the latter temperature the amylocellulose liquefies and then undergoes a degradation into a less complex form. He made the very interesting observation that, by the incomplete degradation of amylocellulose, "artificial starch-grains" can be produced which under the microscope show the peculiar structure of natural starches, and he believes that both natural and artificial starches are mixtures, and that they differ chemically owing to the existence of forms of variable degrees of condensation of the same nucleus.

In the second article he describes three types of artificial starch of different degrees of conversion, which are completely soluble at 150°, 120° and 100°, respectively. The reversion was more rapid, he noted, in the least soluble; 4.3 per cent had reverted in 1 hour in the preparation soluble at 150°; while in the one soluble at 100° the percentage was 1.8. The rapidity of reversion was increased by the presence of acid or alkali in proper proportions. The reverted product could be redissolved only at a temperature not lower than the temperature at which the original starch was soluble and, since it had the same properties as the latter, he holds that it represents a return to the initial state.

In the third article Roux compares the phenomena during saccharification of artificial and ordinary starches. Using maltase at a temperature of 56° for 4 hours, the apparent amounts of maltose formed were on an average 97.9 and 82.3 per cent in the artificial and ordinary starches, respectively. At 67° the percentages were 55.1 and 45 respectively. The dextrans formed from artificial starches, unlike those from natural starches, were almost completely soluble in alcohol.

These investigations were supplemented and extended by Maquenne and Roux (*Compt. rend.*, 1905, cxl, 1303; 1906, cxlii, 124), who found that artificial starches prepared by liquefaction of amylocellulose at 150° and yielding 96 to 98 per cent of maltose,

can by repeated reversion and liquefaction be made to yield 102 per cent of maltose. Ordinary starch-paste, they state, when measured by saccharifying and iodine tests, contains about 80 per cent of amylocellulose or artificial starch. The remaining 20 per cent of the starch is composed of a mucilaginous substance which they refer to as *amylopectin*, to which the gelatinizing property of starch they believe is due.

Amylocellulose they describe as being partly soluble in boiling water and completely soluble at 150°, colored blue with iodine, devoid of gelatinizing power, and entirely converted by diastase into maltose at ordinary temperatures. Amylopectin has the property of gelatinization; it does not give a blue reaction with iodine; and is not saccharified by malt diastase, but merely converted into dextrins. They believe that amylopectin has the property of interfering with the reversion of amylocellulose, both in the starch-grain and in starch-paste. The action of liquefying diastase, they hold, is exerted upon the amylopectin, and consequently that such a diastase should be named *amylopectinase*. The saccharifying diastases, they state, act only on amylocellulose.

In the second contribution these authors record that the conclusions formulated in the previous article must be slightly modified as regards the percentage of amylocellulose, and that starch-pastes in their ordinary condition contain about 90 per cent of amylocellulose and about 10 per cent of amylopectin, unless the latter may be formed by an especially active diastase with an attendant separation of a starch residue.

Maquenne (Compt. rend., 1908, CXLVI, 317) confirmed and extended some of the preceding investigations in proof of a non-colloidal or perfect solution of starch. Demineralized starch, he states, forms absolutely limpid solutions which are not coagulated by electrolysis, and which are transmitted by membranes and through the Chamberland filter, as are salt solutions.

Coombes (Ann. de chim. et phys., 1908, XLXXIX, 280) found that natural starch contains from 80 to 85 per cent of starch and 15 to 20 per cent of amylopectin, the latter being insoluble but gelatinizable in water, not colored blue with iodine, and not at all or but slightly saccharifiable by ordinary diastase. He believes that the group of starches contain substances which do not differ in themselves, but in their degrees of condensation, the less concentrated being stained blue with iodine, being soluble in hot water and in potassium hydrate, and being readily saccharifiable without the production of residual dextrins; but, on the other hand, the most concentrated are not stained blue with iodine and are resistant to the action of diastase.

St. Jentys (Chapter II, page 58), by the agency of tannin, prepared artificial starch-grains which resembled the most beautiful natural grains, such as those found in *Dioscorca* and *Canna*; and by gallic acid he obtained grains resembling those of wheat, buckwheat, and Chinese sokyas.

Crystalline products which do not reduce Fehling's solution and which are formed from starch by bacterial activity were examined by Schardinger (Zentralbl. f. Bakteriol., 1908, xxii, 98). He found that a pure culture of *Bacterium macerans* gives rise to two crystalline bodies which can readily be separated, owing to their marked difference in solubility in cold water. The less soluble crystallized into hexagonal flakes or prismatic crystals, and gave a yellow reaction with iodine. This substance he believes may be identical with Meyer's amylo-dextrin. The more soluble substance crystallized from alcohol in lanceolate needles. He tentatively gave the name "crystallized amylose" to this body. The addition of iodine produced gray needles which in one position appeared blue. Neither body is fermentable with yeast.

#### AMYLODEXTRIN AND MALTODEXTRIN.

The word amylo-dextrin, like the term soluble starch, has been used so indiscriminately that it implies no definite body, simple or compound, and hence has been the cause of considerable confusion. Etymologically it signifies a body intermediate between starch

and dextrin, or a mixture of the two, and it has been used in both senses, but it has also been employed synonymously with soluble starch and maltodextrin, the former being the first dissolution product of ordinary raw starch, while the latter is one of the later products of digestion; and it has been given to various intermediate products between true soluble starch on the one hand and sugar on the other, products giving reactions with iodine ranging from a purple and bluish-violet to wine color, brownish-red, or reddish-yellow, and even to some giving no color reaction. It is obvious, from the foregoing, that the word does not designate any specific individual or mixture, but some indefinite starch-like or dextrin-like or dextrin-sugar substance or compound or mixture that may be formed or exist during the saccharification of starch at any time between the moment of solution of starch-grain and the final stage of dextrin digestion. The same statement (with modifications) holds true in regard to maltodextrin. In quoting the literature the terms amylo-dextrin and maltodextrin will be used as the various authors use them.

It is probable that Jaquelain (*Ann. de chim. et phys.*, 1840, LXXIII, 167) was the earliest observer to specifically note the existence of a body having the characteristics of amylo-dextrin; that is, of a body or compound or mixture having intermediate properties between soluble starch and dextrin, which gives a purple or violet reaction with iodine. He records that after treating starch with water at  $150^{\circ}$ , and then cooling, there separated round grains which gave a purple coloration with iodine. He also makes note of preparing a precipitate in the form of a powder that gave the same reaction. This or a closely related substance was probably described by Musculus (*Compt. rend.*, 1870, LXX, 857), at first under the name of insoluble dextrin. In a later article (*Bull. Soc. chim.*, Paris, 1874, XXII, 26) it is described under the name amidon soluble.

W. Nägeli (*Beiträge z. näheren Kenntnis d. Stärkegruppe*, Leipzig, 1874) seems to have been the earliest investigator to make a serious study of amylo-dextrin; that is, of a body that stands between soluble starch (which gives a blue reaction with iodine) and erythro-dextrin (which gives a red reaction). He treated raw starch with weak acids in the cold for many days, and found that a substance (amylo-dextrin) goes into solution which the acid soon changes into dextrin and sugar. The starch-grains undergo a slow but complete solution, the periphery being the last to dissolve; but the grain residues yield amylo-dextrin as long as they give a color reaction with iodine, even though the reaction be a yellow. Amylo-dextrin crystallizes in small flakes on evaporation, or by freezing, or by the addition of alcohol. According to Nägeli the flakes consist of small needles which are associated in the form of radial aggregates which resemble crystals of inulin. It is insoluble in cold water, but is readily soluble in water at  $60^{\circ}$ , this solution remaining clear upon cooling. A freshly prepared precipitate by means of alcohol is readily soluble in cold water. It does not itself possess the property of dialyzability, but is dialyzable when accompanied by dialyzable substances. It has lower rotatory power than starch, but higher than erythro-dextrin, and it likewise stands between starch and erythro-dextrin in its behavior toward alcohol and baryta-water, and in its affinity for iodine. It consists of two modifications, neither of which in a solid state is colored with iodine, but which when in solution become violet and red, respectively. The rotatory power he gives as  $(\alpha) +175^{\circ} - 177^{\circ}$ . The soluble starch described by Musculus and Gruber (*Zeit. f. physiol. Chemie*, 1878, II, 177) was misnamed and corresponds with the amylo-dextrin of Nägeli, but is not identical. These authors recorded the product as being insoluble in cold water, soluble in water at  $50^{\circ}$  to  $60^{\circ}$ , and in aqueous solution giving a *wine-color* reaction, or when dry a violet, yellow, or brown reaction in the presence of an excess of iodine. The specific rotatory power is given as  $(\alpha) +218^{\circ}$ .

Herzfeld (*Über Maltodextrin*, Halle, 1879) opposed the view of the splitting of the molecules of starch into dextrin and maltose according to the theory of Musculus, and held that there is a consecutive conversion of soluble starch into erythro-dextrin, achroo-dextrin,

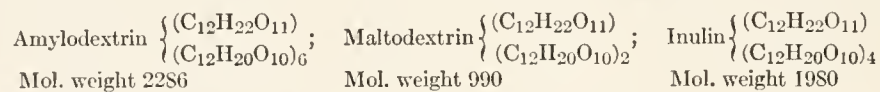
maltodextrin, and maltose. At temperatures lower than  $65^{\circ}$  he states that starch is converted into achroodextrin and maltose, but at higher temperatures erythro-dextrin and maltodextrin also are formed. The maltodextrin he assumes is composed of two dextrin groups and one sugar group, and that it has the formula of  $C_6H_{12}O_6$ . The specific rotatory and reducing powers he gives as follows:  $(a)_J = 71.6^{\circ}$ ,  $K 23.5$ . Much attention was later given to maltodextrin by various experimenters.

In the preparation of soluble starch, Salomon (*Jour. f. prakt. Chemie*, 1883, xxviii, 82) obtained amylo-dextrin. This was prepared by boiling 100 grams of potato starch for  $2\frac{1}{2}$  hours in 1 liter of water containing 5 c.c. of sulphuric acid, then neutralizing, precipitating with alcohol, redissolving and reprecipitating, and, finally, boiling to a syrup and setting aside over night. The preparation after the boiling gave a violet reaction with iodine. After standing over night there was found a precipitate in the form of fine grains, which he states resemble Nageli's amylo-dextrin crystals. A watery solution of this deposit gave a blue or a reddish-violet reaction with iodine, the latter coloration being attributed by Salomon to an impurity in the form of dextrin. The specific rotatory power is recorded as  $(a)_J + 211.50^{\circ} - 211.97^{\circ}$ ,  $(a)_D + 189.98^{\circ} - 190.24^{\circ}$ .

Meyer (*Botanische Zeitung*, 1886, xlix, 697, 713), in studying the nature of starch-cellulose, states that amylo-dextrin constantly originates during the treatment of starch-paste with dilute acids, diastase, pepsin, saliva, and in general all substances which cause a disaggregation of the starch-substance in the presence of water. But if the action of the splitting agent is very energetic from the beginning, the transformation of the amylo-dextrin is so rapid that no skeletons will be formed. The skeletons, he states, consist of pure amylo-dextrin only when they no longer stain violet or blue, but yellowish or reddish brown when allowed to stand in iodine solution for about 5 minutes. He also noted the formation of spherocrystals (occasionally of plates) which resembled starch-grains, except that the interference figure in polarized light was not orthogonal but diagonal; but both saliva and acid skeletons, he records, behave toward polarized light the same as intact grains. The crystals and the skeletons reacted identically to various reagents, including iodine, from which he concludes that they are an identical substance. Carl Nageli (*loc. cit.*) differentiated the soluble granulose from the skeleton-like cellulose by subjecting raw starch to the prolonged action saliva, and Walter Nageli (*loc. cit.*) found that as long as these skeletons showed any color reaction with iodine they were converted into amylo-dextrin. Griessmayer (*Allgem. Bauer. und Hopfenzeit.*, 1887, xxvi, 147), in studying the real nature of starch cellulose, recorded that the skeleton-like substance of the grains is converted by dilute acid into amylo-dextrin. He digested raw starch in a 12 per cent solution of hydrochloric acid for 100 days in the cold. The skeleton-like residue was washed free from acid and other extraneous substances. When dried it represented 30 per cent of the original quantity of starch. It was almost completely soluble in hot water, and by freezing spherocrystals of amylo-dextrin were obtained.

Brown and Morris (*Jour. Chem. Soc.*, 1885, xvii, 526; *Ann. d. Chem. u. Pharm.*, 1885, xxiii, 72; *Jour. Chem. Soc. Trans.*, 1889, lv, 449 and 462) devoted considerable attention to maltodextrin and made, among other studies, comparative investigations of amylo-dextrin, maltodextrin, and inulin. In their earlier contributions they disagree with Herzfeld (page 114) that maltodextrin is a mixture of dextrin and maltose, and they hold that his so-called maltodextrin was impure. They state that a mixture of dextrin and maltose prepared so as to have the same optical activity and reducing power as maltodextrin is separable into its constituents by treatment with alcohol, whereas true maltodextrin is not thus separable; from a mixture of maltose and dextrin maltose may be fermented off, but not so with maltodextrin; when a mixture of dextrin and maltose is subjected to malt extract a residue of dextrin is always left, while with maltodextrin there is no residual dextrin; and when a mixture of dextrin and maltose is subjected to dialysis

it splits into dextrin and maltose, while maltodextrin dialyzes intact. They give maltodextrin the formula  $\left\{ \begin{array}{l} (C_{12}H_{22}O_{11}) \\ (C_{12}H_{20}O_{10})_2 \end{array} \right.$ . In their article published in 1889, they point out the close resemblance between the amylo-dextrin of Nægeli and the maltodextrin described in their earlier contributions, and they state that this substance is neither identical with soluble starch, as held by some; nor is it identical with the starch-cellulose of C. Nægeli, as held by Meyer; nor is it a mixture of dextrin and maltose, as held by Herzberg. They also assert that while the composition of amylo-dextrin can be expressed in terms of a mixture of starch and dextrin, it is really a well-defined chemical substance; that it does not possess the optical properties ascribed to it by its discoverer; that in composition it is analogous to maltodextrin, which may be represented by the formula  $\left\{ \begin{array}{l} (C_{12}H_{22}O_{11}) \\ (C_{12}H_{20}O_{10})_6 \end{array} \right.$ , and constituted by one *amyloin* or maltose group with six *amylin* or dextrin groups; that amylo-dextrin, like maltodextrin, is hydrolyzed immediately into maltose; that it is an entirely different substance from soluble starch, with which it has been confounded; and that the first action of dilute acid on ungelatinized starch in the cold is to convert the starch-substance into soluble starch, which is gradually hydrolyzed into amylo-dextrin, a portion going at the same time into solution and being changed into dextrose; and that there is a great similarity between amylo-dextrin and inulin. The formulæ and molecular weights they give as follows:



The purified amylo-dextrin had the following specific rotatory and reducing coefficients,  $(a)_{D,3.86} = 206.11$ , and  $k_{3.86} = 9.08$ , respectively.

A further study of the maltodextrins ("amyloins") was made by Morris and Wells (Trans. Inst. Brewing, 1892, v, 133) by means of analyses of fermenting yeasts at different stages of fermentation. They found, in experiments with Frohberry and Saaz yeasts, that the properties of these yeasts so differ that one may be used to determine free maltose and the other to determine the fermentable maltodextrin. The Saaz yeast, it was found, does not ferment maltodextrins, but the Frohberry yeast does.

Schiffner (Neue Zeit. Rub. Zuek. Ind., 1892, xxix, 167; Inaug. Diss., Kiel, 1892) endeavored to prepare the maltodextrins of Herzfeld and of Brown and Morris, but without success, and in no case was he able to separate a body which resembled in any way the so-called amyloins (maltodextrins) referred to by the latter. He regards Herzfeld's maltodextrin as being probably a mixture of 26 per cent of dextrin and 74 per cent of isomaltose; and the maltodextrin of Brown and Morris as a mixture of 67 per cent of dextrin and 33 per cent of isomaltose.

The amylo-dextrin (really soluble starch) separated by Lintner and Düll (Ber. d. d. chem. Gesellsch., 1893, xxvi, 2533) was obtained by stopping the action of diastase while the preparation still gave a blue reaction with iodine, and precipitating with hot 40 per cent alcohol. The precipitate thrown down during cooling was purified by repeated fractionation with 40 and 30 per cent alcohol, yielding an extremely light white powder that was slightly soluble in cold water, but very soluble in hot water. It gave a deep-blue reaction with iodine, and its specific rotatory power was  $(a)_D = 196$ , and its formula  $(C_{12}H_{20}O_{10})_{54}$ . From a 20 to 30 per cent solution they obtained spherocrystals.

The products of starch transformation by the actions of several kinds of yeast were studied by Hiepe (Country Brewer's Gazette, 1893 and 1894; Jour. Soc. Chem. Ind., 1894, xiii, 267), but he failed to find any homogeneous precipitate having the properties of a body between dextrin and isomaltose, and he states his belief that the intermediate products between dextrin and sugar (the amyloins of Brown and Morris) consist of a mixture of dextrin, isomaltose, maltose, and glucose.

Meyer (*Die Stärkekörner, loc. cit.*, page 47) studied the microchemical behavior of  $\alpha$ -amylose,  $\beta$ -amylose, and amyloextrin. He prepared what he terms amyloextrin from both amyloses by means of malt extract, dilute acids, and other chemical agents. He noticed that amylose in solution and in crystals, and amyloextrin, have different degrees of affinity for iodine. When crystallized  $\beta$ -amylose (in the form of starch-grains) and amyloextrin are added to a solution of amylose, and a trace of iodine is added, and then gradually more and more iodine, it will be seen, he states, that the solution will be colored first, then the starch-grains, and then the amyloextrin. Pure amylose solution is colored a greenish-blue with a slight excess of iodine; pure amyloextrin, even in a dilution of 1-6000, is colored brownish-red; a mixture of both solutions, according to the relative amount of amylose, will be colored blue, blue-violet, violet, red-violet, pure red, and a brownish-red.

Bülow (*Archiv f. d. ges. Physiologie*, 1895, LXII, 131) obtained what he describes as amyloextrin by subjecting potato starch to the action of a strong solution of potassium hydroxide, heating the paste thus formed in a water-bath, diluting, neutralizing with dilute acetic acid, precipitating with alcohol, dissolving in water, reprecipitating, etc. He also prepared amyloextrin by means of diastase and by dilute sulphuric acid. By precipitation with barium hydroxide he prepared several forms of amyloextrin-barium combinations, which varied in rotatory power from  $191.1^\circ$  to  $205.4^\circ$ , and also in the percentages of the amyloextrin and the barium in the combinations.

Ost (*Chem. Zeit.*, 1895, XIX, 1501), following Lintner and Düll's methods, prepared maltodextrin, but found that it had a higher specific rotatory power than that given by Brown and Morris, and that the method employed by the latter investigators to determine the reducing power yields fallacious results.

Ling and Baker (*Proc. Chem. Soc.*, 1897, CLXXIII, 3) studied in detail the products formed by the limited action of diastase at  $70^\circ$ , and they separated two unfermentable substances which they prepared free from extraneous matters. These they state were maltodextrin  $\alpha$ , which is identical with the maltodextrin of Brown and Morris, but having the properties  $(a)_D = +180$  and  $R = 32.81$ ; and maltodextrin  $\beta$ , identical with Prior's achroodextrin III (see below), and having the properties  $(a)_D = +171.6$  and  $R = 43$ . They believe that starch is broken down by diastase into a series of maltodextrins of decreasing molecular weight and rotatory power, but with increasing reducing power, and which have optical and reducing properties equivalent to varying mixtures of starch and maltose.

According to Prior (*Bayerisches Brauer Jour.*, 1896, VI, 385), the final products of the action of diastase are 3 achroodextrins (I, II, and III) and maltose. Lintner's isomaltose he looks upon as being a mixture of achroodextrin and maltose. The achroodextrins I and II correspond with the same dextrans of Lintner and Düll, but achroodextrin III is a dextrin which he regards as immediately preceding the formation of sugar. This substance he found has a specific rotatory power of  $(a)_D = +171$  and a reducing power of 42.5 per cent of maltose. The dextrans showed differences in fermentability. By the action of yeast, glucose as well as maltose was formed.

Wróblewski (*Ber. d. d. chem. Gesellsch.*, 1897, XXX, 2128; *Chem. Zeitung*, 1898, XXII, 375), in the report of his investigations of soluble starch, calls attention to the difference between soluble starch and amyloextrin. He states that soluble starch is to be regarded as the first product of the hydrolysis of natural starch; that it can be filtered through porcelain; that it does not reduce Fehling's solution; that it is colored blue with iodine; and that amyloextrin is a derivative of soluble starch and has the property of slightly reducing Fehling's solution, and is colored reddish-brown with iodine.

The constitution and oxidation products of maltodextrin were studied by Brown and Millar (*Proc. Chem. Soc.*, 1899, XV, II), who state that while considerable quantities of this substance are present when the decomposition of starch has been arrested at the proper

time, its preparation in a pure state in sufficient quantities is a laborious process. They give it the following rotatory and reducing values :  $(a)_D = +181^\circ-183^\circ$ , and  $R=42$  to  $43$ ; and they state that it is completely hydrolyzed by diastase to maltose, and by acid to *d*-glucose.

The relation of maltodextrin to isomaltose was studied by Pottevin (Ann. de l'Inst. Pasteur, 1899, XIII, 728), who asserts that the maltodextrin of Brown and Morris is not a true compound, but simply a mixture of dextrin and maltose, as previously claimed by Lintner and Düll. By fractional precipitation with alcohol he split up this maltodextrin fraction, and he asserts that the differences between mixtures of dextrin and maltose and maltodextrin in their behavior towards diastase and alcohol, and as regards dialyzability, are readily explained on the basis of differences in the form of the dextrin in the combination. The different dextrans differed in digestibility, solubility in alcohol and dialyzability, and he found that an artificial mixture of maltose with a properly selected dextrin is absolutely identical with Brown and Morris's maltodextrin, which therefore he holds is a mixture and not a true compound.

In studies of the constitution of starch and its derivatives, Syniewski (Ann. d. Chem. u. Pharm., 1899, 282) states that starch as well as the products obtained from it, such as the amylose of Meyer, the amylo-dextrin of Lintner and Düll, and the granulose of Nägeli and various investigators, consists of many molecules of *amylogen* which are joined together in the form of a carbinolanhydrid union (see page 133). When amylogen is hydrolyzed, he assumes, all of the maltose molecules are split off one after another, and dextrin remains. By the continued action of malt extract the dextrin is split into isomaltose and glucose, and the isomaltose finally into glucose. All the products directly obtained from starch by hydrolysis he designates dextrin. Those dextrans which originate from starch by carbinol-hydrolysis, and which therefore do not reduce Fehling's solution, he terms *amylo-dextrin*. The dextrin which originates from amylo-dextrin after the splitting off of all the maltose molecules he terms *grenzdextrin*. All of the dextrans between amylo-dextrin and grenzdextrin (those from which maltose may be split off) he terms *maltodextrin*. The dextrans which originate from grenzdextrin by the splitting off of glucose he terms *glucodextrans*.

In another research, Syniewski (Ann. d. Chem. u. Pharm., 1902, CCCXXIV, 201) studied an iodine compound of amylo-dextrin. He also made a solution of starch by heating starch-paste in the autoclave at  $140^\circ$ , and then subjected the solution to the action of malt that had previously been heated to  $78^\circ$  in the presence of 0.1 per cent of formaldehyde for 216 hours. The starch was completely converted into a body which the author terms grenzdextrin II, and which he states is identical with the maltodextrin of Brown and Morris, and with the  $\alpha$ -maltodextrin of Ling and Baker, and with the achroo-dextrin of Lintner. By hydrolyzing grenzdextrin II with fresh malt extract it was split into a compound corresponding to the formula  $C_{24}H_{42}O_{21}$  and having the following rotatory and reducing powers:  $(a)_D 200=172.17^\circ$ ,  $R=42.7$ . This substance he names  $\gamma$ -maltodextrin. This  $\gamma$ -maltodextrin is in turn resolved into maltose and dextrinose (Lintner's isomaltose).

Hale (Amer. Jour. Science, 1902, XIII, 379) states that amidulin seems to stand between starch and erythro-dextrin in regard to the solubility of its iodide and in the degree of digestibility with saliva.

Ling (Jour. Fed. Inst. Brewing, 1903, IX, 446) suggests that it would be well to use the term maltodextrin instead of dextrin when referring to intermediate dextrinous products of diastatic activity, because this would prevent confusion with dextrin prepared by torrefaction. He refers to the fact that Brown, Morris and Millar have stated that their maltodextrin is completely converted into maltose by diastase, but he finds by subjecting  $\alpha$ -maltodextrin (having the constants  $(a)_D 3.93=+180.5$ , and  $R_{3.93}=36.7$ ) to the action of diastase that there are formed 90 per cent of maltose and 10 per cent of glucose.

In the process of preparation of soluble starch, Ford (Jour. Soc. Chem. Ind., 1904, XXIII, 414) notes that if the treatment by dilute acid be carried too far there is a very con-

siderable production of maltodextrin; that all soluble starches prepared in the heat by acid contain this substance; and it is to its presence, and not to the maltose or glucose, that the copper-reducing power of soluble starches is due. This is proved, he states, by the fact that yeast has no effect in reducing the amount of apparent maltose in a solution of starch which on dialysis does not yield sugar but a body having the properties of amylo-dextrin. He prepared soluble starch from arrowroot by Lintner's method, added water, and dialyzed for 2 days. The dialysate was concentrated, and precipitated twice with alcohol, and finally dissolved in water. The rotatory and reducing powers correspond to  $(a)_{\text{D abs}} = +189$ ,  $R_{\text{abs}} = 14.4$ , which are the values given by Brown and Morris for amylo-dextrin. For practical purposes of diastasi-metry the presence of amylo-dextrin in the starch is of no importance, as it is equivalent to soluble starch.

The degradation products of starch brought about by the action of oxalic acid were studied by Grütters (*Zeit. angew. Chem.*, 1904, xviii, 1169), who obtained achroodextrins I and II, maltodextrin  $\gamma$ , maltose, dextrose, and a small amount of levulose. He found that the products of the action of oxalic acid are the same as those of diastase, except that the maltodextrin  $\gamma$  produced by the acid is replaced by maltodextrin  $\beta$  by the diastase, the latter showing different constants and behaving differently in the presence of diastase. He believes, however, that both forms occur simultaneously, but in varying proportions. He also notes that the divergent behavior of different isomaltose preparations toward malt extract indicates an occasional preponderance of the more resistant maltodextrin  $\gamma$  as the lowest member of the dextrans present.

By the use of chromic acid, Härz (*Beiheft. z. botan. Centralbl.*, 1905; *Woch. f. Brau.*, 1905, xxii, 721) prepared what he records as amylo-dextrin, and he found that (like the original starch) it did not behave as a uniform substance, but seemed to consist of a number of molecular groups which differ in complexity and density of internal structure. The same composite character he noted in erythro-dextrin II that he prepared by the action of a 5 per cent alcoholic solution of hydrochloric acid on starch. When, however, the achroodextrin stage was reached the products seemed to possess homogeneity.

Moreau (*Ann. d. d. Soc. Roy. d. Sc. méd. e. nat. d. Bruxelles*, 1903, xii, 117; 1905, xiv, 64), in following up his earlier work on the isolation of various products of digestion by precipitants, separated the different products by precipitation by barium hydroxide in aqueous or dilute alcoholic media, determining the progress of the reaction and nature of the precipitated bodies by the iodine reactions of the filtrates. Amylo-dextrin and erythro-dextrin were precipitated in aqueous solutions, while achroodextrin and sugar were precipitated only in the presence of more or less alcohol; but the limits of precipitability were sufficiently far apart to permit the separation of the several products by repeated fractionation by barium hydroxide. By this means he determined, in support of Mittel-meyer's theory of starch decomposition, that even in the earliest stages of digestion the starch molecule is immediately broken down into three forms of dextrin and sugar. Detailed directions are given for preparing pure amylo-dextrin from the dextrans of commerce. Such amylo-dextrin, as well as pure erythro-dextrin, were found to be absolutely devoid of reducing power in cupric solutions.

The maltodextrin  $\gamma$ , which was first described by Grütters, was further studied by Rheinfeld (*Woch. f. Brau.*, 1906, xxiii, 510), who finds that its position in the series of disintegration products of starch is between the maltodextrin  $\beta$  of Ling and Baker, or the achroodextrin III of Prior, and maltose. He subjected starch-paste to the action of diastase at a temperature of  $70^{\circ}$ , and allowed the process to go on until a red reaction with iodine no longer occurred. By a series of fractionations he obtained a form of malto-dextrin which had the constants  $(a)_{\text{D}} = +167.7$  and  $R = 60.1$  per cent of maltose. Based upon glucose equivalents the constants were  $(a)_{\text{D}} = +170-173$  and  $R = 61-64$  per cent. He calls attention to the fact that this maltodextrin was fermented to the extent of 50

per cent by Frohberry yeast, while the same form of dextrin prepared by Grütters by means of oxalic acid was fermented only to the extent of 24 per cent. In a subsequent communication (Z. Spiritusind, 1907, xxx, 371) Rheinfeld noted that a certain amount of the products of hydrolysis undergo polymerization and condensation when a solution is repeatedly evaporated. He carried on hydrolysis until no color reaction with iodine was obtained, and by repeated fractionation he prepared 5 specimens of maltodextrin  $\gamma$ . The constants he records as  $(a)_D = +163-167$  and  $R = 58-62$ . Grütters' values were  $(a)_D = +160$  and  $R = 61$ . When determined as Ost's glucose values, when corrected, the values were  $(a)_D = +170-173$  and  $R = 61-64$ .

Reichard (Zeit. ges. Brau. 1908, xxxi, 161) confirmed Syniewski's statement in regard to the formation of a formaldehyde-amylopectin compound by the action of concentrated formaldehyde. He studied the influences of different percentages of formaldehyde in relation to consecutive changes in the starch, and also its influence upon the temperature of gelatinization; a gram of starch was completely gelatinized by 10 c.c. of a 37 per cent unneutralized formaldehyde in 7 to 8 hours at 25°. With weaker or neutralized formaldehyde, and at lower temperatures, the action is slower. At 15° to 16° the starch was gelatinized in 38 per cent formaldehyde in 2 days. The formaldehyde preparations give at first a blue reaction with iodine, but when the gelatinous stage is reached the reaction is brownish-red; and the gelatinized starch dissolved in water yields a yellow reaction, which indicates a further reaction of the formaldehyde.

Castoro (Gas. chim. Ital., 1909, xxxix, 603) heated pea starch for 5 hours in a 2 per cent sulphuric acid, filtered, and precipitated with alcohol. Upon treating the precipitate with water one part was found to go into solution and another to remain undissolved. The latter gave a blue-violet reaction with iodine, and corresponded with the amylopectin of Maquenne and Roux. Upon dialyzing the part in solution two fractions could be obtained, one giving a blue reaction with iodine and the other a violet reaction corresponding to that of amylopectin. By dialyzing a pseudo-solution of potato starch prepared by the agency of 1 per cent sulphuric acid, a diffusible portion was obtained that gave a violet reaction with iodine. He believes that the differences in the color reaction are due to differences in the size of the particles, the large particles becoming blue and the small particles violet. It is pointed out that analogous differences may be observed in gold-colloidal solutions.

#### ERYTHRODEXTRIN, ACHROODEXTRIN, GRENZDEXTRIN, ETC.

Although Vaquélin (Bull. de pharm., 1811, III, 54) noted that when starch is subjected to torrefaction it is converted into a gum-like substance, and Vogel (Schweigger's Jour., 1812, v, 80) that starch is changed by weak acid into gum and sugar, and various experimenters of the following twenty years that starch yields a gummy substance, it remained for Biot and Persoz (Ann. de chim. et phys., 1833, LII, 72) to demonstrate the distinguishing characters of this substance, and to give it the name by which it has continued to be known even to the present day. They believed that it existed as a constituent of the grain, and that it is liberated by boiling in water or by weak solution of acid, which disrupts the outer coating of the grain. From its strong dextro-rotatory action on rays of plane polarized light they named it *dextrin*. The same year, Payen and Persoz (Ann. de chim. et phys., 1833, LII, 73) prepared dextrin by the aid of diastase. They ascertained in these experiments that a number of substances were present in the preparations—one of them was insoluble in cold water, but soluble in hot water, and colored with iodine, and identical with the substance of the interior of the starch-grains; a second substance, which is soluble in both cold and hot water and in weak alcohol, but not colored with iodine, and in the nature of a gum; and a third substance, sugar, etc. This second substance is the body they named dextrin, and corresponds with the achroo-dextrin of the present.

Two years later, Payen (Ann. de chim. et phys., 1836, LXI, 355 and LXV, 225) reported that the rotatory power of dextrin was equal to that of starch; that all starches have the same elementary composition ( $C_6H_{10}O_5$ ); that all parts of the same grain, including dextrin, have the same elementary composition; and that the dextrins formed by dilute acid, diastase, and torrefaction are merely physical modifications of the same substance.

Fürstenberg (Ann. d. Chem. u. Pharm., 1844, LII, 417) discovered that starches of cereals contain dextrin similar to that obtained by the action of dilute acid, or by diastase on starch, and that it is without reducing action on copper solutions. Blondeau de Carolles (Jour. f. prakt. Chemie, 1844, 33, 439), Fehling (Ann. d. Chem. u. Pharm., 1845, LV, 13), and Kalinowsky (Jour. f. prakt. Chemie, 1845, XXXV, 193) found that by the action of sulphuric acid a number of compounds are formed, according to the time of action and strength of acid. Béchamp (Compt. rend., 1856, XLII, 1210) noted that a substance (erythrodextrin) may be formed that is intermediate between starch and dextrin (achroodextrin), giving a reddish coloration with iodine, and which he regards as an inversion product of starch.

The assertion of Payen that the dextrins formed by the action of dilute acid, diastase, and torrefaction, respectively, are merely physical modifications of the same substance was strongly opposed by Mulder (Chemie des Bières, Leipzig, 1858, 166), who asserts that they differ from one another in their reactions, especially with precipitants.

In 1860, Musculus (Compt. rend., 1860, L, 785) refers to a gummy body formed by the action of diastase or acid which does not give a color reaction with iodine, which he did not isolate, but records as dextrin. He believed that the sugar formed during digestion is dextrose; that the sugar is not formed from dextrin, as was at that time and for many years believed, but that dextrin and sugar are formed coincidentally from starch by the action of water, the proportion being 2 of dextrin to 1 of sugar. The following year he reasserted the proportion of dextrin to sugar, and also noted that this ratio exists as soon as the blue reaction with iodine ceases. He believed that the sugar prevents further change in the dextrin, but that at the same time it does not prevent the breaking down of starch. In a later article, Musculus (Ann. de chim. et phys., 1865, VI, 177) studied the characteristics of what he refers to as a "true dextrin," which is a body (achroodextrin) that does not give a color reaction with iodine, does not reduce copper solutions, and is not digested by diastase. In further studies (Compt. rend., 1869, LXVIII, 267) Musculus gives a ratio of 1:1 and states that during the transition of starch by diastase and acid to "colorless dextrin" (non-color reacting dextrin) there is produced a modification, which he calls "insoluble dextrin," which is insoluble in cold water, which gives a violet to a wine-red brown or yellow reaction with iodine, is saccharified by diastase, and yields by acid less sugar than starch.

The assertion of Musculus that the colorless dextrin is not digested by diastase was opposed by Payen (Ann. de chim. et phys., 1866, VII, 382), who to the contrary found it to be readily saccharified. Nasse (Demateriis amtlaceis num in mammalium inveniantur disquisitio, Diss. Halle, 1866) reported a body under the name of "dextrinogen" which corresponds with the so-called colorless dextrin of Musculus.

Three stages must be recognized, writes Griessmayer (Ann. d. Chem. u. Pharm., 1871, CLX, 40), in the process by which starch which has been boiled and set aside is finally transformed into glucose and other products: (1) After a few days the addition of a small amount of weak iodine gives a violet reaction, never a blue. The red of the iodine-dextrin reaction combining with the blue of the iodine-starch reaction makes violet. (2) After standing about 8 days the addition of iodine gives a red reaction, which is attributed to a substance he names *dextrin I*. By carefully adding a moderately concentrated solution of tannic acid, this dextrin can be precipitated, leaving the starch. Besides dextrin I, there is another dextrin present which does not give a color reaction with iodine. (3) After starch has stood for more than 8 days there occurs a time when the addition of iodine causes a red coloration which disappears immediately, but testing with Fehling's solution

gives no sugar reaction. Later, the iodine will not yield a color reaction, while Fehling's solution is reduced. There is in solution, then, a substance that has great affinity for iodine, greater than dextrin I, which has lost its dextrin color-reaction characteristics towards iodine, yet without having acquired the distinguishing marks of sugar. This dextrin he names *dextrin II*. He believed that both dextrans coexist in fresh starch solution in small quantities.

O'Sullivan (Jour. Chem. Soc. Trans., 1872, 579) also noted the existence of two dextrans, which he distinguished as  $\alpha$ - and  $\beta$ -dextrans, respectively, and which correspond with the dextrin I and dextrin II of Griessmayer. O'Sullivan found that both forms are changed into maltose by diastase or dilute acid, and that both have the same rotatory power,  $(a)_D = +213$ . He prepared them almost free of reducing power, and suggests that if pure they would not reduce copper solutions. There appeared at the same time a very elaborate contribution by Brücke (Sitz. d. k. Akad. d. Wissensch., Weín, 1872, LXV, 3 Abth., 126) on the manner of digestion and assimilation of carbohydrates. He noted two dextrans, corresponding with those recorded by Griessmayer and O'Sullivan. The dextrin which gives a red reaction with iodine he termed *erythro-dextrin*, and the one which did not give a color reaction he termed *achroo-dextrin*, which terms have continued in universal use to the present. Besides these dextrans there was found a residue which Brücke named *erythamylum*, which he states consists of Nägeli's cellulose together with a substance which takes a red stain with iodine, which is already present in dry starch, but which is masked by the granulose and its blue reaction with iodine.

A study of amylo-dextrin, together with a comparison of the properties of starch, amylo-dextrin, and dextrin, was made by W. Nägeli (Beiträge z. näheren Kenntnis d. Stärkegruppe, etc., 1874, Leipzig), in which he refers to the existence of two forms of dextrin, one of which is colored red or orange and the other yellow with iodine. He asserts that there is no dextrin which does not become colored with iodine, and also that Musculus's theory of the breaking up of starch coincidentally into dextrin and sugar does not hold. Three dextrans,  $\alpha$ ,  $\beta$ , and  $\gamma$ , were described by Bondonneau (Compt. rend., 1875, LXXXI, 972, 1210). The  $\alpha$ -dextrin corresponds with the dextrin I of Griessmayer, erythro-dextrin of Brücke, and the  $\alpha$ -dextrin of O'Sullivan; while the  $\beta$ -dextrin corresponds with dextrin II of Griessmayer, the achroo-dextrin of Brücke, and the  $\beta$ -dextrin of O'Sullivan. The next year, O'Sullivan (Jour. Chem. Soc., 1878, 1, 479) gave good evidence to show that the  $\gamma$ -dextrin of Bondonneau does not exist, and that the latter was led into error because of the assumption that the sugar product is dextrose, and that the  $\gamma$ -dextrin is merely an expression, not recognized by Bondonneau, of the difference between the reducing powers of maltose and dextrose. Nasse (Archiv f. ges. Physiologie, 1877, XIV, 473) believed by the action of saliva or dilute sulphuric acid that there are formed *dextrinogen* (achroo-dextrin) and also a peculiar form of sugar that is not grape sugar, and which he proposes to distinguish by the name *amylum ptyalose*.

Thus far it had been clearly shown by a number of investigators that two very different forms of dextrin are produced during the saccharification of starch, but Musculus and Gruber (Zeit. f. physiolog. Chemie, 1878, II, 177) were the first to give evidence to lead to the belief that the so-called achroo-dextrin is not an individual, but a mixture of at least three achroo-dextrans which have different optical and reducing powers. Based upon the results of their research, they formulated a definite theory of the processes and products of starch saccharification, which with certain modifications has received a very general acceptance from that time to the present. According to these authors the derivatives of starch by the action of diastase or weak sulphuric acid are as follows:

- (1) *Soluble starch*. [Probably a peculiar form of starch or of erythro-dextrin.] It is insoluble in cold water, and soluble in water at 50° to 60°. An aqueous solution gives a *wine-red* reaction with iodine. Dried in the air it will give a violet, yellow, or brown with an excess of iodine. Its specific rotatory power is  $(a) = +218$ , and its reducing power 6.

- (2) *Erythroextrin*. It differs from starch in that it is soluble in cold water, is not granular, and because in solid form or in solution it gives only a red color with iodine. Both soluble starch and erythroextrin they found to be readily affected by diastase. They did not succeed in obtaining pure erythroextrin.
- (3) *Achroodextrin a*. This does not give a color reaction with iodine. It is more easily converted into sugar by diastase than either soluble starch or erythroextrin. Its rotatory power is  $(\alpha) = +210$ , and its reducing power 12.
- (4) *Achroodextrin β*. It is unaffected by diastase. Its rotatory power is  $(\alpha) = +190$ , and its reducing power 12.
- (5) *Achroodextrin γ*. It also is unaffected by diastase. Its rotatory power is  $(\alpha) = +150$ , and its reducing power 28.
- (6) *Maltose*. Formula,  $C_{12}H_{22}O_{11} + H_2O$ . Rotatory power  $(\alpha) = +150$ , and its reducing power 66. It is not affected by diastase.
- (7) *Glucose*. Formula  $C_6H_{12}O_6 + H_2O$ . Its rotatory power is  $(\alpha) = +56$ , and its reducing power 100. It does not undergo fermentation.

The figures for the rotatory and reducing powers are stated to be only approximate, but they show a decrease of the former and an increase of the latter as decomposition proceeds, with the formation of substances of less molecular weight. Musculus and Gruber write that starch, before it appears in the form glucose, is changed into 5 isomeric bodies, *i.e.*, erythroextrin, achroodextrin *a*, achroodextrin *β*, achroodextrin *γ*, and maltose. They regard the starch substance as having the formula  $n(C_{12}H_{20}O_{10})$ , in which *n* has a value of not less than 5 or 6. Starch by absorption of water, by the addition of diastase or dilute acids, undergoes repeated splitting. At each subsequent splitting there appears besides maltose a new dextrin of less molecular weight than the preceding dextrin, that is, *n* becomes smaller at every stage until achroodextrin *γ* results. The latter, through simple absorption of water, goes over into maltose, and by hydration and splitting this goes into 2 molecules of glucose.

In 1879, Brown and Heron (Proc. Chem. Soc. Trans., 1879, xli, 596; Ann. d. Chem. u. Pharm., 1879, cxcix, 241), while accepting the theory of Musculus and Gruber of the breaking down of the starch-molecule by a series of hydrations and splitting-up processes, stated their belief that the starch-molecule can not have a simpler formula than 10 ( $C_{12}H_{20}O_{10}$ ) and that the first action of diastase is to separate by hydration one of these 10 groups, which is transformed into maltose, while the remaining 9 groups constitute the erythroextrin *a*, or the first of the series of dextrans. By the addition of more water this dextrin is conceived to be split into maltose and another dextrin, erythroextrin *β*, which consists of 8 groups. This in turn by hydration is split into maltose and another dextrin, consisting of 7 groups, and designated achroodextrin *a*, and so on by consecutive splitting and hydration until there occurs ultimately a complete conversion into maltose. According to this theory the number of dextrans is determined by the number of constituent molecules in the starch-molecule. There are therefore 8 possible dextrans, 2 erythrodextrans and 6 achroodextrans, derivable from a starch-molecule having the formula as above stated. As the action proceeds, the rotatory power falls while the reducing power rises, until finally both powers correspond to the properties of maltose, as indicated in table 7. (See Brown and Morris, page 124.)

The results recorded by Brown and Heron received support in the investigations of Squire (Jour. Soc. Chem. Industry, 1884, iii, 397). He states that his experiments confirm

TABLE 7.

No. of transformation.	Specific rotation.	Cu <sub>2</sub> O reduced.	Resulting dextrin.
Soluble starch	216.0	0	
1.....	209.0	6.4	Erythroextrin <i>a</i>
2.....	202.2	12.7	Erythroextrin <i>β</i>
3.....	195.4	18.9	Achroodextrin <i>a</i>
4.....	188.7	25.2	Achroodextrin <i>β</i>
5.....	182.1	31.3	Achroodextrin <i>γ</i>
6.....	175.6	37.3	Achroodextrin <i>δ</i>
7.....	169.0	43.3	Achroodextrin <i>ε</i>
8.....	162.6	55.1	Achroodextrin <i>ζ</i>
9.....	156.3	55.1	Achroodextrin <i>η</i>
Maltose.....	150.0	61.0	

the results of these investigators as far as reaction No. 8 is concerned, namely, that at 60° or 63°, or even below, gelatinized starch undergoes a definite process of hydration which results in the production of practically 80 per cent maltose and 20 per cent dextrin. He states, however, that he did not succeed in obtaining trustworthy evidence of definite reactions representing the other numbers, nor sufficiently concordant results to warrant the assumption that at any given temperature the reaction can always be represented by definite formulæ. The production of maltose he found to be continuous, and not by steps, and that there was never a complete disappearance of dextrin. Squire quotes Southy (Brewing, etc., London, 1877) as having noted that at lower temperatures the proportion of dextrin to sugar is increased.

Almost coincidently with the appearance of Brown and Heron's article, O'Sullivan (Proc. Chem. Soc. Trans., 1879, XLI, 770) reported that he had modified his views regarding the number of dextrans, he having now reached the conclusion that there are formed 1 erythro-dextrin and 3 achroo-dextrans. He states that he separated them in a nearly pure state by means of precipitation with alcohol, and he designates them as follows:  $\alpha$ -dextrin colored brownish-red with iodine;  $\beta$ -dextrin I,  $\beta$ -dextrin II, and  $\beta$ -dextrin III, none of which gives a color reaction with iodine. O'Sullivan believed that the formation of dextrans is not due to the breaking down of the starch-molecule into simpler bodies, but to a rearrangement of the molecules, so that there are formed a series of substances of the same molecular weight, but differing in their behavior, owing to the change in constitution. He thought, therefore, that the entire starch-molecule is affected at once, in contradistinction to the serial degradation according to the theories of Musculus and Gruber and of Brown and Heron.

Still another theory of the processes of saccharification was offered at this time by Herzfeld (Ueber Maltodextrin, Dissertation, Halle, 1879), according to which the transformation of the starch by diastase is by serial actions, giving rise consecutively to amylo-dextrin, erythro-dextrin, and achroo-dextrin, the latter being split into maltodextrin and maltose. Below 65° it is stated that achroo-dextrin and maltose are formed, but at higher temperatures also erythro-dextrin and maltodextrin.

A process for the manufacture of dextrin was devised by Lauga (Jour. Soc. Chem. Industry, 1882, I, 513), which consists essentially of boiling glucose juice with concentrated phosphoric acid until dextrinization, at which time the preparation is cooled to about 50°, neutralized, and filtered under pressure through animal charcoal, etc.

The products of the conversion of starch by organic and inorganic acids were examined by Salomon (Jour. f. prakt. Chemie, 1883, xxviii, 82), who concluded, contrary to other investigators at this time, that only one kind of dextrin results in the saccharification of starch. He took some very pure dextrin that was obtained from a solution from which starch had been removed. The solution of this dextrin gave a brownish-red reaction with iodine and, after purification by resolution in water and precipitation by alcohol, it gave a brownish reaction that disappeared immediately, and it failed to reduce Fehling's solution. He also held that only dextrin and glucose are produced, no maltose.

Schulze (Jour. f. prakt. Chemie, 1883, xxviii, 311) studied the influence of acetic acid on starch by treating starch with 20 per cent acid under pressure, and obtained, as he states, a dextrin like the  $\alpha$ -dextrin of Bondonneau. Heating for 4 hours gave rise almost exclusively to this dextrin, but further heating caused the formation of more or less glucose.

In 1885, Brown and Morris (Ann. d. Chem. u. Pharm., 1885, xxiii, 72; Jour. Chem. Soc., 1885, xlvii, 527) published what they state is to be looked upon as a continuation of the paper by Brown and Heron that appeared in 1879 (page 123). By this extended work they were enabled to add certain facts tending, as they believe, to a better knowledge of the products of the diastatic digestion of starch. The chief results of their investigation may be summarized as follows:

- (1) When starch-paste is acted upon by malt extract above 40° the specific rotatory and reducing powers of the products indicate the existence of maltose and a non-reducing dextrin.
- (2) If the products of transformation are fractionally precipitated by alcohol, the composition of the several fractions, as shown by the optical and reducing powers, is capable of interpretation on the supposition that they consist of maltose and a non-reducing dextrin.
- (3) The authors look upon the foregoing as establishing a criterion of purity for any separated portion of the transformation products, and they ascribe any apparent departures from this rule, as regards substances described by other investigators, as due to impurities or to errors in determination.
- (4) The tendency of all starch transformations when subsequently acted upon by malt extract at 50° or 60° is rapidly to attain a state of equilibrium, which would correspond to the tenth equation  $C_{12}H_{20}O_{11} = 8H_2O = 8C_{12}H_{22}O_{11} + 2C_{12}H_{20}O_{10}$ , corresponding to equation 8 of the series shown in table 8, in which the theoretic amount of maltose contained in 100 parts of dextrans is given at each stage of conversion of starch down to No. 8. When all the products derived from starch are taken together, the reaction corresponds to an optical activity of  $(a)_{3.86} = +162.6$ , and to  $K_{3.86} = 49.3$ , and to a percentage composition of maltose 80.9 and dextrin 19.1.
- (5) The degradation of all of the higher transformations down to this point is due to the hydrolysis of the more complex polymeric dextrans and maltodextrin.
- (6) The dextrin and maltodextrin can be submitted to solution in water, to evaporation, and to frequent precipitation with alcohol without being hydrolyzed.
- (7) This is proved by the fact that the transformation products after such treatment, when subsequently fractionated with alcohol, yield fractions with a mean value of  $(a)$ , and  $K$ , coinciding with that of the original solution and also by the No. 8 equation.
- (8) The separated dextrans have not, as stated by O'Sullivan, different properties as regards their behavior with malt extract from those they possess when in solution with the other transformation products simultaneously with them.
- (9) It follows that it is possible, by submitting a separated dextrin or a mixture of dextrans to the action of malt extract at 50° to 60°, and determining the percentage of maltose they yield, to ascertain the actual or mean position of the dextrin in the polymeric series—the actual position if it be homogeneous, or the average or mean position if it be a mixture.
- (10) By making use of this process it is possible to ascertain by examination of residual products of a beer or similar liquid, after primary fermentation is concluded, the values of  $(a)$ , and  $K$  of the original starch-products as transformed by the mashing process.
- (11) With the exception of No. 8 they always found, on fractionating such preparations with alcohol, that the dextrin is not homogeneous, but belongs partly to a higher and partly to a lower equation.
- (12) It follows from No. 11 that the whole of the starch-product in a transformation is not simultaneously affected, but that some portions are hydrolyzed faster than others and that a sharp line can not be drawn between the equations higher than No. 8.
- (13) The preparation of absolutely non-reducing dextrans is impossible by the mere precipitation with alcohol, even when aided by fermentation, but this can be accomplished by treating the dextrin with alkaline mercuric cyanide.
- (14) The dextrans are not directly fermentable by yeast, but require first to be hydrolyzed.
- (15) When the action of malt extract on starch-paste is limited there is always found, among the products of transformation, besides maltose and dextrin, a third body which has optical and reducing properties corresponding to an apparent composition of 34.6 per cent of maltose and 65.4 per cent of dextrin.

TABLE 8.

Number of the stage of conversion.	Constants of the combined products.		Maltose obtained in 100 of each of the dextrans down to No. 8
	$(a)_{3.86}$	$K_{3.86}$	
Soluble starch....	216	....	84.44
1.....	209	6.4	82.09
2.....	202.2	12.7	79.20
3.....	195.4	18.9	75.39
4.....	188.7	25.2	70.37
5.....	182.1	31.3	63.33
6.....	175.6	37.3	52.77
7.....	169.0	43.3	35.18
8.....	162.6	49.3	00.00

- (16) This body is doubtless the same as that prepared in an impure state by Herzfeld, and described by him as maltodextrin.
- (17) Maltodextrin, they hold, is not a mixture of maltose and dextrin, as is proved by a number of facts.
- (18) While maltodextrin is unfermentable by yeast it is converted into fermentable, crystallizable maltose by malt extract and by certain forms of *Saccharomyces*.
- (19) They believe that maltodextrin is not a mere hydration product of achroodextrin, but that it is produced from starch and the polymeric dextrins by the fixation of a molecule of water upon the ternary group  $(C_{12}H_{20}O_{10})_3$  (of which there can not be less than 5 in the starch molecule) which results in the separation from the dextrin residue maltodextrin  $\begin{cases} (C_{12}H_{22}O_{11}) \\ (C_{12}H_{20}O_{10})_2 \end{cases}$ . This by fixation of two more molecules of water gives rise to a freely fermentable, crystallizable maltose.

In 1889, Brown and Morris (*Jour. Chem. Soc. Trans.*, 1889, LV, 449 and 462) reported the results of further investigations of the constitution of the starch-molecule, and also of the products of transformation. While adhering to their theory of the peculiarities of the groups of the molecule, they believe that the number of groups is distinctly larger than suggested in their previous work. In the first of these articles they study the amylo-dextrin of W. Nägeli in relation to soluble starch, and the relation of amylo-dextrin to maltodextrin (see pages 115 and 116).

In the second article they report their determinations of the molecular weights of carbohydrates, and state that the following hypothesis seems to them to be in accord with the facts: The starch-molecule may be pictured as consisting of 4 complex amylin groups arranged around a fifth similar group which constitutes a molecular nucleus. The first action of hydrolysis by diastase is to break up this complex and to liberate all 5 amylin groups; 4 of these groups when liberated are capable, by successive hydrolyzations through maltodextrins, of being rapidly converted into maltose, while the central amylin nucleus, by closing up the molecule, withstands the influence of hydrolyzing agents and constitutes the stable dextrin of the low equation, which, as is known, is so slowly acted upon by subsequent treatment with diastase. The 4 readily hydrolyzable amylin groups are looked upon as of equal value and in their original state to constitute the so-called high dextrins, which can never be separated completely from the low dextrin by any ordinary means of fractionation. This hypothesis provides for intermediate maltodextrins and amylo-dextrins whose number is only limited by the size of the original amylin group. Each amylin group of the 5 has the formula of  $(C_{12}H_{20}O_{10})_{20}$  and a molecular weight of 6,480, so that the entire starch-molecule, or, more correctly speaking, that of soluble starch, is represented by  $5(C_{12}H_{20}O_{10})_{20}$ , having a molecular weight of 32,400. They state that the dextrins are metameric and not polymeric compounds, as had already been suggested by O'Sullivan.

A special study of dextrins, and with reference to the dextrin products of both enzymic and acid action, was made by Effront (*Moniteur Scientifique*, 1889, 513). He notes that dextrin can be obtained pure by destroying the sugar present by lactic acid fermentation; and for the purpose of the determination of the sugar and dextrin he would destroy the sugars by ammonium hydroxide and sodium hypochlorite, and determine the dextrin by the polariscopic readings before and after the sugar destruction. (See also page 150.)

Improved processes for preparing dextrin were devised by Schumann (*Jour. Soc. Chem. Industry*, 1888, VII, 335; 1889, VIII, 295). By the first process 1 per cent of fixed acid is agitated and allowed to stand for 24 hours with cold starch in milky form. The water is then separated from the precipitated starch, and the latter is washed with fresh water until free of acid. The washed starch is once more reduced by water to a milky state at 15° Baumé, and then boiled under pressure of 3 to 4 atmospheres with 0.5 per cent of saturated sulphurous acid solution until the first trace of glucose can be detected in the product. The reaction is then stopped, the slight trace of sulphuric acid formed is fixed, and the syrup is filtered through animal charcoal and evaporated. In the second process

the starch is mixed to a thick cream with cold water and then treated with 1 per cent of its weight of sulphuric, hydrochloric, or nitric acid for 24 hours. The preparation is then washed free from acid. This prepared starch is either dried or again mixed with water to a cream, and heated to 160° to 170° in an oil bath, or by means of superheated steam, until all the starch is converted. The solution is then refined.

The percentages of dextrin, maltose, starch, etc., in commercial dextrin were studied by Hanofsky (Mittheil. d. k. k. Tech. Gew.-Museums, 1889, 56), the essential results being given in table 9.

TABLE 9.

Sample No.	Maltose.	Dextrin.	Starch.	Moisture.	Ash.	Other organic matters.	Acidity of 100 grams in c. c. of decinormal potash solution.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
1	4.25	47.78	35.55	10.11	0.27	2.04	40.0
2	10.90	36.75	43.20	7.02	0.39	1.74	26.6
3	3.75	29.46	58.00	6.85	0.60	1.34	25.3

Contrary to the findings of a number of investigators mentioned in the preceding pages, Flourens (Compt. rend., 1890, cx, 1204), from experiments on the products of the saccharification of starch with acids, was led to the conclusion, from the entire agreement of the rotatory and reducing powers at the various stages of the process, that only one and not several dextrans are formed; and also glucose, but not maltose.

In an article on the chemistry of starch and the nature of dextrans, Scheibler and Mittelmeier (Ber. d. d. chem. Gesellsch., 1890, xxiii, 3060), it is stated that dextrin could be readily purified by repeated precipitation with alcohol, or by osmosis. The dextrin thus obtained they look upon as a mixture of several dextrans. They prepared the hydrosazone by dissolving the dextrin in the cold in the presence of phenylhydrazine. The hydrosazone is hydrolyzable by diastase, and is quite soluble in water. When a cold solution of dextrin was treated with sodium amalgam a product can be precipitated by alcohol which does not reduce Fehling's solution, nor dissolve in phenylhydrazine. This body, which appears in the form of a white powder, they named *dextrite*. It is saccharified by diastase or strong acids; it is not precipitated by basic acetate of lead or by lime-water; it does not reduce Fehling's solution, but acquires reducing power by treatment with diastase or dilute acid; its solution reddens litmus; it decomposes calcium carbonate; and it is soluble in phenylhydrazine. They call attention to the fact that a number of observers have made use of oxidizing agents to destroy sugars in the preparation of dextrans upon the erroneous assumption that the dextrin itself is not affected. The non-reducing dextrin obtained in this way they believe is probably a carboxylic acid of dextrin. They also state, in support of some observers and contrary to others, that pure dextrin reduces Fehling's solution.

Leitner (Zeits. f. angew. Chem., 1890, 546) noted that permanganate brings about a conversion of starch into gummy substances which he states differ from dextrans by their acid reaction and by their yielding precipitates with basic lead acetate and barium hydroxide.

Glycerine has been employed to prepare soluble starch by restricting its action, and by further action to convert the soluble starch into dextrans or dextrin-like bodies. Zulkowski (Ber. d. d. chem. Gesellsch., 1891, xxiii, 3295) subjected starch to hot glycerol, and found that it was soon completely broken down into erythrodextrin, achroodextrin, and a number of bodies of increasing solubility as the reaction proceeded. These various substances were separated by means of their different solubilities, but it was found difficult to remove the last traces of glycerine. In a later investigation by Zulkowsky in association with Franz (Ber. d. österr. Gesellsch. z. Forderung chem. Ind., 1894, xvi, 120),

Zulkowsky's previous work was confirmed and extended. It was found, by heating starch-paste to  $190^{\circ}$ , that soluble starch was formed having a specific rotation of 188.3; at  $200^{\circ}$  erythro-dextrin appeared. It gives a cherry-red reaction with iodine, and had a specific rotation of 181. At  $210^{\circ}$  another dextrin is formed, which is recorded as giving a brownish-yellow reaction with iodine and having a specific rotatory power of  $173.5^{\circ}$ . Both dextrins were found to reduce Fehling's solution. When the preparation was heated at  $210^{\circ}$  for a long time two other products were formed, one of which resembled gum arabic. Ost (Chem. Zeit., 1895, XIX, 1501) prepared the dextrinous product according to Zulkowski's process and found a glucose value of 90 per cent. He concluded that this substance is not a true dextrin.

*Bacillus amylobacter* was used by Villiers (Compt. rend., 1891, CXII, 435, 536) to convert starch into dextrins. Potato starch was made into a paste with tap-water; the paste put in a flask which it nearly filled; the starch inoculated at  $100^{\circ}$ ; the flask closed with sterilized cotton, and placed in an oven at  $40^{\circ}$ . Usually liquefaction had occurred by the end of 24 hours. After 2 to 4 days or more a red reaction with iodine was obtained. The preparation had the odor of butyric acid, of which it contained about 0.3 part for each 100 of starch. The chief product was dextrins. He states that it remains to be shown whether or not these dextrins are identical with those formed by the action of diastase or acid, and that it is remarkable that they are formed without an accompanying formation of either maltose or glucose. Villiers prepared the dextrins by filtration, drying, resolution, and precipitation with alcohol. They occur in the form of a white, friable, hygroscopic mass, which consists of several dextrins having different rotatory and reducing powers, and different reactions with iodine. The dextrin having the highest rotatory power has the lowest reducing power and gives a red reaction with iodine, while those having lower rotatory powers and higher reducing powers do not give a color reaction with iodine. In Villiers's second article he states that besides the dextrins there is formed about 0.3 per cent of a carbohydrate that is dissolved in the alcohol used to precipitate the dextrins; that this is somewhat soluble in hot water and that it may be deposited in the form of fine crystals having the composition of  $C_{12}H_{20}O_{10} + 3H_2O$ . It is dextro-rotatory,  $(a)_D = +159.4^{\circ}$ ; it is unfermentable; it does not reduce Fehling's solution; and it does not form an osazone. Another product had the properties of cellulose, and was converted into glucose by warm, dilute mineral acids.

Dextrin-like products were obtained by Berge (Jour. Soc. Chem. Industry, 1892, XI, 448) by subjecting starch and various amylaceous substances in a dry state to the action of sulphurous anhydride. The substances are placed in a closed vessel into which sulphurous anhydride is introduced until all of the air is expelled, and the vessel then heated on an oil bath to a temperature of  $120^{\circ}$  to  $190^{\circ}$ , depending upon the nature of the product desired. Upon the completion of the conversion the gas is permitted to escape.

As far back as 1846 Magendie showed that the blood has the power of converting starch into sugar, and more or less attention was given to this property by Claude Bernard, Hensen, Schiff, von Wittich, and other investigators. In 1892 Bial, under the guidance of Röhmann, and also Röhmann himself, studied the diastatic actions of the sera of the blood and lymph. Bial (Archiv f. ges. Physiologie, 1892, LII, 137; 1893, 156) found that these sera reduce starch to dextrin, maltose, and glucose. Röhmann (Archiv f. ges. Physiologie, 1892, LII, 157; Ber. d. d. chem. Gesellsch., 1892, XXV, 3652) subjected 100 grams of potato starch converted by heat into a paste with 5 liters of water, after cooling, to the action of 1 liter of bullock's serum, to which was added 100 c.c. of a 10 per cent alcoholic solution of thymol to prevent bacterial action. This preparation was kept at  $32^{\circ}$  for 24 hours, at the end of which time there were present dextrin, giving a brown reaction with iodine, achroo-dextrin, glucose, and possibly maltose. After 10 hours' action a mixture of dextrin was present (which Röhmann calls porphyrodextrin), which gave a brown reaction with iodine,

together with some soluble starch. He states that mixtures of this body and soluble starch give iodine reactions ranging from a reddish-blue to a bluish-red, and that the so-called erythroextrin is not an individual, but a mixture of porphyrodextrin and soluble starch.

Schiffner (Inaug. Diss., Kiel, 1892) unsuccessfully attempted the preparation of the so-called maltodextrins of Brown and Morris, but in no case did he succeed in obtaining any substance that had any resemblance to the amyloins of these authors (page 115); but he obtained dextrins, isomaltose, and maltose. Dextrins, he records, are not fermentable, but they reduce Fehling's solution, and he believes that their number is not more than 2, and probably only 1 (achroodextrin), which does not give a color reaction with iodine. He thinks that other dextrins are mixtures of this with varying proportions of soluble starch, but his experiments failed to conclusively demonstrate this point. Röhmann had found that such mixtures always give a blue reaction, and therefore that erythroextrin could not be made up of such a mixture (see above).

Lintner and Düll (Ber. d. d. chem. Gesellsch., 1893, xxvi, 2533) prepared amyloextrin, erythroextrin, achroodextrin, isomaltose, and maltose. They hold that the theory of Brown and Morris of the breaking down of amylin groups is untenable, and they contend that the process is serial, amyloextrin being the first product, this being split into erythroextrin, this in turn into achroodextrin, and this into isomaltose, and this finally into maltose. Amyloextrin  $(C_{12}H_{20}O_{10})_{54}$  is precipitated, they found, by alcohol in the form of a white powder, and that it can be obtained from a 20 to 30 per cent aqueous solution in the form of spherocrystals. It is easily soluble in hot water. Even a 10 per cent solution does not reduce Fehling's solution. Iodine gives with it a blue reaction. Its rotatory power is  $(a)_D = +196$ . Amyloextrin, they state, is broken up into 3 molecules of erythroextrin  $(C_{12}H_{20}O_{10})_{54} + 3H_2O = 3(C_{12}H_{20}O_{10})_{17} \cdot (C_{12}H_{22}O_{11})$ . This is easily soluble in water, and hardly soluble in hot 50 per cent alcohol. It separates from a hot solution in dilute alcohol in the form of spherocrystals. It reduces Fehling's solution, and gives a reddish-brown reaction with iodine. The rotatory power is given as  $(a)_D = +196$ . Erythroextrin is in turn broken down into 3 molecules of achroodextrin  $3(C_{12}H_{20}O_{10})_{17} \cdot C_{12}H_{22}O_{11} + 6H_2O = (C_{12}H_{20}O_{10})_5 \cdot C_{12}H_{22}O_{11}$ . It is easily soluble in water, and almost insoluble in 70 per cent alcohol. Spherocrystals may also be obtained. Fehling's solution is reduced,  $R=10$ . No color reaction occurs with iodine. The rotatory power is  $(a)_D = +192$ . It has a slightly sweetish taste. Achroodextrin is broken down into isomaltose according to the following reaction:  $9[(C_{12}H_{20}O_{10})_5 \cdot C_{12}H_{22}O_{11}] + 45H_2O = 54 C_{12}H_{22}O_{11}$ . It was not obtained in spherocrystals. It is readily soluble in water, and soluble in 80 per cent alcohol and methyl alcohol, but insoluble in 95 per cent hot alcohol. The latter will dissolve 5 per cent of maltose. It tastes sweet. Concentrated solutions heated in water-bath turn yellow. Its rotatory power is  $(a)_D = +140$ , and its reducing power,  $R=80$ . It ferments with yeast and malt with difficulty, and is convertible into maltose. The melting-point of its osazone is  $150^\circ$  to  $153^\circ$ .

The four stages of decomposition, Lintner and Düll hold, go on simultaneously, the energy of the diastatic process decreasing so that at a definite stage of the reactions, even at favorable temperatures, *i.e.*, when two-thirds of the achroodextrin has been converted into maltose, no more maltose is produced. They believe that the stoppage of the conversion of dextrin into sugar at a temperature of  $70^\circ$  is owing to the formation of a modified form of achroodextrin that withstands diastatic action. This has, however, been explained by Pottevin, Effront, and others by a modification of the properties of the enzyme when heated to this temperature. They hold that the amyloins, or maltodextrins, of Brown and Morris, etc., are merely mixtures of dextrin and isomaltose, and sometimes really identical with the latter, and that dextrins as well as starch are composed of isomaltose groups. In a later contribution (Zeitschr. f. Brau., 1894, xvii, 339) they describe a form of achroodextrin (achroodextrin II) which is similar to the maltodextrin

of Brown and Morris, which has the formula  $(C_{12}H_{20}O_{10})_3 + H_2O$ , a specific rotatory power of  $(a)_D = +183$ , and a reducing power,  $R = 26.5$  to  $26.8$ . These figures do not agree with those of Brown and Morris.

Commercial amyloins were examined by Hiepe (*The Country Brewer's Gazette*, 1893 and 1894; *Jour. Soc. Chem. Industry*, 1894, **xiii**, 267) with especial reference to their fermentability, the percentages of dextrin and sugars, and the existence of the so-called maltodextrin. After subjecting the amyloins to fractional precipitation by alcohol, they found dextrin, maltose, isomaltose, and glucose, but no substance having the properties of maltodextrin.

Dextrin-like products of starch were investigated by Bülow (*Archiv f. ges. Physiologie*, 1895, **lxii**, 131), who prepared baryta compounds of amylo-dextrin, erythro-dextrin, and achroo-dextrin, and determined their specific rotatory and reducing powers. A number of each of these compounds were studied. Various methods were pursued to obtain pure achroo-dextrin, such as treatment with phenylhydrazine, heating in alkaline copper solution, precipitation with iron, fractional precipitation with barium hydroxide, and dialysis. Quite a number of erythro-dextrins, having different rotatory and reducing powers, are recorded as having been obtained by these means.

Meyer (*Die Stärkekörner*, etc., *loc. cit.*) prepared by means of oxalic acid an erythro-dextrin that had a rotatory power of  $(a)_D = +192^\circ$ , and a reducing power  $R = 10$ .

Lintner and Düll (*Ber. d. d. chem. Gesellsch.*, 1895, **xxviii**, 1522) investigated the products of the conversion by oxalic acid by the methods they had previously employed in diastatic digestion. The chief difference noted was in the formation of dextrose as the final product of the action of acid, while maltose was the final product of diastase. With acid they obtained amylo-dextrin, erythro-dextrin I, erythro-dextrin II  $\alpha$ , erythro-dextrin II  $\beta$ , achroo-dextrin I, achroo-dextrin II, isomaltose, and dextrose. With diastase they recorded amylo-dextrin, erythro-dextrin I, achroo-dextrin I, achroo-dextrin II, isomaltose, and maltose. The following were the color reactions of the dextrins with iodine: Erythro-dextrin I, red-violet; erythro-dextrin II  $\alpha$ , red-brown with dilute iodine solution, but blue if the iodine is concentrated; erythro-dextrin II  $\beta$ , red-brown, even with concentrated iodine; achroo-dextrins, no color reaction.

Ling and Baker (*Proc. Chem. Soc. Trans.*, 1895, **lxvii**, 702, 739) obtained, by the action of diastase, a substance which in its rotatory and reducing properties resembled Brown and Morris's maltodextrin more closely than Lintner and Düll's achroo-dextrin II. They suggest that Lintner's isomaltose may consist of maltose and a simple dextrin,  $C_{12}H_{20}O_{10} + H_2O$ . They isolated a substance having the optical and reducing properties of "isomaltose," which fermented slowly with beer yeast, leaving a residue which was not altered by diastase. This residue they state is a simple dextrin.

Ost (*Chem. Zeit.*, 1895, **xix**, 1501), in following Lintner and Düll's methods, found that a mixture of maltose and dextrin had properties corresponding to Lintner's isomaltose, and he therefore concludes that Lintner's isomaltose is an impure maltose. Ost believes it uncertain whether Lintner and Düll's erythro-dextrin and achroo-dextrin are homogeneous substances; and he calls attention to the fact that the specific rotatory power of the so-called maltodextrin and achroo-dextrin II are not the same according to different observers, and he states that Brown and Morris's non-reducing dextrin does not exist. Ost believes that the dextrinous substance obtained by Zulkowski (page 108) is not a true dextrin, and he supports the statement of Musculus and Meyer, but in opposition to Röhmann and Schifferer, that erythro-dextrins are mixtures of achroo-dextrin and starch.

Prior (*Bayerisches Brauer Jour.*, 1896, **vi**, 385) found in experiments with diastase 3 dextrins and maltose; and in some experiments with yeast also dextrose. He believes Lintner's isomaltose to be a mixture of achroo-dextrin and maltose.

Chlodounsky and Sulc (Sitzungsber. d. k. böhm. Gesellsch. d. Wissensch., 1896; Jahr. ii. d. Fort. d. Tierchemie, 1896, xxvi, 67) subjected starch-paste to the action of an extract of pancreas for 18 days at 38°. The greater part of the starch was unchanged. The solution was filtered and concentrated to a syrup, and then precipitated with 80 per cent alcohol, the precipitate being designated *dextrin I*. The alcohol was evaporated, and the solution again reduced to a syrup, and then precipitated with 90 per cent alcohol, yielding *dextrin II*. By subsequent treatment osazones were obtained. In the *dextrin I* fraction only achroodextrin could be found; and from the *dextrin II* fraction no dextrin could be obtained that was suitable for experiments, even after repeated purification. In other preparations with the glycerine extract of pancreas they state that erythro-dextrin could be detected.

A method for preparing pure commercial dextrin was reported by Berge (Bull. Assoc. Belge d. Chimistes, 1879, x, 444), which consists of subjecting raw starch to a temperature between 80° and 115° in an atmosphere of gaseous sulphur dioxide. By this means Berge prepared dextrin containing as little as 0.95 per cent of sugar. He also used sulphurous acid in a liquid state, when he found that saccharification begins at about 100°, the most favorable conditions being a temperature of 135° to 140°, a pressure of 6 atmospheres, and 25 per cent of starch in a 3 to 6 per cent solution of sulphurous acid. Saccharification was complete in about an hour.

Petit (Compt. rend., 1897, cxxv, 309, 355) prepared dextrin by subjecting boiled starch to the action of diastase at 70° for about half an hour, when it yielded a constant red reaction with iodine. He obtained the dextrin in the form of a white powder, which was non-hygroscopic and did not yield an osazone. This dextrin by further treatment was converted into saccharine products which differ somewhat according to the decomposing agent, the period of action, and the temperature.

Lintner (Chem. Zeit., 1897, xxi, 737, 752) extended his previous investigations on the chemistry of starches, especially with reference to the dextrans and their isolation. He goes on to state that the purity of the product can be fairly well determined by the application of cryoscopic and osazone tests, together with the rotatory and reducing powers, and the iodine reactions. Dilute alcohol he regards as the best agent for the isolation of the different dextrans, and he found that alcoholic barium hydroxide and alcoholic calcium-hydroxide solutions tend to bring about decomposition. The lower the molecular weight of the dextrin the greater the solubility, as a rule; but the dextrans were found to react upon one another when in solution, affecting each other's solubility. When strong alcohol is to be used he advises that only some of the total amount of alcohol be added to a hot dextrin solution, and that to this mixture be added, with brisk agitation, hot alcohol of definite volume and strength, and that the preparation then be set aside to cool to room temperature.

The products of the restricted action of diastase when boiled starch is acted upon at 70° were studied by Ling and Baker (Proc. Chem. Soc., 1897, clxxiii, 3; Jour. Chem. Soc., lxxi, 1897, 508). Besides maltose they separated a substance which was isomeric with maltose (which they believe probably consists of maltose and a simple form of dextrin already described by them), together with two forms of maltodextrin. One of the maltodextrans they look upon as being identical with the maltodextrin of Brown and Morris, while the other they identify with Prior's achroodextrin II. They hold that there is ample evidence to justify the conclusion that starch is broken down into a series of maltodextrans of decreasing rotatory power and molecular weight and increasing reducing power, all of these bodies having optical and reducing powers equivalent to mixtures of starch and maltose.

A number of erythro-dextrans were obtained by Young (Journal of Physiology, 1897-8, xxii, 401) by subjecting soluble starch to the actions of dilute acids or enzymes, arresting

the action at the proper time, and then precipitating the special dextrin by means of a proper neutral saline. Preparations of different dextrans were also made from commercial dextrin. Three forms of erythro-dextrin were investigated. Erythro-dextrin I was prepared from either commercial dextrin or a starch-digestion by saturating with magnesium sulphate, washing the precipitate with a saturated solution of this salt, dissolving the precipitate in water, and then removing any soluble starch that may be present by half saturation with ammonium sulphate. The filtrate gives a bright reddish-purple (almost magenta) without any preliminary blue, irrespective of the quantity of iodine. This iodide of dextrin, they state, is thrown down upon saturation with ammonium sulphate in the form of a darker reddish-purple precipitate. Erythro-dextrin II was obtained by saturating a solution of the mixed products with magnesium sulphate, filtering off the soluble starch and the erythro-dextrin I that are precipitated, and then saturating the filtrate with sodium sulphate at 33°. A solution of this last precipitate gives a reaction with iodine varying from a bright reddish-purple to mahogany-red. Young states that this dextrin may be made up chiefly of erythro-dextrin I, but that this is improbable. Erythro-dextrin III was prepared by the spontaneous precipitation of the filtrate obtained after saturation as above of a solution of commercial erythro-dextrin or a starch-digestion. This substance gives a red or purple reaction with iodine. If the salt had not dialyzed away previously and the iodine had been added, the filtrate, which at first is reddish-brown, becomes opaque, turbid, and brownish-black or of even a greenish tinge, and on standing a dark-blue precipitate is thrown down. If this precipitate is collected on a filter and dried it becomes reddish-brown; and if dissolved in water it forms a blue solution, which instantly changes to the red-brown characteristic color of this erythro-dextrin, the solution now behaving as an ordinary solution of iodide of erythro-dextrin III.

Young notes that glycogen gives a color reaction very closely similar to that of erythro-dextrin III, but that it can readily be distinguished from this dextrin by differences in its behavior with neutral salts. Achroodextrin was prepared by allowing diastase or dilute acid to act on starch-paste to a stage beyond the achromic point in relation to iodine. The action was stopped by boiling or by neutralization. The dextrans were precipitated by alcohol, collected, washed, dissolved in water, and then boiled on a water-bath to expel the alcohol. The solutions were cooled and treated in the usual way by saturation with neutral saline. Achroodextrin gives a very slight precipitate on saturation with ammonium sulphate, but it is doubtful, it is stated, if the precipitate is actually achroodextrin. Young notes that erythro-dextrin so-called is really a series of bodies quite distinct from either starch or the achroodextrin, and that the three products he distinguishes as erythro-dextrin I, II, and III give reactions closely similar to the corresponding products described by Lintner and Düll and obtained by other methods.

A method for separating dextrin from soluble starch was reported by Hefelmann and Schmitz-Dumont (*Zeit. f. offentl. Chem.*, 1898; *Chem. Centralbl.*, 1898, II, 561) by which, for instance, 5 grams of commercial dextrin are dissolved in 250 c.c. of cold water and placed in a flask of slightly greater capacity, to which solution is added 18 c.c. of ether, and the flask stoppered and shaken. The contaminating starch separates as a flocculent precipitate, while the dextrin remains in solution. If sugar and soluble salts are present they will remain in solution with the dextrin.

Starch-paste was liquefied and converted into dextrin without the formation of sugar by Pottevin (*Compt. rend.*, 1898, CXXVI, 1218) by the action of malt extract that had been kept for 15 to 20 minutes at a temperature of 79° to 80°. A 10 per cent solution of starch was subjected for 12 hours at 60° to malt extract that had thus been heated; and the solution was fractionated with alcohol into 3 portions. The first portion, or least soluble, gave with a trace of iodine a blue reaction, but a violet-brown with an excess; the second portion gave a reddish tint; and the third portion no coloration. In none was any sugar

present. Pottevin states that the dextrans differ only physically, and that the denser parts of the starch-grains yield a dextrin more difficult to saccharify than the other parts.

A reversion product was obtained by Syniewski (Ber. d. d. chem. Gesellsch., 1898, xxxi, 1791) in his experiments with soluble starch obtained by the action of sodium peroxide, which he believes is probably identical with a substance formed by the action of high pressure on potato starch. He noted that solutions containing more than 12.5 per cent of soluble starch deposit a white precipitate that is insoluble in cold water, and which when washed with water, alcohol, and ether, showed upon analysis that it is a derivative from soluble starch by the removal of water. In a later communication (Ann. d. Chem. u. Pharm., 1899, cccix, 282) he holds:

- (1) The starch-grains consist of a homogeneous substance having the formula  $C_6H_{10}O_5$ .
- (2) That two forms of hydrolysis occur, carbinolhydrolysis and carbonylhydrolysis, according as when in the combination with water there goes into solution an anhydrid union between two carbinol groups, or between two groups, one of which is carbonyl.
- (3) That the products from potato starch which do not reduce Fehling's solution are the results of carbonylhydrolysis.
- (4) That the simplest product of carbinolhydrolysis is amylogen ( $C_{54}H_{96}O_{48}$ ), and that the starch-molecule, as well as products of carbonylhydrolysis between starch and amylogen, consists of an unknown number of amylogen molecules in some form of an anhydrid union.
- (5) That amylogen consists of 3 maltose groups in combination with 1 dextrin residue containing 18 atoms of carbon, and that the latter is composed of 3 sugar residues, of which 2 are isomaltose residues.
- (6) That during the first stage of the hydrolysis of amylogen the maltose molecules gradually separate, and the dextrin residue remains behind, but that this finally splits into isomaltose and glucose, and the isomaltose finally into glucose.
- (7) That the diastatic hydrolysis of amylogens gives rise to intermediate products, the different stages being expressed as follows: amylogen = dextrin residue I = dextrin residue II = dextrin residue III = isomaltose = glucose.
- (8) That all the products obtained from starch by hydrolysis are to be designated dextrans. Those which originate by carbinolhydrolysis, and which do not reduce Fehling's solution he terms amylo-dextrin. The dextrin which originates from amylo-dextrin after the splitting off of all the isomaltose molecules he names grenz-dextrin. All dextrans between amylo-dextrin and grenz-dextrin, those therefore from which isomaltose can be split off, he names maltodextrin. The dextrin which originates from grenz-dextrin by the splitting off of glucose he names glueo-dextrin. (See pages 118, 135, and 147.)

In studies of the saccharification of starch by the amylase of malt, Pottevin (Compt. rend., 1898, cxxvi, 1218) dissents from the view of Brown and Morris that dextrin and maltose are formed coincidentally and not successively, and that all dextrans have the same composition, molecular weight, rotatory power, and reducing power; but he supports Duclaux in the theory that dissimilarities of dextrans are due to physical differences. By heating malt extract for 20 minutes at  $79^\circ$  to  $80^\circ$ , and treating starch-paste with it, the paste was liquefied quickly, the greater part going over into dextrin without forming any reducing sugar. The paste was prepared by subjecting 10 grams of starch in 1 liter of water for half an hour at a temperature of  $90^\circ$ , and then for the same length of time in an autoclave at  $120^\circ$ . The paste thus obtained was sufficiently transparent to allow of the determination of its rotatory power,  $(a)_D = +197.6^\circ$ .

Two liters of the paste were treated with 30 c.c. of the heated malt extract at  $60^\circ$  for 12 hours. Allowing for the correction required by the malt, the rotatory power was unchanged. A small quantity of iodine gave a violet reaction; but with an additional amount of the reagent the color became a reddish-brown. The fluid was concentrated until it contained 10 per cent of solids, and it was then fractionally precipitated with alcohol. A 63 per cent alcohol precipitated a dextrin that was colored blue at first and then brownish-

violet, and was then converted into maltose by malt extract at 63.7°. Seventy per cent alcohol precipitated erythro-dextrin from the filtrate. This dextrin was at first colored light red with iodine, and then a brownish-red, and yielded 82 per cent of maltose. In the filtrate achroo-dextrin was found, 95 per cent of which was converted into maltose.

According to Pottevin, starch is changed into dextrin, and dextrin into sugar. He explains the simultaneous presence of dextrin and sugar in certain stages of the reaction by the fact that starch is composed of several constituents having varying degrees of saccharifiability. He treated raw wheat starch with different portions of malt until 10 per cent of the original amount was left, and then prepared starch-paste from this, and also from the normal raw starch, and found when both were treated in the same manner with diastase that the former yielded only 44 per cent of maltose, while the latter gave 75 per cent.

In another contribution (Ann. d. l'Inst. Pasteur, 1899, XIII, 728) Pottevin reports on the non-homogeneity of starch-paste, and he states that the interior more labile parts of the starch-grain are transformed almost instantly into dextrans which are wholly converted into sugar, and which are soluble in 60 to 70 per cent alcohol, while the more resistant portions of the grains yield dextrans that are only partially convertible into maltose and which are insoluble in strong alcohol. He shows that the diffusibility of various dextrans differs, and also that, owing to this, some of Brown and Morris's deductions regarding their maltodextrans are erroneous. He prepared mixtures of a properly selected dextrin and maltose which were absolutely identical with the maltodextrin of Brown and Morris, which is claimed by them to be an individual, but which, however, do not exist in chemical combination.

Pottevin does not admit the existence of Lintner's isomaltose, and he holds that this body is merely a mixture of maltose and dextrin. In a third communication (Ann. d. l'Inst. Pasteur, 1899, XIII, 655) he maintains his statement in a former article that the starch-grain and also starch-paste are not homogeneous; that the less dense portions are readily converted into dextrin and this into sugar, while the more dense particles are converted slowly and not completely, and that there is always present at the end of saccharification a residue of stable dextrin. Pottevin studied especially the different phases of the reaction with reference to the properties of the enzyme used, and he holds that the enzyme is to be regarded as being a mixture, dextrin-forming and sugar-forming, the one converting starch into dextrin and the other converting dextrin into sugar.

Stable dextrin in its relations to maltodextrin and soluble starch was studied by Brown and Millar (Proc. Chem. Soc., 1899, xv, 13). They ascertained that when starch is transformed by active diastase at a temperature of 60° the reaction goes on very rapidly until a stage is reached when the rotatory power corresponds to  $(a)_D = +153$ , and the reducing value  $R = 80$ , at which time there is present maltose and a resistant dextrin having properties corresponding to  $(a)_D = +195.7$  and  $R = 5.7$  to  $5.9$ . The reducing power they believe to be inherent in the dextrin, as is indicated by the fact, as they state, that oxidation of the dextrin gives rise to dextrinic acid, a polysaccharid acid; and they believe that both the stable dextrin and the dextrinic acid are built up of  $C_6$  groups, and not  $C_{12}$  groups, as in the case of maltodextrin. They state that the dextrin molecule may be regarded empirically as composed of 39  $C_6H_{10}O_5$  groups in combination with a terminal  $C_6H_{12}O_6$  group, or, more correctly, as a condensation of 40 glucose molecules with the elimination of 39  $H_2O$ .

Petit (Compt. rend., 1899, cxxviii, 1176) supplemented the investigations above referred to. He prepared dextrin by the action of a 1 per cent of malt diastase on starch paste at 70°. The molecular weight of the dextrin he estimated to be 485, corresponding to the formula  $(C_6H_{10}O_5)_3$ . Its rotatory power was  $(a)_D = +166.6$ , and its reducing power,  $R = 18$ . Subjected to diastase at 50° to 55°, it was broken down into maltose and a residue

of the same dextrin. In a still later contribution (Compt. rend., 1900, cxxxI, 453) Petit studied the dextrans formed at 50°, 60°, and 70°, respectively, which were separated by successive precipitations with alcohol.

The figures given in table 10 are recorded.

TABLE 10.

	Reducing power.	Glucose equivalent.	Rotatory power ( $\alpha$ ) <sub>D</sub> =	Molecular weight (by freezing).
At 50°	11.8	103.5	+183°	1,096
At 60°	11.2	103.0	+185°	1,310
At 70°	16.3	103.1	+197°	723

The first two he regards as being probably identical, but quite different from the third.

Differences in the products of diastatic and acid action were recorded by van Laer (Jour. Fed. Inst. Brew., 1900, VI, 162), who notes that not only are the sugar-products not identical, but also the intermediate bodies. (See page 152.) Acetyl derivatives were studied by Pregl (Monatsch. f. Chem., 1901, xxII, 1049), who found a substance that gave a red reaction with iodine, and which he believes is an erythro-dextrin. It is stated to reduce Fehling's solution and to have a reducing power equal to about 12.5 per cent of that of glucose. He could not identify it with any known dextrin, but states that it resembles a dextrin described by Syniewski, which was obtained by the action of malt extract.

Baker (Jour. Chem. Soc., 1902, xvIII, 134) investigated the actions of the diastase of ungerminated barley on soluble starch, in which the reaction was caused to continue for 1½ hours at 50°, when it was found that dextrans and maltose were the only products. One of the dextrans obtained by precipitation with alcohol is recorded as giving a blue reaction with iodine and to be acted upon by the barley diastase very slowly. After digestion for 90 hours at 45° to 50°, the product is stated to yield a blue reaction with iodine and to consist of unaltered dextrin with maltose and glucose. After 18 hours at 55°, by the action of *malt* diastase, this dextrin no longer gave a blue reaction with iodine, and the products consisted of achroodextrin and a considerable quantity of glucose. The dextrin formed by barley diastase differed from Nägeli's amylo-dextrin, and he proposes, owing to its general behavior, to name it *r-amylo-dextrin*.

Erythro-dextrin was described by Hale as being one of the products formed by the primary action of iodine and oxidizing agents on starch (Amer. Jour. Science, 1902, xIII, 379). By titrating an arsenite or tartar emetic solution with iodine and impure starch, a loss of iodine occurs and a compound forms that gives a red reaction with iodine. This compound behaved like erythro-dextrin, but it was not identical with it. Impure starch yielded also another substance which seemed to be intermediate between starch and erythro-dextrin. He states that amidulin stands between soluble starch and erythro-dextrin and that the last is the first product of amidulin.

In a further report on the constitution and decomposition products of starch, Syniewski (Rozfrawy ahademji umiejetnosci, Krakau, 1902; Jahr. ü. d. Fort. d. Tierchemie, 1902, xxxII, 98, 100) subjected a 5 per cent solution of potato-starch to a temperature of 138° to 140° for 12 hours, and obtained amylo-dextrin. The hydrolysis of amylo-dextrin by malt extract at room temperature yielded a dextrin which he named *grenzdextrin I*. Malt extract acting on this dextrin produced maltose and an isomer of maltose which he named *dextrinose*. By heating malt extract for 15 minutes and then adding amylo-dextrin solution, and stopping the action at the point when the solution no longer gave a blue reaction with iodine, the amylo-dextrin was found to have been converted into a form distinguished as *grenzdextrin II*. This he believes is identical with Brown and Morris's maltodextrin,

with Ling and Baker's  $\alpha$ -maltodextrin, and with Lintner's achroodextrin II. Grenz-dextrin II is hydrolyzed by malt extract into maltose and a compound named  $\gamma$ -maltodextrin, which in turn may be broken down into maltose and dextrinose.

From this and his previous investigations, Syniewski holds that the starch-molecule consists of 4 amylogen groups, its formula being  $C_{216}H_{360}O_{180}$ , each group consisting of 9 glucose groups united through their 9 carbonyl groups, thus proving that only monocarbonyl groups exist among the glucose groups. The glucose groups, he conceives, are arranged into 1 dextrin group consisting of 3 rings of glucose groups linked together, and 3 maltose groups united to the dextrin group. The amylogen groups are conceived to be so combined in the starch-molecule that each is united to three others through 6 carbinol linkages, the carbinol linkage existing between the dextrin groups (*d*-carbinol linkage) as well as between the maltose groups (*m*-carbinol linkage) of the amylogen groups. Amylodextrin, he states, is derived from the starch-molecule by the decomposition of *m*-carbinol linkage on the addition of 6 molecules of water. Heating starch-paste at  $140^{\circ}$  will not cause a decomposition of starch, but such decomposition can be brought about, he found, by diastase. Malt extract, by bursting the dextrin ring at first, releases a carbonyl combination between the two glucose groups of each dextrin group, and then gradually all the maltose groups are separated from the dextrin group, producing grenzdextrin I, which was made up of dextrin groups united through *d*-carbinol linkages. The further action of diastase, he writes, causes the separating of one maltose group from each dextrin group of the grenzdextrin, finally resulting in maltose and its isomer dextrinose, in the molecule of which the glucose groups are united by *d*-carbinol bonds. Malt extract at  $78^{\circ}$  likewise breaks the carbonyl bonds, primarily those which bring the glucose groups to the dextrin ring, thus forming grenzdextrin II, which is put down as being composed of 2 complexes ( $C_{18}$ ) united by *d*-carbinol linkage consisting of 3 glucose groups linked together through carbonyl bonds. It is conceivable, he states, that the separation of a maltose residue from this dextrin will produce  $\gamma$ -maltodextrin and that dextrinose results from the further splitting of the maltose group. Although the carbinol linkage seems to have great resistance to the hydrolytic action of diastase, dextrinose nevertheless finally breaks up into 2 molecules of glucose.

Another communication by Syniewski (*ibid.*, page 109) reports the changes brought about in starch by subjection to the action of 40 per cent formaldehyde for 2 months. He investigated especially the iodine combinations of amylodextrin and concluded that each amylogen residue of the amylodextrin takes up 3 atoms of iodine, these atoms probably replacing the hydroxyls of the primary alcohol group  $CH_2(OH)$ , each amylogen residue containing 3 such hydroxyls. That the hydroxyls of the primary and not the secondary groups are replaced by the iodine is, he states, confirmed by barium compounds of amylogen, in which undoubtedly the primary hydroxyls are replaced by barium, the barium compound not combining with iodine, inasmuch as it shows no iodine reaction. This conception of the composition of iodine-amylodextrin lends probably, Syniewski holds, to the theory that the same alcohol groups of the amylogen residues also take part in the reaction with formaldehyde, the primary alcohol groups evidently being replaced by formaldehyde groups in the compound of amylodextrin and formaldehyde. By the hydrolytic action of added water, or by the addition of acids, one hydroxyl group after another will be set free, giving rise to color manifestations induced by the addition of iodine. This change of color in the iodine reaction, which occurs also in the diastatic hydrolysis of starch, is due, he holds, to the gradual breaking away of the dextrin molecules from the maltose group, containing primary alcohol groups. It is therefore probable that the dextrans I and II, described in 1899, would show a red or brown color with iodine.

In experimental studies of the processes concerned in the conversion of starch into sugar, Moreau (Ann. d. d. Soc. roy. d. Sc. méd. e. nat. d. Bruxelles, 1903, xii, 117; Jahr.

ü. d. Fort. d. Thierchemie, 1903, xxxiii, 106) ascertained that precipitation of the products of digestion by an aqueous solution of barium hydroxide does not throw down achroodextrin, while with an alcoholic solution of barium hydroxide the achroodextrin can not be separated from the erythro-dextrin; but by precipitating the amylo-dextrin and erythro-dextrin with an aqueous solution, and subsequently the achroodextrin in an alcoholic solution, the dextrin may be separated, and also a minute quantity of an undetermined dextrin-like substance. Repeated precipitation with barium hydroxide was found to free the dextrans from adherent sugars. Prolonged digestion of starch always gave a residue that was not starch, dextrin, or cellulose. According to Moreau, dextrans do not exist as such in the starch molecule, nor are they produced by boiling a 1 per cent starch-paste, as maintained by Griessmayer. Moreau is of the opinion that Griessmayer's results were due to the presence of micro-organisms.

In order to avoid misunderstanding in the use of terms applied to the intermediate products of the saccharification of starch, Ling (*Jour. Fed. Inst. Brewing*, 1903, ix, 446) proposes to discontinue the use of the word dextrin as applied to the products of diastatic and acid digestion, and substitute the word maltodextrin, which he thinks would not only specifically indicate the mode of origin, but also distinguish those products from torrefaction dextrin.

Achroodextrin was prepared from peat, lichens, and moss by Reynaud (*Jour. Soc. Chem. Industry*, 1903, xxii, 567). A comparison of the products of potato starch with those of cereal starches was made by O'Sullivan (*Proc. Chem. Soc.*, 1904, xx, 65), who states that no quantitative relationships were found between the percentages of maltose and dextrin of the former with those of the latter (see p. 148).

The rapidity of hydrolysis by the action of dilute acids upon dextrin and maltose was studied by Noyes and several associates (*Jour. Amer. Chem. Soc.*, 1904, xxvi, 266), who recorded that dilute hydrochloric acid will hydrolyze dextrin only half as rapidly as it will maltose. A 2.5 per cent hydrochloric acid hardly affected the reducing power of dextrin, but the hydrolysis of maltose at 100° reached 90 per cent in an hour, and somewhat less than 95 per cent in 2 hours.

The behavior of starch, amylo-dextrin, and erythro-dextrin towards chromic acid was investigated by Hänz (*Beiheft. z. Botan. Centralbl.*, 1905; *Woch. f. Brau*, 1905, xxii, 721). The author states that chromic acid forms combinations with these three substances that are analogous to those formed by iodine; and that all three behave in such a way as to indicate that not one is a uniform substance, and that they really consist of a number of groups which differ in density or complexity of their molecular structures. Achroodextrin seemed to be of apparently uniform composition.

The method employed by Bülow (p. 130) for the separation of the several products of digestion by aqueous and alcoholic solutions of barium hydroxide was improved by Moreau (*Ann. d. d. Soc. roy. Se. méd. nat. d. Bruxelles*, 1905; *Woch. f. Brau*, 1905, xxii, 37). The progress of the reaction was determined by iodine. He found that amylo-dextrin and erythro-dextrin were precipitated from aqueous solutions, but not achroodextrin or sugar, both of which were precipitated in the presence of alcohol. The limits of precipitation were sufficiently far apart to permit of a complete separation of the several constituents by successive fractional precipitation by barium hydroxide. By this method he found in the digestion of starch by diastase, ptyalin, pancreatin, serum, or mineral acids that even during the very earliest stages of the decomposition processes all three forms of dextrin, as well as sugar, were present. He states that this confirms Mittelmayer's theory of starch conversion, that is, that the starch molecule is at once broken down into these products, and that there may be some subsequent hydrolysis of the dextrans first formed. Moreau gives in detail processes for preparing amylo-dextrin, erythro-dextrin, and achroodextrin from commercial dextrin. The first two he prepared

absolutely free of reducing power, but the achroodextrin retained a slight reducing power, even after many successive precipitations. Because of the absence of reducing power in amylo-dextrin and erythro-dextrin, Moreau believes that sugar does not constitute a part of the dextrin molecule.

Many investigators have referred to the existence of a dextrin residue that is not converted into sugar, but Fernbach and Wolff (Compt. rend., 1906, CXLII, 1216) found that if the residual dextrin is separated it may be saccharified by malt extract, and therefore that if any dextrin exists which is not convertible into maltose it must be but an infinitesimal part of the original starch.

The dextrinizing action of formaldehyde was referred to on page 109, in connection with Syniewski's investigations, the results of which have received confirmation in the investigations of Reichard (Zeit. ges. Brauw, 1908, XXXI, 161), who noted the succession of different color reactions with iodine as the processes proceeded. Erythro-dextrins were prepared by Tanret (Compt. rend., 1909, CXLVIII, 1775) from a weak alcoholic solution of the insoluble precipitate (reverted starch) that formed in soluble starch on cooling, the quantity of erythro-dextrins obtained representing about 8 per cent of the total matter dissolved in alcohol.

A colorimetric method for determining the molecular weights of starch and its decomposition products and related carbohydrates was reported by Wacker (Ber. d. d. chem. Gesellsch., 1908, XLI, 266; 1909, XLII, 2675). From these observations both erythro-dextrin and achroodextrin have the values of 4 hexose groups.

#### ISOMALTOSE, MALTOSE, GLUCOSE, SACCHAROSE, ETC.

The discovery of sugar as a product of the activity of weak acids on starch was made by Kirehloff in 1811 (Schweigger's Journal, 1815, XIV, 389), and 3 years later he found that gluten and germinating barley also gave rise to sugar. He and his contemporaries referred to this sugar as glucose. Over 30 years later Dubrunfaut (Ann. de chim. et phys., 1847, XXI, 178) isolated this body, and from a study of its properties he showed that it is not identical with glucose. This discovery seems, however, to have made no impression, since the literature of the subject shows that various investigators up to 1872 refer to this sugar as glucose; and even Musculus based his theory of the hydrolysis of starch into dextrin and sugar upon the assumption that he was dealing with glucose. O'Sullivan (Jour. Chem. Soc., 1872, x, 579) rediscovered the sugar described by Dubrunfaut, and found that it had only 65 per cent of the reducing power of glucose, and named it *maltose*. Petit (Bull. d. l. Soc. chim., 1875, XXIV, 519) records, after digesting starch-paste with diastase, that the preparation contained 5 per cent of dextrin and two kinds of sugar, one of which he states reduces an alkaline solution of copper oxide, while the other is without influence; the latter, after being boiled with 1 per cent sulphuric acid, is saccharified, the resulting sugar being composed of about three-fourths of this non-reducing sugar.

Sachsse (Sitzungsber. d. Naturf. Gesellsch., Leipzig, 1877; Jahr. ü. d. Fort. d. Tierchemie, 1877, VII, 60), in discussing the formula usually given for starch that was objected to by W. Nägeli, who proposed for the accepted  $C_6H_{10}O_5$  the formula  $C_{36}H_{62}O_{31}$ , states that it is the same as  $6(C_6H_{10}O_5) + H_2O$ , and that this slight alteration is of interest analytically, as it takes into consideration the amount of starch in the relative determination in the conversion into sugar. If the formula read  $C_6H_{10}O_5$  (mol. weight 162), 180 molecules of dextrose would be derived from 162 of starch, or 100 : 90; but if it read according to Nägeli, there would be 1,080 molecules of dextrose from 990 of starch, or 108 : 99. Correct results, Sachsse states, are obtained by changing the formula to  $C_{36}H_{62}O_{31}$ , while in using the other formula  $C_6H_{10}O_5$  there was always an inexplicable difference of 1 to 2 per cent.

In an investigation of the products of activity of salivary and pancreatic enzymes in the digestion of glycogen, Seegen (Centralbl. f. med. Wissensch, 1876, xiv, 849) found that even though the glycogen was completely digested there was formed a much smaller amount of glucose than should be expected, only from 34 to 41 per cent of the calculated amount in the case of saliva, and from 45 to 48 per cent in case of the pancreatic extract; and he believes that some other kind of sugar is produced, or some other form of decomposition product.

Nasse (Archiv f. ges. Physiologie, 1877, xiv, 473) confirmed Seegen's work and extended the investigations to starch. Nasse digested boiled arrowroot starch with filtered human saliva on a water bath at 40°, varying the quantity of starch and the period of digestion. In most cases the reducing power of the product amounted to only 48 per cent of that called for. Even when 0.3 gram of starch in 20 c.c. of water and 15 to 20 c.c. of saliva were digested from 6 to 7 hours, the reducing power was below 45 per cent of what should be expected. He holds that animal diastatic ferments do not convert starch into glucose, but into a form of sugar which he names *amylum ptyalose*, which when boiled with dilute sulphuric acid has its reducing power doubled. He also found aehroodextrin. In experiments with glycogen he determined the presence of both ptyalose and aehroodextrin.

Musculus and Gruber (Zeit. f. physiolog. Chemie, 1878, ii, 177) record both maltose and dextrose. The former, having the formula  $C_{12}H_{22}O_{11}$ , a rotatory power of  $(\alpha) = +150^\circ$ , and a reducing power of 66, is believed by them to be formed from aehroodextrin and to be convertible into glucose, each molecule being transformed by hydrolysis and cleavage into 2 molecules of glucose according to the following:  $C_{12}H_{22}O_{11} + H_2O = 2(C_6H_{12}O_6)$ , glucose having a rotatory power of  $(\alpha) = +56$  and a reducing power of 100.

Nasse's assertion that a special form of sugar, and not glucose, results from the salivary digestion of starch was shown to be erroneous by Musculus and von Mering (Zeit. f. physiolog. Chemie, 1878, ii, 403), who ascertained that the conversion of starch by saliva is analogous to that by diastase, giving rise to aehroodextrin, maltose, and glucose. After repeated experiments they determined that under ordinary circumstances the quantities of maltose and glucose formed by the action of saliva or diastase are 70 and 1 per cent, respectively, of the original quantity of starch.

Brown and Heron (Ann. d. Chem. u Pharm., 1879, cxcix, 241) using malt-extract recorded the production of maltose, and state that the results of their experiments indicate that even by the continued action on starch no glucose is formed, and that maltose is the end product, undergoing no further change. Pancreatic extract gave rise to both maltose and glucose. The contradictory results of Musculus and von Mering on the one hand by diastase, and of Brown and Heron on the other, as regards the formation of glucose by diastase, were discussed by von Mering (Zeit. f. physiolog. Chemie, 1881, v, 185), who attempted an explanation of the disagreement. Von Mering treated potato starch-paste with diastase for 4 hours at 60° to 70°, and then for 20 hours at room-temperature, and found by ordinary fractional precipitation of an alcoholic solution with ether that glucose was present. The prolonged action of diastase increased the amounts of both glucose and strongly reducing dextrins. Shorter action produced maltose, but no glucose. Maltose remained unchanged after 2 hours' treatment with diastase at 60°, but after 24 hours the reducing power was increased and the rotatory power decreased, indicating glucose formation. Putrefaction, watery yeast-solution, and emulsin did not convert maltose into glucose; but by the prolonged action of saliva or pancreatic extract both maltose and glucose were formed, the pancreatic extract being energetic in the conversion of maltose into glucose. He states that diastase and saliva will convert starch-paste into 2 dextrins, one of which is broken down into maltose and a secondary glucose, while the other remains unchanged by the same ferments.

Jaquelain in 1840 (*loc. cit.*) recorded that when starch is subjected to a temperature of  $160^{\circ}$  it is converted in part into dextrin and glucose. This statement was confirmed or contradicted by different observers so that Soxhlet (Reportorium f. analyt. Chemie, 1881; *Jahr. ü. d. Fort. d. Thierchemie*, 1881, xi, 86) was led to repeat the experiments to determine the cause of the disagreement. Soxhlet found by a preliminary test that a given kind and quantity of starch at  $149^{\circ}$  yielded a larger amount of sugar the less the quantity of water present, from which he concluded that commercial starch contains a saccharifying substance whose power is lessened by dilution. He also noted that by subjecting specimens of the same starch for 5 hours at  $149^{\circ}$  entirely different proportions of sugar were formed in accordance with the amount of acid present. Potato starch and wheat starch showed acidity, 100 grams having an acidity equal to from 0.06 to 0.4 gram per 100 of sulphuric acid; but rice and corn starch usually exhibit an alkaline reaction. He notes also that even commercial starch has some reducing action, which may be due to the presence of preformed sugar.

Salomon (Reportorium f. analyt. Chemie, 1881; *Jahr. ü. d. Fort. d. Thierchemie*, 1881, xi, 86) does not agree with Sachsse's statement regarding the formula for starch and the percentage of sugar obtainable (page 138). He found that the proportion of sugar conforms best with the formula  $C_6H_{10}O_5$  for starch. In the conversion of starch into dextrose, according to the equation  $x(C_6H_{10}O_5) + H_2O = C_6H_{12}O_6$ , the yield of glucose should be 111.11 parts of dextrose to 100 parts of starch, whereas according to Nägeli's formula the amount would be 109.09. Salomon saccharified potato starch by boiling with dilute acid according to the method of Sachsse, and obtained 111.16 and 111.11 per cent of glucose. In other communications (*Jour. f. prakt. Chemie*, 1882, xxv, 348, and xxvi, 324) he notes that while he obtained the proportion required on the basis of the formula being  $C_6H_{10}O_5$ , the percentages found by other investigators in acid hydrolysis were lower—by Brunner 107, de Saussure 110, and von Allihn only 106 to 107. He reasons that these differences may be due to incomplete desiccation of the starch or to faulty methods in determining the quantities of sugar. While he obtained the required theoretical quantity with potato starch, he recorded only 106.8 per cent with rice starch, which difference he attributes to incomplete saccharification owing to an injurious effect of alkali upon the starch during the process of preparation. Salomon (*Jour. f. prakt. Chemie*, 1883, xxviii, 82) in a later investigation asserts his belief that there is only one kind of dextrin formed during saccharification of starch; and that glucose, but not maltose, is formed by the action of sulphuric, oxalic, citric, or tartaric acid, the conclusion as regards sugar being based upon the determination of the specific gravity and rotatory and reducing powers.

The results of a study of wheat starch by Schulze (*Jour. f. prakt. Chemie.*, 1883, xxviii, 311) support Salomon's conclusions regarding the formula of starch, the per cent of glucose, and of glucose being the sole sugar product. Schulze dried wheat starch and saccharified it by dilute hydrochloric acid, and obtained by von Allihn's method (copper reduction) 110.986 of glucose per 100 of starch, corresponding almost exactly to the formula  $C_6H_{10}O_5$ . By the specific-gravity method he recorded 111.4; and by the polariscope method, 111.85. By treatment with acetic acid under pressure he obtained a dextrin corresponding to the  $\alpha$ -dextrin (erythrodextrin) of Bondonneau. Heating for 4 hours produced almost exclusively this dextrin, but by continued heating the dextrin partially goes over into glucose.

The maximum conversion of starch into sugar under varying conditions was investigated by von Allihn (*Zeit. d. Ver. f. d. Rubenzucker-Industrie*, 1883, 786; *Dingl. Polytech. Jour.*, 1884, ccl, 554). Ten grams of anhydrous starch, containing 0.9 per cent of ash and 0.3 per cent of insoluble residue, were subjected to 100 c.c. of dilute hydrochloric acid of strengths varying from 1.33 to 10 per cent, and boiled for periods ranging from 2 minutes to  $2\frac{1}{2}$  hours. Table 11 is a statement of his results.

TABLE 11.

Duration of boiling.	Per cent of hydrochloric acid.					Percentage of starch converted into sugar by boiling with the dilute acid.
	10	5	3.33	2	1.33	
2 minutes	92.6	....	....	....	....	}
5 "	92.1	....	....	....	....	
10 "	....	90.6	....	....	....	
15 "	91.7	....	....	....	....	
30 "	89.6	94.3	93.27	81.91	....	
50 "	87.4	93.3	....	....	....	
60 "	....	....	94.65	93.68	87.85	
90 "	....	....	94.49	95.05	92.87	
105 "	....	....	....	94.89	....	
120 "	....	....	....	....	93.84	
150 "	....	....	....	....	94.65	

Von Allihn recommends a 2 per cent solution of acid, and that the glucose, if wanted pure, be recrystallized from methyl alcohol of a specific gravity of 0.816.

Starch syrup was found by Sieben (Zeit. d. Vereins f. d. Rubenzucker-Industrie, 1884, 837) to contain 21.70 per cent of dextrose, 15.80 of maltose, 41.96 of dextrin, 20.10 of water, and 0.3 of ash. Incidentally it is of interest to note that genuine honey contains neither dextrin nor maltose, and that Sieben found 34.71 per cent of glucose, 39.24 of levulose, 1.08 of saccharose, 19.98 of water, and 5.02 of non-saccharine matter, and total per cent of sugar 75.02.

Brown and Morris (Ann. d. Chem. u. Phar., 1885, ccxxxI, 72; Jour. Chem. Soc., 1885, XLVII, 527), by the action of malt extract at 50° to 60°, recorded at the end of digestion 80.9 per cent of maltose and 19.1 per cent of dextrin, and they hold that the properties of the products of the diastatic digestion of starch can be fully accounted for by the presence only of maltose and a non-reducing dextrin. Lintner (Jour. f. prakt. Chemie, 1887, xxxvi, 481) looks upon maltose as being the sugar-product of starch digestion by diastase. Bourquelot (Compt. rend. 1887, civ, 71, 576) asserts that both maltose and glucose are products of enzymic activity.

An important discovery bearing upon the explanation of the presence or absence of glucose among the products of digestion was made by Cuisinier (Chem. Centralbl., 1886, xvii, 614; Moniteur scient., 1886, 718), who noted that since glucose is formed in both normal barley and barley malt and in certain other cereals but not in others, there must be a special enzyme present which forms glucose. This hypothetical enzyme he named *glucase*. He found upon macerating corn-meal in water at a temperature of 50° that sugar was formed, and in order to determine the nature of the saccharifying agent, and also the character of the sugar, he carried out a series of experiments in which he found that corn contains an enzyme which converts starch into dextrans and glucose, and that the dextrans themselves are finally changed to glucose. He also showed that this enzyme converted maltose into glucose, and that it acts upon starch-paste as well as upon raw starch. It has since been rendered evident that Cuisinier's enzyme was a mixture of diastase and glucase.

The existence of such an enzyme as glucase was foreshadowed by the work of Brown and Heron (1880), who found that while diastase gave rise to maltose, extract of the pancreas or of the small intestine not only formed maltose but also had the power of converting maltose into glucose. Von Mering (1881) confirmed Brown and Heron's statement of the action of extract of the pancreas. Bourquelet (1883) added further confirmation in experiments with extracts carried on under aseptic conditions; and he also obtained proof that the yeast plant and certain other low organisms secrete an enzyme which hydrolyzes maltose into glucose. Since the announcement of Cuisinier, it has been shown that glucase has probably a wide distribution in plant life.

Geduld (Wochenschrift f. Brauerei, 1892, VIII, 620) also extracted glucase from corn by soaking the grist in cold water, filtering, and precipitating with alcohol. At temperatures from 57° to 60°, 1 part converted 100 parts of maltose into glucose. This enzyme was prepared later by Beijerinck and others. The same or a similar enzyme has been shown to exist in the sera of the blood and lymph, in the saliva and pancreatic juice and succus entericus, and in yeast and malt, and in emulsin and certain other enzymes. Its presence or absence, as well as the amount present, serve at times to explain many discrepancies in the results of various observers as to the kind or kinds of sugar formed. In fact, diastatic enzymes as ordinarily prepared, especially animal enzymes, are apt to contain more or less glucase, and hence to give rise to more or less conversion of maltose into glucose (see page 143).

Differences in the sugar product by the actions of enzymes and dilute acids, respectively, were noted by Effront (Moniteur scient., 1887, 513). He states that maltose is always formed by the actions of enzymes and acids; that in the saccharification by malt the production of glucose is variable; that by the action of acid glucose is always formed with maltose in the earliest stages; and that in the earliest stages there is produced an almost constant ratio between the percentages of maltose and glucose (34 to 38 of maltose to 100 of glucose).

Griessmayer (Allgem. Brauer- und Hopfenzeit., 1887, xxvi, 147), in his studies of the nature of the starch-cellulose of C. Nägeli, refers to the presence of maltose and glucose after treating starch-grains with dilute acid until the skeleton-like substance (starch-cellulose) remained. Hanofsky (Mittheil. d. k. k. Tech. Gew.-Museums, 1889, 56), in analyses of commercial dextrin, makes note of the presence of maltose and also of the conversion of this sugar into glucose by further action of dilute acid. Brown and Morris (Jour. Chem. Soc. Trans., 1889, LV, 462) refer to maltose as the final product of the action of diastase. Lintner (Brauer u. Malzerkalender, 1890, XIII, 83), in his examinations of the action of diastase on raw starch from different sources at different temperatures, filtered the preparations and changed the dissolved substances into glucose by hydrochloric acid. Flourens (Compt. rend., 1890, cx, 1204) subjected starch to the action of dilute sulphuric acid and found that the properties of the products of digestion as determined by their rotatory and reducing powers are in accord with the belief that only one dextrin together with glucose is formed, but no maltose.

The synthesis of a new glucobiose, now known as isomaltose, which is of importance as one of the end-products of the saccharification of starch, was reported by Fischer (Ber. d. d. chem. Gesellschaft., 1890, XXIII, 3687; 1895, XXVIII, 3024). This sugar was obtained by subjecting 100 grams of glucose in 400 grams of hydrochloric acid of a specific gravity of 1.19 for 15 hours at a temperature of 10° to 15°. By precipitation with alcohol, a flocculent deposit was obtained which contained a small amount of isomaltose together with other substances. From the filtrate a precipitate was thrown down by ether, which consisted of glucose, isomaltose, and another substance. By appropriate methods isomaltose was isolated and its properties studied.

A body was prepared by Schiebler and Mittelmeier (Ber. d. d. chem. Gesellsch., 1890, XXIII, 3060; 1890, XXIV, 301) from the unfermented residue of starch sugar, which they state is identical with the isomaltose of Fischer that was prepared from glucose by the action of acid. This residue, referred to as *gallisin*, was purified by repeated precipitation with strong alcohol, and they found that it yielded an osazone which resembled the osazones of saccharoses, from which they concluded that *gallisin* contains a sugar. At first it was thought that this sugar was an intermediate product between starch-cellulose and glucose, but in their second contribution they recognize that the new sugar is formed from glucose. They subjected glucose to the action of 2.5 per cent sulphuric acid for 12 hours on a water-bath. The acid was then gotten rid of by barium hydrate, and the solution heated with phenylhydrazine acetate, when a large quantity of glucosone separated;

and on cooling the filtered solution another osazone was obtained which was identical with the osazone prepared from gallsin. Frequent mention is made of isomaltose in the subsequent literature on the products of starch digestion.

Tollens (Ann. d. Chem. u. Pharm., 1890, cclvii, 150) prepared from corn a syrup from which a sugar crystallized, which he states had all the properties of *saccharose*. In analyses of sweet corn at different stages of growth he found *saccharose*, glucose, dextrin, and other bodies. By the action of butyric acid ferment (*B. amylobacter*), Villiers (Compt. rend., 1891, cxii, 536) found among other products a small amount of a crystalline carbohydrate which was converted into glucose by warm hydrochloric acid; and there was also another product which resembled cellulose, which was converted into glucose by acid. Lintner (Zeit. f. d. ges. Brauwesen, 1891, 281), in experiments with beer-worts, to which he added phenylhydrazine acetate, recorded that an osazone was formed which in crystalline properties and melting-point was identical with the isomaltose of Fischer. Later, he and Düll prepared isomaltose from the products of the action of diastase on starch.

The saccharine products of the fermentation of glucose by means of pure cultures of *Sacch. cerevisiæ* and *S. apunculatus* were examined by Reinke (Zeit. f. Spiritusind., 1892, 79). His results were such as to indicate that only glucose was fermented. The dextrin present was determined by inversion with hydrochloric acid, while the percentage of isomaltose was determined by the residual reducing power after fermentation. The figures are recorded in table 12.

TABLE 12.

	Solid glucose.	Syrup A.	Syrup B.
Dextrose (fermentable) . . . . .	62.38	30.10	32.76
Isomaltose (reducing-power) = 84 per cent of that of maltose	13.67	22.48	22.64
Dextrin . . . . .	3.22	26.91	25.13

The existence of Cuisinier's glucose (p. 141) was denied by Lintner (Wochenschr. f. Brauerei, 1889, v, 1038) because he failed to find glucose as well as maltose in experiments similar to those of Cuisinier. In a later research, Lintner (Zeit. ges. Brauw., 1892, xv, 123) acknowledges his error, and notes that while barley and wheat contain but little glucase, corn contains much. He prepared glucose by mixing a thin starch-paste with corn-meal, and keeping the mixture at 60° for 30 to 48 hours. The filtrate if concentrated will crystallize. The amount of glucose, he states, is small in comparison with maltose. In another article published at the same time, Lintner and Düll (Zeit. ges. Brauw., 1892, xv, 145) give a detailed method for the preparation of isomaltose, by which a pure isomaltose can be obtained, and in quantity representing 20 per cent of the original starch. This sugar they found to ferment with yeast much more slowly than maltose. It had the same specific rotatory power as maltose ( $a_D = +140^\circ$ ), but its reducing power was found to be only 83 per cent of that of maltose. Its osazone melted at 150° to 153°, while that of maltose melts at 206°.

The sole products of the action of diastase on starch are, according to Schifferer (Neue Zeit. Rub. Zuck. Ind., 1892, xxix, 167), dextrin or dextrins, isomaltose, and maltose. The maltodextrin of Brown and Morris he believes is probably a mixture of 67 per cent of dextrin and 33 per cent of isomaltose, while the maltodextrin of Herzfeld he regards as consisting of 26 per cent of dextrin and 74 per cent of isomaltose, and he thinks that Herzfeld must have unknowingly fractionated isomaltose from his so-called maltodextrin. Schifferer states that isomaltose is always formed during the saccharification of starch as long as dextrin is present; and that at the limit of the reaction the preparation does not have a reducing power corresponding to 80 to 81 per cent of maltose, but to 60 to 68 per cent.

Glucase was investigated by Geduld (Wochensch. f. Brauerei, 1892, vii, 620), who found that this enzyme exists in ungerminated cereals in both soluble and insoluble forms, and in germinated grains in an insoluble form; that it does liquefy starch-paste; that it has only a very slight action on starch, a greater action on dextrins, and a very energetic

action on maltose, the end-product being dextrose; that the optimal temperature is between  $56^{\circ}$  and  $60^{\circ}$ , and that above  $70^{\circ}$  it is inert; and that it is probably widely distributed and an important diastase. Geduld's results received support in the investigations of Jalowetz (Wochensch. f. Brauerei, 1892, VIII, 1264), who found evidence of the existence of glucase in cereals other than barley and corn.

Morris (Trans. Inst. Brew., 1893, v, 132), however, failed to note any formation of glucose in experiments with maltose solution and aqueous extracts of corn, barley, rye, oats, and wheat, both malted and unmalted, with the exception of corn malt. He looks upon the results of Lintner and Jalowetz as fallacious because they ignored the existence of glucose and other sugars in the extracts they employed, and he believes that the enzyme is peculiar to corn and possibly other cereals as yet not examined, and that it does not occur as a normal or frequent constituent of barley or barley malt.

Lintner and Düll (Ber. d. d. chem. Gesellsch., 1893, xxvi, 2533), in an investigation of the degradation of starch by the action of diastase, record that the products of digestion are amyloextrin, erythroextrin, achroodextrin, isomaltose, and maltose. The so-called amyloins of Brown and Morris (page 115) they hold consist partly of mixtures of dextrin and isomaltose and in part identical with isomaltose. Isomaltose they found has a reducing power equal to 80 per cent of that of maltose; its rotatory power they give as  $(\alpha)_D = +140^{\circ}$ , and the melting-point of its osazone  $150^{\circ}$  to  $153^{\circ}$ . Maltose they believe is formed from isomaltose, which they think leads to the conclusion that dextrans and starch are composed of isomaltose groups.

The sugar products formed by the actions of animal enzymes were studied by Külz and Vogel (Zeit. f. Biologie, 1895, xxxi, 108). They used a 5 per cent solution of rice starch, and determined the sugars present by means of their osazones. They found that human parotid saliva formed isomaltose; that mixed human saliva formed isomaltose at first, then maltose, with traces of glucose; that dog's saliva formed isomaltose; and that pancreas of the ox also formed isomaltose. With liver glycogen instead of starch, parotid saliva formed 1 part of isomaltose to 2 parts of maltose; with muscle glycogen, saliva in small quantity yielded isomaltose with a little maltose and glucose, but a larger quantity gave only maltose. With liver glycogen, pancreas gave isomaltose and a trace of maltose; and with muscle glycogen, there were formed isomaltose with a trace of glucose.

Glucose, isomaltose, and maltose were found to be the sugar products of starch decomposition by Hiepe (The Country Brewer's Gazette, 1893, 1894; Jour. Soc. Chem. Industry, 1894, xiii, 267). Hiepe used several types of yeast, and noted that the percentages of isomaltose formed varied widely in relation to the kind of yeast. He shows that the amyloins are not individuals but mixtures. One of these bodies subjected to an elaborate process of fractional precipitation by alcohol showed the presence of glucose, maltose, isomaltose and dextrin.

In a study of the differences in the products of starch conversion by the action of acids and diastase, Lintner and Düll (Ber. d. d. chem. Gesellsch., 1895, xxviii, 1522) ascertained that isomaltose and glucose were produced with acid, while with diastase there were isomaltose and maltose. Lintner (Zeit. f. ges. Brauw., 1895, xvii, 173, 414) investigated the action of enzymes on isomaltose and maltose. He found that the conversion of isomaltose into maltose did not go on to completion, sometimes only 30 per cent being changed. He also notes that yeast powder gave rise to a very active formation of glucose, that a watery extract is less effective, and that isomaltose is more readily affected than maltose. He believes with Bau (Wochenschrift f. Brauerei, 1894, x, 1366; 1895, xi, 431) and Munche (*ibidum*) that isomaltose may consist of two stereoisomeric forms of isomaltose which differ in fermentability. Prior (Bayerisches Brauer-Journal, 1895, 193) holds, to the contrary, that there are not two isomerides, and that Lintner's isomaltose is a homogeneous substance. Lintner is of the opinion that the ferment which hydrolyzes maltose

and isomaltose is not the same as the invertase which is also found in yeast, as it is less soluble than invertase and more nearly resembles glucase.

Certain statements of Fischer, and of Lintner and Düll, were opposed by Ost (*Chemiker Zeit.*, 1895, XIX, 1501, 1727). Ost investigated Fischer's isomaltosazone and concluded that it is a maltosazone, and he believes that maltose and not isomaltose is formed by hydrochloric acid, as in Fischer's experiments. He followed Lintner and Düll's method for obtaining and purifying isomaltose, and concluded that the substance was not homogeneous and that it is the nature of an impure maltose. Ost determined the rotatory power of maltose to be  $(\alpha)_D = +136.95^\circ$  at  $20^\circ$ . He notes that this value differs from those of Meissl, Brown and Heron, Effront, and Herzfeld, but agrees with that of Parcus and Tollens. Ulrich (*Chemiker Zeit.*, 1895, XIX, 1523), under Ost's direction, arrived at the same conclusion as Ost regarding the conversion of starch into maltose and not into isomaltose; and he also states that the melting-point of the maltosazone is modified by the method of preparation.

Ling and Baker (*Proc. Chem. Soc. Trans.*, 1895, XLVII, 702, 739) also investigated Lintner's isomaltose, and obtained a substance yielding crystals resembling those of maltose and having properties resembling those of Lintner's isomaltose. The rotatory power was found to be  $+142.6^\circ$  to  $+143.8^\circ$ , and the reducing power 81.52 to 81.81 per cent of that of maltose. The melting-point of the osazone was  $160^\circ$  to  $170^\circ$ . In another instance an alcoholic extract of the products of diastatic digestion at  $70^\circ$ , upon treatment with phenylhydrazine, yielded a small quantity of glucosazone, and also an osazone that had the properties of Lintner's isomaltosazone. The latter osazone had a melting-point of  $151^\circ$ . They thought at first that this osazone was derived from a hexatriose, but in their second paper they withdraw this suggestion.

Brown and Morris (*Proc. Chem. Soc. Trans.*, 1895, LXVII, 709) agree with Ling and Baker that Lintner's isomaltose is not a chemical individual. They state, moreover, that Lintner gives no evidence in any of his publications that the isomaltose prepared by him is identical with that of Fischer. They hold that Lintner's isomaltose can be split by fractionation with alcohol, or by fermentation, in such a manner as to indicate a mixture of maltose and dextrinous compounds of the maltodextrin or amyloin class; that the isomaltosazone prepared by Lintner, upon which mainly he based his belief in the existence of isomaltose, is nothing but maltosazone modified by the presence of small but varying quantities of another substance, such as dextrinous compounds; and that the only product of diastatic digestion capable of yielding an osazone is maltose.

Jalowetz (*Chemiker Zeit.*, 1895, XIX, 2003) confirmed the statement of Brown and Morris that by mixing variable proportions of dextrin with maltose, maltosazones can be obtained which differ in melting-point, crystalline form, and general characteristics.

In comparative studies of the actions of salivary, pancreatic, and intestinal enzymes, Hamburger (*Archiv f. ges. Physiologie*, 1895, LX, 543) showed that under certain conditions each will produce isomaltose, maltose, and glucose. He here found that two enzymes, diastase and glucase, are present in different proportions in the salivary, pancreatic, and intestinal secretions, and also in the blood. More diastase and glucase are found in the pancreatic juice than in the saliva, and less glucase than in the blood. In the saliva more diastase is found than in the blood and intestinal ferment, but less than in the pancreatic juice. Glucase predominates in the blood ferment, and therefore larger amounts of glucose than isomaltose and maltose are obtained by saccharification (see page 141). By the action of extract of pancreas on starch-paste, Chlodounsky and Sulz (*Sitz. d. k. böhm. Gesellsch. d. Wissensch.*, 1896; *Jahr. ü. d. Fort. d. Tierchemie*, 1896, XXVI, 67) found that a sugar was formed which yielded an osazone that was identified with glucosazone.

In three contributions during this and the following year, Brown, Morris and Millar (*Proc. Chem. Soc. Trans.*, 1896, XLVIII, 242, 243; 1897, XLIX, 4) take up a technical study of the specific rotatory and reducing powers of glucose, maltose, levulose, and invert sugar.

Further studies of the actions of diastase on starch were made by Ling and Baker (Proc. Chem. Soc., 1897, CLXXIII, 3; Jour. Chem. Soc. Trans., 1897, LXXI, 508), in which they describe a substance isomeric with the isomaltose of Lintner. Its rotatory power was  $(\alpha)_D = +156^\circ$  and the reducing power 62.5. They believe that it might consist of a simple dextrin together with some maltose. Petit (Compt. rend., 1897, CXXV, 1899, CXXVIII, 1176) found in experiments in which starch was subjected to a temperature of  $70^\circ$  with 1 per cent of diastase that a dextrin was formed which upon being treated with boiling dilute hydrochloric acid is within 3 hours completely converted into glucose, but if the reaction is arrested at the end of a half hour, two osazones can be obtained by treating with phenylhydrazine and sodium acetate, one being glucosazone and the other corresponding to a diose, the latter being convertible into glucose by continued action. By further treatment with 1 per cent diastase at  $50^\circ$  to  $55^\circ$ , the dextrin formed yields two substances which differ in rotatory and reducing powers, the proportion of one being twice that of the other. Syniewski (Ber. d. d. chem. Gesellsch., 1898, XXXI, 1791), upon subjecting soluble starch prepared by sodium peroxide to the action of hydrochloric acid, found a quantity of glucose equivalent to 99.3 per cent of the starch, assuming, he states, that soluble starch has the formula  $C_{18}H_{32}O_{16}$ . With malt extract at  $65^\circ$  for  $1\frac{1}{2}$  hours a yield of maltose equal to 82.7 per cent was recorded.

Some of the discrepancies between the sugar determinations of different observers are due to a failure to recognize that sugars of various kinds may be present in the grains or seeds or in the diastatic extracts. In 1875, Kuhnemann (Ber. d. d. chem. Gesellsch., 1875, VIII, 202, 387) recognized in both germinating and ungerminated barley the presence of reducing sugars. He also isolated cane sugar and found that the quantity of sugar increased in germinating grains. His observations have had confirmation in the records of a number of investigators. O'Sullivan (Trans. Lab. Club, 1890, III, 5) extracted barley with alcohol, and also extracted raffinose, and he showed that it is probable that both germinating and ungerminated grains may contain maltose, levulose, glucose, and saccharose. Brown and Morris (Jour. Chem. Soc. Trans., 1890, LVI, 458) found that during the germination of barley the "secretion diastase" converts some of the starch into maltose, and that maltose is converted by the epithelium into *saccharose*. They also showed that the excised embryos placed in a solution of maltose changed maltose into cane-sugar.

Grüss (Wochenschr. f. Brau., 1898, XIV, 81), in investigations of the sugars in barley and malt, ascertained that during the early stage of germination no erosion of the starch-grains occurs and that the grains are conserved for a time at the expense of stored-up sugars. Reducing sugar was found to be present in the aleurone layer under the furrow of the germinating grain. Cane-sugar was not found in the aleurone layer until after the beginning of germination, when it was detected there in larger quantity than in any other part of the grain. The reducing sugars in the endosperm were found to be in greatest quantity in the central part, and least in the cells contiguous to the aleurone layer. Grüss believes that reducing sugar is converted into cane sugar in or near the aleurone cells. In another investigation, Grüss (Wochenschr. f. Brauerei, 1899, XVI, 519) made studies of the actions of two groups of oxidases and one group of diastases upon transitory starches. He notes among other things the formation, in the presence of *air*, of starch from *saccharose*.

Ling (Jour. Fed. Inst. Brew., 1898, IV, 187) determined the preformed sugars of malt by extraction with an alkaline solution. By treatment with phenylhydrazine he obtained an osazone which was probably glucosazone, and another which he records as being probably a maltosazone.

Differences in the products of the actions of acids and enzymes were pointed out by Johnson (Jour. Chem. Soc. Trans., 1898, LXXIII, 490), both in regard to final and intermediate products (see page 151). Pottevin (Compt. rend., 1898, CXXVI, 1218) treated wheat starch with successive portions of malt extract until 10 per cent of the original

amount of starch was left. He made starch-paste of this residue and also of the whole starch, and subjected both pastes to the action of diastase under the same conditions. The former yielded 44 per cent of maltose and the latter 75 per cent. In another article (Ann. d. l'Inst. Pasteur, 1899, XIII, 728, 796) he discusses especially maltodextrins and Lintner's isomaltose. Pottevin had previously found that the starch-grain is not homogeneous, and that owing to this the dextrins yielded by different parts of the grain are not identical, the more resistant portions of the starch yielding dextrins which are only partially convertible into maltose. He found by mixing the proper kind of dextrin in proper proportion with maltose that a mixture could be obtained which seemed to be absolutely identical with the maltodextrin of Brown and Morris, and he therefore holds that the maltodextrin is not a chemical individual. He also contends with Brown and Morris, and with Ling and Baker, that Lintner's isomaltose is not an individual. He states that it is merely a mixture of a non-reducing dextrin and maltose.

In other communications during the same year (Ann. d. l'Inst. Pasteur, 1899, XIII, 665; Wochenschr. f. Brauerei, 1899, XVI, 641) Pottevin studied the phases of the processes of starch conversion, the heterogeneous character of the starch-grain, the differences in the dextrin and sugar products caused by variations in the constitution of starch, and the degree of digestibility of the several components of the grains. Ground wheat starch was subjected for an hour to the action of malt extract, the solution filtered off, and fresh malt extract added to the undigested starch, this solution filtered off, and this process repeated four times, yielding four solutions. Examinations of these solutions showed that each successive portion of the dissolved products was more resistant to diastase. Differences in the starch-residues were also noted. Compared with the paste made from the whole starch, which yielded 80 per cent of maltose, a paste made from a residue representing 9.5 per cent of the original starch yielded only 43 per cent of maltose. The denser the part of the grain the less digestible the starch, the less digestible the dextrin product, and the less the amount of the final sugar product.

Notwithstanding that considerable evidence had already been offered to show positively that maltodextrins are merely mixtures of dextrins and maltose, Brown and Millar (Proc. Chem. Soc. Trans., 1899, xv, 13) continued to study maltodextrin as though it were an individual. They obtained a product of oxidation of maltodextrin which they term maltodextrinic acid. In an appendix they gave the methods they employed to determine the yield of glucose by the hydrolysis of starch, maltose, maltodextrin, and maltobionic acid by oxalic acid. (See page 152.)

Syniewski (Ann. d. Chem. u. Pharm., 1899, CCIX, 282), in his studies of the constitution of starch, holds, as already stated (page 133), that the simplest product of carbinol-hydrolysis is amylogen; that amylogen consists of 3 maltose residues with 1 dextrin residue, which latter in turn is composed of 3 glucose residues, of which 2 are isomaltose residues. In the first stage of hydrolysis of amylogen, all the maltose molecules gradually separate and the dextrin residues remain behind. By the prolonged action of malt extract this dextrin residue is split into isomaltose, and the isomaltose is finally converted into glucose. In a later article (Rozprawy akademji umiejtnosci, Krakau, 1902; Jähr. u. d. Fort. d. Thierchemie, 1902, XXXII, 98) he records that malt extract acting on grenzdextrin I produced maltose and another sugar, an isomer of maltose, which he terms *dextrinose*. It was found to have a rotatory power of  $(\alpha)_{D20} = +141.41$ , and a reducing power of 84.5 per cent of maltose, and its osazone had a melting-point of  $152^{\circ}$  to  $153^{\circ}$ . He states that this sugar differs from Fischer's maltose and isomaltose, that it is probably identical with Lintner's isomaltose, and that it finally breaks up into glucose.

Baker (Proc. Chem. Soc. Trans., 1902, XVIII, 1177) studied the products of the action of barley diastase on starch. Barley diastase produced at  $50^{\circ}$  only dextrin and maltose during the first  $1\frac{1}{2}$  to 2 hours, but after 24 hours there was evidence of the presence of

glucose. The dextrin isolated by precipitation with alcohol was acted upon slowly by barley diastase. At the end of 90 hours at 45° to 50° the product consisted of unaltered dextrin, maltose, and glucose. Barley diastase was found to be without action on maltose; therefore the glucose must have come from dextrin.

Ling (*Jour. Fed. Inst. Brewing*, 1903, ix, 446) noted that glucose is formed by the prolonged action of diastase that had been heated to 65°, and that the quantity formed by a restricted diastase did not exceed 12 per cent of the total products of hydrolysis. He also made the very interesting observation that upon continuing digestion the amount of glucose diminishes. He believes that a reversion of the glucose occurred through a synthetic action of the enzyme. Lintner's isomaltose, he suggests, may be a reversion product from glucose.

Moreau (*Ann. d. l. Soc. roy. d. Sc. méd. e. n. d. Bruxelles*, 1903 xii, 117), by employing malt extract, saliva, pancreatic extract, blood serum, or dilute hydrochloric acid as a hydrolyzer of starch and dextrin, found both maltose and glucose as sugar products.

Both Rolfe and Geromanos (*Jour. Amer. Chem. Soc.*, 1903, xxii, 1003) and Rolfe and Haddock (*ibid.*, 1015) record that through hydrochloric acid hydrolysis both maltose and glucose are formed.

Dierssen (*Zeit. angew. Chem.*, 1903, xvi, 122), in experiments with oxalic acid, found glucose and levulose, and also a disaccharid, but never maltose, and he states that if maltose is formed it must be converted immediately into glucose. The disaccharid corresponded with the properties of Lintner's isomaltose, excepting that it, unlike Lintner's isomaltose, is not converted by diastase into maltose. He does not believe it identical with Fischer's isomaltose, because it is fermentable, while Fischer's is not.

Grüters (*Zeits. angew. Chem.*, 1904, xvii, 1169) also studied the products of oxalic acid hydrolysis. According to him these substances consist of achroodextrins, maltodextrin, maltose, glucose, and a small amount of levulose. He failed in attempts to prepare a pure isomaltose, and found that mixtures of dextrin and maltose yielded osazones having the properties of isomaltosazone. He states his disbelief in the "isomaltose theory."

A comparison of the products of the decomposition of potato starch with starches from cereals was made by O'Sullivan (*Proc. Chem. Soc. Trans.*, 1904, xx, 65), who found that the percentages of maltose and dextrin obtained from potato starch bear no quantitative relation to those recorded in the experiments with cereal starches. This is in opposition to his previous finding (*Jour. Chem. Soc. Trans.*, 1878, ii, 1410), and his statement has been shown by Ford and Guthrie (*Jour. Soc. Chem. Ind.*, 1905, xxiv, 605) to be erroneous, and due to errors of experiment (see page 193).

The hydrolysis of maltose and dextrin was investigated by Noyes and his associates (*Jour. Amer. Chem. Soc.*, 1904, xxvi, 266). They found that while the reducing power of glucose is scarcely affected by heating with a 2.5 per cent solution of hydrochloric acid, the products formed by the hydrolysis of maltose show a maximum reducing power after about 1 hour at 100° or after 20 minutes at 111°, and that further heating causes a decrease. The maximum reducing power corresponded to a hydrolysis of 96 to 98 per cent. Hydrolysis seemed to be more complete in 2.2 to 4 per cent solution of product than in a 0.5 per cent solution. The rate of hydrolysis of dextrin was about half that of maltose. The hydrolysis reached 90 per cent in an hour. The reducing power of the products obtained by the action of malt indicated a composition of 74 to 78 per cent of maltose and 26 to 22 per cent of dextrin. Such a mixture, from the results recorded, should, after 1 hour at 100°, give about 96 per cent of the copper oxide, which would correspond to complete hydrolysis. The amount actually found was 96.4 per cent for the 0.5 per cent solution, and 97.1 for the 2 per cent solution. Practically, the maximum effect is reached in about 1 hour at 100°. By direct treatment of corn starch with 2.5 per cent hydrochloric acid they obtained in a 0.5 per cent solution a hydrolysis of 97 per cent in 1 hour and 98 per cent in 4 hours.

Roux (Compt. rend., 1905, cXL, 1259) made comparative studies of the saccharification of "artificial starches" (page 112) and ordinary starch-paste by malt extract at various temperatures. After 4 hours digestion he found dextrin and maltose, but no glucose. At 56° the average apparent amount of maltose from the artificial starch was 97.9 per cent, while from the ordinary paste it was 82.3 per cent. By fractionation the former yielded 30.4 per cent of crystallized maltose and the latter 30.4 per cent. At 67° the percentages of maltose from artificial and normal starches were 55.1 and 45, respectively. At 80° the artificial starch was unaffected. The dextrans of artificial starch, unlike those from ordinary starch, were almost completely soluble in alcohol.

Fernbach and Wolff (Compt. rend., 1905, cXL, 1067) found that the energy of saccharification is influenced materially by the condition of the starch, whether in liquefied or in paste form. Under the same condition of experiment the conversion products of liquefied starch that had been obtained by heating to 140° to 145° were greater than those of boiled starch as ordinarily prepared. They note that barley extract has a diastatic but not a liquefying power. Starch liquefied by a minute amount of malt extract heated to 70° behaved the same as starch liquefied at high temperatures; therefore, the addition of a minute amount of malt extract (sufficient to cause liquefaction) to barley extract caused very energetic saccharification.

A modified form of dextrose, provisionally termed  $\delta$ -*dextrose*, was described by Roessing (Chemiker Zeit., 1905, xxix, 867), and obtained by him from starch syrups or pure dextrose that had been subjected under pressure to the action of dilute hydrochloric acid at temperatures of 130° or higher. When sulphuric acid is used instead of hydrochloric, the temperature might be raised to 160° without giving rise to this abnormal product.

Maquenne and Roux (Compt. rend., 1906, cXLII, 142) recorded that if a starch-paste and malt-extract mixture be nearly neutralized, a higher yield of maltose may be obtained. Fernbach and Wolff (Compt. rend., 1906, cXLII, 1216) showed, however, that the increased quantities can also be obtained without neutralization if a longer time of action be permitted. Neutralization, they state, therefore merely increased the velocity of the reaction. They record that any dextrin present, if separated, is saccharifiable, and that if there is present any dextrin that is not convertible into dextrose, it must represent only an infinitesimal fraction of the original starch.

Croft Hill (Jour. Chem. Soc. Trans., 1898, LXXIII, 634), in experiments on reversible hydrolysis, states that glucose can under proper conditions be synthesized into maltose together with another sugar which he termed *revertose*. Emmerling (Ber. d. d. chem. Gesellsch., 1901, xxxiv, 600, 2206, 2207, 3810, 3811) showed that revertose is isomaltose. This was confirmed by Armstrong (Proc. Roy. Soc., 1905, LXXVI, B., 592; The Simple Carbohydrates and Glucosides, 1910).

Emulsin, an enzyme that forms isomaltose, was found in yeast by Henry and Auld (Proc. Roy. Soc., 1905, LXXVI, B, 568). Since yeast also contains maltase, which forms maltose, the occurrence of both isomaltose and maltose can be accounted for by the independent actions of the two enzymes, and not, according to Lintner and Düll, to the conversion of isomaltose into maltose (page 144). In fact, yeast extract may contain at least five diastatic enzymes, and emulsin at least three.

#### DIFFERENCES IN THE PRODUCTS OF ACID AND ENZYMIC ACTION.

The striking analogy that exists between the processes and products of enzymic and acid activity has not infrequently led to the assumption of a closer likeness than really exists. It is assumed that both cause a degradation of the starch-molecule, and of the intermediate products of saccharification, by successive stages of hydrolysis, and that the stages as well as the intermediate and final products are essentially the same. But there are abundant reasons for believing that there may not only be certain specific differ-

ences in the products, but also more or less marked differences in the stages, at least in certain cases. In fact, not only are dissimilarities to be observed between the effects of enzymes and acids, but also between the effects of different enzymes and different acids.

Different enzymes might be expected to yield somewhat different results, owing in part to differences in origin in plant and animal life respectively, but chiefly because enzymes, as ordinarily used or prepared, are seldom individuals, but composites of several distinct enzymes, varying in regard to kind and quantity and in their specific actions. If the progress of the action of *animal* enzymes (ptyalin and amylopsin) on starch be traced by testing from time to time with a very dilute iodine solution (1 per cent Lugol's solution), the blue reaction noted at the very beginning of digestion gives way to a purple, and this to a *violet*, this color persisting but becoming weaker and weaker, up to the time of the final disappearance of all color response with the iodine. With *plant* enzymes and with *acid* the blue reaction gives way quickly to a *red* or *brownish-red* reaction which may ultimately pass into a reddish yellow, gradually fading away. This certainly indicates some differences in the intermediate products. Moreover, whether there is produced by enzymic action only maltose, or both maltose and isomaltose, or maltose and isomaltose and dextrose, etc., will be found to depend solely or almost entirely upon the composition of the enzyme preparation. With a given enzyme maltose may be the sole sugar product, with another there may be isomaltose, or maltose and glucose, or isomaltose and glucose, etc., depending upon whether there be a pure maltase present, or emulsin, or maltase with glucase, or maltase with emulsin and glucase, etc., maltase converting dextrin into maltose, emulsin forming isomaltose, and glucase converting maltose and isomaltose into glucose, etc.

The nature of the products formed by acid hydrolysis are modified by the nature of the acid, the temperature of digestion, etc.; moreover, the products compared with those formed by enzymes and other agencies are by no means identical. Mulder (*Chemie des Bieres*, Leipzig, 1858, 166; quoted by W. Nägeli in *Die Stärkegruppe*, etc., *loc. cit.*) found that the dextrans formed by the action of malt extract, sulphuric acid, and torrefaction, respectively, differed from one another in their reactions, especially in relation to certain precipitants.

Soxhlet (*Zeit. Spiritindustrie*, 1884; quoted by Johnson, *Jour. Chem. Soc. Trans.*, 1898, LXXIII, 490) states that the dextrans resulting from the actions of acids are quite different from those produced by diastase. Diastase, for instance, had no action on the dextrans formed and digestible by acid. Johnson (see page 151) has, however, shown that there is a slight action. Effront (*Monit. Scient. f.* 1887, 513; *Jour. Soc. Chem. Ind.*, 1889, VI, 733) investigated the products of the saccharification of starch by extract of malt and by acid, and reached the following conclusions:

- (1) The course of the conversion of starch into dextrin and sugar is not the same with malt and acid. The saccharification by malt is attended by the decomposition of the starch-molecule into dextrin and maltose, while with acid the conversion is into dextrin and maltose, which in turn are converted into glucose.
- (2) The dextrans formed by malt and acid are not identical; those formed by malt are polymeric, while those formed by acid are not.
- (3) The dextrans in both instances have the same rotatory power.
- (4) Maltose is always formed in the saccharification of starch by acid, and the quantity increases as saccharification proceeds. In the earlier stages of saccharification there is an almost constant ratio between the quantities of maltose and glucose formed. This is about 34 to 38 of maltose to 100 of glucose.
- (5) In the saccharification of starch by malt the formation of glucose is inconstant. While glucose almost always occurs in solutions of high gravity, it is only formed in liquids of low gravity if the malt extract employed be turbid.

In another publication (*Enzymes and their Applications*, Trans. by Prescott, 1902) Effront states that the dextrans formed by the action of acid have a very low nutritive

value because pancreatic juice has a scant and incomplete action on them, while on the other hand the dextrans formed by malt are easily transformed by diastases, as shown by Soxhlet and Stutzer.

A comparative study of the products of diastatic and acid degradation of starch was made by Lintner and Düll (Ber. d. d. chem. Gesellsch., 1893, xxvi, 2533; 1895, xxviii, 1522). In the first research they subjected starch to the action of air-dried malt, varying the amount, and stopping the digestion at the necessary point by boiling, and then repeatedly fractionating the product with alcohol. In the second investigation they used oxalic acid instead of enzyme. Table 13 is a statement of the products obtained in the two cases.

They state that, judging from their experiments, acid and diastatic products of the same molecular weight would yield the same products and behave similarly upon the addition of more acid or enzyme. Even though the dextrans are alike, and even though the erythro-dextrin II  $\alpha$  and II  $\beta$  were also obtained with diastase, the only difference between the two hydrolytic processes would be that in the acid process no maltose but isomaltose and glucose appears as the end-product, and with the other maltose appears as the end-product.

Rolfé and Defren (Jour. Amer. Chem. Soc., 1896, xviii, 869) found that only three carbohydrates are present during acid hydrolysis, namely, dextrin, maltose, and glucose. They believed that the products of acid and enzymic activities are the same.

Petit (Compt. rend., 1897, cxxv, 309, 355) records that the saccharine products of dextrin digestion differ somewhat according to the decomposing agent, the period of action, and the temperature.

The torrefaction dextrans of commerce can be distinguished, according to Henderson (Inaug. Diss., Munich, 1897; Jour. Soc. Chem., 1898, xvii, 591), from those obtained by the actions of diastase and acids by their containing a more or less large proportion of substances of low rotatory and reducing power, so that the dextrans formed by these methods can not be considered identical. Henderson states that generally speaking the same dextrans are formed by the actions of diastase and acids, although with diastase dextrans are produced which resist further action of diastase. These dextrans, he believes, have the composition of achroodextrins I and II of Lintner and Düll. The possibility of isomaltose being included among the resistive products, he states, is to be recognized.

The reactions with acids were found by Syniewski (Ber. d. d. chem. Gesellsch., 1898, xxxi, 1791) to proceed farther in the formation of glucose than with enzymes. Experimenting with soluble starch prepared by the agency of sodium peroxide, he noted that with hydrochloric acid the amount of glucose obtained was equivalent to 99.3 per cent, whereas with freshly prepared malt extract it was 82.7 per cent of maltose.

A comparative study of the products of acid and enzymic hydrolysis was made by Johnson (Jour. Chem. Soc. Trans., 1898, lxxiii, 490), who ascertained that the products in the two cases differ to a marked degree. He states that when the products of diastatic conversions are fractionated by means of alcohol, the precipitated portions have specific rotations which may vary from about  $(a)_{D_{3-86}} = +150^\circ$  to about  $190^\circ$ . These fractions are reduced to represent the following peculiarities: They are unfermentable by Saaz yeast, and their cupric-reducing powers and specific rotations can be expressed in terms of dextrin and maltose. Dextrin has a specific rotation of  $(a)_{D_{3-86}} = +195^\circ$ , and maltose  $(a)_{D_{3-86}} = +135.4^\circ$ , so that no substance resulting from diastase hydrolysis can have a special rotation

TABLE 13.

With diastase.	With oxalic acid.
Amylodextrin	Amylodextrin
Erythro-dextrin I	Erythro-dextrin I
.....	Erythro-dextrin II $\alpha$
.....	Erythro-dextrin II $\beta$
Achroodextrin I	Achroodextrin I
Achroodextrin II	Achroodextrin II
Isomaltose	Isomaltose
Maltose	.....
.....	Glucose

of less than  $135.4^\circ$ . When, however, fractions from the products of acid hydrolysis are separated the specific rotations vary between about  $(a)_D = +190^\circ$  and  $(a)_D = +80^\circ$ . These fractions contain no free glucose, because they are unfermentable by Saaz yeast; moreover, they can not be molecular aggregates of maltose and dextrin, since the specific rotation of a maltodextrin can not fall much below  $(a)_D = +150^\circ$ , and we are in the presence here of fractions whose rotations can fall as low as  $80^\circ$ . There are therefore present in acid reactions intermediate substances which do not exist in diastase conversions. In the results of fermentation and dialysis tests he gives evidence to show that the fractions are definite compounds and not mixtures of glucose and other carbohydrates. Further proof that the intermediate products of acid and diastatic hydrolysis are not identical is shown by the difference in their behavior to diastase and acids. Soxhlet had already reported that diastase has no action on the dextrans produced by acid, but Johnson shows that it has a slight action, which he explains by the assumption that it may attack molecules of starch that have not been acted upon by acid, although he admits that it attacks conversions which do not give a blue reaction with iodine. Diastase has no action on fractions having rotations of  $114^\circ$  and lower, but such dextrans do not offer much resistance to acids.

In studies of the oxidation products and constitution of maltodextrin, Brown and Millar (Proc. Chem. Soc., 1899, xv, 11) found that this substance is completely hydrolyzed by diastase into maltose, and by acids into *d*-glucose. When maltodextrin is oxidized with mercuric oxide and barium hydroxide, the greater part of the product appears as a barium salt of a complex carboxylic acid, which they provisionally term *maltodextrinic acid A*. When maltodextrinic acid A is subjected to diastase it yields 40 per cent of maltose and 60 per cent of *maltodextrinic acid B*. When acted upon by dilute oxalic acid, maltodextrinic acid A yields 85.8 per cent of *d*-glucose and a simpler form of maltodextrinic acid.

Van Laer (Jour. Fed. Inst. Brewing, 1900, vi, 162) notes that the differences in the products of acid and diastase are of importance in brewing. He states that acid hydrolysis differs from enzymic hydrolysis not only in that the end-product of the former is glucose and that of the latter maltose, but also in that the intermediate products differ. In order to meet certain conditions favorable to brewing he proposes to partially saccharify grits by dilute acid, then neutralize, and finally complete saccharification by malt.

In the saccharification of starch by acid it was found by Effront, as stated, that maltose is formed, but Lintner and Düll (*loc. cit.*) did not obtain maltose, and they look upon the absence of maltose in acid hydrolysis as being the essential distinctive feature to differentiate the products of acid and enzymic hydrolysis. Rolfe and Defren (*loc. cit.*) found maltose present in both acid and enzymic hydrolysis. Rolfe and Haddock (Jour. Amer. Chem. Soc., 1903, xxii, 1015) and Rolfe and Geromanos (*ibid.*, 1003) support Rolfe and Defren. Rolfe, in a large number of determinations of the products of acid hydrolysis of corn starch and potato starch, found conclusive evidence of the production of a reducing body other than dextrose, and Rolfe and Haddock made alcoholic fractions of a commercial glucose that had been made by the action of hydrochloric acid at a pressure of 2 atmospheres, and having a specific rotation of  $126.5^\circ$  and a reducing power of 0.575. From these fractions they prepared crystals of maltosazone and dextrosazone.

Dierssen (Zeit. angew. Chem., 1903, xvi, 122; Jour. Soc. Chem. Ind., 1903, xxii, 312) used oxalic acid, and he agrees with Lintner and Düll in regard to the absence of maltose. Dierssen concluded that in acid hydrolysis glucose, levulose, and a disaccharid are formed. The rotatory and reducing powers of the latter, and the solubility, appearance, and melting-point of its osazone correspond with Lintner's isomaltose, but it is not affected by diastase, while Lintner's isomaltose (which was prepared by diastase) was converted by this enzyme into maltose. Whether or not the isomaltose obtained by Lintner by acid hydrolysis would have been converted by diastase was not noted. He states that it can not be identi-

cal with Fischer's isomaltose because Fischer's isomaltose was not fermentable, while this sugar is fermentable; and, moreover, Fischer's was lævo-rotatory, while his was dextro-rotatory. Dierssen goes on to state that from the results obtained no conclusions can be drawn with regard to the course of the diastatic hydrolysis of starch, but the fact is emphasized that the products in this case differ considerably from those yielded by acid hydrolysis. Additional evidence is also afforded by the tendency of glucose to form double compounds with other sugars, one molecule of the syrupy isomaltose obtained by Dierssen yielding crystalline double compounds with any number of molecules of glucose from one upward.

Grüters (*Zeit. angew. Chem.*, 1904, xvii, 1169; *Jour. Soc. Chem. Ind.*, 1904, xxiii, 875) also studied the final degradation products of the action of oxalic acid on starch. He states that these substances include achroodextrin I and II, maltodextrin  $\gamma$ , maltose, glucose, and a small proportion of levulose. These he notes are the same as the products yielded by diastatic action, except that in the latter case maltodextrin  $\gamma$  is replaced by maltodextrin  $\beta$ , which exhibits different constants and also behaves differently towards diastase. He believes that both these dextrans occur simultaneously, but in varying proportions, this view being supported by the fact that the conversion into maltose is sometimes very imperfect, and at other times almost complete. The divergent behavior of various isomaltose preparations towards malt extract is also regarded as indicating that the more resistant maltodextrin  $\gamma$  occasionally preponderates as the lowest member of the dextrin series. Like Lintner and Dierssen, he failed to isolate maltose in the pure crystalline state from syrups; nor could he accomplish this from mixtures of maltose and dextrin, which, moreover, gave osazones resembling isomaltosazone.

Differences in conditions under which hydrolysis occurs may decidedly modify the products, as shown by Skraup (*Ber. d. d. chem. Gesellsch.*, 1899, xxxii, 2413). He noted, for instance, that usually, on acetylation of starch with mixtures of acetic and sulphuric acid, products of greater complexity are produced by little acid and low temperatures than under opposite conditions.

Of incidental interest in this connection, and in support of differences in the products of acid and enzymic hydrolysis, are the results of the experiments of Tebb (*Jour. Physiology*, 1897-8, xxii, 423) with glycogen, and of Loewi (*Archiv f. exp. Path. u. Pharm.*, 1902, xlviii, 303), Henriques and Hansen (*Zeit. f. physiol. Chemie*, 1905, xliii, 417), and Abderhalden and Rona (*Zeit. f. physiol. Chemie*, 1904, xlvii, 530 and 1905, xlv, 200) with proteins. Tebb found in the acid hydrolysis of glycogen that the products are soluble glycogen, erythro-dextrin, achroodextrin, and glucose, and that prolonged hydrolysis by saliva, pancreatic extract, and malt extract the only dextrans that could be separated in amount sufficient to work with subsequently were of the achroodextrin variety, although evidence of erythro-dextrin was noted. By liver enzyme the intermediate dextrans resembled those produced by the amylolytic enzymes, except that a small amount of erythro-dextrin is constantly found in the earlier stages of the hydrolysis of glycogen. The reaction of liver enzyme differed from the other enzymes used in the nature of the final product obtained, which is, chiefly at least, glucose and not maltose. Loewi found that an animal fed on the products of the prolonged self-digestion of pancreas may not only thrive but gain in weight. Henderson and Dean fed a bitch on the non-protein products of acid hydrolysis of lean beef together with lard and arrowroot, and record that their numerical results are essentially similar to those obtained by Loewi. From the ninth to the thirteenth day of feeding, the animal was in nitrogenous equilibrium, and the body weight was maintained. In opposition to the statements of Henderson and Dean, both Henriques and Hansen and Abderhalden and Rona found that while animals may thrive on the enzymic products of protein digestion they do not live longer on the products of the acid hydrolysis of proteins than animals fed on a non-nitrogenous diet.

## UNUSUAL PRODUCTS OF THE DECOMPOSITION OF STARCH.

The usual products of the decomposition of starch through the agency of enzymes, dilute acids, bacteria, and other agents which bring about saccharification are erythro-dextrin, achroodextrins, maltose, isomaltose, and glucose, which products are variable quantitatively and qualitatively in relation to the conditions of experiment. The so-called amylo-dextrins and maltodextrins we are justified, from the literature of the subject, in regarding as mixtures. Such dextrins and sugars, as noted, may be looked for with certainty under ordinary conditions of experiment with most digestive agents, but certain unusual products may be formed under modified conditions or by certain agents. A number of references have been made to such products, as, for instance: To Leitner (page 127), who found through the action of permanganate that gummy substances are formed which differ from dextrins; to Zulkowsky and Franz (page 127), who found by the prolonged action of high temperature with moisture that a substance is formed which resembles gum arabic; to Zulkowsky (page 127), who by using hot glycerol found erythro-dextrin, achroodextrin, and a number of bodies of increasing solubility as the reaction proceeded; to Ost (page 128), who by the use of high moist heat obtained a dextrinous product that was not a true dextrin; to Roessing (page 149), who obtained from starch syrup by the action of dilute hydrochloric acid at high temperatures a modified form of dextrose which he provisionally named  $\delta$ -dextrose; and to Roux (page 149), who prepared from artificial starch dextrins that were almost completely soluble in alcohol. Abnormal dextrins have been reported as existing in certain beers at times, and occasionally peculiar sugars or saccharine bodies have been recorded. Finally, if there be present contaminating enzymes a number of non-saccharine products of varied characters, ranging between sugars and  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , may be formed.

## DIFFERENCES IN THE DECOMPOSITION PRODUCTS OF DIFFERENT STARCHES.

Sufficient evidence has been presented in Chapter II and in this chapter to justify the conclusion that starch is not a uniform substance, and that it exists in many isomeric forms which differ in different species, different parts of the same plant, and even in the individual mature grains, etc. It is therefore reasonable to suppose that the higher decomposition products, especially the so-called soluble starch and the dextrins, would be produced in corresponding differentiated homologous forms; in other words, that each form of starch-substance would yield peculiar constitutional forms of decomposition products which would bear specific stereochemical relationships to the constitutional structures of the initial substances. Evidence in support of such a conception is found, for instance, in the constitutional differences of the inner and outer parts (the "granulose" and "cellulose") of the mature starch-grain as exhibited in the changes which ensue when the grain is subjected to gelatinization and pseudo-solution by heating in water, when comminuted grains are macerated in water, when the grains are subjected to certain aniline dyes and to digestive and various other reagents, and when normal and reverted starches are digested, etc.

When raw starch is heated in water to a proper temperature in relation to the kind of starch, swelling occurs, and in the course of time the grain becomes divided into two parts, constituting an outer portion that appears in the form of a sac or capsule, and an intracapsular semifluid substance. After a time the capsule ruptures at one or more places, permitting the escape of the inner substance. By continued heating for some minutes, the length of time varying with the kind of starch, the sac undergoes a complete breaking down. The inner, more soluble, less dense part in case of all forms of starch, which yields a blue or purple or violet reaction in the normal state, yields an intense indigo-blue coloration with iodine, whereas the sacs almost always become a bluish-violet to a red-violet, showing of course a chemical difference between the inner and outer parts. The reaction of the inner

part is the typical reaction of pure starch as universally understood, but the explanation of the different reaction of the outer part might not be the same in accordance with variable conceptions of starch components and decomposition products. If one assumed that there exists an individual body between starch and erythro-dextrins which is intermediate in character, and which possesses properties of both, the violet reaction can readily be accounted for, and as a corollary the outer or capsule part of the grain might with sufficient reason be regarded as a transitional non-starch substance. There is, however, no necessity for the assumption of the existence of such an intermediate body, but there is sufficient evidence to warrant the conclusion that such bodies as have been so described are modified forms of starch or mixtures of starch and dextrin. The violet reaction might satisfactorily be accounted for, as experimental observations have shown, and which is in accord with our views of the processes and products in the synthesis and analysis of starch, upon the basis of the presence of a small and variable amount of erythro-dextrin in the outer coat. Erythro-dextrin is, as far as we know, the nearest individual to starch, and it is a natural conclusion that, since the grain grows by external accretion, there may be present in the outer layer variable amounts of erythro-dextrin in the course of transition into starch. Erythro-dextrin gives a red reaction with iodine, and starch a blue reaction, and a combination of the two a purple, blue-violet, or red-violet, or intermediate gradations, in accordance with the proportions of these substances. The difference in color reactions between the inner and outer parts of the grain can not therefore of itself be taken to indicate any specific difference in the composition of these parts. There are, however, a number of facts which very strongly suggest constitutional differences, as, for instance, the differences in solubility, the differences in the reactions to aniline and other coloring agents, and differences in the degree of digestibility and in the products of digestion.

When comminuted grains are macerated in water the inner part goes into pseudo-solution, while the outer part remains undissolved in the form of suspended flakes, etc., and when raw starch is subjected to the prolonged action of weak acid, etc., the inner part is dissolved, leaving skeletons of the grains which from their polariscopic properties may be totally unchanged. This difference in solubility is in accord with the phenomena observed when the grains are subjected to moist heat, and it would seem that it can not be due in the least to the contaminating erythro-dextrin, which is certainly more soluble than starch; nor is there evidence that it may be due to the presence of other substances. Differences in the reactions with aniline and other coloring agents have been referred to particularly on pages 55, 56, and 58 and will be considered further in Chapters IV and VI.

Pottevin's experiments (pages 134 and 147) furnish strong evidence of differences in the constitution of the starch-substance in different parts of the starch-grain. He found, as already stated, that the denser external parts of the grain are less digestible than the less dense inner parts, and that the dextrin yielded by them is different, the dextrin from the outer part being more difficult to saccharify and yielding a very much lower percentage of sugar under the same conditions of experiment, and in being insoluble in strong alcohol, and also differing in the degree of diffusibility. Roux (see pages 46, 112, and 149) recorded that not only were reverted and normal starches different in the degree of digestibility under the same conditions of experiment, but also that the dextrins of reverted starch, in accord with Pottevin, are, unlike those from ordinary starch, freely soluble in strong alcohol. (See on reverted starch, page 111.) Further evidence of differences in constitution that are suggested by variations in digestibility, etc., will be found in Chapter IV.

Here it might be noted incidentally that in experiments by O'Sullivan it was found that the percentages of dextrin and maltose yielded by potato starch do not correspond with those from the starches of malt, barley, corn, and rice. It was shown, however, by Ford and Guthrie (page 193) that the differences were due to errors of experiment.

Admitting the existence of stereoisomeric forms of starch-substance, and that *a priori* there should be corresponding specific stereoisomeric forms of dextrans, we meet with a very obvious difficulty in the study of the specificities of these hypothetical decomposition products in relation to genera, species, etc., in the total lack of exact knowledge of the precise dextrinous products of digestion. It is manifest from the literature quoted in this and other chapters that our information as to the *precise* nature of the dextrans formed is *nil*; that most if not all experimenters have been working with impure bodies or mixtures; that our methods of distinguishing different erythro-dextrans and different achroo-dextrans obtained from the same preparation, if such really have existence, are by no means certain; and that it is therefore useless under present conditions to attempt the differentiation of corresponding dextrans from the starches of different species, except in a crude and inconclusive way, as, for instance, as done by Pottevin, Roux, and others, by taking the dextrinous products as a whole.

#### THE SYNTHESIS OF STARCH.

According to the well-known hypothesis of van't Hoff (*Zeit. f. anorgan. Chemie*, 1898, XVIII, 1) an enzyme gives rise only to such products in the analysis of a given substance as it will under appropriate conditions combine in synthesizing the same substance. In other words, if a given enzyme or mixture of enzymes acts upon starch to decompose it through a series of actions into a series of products such as erythro-dextrin, achroo-dextrin, maltose, and glucose, it or they will under properly modified conditions reverse the operations and thus synthesize starch. The results of modern research lead unquestionably to the belief that the activities of enzymes underlie vital processes. Various plant and animal enzymes have been used to break down starch into the products mentioned, and these products correspond with substances found in plants during the formation of starch; hence they are presumably intermediate bodies between starch and the immediate antecedents of glucose that are synthesized from carbon dioxide and water. Moreover, syntheses representing at least two of these hypothetical steps in starch formation have been carried out *in vitro* by Croft Hill (*Jour. Chem. Soc. Trans.*, 1903, LXXXIII, 578) and E. F. Armstrong (*Proc. Roy. Soc.*, 1905, LXXVI, B. 592), in which the maltose-glucose reaction was reversed, and by the author (*Univ. Penna. Medical Bull.*, 1910, XXIII, 57; *Proc. Soc. Exper. Biology and Med.*, 1910), in which the starch-erythro-dextrin reaction was reversed, with slight evidence of an achroo-dextrin-maltose reversion and also of a maltose-glucose reversion. Ling (page 148) suspected a glucose reversion.

Until comparatively recent years the energies of the chemist in the study of bodies which occur naturally only in plants and animals have been chiefly in the direction of analysis, so that he has worked essentially in a direction opposite to those in living matter in the syntheses of these substances; but during the last ten or twelve years especially he has come in touch with Nature's processes, owing to the epochal investigation of Croft Hill, with the result of successes in the syntheses of carbohydrates, fats, and proteins which scarcely more than two generations ago would generally have been regarded as being improbable or impossible; and he has at the same time thrown extremely important light on the analytic and synthetic processes that take place in living matter. The important discovery of Wöhler, in 1828, that he had made urea by the interaction of ammonia and lead acetate, is the first on record of the synthesis *in vitro* from inorganic substances of an organic substance that is inherently peculiar to living matter. Wöhler evidently appreciated the great fundamental importance of his discovery, but it seems that this was far from being the case with his contemporaries, and that, as is often the case, an epochal investigation remains wholly unappreciated for years until new inquiries bring it to the forefront. Even his teacher Berzelius failed to realize its importance. The progress in the synthesis of plant and animal substances from this period up to the present has been

astonishingly slow and insignificant in comparison with the tremendous progress in other lines of chemical investigation, and largely because of the violent methods generally employed in such work. Through ignorance or misconception of the processes in animals and plants the chemist was led too far away from the methods of Nature, but owing primarily to the basic work of Croft Hill the way has been opened for an endless amount of investigation which must bring results of incalculable value in the explanation of protoplasmic processes.

Apart from the work of Wöhler, it seems that it was the discovery of Butlerow (Ann. d. Chem. u. Phar., 1861, cxx, 195; Compt. rend., 1861, LIII, 145) that a saccharine substance (methylenitan,  $C_7H_{17}O_6$ ) could be formed by the reaction of a solution of trioxymethylene and lime-water, that led to Bayer's important statement (Ber. d. d. chem. Gesellsch., 1870, III, 68) that formaldehyde may be formed from carbon dioxide and water, and that theoretically by the polymerization of 6 molecules of formaldehyde there would result a hexose. Various more or less important modifications of Bayer's hypothesis and conceptions have been suggested, particularly by Erlenmeyer (Ber. d. d. chem. Gesellsch., 1877, x, 634), Bach (Compt. rend., 1893, cxvi, 1145, 1389; 1898, cxxv, 479), Pollacci (Bot. Centralbl., 1904, xcv, 425, and xcvi, 473; 1905, xcvi, 247), and Usher and Priestly (Proc. Roy. Soc., 1905-06, LXXVII, B, 369; 1906, LXXVIII, B, 318). Since Bayer's investigations, interest has been aroused as to whether or not formaldehyde is to be regarded as a primary assimilative product; whether formaldehyde can be formed from carbon dioxide and water *in vitro*; whether sugar can be produced from formaldehyde *in vitro*; whether a plant can thrive and take up formaldehyde from its ambient medium; and whether connection can be traced between formaldehyde and the synthesis of sugar, dextrin, starch, and glycogen in the plant.

Associated with these inquiries studies have been made, both in the plant and *in vitro*, in connection with the various saccharine and dextrinous substances which are conceived to represent the main bodies in the analyses and syntheses of starch and glycogen. The mere fact that during the syntheses of starch or glycogen there are present intermediate bodies between carbon dioxide and water on the one hand and these polysaccharoses on the other, does not prove of course that such intermediate substances are utilized in the syntheses, or that starch might not be formed at a single step from carbon dioxide and water, or that because of their seeming absence they may not actually be necessary and that they are made and instantly transformed; but the fact that a serial decomposition occurs in the organism and *in vitro*, and that the intermediate bodies formed during analysis correspond with those found in plants during the syntheses of polysaccharoses, is strong evidence of the formation of starch by the production of a definitely related series of intermediate bodies which show progressively higher and higher molecular weight and complexity of structure, and that such intermediate bodies as are found in plants may be regarded as representing specific steps in analysis or synthesis, in accordance with whether the plant is consuming or making starch or glycogen.

If, therefore, formaldehyde can be formed *in vitro* from carbon dioxide, and sugar formed from formaldehyde, and if at the same time it can be shown that in the plant carbon dioxide and water are primary substances in synthesis, and that formaldehyde is a normal constituent of plants, and that a plant may thrive in a solution or in an atmosphere containing appreciable quantities of formaldehyde and even assimilate this substance, it seems that we have reached the point where it is not a question as to whether or not formaldehyde is a primary assimilative substance and an intermediate body in the synthesis of certain saccharoses, but to seek for the evidence to prove it.

Attempts to produce formaldehyde *in vitro* from carbon dioxide and water were made by Maly (Ann. d. Chem. u. Pharm., 1865, cxxxv, 119), Royer (Compt. rend., 1870, LXX, 731), Bach (Compt. rend., 1893, cxvi, 1145, 1389; Chem. Centralbl., 1898, II, 42), Lieben (Ann. d. Phys. u. Chem., 1895, XIX, 463), Cohen and Jahn (Ber. d. d. chem. Gesellsch.,

1904, xxxvii, 2836), Berthelot and Gaudcehon (Compt. rend., 1890, cx, 1690), Usher and Priestly (*loc. cit.*), and Fenton (Jour. Chem. Soc., 1907, xci, 687). In the investigation of Bach, Berthelot and Gaudcehon, Usher and Priestly, and Fenton formaldehyde was found, and in several formic acid was noted. Various other substances have been used by chemists in the synthesis of formaldehyde, and other instances might be given which have bearing upon aldehyde formation in plants.

If sugar can be reduced to aldehyde *in vitro* we are justified in assuming that the reverse may be brought about under proper conditions. This finds confirmation in the investigations of König, Spieckmann and Olig (Jour. Chem. Soc., 1903, lxxxiv, 386), who found that bacteria (belonging to the type of *Bacillus coli communis*) in a solution of glucose actually form acetic aldehyde and other compounds; and in those by Renard (Ann. d. chim. e. phys., xvii, 321), who records that glucose when subjected to electrolysis in dilute sulphuric acid yields among its products trioxymethylene, which in turn is changed into formaldehyde, and that a reversal can be brought about so that formaldehyde is transformed into sugar.

Other successful experiments in the synthesis of sugar *in vitro* have been reported by a number of investigators, as for instance, Loew (Jour. f. prakt. Chemie, 1886, xxxiii, 321; Ber. d. d. chem. Gesellsch., 1889, xxii, 475), Fischer (Untersuchungen u. Kohlenhydrate ü. Fermente, Berlin, 1909—a collection of papers that appeared in the Ber. d. d. chem. Gesellsch., from 1884 to 1908), Löb (Zeit. f. Electrochemie, 1907, xii, 282, Biochem. Zeit., 1907, xii, 78), Euler (Ber. d. d. chem. Gesellsch., 1906, xxxix, 39,45), Slosse (Bull. d. l'Acad. roy. d. Belg., 1906, xxxv, 547), and Berthelot (Compt. rend., 1903, cxxvi, 610). In some of these investigations intermediate bodies, such as *a*-acrose, *a*-acrosazone, *i*-fructose, *i*-mannitol, *i*-mannose, *i*-mannonic acid, *d*-mannonic acid, *l*-gluconic acid, and *d*-gluconic acid are recorded in the synthesis of glucose. The evidence, then, is conclusive that not only may saccharine substances be formed from formaldehyde, but also sugar that corresponds with decomposition products of starch and glycogen. Glucose, as already stated, has been converted into isomaltose. Moreover, having glucose as an initial substance, one may obtain from it by appropriate methods *in vitro* levulose, mannose, and other saccharine substances.

Of incidental interest is the fact discovered by Mayer (Zeit. f. physiolog. Chemie, 1903, xxxviii, 135) that glycollic aldehyde is eliminated by rabbits in the form of glucose. The fact that aldehydases which oxidize aldehydes have been found in plants and also in the liver and other organs of animals is not without significance. Gautier suggested years ago that certain glucosides, such as arbutin and salicin, may be derived from formaldehyde by the addition of hydrogen by molecular condensation and dehydration.

It has also been shown that plant substances may be broken down with the formation of aldehyde, as for instance, salicin into heletin and this into salicylic aldehyde or glucose, and amygdalin into benzoic aldehyde, glucose, and hydrocyanic acid. Acetic aldehyde is produced in acetification, and in wines, etc.; and it is formed in the animal body as a decomposition product. There are quite a number of both plant and animal substances which by oxidation yield forms of aldehyde. In fact, instances of this character which have for their indication a suggestion that aldehydes are among the plant metabolites utilized in carbohydrate and other forms of metabolism might be considerably multiplied. In the animal organism starch is not merely reduced to the glucose stage, but ultimately to carbon dioxide and water. In other words, when diastatic enzymes cease their decomposing actions other enzymes which cause further hydrolysis, or simple molecular splitting, oxidation, etc., come into play, by the agency of which a considerable number of products are formed, among which are included alcohol and its aldehyde.

Whether or not aldehyde is formed in plants is a matter yet under discussion. Evidence in favor of the presence of this substance in plants has been offered in the investigations

of Loew and Bokorny (Archiv f. ges. Physiologie, 1881, xxv, 150; xxvi, 50), Reinke (Ber. d. d. chem. Gesellsch., 1881, xiv, 2144), Mori (Nuovo. Gio. Bot. Ital., 1882, xiv, 147), Pollacci (Atti d. instit. Bot. d. Univ. d. Pavia, 1900, vi, 45; Atti d. Real. Accad. d. Lincei, 1902, xvi, 199), Grafe (Osterr. bot. Zeitschr., 1906, xlvi, I), Kimpelin (Compt. rend., 1907, cxliv, 148), Usher and Priestly (*loc. cit.*), Gibson and Titherly (Annals of Botany, 1908, xxxii, 117), Bokorny (Archiv f. ges. Physiologie, 1908, cxxv, 467, and 1909, cxxviii, 565), and Schryver (Proc. Roy. Soc., London, B., 1910, lxxxii, 226). Opposition to the conclusions of some of the experiments has been offered by Loew and Bokorny (*loc. cit.*), Plancher and Ravenna (Atti d. Real. Accad. d. Lincei, 149, xiii, 459), Czapeck (Bot. Zeit., 1900, lviii, 153), Euler (Ber. d. d. chem. Gesellsch., 1905, xxxvii, 341), and Bokorny (*loc. cit.*). While there may be reasonable doubt whether the presence of formaldehyde in plants in detectable quantities has been conclusively demonstrated, it seems certain that it is formed, and that inasmuch as it is not in the nature of a storage substance it is at once transformed into a saccharine body, and therefore that scarcely more than traces could be expected to be found at any time.

Another step in favor of formaldehyde constituting one of the primary metabolites used by plants in the elaboration of saccharoses has been demonstrated in the power of a plant to thrive in a medium containing formaldehyde and in its power to assimilate it. Work of this kind in showing one or the other or both of those phenomena has been carried out by Bokorny (Ber. d. d. chem. Gesellsch., 1888, xxi, 119; Archiv f. ges. Physiologie, 1908, cxxv, 467, and 1909, cxxviii, 565), Bouillac and Giustiniana (Compt. rend., 1903, cxxxvi, 1155), Tréboux (Flora, 1903, lxxxii, 49), Grafe and Vieser (Ber. d. d. bot. Gesellsch., 1894, xxvii, 431), and Usher and Priestly (*loc. cit.*). While it has been clearly shown that plants may thrive in a medium containing very small quantities of formaldehyde, and that some of the investigators also show that formaldehyde is absorbed, and also that the plants may even thrive better in an atmosphere free from carbon dioxide if it contain formaldehyde than when it does not, it has only been inferentially demonstrated that formaldehyde is used in the plant in the synthesis of saccharoses.

The several links of the chain constituting the synthesis of starch and glycogen may be formulated hypothetically as follows: Carbon dioxide and water by deoxidation yield formaldehyde, and perhaps other aldehydes; aldehyde by polymerization and atomic rearrangement yields sugars in the form of monosaccharoses; monosaccharoses by dehydration yield disaccharoses; and these in turn by the same process yield polysaccharoses in the form of aehroodextrins; aehroodextrins by some form of intramolecular rearrangement yield erythro-dextrins; erythro-dextrins by some form of intramolecular rearrangement yield starches or glycogens. Dextrins, glycogens, and starches are closely related polysaccharoses. Of the members of this chain the substances that are formed for storage purposes, and therefore such as might be expected to be found in large quantities in plants, are sugar, aehroodextrin, erythro-dextrin, glycogen, and starch. Of these, sugar and starch are quantitatively preëminently important, but in certain plants they seem to be replaced by glycogen or erythro-dextrin. This scheme is, of course, modifiable in many ways, inasmuch as the exact processes and primary substances in starch formation can not be identical in all plants, or probably even at all times in the same plant. For instance, when saccharose is utilized in this synthesis the process can not be identical with that when maltose is used. The formation of saccharose must be on a somewhat different plan, inasmuch as this sugar is of a distinctly different constitution, as is instanced in it not giving many of the sugar tests, in its non-fermentability, and in its not reacting with phenylhydrazine.

There are various reasons for believing that in the synthesis of starch there are formed thousands or even millions of stereoisomeric forms, each representing a homologue which differs from the others. In the first place, the differences in the plastids of different species

of plants and plant-parts justifies the assumption of Meyer (page 51) that in accordance with these differences we have different biologic mechanisms. Hence it would seem to follow, for reasons stated on page 11, that corresponding metabolites should be modified in accordance with the modifications of the mechanisms which produce them. In the second place, it has been shown that saccharose, or cane sugar, can be obtained from starch as one of the products, and that the plant may utilize saccharose in the synthesis of starch. This holds good for maltose and other sugars which have a very different chemical constitution from saccharose. If, therefore, sugars differing so in chemical constitution are used in the synthesis of starch, it seems probable that such constitutional differences tend to be carried throughout the whole series of synthetic bodies and to be present ultimately in the starch or glycogen molecule. One can readily conceive how, through the operations of these two factors (differences in the *living mechanism* and differences in the *building material*), each species of plant, or even each individual, may produce a specific kind of starch; and, moreover, that this product may be modifiable under normal or abnormal conditions, owing to temporary or permanent modifications of the plastids, or of the food supply, etc. An instance of such a modification is found in the peculiar form of glycogen found by Claude Bernard in paralyzed muscles—the glycogen of normal muscles yields a reddish or port-wine color reaction with iodine, while the abnormal glycogen yielded a blue reaction. Even after a given form of starch is produced it may undergo a spontaneous change into another stereoisomeric form (see page 9).

#### SUMMARY AND CONCLUSIONS.

One can not review the literature of the decomposition products of starches without a realization that our knowledge is exceedingly inexact and that we have scarcely reached the threshold of accurate information of the characteristics of the various homologous forms of starch, of the actual substances that are formed as intermediate products during the process of saccharification, and of the precise chemical processes involved in the several reactions. The available methods for the differentiation of homologous forms of starch have been used to but a very limited extent and (as in case of the differentiation of similarly related bodies) they are for the most part crude and almost wholly quantitative. The methods of measurement of the progress of the various steps in the saccharification of starch and the methods of separation of the intermediate bodies and the results of their study are quite as unsatisfactory. Exactly when any one stage begins and ends, when the last molecule of starch disappears and when the first molecule of achroodextrin appears, whether or not there exists any form of dextrin that will reduce copper solutions or which through some obscure relationship with maltose will affect its reactions with copper, and whether there are multiple forms of erythro-dextrin and of achroodextrin, are but a few of the fundamental questions that require the final answer.

In a word, the determination of the sequence of events and their relations and the exact products formed remains to be demonstrated, for as yet the only means we have of ascertaining the degree of progress of enzymic saccharification that has even an approach to accuracy is the estimation of the amount of maltose, as for instance by the copper test, which is the best; but this may be attended by more or less fallacy because of the existence of an achroodextrin or other non-saccharine body which may take part in the reaction, or because of the presence of variable amounts of glucose which has a very different reducing value, in addition to other distinctive properties. Finally, the assumption that the processes are those of successive hydrolysis does not take into account the fact that the liquefaction of raw starch or starch-paste is doubtless not one of hydrolysis but of adsorption; or that the conversion of starch into dextrin, and of a higher form of dextrin into a lower form, is in effect a depolymerization; or that oxygen may be essential during a part of or throughout the entire group of reactions.

With so unstable a foundation one can not go far in formulating conclusions that can have more than a tentative value, and with this idea in view the following synopsis of certain especially important points may be made:

(1) It may be conceded, from the literature herein cited, that the starch-substance is not a uniform body and that it exists in many stereoisomeric forms which vary in the starches of different plants, of different parts of the same plant, of different grains of the same starch, and even of different parts of the same grains; and that in accordance with these variations corresponding differences may be expected in the intermediate derivatives and reversion products in accordance with the stereochemic differences of the homologues.

(2) That the first step ordinarily, though perhaps not essential, in the saccharification of starch is a liquefaction, and that this is followed serially by the production of erythro-dextrin, achroo-dextrin, maltose or isomaltose, and glucose, all of which processes, when once under way, go on together, the disappearance of one substrate after another eliminating the corresponding process, until an equilibrium of solution of maltose and glucose is attained; and that not only may the progress of the reaction be stopped at will, but also, by modifications of the processes, the stages of the degradation of the starch-molecule may be so specifically limited that they do not go beyond the liquefaction of starch, or the formation of dextrans, or the formation of maltose; in other words, these substances are the essential end-products of the reactions in the several cases, so that we may have liquefied starch without the formation of any dextrin, or dextrans without a trace of maltose, or maltose without a trace of glucose. This serial action, which results in the formation of an individual substance at each step, finally disposes of the theory of Musculus of the coincident formation by hydrolysis of two substances in the form of dextrin and sugar, and their subsequent cleavage; and it is in support of the theory of Lintner and Düll. We may regard these markedly differentiated steps as representing the major stages, and assume that there are a number of substages.

(3) That the processes involved in these major stages, as in the case of acids, are due to different functional properties of a single agent; or, as in case of enzymes, either to different functional properties of a given enzyme or to individual properties of different coöperative enzymes, or to both. Sufficient evidence has been offered to show that, at least in some instances, as in the reduction of starch to maltose and of maltose to glucose, two specific enzymes are required, one to yield the maltose and the other to convert this sugar into glucose. It seems likely that four enzymes (or four independent specific properties) are necessary—one to liquefy the starch, one to convert the starch into dextrin, one to convert the dextrin into maltose, and one to convert the maltose into glucose. Were we to follow the decomposition processes in the body to the ultimate conversion of the sugar molecules into  $\text{CO}_2$  and  $\text{H}_2\text{O}$  we should find with certainty that still other enzymes are involved, some carrying on very different chemical processes from those stated.

(4) That all of the processes in acid and enzymic saccharification are those of hydration, but not necessarily of hydrolysis. The swelling or gelatinization and the formation of pseudo-solution and true solution can be fully accounted for on physico-chemical grounds upon the basis of the adsorption of water, which involves absolutely neither enzymic nor acid activity, nor hydrolysis, and no hydration in the strictly chemical sense. The reversion of starch when in pseudo-solution or true solution to less soluble forms may be regarded as simply a manifestation of a reversal of the adsorption process and accompanying changes which we find paralleled in other of the so-called colloids, and which we observe even in living colloidal matter, as in the case of the giving off and taking in of water by the muscle substance, which is assumed by some physiologists to be the essential mechanical part of the phenomena of contraction and relaxation.

The conversion of starch into erythro-dextrin, and of erythro-dextrin into achroo-dextrin, if it be a process of hydrolysis, is of such a character that there is no permanent addition of water in the reaction, or, if the product be in the nature of a hydrate, the change is due to a

rearrangement of the atoms of the molecules so that in either case the product is of a depolymeric form. The conversion of dextrin into maltose, and of maltose into glucose, we are certainly justified in regarding as being due to hydrolysis, by which molecules of water are taken up and constitute not only a part of the process but also a part of the product.

Starch is classed among the most typical of the so-called colloids, yet, like many or probably all of the so-called colloids, it may exist in crystalline form; likewise are many simple inorganic substances colloidal, as for instance silicic acid, platinum, gold, silver, ferric hydroxide, arsenious sulphide, aluminum hydroxide, etc. In fact, it seems probable from the advances of physical chemistry that any substance, according to conditions, may exist in either a colloidal or crystalloidal state. Hemoglobin in the erythrocyte exists in a colloidal state, yet when freed it is usually easily obtained in a crystalline state; egg albumin and serum albumin are under normal conditions typical colloids, yet they may be crystallized. Boiled starch is a typical colloid, but when demineralized and rendered by high moist temperature into a true solution it is thus transformed into a crystalloidal state or phase. The transition of colloidal into non-colloidal forms of starch and of dextrin into maltose or some other form or forms of sugar are instances of the readiness with which an alteration from one state to another can be brought about in the transition of intimately related substances, one into the other. It seems therefore, as is being generally recognized, that we are not dealing with colloidal or crystalloidal *substances*, but with corresponding *states, phases, conditions, or forms*. Hence, the transition of a typical colloidal state of starch into a crystalloidal state, and the reversal, are phenomena that were to be expected. In fact, the line of demarcation between these two classes of substances, or better, between these two states, is by no means so definitely defined as is generally believed, for not only are there substances which may be so arranged that every transitional stage may be filled in between the most typical colloid and the most typical crystalloid, but also it is found that the transition from one state to the other occurs with such apparent ease that the two states can not be so far separated as it would seem upon superficial investigation. This facility of transition is well illustrated in the various steps in the saccharification of starch: The starch-grain has a crystalline structure as well defined as the structure of spherocrystals of many typical inorganic crystalloids; by the adsorption of water the crystallized starch is converted into a typical colloid that forms a typical *pseudo-solution*; by demineralization of the pseudo-solution and subjection to high moist heat, a *true* solution is formed, and hence the colloidal starch is transformed into a crystalloidal form; by the action of diastatic enzymes the starch in this crystalloidal state is converted into erythro-dextrin, another colloidal substance normally existing in a colloidal state, but having certain colloidal properties which distinguish it from starch; by further action of the enzyme this colloid erythro-dextrin is converted into another colloid in the form of achroo-dextrin with the assumption by this substance of a further modification of properties; from this dextrin normally existing in a colloidal state there is formed maltose, a typical crystalloid; and from maltose there may be derived glucose, which as we are accustomed to see it is a crystalloid, yet (like iron, etc.) it may exist in colloidal form in combination with certain proteins. It goes without saying that, inasmuch as the processes of life are inseparably associated with protoplasm, and hence with the colloidal state of matter, that the interrelations of colloidal and crystalloidal states, together with the surprising readiness with which transitions from one state to another can be brought about, are phenomena of the greatest fundamental importance, and that every advance along these lines must bring us closer to the mechanics of protoplasm. Of no less fundamental importance are the transient and permanent effects of changes of external conditions on the constitution of starch, gelatin, fibrin, and such substances when in a colloidal state (page 96), for it seems that in these effects we have a primitive expression of the influence of changes in internal and external conditions upon the constitution of protoplasm, and hence upon its reactions;

in other words, that through the evolution of this basic property there have been brought about, and there is being brought about continually in all living matter, those various modifications of responsivity or irritability which we can readily observe if not explain.

(5) That there is no justification for the assumption of the existence of amyloextrin and maltodextrins as individual substances which have properties intermediate between starch and dextrin, and dextrin and maltose, respectively. It seems to have been shown quite clearly that they are merely mixtures of variable composition depending upon the particular kinds of substances entering into them. For reasons that are perfectly obvious the use of the words amyloextrin and maltodextrin should be discontinued, and especially the former, because of its indiscriminate use in designating soluble starch, special forms of starch, mixtures of starch and dextrans, etc. Likewise a clearer understanding of the dextrinous products of starch will be had by dropping multiple names for dextrans (nearly all or all of which are names for impure substances and unknown mixtures) and using exclusively erythroextrin and achrooextrin, until at least these latter bodies have been satisfactorily studied.

(6) That under various modifications of experiment abnormal forms of dextrin, and also gums, and also unusual saccharine products, etc., may be formed and that the processes may be carried beyond the sugar stage by the presence of certain enzymes, bacteria, etc.

(7) That the products of decomposition by high and dry heat, and by dilute acids and enzymes, and different acids, and different enzymes are not in all respects identical. The dextrans formed by torrefaction, acid action, and enzymic action are not absolutely identical, and especially interesting is the comparison of the products of acid and enzymic action with each other, and of these with those of torrefaction. In fact, it seems probable, had we adequate methods for differentiating the vast number of hypothetical stereoisomeric forms of starches and of dextrans, that it would be found that corresponding dextrans, as for instance some specific kind of erythroextrin that is produced by mineral acid, organic acid, enzyme, or heat, would exhibit such differences as to show that we have as many stereoisomeric forms as we have types of means of producing them. In fact, one might go further and hold that the product of the plant enzyme will differ from that of the animal enzyme, and that as plant and animal enzymes differ in essential respects from one another, the product will be correspondingly modified. The conception held by many that dilute acids and enzymes merely increase the velocity of reactions seems disproved by the fact that the products of the reactions of these two classes of decomposing agents differ not merely quantitatively but also qualitatively. It seems obvious, if the effects were merely those of an energizer, that no qualitative differences such as have been reported would be noted in the digestions by acids and enzymes. That they increase the velocity of reactions must be admitted, and likewise that *any substance* under appropriate conditions may act catalytically, but there are certain special properties which are attached to each catalytic body and its modes of action which must not be ignored.

(8) Accepting the hypothesis of van't Hoff that an enzyme gives rise only to such products in the analysis of a given substance as it will under appropriate conditions combine in the synthesis of the same substance, and coupling this hypothesis with the conception that the synthesis of starch in the plant is essentially fundamentally through the actions of enzymes, it is obvious that accurate knowledge of the processes in plants during this synthesis, and accurate knowledge of the processes in the analysis by the same enzymes *in vitro*, would be mutually helpful and corrective, the one checking the other. Hence the importance of comparative investigations and of advances in one investigation foretelling identical advances in the other. Thus, if *in vitro* by the agency of plant enzymes sugar may be reduced to aldehyde and this to  $\text{CO}_2$ , and  $\text{H}_2\text{O}$ , we may with confidence look for the reverse processes in the plant, which is essentially synthetic in contradiction to enzymic processes *in vitro* which are under the usual conditions of experiment essentially analytic; but the latter we may have reversed under appropriately altered conditions.



## CHAPTER IV.

### THE DIFFERENTIATION OF THE STARCHES FROM DIFFERENT GENERA, SPECIES, ETC.

The differences in the histological characteristics of starches from different kinds of plants attracted the attention of some of the earliest workers, and as far back as 1834 Fritzsche (page 64) noted that not only were the forms very various, but *often* were so characteristic as to indicate the genus or family, or even the species. This observation received more or less confirmation in the investigations of Schleiden and others. In fact, the differences in the forms of the grains of certain of the familiar articles of commerce, such as cereals, beans, peas, potato, etc., are so marked as to have given the impression to superficial observers that the microscopical appearance is typical for each individual starch, whatever its source.

That the starch of each species is specific in certain of its characters is obvious from the results of the present investigation, but it would be hazardous, as shown by the records of Nägeli and others (Chapter V), to rely solely, or even often, in any important measure, upon the histological features of the grains. In Plates 13, 51, 68, 75, and 78, figs. 77, 301, 407, 441, and 465, starches are pictured from species belonging to entirely different genera. If such grains were mixed it does not seem likely that the microscopist would detect that it is a mixture, yet such could readily be shown by other means. In other words, the morphological method by itself may be entirely misleading, yet when coupled with other methods may have very great value. By the histological peculiarities it might be possible to state that this or that starch did not come from a certain species or genus, yet it might be impossible to assign it to its proper source, because of its seeming histological identity with certain other starches which may not have even the remotest relationship.

The investigations along various independent lines of inquiry have demonstrated that we have a number of means which collectively will not only enable us to differentiate the starches of different genera, species, varieties, and hybrids, but also of different parts of the same plant, indicating thereby a specific biologic relationship between the peculiarities of the starch-grains and the specialized plastids which form them, so that even in the same individual, if there are a number of groups of these starch-forming cells, each group being differentiated from the others, each will produce a form of starch which logically should have individual distinctive histological characteristics; but such characteristics would not of themselves necessarily imply stereochemic differences.

Comparative investigations of starch have been pursued by various methods, such as the following:

- (1) The histological method, by which are studied the form and size of the grains, position and character of the hilum, characteristics of the lamellæ, orientation, etc.
- (2) The proximate constituents and other features as regards general chemical composition.
- (3) Color reactions with various reagents.
- (4) Reactions with aniline dyes.
- (5) Reactions with swelling reagents.
- (6) Temperature of gelatinization.

- (7) The refractive index.
- (8) The reaction in polarized light.
- (9) Characters of the starch-paste and starch-solution.
- (10) The phenomena of digestibility.

#### THE HISTOLOGICAL METHOD.

The findings by the histological method have been referred to in the preceding chapters and will be quite fully reviewed in subsequent chapters, especially in Chapter V, so that nothing need be stated at this place.

#### PROXIMATE CONSTITUENTS AND OTHER FEATURES OF GENERAL CHEMICAL COMPOSITION.

##### THE PROXIMATE PRINCIPLES.

Starch-grains consist approximately of from 80 to 85 per cent of starch-substance, 15 to 20 per cent of water, and small and variable amounts of organic and inorganic substances, including fat, cell residues, cholesterin, dextrin, sugars, protein matters, phosphates, tannin, copper, and other substances. The figures in table 14 are by König (Die menschl. Nahrungs- u. Genussmittel; Die Rohstoffe des Pflanzenreiches, Wiesner, Leipzig, 1903, Bd. I, 580). (See Jessen, page 29.)

TABLE 14.

Kind.	Starch substance.	Water.	Ash.	Fat.	Cell residues.
Wheat.....	83.3	14	0.4	0.2	0.3
Corn.....	84.1	14	0.4	....	....
Arrowroot.....	84.1	15.7	0.2	0.1	0.05
Sago.....	82.8	12.9	0.4	....	....
Tapioca.....	84.8	14.4	0.25	....	....
Potato (a).....	79.6	19.2	0.3	0.04	0.1
Potato (b).....	80.8	17.2	1.0	....	....

Commercial starches, and starches as ordinarily prepared in the laboratory, contain large percentages of water and more or less impurities that can be removed by successive treatment with dilute hydrochloric acid, alcohol, and ether, and subsequent drying. Salomon (Reportorium f. Analyt. Chem., 1881, 274; Jour. f. prakt. Chemie, 1882, xxv, 348, and xxvi, 324) recommends that desiccation be carried on at 120°, because at lower temperatures water is retained, while at higher temperatures the starch becomes discolored and decomposition processes set in.

##### THE STARCH-SUBSTANCE.

The starch-substance may be regarded, in the light of the more recent investigations, as consisting of a number of modified forms of a single substance, and it is probable that not only are certain of these modifications peculiar to the species, etc., but also that variations exist in the kinds and proportions of these modifications in different grains, and even in a given grain from the starch of a given plant. Meyer (*loc. cit.*) records that the percentages of  $\alpha$ -amylose differ in different starches, as follows: potato 0.6, rice 0.9, corn 1, wheat 0.5, and arrowroot 2.5. Day (U. S. Dept. Agriculture, Office Expt. Stat. Bull. 202, 1908, 40) states that blue-amylose represents the entire inside of potato, arrowroot, tapioca, and sago starches, and 90 per cent or more of wheat, corn, rice, and barley starches; that red-amylose constitutes the outer layer of starch-grains; and that rose-amylose forms about 10 per cent of the inner part of wheat, corn, rice, and barley starches, but is absent from potato, arrowroot, tapioca, and sago starches (see page 58). See also references to the investigations of Kraemer, Salter, Denuiston, and others in Chapter II.

## THE WATER OF STARCH.

Starch is very hygroscopic, and the per cent of water is variable in relation to temperature, the amount of moisture in the air, and the character and amount of foreign substances present. According to table 14 the percentages range from 12.9 to 19.2 for starches of different kinds, sago starch having the lowest and preparation *a* of potato starch the highest. Payen found that potato starch, after exposure for several days to an atmosphere saturated with moisture, contained 35 per cent of water. The freshly prepared starch may have 45 per cent of water. Soxhlet (Centralb. f. Agrikultur-Chemie, 1881, 554) recorded for air-dried potato starch 20 per cent of water, and for wheat and corn starch 16 per cent. Salomon (Jour. f. prakt. Chemie, 1882, xxv, 348; xxvi, 324) gives for air-dried rice starch 22.98 per cent of water. Saare (Zeit. Spiritusind., 1901, xxiv, 502, 512) found for wheat starch 9.9 to 15.3 per cent (mean 13.2) and about the same for corn. Hoffmann and Philippe (Woch. f. Brauerei, 1905, xxii, 71) give for air-dried potato starch 14.38 per cent. According to Dubose (Dingl. Polyt. Jour., 1892, cclxxxv, 213) the starch of the sago palm contains 12 per cent of water. Nossian (Jour. f. prakt. Chemie, 1861, lxxxiii, 41) showed the marked hygroscopic properties of starch by subjecting starches from various sources, that had been dried at 100°, to atmospheres containing 73 and 100 per cent of moisture respectively at 17° to 20° (table 15).

TABLE 15.

Kind.	Air containing 73 per cent of moisture.	Air containing 100 per cent of moisture.
Wheat.....	6.94	18.92
Rye.....	10.01	19.36
Potato.....	10.33	20.92
Corn.....	10.53	19.55
Buckwheat.....	10.85	20.02
Rice.....	10.89	10.84
Acorn.....	11.96	22.98

Per cent of water  
in the starch.

Archbold (Jour. Soc. Chem. Industry, 1887, vi, 83) gives the following data:

Starch dried at 100° *in vacuo* is completely dehydrated.  
 Starch dried at 15.5° *in vacuo* contained 10 per cent of water.  
 Starch dried at 20° in air containing 0.6 per cent of moisture contained 18.6 per cent of water.  
 Starch dried in air saturated with moisture contained 35.7 per cent of water.

Carl Nägeli (Die Stärkekörner, etc., *loc. cit.*) noted that heat is developed upon the addition of water to dry starch. Fischer (Beihefte z. botan. Centralbl., 1902, xii, 227) states that the phenomenon is due to the chemical affinity of the starch-substance for water. Ullik (Zeit. f. d. ges. Brau., 1891, 565) dried potato starch at 120° and found that it had contained 12.1 per cent of water. Starch after having been exposed in an atmosphere saturated with moisture at 16° to 20° contained 37 per cent of water, and when mixed with water no rise of temperature occurred. When placed in an indifferent solution, such as a solution of sucrose, anhydrous starch took up 23.75 to 24.58 per cent of water, while the air-dried preparation absorbed from 18.98 to 19.1 per cent. The drier the starch the larger the quantity of water taken up and the greater the rise of temperature. Ullik, in experiments with 20-gram quantities of dried starch mixed with the same weight of water, recorded the changes in temperatures shown in table 16.

TABLE 16.

Preparation.	Increase of temperature.
1. Anhydrous starch, dried at 120°...	13.8°
2. Starch, dried at 90°.....	12.0°
3. Starch, dried over sulphuric acid..	8.8°
4. Starch, air-dried.....	3.0°

Ullik's results received confirmation in the investigations of Hoffmann and Philippe (*loc. cit.*) and of Emslander and Freulich (quoted by Hoffmann and Philippe).

## THE ASH.

The phosphates of the starch-grains have attracted especial attention because of their giving, it is believed, an acid reaction to certain kinds of starches; because of their assumed effect upon the rapidity of the decomposition of starches by enzymes; and, finally, because of significant different percentages in starches from different sources and in different grains and parts of the same grain. The relation of the phosphates to the reaction of the grains will be referred to in a subsequent paragraph. The asserted effect upon enzymic processes has been a matter of dispute.

Effront (*Enzymes and their Applications*, trans. by Prescott, 1902, 118) records that the addition of 0.5 gram of acid calcium phosphate to 100 c.c. of boiled starch increased the saccharifying power of an infusion of malt 5.3 times, and that 0.7 gram of ammonium phosphate caused an increase of 6 times. It has been noted by a number of investigators that the addition of very small amounts of certain acids or acid salts is favorable to enzymic activity.

Fernbach (*Woch. f. Brau.*, 1900, xvii, 35; *Jour. Soc. Chem. Ind.*, 1900, xix, 260) notes that Ling and other investigators found that diastatic activity is increased by small quantities of acid and that a distinction must be made between acid salts and free acids. In comparative experiments with increasing additions of acids, it was found that excepting small quantities of acid phosphate the effect was injurious. Phosphates, he records, have a specific action on the velocity of saccharification. Neutral (dibasic) phosphates are alkaline to methyl orange, and they have a harmful effect on saccharification. Diastase was found to act best in a medium that has a neutral reaction to methyl orange, therefore acid phosphates (monobasic) are favorable, while free acid is harmful.

Fernbach and Hubert (*Compt. rend.*, 1900, cxxxI, 293) found that the primary action of alkaline phosphates is to increase the activity of malt diastase, and the secondary action is to decrease activity. They ascertained that during both primary and secondary actions the reaction of the preparations was acid to phenolphthaleine and alkaline to methyl orange.

Ford (*Jour. Soc. Chem. Industry*, 1904, xxiii, 414), in reviewing the subject, states that in his opinion the favorable effect of the acid is not due to the acid alone, but to its neutralizing alkaline impurities, which have a powerful inhibiting effect on diastatic action, and that when these are more or less neutralized the action approaches its normal maximum, which takes place in a neutral medium, or at least one in which the free hydriions or hydroions are at a minimum. Ford found that ordinary preparations of soluble starch invariably contain phosphates and possible traces of organic phosphorus compounds which are not removed by prolonged treatment with dilute alkali or acid, or washing with water. He holds that the acid phosphates constitute a negligible amount of acidity to starch-solutions, and that they may be looked upon as neutral salts. Maize, wheat, and rice starch contain less or are more easily freed from phosphorus compounds than potato starch, and Ford states that he had prepared from maize specimens which are close approximations to the pure substance.

An especial degree of importance seems to have been attached to the phosphorus content of starch by the investigations of Fernbach (*Compt. rend.*, 1904, cxxxviii, 428), who found that large grains and small grains of potato starch differ materially in the percentage of phosphorus. For every 100 grams of large and small grains he found the following proportions of  $P_2O_5$ , expressed in milligrams: 160-199, 143-158, 159-185, 160-194, 178-226, and 138-215, the proportions in the larger heavier grains to the smaller lighter grains being 100 : 110 to 115. Regarding the smaller grains as being young grains (see page 180) he arrives at the conclusion that the mature starch-grain consists of a nucleus that is relatively rich in phosphorus which becomes during the course of growth covered with layers free from phosphorus, and which constitute the larger portion of the grain. The differentiation of the small young grains from the later deposited layers has also been shown by means of aniline dyes (page 57).

## THE REACTION OF STARCHES.

The reactions of commercial starches differ widely, owing chiefly to different methods of preparation and variable contaminations. Soxhlet (Reportorium f. analyt. Chemie, 1881; Jahr. ii. d. Fort. d. Thierchemie, 1881, xi, 86), by titrating commercial starches with a standard solution of sodium hydroxide, determined that for every 100 grams of potato or wheat starch the acidity was equal to 0.06 to 0.4 gram of sulphuric acid. For potato starch from 15 to 85 c.c. of a N/10 NaOH solution, and for wheat starch from 13 to 19 c.c. were required for neutralization. Rice and corn starches were, on the other hand, usually markedly alkaline.

Meyer (Die Stärkekörner, *loc. cit.*) states that the reaction of commercial starches varies, generally being acid, but sometimes alkaline. He records that he has not found a neutral preparation. Saare (Zeit. Spiritusind, 1901, xxiv, 502) noted that both wheat and corn starches are acid, wheat being the more acid.

Ford (Jour. Soc. Chem. Ind., 1904, xxiii, 414) observed that the reactions of starch preparations, as determined by color indicators, are variable in accordance with the indicator. A solution of ordinary starch may be *neutral* to rosolic acid, *acid* to phenolphthaleine, and *alkaline* to methyl orange. Therefore, in referring to starch as being neutral, acid, or alkaline, it is necessary to define in what sense the word is used. Litmus paper and litmus solution, he states, are useless for testing starch. A number of starches from different sources were prepared by successive treatment with alkali and dilute acid, well-washed, and dried. Given quantities (15 to 20 grams) were gelatinized by heat, and upon cooling were liquefied at 79° to 80° by a trace of precipitated diastase, and then boiled. The preparation was

made up to 500 c.c., then 1 c.c. of malt extract was added to each 70 c.c. of preparation and the mixture digested at 40° for an hour, and then boiled and made up to 100 c.c. The reactions of 10 grams of preparation were found to be as given in table 17.

From similar preparations of the same starches made by Lintner's method, the results appearing in table 18 were recorded.

TABLE 17.

Kind of starch.	Reaction with rosolic acid.
Arrowroot, Natal.....	Neutral
Rice.....	Acid; 0.17 c.c. N/100 NaOH
Wheat.....	Acid; 0.17 c.c. N/100 NaOH
Corn.....	Alkaline; 0.10 N/100 H <sub>2</sub> SO <sub>4</sub>
Potato.....	Neutral
Barley.....	Acid; 0.17 c.c. N/100 NaOH
Arrowroot, unpurified.....	Alkaline; 0.30 c.c. N/100 H <sub>2</sub> SO <sub>4</sub>
Arrowroot, Lintner's soluble	Neutral

TABLE 18.

Kind of starch	Reaction with rosolic acid.	Reaction with phenolphthaleine.
Arrowroot <i>a</i> .....	Acid; 0.8 c.c. N/100 NaOH	Acid; 5 c.c. N/100 NaOH
Rice.....	Acid; 3.7 c.c. N/100 NaOH	Acid; 27 c.c. N/100 NaOH
Wheat.....	Acid; 5.2 c.c. N/100 NaOH	Acid; 27 c.c. N/100 NaOH
Corn.....	Acid; 4.6 c.c. N/100 NaOH	Acid; 25 c.c. N/100 NaOH
Potato.....	Acid; 1.5 c.c. N/100 NaOH	Acid; 15 c.c. N/100 NaOH
Arrowroot <i>β</i> .....	Acid; 0.5 c.c. N/100 H <sub>2</sub> SO <sub>4</sub>	Acid; 5 c.c. N/100 NaOH
Arrowroot <i>γ</i> .....	Alkaline; 1.0 c.c. N/100 H <sub>2</sub> SO <sub>4</sub>	Acid; 10 c.c. N/100 NaOH
Potato I.....	Alkaline; 2.0 c.c. N/100 H <sub>2</sub> SO <sub>4</sub>	.....

The acid property of starch was noted by Blondeau (Compt. rend., 1864, LIX, 403), who states that starch forms with ammonia a combination having the properties of a weak base. Rice starch demineralized by dilute hydrochloric acid and washed free from acid was found by Demoussy (Compt. rend., 1906, cxlii, 933) to have properties of a weak acid comparable to carbonic acid. He reports that it forms compounds with metallic hydroxides, ammonia, and alkaline carbonates which are dissociable by water. He also

noted that it has the property of absorbing neutral salines, etc. The asserted acid character of starch is in accord with the observation of Ford and Guthrie (Chem. Soc. Trans., 1906, LXXXIX, 76), who state that soluble starch freed from impurities possesses feebly acid properties, as is shown by the fact that it is capable of combining with sodium hydroxide to such an extent that the molecular conductivity of the sodium hydroxide solution is reduced very considerably upon the addition of soluble starch.

## MISCELLANEOUS CHEMICAL DIFFERENCES.

The proportions of insoluble residues are variable in relation to different kinds of starches, as will be noted by table 13 on page 166. W. Nägeli (Die Stärkegruppe, etc., *loc. cit.*) states that potato starch leaves a larger quantity of insoluble residue than wheat starch, which is the reverse of the figures in König's table. Parow and Neumann (Zeit. Spiritsind., 1907, xxx, 561) worked out a simple method for the determination of the quantity of starch in commercial products. One of the best-known methods is to digest the starch with malt extract, finish saccharification by dilute hydrochloric acid, and determine the dextrose by Fehling's solution, using the theoretical factor 0.9 for calculating the dextrose into terms of starch. Parow and Neumann not only found this factor too low (0.95 being better), but also that the determination could be made by a much simpler method by which dextrose can be determined polariscopically. In these experiments they ascertained that each of the starches examined has a specific factor, the differences depending upon degrees of saccharification, which of course are indexes of the quantities of undigested residue. The factors given in table 19 were recorded.

TABLE 19.

Kind of starch.	Polarization factor for dry starch.	
	Soliel-Ventzke scale.	Circular degrees.
Potato.....	2.872	8.288
Corn.....	2.938	8.478
Rice.....	2.944	8.497
Wheat.....	2.918	8.420

Parow (Zeit. Spiritusind., 1908, xxxi, 286) states that corn starch contains 0.44 per cent of protein, potato starch 0.26, and cassava starch 0.23. Little or no importance is to be attached to such data beyond the fact that they indicate the degree of contamination rather than specific differences in the composition of starches from different sources.

Various laboratory procedures were pursued by Bloemendal (Pharmac. Weekbl., 1906, XLVIII, 1249; Wochschr. f. Brau., 1909, xxxiii, 436; Chem. Ab. Amer. Chem. Soc., 1909, iii, 1102). He studied the properties of potato, rice, wheat, and arrowroot starches as regards chemical composition, content of pentosans, heat of combustion, and specific gravity. The C and H contents of the four starches were remarkably constant, varying from 44.03 to 44.54, and 6.02 and 6.45, respectively, these figures corresponding to the familiar mean values,  $C_{44.4} H_{6.2} O_{49.4}$ . The C and H values were the same in the small grains as in the large. The furfural contents in the form of phloroglucids obtained by boiling with hydrochloric acid were 0.025 for arrowroot and 0.032 for rice for each 2.5 grams of starch. The quantity of pentosans was very small. Unimportant differences were noted in specific gravity, water-content, and heat values.

## COLOR REACTIONS.

## IODINE.

The use of iodine as a color-test for starch dates from the earlier observers, and the blue reaction is regarded as being specific, although Claude Bernard showed that an abnormal glycogen may be formed in paralyzed muscles which may give the same reaction, while others have reported that some forms of plant mucus and of cellulose also gave a blue reaction. The rapidity and intensity of the reaction to starches from different sources

and also to different grains of the same starch, and even of parts of the same grain, have long been known. Boiled starch and soluble starch with very rare exceptions (p. 53) always yields a blue reaction, but starch-grains, and the grain-sacs after boiling starch, may give a blue, violet, or reddish reaction, depending upon the source, or upon the amount of iodine used and other conditions. It is quite common, after boiling, for the capsular part of the gelatinized grain to give a violet reaction, while the inner part becomes an indigo blue. A weak iodine-iodide of potassium solution is usually employed, preferably a 1 to 2 per cent Lugol's solution. The differences in the behavior of starch, erythro-dextrin, and achroo-dextrin with iodine has rendered this agent of great value in the recognition and differentiation of these substances. The relatively greater intensity of the blue reaction over the red with a mixture of starch and erythro-dextrin may cause the color reactions of the latter to be masked, unless the iodine be used cautiously.

#### IODINE VAPOR.

The use of iodine vapor to distinguish different kinds of starch was adopted by Dubose (Chem. Zeit., 1904, xxviii, 1149). Iodine crystals are placed on a watch-glass which is put on a glass plate, on which also is the starch, and the whole is covered with a bell-jar and set aside for 24 hours. Corn starch was colored a blackish-violet, wheat starch a bluish-gray, sago starch a brownish-gray, and potato starch a yellowish-gray, the intensity of the yellow being proportional to the amount of foreign matter.

#### IODINE-CHLORAL HYDRATE.

A modification of the iodine reagent as ordinarily employed is prepared by saturating a saturated solution of chloral hydrate with iodine. The chloral hydrate usually causes swelling and bursting of the starch-grains, which is coupled with the iodine reaction. Green (The Soluble Ferments and Fermentation, Cambridge, 1901, 59) made use of this reagent to trace certain changes in the reserve starch in germinating pollen-grains during the development of the pollen-tube. Green writes that the plant whose pollen gave the most satisfactory results was *Lilium pardalinum*. The ripe pollen-grains when treated with a solution of iodine-chloral hydrate, are rendered transparent and the starch-grains are stained blue. Mixed with them here and there were a few grains staining like erythro-dextrin. As the tube was put out from the grain these granules were gradually carried over into the protruding portion, and they flowed slowly down the tube as it extended. When the tube was as long as twice the diameter of the grain, if the iodine-chloral hydrate solution was added, the grains were found to be somewhat different in color, becoming slightly purple with iodine. With longer tubes, the grains, still traveling forward, showed this change more and more markedly, particularly near the tip of the tube. When a tube which had attained a length of 20 to 30 times the diameter of the grain was treated in the same way, the general effect of the iodine was very different. There were but few blue granules, which were in the part nearest the pollen-grain. The greater part of the length of the tube was studded with purple grains, and towards the tip they became nearly red. The starch was evidently in the process of digestion under the action of diastase, which other experiments had shown the same pollen to contain. The starch-grains did not change their shape nor show signs of corrosion even when seen under high magnification. The amount of chloral hydrate was not sufficient to cause the swelling that is observed with strong solutions.

#### IODINE-LACTIC ACID.

By the addition of iodine to hot syrupy lactic acid, Lagerheim (Svensk. Farm. Tidskr., 1901; Jour. Soc. Chem. Ind., 1901, xx, 1245) prepared a reagent which, like the iodine-chloral hydrate combination, renders the plant tissues transparent and at the same time colors the starch, by which means he proposes not only to determine the presence of starch *in situ*, but also to differentiate between exhausted and non-exhausted tea-leaves.

## PHENOLS WITH SULPHURIC OR HYDROCHLORIC ACID.

It was found by Ihl (Chem. Zeit., 1887, XI, 19; Zeit. d. Alpinen. Oesterr. Apotheker-Vereins, 1888; Jour. Soc. Chem. Ind., 1887, VI, 306, and 1888, VII, 511) that phenols with sulphuric or hydrochloric acid give with carbohydrates brilliant color reactions. If starch be moistened on a watch-glass with an alcoholic  $\alpha$ -naphthol solution, and if then a few drops of warm sulphuric acid are added, the starch is colored red-violet. Thymol, cresol, guaiacol, and pyrocatecol produce a splendid vermilion-red; resorcinol and orcinol give a yellow-red; whereas phloroglucinol gives a yellow-brown. The different kinds of gum behave on the whole like starch. The actions of phloroglucinol on arabin is very characteristic. If arabinose be boiled with an alcoholic solution of this substance and hydrochloric acid, a fine cherry-red is produced.

## REACTIONS WITH ANILINE DYES.

Starch seems to have affinities for many aniline dyes. Investigations in this connection have been referred to particularly on pages 55 to 58, and sufficient has been shown to prove that starch from different sources and even parts of the same grain do not react identically with a given stain.

## REACTIONS WITH VARIOUS AGENTS, WITH ESPECIAL REFERENCE TO THE DEMONSTRATION OF THE STRUCTURE AND COMPOSITION OF THE STARCHES FROM DIFFERENT SOURCES.

Quite a number of reagents, such as potassium hydroxide, tannin, sulphuric and other mineral acids, acetic acid, concentrated solution of chloride of zinc, glycerine, etc., were used by the investigators prior to the seventies in studying the properties of starch-grains. Meyer (Die Stärkekörner, *loc. cit.*) made use of a number of such reagents besides enzymes, to bring out the microchemical properties of  $\alpha$ -amylose,  $\beta$ -amylose, and amyloextrin, including among these potassium hydroxide, calcium nitrate, 25 per cent hydrochloric acid, and 3 per cent acetic acid. Kraemer (Jour. Amer. Chem. Soc., 1899, XXI, 650; Amer. Jour. Pharm., 1899, 174; Botanical Gazette, 1902, XXXIV, 341) made a comparative study of the structure of wheat, corn, and potato starches by means of the following agents:

- |  |  |
|--|--|
| <ol style="list-style-type: none"> <li>1. Chloral-iodine+iodine solution (equal parts of each).</li> <li>2. Chlor-zinc-iodide solution.</li> <li>3. Chloral solution (saturated), water, and glycerine (equal parts of each, to which iodine is added to saturation).</li> <li>4. Calcium nitrate solution, 30 per cent.</li> <li>5. Chromic acid solution, 15 per cent.</li> <li>6. Saliva.</li> <li>7. Taka-diastase.</li> </ol> | <ol style="list-style-type: none"> <li>8. Silver nitrate solution, 2 per cent.</li> <li>9. Sulphuric acid, C. P. with 10 per cent of water.</li> <li>10. Sodium acetate solution, 50 per cent.</li> <li>11. Potassium hydroxide solution, 0.1 per cent.</li> <li>12. Potassium nitrate solution, saturated.</li> <li>13. Potassium phosphate solution, saturated.</li> <li>14. Tannin solution.</li> <li>15. Hydrochloric acid, 5 per cent.</li> <li>16. Water.</li> </ol> |
|--|--|

The results of his comparative study of wheat and corn starch are presented in the following quotation:

## WHEAT.

(1) Chloral-iodine+iodine solution causes the grains to become at first uniformly blue in color; swelling of the grains soon takes place and finally alternate light blue and blue layers are observed.

(2) Chlor-zinc-iodide behaves similarly to the preceding reagent.

## CORN.

(1) Chloral-iodine+iodine solution causes some of the grains to swell in 5 hours and others to show a tricheten arrangement of the layers; the grains do not appear to be swollen to the extent that the wheat grains are, and therefore show apparently a deeper color with the iodine.

(2) Chlor-zinc-iodide brings out immediately the fissures or point of growth, which is in marked contrast to the wheat starch; in the course of several hours the grains swell at one end, the portion showing the swelling becomes light blue, and finally almost colorless, while the other portion remains of a deep blue color; some of the grains finally disintegrate into several portions.

## WHEAT.—Continued.

(3) Chloral and glycerine solution behaves similarly to No. 1, but the grain is not colored and the lacunæ or fissures are more pronounced.

(4) Calcium nitrate produces in 15 minutes a strong corrosion of some of the grains, and those not acted upon by the reagent in this manner swell in 1 hour very perceptibly, then show a tricheten-like\* development, and in 5 hours swell enormously, and finally burst.

\* Referring to the trichetes of Meyer (p. 77).

(5) Chromic acid produces a similar effect upon the grains, but appears to be more pronounced in its action.

(6) Saliva causes in some grains the development of prominent radiations and lamellæ, in others a tricheten-like structure is developed; in 5 hours the grains give indications of corrosion, which in the course of 17 to 24 hours is very pronounced.

(7) Taka-diastrase, on the other hand, acts very slowly in comparison. In 5 hours there is little or no effect observable, the tricheten-like structure developing after this length of time and corrosion finally taking place.

(8) Silver nitrate has but little action upon the grains at first; in 5 hours the tricheten-like development appears, and later the grains swell and disintegrate.

(9) Sulphuric acid acts almost immediately, causes the grains to become nearly transparent and irregular in outline, and a rapid solution takes place.

(10) Sodium acetate causes some of the grains, in the course of 5 hours, to swell and others to become very much corroded.

(11) Potassium hydroxide very soon produces a swelling and rupture of some of the grains and in others the development of a prominent tricheten-like structure, and finally in both a slow corrosion.

(12) Potassium nitrate causes almost immediately a swelling and rupture of the grains, or a strong corrosion.

(13) Potassium phosphate produces prominent fissures with the subsequent development of rather numerous tricheten-like layers in some grains, in others there is a swelling and rupture of the grains, with finally a gradual corrosive action on both.

(14) Tannin produces a swelling of the grains, together with the development of rather large irregular lacunæ, and in 5 hours the grains become very much swollen and of irregular shape, after which disintegration and solution takes place.

(15) Hydrochloric acid causes the appearance of prominent tricheten in some of the grains in a few hours, in others there is a tendency to swell, and both kinds finally divide into two or more parts.

(16) Water at a temperature between 50° and 70° C. produces a marked effect upon the grains; those digested at a temperature between 50° and 55° C. for several hours, are swollen, and in many cases even ruptured; at 60° C. they show a prominent tricheten-like structure, which is scarcely visible at 65° C. and after digestion at 70° for 1 hour the grains become very irregular and swollen and are apparently not further affected by a temperature between 70° and 95°.

## CORN.—Continued.

(3) Chloral and glycerine solution causes lenticular, somewhat irregular, or more or less star-shaped and prominent lacunæ or fissures, and in the course of 24 hours in some grains prominent radiations are developed, whereas in others a marked swelling takes place.

(4) Calcium nitrate makes the point of growth more visible as with previous reagents, then strong radiations or a tricheten-like structure develops in some grains, whereas in other grains the fissures develop into large radiating canals, which extend to the margin of the grain, the swelling continuing so that in 5 hours only the outline of the grain is visible.

(5) Chromic acid causes a prominent swelling of the more or less star-shaped point of growth, which continues to such an extent in some cases as to produce a rupture of the grain at one of the angles; in other cases there are numerous radiations, or a tricheten-like structure, developed around the swollen fissures, which finally disappear as the grain swells and breaks down.

(6) Saliva acts upon the grain very much like chromic acid and calcium nitrate, only instead of a swelling of the grain we have a rather slow corrosion in the course of 48 hours, following the pronounced development of fissures.

(7) Taka-diastrase behaves like saliva, only the corrosive action is more rapid.

(8) Silver nitrate causes the formation of prominent and angular fissures, which become more or less circular in outline, and near the periphery prominent radiations may develop.

(9) Sulphuric acid produces in some of the grains marked development of an angular fissure which becomes circular to radiating in outline, whereas in others a corrosive action appears to begin at the periphery of the grain, followed by gradual solution of the entire grain.

(10) Sodium acetate behaves very much like calcium nitrate.

(11) Potassium hydroxide acts similarly on the grain to calcium nitrate and sodium acetate.

(12) Potassium nitrate differs very markedly in its action on corn from that on wheat, in that there is a strong development of radiating fissures which extend in many cases to the periphery, whereas in wheat there is a more pronounced swelling of the grains and an irregular corrosive action.

(13) Potassium phosphate causes the development of a prominent lenticular or star-shaped fissure, which increases in size and in 17 to 24 hours there is a complete breaking down of the grain.

(14) Tannin causes also the immediate production of prominent fissures which in 5 hours develop into large canals, or circular portions, and there is finally a separation of the grain into several parts.

(15) Hydrochloric acid causes in some cases the development of large star-shaped or lenticular fissures, and in other cases in the course of but 20 minutes there is a marked swelling of the grain at one point, which continues until disintegration takes place.

(16) Water between the temperature of 50° and 70° C. causes certain characteristic features to be developed; the grains when heated for 90 minutes at 50° C. develop in most cases a rather pronounced circular fissure; on other grains there may be a swelling or lenticular or star-shaped fissure; at 55° to 60° C. the swelling of the grain is more pronounced, and at 65° C. the remainder of the grains show a marked one-layered tricheten-like structure; at 70° C. the markings have disappeared and the grains have become swollen to angular and irregular masses.

## POTATO.

The behavior of *potato* starch toward these reagents may be briefly summarized as follows: The first effect of chromic acid and other swelling reagents is to make the lamellæ more distinct; this is followed by the development of the crystalloidal character of the lamellæ, which is most pronounced in those colored blue with iodine; this is followed by the production of small tracts or channels connecting the contiguous lamellæ, particularly in the middle of the grain; succeeding this there is the formation of channels which are larger and plume-like in appearance, the grain meanwhile swelling quite perceptibly, the middle portion becoming clearer and assuming a zigzag outline, between which and the periphery of the grain a number of crystalloidal lamellæ arise; the grain now becomes spherical and marked by a number of concentric lamellæ near the periphery; the latter finally ruptures and then follows a gradual solution of the grain, the peripheral layer sometimes recurring like the cutin layer of an epidermal cell on treatment with sulphuric acid.

## WHEAT.

In *wheat* starch the development of the crystalloidal character of the lamellæ is followed by the formation of narrow, interrupted or continuous, radial channels near the periphery of the grain, which are sometimes connected with lamellæ occurring near the middle of the grain; the grain meanwhile swells quite perceptibly, the center becomes clearer, the contents are crowded into crescent-shaped halves, which are still connected at the poles; the contents of each of the halves of the grain consist of crystalloidal lamellæ in which are then produced small tracts or channels connecting the continuous lamellæ; the halves in some instances finally separating and slowly dissolving. In some cases, on the other hand, there is a corrosion of the grain at the periphery, followed by gradual disintegration without the separation into halves.

## CORN.

The first effect of reagents upon the *corn* starch-grain is to bring out the point of origin of growth; the latter becomes larger and in some cases more or less zigzag in outline; between this and the periphery of the grain arise more or less interrupted or continuous radial channels (usually the latter); the crystalloidal structure of this grain develops slowly and is most pronounced when the grain has swollen to two or three times its normal size; at this stage we find that the center of the grain has become clear and the point of origin of growth has become obliterated in some cases, and between it and the periphery occur numerous crystalloidal lamellæ similar to those observed in the potato starch; finally the peripheral layer ruptures and there is a gradual disintegration of the grain. Sometimes the grain appears to separate into as many parts as there were arms to the point of origin of growth, particularly when acted upon by saliva or diastase.

The behavior of starch towards chromic acid was studied by Hürz (Beiheft. z. bot. Centralbl.; Woch. f. Brau., 1905, xxii, 721), who found that with chromic acid alone colors are obtained, varying from a yellowish-grayish-green to an olive-green, and finally a golden-yellow, according to the concentration of the solution. With a mixture of chromic and sulphuric acids the colors were shades of green. The different kinds of starch, and also different grains of the same starch, varied greatly in their behavior, so much so that starch can not be regarded as a physically uniform substance, the grains differing from one another according to the denser or looser constitution of their ultimate complexes.

Sodium salicylate has been reported by Lenz (Seventh Inter. Congress Appl. Chem., London, 1909; Jour. Soc. Chem. Ind., 1909, xxviii, 731) as a reagent to be employed in the microchemical differentiation of starches of different kinds. He states that if a trace of rye starch, in a hanging drop of a solution of 1 part of sodium salicylate in 11 parts of water, is examined under a magnification of 200, at the ordinary temperature, it will be found that after the lapse of an hour (more distinctly after 24 hours), most of the large granules have swollen; only a small part resists the action of the salicylate and still shows the polarization cross between crossed nicols. In the case of wheat starch, only a few of the large granules become swollen; after 1 to 24 hours the outline of the unswollen wheat starch-granules is sharply defined, and the granules, unlike those of rye starch, do not become flattened (starch of any kind which has been altered by storage in a moist condition swells on treatment with the salicylate solution). Barley and millet starches swell to a small extent only. But few of the grains of oats, maize, rice, potato, bean, pea, lentil, and arrowroot starches become swollen.

## TEMPERATURES OF SWELLING AND GELATINIZATION.

When starch-grains are heated in water they begin to swell at temperatures usually between 45° and 55°, sometimes higher and sometimes lower, according to the source of the starch. As the temperature increases the grains lose their form and become gelatinous,

forming ultimately a paste or a pseudo-solution of varying degrees of viscosity, according to the percentage and kind of starch and various attendant conditions. Lippmann (Jour. f. prakt. Chemie, 1861, LXXXIII, 51) studied the temperatures at which swelling and at which gelatinization begin, and at which gelatinization is complete. The starch and water were put in a beaker in a water-bath and slowly heated, and the preparation was subjected to microscopical examination at proper intervals. His results are given in table 20.

TABLE 20.

Kind of starch.	Swelling begins.	Gelatinization begins.	Gelatinization complete.
Rye.....	45.00	50.0	55.0
Corn.....	50.0	55.0	62.5
Horse chestnut ( <i>Esculus hippocastanum</i> ).....	52.5	56.25	58.75
Barley.....	37.5	57.5	62.5
Chestnut ( <i>Castanea vesca</i> ).....	52.5	58.75	62.5
Potato.....	46.25	58.75	62.5
Rice.....	53.75	58.75	61.25
<i>Arum maculatum</i> .....	50.0	58.75	62.5
Arrowroot ( <i>Maranta arund.</i> ).....	66.25	66.25	70.0
Tapioca ( <i>Jatropha utilis</i> ).....	.....	62.5	68.75
<i>Arum esculentum</i> .....	45.0	63.75	68.75
Sago ( <i>Sagus rumphii</i> ).....	.....	66.25	70.0
Buckwheat.....	55.0	68.75	71.25
Acorn.....	57.5	77.5	87.5
Wheat.....	50.0	65.0	67.5

Lintner (Tollen's Handb. d. Kohlenh., II, 207) records that potato starch suddenly goes into a paste at about 62° to 64°, while cereal starches undergo the same change at 75° to 80°. (See table 23, page 178.)

According to Dafert (Meyer, Die Stärkekörner, loc. cit. p. 134) rice starch gelatinizes at 73°. Whympfer (Seventh Int. Cong. Appl. Chem., London, 1909; Jour. Soc. Chem. Ind., 1909, xxviii, 806) recorded the temperatures of gelatinization of barley, corn, rye, potato, rice, wheat, and tapioca starches by subjecting them to a gradually rising temperature and examining them microscopically. The values differed in most cases from those of previous observers, and also varied with the state of maturity of the grains. The larger granules of any given starch were found to almost invariably succumb more quickly than the smaller granules to both wet and dry heat, and to diastase and mineral acids.

Gelatinization may be brought about by various chemicals, such as potassium hydroxide, chloral hydrate, chromic acid, etc. (see page 172).

## REFRACTIVE INDEXES OF STARCHES.

The refractive indexes of starches from different sources were determined by Ott (Osterr. bot. Zeitschr., 1899, xxxix, 313). The figures recorded are given in table 21.

TABLE 21.

Kind of starch.	Refractive index.	Kind of starch.	Refractive index.
Fritillaria.....	n = 1.5040	Barley.....	n = 1.5220
Potato.....	1.5135	Corn.....	1.5222
Canna.....	1.5200	Wheat.....	1.5245
Sago.....	1.5208	Maranta.....	1.5247
Rye.....	1.5212	Tapioca.....	1.5293
Rice.....	1.5219		

## REACTIONS IN POLARIZED LIGHT.

The behavior of starch-grains toward polarized light seems to have been discovered by Biot (Compt. rend., 1844, xviii, 795). Since then many observers have noted the differences in the form and distinctness of the interference figure or "cross"; and also differences in the degree with which light is transmitted by grains of the same and of different starches,

and in the colors with a selenite plate. The point of intersection of the two parts of the cross usually corresponds to the position of the hilum, or in compound grains to each of the many hila. In grains having a centric hilum, the form of the cross corresponds with that of the Greek cross or cross of St. George, but in grains with eccentric hila, the figure corresponds with that of the Latin cross, the cross-arm being in any position between a short distance from the center and the almost extreme end of the part corresponding to the upright, and in such grains the arms become curved, sometimes so much so as to resemble the hanging branches of the weeping-willow tree, losing the appearance of a cross. Many illustrations will be seen in the accompanying plates. Muter (*loc. cit.*) found that the following starches give a well-marked cross: Potato, canna, maranta, Natal arrowroot, turmeric, mother-cloves, sago, tapioca, and cinnamon. The following do not give a well-marked cross: Wheat, barley, rye, acorn, and cacao. The following give a faint cross: Ginger, banana, nutmeg, sorghum, oat, and corn. The following give an indistinct cross: Pea, bean, and lentil. Rice gives a cross that is distinct and well-marked, and pepper shows a cross by high magnification.

The polariscopical properties are destroyed by all agents which cause a swelling of the grains, but they are not affected after prolonged subjection of grains to dilute acid, which renders the grains soluble without destroying their skeletal structure.

The employment of polarized light for the microscopic detection of the presence of foreign starches has been proposed by Gastine (*Compt. rend.*, 1907, cXLIV, 35; *Jour. Soc. Chem. Ind.*, 1907, xxvi, 108). A small quantity of the starch is suspended in a drop of water, placed on a microscopic slide, and dried by exposure at room temperature and finally by heating for a few moments at 120° to 130°. The preparation is then mounted in Canada balsam and examined in ordinary polarized light, and also with the addition in the polariscope of a selenite plate. With ordinary polarized light, the grains of rice starch with a magnification of 300 appear brilliantly illuminated in the dark field and present a granitic structure. In the chromatic polarized light obtained by means of selenite, the blue and orange tints present a characteristic network of lines. Corn starch gives a similar network, but its meshes are much larger than those obtained with rice starch; corn starch also polarizes brilliantly. Millet, buckwheat, and rye starches, and many others, show appearances similar to those of rice and corn starches. The characteristic form of the grains of bean and pea starch and their very brilliant polarization render their determination easy. In the case of wheat starch, the groups formed of these grains are of very varied size and irregularly placed, giving a very characteristic appearance under the microscope; seen in chromatic polarized light they do not present a symmetric network of lines.

#### CHARACTERS OF STARCH-PASTE AND PSEUDO-SOLUTIONS FORMED BY STARCHES FROM DIFFERENT SOURCES.

The characters of the starch-paste and also of the pseudo-solution vary with different kinds of starches. This has been recognized in technical trades for many years, and on this account preference is given to certain starches in the stiffening, sizing, and finishing of fabrics. Potato starch yields a preparation by boiling that is poorly adapted to these purposes, whereas wheat and corn starch yield an excellent product; but where a starch is normally not of high value in this respect it may be rendered so by various means, as, for instance, by the process suggested by Bellmas (*Osterr. Chem. Zeitung.*, 1902, 366), which is to digest potato starch at 55° in a 2 per cent hydrochloric acid, then forming a limpid preparation by boiling. Various of the methods employed for making "soluble starch" and liquefying starch-paste may be used (see page 101). In the experiments of Saare and Martens (*Zeit. Spiritusind.*, 1903, xxvi, 436) it was found that the length of time the starch was heated at the boiling-point had a considerable influence upon the stiffening power of

the preparation. Wheat and corn starches did not attain their maximum stiffness until after boiling for some minutes; on the other hand, potato starch had its maximum stiffness when the boiling-point was reached, and lost in power very considerably by further heating.

The non-homogeneity of starch-paste was particularly referred to by Pottevin (Ann. d. Inst. Pasteur, 1899, XIII, 728) (see page 134). Ling (Jour. Fed. Inst. Brew., 1903, IX, 446) found considerable difference in the conversions of starch-pastes and also of raw starch at temperatures below the point of gelatinization.

The relative stiffening strength of starches prepared under the same conditions is, according to the Scientific American Cyclopedia of Receipts, taking 100 as the standard of comparison: Pure dry rice starch, 100; rice starch No. 1, 95; rice starch No. 2, 91; pure dry corn starch, 87; corn starch, 85; rye starch, 81; oat starch, 80; acorn starch, 80; wheat starch, 80; barley starch, 78; Bermuda arrowroot, 75; Natal arrowroot, 73; pure potato starch, 68; and potato farina, 65.

#### PHENOMENA OF DIGESTIBILITY.

##### RAW STARCHES.

It has long been established that starch-grains are in the nature of a reserve food of the plant and that they undergo ready solution *in situ* (presumably by the action of enzymes) when their derivatives are required for nutritive purposes. While such dissolution takes place with ease in the plant, in seeds, in bulbs, etc., such is not the case *in vitro*, even though presumably the same enzymes are present, unless the outer protective coatings of the grains are injured so as to expose the starch-substance, especially the innermost part. It is true that there are many records to indicate that various kinds of raw starch are digestible *in vitro* with various degrees of ease or difficulty, but it is quite clear that when bacterial action has been prevented, and when the grains are uninjured, absolutely no digestion occurs. If, however, the grains have been eroded by bacterial or other action, or if the grains be fissured or broken so as to permit contact of the enzymes with the intracapsular part of the grain, digestion proceeds with variable degrees of rapidity according to attendant conditions. So long as the grains are perfect, the coating, which varies in resistiveness in starches of different kinds, and also in grains of the same starch, serves as a perfect protective against the influences of diastatic enzymes *in vitro*, if bacterial action is prevented. The writer has subjected various kinds of starches to the actions of ptyalin, pancreatin, Taka-diastase, and malt diastase in a 1 per cent chloroform solution for over 12 months at optimal temperatures, without evidence of erosion or digestion of the grains, except in the case of injured grains; yet similar solutions of the same enzymes caused a practically complete saccharification of the boiled starches in 6 hours. It seems that the virtually absolute indigestibility of perfect starch-grains *in vitro* in the presence of enzymes is owing to the absence of some factor in the plant that in some way gets rid of the barrier presented by the coating.

The non-digestibility of raw starch by enzymes was recorded as far back as 1835. Guérin-Varry (Ann. d. chim. e. phys., 1835, LX, 32) set aside a preparation of potato starch and malt extract in a sealed tube, at room temperature, for 63 days. At the end of this period there was not a trace of sugar, nor did the grains show any microscopic changes. Dubrunfaut (Ann. d. chim. e. phys., 1847, XXI, 178) noted that while raw potato starch is unaffected by malt diastase, wheat, barley, and rice starches are affected. C. Nägeli (Die Stärkekörner, *loc. cit.*), in his digestion experiments with saliva, by which he differentiated the so-called amylose and cellulose, found that the amylose was digested, leaving the skeleton cellulose. In fact, as the author has found, saliva becomes absolutely inert before any such action can take place. Hence it was not the saliva, but some other agent, that caused the effects noted. The influences of saliva on raw starch were also studied

by Hammarsten (Jahr. ü. d. Fort. d. Tierchemie, 1871, I, 187), who writes that the variation in the time required for changing starch-granules into sugar is due to differences in the starches, and also according as to whether the grains were whole or broken or gelatinized. Thus, in experiments made with human saliva, sugar was formed from the raw starches as shown in table 22.

Hammarsten states that since the use of starch-paste made from these several starches showed no difference in the sugar-forming time, it may be presumed that the unequal deposition of cellulose in different starches presupposes unequal resistance to the influence of the saliva and that it was therefore natural to expect that starch-granules, which in a raw state were converted into sugar with difficulty, would react readily if after being pulverized they were treated with saliva.

This supposition was confirmed, inasmuch as finely powdered potato starch was found to be rich in sugar after 5 minutes. Chewing starch-granules was then tried, with the result that sugar was formed in all of the above-mentioned starches in 1 to 4 minutes. The refractivity of raw potato starch to digestion was also noted by O'Sullivan (Jour. Chem. Soc. Trans., 1878, II, 125), who records that in the presence of fresh malt extract the granules were unaffected after 24 hours.

Baranetzky (Die Stärkeumbildenen Fermente in den Pflanzen, Leipzig, 1878, 40) places the order of digestibility of raw starches as follows: Buckwheat, wheat, bean, acorn, chestnut, potato, and rice. This does not accord with Hammarsten's records. Brown and Heron (Ann. d. Chem. u. Phys., 1879, CLIX, 206) ascertained that starch-grains in the presence of malt extract do not undergo any change even after a considerable time although when *in situ* in germinating seeds they undergo more or less rapid solution. When, however, the grains were crushed by grinding in rough sand or broken glass, rapid solution occurred. The marked solubility of the broken grains was noted by Hammarsten and also by certain of the earlier investigators of starch. Recently Maquenne (Compt. rend., 1904, CXXXVIII, 375) found that fully comminuted grains yield as much soluble matter by digestion at 55° with malt extract as starch-paste, and therefore that rupturing the grains is as effective as gelatinization by heat in rendering the raw starch digestible.

Lintner (Brau. u. Malzkalender, 1890, XIII, 83) carried out a series of experiments with various starches with diastase at different temperatures. In each experiment 2 grams of air-dried starch were subjected to the action of 50 c.c. of malt extract for 4 hours. Each preparation was then made to 100 c.c. by the addition of water, and then filtered to remove the undigested starch. The filtrate was saccharified by weak hydrochloric acid, and the sugar determined by Fehling's solution. From the sugar determinations the figures given in table 23 were estimated as showing the percentages of starch digested.

TABLE 22.

Kind of starch.	Time of appearance of sugar.
Potato.....	2 to 4 hours.
Pea.....	1½ hours.
Wheat.....	30 to 60 minutes.
Barley.....	10 to 15 minutes.
Oat.....	5 to 7 minutes.
Rye.....	3 to 6 minutes.
Corn.....	2 to 3 minutes.

TABLE 23.

Kind of starch.	Temperature of digestion.				Temperature of gelatinization.
	50°	55°	60°	65°	
Potato.....	p. ct. 0.13	p. ct. 5.03	p. ct. 62.68	p. ct. 90.34	65
Rice.....	6.68	9.68	19.68	31.14	80
Barley.....	12.13	53.30	92.81	96.24	80
Wheat.....	.....	62.23	91.08	94.58	75 to 80
Malt (fresh).....	29.70	58.65	92.13	96.26	..
Malt (cured).....	13.07	56.02	91.70	93.62	..
Corn.....	2	....	18	54	75
Rye.....	25	....	40	94	..

The effects of enzymic action on raw starch were studied by Meyer (Die Stärkekörner, *loc. cit.*), who records that diastase as well as saliva can penetrate the porous starch-grains, as shown by the following observations: Fresh starch-grains from the endosperm of *Hordeum distichum* when placed in malt extract form many cracks which radiate from the center of the grains. The presence of these cracks is explained upon the assumption that the penetrating ferment causes a swelling of the grain. Starch-grains from *Dieffenbachia*, treated for 3 weeks with malt extract, when compared with untreated grains, show a degree of transparency which indicates that some of the substance has been lost, or that the grains have undergone some internal change. Diastase, he states, has the capacity to dissolve every layer of every starch-grain at ordinary room temperature if the action continues for a sufficient length of time. At 40° the ferment action is more powerful on intact grains than at 17°. At 40°, after 24 hours all the grains acted upon were dissolved. A pressure of 3 atmospheres on a preparation consisting of wheat starch and a solution of diastase did not accelerate the solution. In another place Meyer notes that perfect potato-starch grains are dissolved away centripetally in successive layers, gradually forming long, spindle-shaped grains; but grains having clefts or fissures were acted upon so as to give rise to channels, pits, cavities, etc. Similar effects were recorded with the starch of *Dieffenbachia seguine*.

The non-uniform composition of different starch-grains was pointed out by Pottevin (Woch. f. Brau., 1899, xvi, 641) to be shown by the behavior of starch-grains acted upon by diastase at ordinary temperature when the process is carefully observed under the microscope. Wheat starch, he found, is attacked by diastase at ordinary temperature. Pottevin observed that the thickest grains were less rapidly affected than the thinner grains, and that the smallest polyhedral grains were not at all attacked. The outer layers were not digested, remaining as residues. He also obtained a number of residues by digesting starch-grains in the cold for an hour, obtaining the residues of undigested matter, at specified periods, and subjecting these residues in turn to digestion. He thus obtained a number of residues, each successive residue being more resistant than the preceding to diastase. All of these residues were gelatinized with hot water, and in comparison with whole starch yielded only little more than one-half the proportion of maltose. He believes that the starch-grain is heterogeneous, and that therefore starch-paste is heterogeneous, the part of the paste formed from the less dense parts of the grains being easily digested and the denser parts digested with difficulty and after prolonged action, the dextrin from the latter constituting the "residual dextrins."

Effront (Enzymes and their Applications, trans. by Prescott, 1902, 128) states that a granule of starch is irregularly attacked by diastase, corrosion occurring in very different directions and places. The manner of corrosion, he found, arises from the inequality of resistance of the surface of the grains, so that the difference existing in the compactness of the various parts of the grain is, on the whole, the initial cause of the variations in the resistance to diastatic action. Potato and barley starch he writes are both composed of non-homogeneous grains whose layers differ in degree of compactness; but in potato starch more resisting layers are found than in barley starch. Gelatinization, he records, does not change the properties of starch, which are owing to the variations in the compactness of the layers; therefore the more coherent particles form a paste that is more difficult to liquefy, and yield a dextrin of greater resistance than the less coherent particles.

Ling (Brit. Assoc. Report, 1903; Jour. Soc. Chem. Ind., 1903, xxii, 1058) found in his investigations of the action of diastase on starch-grains of raw and malted barley that the starches of cereals differ from the starch of potato in being readily attacked by diastase in an ungelatinized condition. He carried out a series of experiments with mashes of barley and malt starches of various origin, the starches being mixed with the diastase preparation in the dry state and mashed with water at various temperatures for 2 hours. The figures in table 24 illustrate the results.

TABLE 24.

Kind of starch.	Mashing temperature.	$(a)_D = 3.93$		Kind of starch.	Mashing temperature.	$(a)_D = 3.93$	
		$^{\circ} E.$	$p. ct.$			$^{\circ} E.$	$p. ct.$
Barley, 1899 sample..	60	140.8	88.7	Kilned malt sample..	71	161.3	67.2
Do.....	65.5	143.4	88.2	Low-dried malt.....	60	151.6	85.3
Do.....	71	145.1	80.6	Do.....	65.5	152.5	83.3
Barley, 1902 sample..	60	150.7	84.2	Do.....	71	165.7	66.6
Do.....	65.5	152.3	79.0	Barley starch (paste)	65.5	168.6	52.4
Do.....	71	163.8	77.8	Potato starch (paste)	60	154.5	81.8
Kilned malt sample..	60	150.9	84.4	Do.....	65.5	155.6	71.5
Do.....	65.5	156.1	80.6	Do.....	71	167.1	55.3

Fernbach (Compt. rend., 1904, cxxxviii, 428) ascertained that the small, light grains as compared with the large, heavy grains of potato starch contain a higher percentage of phosphates, and he concluded that the grains consist of a nuclear portion relatively rich in phosphorus, upon which are superposed layers free from phosphorus. In digestion experiments he noted differences in the behavior of the grains and parts of grains in accordance with this peculiarity. Whole starch-grains were found by Maquenne (Compt. rend., 1904, cxxxviii, 375) to be but little affected by malt diastase. Only 2.8 per cent of the raw starch was dissolved after treatment with malt extract at 55°. The same amount of starch after boiling and subjected to the same quantity of malt, the same temperature and period of time, was completely saccharified. Under the latter conditions of experiment, finely comminuted raw grains were saccharified to the extent of 94.8 per cent, or almost equivalent to gelatinized starch. Day (U. S. Dept. Agriculture, Office Expt. Stas. Bull. 202, 1908, 24), in experiments with wheat, corn, barley, rice, potato, and arrowroot starches, found that raw starch digests slowly; that the cracked grains digest more rapidly than the solid grains; that the outer layer is less digestible than the inner; that the six kinds of starch fall into two divisions according to digestibility, the starches of wheat, corn, barley, and rice being more digestible than those of potato and arrowroot. (See pages 166, 189, 190, and 192 for further reference to the investigations of Day.)

Hanausek (The Microscopy of Technical Products, trans. by Winton, 1907, 37) describes erosion changes in potato-starch grains such as may be observed in a sprouting potato. The specimen examined he thinks had been acted upon by diastase. Some of the grains were marked by clefts and fissures which passed through or radiated from the hilum; others had an irregular sac-like nucleus; and others were corroded or finely pitted, displaying cavities, channels, etc. In a specimen of wheat starch he found grains which were partly eaten away, mostly on one side, but the appearance was different from that of the sprouted grain caused by diastase. He attributes the erosion to the action of acid used in the preparation of the starch. Eroded grains *in situ* he states display delicate concentric rings and more or less distinct central fissures. Often the rings in parts of the grain (in the same radial direction) are very distinct, while in the intermediate parts they are scarcely evident. After long-continued action of the ferments the grains become hollowed in the center and show numerous canals resembling the burrows of insects. Hanausek also observed the grains from sprouted rye, which he states present a very different appearance. The outer layers, he records, are radially striated, and separated from the inner by a marked circular crack, while the central portion is irregularly fissured, the original clefts about the hilum being enlarged.

It seems to be generally believed that starch-grains of different sources undergo erosion in specific ways peculiar to the plant source. If the character of erosion is peculiar to each kind of starch, it indicates specific forms of non-homogeneity of the granules which are attributable to peculiarities of the metabolic processes which give rise to the starch-grain; hence, is a matter of considerable importance in this research.

Several observers have studied the phenomena of erosion of the starch-grains *in situ*, and a number have made comparisons of the changes which occur *in situ* and *in vitro*, and there are also records of comparisons of the differences in the erosion phenomena exhibited by the grains of different plants. Baranetzky (Die stärkeumbildenen Fermente in den Pflanzen, Leipzig, 1878) studied the starches of *Polygonum fagopyrum*, *Phaseolus multiflorus*, *Mirabilis jalapa*, *Quercus pedunculata*, *Æsculus hippocastanum*, *Solanum tuberosum*, *Fagopyrum esculentum*, *Triticum sativum* var. *vulgare*, and *Oryza sativa*. He records that these starches differed in their susceptibility to the action of ferments. The grains of buckwheat were the most easily affected; wheat and bean coming next; those of potato, and especially rice, being the least easily affected. None was found to entirely resist the influence of ferments. A given kind of starch was noted to be affected not only by the ferments of the same plants but also by any kind of a starch-reducing ferment, all kinds affecting it similarly.

The experiments by Baranetzky include the actions of ferments of barley-malt, of germinated seeds of *Polygonum fagopyrum*, *Mirabilis jalapa*, *Phaseolus multiflorus* (cotyledons), *Æsculus hippocastanum* (cotyledons); of sprouting tubers of *Solanum tuberosum* and *Beta vulgaris*; of stems and leaves of *Phaseolus multiflorus*; and of leaves of *Eriobotrya japonica*, *Acanthus cordifolia*, and *Tradescantia discolor*. The solution of the starch-grains by the action of ferments was found to run parallel with the formation of starch-paste. Since various starches differ in their resistance to the action of ferments, weak ferments are limited in their action to grains that are easily affected, while energetic ferments are required to influence such ferment-resisting grains as those of *Solanum* and *Oryza*. The phenomena of the solution of the starch-grains are stated to refer only to the actions of ferments, and not to any other influence. Bacteria, he notes, will naturally form in a solution that has been allowed to stand, yet in the easily affected grains signs of erosion appear in about 24 hours, at the time when, as he states, the fluid is still free from organisms. Nor can the solution of the grains be due to the action of acids, because grains of potato starch subjected to the macerating action of weak hydrochloric or acetic acid for 33 days were entirely unchanged. Although the grains of horse-chestnut were rendered transparent in a month after treatment with very weak acetic acid, this change only slightly resembled that produced by the action of ferments. Baranetzky writes that up to that time the changes which the starch-grains undergo by the action of ferments have been observed only in the living plant, and that experimenters had not been able to produce them artificially.

#### PHASEOLUS MULTIFLORUS.

Baranetzky records that the dissolution *in vitro* of the starch-grains of *Phaseolus multiflorus* always proceeds from the inside toward the periphery. After the ferment has been allowed to act for 24 hours, it will be observed that some grains have a sort of furrow, having the same shape as the grain, and which at first appears to be filled with a granular mass. This furrow widens and becomes entirely transparent, and its walls (lamellæ) break at several places, forming an outlet. It now becomes evident that this apparent furrow is composed of a uniform, very transparent substance which remains in the place of the dissolved starch-grains. The unchanged, dense mass of the grain gradually grows less until finally only tiny flakes and streaks are left, which are irregularly distributed on the periphery of the remaining skeleton. This skeleton is a clear, transparent disk, with a delicate but distinct contour, and completely retains the form and size of the unchanged grain, and the lamellæ are frequently even more marked than in the intact grain.

In treating starch-grains in this state with iodine, Baranetzky found that all the denser parts give the usual iodine reaction, while the transparent parts remain colorless, even after the prolonged action of the reagent; that the skeletons therefore no longer con-

tain "granulose," and that it is easy to prove that they consist of pure "cellulose," for if Lugol's solution be used instead of iodine the skeletons in a short time become brownish, then a copper-red, and finally gradually go over into violet. Thus, he states, the actions of the plant ferment, like those of saliva, at first cause the granulose of the starch-grains to dissolve. The cellulose of the starch-grain of the bean was not so insoluble, because after prolonged action of the ferment the grains become more transparent and delicate, so that the grains finally can be distinguished only when stained; their contours, which at first are distinct, become obscure, and the skeletons finally disappear altogether. Powerful ferments brought this about in 4 to 5 days.

In the germinating seed of *Phaseolus multiflorus* the same phenomena were observed, except that frequently some of these grains were found which did not dissolve from the inside but were dissolved evenly, so that the transparency of their skeletons gradually became visible throughout the entire grain. Similar appearances were also observed in artificially dissolved grains where the action of the ferment was slow and weak, although an actual formation of a skeleton never took place. This deviation from the ordinary process of solution, Baranetzky believes, is probably due to a non-penetration of the ferment evenly into all parts of the grain. The fresh starch-grains of the scarlet bean almost always show, he states, a complicated system of furrows and fissures, of which several extend to the periphery. In ordinary cases the ferment probably forces its way through these fissures into the inside of the grains and begins its action before the outside layers have been in the least affected by it. Accordingly, it is found that the inner furrow usually follows the direction of the fissure, and widens toward the inside to reach the periphery of the grain at these places.

While the dissolution of the granulose takes place almost uniformly from within outward, the grains of some of the seeds of *Phaseolus* deviated from this rule. In these cases the granulose disappeared very unevenly, and the remaining skeletons, although consisting of pure cellulose, were nevertheless usually substantial, and their contours almost as well-defined as those of the intact grain. These skeletons, however, were finally dissolved by the prolonged action of the ferment. Baranetzky states that this would seem to prove that the starch-grains of the same plant may differ in their cellulose content, since it does not seem likely that these deviations can be attributed to the differences in the solubility of the granulose and the cellulose of the different starch-grains.

#### QUERCUS PEDUNCULATA.

This starch behaves similarly to that of *Phaseolus*. The process always begins in the inside of the grain, with the difference that the outlines of the gradually increasing clear spot are not distinct, and gradually pass into the grain substance. As the process of solution reaches the periphery, only the granulose disappears, the cellulose remaining behind in the form of a sharply outlined skeleton, as in *Phaseolus*. The cellulose reaction is not so easily produced as in *Phaseolus*. Lugol's solution causes a yellowish color at first, which finally goes over into copper-red and violet. The cellulose skeletons are also completely dissolved.

#### SOLANUM TUBEROSUM.

In the grains of potato starch the process of solution begins at the periphery and extends to the inside of the grains, where small round or irregular clearly outlined spots make their appearance. Seen in profile, these spots appear like canals entering into the inner part of the grain and are filled with a soft transparent substance. Fine but distinct lamellæ may often be seen in the latter. Sometimes those places on the outside of the grain at which erosion takes place simultaneously are numerous and irregular, and gradually merge into each other, so that the surface of the grain is covered with complicated markings. The eroded places grow deeper toward the inside of the grain, and then widen

out, so that the process of solution may now take place in the opposite direction—that is, from the inside to the periphery. Otherwise, the behavior of potato starch is like that of *Phaseolus* grains. The remaining cellulose skeleton shows sharp outlines at first, and more or less distinct lamellæ, especially in the larger grains; the cellulose skeletons appear to be more substantial, and the outer layers are decidedly more dense than those in the inside of the grain. The cellulose reaction with Lugol's solution is not so easily obtained with the skeletons of potato starch. At first they are stained only a pale yellow, and it takes 30 to 60 minutes to produce the violet reaction. The outside dense layers are less easily stained than the other parts, and usually continue to show a brownish color when the violet has already begun to appear in the inner parts. The solution of the cellulose skeletons appears to take place more rapidly here. Some grains were noticed in which one part was still unchanged, while the skeleton of the other part had almost entirely dissolved.

In the few sprouting tubers of potato examined it was found that solution seems to begin in the center of the grain, and the process to go on evenly from this point, so that the grain is changed into a thin-walled shell, with the inner layer of the wall indistinct. These deviations are stated to be unimportant in the process of solution.

#### ÆSCULUS HIPPOCASTANUM.

The starch-grains of the horse-chestnut are changed outside the cell in the following manner: At that part of the starch-grain farthest removed from the hilum, where the otherwise not very distinct lamellæ appear particularly distinct and numerous, a transparent triangular space is developed which penetrates deeper into the inner part of the grain, and reaches and surrounds the hilum; in the meanwhile, some single lamellæ or groups of lamellæ dissolve more quickly than others, causing the latter to appear as dark cross-stripes, and also giving the grain a peculiar striated appearance. The more dense and unchanged portions belong to the periphery of the grain, so that after the process of solution in the inner part has sufficiently progressed, the grain floats in the solution and appears like a bubble whose walls are riddled with numerous irregular holes. Finally, only isolated portions of the latter are left, which, however, retain their former position, so that the outlines of the former starch-grain can still be recognized. This goes to show, writes Baranetzsky, that in this case also, after the granulose has dissolved, a residue is left which undoubtedly is cellulose, but which is so delicate that it is hardly visible. The interstices sometimes take a violet color with Lugol's solution and sometimes remain colorless.

In the cotyledons of germinating seeds of *Æsculus hippocastanum*, starch-grains are very often found which, while unchanged on the inside, have a sort of pitted appearance on their outer surface. Seen from the flat side the grains usually show a small cavity at the end opposite the hilum, and from which the starch-substance has entirely disappeared. Such cavities also sometimes develop on the flat side of the grain, and when seen from above look clear and round. Seen from the side of the grain, the contours of these cavities are always distinct, proving that the solvent action of the ferment is merely a superficial one and does not penetrate into the substance of the grain. Very often conditions are found in which the greater part of the grain has disappeared while the remaining clearly outlined part is unchanged. How this superficial process of solution continues was not determined. During germination of such seeds as those of *Æsculus hippocastanum*, only a very small part of the grain is used up. It would therefore seem that grains which so energetically resist the action of the ferment undergo no change. Usually, however, the germinating seeds of the horse-chestnut dissolve from the inside, resembling the process described in the grains of potato starch. The starch-grains of one and the same seeds are not always affected in the same way, so that it would be necessary to examine a number of seeds in order to observe typical forms of the two extremes.

## POLYGONUM FAGOPYRUM.

The small grains of this plant were not easily observed. Under magnification of 300, in the first stages of erosion the grains have a striped appearance, with fine, dark, dotted lines radiating from the center to the periphery. Magnified 500 times, it can clearly be seen that the change begins on the outside. Numerous narrow canals are found on the periphery which penetrate into the center of the grain; the canals appear lighter than the other parts, due to the lamellæ of the grain, which do not show under a lower power. The substance in the center of the grains dissolves more easily, and the canals therefore run together here into a small hollow, and the process of solution now continues centrifugally. Finally, only a ring of isolated small grains is left which is evidently held together by the remaining invisible mass of cellulose.

## FAGOPYRUM ESCULENTUM.

The same process takes place in the germinating seeds of buckwheat, but among all the seeds examined those of buckwheat were most easily affected by the ferment. Only small portions of the grains were left after 24 hours' action of the ferment treated with strong formic acid, and in 48 hours no trace of the starch-grain was left. The minimum of the time reaction is probably only a few hours.

## TRITICUM SATIVUM VAR. VULGARE.

Baranetzky found that the starch-grains of wheat are almost as easily affected as those of buckwheat. As is well known, there are two kinds of starch-grains in the seeds of wheat, the large, flat, crescent-shaped and the small, more or less spherical. The erosion process described is that of the large grains. The first changes are like those of *Polygonum*, the same striped appearance, and the same radiating lines, but the latter not always extending to the center. Similar canals also run from the flat sides into the inner part of the grain; on the surface of the grain these canals appear as small, clearly outlined circles, greatly resembling the round tufts of the parenchymatous cells. In the meantime the clear parts of the grain grow wider, so that the unchanged portions have the appearance of dense radial bands in the clear, concentric-layered grain. At the same time the clear portions begin to melt away from the outside, but the bands do not keep pace with them, the ends of the latter usually protruding more or less freely. After these bands are finally absorbed only a small disk remains, which is formed from the central part of the grain where the lateral canals did not penetrate. This disk now dissolves at its upper surface and at the walls of the canals which have penetrated from the sides of the grain into its center. At last only an irregular lump, riddled with holes, is left of the entire starch-grain, and this also finally dissolves. Very often the process of solution begins with the formation of concentric spaces which evidently correspond to the dissolved single groups of lamellæ. In such cases the grain usually dissolves from the outside, without previous formation of canals. Usually the formation of the centric spaces combines in different ways with the radial canals, producing varied and complicated appearances in the process of erosion. In rare cases, starch-grains may be found which dissolve equally in all parts. They then become very transparent and show 2 to 3 concentric zones of varying density, gradually merging into one another. The final disappearance of such grains takes place either by a melting away from the outside or by a gradual complete solution.

No cellulose skeleton was found in any of the starch-grains of *Triticum* after the granulose had been extracted. Watery solutions of iodine always have a violet reaction, even in those parts of the grains which were on the point of disappearing. This, Baranetzky states, need not necessarily mean that the grains of *Triticum* contain no cellulose, for the same results should be obtained if the cellulose were more soluble, or at least equally as soluble, as the granulose of the grains in question.

The starch-grains of germinating seeds of wheat were found to be variously affected, so that all of the phenomena described above could be observed in them. It is worthy of remark that in one and the same seed the process of solution takes place in a definite way, the usual course being as follows: A concentric fissure develops at a distance from the rim of the disk; in the middle of the space surrounded by this fissure a system of irregular, branched fissures arise, giving this portion the appearance of falling apart, while the ring of the surrounding border is unbroken. The latter is absorbed in its entirety, and radial stripes may frequently be noticed in it. The deviations from the usual course exhibited by some grains explains the contradictions by Sachs (Botan. Zeitung., 1862, page 147) and by Gris (Annales de science naturelles (Botanique), 1860, XIII, 110) in their descriptions of the same process. Sachs's statement that the affected grains first lose their granulose, leaving a skeleton which becomes wine-colored when treated with iodine, could not be verified. In the germinating seeds, as well as in the tests outside the cell, the iodine reaction was always the same, *i.e.*, a blue-violet color was obtained until the very disappearance of the grains.

#### MIRABILIS JALAPA.

As is well known, these grains consist of numerous part-grains (p. 273) which separate easily, and in their isolated state look like very minute spheres showing a Brownian movement. It is hardly possible to follow the process of erosion in such grains, yet on the whole it is safe to assume that they also dissolve through the action of the ferment. Their contours become vague and indefinite, and the single part-grains gradually change into indistinguishable dark points, which finally become invisible. In the grains or their fragments that have not fallen into the single minute grains, the part-grains lying on the periphery of the groups are affected first, while the others remain unchanged. If such groups are not very large, then all their part-grains finally are changed, and the whole fragment takes on the appearance of a fine, granular, semi-transparent mass.

#### ORYZA SATIVA.

In size and form, Baranetzky writes, these grains closely resemble those of buckwheat, but show an extreme difference in their solubility, being the least easily affected by ferments. The erosion process here appears to begin in the middle of the grain, and solution takes place peripherally, the last stages in the process thus resembling those of buckwheat grains.

Baranetzky notes that close observation of the process of erosion of the grains of different starches at once shows variations in their structure. In most of the starches examined (bean, buckwheat, potato, and rice) erosion takes place more readily in the inner layers than in the outer ones, agreeing perfectly with Nägeli's theory on the structure of the starch-grain. When the soft hilum is eccentric the inner cavity usually spreads toward the hilum side. In wheat starch the structure of the grain seems to be symmetrical, as is shown by the concentric layers which make their appearance as the grains dissolve, the soft hilum being in the exact or mathematical center. Nevertheless, the central part of round starch-grains does not dissolve as easily as the outer part. The radial canals in which erosion begins usually stop at a distance from the center of the grain, final solution usually taking place by the gradual melting away from the outside. The greater solubility of the outer part of the grain might be due to a difference in chemical composition, but the presence of cellulose could not be proved in this case, and there would be no other reason for supposing any chemical modification in the granulose.

Baranetzky records that the formation of the canals by which the ferments reach the inner part of the grain is worthy of notice. The possibility alone of this formation proves, he states, that not only different concentric layers, but also different parts of the

substance of the starch-grains, may be unequally affected by ferments from the outside toward the inside, and that therefore they must differ in their density. Special emphasis, he believes, should be laid on the fact that the contours of these canals are sharply outlined, and that they usually remain narrow, while in the meantime the concentric layers successively disappear. This may be clearly seen, especially in the seeds of wheat, where different parts of the grain remain unchanged while others are already transformed into a cellulose skeleton. In the ferment-resisting grains of potato and rice starch, after the action for 24 hours of a strong ferment, single eroded grains can nearly always be found, the number increasing after a few days; but the majority of the grains remain unaffected in spite of repeated renewal of the ferment. This is most strikingly exhibited in the scarlet bean and horse-chestnut, where a few single grains were found to be entirely intact at a time when all the others were on the point of disappearing.

It is an interesting fact, writes Baranetzky, that the structure of the grain varies in single seeds of the same species of plants, as is undoubtedly proved by the differences of the grains in their processes of erosion. It would seem, however, that during germination the majority of starch-grains in one and the same seed are affected alike, but differently in different seeds. It seems of little moment, he found, whether the entire substance of the starch-grain is permeated by the ferment, or whether the ferment action is merely a superficial one. It is more than likely that the process of solution depends on the concentration of the ferment solution. A highly concentrated ferment, he believes, will act so energetically that the outer layers of the grain are affected before the inner parts have been permeated by ferment; the character of the colloidal ferment must necessarily retard the latter procedure. On the other hand, if the mother-liquor surrounding the starch-grain contains, as is usual, only a small amount of ferment in the living cell, then the ferment gradually permeates the entire mass of the starch-grain and the central soft portion is the first to come under the influence of the dissolving agent. The unequal power of resisting ferments exhibited by different starch-grains can not, he believes, be attributed to their cellulose content, for *Phaseolus* grains are an example of grains which dissolve easily and leave a skeleton of cellulose behind after the granulose has been dissolved out of them. On the other hand, the more resisting grains of *Æsculus hippocastanum* appear to contain much less cellulose. Baranetzky concludes that the differences in the behavior of different grains toward ferments must be due to specific differences in the structure of the starch-grains of different plants.

Important in this connection is an investigation of Wortmann (Zeit. f. physiolog. Chemie, 1882, vi, 287) on the actions of bacteria on raw starch. His conclusions are as follows: Bacteria possess the power of producing the same changes as diastase in starch-grains, starch-paste, and soluble starch; they dissolve different starches with varying rapidity; they will attack starches only in the absence of other carbohydrate nutrients, and only when not deprived of air; the action of bacteria on starches is due to a ferment secreted by them for this purpose, which, like diastase, can be precipitated with alcohol and is soluble in water; the action of this ferment is purely diastatic; the ferment in itself is capable of exerting its influence in the absence of acids; bacteria also secrete the ferments which are active in neutral starch-solutions; they act more energetically in weak acid solutions; the manifestations of erosion agree exactly with those described by Baranetzky, as being caused by diastase. Wortmann's basic experiments were made with wheat starch in a preparation consisting of 1 or 2 drops of liquid from putrefying beans or potatoes (in which the *Bacterium termo* is abundant) in a 1 per cent solution of equal parts of sodium chloride, magnesium sulphate, potassium nitrate, and acid ammonium phosphate. Erosion begins at room temperature in 5 to 7 days.

Another, and perhaps the most important, investigation on the phenomena of erosion of the starch-grain was made by Krabbe (Jahr. f. wissensch. Botanik, 1890, xxi, 520), in

which were studied the alterations of starches of various kinds *in situ* and *in vitro*. The following data are from Krabbe's article:

TRITICUM SATIVUM VAR. VULGARE.

During the process of erosion of the starch-grains of wheat, wedge-shaped and conical indentations are formed with their bases turned toward the circumference of the grain. They are distinguished from the rest of the substance by their weaker refractive power, and when seen in profile appear to have more or less dense lamellæ, which can not be seen in the intact grain. These indentations are in reality pore-canals of unequal length penetrating to the center of the grain. The formation and successive lengthening of these canals is not caused by the dissolving action of the diastase, as is proved by the distinct outlines of the pores in all stages of development. The starch-substance remains unchanged during the formation of the canals; swelling agents and iodine affected the eroded grain the same as the intact one. Ferments therefore can not penetrate into the intercellular spaces of the starch-grain, and there can be no question of their dissolving action. As the pore-canals lengthen they usually become branched, thus setting up intercommunication at various places in the cell. Observation proves that these canals originate in the outer surface of the grain, the canals within the grain being secondary branches of older canals which have an opening on the outside of the grain. Superficial observation of grains about to dissolve often revealed some grains which looked as though large portions of the circumference had been absorbed, but closer examination showed that this appearance is due to the same factors which cause the formation of the pore-canals, and not to the action of the ferment. In cross-section the wedge-shaped and conical canals on the circumference of the grains are at first circular, but as they widen they become elliptical. This is due to the lateral merging of two hitherto parallel pores, a phenomenon which can be observed in all its stages in the starch-grains of *Triticum*. In consequence of this, fissures from the periphery of the grain, encircling about one-third of the circumference, give rise to the mistaken supposition that erosion had taken place here. There were evidently such starch-grains, in which similar coalescence had taken place, which led Baranetzky to the erroneous conclusion that the diastatic ferment penetrates the starch-substance and acts as a dissolving agent.

Other phenomena may be observed in *Triticum*, which also might lead to incorrect inferences. In some grains passages running parallel to the concentric layers arise which look as though they had their origin in the inner part of the grain without regard to the outside of the grain, but it can be seen that these passages communicate with openings on the outer surface. The origin of these spaces can be traced to ferment passages which come from outside. Since these latter are capable of entering the grain in all directions, it will not be surprising to find now and then some of these passages running parallel to the concentric lamellæ. Thus the different manifestations in processes of erosion in *Triticum* depend primarily on the width, the branching, and the manner of intercommunication of the pore-canals, and in a measure on the unequal distribution of the pore-canals in the eroded grain. As a rule, the pores on the margin develop earlier and in greater numbers on the flat side of the grain, but any number of variations from this rule may occur; and it is easy to conceive that the unequal arrangement and distribution of the pores in different grains can produce widely deviating characteristics in the process of erosion.

HORDEUM VULGARE AND SECALE CEREALE.

The process of erosion is similar to that in *Triticum*. No appearance of diastase penetrating the substance could be determined; and the very distinct outlines of the pore-canals and the rarer occurrence of lateral coalescence of pores than in *Triticum* confirm the theory that diastase does not act in the manner of dilute acids.

## ZEA MAYS.

As in the above, canals also form on the flat sides of the grain, which communicate with those coming from the margin of *Zea mays* grains, and like barley and wheat starch grains, the grains are ruptured by these numerous canals, which finally cause the complete dissolution of the grain. This is true not only of *Graminaceæ* but also of a large number of other plant orders. In *Polygonum fagopyrum*, *Rheum rhaponticum*, *Polygonum bistorta*, *Convolvulus* (root), *Adoxa moschatellina* (rhizomes), *Galanthus nivalis* (scales of bulbs), *Narcissus poeticus*, *Tulipa*, and *Gesneriana* (scales of bulbs), the dissolution of the starch-grain by pore-canals may be observed.

## HYACINTHUS ORIENTALIS.

The distinguishing characteristic of erosion in these starch-grains seems to be that the formation of pore-canals may be combined in various ways with the development of inner cavities, each grain thus presenting a different picture. While some grains are eroded by numerous ramified passages, almost equal in width, others show merely distinctly outlined cavities of various shapes which naturally have an opening for the ferment at any indifferent part of the surface of the grain. These grains are a striking example of the fact that the diastatic ferment during its action on the starch-grain does not penetrate the starch-substance. The skeleton remaining after the action of the ferment, as well as the tiny broken particles resulting from the breaking up of the grain, show all the characteristics of normal starch-substances.

## PHASEOLUS MULTIFLORUS.

In these grains also the diastatic ferment enters the grain from without, and the solution of the starch-substance takes place from within after the ferment has passed through these canals into the inner part of the grain. Before germination has begun, the large grains of the cotyledons of *Phaseolus* have a distinct inner cavity, formed by the basal parts of radial fissures. These fissures differ from ferment passages in that they usually end in a sharp point on the margin of the grain, while the latter, as a rule, have a rounded apex. When the fissures begin to widen and their ends become round we know that the ferment has penetrated the inner part of the grain. They perform as ferment passages the same function in the erosion process as soon as communication has been established between the two. In grains with numerous fissures, the process of erosion may at once appear to be determined by them, inasmuch as they take up the ferment from outside and conduct it in definite passages. This is the case with *Phaseolus multiflorus*. When this has reached the inner cavity it naturally spreads, and as the hole enlarges dissolution must take place from within. Starch-grains in which erosion takes place from the outside are usually comparatively large, with an eccentric hilum and distinct lamellæ, the latter less numerous at the hilum than at the latter end. The watery lamellæ at this part of the grain usually end blindly at the sides, wedging themselves in between the more dense lamellæ, usually at some distance from the outer surface, and never known to have free ends.

## LILIUM CANDIDUM.

The ferment attacks the entire outer surface of the grain with equal intensity, not affecting the broad, densely lamellated end more than the hilum end. As soon as ferment action begins, the lateral increase of the outer lamellæ must cease, and we then have starch-grains with more or less substantial lamellæ and free ends, according to the intensity of the diastatic action. The sides of these grains exhibit the same characteristics of erosion as those described in *Graminaceæ*, the ferment coming from the periphery and acting more energetically on the watery layers than on the dense ones, producing similar bands and protuberances.

In germinating seed of *Lilium candidum*, *Phaius*, *Lathraea*, and *Orobanche* not all of the grains dissolve from the outside. In many grains the margin remains intact until

the development of the pore-canals. This is very often accompanied by the formation of an inner cavity. In all such cases the starch dissolves from within, as is usually observed in the smaller grains.

The characteristics marking the processes of erosion in *Lilium candidum*, *Lathraea clandestina*, *Orobanche*, and *Phaius grandiflorus* may be grouped as follows: (1) Erosion usually takes place from the outside; (2) in large grain special local points of erosion often occur in the form of grooves or crater-like depressions; (3) in contradistinction to the above, the small grains usually dissolve from within, inasmuch as pore-canals are developed which by extending to the inner part of the grain often produce a cavity.

#### EROSION OF STARCH-GRAINS IN NON-TYPICALLY RESERVE-STORING PARTS OF THE PLANT.

In parasitic plants like *Nicotiana glauca* and different species of *Lathraea* and *Orobanche*, during the development of the buds a large amount of starch develops in the cells of the flower-stalks. As soon as fructification has taken place and the seed begins to develop, this starch disappears from these parts in the same way as from the true reserve receptacles. In the rather small grains of *Nicotiana glauca* the grains are usually attached by pore-canals and are broken by ramification of the same. In *Lathraea* and *Orobanche* the process is as previously described. In non-parasitic plants, as well as in those having oily seeds, such as *Linum catharticum*, *Sinapis*, *Papaver*, etc., the same erosion characteristics appear in more or less marked degree.

#### EROSION OF STARCHES OUTSIDE THE PLANT BY ACTION OF WATERY EXTRACTS OF DIASTASE AND BY BACTERIAL FLUIDS.

Baranetzky (page 181) has recorded that most starches dissolve outside the plant in watery solutions of diastase, and Wortmann (page 186) has proved that bacteria are capable of attacking starch-grains. In *Graminaceæ* Krabbe found that erosion is alike in germinating seeds in extracts of diastase and in bacterial fluids. Slight deviations were noticed in the bacterial fluids. In potato starch, on the other hand, the process of erosion in extracts of diastase and in bacterial fluids differs from that in sprouting tubers. Uniform dissolution of the grains from without, which is a rule in tubers, seems never to take place outside the plant in extracts of diastase or bacterial fluids. In the bacterial fluid the starch-grains develop depressions like holes bored into the outer surface of the grain, and these depressions increase in circumference and depth with the continued action of the bacteria; they naturally merge into each other as the starch-substance separating them gradually dissolves, thus producing large irregular depressions on the outside of the grain. The action of extracts of diastase outside the plant produces superficial irregular points of erosion which soon deepen and enter the inner part of the grain and assume different forms. As a rule, the eroded grains are filled with a network of canals, and inner cavities are also usually formed. Sometimes superficial fissures have been observed in the diastatic action in the grains of potato starch, the ramification of which gave the grains a reticulated appearance. Such branched fissures were observed in the eccentric and distinctly lamellated grains of *Fritillaria imperialis*. The fissures begin at the wide end of the grain and lengthen and ramify until they completely cover the outer surface. As they lengthen they also become deeper, and if the diastase is allowed to act long enough they finally cut the grains into pieces.

Day (*loc. cit.*) observed the effects of unfiltered saliva on potato, arrowroot, corn, rice, wheat, and barley starches. After 14 hours at 40° (the preparations each containing a few drops of thymol solution) in most of the corn and rice grains the center part was apparently all dissolved, leaving a thick shell having deep clefts along what were the edges of the polyhedral grain; in many of the barley and wheat grains there was no change except corrosion of the surface, but in many others there remained nothing but hollow

shells, thinner than in the corn, and with pitted surfaces and cracks around the edge, much like the openings of oyster shells. At the end of 38 hours the arrowroot and some potato grains showed surface erosions. Some of the potato grains showed change at the small end, this part not reacting with iodine, while the remaining part stained a deep blue.

#### BOILED STARCHES.

The results obtained by various investigators in their digestion experiments with boiled starch are quite as conflicting as those with raw starch. It has long been believed that certain kinds of boiled starches are more digestible than others, and it is owing chiefly or solely to this that arrowroot starch is given preference by the physician over other starches in infant feeding and in the diet of the sick-room. Its superior qualities, however, as will be noted from the following pages, are not owing to the factor of digestibility, but to the absence of certain contaminations which render certain other starches of commerce, notably potato starch, unpalatable. In a word, all kinds of boiled starches as ordinarily prepared and subjected to the same conditions of experiment are of equal digestibility and yield the same products, quantitatively and qualitatively.

In the experiments by Hammarsten (*loc. cit.*) with saliva on normal raw starch there were material variations in the time of the formation of sugar in the case of different kinds of starch, the interval ranging from 2 to 3 minutes to the other extreme of 2 to 4 hours. When, however, the grains were comminuted by chewing, which is equivalent, as previously shown, to partial or complete gelatinization in proportion to the degree of comminution, sugar was formed in all of the starches in 1 to 4 minutes. Starch-pastes were found by Hammarsten to show no difference in sugar-forming time. Levberg (*Inaug. Dissert.*, St. Petersburg, 1874; *Ber. d. d. chem. Gesellsch.*, 1874, x, 76) gives the order of digestibility of boiled starches, as determined by the quantity of glucose, as follows: Arrowroot, potato, wheat, and rice—the first being the most digestible. He made one series of experiments in which he determined the quantity of glucose formed when given quantities of saliva and starch were used. With 9 c.c. of saliva he obtained from potato 60.3 per cent and from arrowroot 59.62 per cent; with 10 c.c. of saliva he records from rice 55.76 per cent; and with 16 c.c. of saliva with wheat starch the yield was 62.87 per cent. In other observations he found, with standard quantities of saliva and starch, that the maximum results were recorded as follows: Arrowroot 8 hours, potato 9 hours, wheat 12 hours, and rice 14 hours. Salomon (*Jour. f. prakt. Chemie*, 1882, xxvi, 324), upon the basis of the amount of sugar formed by the digestion of potato and rice starches, found that these starches are identical in degree of digestibility.

O'Sullivan (*Jour. Chem. Soc. Trans.*, 1878, II, 141) conducted most of his experiments with potato starch. In his earlier experiments he satisfied himself that different boiled starches (potato, rice, wheat, corn, etc.) give the same results, quantitatively and qualitatively, but in a comparatively recent article (*Proc. Chem. Soc. Trans.*, 1904, xc, 65), in which he reports the results of six series of experiments with potato, malt, barley, corn, and rice starches, using extract of malt or diastase, it is stated that the percentages of dextrin and maltose yielded by potato starch do not correspond with those recorded with the other starches, and therefore that the products of potato starch can not be taken to indicate the products of the other starches. It has since been found that the difference noted by O'Sullivan is due to errors of experiment (see Ford, page 193).

The digestibility of all kinds of starch is stated by Butyozin (*Inaug. Dissert.*, St. Petersburg, 1887) to be increased by the length of time they are boiled, and that under given conditions the starches of millet, buckwheat, rice, and peas show ease of digestibility in the order given. In opposition to this author, Day (*loc. cit.*) records that potato, arrowroot, and probably tapioca and sago starch-pastes are not rendered more digestible by prolonged cooking; and in confirmation, that cereal starches (wheat, corn, rice, barley)

become more digestible. According to Day, the raw starch-grains contain three substances which, owing to their different color reactions with iodine, may be distinguished as *blue-amylose*, *red-amylose*, and *rose-amylose*. Blue-amylose is identical with the substance known as granulose or the  $\beta$ -amylose of Meyer; red-amylose constitutes the outer layer of the starch-grain and is more difficult of digestion than blue-amylose; rose-amylose forms about 10 per cent of the inside of cereal starches, and is not found in potato, arrowroot, tapioca, and sago starches, and is the least digestible of the three. Blue-amylose constitutes the entire inside of potato, arrowroot, tapioca, and sago starches, and 90 per cent or more of the inside of cereal starches, and is not rendered more digestible by boiling. The digestibility of red-amylose is not increased by boiling, but rose-amylose is slowly changed into blue-amylose by cooking and thus rendered more digestible. Day calls attention to the fact that the skin formed in boiled starch contains reverted starch that gives no color reaction with iodine, and which is very slow of digestion. Finally, starch-paste made below the boiling temperature of water is as easily digested as that which has been boiled for a few minutes.

The use of impure gluten for the saccharification of starch, which dates back to Kirchoff (1814) and Mathieu de Dombasle (1814), was resumed by Reychler (Ber. d. d. chem. Gesellsch., 1889, xxii, 414), who made preparations of what he termed "artificial diastase." These were prepared by means of dilute acids or acid salts (hydrochloric, phosphoric, acetic, tartaric, and lactic acids, and acid sulphate of potassium, and acid phosphates of alkalis were found most desirable). Well-washed, fresh gluten was subjected to the action of the solvent at 30° to 40° for 4 to 5 hours. Reychler notes that the preparations of wheat flour act like diastase, and that barley contains a diastatic constituent. In a number of experiments with barley, wheat, and corn, the saccharifying action was found to be greatest in the case of barley, and the intensity of action was increased by the addition of potato starch.

Grierson (Pharm. Jour. Trans., 1892, xxiii, 187) subjected given amounts of boiled starch, water, and pancreatic extract to a temperature of 37° to 38°, and determined the period of the reaction with iodine to show the differences in the degrees of digestibility of different starches. Corn starch yielded a blue reaction after 20 hours, and wheat and rice starch after 2 hours. Tapioca was colored a weak green after 30 minutes; but *tous-le-mois* (from *Canna edulis*), Bermuda and St. Vincent arrowroots, and potato starch showed no reaction with iodine after 19 minutes. Oat and wheat flour gave a starch reaction after 80 minutes. He concludes that *tous-le-mois*, arrowroots, and potato starch are best for patients with weak digestions.

A line of investigation similar to that followed by Grierson, but covering a somewhat wider field, was pursued by Stone (The Carbohydrates of Wheat, Maize, Flour, and Bread, and the Action of Enzymic Ferments upon Starches of Different Origin, Bull. No. 34, Office Expt. Sta. U. S. Dept. Agriculture, 1896), who carried out a plan to yield comparative rather than absolute results. Care is stated to have been taken to secure identically the same physical condition of all the starches, exposure under constant conditions of temperature and dilution, and to a uniform solution of diastase. As an index of the energy of the action of the enzyme, a solution of iodine-iodide of potassium was employed, the test being made by removing 0.5 c.c. of the starch preparation to a watch-glass or porcelain test-plate, and adding a drop of iodine. When the blue reaction could no longer be obtained after repeated tests the end of the experiment was recorded. Malt diastase, salivary enzymes, pancreatic enzymes, and Taka-diastase were used, together with potato, sweet potato, corn, rice, and wheat starches. The main conclusions derived from his investigations are as follows:

- (1) The starches of sweet potato, maize, rice, and wheat vary greatly in their susceptibility to the action of enzymes.
- (2) This variation reaches such a degree that under precisely the same conditions certain starches require 80 times as long as others for complete solution or saccharification.

- (3) This variation is exhibited toward all of the common enzymic ferments studied, *viz*, diastase, ptyalin, pancreatin, and Taka-diastase, in the same relative order, with slight exception.
- (4) This order, beginning with the starch which is most easily changed, is, for *malt extract*, sweet potato, potato, wheat, and maize; for *saliva*, potato, sweet potato, maize, rice, and wheat; for *pancreatic fluid*, potato, sweet potato, and maize, with wheat and rice unchanged; for *Taka-diastase* the potato was more quickly changed than any other.
- (5) It seems reasonable to assume that the same relative degree of susceptibility exhibited by these starches in the experiments described would still obtain when they are subjected to the action of the same enzymes in the process of digestion.

Lindet (Bull. Soc. chim., 1902, xxvii, 634) records that the starch of stale bread is less digestible than that of fresh bread. Roux (Compt. rend., 1904, cxxxviii, 1356), in his studies of reverted starch, states his belief that stale bread may contain this substance, and Day (*loc. cit.*) several years later reports its presence in the skin formed on boiled starch.

Effront (Enzymes and their Applications, trans. by Prescott, 1902, 128) assumes that both raw and boiled starches of different kinds differ in digestibility because of inherent differences in the starch, which are owing to differences in the compactness of the layers. He states that potato starch and barley starch are both composed of non-homogeneous granules differing in degree of compactness of the layers which compose them. In the granules of potato starch more resisting layers are found than in the granules of barley starch, and from different kinds of starch there are obtained pastes which saccharify with more or less difficulty. It must therefore be assumed that the difference in compactness between parts of the same granule does not disappear when the starch gelatinizes. The more coherent parts of the granules will form a paste more difficult to liquefy (see page 179).

Liquefied starch was found by Fernbach and Wolff (Compt. rend., 1905, cxl, 1067) to yield a larger quantity of products of digestion than ordinary starch-pastes. The pastes of cereal starches, they found, were more readily saccharified than the paste of potato starch. In another contribution (*ibid.*, page 1547) they state that the starch from green peas differs from other starches by a high percentage of amylocellulose that is not saccharified. They found that if the freshly boiled starch is at once subjected to digestion, complete saccharification occurs, but if allowed to stand an unsaccharifiable amylocellulose is thrown down. Pea starch in its natural condition, they state, is analogous to potato starch that has been coagulated by amylocoagulase. Roux (Compt. rend. 1906, 95) subjected starch-pastes prepared at 100°, 120°, and 150° to the action of malt extract at 56°, and found that the yields of maltose from different starches were not the same. Calculated on the basis of dry starch, the quantities were: Potato 83.0 per cent, corn 83.3 per cent, wheat 87.1 per cent (prepared at 150°), rice 85.2 per cent, pea 83.8 per cent, tapioca 81.5 per cent. The yields from starches prepared at 100°, 120° and 150° differed but slightly from each other. Ling (Jour. Fed. Inst. Brewing, 1903, ix, 446) found that soluble starches prepared under different conditions do not yield identical results under the influence of diastase.

In an investigation of soluble starch of various origins, means of preparation, and properties, Ford (Jour. Chem. Ind., 1904, xxiii, 414) recorded that there is no doubt that preparations of soluble starches do differ in certain physical characteristics, and that he is of the opinion that when different specimens give different maltose productions with diastase, it is not the starch which causes the variations, but the impurities present in the specimen. Solutions of soluble starch, from whatever source, when equally pure, will give the same maltose production when acted upon by equal amounts of diastase under the same conditions of temperature, etc. The starches experimented with, except barley, were bought commercially and purified by treatment with dilute alkali and acid, being well washed and air-dried. Portions of 15 to 20 grams were gelatinized, and then liquefied at 79° to 80° by means of a trace of precipitated diastase, boiled when limpid, and then

made up to 500 c.c. To each 70 c.c. of each starch-solution was added 1 c.c. of malt extract at 40°, and the preparation kept at 40° for 1 hour. The preparation was then boiled and made up to 100 c.c. The results in all cases, corrected for reduction of starch-solutions and malt extract, are shown in table 25.

TABLE 25.

Kind of starch.	CuO per 100 c.c.
	<i>gram</i>
Arrowroot, natal.....	0.53
Rice.....	0.52
Wheat.....	0.52
Maize.....	0.52
Potato.....	0.54
Barley.....	0.52
Arrowroot, unpurified.....	0.47
Arrowroot, Lintner's soluble	0.54

TABLE 26.

Kind of starch.	CuO per 100 c.c.
	<i>gram</i>
Arrowroot $\alpha$ .....	0.54
Rice.....	0.53
Wheat.....	0.50
Maize.....	0.52
Potato.....	0.53
Arrowroot $\beta$ .....	0.54
Arrowroot $\gamma$ .....	0.47
Potato I.....	0.44

It is evident, as Ford states, that the origin of the starch has, under these conditions, no influence on the results when the starches are equally purified. From the same starches soluble starches were prepared according to Lintner's method, and similarly tested, with the results shown in table 26.

The specimen designated potato I was made from a "purest commercial farina" by gelatinization and subsequent liquefaction with a trace of diastase. The solution after concentration was precipitated and washed with tap-water and twice with alcohol. The result, Ford states, shows well how impurities cling to the starch. The slight differences shown in the table, he holds, may be accounted for by variations in the impurities, and one may safely infer that equally purified preparations of Lintner's soluble starch from starches of different origin will give the same maltose production with equal quantities of diastase. The above preparations, though exhaustively washed with distilled water, were by no means pure.

Further studies of the quantitative identity of the decomposition products of starches of various origins were made by Ford and Guthrie (*Jour. Soc. Chem. Ind.*, 1905, xxiv, 605). The starches were prepared by these investigators, with the exception of arrowroots, which were bought and then subjected to purification in the usual manner. All of the starches were purified by treatment with 0.2 per cent caustic soda and hydrochloric acid, washed with distilled water, and extracted with alcohol and ether when necessary; then air-dried and further dried at 25° to 30°. In each experiment 5 grams of starch were gelatinized as usual, cooled to 65°, and converted during 1½ hours with 25 c.c. of malt extract that had previously been heated for 10 to 15 minutes at 65°. The converted preparations were made up to definite volumes and the specific gravity, optical rotation, and copper-reducing power were observed. Their experience convinced them of the validity of "the law of relationship" pronounced by Brown, Morris, and Millar (*Jour. Chem. Soc.*, 1897, LXXI, 115); hence, determinations of the solids and their optical rotation is all that is necessary. The following rotatory values were recorded (table 27):

TABLE 27.

Kind of starch.	( $\alpha$ ) <sub>D<sub>3</sub>,93</sub>	Kind of starch.	( $\alpha$ ) <sub>D<sub>3</sub>,93</sub>	Kind of starch.	( $\alpha$ ) <sub>D<sub>3</sub>,93</sub>
Potato (controls of same specimen).....	161.0 160.7 159.5 160.7	Corn.....	160.0	Potato (commercial).....	163.4
Pea.....	161.0	Tous-les-mois ( <i>Canna edulis</i> ).....	159.7	Potato (purified).....	161.0
Barley.....	160.8	Arrowroot ( <i>Maranta</i> ).....	161.0	Rye.....	163.2
Wheat.....	161.0	Rice.....	161.5	Buckwheat.....	164.0
Oats.....	162.0	Lentil.....	160.0	Chestnut.....	166.9
		Banana.....	159.7	Pari.....	163.7
		Barley, malted.....	160.7	Millet.....	162.6
		Barley, malted.....	160.8		

They state that the last five starches were obviously impure, and that they did not yield homogeneous pastes. Successive purifications lowered the angle in each case. The chestnut starch contained evident traces of something other than starch.

In another series of experiments they used precipitated diastase in the proportion of 0.2 gram per 5 grams of starch at 60° for 1 hour, with the results shown in table 28.

TABLE 28.

Kind of starch.	(a) <sub>D<sub>3.93</sub></sub>	R = 3.93
Maize.....	174.7	40.5
Wheat.....	175.6	43.8
Potato.....	174.0	43.8
Arrowroot.....	175.4	40.7
Barley, malted...	174.4	45.0
Rice.....	175.0	44.0
Potato.....	174.5	43.9

In another series, with 0.4 gram of precipitated diastase to 5 grams of starch for 2 hours at 60°, they recorded the results shown in table 29.

TABLE 29.

Kind of starch.	(a) <sub>D<sub>3.93</sub></sub>	R = 3.93
Babana.....	153.1	74.0
Oat.....	155.4	72.6
Barley.....	154.3	74.5
Potato.....	154.2	73.3
Pari.....	156.4	74.4

In an earlier investigation, Ford showed that metallic and other impurities influence amylolytic activity, and that dried starch readily absorbs substances which may interfere with hydrolysis. Ford and Guthrie state that the contradictory results of O'Sullivan and themselves are to be attributed to impurity of the potato starch used by O'Sullivan. A specimen of this starch was obtained from O'Sullivan, and was found to contain copper to the extent of 0.035 per cent. They determined experimentally that this amount of copper is sufficient to bring about a marked reduction in maltose formation. It seems, therefore, quite clear from the results of the investigations of both Ford, and Ford and Guthrie, that the source of starch is without influence on the products of decomposition if the starches are sufficiently purified and freed from substances which influence amylolytic action.

That all boiled starches, whatever the source, yield the same decomposition products, quantitatively and qualitatively, under the same conditions of experiment, has been shown by the author's work. To obtain uniform results it is essential to secure as nearly as possible the same conditions of experiment. It seems to be a universally accepted belief that enzymes in a 1 to 2 per cent solution of chloroform, or in other antiseptic solutions which are inert towards the enzyme, do not undergo rapid impairment in energy. While this is in a large measure justified in regard to plant enzymes, such is not the case with amylase and ptyalin and probably all animal enzymes. Even within 24 hours such diastatic enzymes show deterioration, which steadily increases with time. Therefore, in making comparative experiments the solution of enzyme should be made immediately before using. It is also of importance that none of the starch be lost after boiling, by adherence to the sides of the vessel, and that other specific standard conditions be maintained. A large number of experiments were carried out with Merck's "pure ptyalin," "pure pancreatin," and "medicinal malt diastase," and also with Parke, Davis & Company's Taka-diastase. These enzymes showed marked differences in energy, Taka-diastase being the most energetic, pancreatin a shade less energetic, malt diastase distinctly less energetic than the foregoing, and ptyalin comparatively weak. It is, therefore, manifest that, in making comparative studies of different starches, a given enzyme must be used throughout. Moreover, as the different preparations of the same enzyme differ widely in energy and also in their content of glucose, etc., the same preparation must be used throughout for control experiments made to permit of proper corrections.

A number of experiments were made with starches prepared from *Solanum tuberosum*, *Zea mays*, *Batatas edulis*, *Canna roscoeana*, *Canna warszewiczii*, *Canna musafolia*, *Canna edulis*, *Maranta arundinacea*, *Freesia refracta* var. *alba*, *Dieffenbachia seguine*, and other starches derived from widely separated genera, orders, etc. In each experiment 0.5 gram of starch was boiled in 35 c.c. of water for 1 minute, the preparation cooled, the starch

adherent to the walls of the beaker washed out, and the preparation made up to 50 c.c., and then warmed in a water-bath to 37°. To 5 c.c. of water was then added 0.75 gram of pancreatin rubbed up to a paste in a mortar, and water gradually added to make 25 c.c., and this preparation likewise placed in a water-bath warmed to 37°. The starch and enzyme preparations were then mixed and placed in a moist chamber having a maintained temperature of 37°. At 15-minute intervals during the first hour the preparation was shaken, and 5 c.c. removed, quickly boiled, and tested with a 2 per cent Lugol's solution and with Purdy's solution. Two immediately subsequent observations were made at half-hour intervals, and subsequently at 1-hour, and later at 2-hour intervals, the entire period of actual experiment covering 6 hours. The record given in table 30 may be taken as being practically absolutely identical with that of every starch, no matter what its source, the deviations from this being absolutely insignificant, as shown by the sugar determinations, which vary only in the third decimal figure. The sugar determinations were made in terms of maltose. The pancreatin contained an appreciable amount of glucase, giving rise to the conversion of some of the maltose into glucose, thus yielding higher values than had the sugar consisted solely of maltose.

TABLE 30.

Conditions of experiment.	Time from beginning of experiment.		Color reaction with iodine.	Weight of sugar (maltose + glucose) in terms of maltose.
Kind of starch: Solanum tuberosum. 50 c.c. of 1 per cent boiled starch, with 0.75 gram of pancreatin in 25 c.c. of water, both having been heated to 37°, and then mixed, and placed in moist chamber at 37° constant temperature. Sugar determination by Purdy's solution.	<i>hrs.</i>	<i>min.</i>		<i>gram.</i>
	0	15	Weak violet.....	0.334
	0	30	Very weak violet.....	0.362
	0	45	Faint violet.....	0.396
	1	00	Fainter violet.....	0.409
	1	30	Trace of violet.....	0.423
	2	00	Barest trace of violet..	0.436
	4	00	Do.....	0.488
6	00	No color reaction.....	0.528	

These results coupled with those of Ford, and of Ford and Guthrie, leave no doubt that under the same conditions of experiment all boiled starches, from whatever source, are practically absolutely identical in digestibility.

## SUMMARY AND CONCLUSIONS.

Of the many methods and reagents used in the differentiation of different starches, different grains of the same starch, and different parts of the same grain, it is obvious that their relative values extend within very wide limits. Many of the results recorded are fallacious, owing to the presence of foreign matter or of other incidental conditions; and very frequently the reports of one observer are not confirmed, or are absolutely contradicted, by those of others, even when the same method or reagent had been employed. In many instances the cause of the different findings has been rendered quite obvious, as in the discrepancies in the reports on the digestibility of boiled starches; but in others it is not clear, as, for instance, in the repeated statements of the digestibility of raw starch under conditions in which it is asserted bacterial action had been prevented. There will be found, therefore, in the following paragraphs several statements which in the light of these contradictions may be regarded as being tentative. The following statements cover in a brief way the more essential points embraced in the literature referred to in this chapter:

(1) That the histological method is of great value in the differentiation of starches from different sources, different grains of the same starch, and different parts of the same grain, but that this method of itself, if solely depended upon, to diagnose different kinds

of starch, may be insufficient and even absolutely misleading. Therefore, for the positive identification of any given kind of starch other methods of differentiation should be coupled with the histological method.

(2) That young grains and mature grains may show differences not only in their histological characteristics, but also in their chemical and other reactions. The young grain serves as a nucleus for the deposition of starch during the development of the mature grain, it lacks lamellar structure, it shows a higher phosphorus content, it is more resistant to the actions of both dry and moist heat, it is more resistant to enzymes and dilute acids, and it exhibits differences in behavior towards iodine and aniline dyes. Likewise, the outer coat of the starch-grain shows differences from the inner part.

(3) That the starches from different sources, when subjected to the ordinary laboratory procedures of differential study, show no differences in elementary composition; that different starches show different percentages of water, starch-substance, kinds of starch-substance, digestible matter, fat, furfural contents, protein, ash, etc.; that unimportant differences may be observed in specific gravity and in heat values; that starches differ in the degree of acidity, and that the reaction is variable in accordance with the indicator, as, for instance, acid to phenolphthaleine, alkaline to methyl orange, and neutral to rosolic acid, and that different starches may react differently with the same reagent; that phosphates constitute a more or less important element in the digestibility of starches, not only of themselves directly, but also in the neutralization of alkaline impurities.

(4) That the color reactions of different starches, different grains of the same starch, and different parts of the same starch-grain may not be the same.

(5) That the different swelling reagents show not only quantitative and qualitative differences in relation to different kinds of starches, and even to different grains of the same starch, but also that some at least of these possess individual characters by which their reactions may be distinguished from those of others.

(6) That the starches of different kinds show quite a range of temperature at which gelatinization begins and at which it is complete, and that in a given specimen the larger grains tend to gelatinize at lower temperatures than the smaller grains.

(7) That the reactions in polarized light, without and with a selenite plate, may vary markedly, not only in starches from different sources, but also in different grains and in different aspects of the same grain.

(8) That the starch-pastes and pseudo-solutions obtained from different starches are not identical in their stiffness and in their penetrability in relation to fabrics; that the stiffness of the paste is affected by the length of the period of boiling, a longer period increasing the stiffness of the pastes of the certain starches, but decreasing it in others; and that gelatinized starch tends to undergo reversion, and therefore becomes less digestible.

(9) That if uninjured starch-grains are subjected *in vitro* to the actions of enzymes under strictly aseptic conditions, absolutely no digestion occurs; but if the grains be broken, eroded, or cracked, etc., or if bacterial action is not prevented, a more or less rapid erosion takes place, which is more or less peculiar to the kind of starch and corresponds with the peculiarities of the erosion phenomena observed when the starch is *in situ*; that oxygen may be necessary in the saccharification of starch, as has been indicated in enzymic and bacterial processes.

(10) That boiled starches, whatever their source, are of equal digestibility, yielding quantitatively and qualitatively the same saccharine products, provided the conditions of experiment are the same. Different starches may nevertheless have different values as articles of diet, owing to the presence in some of unpalatable or other forms of contamination. Corn starch, for instance, should be equally as good as the comparatively expensive arrowroot, but potato starch, which is of equal digestibility, has a comparatively low value because of its being or becoming unpalatable.

## CHAPTER V.

### A SYSTEMATIC SUMMARY OF THE GROSS HISTOLOGICAL PROPERTIES OF STARCHES FROM VARIOUS SOURCES.

#### NÄGELI'S CLASSIFICATION OF STARCHES FROM DIFFERENT SOURCES.

Nägeli, in his elaborate memoir on *Die Stärkekörner* (*loc. cit.*), recorded the results of the histological examinations by Raspail, Payen, Soubeiran, Schleiden, Bischoff, Crüger, Walpers, Berg, Münter, Fritzsche, Harting, and others, including his own observations, of over 1200 starches from different sources. These starches he arranged in 3 classes, 5 subclasses, and 17 types. As this publication has long since been relegated to the antiquaria, as virtually no notice is to be found in recent works of this part of his very laborious investigations, as the memoir is a rarity even in the largest of our libraries, and as this data has especial importance apropos of the present research and is of general botanical and biological interest, it seemed very desirable to include these valuable records in the present memoir. In recent literature a large number of descriptions of the forms of different starches will be found, but as such publications are generally readily available these references have not been included. The following text is a free translation of Chapter XII, almost in its entirety, of Nägeli's memoir; but many changes have been made in the Latin names, in part in the correction of typographical errors, and in part to bring the matter up to date.

Nägeli states that the different types of starch-grains merge almost imperceptibly into one another, and therefore that the boundaries are necessarily arbitrary ones; and, moreover, that very similar starch-grains are often placed far from one another in the different types. The author states that the grains might always have been arranged systematically within the individual classes according to their shape and structure, but that such a comparative classification would scarcely be possible without a complete collection of microscopical preparations. He therefore thought it better to make a classification according to other principles. In each group the seeds were considered separately from other parts of the plants and the latter were enumerated in systematic order. The natural relationships often determined to what type the grains belong. Thus, the starch-grains from all the *Bromus* seeds, although rather varied, are placed together; the grains of the seeds of *Alismaceæ* are classified in one type, while those of *Butomaceæ* are placed in another, although their grains in general are very similar. Even if the starch of the species of one order completely agrees with that of the other order they are thus classified, because on the whole the grains of *Alismaceæ* follow more closely a given type, and those of *Bulomaceæ* another type. It follows from this, he states, that the descriptions are somewhat more diffuse, less sharply drawn, and correspondingly less diagnostic than they would be in case of a comparative systematic arrangement. They have throughout not the significance of differential characters, and this the less so since it has not been determined which are specific and which are merely individual characteristics. Especial consideration has been given to the relative size, mostly to linear dimensions.

The following is Nägeli's classification of starches from different sources:

#### A. GRAINS SIMPLE.

I. *Centric*. Hilum in the mathematical center; lamellæ always equal at two corresponding diametrically opposite points.

Type 1. *Spherical*. When the grain is free both hilum and grain are spherical.

## A. GRAINS SIMPLE—continued.

I. *Centric*—continued.

Type 2. *Lenticular*. When the grain is free both hilum and grain are rounded; grains compressed;\* sometimes circular or ovoid; sometimes triangular or quadrangular.

Type 3. *Oval*. When the grain is free both hilum and grain are oval to lanceolate-oval; occasionally kidney-shaped or somewhat curved; when on end they appear circular or somewhat compressed.

Type 4. *Spindle-shaped*. Grain linear or lanceolate, tapering towards the pointed ends, or of equal-width blunt ends; when on end they appear almost circular.

Type 5. *Bone-shaped*. Grain elongated and compressed from the narrow aspect, but linear spindle-shaped from the broad aspect, with enlarged laminated ends.

II. *Eccentric*. Hilum usually more or less removed from the mathematical center of the grain; lamellæ coarsest and finest at opposite ends of the grain, respectively.

Type 6. *Inverted cone-shaped*. Grain on end almost circular; more slender at the hilum end.

Type 7. *Cone-shaped*. Grains on end almost circular; decidedly thicker and broader at the hilum end.

Type 8. *Wedge-shaped or compressed*. Grain compressed,\* of equal thickness throughout, or thicker but narrower at the hilum end than at the distal end.

Type 9. *Rod-shaped*.

III. *Grains simple and structure obscure*.

Type 10. *Structure not fully developed or not identified, owing to diminutive size of the grains*. Lamellæ, hila, cavities, fissures, and clefts seldom observed.

## B. GRAINS SEMICOMPOUND.

Type 11. *Grains semicomponent*. The component part-grains are enveloped by a common substance.

## C. GRAINS COMPOUND. The component part-grains not enveloped by a common substance.

I. *Composed of fused part-grains*.

Type 12. *Composed of fused part-grains*. The part-grains are not separated by fissures, and even different grains may be fused with one another.

II. *Composed of separated part-grains*. The part-grains separated by fissures.

Type 13. *Grains in 1 or 2 rows*. From 3 to 11 components arranged in 1 or 2 rows.

Type 14. *Equally divided grains of few components*. From 2 to 10 or more almost equal sized part-grains which, when separated, have one curved surface and one or more pressure facets.

Type 15. *Unequally divided grains of few components*. From 2 to 10 or more unequal sized, firmly united part-grains, which when separated have one curved surface and several flattened pressure facets.

Type 16. *Multiple grains*. From 20 to many thousand firmly united part-grains which, when separated, are covered with pressure facets.

Type 17. *Hollow spherical grains*. The part-grains are arranged in a spherical layer, as if a globular shell had been divided radially.

For further details of the characteristics of the various types, see as follows: Type 1, page 198; type 2, page 203; type 3, page 207; type 4, page 212; type 5, page 213; type 6, page 213; type 7, page 214; type 8, page 221; type 9, page 229; type 10, page 232; type 11, page 251; type 12, page 252; type 13, page 254; type 14, page 255; type 15, page 268; type 16, page 273; type 17, page 293.

## TYPE 1. GRAINS SIMPLE, CENTRIC, SPHERICAL.

Grains spherical or oval-spherical, and more or less polyhedral when crowded. Hilum in center, spherical. Lamellæ of equal thickness; striae of the dried grain radiate in all directions. As far as known, Nägeli states, these grains exist only in seeds. In the other parts of the plant, the underground parts for example, spherical grains with a central hilum may occur. Judging from their

\* Nägeli, in his descriptions, uses the words *zusammengedrückt* (compressed) and *abgeplattet* (flattened), but since they are both applied to the effect of pressure from *above* they may be regarded as synonymous, and this has been adopted at times in the translation.

rather diminutive size, they may be undeveloped forms of an eccentric type, and the fact that eccentric grains are found in related plants makes the likelihood probable. They are therefore classified as grains of indefinite structure. Single compound grains are often found along with centric, spherical ones, their components being arranged either spherically or lineally.

*Pharus seaber* Humb., Bonp. (*Graminaeae*.) *Dry seed*.—Grains rounded-angular to polyhedral, with small central cavity and several radial fissures. Size about  $25\mu$ .

*Zea mays* Linn. (*Graminaeae*.) *Fresh seed*.—Grains during early development at first spherical, seldom oval, most of them being twice as long as broad; later, mostly polyhedral with sharp edges; hilum distinct, very rarely with delicate lamellæ surrounding it. Size about 16 to  $21\mu$ . The dried grain usually has a central cavity with radial fissures. In the fresh, not fully developed grains several short radial fissures also occur, as well as grains with granular outer surfaces. Irregular compound grains of 2 to 6 part-grains are often found among the simple ones. The grains in the outer part of the seed are somewhat smaller (about  $16\mu$ ) than in the inner part (about  $21\mu$ ). According to Payen (*Ann. Sc. Nat.*, 1838, 11, p. 23), the starch-grains of maize average  $30\mu$  and the horn-like part of the seed contains crowded polyhedral grains, while the inner mealy parts inclose more rounded, loosely arranged ones.

*Coix laeryma* Linn. (*Graminaeae*.) *Dry seed*.—Grains spherical, frequently more or less angular owing to pressure; no lamellæ; solid or with a small central cavity having sometimes numerous fissures radiating from it. Size about 12 to  $16\mu$ .

*Paspalum dilatatum* Poir. (*Graminaeae*.) *Dry seed*.—Grains rounded or oval, many of them angular or polyhedral owing to pressure; no lamellæ; sometimes with small central cavity. Size about 6 to  $7\mu$ .

*Paspalum platycaule* Poir; *P. complanatum* Nees. (*Graminaeae*.) *Dry seed*.—Grains as in *P. dilatatum*, the larger ones with central cavity from which several short fissures radiate. Size about  $8\mu$ , rarely up to  $12\mu$ .

*Paspalum stoloniferum* Bosc.; *Maizilla stoloniferum* Schlecht. (*Graminaeae*.) *Dry seed*.—Grains angular with rounded corners to polyhedral, with sharp edges and angles; the larger ones with a central cavity from which short fissures sometimes radiate. Size about  $12\mu$ . The grains which have fallen out of the cell are often clumped together, resembling compound grains.

*Amphicarpum purshii* Kunth. (*Graminaeae*.) *Dry seed*.—Grains spherical to almost polyhedral; no lamellæ; the majority with single short fissures radiating from the center. Size about  $19\mu$ .

*Olyra paniculata* Swartz. (*Graminaeae*.) *Dry seed*.—Grains polyhedral, filling the cells; the larger ones mostly with a large or a small cavity, and rarely with single, delicate fissures. Size about  $12\mu$ .

*Oplismenus colonus* Humb., Kunth.; *Panicum colonum* Linn. (*Graminaeae*.) *Dry seed*.—Grains rounded, sometimes angular owing to pressure, with small central cavity from which fissures radiate. Size about  $10\mu$ .

*Oplismenus frumentaceus* Kunth.; *Panicum frumentaceum* Roxb. (*Graminaeae*.) *Dry seed*.—Grains spherical or oval-spherical, sometimes angular owing to pressure; the largest ones sometimes somewhat shrunken; with a central cavity, rarely with short radiating fissures. Size about 14 to  $18\mu$ .

*Setaria glauca* Beauv.; *Panicum glaucum* Linn. (*Graminaeae*.) *Dry seed*.—Grains spherical or oval-spherical, often angular owing to pressure; often with small central cavity, with radiating fissure; sometimes appears to be split into several part-grains by these fissures. Size about  $8\mu$ . Single grains with granular surfaces.

*Setaria italica* Beauv.; *Panicum italica* Linn. (*Graminaeae*.) *Dry seed*.—Grains during early development spherical or oval-spherical, but later polyhedral with sharp edges; no lamellæ; often with distinct central hilum. Size about  $14\mu$ ; according to Payen sometimes  $16\mu$ . Frequently some of the grains have granular surfaces, probably the result of abnormal solution. Compound grains of 2 to 3 part-grains appear with the single ones; these can best be observed in not fully developed seeds.

*Setaria flava* Kunth; *Panicum alopecuroideum* Schreb. (*Graminaeae*.) *Dry seed*.—Grains as above; increasing in size from the surface to the middle of the endosperm.

*Isachne australis* R. Br.; *Panicum antipodum* Spreng. (*Graminaeae*.) *Dry seed*.—Grains angular with roundish corners to polyhedral (filling the cells); the larger ones usually with central cavity and frequently with radial fissures. Size about  $13\mu$ .

- Panicum miliaceum* Linn. (*Graminaceae*.) *Dry seed*.—Grains spherical or oval-spherical, frequently somewhat polyhedral owing to pressure; usually with small central cavity from which sometimes a few fissures radiate. Size about  $7\mu$ ; according to Payen,  $10\mu$ .
- Panicum acuminatum* Swartz. (*Graminaceae*.) *Dry seed*.—Grains angular, with rounded corners, to polyhedral (filling the cells); usually with a cavity and sometimes with radial fissures, giving the single grains the appearance of compound grains. Size about 12 to  $15\mu$ .
- Panicum tonsum* Steud.; *Tricholæna tonsa* Nees. (*Graminaceae*.) *Dry seed*.—Grains angular with rounded corners, or polyhedral with blunt, or, more often, sharp corners and edges; the larger ones hollow in the center. Size about  $12\mu$ .
- Panicum hoffmannseggii* R. and Sch. (*Graminaceae*.) *Dry seed*.—Grains angular with rounded corners, or polyhedral; frequently with large or small angular cavity. Size about  $10\mu$ .
- Helopus annulatus* Nees. (*Graminaceae*.) *Dry seed*.—Grains spherical or somewhat angular owing to pressure; with central cavity from which fissures sometimes radiate. Size about 7 to  $8\mu$ .
- Pennisetum longistylum* Hochst. (*Graminaceae*.) *Dry seed*.—Grains roundish, frequently somewhat angular owing to pressure; usually with a smaller or larger central cavity from which delicate short fissures radiate. Size about 15 to  $20\mu$ .
- Pennisetum cenchroides* Rich. (*Graminaceae*.) *Dry seed*.—Grains spherical, sometimes angular, often with a central cavity and delicate radial fissures. Size about  $15\mu$ . Some have a granular surface. Other seeds designated *Gymnothrix cenchroides* R. and Sch., have spherical to rounded-oval, frequently somewhat angular, grains with central cavity and numerous fissures. Size about  $11\mu$ .
- Penicillaria spicata* Willd.; *P. pluckenetti* Link; *Pennisetum typhoideum* Rich. (*Graminaceae*.) *Dry seed*.—Grains usually spherical, more or less polyhedral, owing to pressure; frequently with a small central cavity, rarely with radial fissures. Size about 12 to  $15\mu$ .
- Antheplora elegans* Schreb.; *Cenchrus lavigatus* Trin.; *Tripsacum hermaphroditum* Linn. fil. (*Graminaceae*.) *Dry seed*.—Grains spherical, usually angular owing to pressure, and sometimes polyhedral with sharp corners and edges; sometimes delicate concentric lamellæ; usually a small, central cavity from which some or at times numerous deep fissures radiate. Size about  $36\mu$ , rarely as much as  $51\mu$ . The grains are rarely compressed, sometimes the larger ones fall to pieces. Among them are found some compound grains with few divisions, and some grains that have separated. The separated grains have one hemispherical and one or two plane surfaces, which distinguish them from the simple, polyhedral grains, which are more or less flattened on all sides.
- Lappago racemosa* Willd. (*Graminaceae*.) *Fresh endosperm*.—Grains polyhedral, with rather sharp edges and angles; the larger ones hollow. Size about  $9\mu$ . Although the grains are sometimes clumped together, they nevertheless appear to belong to the simple type.
- Lopholepis ornithocephala* Deesn. (*Graminaceae*.) *Dry seed*.—Grains angular, with rounded corners to polyhedral; with, small, rarely large, usually stellate angular cavity. Size about  $11\mu$ . The protoplasmic cells are crowded with starch-grains; there are no indications of compound grains.
- Centotheca lappacea* Beauv.; *Cenchrus lappaceus* Linn. (*Graminaceae*.) *Dry seed*.—Grains spherical, sometimes slightly angular owing to pressure; with or without indistinct lamellæ; with central cavity from which fissures usually radiate. Size about 25 to  $30\mu$ .
- Beekera petiolaris* Kochst. (*Graminaceae*.) *Dry seed*.—Grains rounded to nearly polyhedral; with central cavity and radial fissures. Size about  $11\mu$ .
- Ampelodesmos tenax* Link.; *Arundo tenax* Vahl. (*Graminaceae*.) *Dry seed*.—Grains spherical, angular with rounded corners, or polyhedral; usually with small or large central cavity, and sometimes with radial fissures. Size about 18 to  $21\mu$ .
- Pappophorum nigricans* R. Br. (*Graminaceae*.) *Dry seed*.—Grains circular or ovoid; the smaller ones spherical; the larger ones compressed to about one-half their width; with a central cavity and radial fissures. Size about 25 to  $30\mu$ . Notwithstanding the pressure facets the larger grains belong to the spherical rather than to the lenticular type, because they show the same radial fissures in their narrow aspect as in the broad one.
- Gymnopogon foliosus* Nees. (*Graminaceae*.) *Dry seed*.—Grains angular with rounded corners to polyhedral; with a small or large square cavity or one with radial fissures. Size about  $16\mu$ . These starch-grains doubtless belong to the simple spherical type. No indications of compound grains were present even within the cells.

- Uniola latifolia* Michx. (*Graminaceæ*.) *Dry seed*.—Grains rounded, usually more or less polyhedral owing to pressure, slightly if at all compressed, usually hollow. Size about 20 $\mu$ .
- Chusquea cuningii* Nees. (*Graminaceæ*.) *Dry seed*.—Grains rounded, angular with rounded corners to polyhedral; the larger ones with central cavity and some with radial fissures. Size about 15 $\mu$ . No indications of compound grains within the cell.
- Orthoclada laxa* Beauv. (*Graminaceæ*.) *Dry seed*.—Grains angular with rounded corners, or polyhedral; lamellæ, if any, delicate, homogeneous, or very slight. Size about 35 to 40 $\mu$ . The protoplasmic cells, the outlines of which are often quite indistinct, are filled with two kinds of grains of unequal size, the smaller lying in between the larger.
- Hemarthria fasciculata* Kunth. (*Graminaceæ*.) *Dry seed*.—Grains spherical or oval-spherical, usually polyhedral, or sometimes angular owing to pressure; the larger ones with usually a small central cavity and several radial fissures. Size about 15 $\mu$ .
- Rottbølla arundinacea* Hochst. (*Graminaceæ*.) *Dry seed*.—Grains spherical or oval-spherical, sometimes angular owing to pressure; the larger ones usually with a small central cavity, and frequently with several radial fissures. Size about 10 $\mu$ . Some compound grains of few components (type 14).
- Manisuris granularis* Swartz. (*Graminaceæ*.) *Dry seed*.—Grains rounded or angular with rounded corners, frequently spherical or oval-spherical; the larger ones with a central cavity, and usually with a few radial fissures. Size about 12 $\mu$ .
- Andropogon dissitiflorus* Michx. (*Graminaceæ*.) *Dry seed*.—Grains spherical or oval-spherical, and angular with rounded corners; with a central cavity and radial fissures. Size about 15 $\mu$ . Single separated-grains with one curved surface and one to three pressure facets indicate the former existence of compound grains.
- Andropogon contortus* Linn.; *Heteropogon contortus* R. and S. (*Graminaceæ*.) *Dry seed*.—Grains rounded or angular to polyhedral owing to pressure; with a small central cavity, and some radial fissures. Size about 15 to 21 $\mu$ .
- Andropogon diversiflorus* Steud. (*Graminaceæ*.) *Dry seed*.—Grains angular, with rounded corners, to polyhedral; with small central cavity and a few radial fissures. Size about 16 to 21 $\mu$ .
- Andropogon leucostachyus* Humb., Bonp.; *Ilypogonium campestre* Nees. (*Graminaceæ*.) *Dry seed*.—Grains spherical, rounded-oval, or angular with rounded corners; with central cavity and radial fissures. Size about 20 $\mu$ . Among them isolated compound grains consisting of a few components may be found; also separated-grains with one curved and 1 to 3 plane surfaces; cavity with single radial fissures.
- Andropogon ischaemum* Linn. (*Graminaceæ*.) *Dry seed*.—Grains spherical, or with rounded corners, or polyhedral; with small central cavity and a few radial fissures. Size about 13 $\mu$ . No compound grains; a few separated-grains, as in *Andropogon leucostachyus*, appear to be present.
- Andropogon umbrosus* Hochst. (*Graminaceæ*.) *Dry seed*.—Grains rounded, more or less angular to polyhedral, owing to pressure; with small central cavity and single radiating fissures. Size about 15 to 19 $\mu$ .
- Andropogon laguroides* DC. (*Graminaceæ*.) *Dry seed*.—Grains spherical, oval-spherical, and angular with rounded corners; with large or small cavity and radial fissures. Size about 15 $\mu$ . Some separated grains with 1 to 3 pressure facets appear to be present.
- Andropogon argenteus* DC. (*Graminaceæ*.) *Dry seed*.—Grains spherical, frequently somewhat angular owing to pressure; with rather large cavity. Size about 14 $\mu$ . Seeds not entirely ripe.
- Andropogon cernus* Roxb.; *Sorghum cernus* Willd. (*Graminaceæ*.) *Dry seed*.—Grains as in above, solid or with small central cavity and single radiating fissure; sometimes split by the fissures into what appear to be separate grains. Size about 13 $\mu$ . Some grains have a granular surface.
- Andropogon sorghum* Brot.; *Sorghum vulgare* Pers. (*Graminaceæ*.) *Dry and fresh seed*.—Grains spherical or oval-spherical, sometimes more or less polyhedral owing to pressure; fresh, with distinct central hilum, rarely with radial fissures; dry, usually with central cavity from which fissures radiate. Size about 11 to 15 $\mu$ . According to Payen, the diameter is 30 $\mu$ . Among these are found compound grains of 2 to 4 or 5 part-grains.
- Andropogon aciculatus* Retz.; *Chrysopogon aciculatus* Trin. (*Graminaceæ*.) *Dry seed*.—Grains spherical or rounded-oval; with large or small central cavity from which frequently fissures radiate. Size about 14 $\mu$ . Among these are found some doublets and triplets.

- Andropogon nepalensis* Hort. berol. (*Graminaceæ*.) *Dry seed*.—Grains spherical, occasionally by means of pressure somewhat angular; with small central cavity from which single or several fissures radiate. Size about 13 to 17 $\mu$ .
- Anthestiria cymbaria* Roxb.; *Andropogon cymbarius* Linn. (*Graminaceæ*.) *Dry seed*.—Grains rounded, or rounded-oval, sometimes somewhat angular to polyhedral owing to pressure. Size about 20 to 25 $\mu$ .
- Anthestiria pseudo-cymbaria* Steud.; *Andropogon cymbarius* Hochst. (*Graminaceæ*.) *Dry seed*.—Grains rounded, angular with rounded corners to polyhedral; with central cavity and radial fissures. Size about 32 to 37 $\mu$ .
- Anthestiria laxa* Andr. (*Graminaceæ*.) *Dry seed*.—Grains spherical to oval, often more or less angular owing to pressure; with small central cavity and marked radial fissures. Size about 16 to 20 $\mu$ .
- Androscepia gigantea* Brongn.; *Apluda gigantea* Spreng. (*Graminaceæ*.) *Dry seed*.—Grains spherical or oval-spherical, many of them with rounded corners, or even polyhedral, owing to pressure; usually with a small cavity and several radial fissures. Size about 20 to 26 $\mu$ .
- Imperata arundinacea* Cyrill. (*Graminaceæ*.) *Dry seed*.—Grains spherical or oval-spherical, angular with rounded corners; with central cavity and radiating fissures. Size about 13 to 17 $\mu$ . Among these are found separated-grains with one curved and one to four plane surfaces; hollow, and with several radial fissures, size about 3 to 11 $\mu$ . Compound grains of 2 to 5 equal part-grains are also found.
- Saccharum spontaneum* Linn. (*Graminaceæ*.) *Dry seed*.—Grains spherical or oval, or rounded-angular; with a large or small cavity. Size about 9 $\mu$ . Among these are some separated-grains with one curved and one to three plane surfaces; also hollow. Size about 7 $\mu$ . Compound grains of few equal-sized part-grains are occasionally present. The seeds were not quite ripe.
- Erianthus ravennæ* Beauv.; *Saccharum ravennæ* Murr. (*Graminaceæ*.) *Dry seed*.—Grains spherical or oval-spherical, some of them almost polyhedral owing to pressure; with central cavity, usually large, and radial fissures. Size about 21 $\mu$ . Among them numerous separated-grains with one curved and 1 to 4 plane surfaces; also with a cavity and fissures. Size 4 to 15 $\mu$ . Few compound grains of 2 to 5 equal-sized part-grains are occasionally present.
- Zoysia tenuifolia* Willd. (*Graminaceæ*.) *Dry seed*.—Grains spherical, oval-spherical, angular with rounded corners or almost polyhedral; usually with a central cavity, and frequently with radial fissures. Size about 15 $\mu$ .
- Hohenbergia strobilacea* Schult. fil. (*Bromeliaceæ*.) *Dry seed*.—Grains spherical or oval-spherical, frequently somewhat angular owing to pressure, sometimes irregular; no lamellæ; often with a small central cavity without fissures. Size about 15 $\mu$ . Among them compound grains of few, equal-sized part-grains (type 14).
- Billbergia zebrina* Lindl. (*Bromeliaceæ*.) *Dry seed*.—Grains rounded, sometimes irregular; no lamellæ; with central cavity from which fissures usually radiate. Size about 15 $\mu$ . Among them compound grains of few equal-sized part-grains (type 14).
- Pitcairnia albucafolia* Schrad.; *P. punicea* Lindl. (*Bromeliaceæ*.) *Dry seed*.—Grains more or less spherical, occasionally somewhat irregular; without lamellæ, mostly with large central cavity from which fissures sometimes radiate; some of them somewhat shrunken, due to drying. Size about 14 to 18 $\mu$ . Among them some compound grains of few parts (type 14).
- Pterostegia drymarioides* F. M. (*Polygonaceæ*.) *Dry seed*.—Grains rounded, more or less angular owing to pressure; with small or large central cavity. Size about 10 $\mu$ .
- Oxyria digyna* Campd. (*Polygonaceæ*.) *Dry seed*.—Grains angular with rounded corners to polyhedral with rather sharp corners and edges; the small ones solid, the larger ones with small central cavity, and the largest ones with single radial fissures. Size about 10 $\mu$ . The grains as they come from the cell frequently adhere to each other in groups or in rows.
- Rheum hybridum* Ait.; *Rheum rhaponticum* Linn. (*Polygonaceæ*.) *Dry seed*.—Grains exactly spherical, sometimes somewhat angular owing to pressure; without lamellæ; with small central cavity, the largest sometimes have a few very short radial fissures. Size about 13 $\mu$ .
- Polygonum tinctorium* Lour. (*Polygonaceæ*.) *Dry seed*.—Grains rounded, somewhat angular owing to pressure; with large or small hole. Size about 13 $\mu$ .

- Polygonum orientale* Linn. (*Polygonaceæ*.) *Fresh seed*.—Grains spherical, or somewhat angular owing to pressure; hilum usually distinct. Size about  $10\mu$ . Groups of adhering grains and also compound grains occur, the latter not easily distinguished from the former. Some semicompound forms with several hila were also observed.
- Fagopyrum cymosum* Meisn. (*Polygonaceæ*.) *Fresh seed*.—Grains spherical or oval-spherical, usually more or less polyhedral owing to pressure; no lamellæ; the larger grains with a distinct central hilum; when dry, with a cavity, not often with radial fissures. Size of the spherical about  $17\mu$  and that of the oval about  $21\mu$ .
- Fagopyrum esculentum* Moench.; *Polygonum fagopyrum* Linn. (*Polygonaceæ*.) *Dry seed*.—Grains spherical or oval, frequently more or less angular or even polyhedral owing to pressure; with small central cavity from which, at times, single short fissures radiate. Size about 10 to  $12\mu$ . Among them compound grains with few part-grains in one or two rows.
- Emex spinosa* Cambess. (*Polygonaceæ*.) *Dry seed*.—Grains spherical, or angular with rounded corners owing to pressure; the larger ones with a small central cavity from which frequently delicate fissures reach the surface. Size about  $8\mu$ , rarely  $10\mu$ .
- Rumex ratiientia* Linn. (*Polygonaceæ*.) *Dry seed*.—Grains spherical, or more or less slightly angular owing to pressure; usually with large cavity. Size about 12 to  $14\mu$ .
- Tragopyrum lanceolatum* Biebrst. (*Polygonaceæ*.) *Dry seed*.—Grains rounded, almost angular, occasionally with small central cavity. Size about  $9\mu$ .
- Atraphaxis spinosa* Linn. (*Polygonaceæ*.) *Dry seed*.—Grains spherical, frequently more or less angular owing to pressure; with small central cavity. Size about 6 to  $8\mu$ .
- Antigonon species from Guatemala*. (*Polygonaceæ*.) *Dry seed*.—Grains spherical, rarely oval, usually more or less polyhedral owing to pressure; some with small central cavity, and single short radial fissures. Size about 13 to  $17\mu$ . Among them compound grains consisting of 3 to 5 part-grains disposed in rows.
- Pisonia aculeata* Linn. (*Nyctaginiaceæ*.) *Dry seed*.—Grains spherical or polyhedral, frequently with one curved and several pressure facets; the larger with a central cavity, and at times with several short radial fissures. Size about  $13\mu$ . Undoubtedly simple grains. A few compound grains composed of 2 to more than 12 part-grains are present.
- Nepenthes destillatoria* Linn. (*Nepenthaceæ*.) *Dry seed*.—Grains spherical, or with rounded corners to almost polyhedral; with a large or small central cavity, and often with radial fissures. Size 10 to  $13\mu$ .
- Acanthus mollis* Linn. (*Acanthaceæ*.) *Fresh and dry cotyledons*.—Grains spherical to oval, not at all or slightly compressed; with delicate, indistinct lamellæ; fresh, with central spherical hilum; dry, with marked radial fissures, which extend almost to the periphery, the fissures being equally marked in all aspects. Size about  $60\mu$ . Among them are semicompound and compound grains with few equal or unequal size part-grains due to the splitting of the hilum as well as to the breaking away of corners. The starch-grains and cell-tissue of *Acanthus*, like the seeds, resemble the starchy seeds of the *Papilionaceæ*.
- Drosera longifolia* Linn. (*Droseraceæ*.) *Dry seed*.—Grains spherical or oval-spherical, rarely oval, many of them more or less polyhedral owing to pressure; with a central cavity and several marked radial fissures, some of which frequently are short, others extend to the periphery, and appear to split the simple grain into part-grains. Size about  $18\mu$ . In unripe seeds rounded grains only occur, while in the ripe seeds many polyhedral grains are seen along with them.
- Drosophyllum lusitanicum* Spreng. (*Droseraceæ*.) *Dry seed*.—Grains spherical or nearly oval, but rarely oval, and many of them more or less polyhedral owing to pressure; with small central cavity and radial fissures. Size about 15 to  $19\mu$ .

## TYPE 2. GRAINS SIMPLE, CENTRIC, LENTICULAR.

Grains circular, rounded-oval, kidney-shaped, or triangular, or 3 to 4 angular with rounded corners and pressure facets; more or less polyhedral when crowded. Hilum in the center, usually shaped like the grain, but much thinner in comparison. Lamellæ usually of equal thickness at points opposite to hilum, but thickest toward the margin of the grain.

During the process of drying a fissure coinciding with the largest plane is nearly always formed, and from which radial fissures issue, which are usually invisible on the broad aspect but appear as

dark stripes on the narrow side. These grains are usually symmetrical on all sides, although the two sides of the margin sometimes develop unequally. This type passes over into the centric-spherical as well as into the centric-oval type. It is found with certainty only in spores and seeds. Although round grains which have been pressed together do occur in the underground parts of the plant, the absence of a hilum and of lamellæ makes their structure doubtful. Compound grains are never or very rarely found along with the simple ones.

*Edogonium landsboroughii* Hass., Kutz. (*Algæ.*) *Dry spores.*—Grains rounded, triangular with rounded corners, or oval, frequently irregular; compressed to about one-half or more of the width, with a longitudinal slit on the narrow aspect. Size about 12 $\mu$ . Similar or somewhat smaller starch-grains sometimes form a partial or complete wall within the vegetative cells. Some grains approach the centric-oval (type 3).

*Edogonium vesicatum* Vauch., Link. (*Algæ.*) *Dry spores.*—Grains rounded or rounded-oval, frequently somewhat angular or irregular; the wider ones compressed to a little more than one-half of their width. Size about 8 $\mu$ . The more starch the less oil present.

*Edogonium cehinospermum* A. Braun. (*Algæ.*) *Dry spores.*—Grains as in preceding. Size about 7 $\mu$ .  
*Bulbochate sphaerocarpa* A. Braun. (*Algæ.*) *Dry spores.*—Grains circular to oval, frequently somewhat angular or irregular; the larger ones compressed to about one-half or more of their width, with a long slit on the narrow aspect. Size about 10 to 13 $\mu$ . Approaching the centric-oval (type 3). The grains in the cell are almost equal in size and are arranged close together in a single layer along the primordial sheath, within which the lumen is filled with red oil, but very often it is not visible until part of the oil has been expressed. Similar grains, but somewhat smaller in size, often form a partial or complete wall within the vegetative cell.

*Bulbochate setigera* Roth. (*Algæ.*) *Dry spores.*—Grains rounded, mostly polygonal; the larger ones compressed to about one-half or more of their width; spindle-shaped when seen from the narrow aspect. Size about 11 $\mu$ .

*Nitella*. (*Characeæ.*) *Spores.*—Grains rounded, usually irregularly angular, the angles sometimes appearing almost lobular; seldom regularly 4 to 6 sided or compressed to about one-half to one-third of their width; rarely with delicate lamellæ, and a central hilum; dry, without fissures. The grains in a spore are, as a rule, of equal size, and arranged in a simple layer within the inner surface of the membrane, and so crowded that by pressure they become polygonal.

*Nitella syncarpa* Kutz. *Fresh.*—Size about 70 $\mu$ .

*Nitella flexilis* Ag. *Dry.*—Size 17 to 50–60 $\mu$ .

*Nitella fasciculata* A. Braun. *Dry.*—Size about 14 to 56 $\mu$ .

*Nitella hyalina* Kutz. *Dry.*—Size about 15 to 45 $\mu$ . The fissures, which are seen from both aspects, are undoubtedly due to pressure.

*Nitella translucens* Pers. *Dry.*—Size about 7 to 40 $\mu$ .

*Nitella batrachosperma* A. Braun. *Dry.*—Size 10 to 45 $\mu$ .

*Nitella tenuissima* Kutz. *Dry.*—Size about 13 to 42 $\mu$ .

*Nitella gracilis* Ag. *Dry.*—Size about 10 to 40 $\mu$ .

*Nitella exilis* A. Braun.; *N. flabellata* Kutz. *Dry.*—Size about 15 to 45 $\mu$ .

*Chara*. (*Algæ.*) *Spores.*—These contain a wall of starch which consists of two kinds of grains—large, rounded and compressed grains, and small grains of indistinct structure (type 10). The larger grains are arranged with their broad side along the wall, and form a simple layer, while the smaller grains fill in the spaces between the larger ones. On crushing the spore the oil within comes out first, then the small starch-grains. Increased pressure will finally release and expel the larger grains. For this reason the latter very often are injured, and have a number of more or less marked fissures radiating from the center. In order to obtain them uninjured it is best to cut the spores.

*Chara fatida* A. Braun. *Fresh.*—Grains rounded, at times somewhat irregular; thickness two-thirds their width; lamellæ numerous, usually 3 of them very distinct at regular intervals; hilum large, circular when seen on one side, and elliptical on the other. Size about 65 $\mu$ .

*Chara hispida* Linn. *Dry.*—Grains almost round, rounded-oval, often exactly circular, compressed to about one-half or more of the width; when seen from the narrow side oval, frequently with a longitudinal slit; lamellæ numerous, very delicate; hilum circular, when seen from the broad aspect, but elongated from the narrow aspect. Size about 80 to 100 $\mu$ .

- Chara baueri* A. Braun. *Dry*.—Size of the grain about 65 $\mu$ .
- Chara alopecuroidea* Del. *Dry*.—Grains circular, or angular with round corners; compressed to about half their width. Size about 60 $\mu$ .
- Chara barbata* Meyen. *Dry*.—Grains circular; compressed to about half or more of their width; with numerous distinct lamellæ. Size about 90 $\mu$ . In some of the grains fungi threads were found which affected the starch-grains.
- Chara fragilis* Desv. *Dry*.—Grains rounded or circular; usually with beautiful, delicate lamellæ. Size about 98 $\mu$ .
- Chara aspera* Willd. *Dry*.—Size about 100 $\mu$ .
- Chara contraria* A. Braun. *Dry*.—Size about 70 $\mu$ .
- Chara gymnophylla* A. Braun. *Dry*.—Size about 68 $\mu$ .
- Chara crinita* Wallr. *Dry*.—Size about 70 $\mu$ .
- Chara coronata* Ziz. *Dry*.—Size about 66 $\mu$ .
- Pilularia globulifera* Linn. (*Marsiliaeae*.) *Dry gymnosporous*.—Grains rounded, rounded-oval, rarely somewhat irregular; compressed to a little more than one-half; seen in the narrow aspect, a longitudinal slit or an elongated hilum, around which lamellæ can rarely be distinguished. Size about 24 to 50 $\mu$ . The grains approach the centric-oval (type 3).
- Pilularia minuta* Du Rieu. (*Marsiliaeae*.) *Dry gymnosporous*.—Grains rounded to almost oval, the wider ones compressed to about one-half or more of their width; sometimes with a longitudinal slit seen in the narrow aspect; rarely with several indistinct lamellæ. Size about 17 to 56 $\mu$ . The grains very closely approach type 3.
- Ephedra distachyia* Linn. (*Gnetaeae*.) *Dry seed*.—Grains circular, sometimes rounded-oval, kidney-shaped, or triangular with rounded angles; compressed to about two-thirds their width; a marked lengthwise slit on the narrow side; no lamellæ. Size about 17 $\mu$ .
- Ephedra alata* Desne. (*Gnetaeae*.) *Dry seed*.—Grains circular to oval, often triangular with rounded angles, or oval pear-shaped; about five-sixths to as broad as long; two-thirds to three-fourths as thick as long; no lamellæ; in the wide aspect sometimes seen with indistinct radial fissures; on the narrow side, with a long slit from the two ends of which single oblique fissures radiate, or with 2 curved fissures which are joined at their convex curvatures. Size about 27 $\mu$ .
- Ephedra fragilis* Desf. (*Gnetaeae*.) *Dry seed*.—Grains as in the preceding, though somewhat larger, and frequently with distinct radial fissures in the broad aspect. Size 30 $\mu$ .
- Stipa gigantea* Lagase. (*Graminaeae*.) *Dry seed*.—Grains circular to rounded-oval; compressed to one-half and two-fifths of their width; no lamellæ; sometimes with a longitudinal slit in the narrow aspect. Size about 28 $\mu$ . These grains seem to occur more frequently in the inner part of the large cells of seeds. On the whole the principal bulk of starch consists of small, broken separated-grains (type 16).
- Heteranthelium piliferum* Hochst. (*Graminaeae*.) *Dry seed*.—Grains circular and triangular with rounded angles to kidney-shaped and oval; lamellæ very delicate, if any; the circular ones compressed to a little more than or over one-half their width, with a longitudinal slit when viewed from the narrow aspect. Size 20 to 25 $\mu$ . Among them numerous small, rounded grains, as in the *Hordeaeae*.
- Hordeaeae*. *Seed*.—The inner tissue contains numerous small grains along with large centric-lenticular ones; the outer cells are completely filled with the small grains (type 10).
- Triticum turgidum*. (*Graminaeae*.) *Fresh seed*.—Grains circular, oval, or irregular; from three-fourths to just as broad as long; one-third to one-half as thick as broad; sometimes with distinct hilum; rarely with lamellæ; lanceolate, elliptical, or plano-convex, when seen in the narrow aspect. Size about 24 $\mu$ . At times the outer surface has a reticulate appearance caused by erosion of the grain.
- Triticum monococcum* Linn. (*Graminaeae*.) *Dry seed*.—Grains as in the preceding, but somewhat smaller. Size about 30 $\mu$ .
- Triticum dicoccum* Schrank.; *T. amyleum* Sering. (*Graminaeae*.) *Dry endosperm*.—Grains as in the preceding. Size 27 $\mu$ .
- Agropyrum rigidum* R. and S.; *A. cristatum* R. and S.; *Triticum rigidum* Schrad; and *T. cristatum* Schreb. (*Graminaeae*.) *Dry seed*.—Grains rounded or oval; compressed to about one-half to two-fifths their width; without distinct lamellæ; sometimes with a longitudinal slit in the narrow aspect. Size 33 $\mu$ .

- Secale cereale* Linn. (*Graminaceæ*.) *Dry seed*.—Grains circular, oval or somewhat irregular; from three-fourths to as broad as long; one-half to one-third as thick as broad; rarely with single lamellæ, or with distinct hilum; elliptical or oval in the narrow aspect. Size  $48\mu$ . Some grains with reticulated outer surfaces.
- Elymus engelmanni* Hort. (*Graminaceæ*.) *Dry seed*.—Grains circular, rounded-oval or kidney-shaped; three-fourths to as broad as long; the larger ones three-fifths to three-fourths, those of medium-size two-fifths to one-half as thick as long; without lamellæ; sometimes with a small oblong central cavity. Size  $29\mu$ .
- Elymus hystrix* Linn.; *Asprella hystrix* Willd. (*Graminaceæ*.) *Dry seed*.—Grains rounded, homogeneous and solid; compressed. Size  $22\mu$ . The outer surface frequently shows reticulate or nodule-like markings.
- Hordeum vulgare* Linn. (*Graminaceæ*.) *Dry seed*.—Grains circular or oval; three-fourths to as broad as long; one-half to three-fifths as thick as broad; homogeneous and solid. Size  $35\mu$ .
- Hordeum bulbosum* Linn. (*Graminaceæ*.) *Dry seed*.—Grains circular, rounded-oval, triangular to quadrangular; one-half to three-fifths as thick as broad; without lamellæ; rarely with distinct, small hilum. Size 20 to  $23\mu$ .
- Hordeum himalayense* Ritter; *Critho ægicras* E. Meyer. (*Graminaceæ*.) *Dry seed, endosperm*.—Grains rounded, rarely kidney-shaped or oval, frequently irregular; compressed; no lamellæ; sometimes with a small, rarely a large, central cavity. Size 26 to  $33\mu$ .
- Ægilops caudata* Linn. (*Graminaceæ*.) *Dry seed*.—Grains circular, rarely oval, lenticular-shaped; compressed; homogeneous and solid. Size  $30\mu$ .
- Ægilops triuncialis* Linn. (*Graminaceæ*.) *Dry seed*.—Grains as in the preceding, sometimes with small central cavity, and with a single lamella. Size  $28\mu$ .
- Braconnotia clymoides* Goodr. (*Graminaceæ*.) *Dry seed*.—Grains rounded or oval; compressed to a little more than one-half the width or over; homogeneous and solid. Size  $28\mu$ .
- Lilac subulata* Humb., Bonp. (*Naiadaceæ*.) *Dry embryo*.—Grains rounded-oval, or oval, and almost terete; two-thirds to almost as broad as long; oval or elliptical when seen in the narrow aspect, and with a distinct longitudinal slit. Length  $13\mu$ , width  $11\mu$ , and thickness 7 and  $8\mu$ . The grain approaches the centric-oval type (type 3). Much protoplasm and some oil may be found with the starch.
- Triglochin barrelieri* Lois. (*Naiadaceæ*.) *Dry embryo*.—Grains usually oval, the wider ones compressed; homogeneous. Size about  $6\mu$ . The grains approach the centric-oval type (type 3). Little starch, much oil.
- Scheuchzeria palustris*. (*Naiadaceæ*.) *Dry embryo*.—Grains circular or oval, sometimes irregular; compressed to about one-half to one-third their width; homogeneous. Grains  $9\mu$ . Starch, oil, and protoplasm.
- Alisma ranunculoides* Linn. (*Alismaceæ*.) *Dry embryo*.—Grains rounded-oval, or oval three-fifths to almost as broad as long; two-fifths to three-fifths as thick as long; without lamellæ; sometimes with a distinct hilum; when seen from the narrow side, slightly contracted in the middle, rarely with a lengthwise slit. Size  $20\mu$ . The grains approach type 3. Some oil with the starch.
- Sagittaria sagittifolia* Linn. (*Alismaceæ*.) *Dry embryo*.—Grains rounded, or triangular with rounded angles, or oval, and oval pear-shaped, the wider ones compressed at about one-half their width; many with a longitudinal slit in the narrow aspect. Size  $25\mu$ . Among them some doublets. Little oil with the starch.
- Actinocarpus damasonium* Smith. (*Alismaceæ*.) *Dry embryo*.—Grains rounded or oval, occasionally kidney-shaped; two-thirds to almost as broad as long; the wider ones compressed at two-thirds or over; rarely with few lamellæ and with distinct hilum; a few very delicate fissures are sometimes visible in the broad aspect, and an indistinct slit can frequently be seen in the narrow aspect. Length 20 to  $24\mu$ , and width  $16\mu$ . The grains closely approach type 3.
- Luzula* and *Juncus*. (*Juncaceæ*.) *Seed*.—Dry and ripe seeds only were examined. Their starch-grains are undoubtedly simple, they can be seen within the cell equally distributed throughout the lumen, and there are no indications of compound ones. The grains are compressed and occasionally a central cavity can be distinguished. Therefore they most likely belong to the centric-lenticular type. The surface is more or less polyhedral, and frequently very irregular. This is no doubt due to the shrinking which the substance, being very soft in a fresh state, undergoes in drying, and also to the flattening caused by the crowding of the grains.

- Luzula nivea* Desv. *Dry seed*.—Grains rounded, more or less angular, frequently with sharp angles; homogeneous; the larger ones compressed at about one-third their width. Size 11 to 13 $\mu$ .
- Luzula forsteri* Desv. and *L. multiflora* Lejeun. *Dry seed*.—Grains rounded, occasionally somewhat angular, compressed. Size 20 $\mu$ . Thickness sometimes only 1.5 and 2 $\mu$ .
- Juncus balticus* Dethard. *Dry seed*.—Grains rounded, many of them somewhat angular, with blunt, rarely sharp, angles; somewhat compressed, extending halfway; homogeneous or granular; sometimes with small central cavity. Size 9 to 12 $\mu$ .
- Juncus glaucus* Ehrh.; *J. effusus* Linn. *Dry seed*.—Grains rounded, quadrangular with rounded corners, or polyhedral with sharp angles, usually irregular; compressed at about one-third; homogeneous or granular; sometimes hollow. Size 9 to 12 $\mu$ .
- Juncus bulbosus* Linn.; *J. compressus* Jacq. *Dry seed*.—Grains rounded or roundish-angular, or often irregular with blunt or sharp corners; compressed; homogeneous or granular. Size 13 $\mu$ .
- Juncus acutiflorus* Ehrh.; *J. sylvaticus* Reichard, var. *macrocephalus*. *Dry seed*.—Grains rounded, frequently more or less angular; compressed, sometimes to about one-half the width; homogeneous or granular. Size about 13 $\mu$ .
- Salsola soda* Linn. (*Chenopodiaceæ*.) *Dry cotyledons*.—Grains rounded to oval, usually angular and irregular; the broad ones laterally compressed at about one-half or over; homogeneous; with a long slit when seen from the narrow side. Size 11 to 13 $\mu$ . The grains approach type 3. Among them some compound grains of two or many part-grains. Seeds obtained from various places were examined. Those whose starch-grains have just been described were green, as were also the tissues of the cotyledons. Some of the starch-grains were also green, proving that they were formed in the chlorophyl. These seeds were rich in starch, and also contained much oil. On the other hand, in seeds of a brown color with colorless tissues in the cotyledons, considerably more oil and remarkably little starch was found. The latter are a little smaller (up to 8 $\mu$ ), rounded oval, or angular with rounded corners, and are compressed. The seed of other species of *Salsola soda* examined contained no starch, possibly oil replacing the starch in ripe *Salsola soda*.
- Trapa natans* Linn. (*Onagraceæ*.) *Dry cotyledons*.—Grains circular, rounded-oval to triangular to quadrangular; one-third to one-half as thick as broad; usually with indistinct and scant lamellæ; frequently with a small central cavity from which delicate fissures radiate; with a marked slit seen in the narrow aspect. Size to 32 $\mu$ .

## TYPE 3. GRAINS SIMPLE, CENTRIC, OVAL.

Grains oval or oblong, with equal blunt ends, rarely with unequal or pointed ends; seen on end, circular or compressed; more or less angular when crowded. Hilum in the center, and in a general way of the same shape as the grain, but relatively much longer and more compressed. Lamellæ, as a rule, alike at two opposite parts, most marked at the ends. The true terete grains, when dried, show fissures which radiate in all directions from the longitudinal axis; the compressed forms, almost without exception, show a fissure which coincides with the largest plane, which fissure when seen from the narrow side appears as a dark median line, and from the broad aspect has a very indistinct or almost invisible border. The fissure is sometimes crossed at right angles by another, but more frequently only cross-fissures radiate from it. The grains are usually symmetrical on all sides, but the compressed forms show a decided inclination, which is more marked along one margin than along the opposite, and they therefore become plano-convex, kidney-shaped, elliptical, or triangular with rounded angles. This type, going over on one side to the centric-spherical and on the other to the centric-lenticular, is found with certainty only in spores and seeds, and seems to be entirely wanting in other parts of the plant. Semicompound and compound grains are occasionally found among the simple ones, and they usually consist of few, and, almost without exception, part-grains of equal size.

*Chara aspera* Willd. (*Algæ*.) *Dry bulbils of the lowest nodes of the stem*.—The bulbils consist of a single cell, with a structureless membrane, and filled with two kinds of starch-grains:

- (1) Large grains, spherical or oval; two-thirds to as broad as long; the widest ones compressed to three-fourths of the grain; usually without distinct lamellæ; with central, spherical, or oblong hilum; usually with marked fissures radiating from the center; the compressed forms have a slit coinciding with the plane. Length 40 to 100 $\mu$ , width 70 $\mu$ , thickness to 50 $\mu$ .

- (2) Small grains rounded, more often oblong or oval; usually distinctly convex at one end and less so or somewhat concave at the other; occasionally reniform or triangular; the wide forms are slightly compressed. Size 4 to 30 $\mu$ . The classification of these grains is somewhat uncertain. The lamellæ of the spherical grains are mathematically centric. The structure of the large oval grains agrees with that of the *Lcguminosæ*. The smaller grains resemble the largest in form at least, but the almost central hilum seems to be more spherical. Perhaps the larger and smaller grains of *Lcguminosæ* also differ as in the case with the seeds of *Chara*. Among the grains described doublets occur which are composed of one large and one small component.
- Marsilea pubescens* Ten. (*Marsiliaceæ*.) *Dry gymnosporcs.*—Grains oval, or rounded triangular to oblong, and pear-shaped, at times somewhat irregular; the wider ones compressed to about one-half; hilum oblong, lamellæ distinct, frequently with a longitudinal slit that is seen distinctly in the narrow side, and from which cross-fissures frequently radiate. Length 30 to 110 $\mu$ , width 60 $\mu$ , thickness 35 $\mu$ . Among them some compound grains (type 11).
- Marsilea pubescens* Ten. (*Marsiliaceæ*.) *Dry androspores.*—Grains elliptical, rounded-triangular to oblong, and pear-shaped; two-thirds to as broad as long, to 4 times as long as thick, the broader ones compressed to about one-third; sometimes with a slit seen in the narrow aspect. Length 10 $\mu$ , breadth 7 $\mu$ .
- Globularia pilulifera* Linn. (*Globularisaccæ*.) *Dry androspores.*—Grains rounded, or rounded-triangular to oval, or pear-shaped; compressed to about one-half or more; a longitudinal slit in the narrow aspect. Size 7 $\mu$ .
- Butomus umbellatus* Linn. (*Alismaceæ*.) *Dry embryo.*—Grains rounded, reniform to oval, and pear-shaped; the wider ones compressed to about one-half; a longitudinal slit on the narrow aspect. Length 11 $\mu$ , width 10 $\mu$ . The grains closely approach the centric-lenticular type (type 2).
- Limnocharis plumicri* Rich. (*Alismocæ*.) *Dry embryo.*—Grains circular to oval, occasionally somewhat irregular; compressed to about one-half the width, without lamellæ; frequently with distinct hilum; on the broad aspect, usually with 2 to 7 marked fissures radiating from the center to the margin; a lengthwise slit is often seen on the narrow side. Length 21 $\mu$ , width 17 $\mu$ , thickness 8 $\mu$ . The grains approach type 2.
- Philydrum lanuginosum* Gaert. (*Philydaccæ*.) *Dry seed.*—Grains elliptical or ovoid, sometimes almost reniform, frequently somewhat irregular; one-half to equally as broad as long; sometimes slightly compressed; no lamellæ; usually with an indistinct slit which in the compressed grains coincides with the greatest plane, and from which indistinct cross-fissures sometimes radiate. Length 22 $\mu$ , breadth 14 $\mu$ , thickness 12 $\mu$ . Doublets and triplets are often found.
- Reussia triflora* Endl. (*Pontederiaceæ*.) *Dry seed.*—Grains oval to lanceolate, very often more or less curved, usually elliptical or spindle-shaped, sometimes drawn into sharp points, and more or less regular; 1.5 to 4 and 6 times as long as broad; the broad ones compressed, the smaller ones terete; no lamellæ; an indistinct longitudinal slit is seen in the narrow, and at times also in the broad aspect. Length 30 to 45 $\mu$ . A few compound grains have small number of part-grains.
- Heteranthera limosa* Vahl. (*Pontederiaceæ*.) *Dry seed.*—Grains elliptical to oblong, usually somewhat curved or reniform, sometimes triangular, and frequently irregular with protruding angles or humps; occasionally pointed at one or at both ends; 1.33 to 3 times as long as broad; the broader ones compressed; with a central cavity or fissure. Length 16 to 22 $\mu$ , breadth 10 $\mu$ , thickness 8 $\mu$ . The grains resemble those of *Reussia*, but are shorter, more compact, and more torulose.
- Pontederia* sp. (*Pontederiaceæ*.) *Dry seed.*—Grains as above, but shorter, less pointed, and more polyhedral.
- Eichhornia trieolor* M. Seubert. (*Pontederiaceæ*.) *Dry seed.*—Grains as in *Pontederia*.
- Stratiotes aloides* Linn. (*Hydrocharitaceæ*.) *Dry embryo.*—Grains elliptical, oblong, rarely curved, or almost reniform; 1.5 to 3 times as long as thick; the majority compressed; usually with a longitudinal slit seen in the narrow aspect. Length about 13 $\mu$ , thickness about 8 $\mu$ .
- Damasonium indicum* Willd.; *Ottelia alismoides* Pers. (*Hydrocharitaceæ*.) *Dry embryo.*—Grains oval or elliptical, frequently somewhat kidney-shaped; one-half to almost equally as broad as long, the broad ones laterally compressed to about one-half, the narrow ones slightly or not at all compressed; no lamellæ; frequently with a longitudinal slit on the narrow aspect. Length about 20 to 27 $\mu$ , breadth 18 $\mu$ . The grains approach the centric-lenticular form (type 2).

- Hydrocharis morsus-ranæ* Linn. (*Hydrocharitaceæ*.) *Dry embryo*.—Grains rounded to oval. Length  $10\mu$ . Approaching centric-lenticular form (type 2).
- Lachnauthes tinctoria* Ell. (*Hamodoraceæ*.) *Dry seed*.—Grains elliptical to lanceolate, frequently slightly curved, sometimes triangular or reniform, usually more or less irregular and torulose; one-fifth to almost as broad as long; the broad ones slightly compressed; no lamellæ; with frequently a very large cavity with a longitudinal slit. Length 20 to  $28\mu$ , breadth  $11\mu$ . It is doubtful whether these grains belong to this type.
- Naias major* Roth.; *N. marina* Linn. (*Naiadaceæ*.) *Dry embryo*.—Grains oval, three-fifths to four-fifths as broad as long; the broad ones compressed; without lamellæ; a longitudinal slit from which several fissures usually radiate may be seen from the narrow aspect, and sometimes also from the broad aspect. Length  $30\mu$ .
- Zostera marina* Linn. (*Naiadaceæ*.) *Dry embryo*.—Grains pressed into true polyhedrons having sharp edges and angles, filling the cells like a parenchyma; one-half to as thick as long; no lamellæ; with fissures, which in the isodiametric grains usually radiate from a central cavity and in the oblong ones proceed from a longitudinal slit at right angles to it. Length 30 to  $35\mu$ . The grains approach centric-spherical (type 1). The grains in the outer parts are of less size, those in the outermost being only 4 to  $6\mu$ . They are rounded or oval, and more or less angular. These grains show very distinctly that they belong to the simple type.
- Ruppia maritima* Linn. (*Naiadaceæ*.) *Dry embryo*.—Grains rounded to rounded-oval, frequently somewhat angular; without lamellæ; with a rounded, oblong, or irregular cavity, and usually with irregular radial fissures. Length  $25\mu$ . Seems closely to approach the centric-spherical type (type 1). Compound grains of few and usually irregular part-grains are also found (type 15).
- Zannichellia pedicellata* Fries. (*Naiadaceæ*.) *Dry embryo*.—Grains rounded to oval; half to as broad as long; the broader ones compressed to about one-half and one-third their width; without lamellæ; longitudinal slit marked in the narrow side, also sometimes shows indistinctly in the broad aspect. Length  $18\mu$ , breadth  $15\mu$ .
- Althenia filiformis* Petit. (*Naiadaceæ*.) *Dry embryo*.—Grains elliptical to oval, sometimes reniform; half to as broad as long; the broader ones laterally compressed to about half or more of their width; lamellæ indistinct; longitudinal slit is seen in the narrow aspect. Length  $18\mu$ , breadth  $15\mu$ .
- Potamogeton natans* Linn. (*Naiadaceæ*.) *Dry embryo*.—Grains oval or elliptical; three-fifths to three-fourths as broad as long; the broader ones compressed to about half their width; the narrower slightly compressed; no lamellæ; with a longitudinal slit on the narrow side. Length  $30\mu$ , breadth  $38\mu$ .
- Potamogeton prælongus*. (*Naiadaceæ*.) *Dry embryo*.—Grains elliptical or oval; compressed; no lamellæ; sometimes with a longitudinal slit on the narrow side. Length  $15\mu$ , breadth  $15\mu$ .
- Calla palustris* Linn. (*Aroideæ*.) *Dry seed*.—Grains spherical or oval, frequently irregular; sometimes slightly compressed; half to as thick as long; with a small central cavity, which in the spherical form is almost round, in the oval forms oblong. The grains approach the centric-spherical (type 1).
- Anthurium acaule* Sweet.; *Pothos acaulis* Linn. (*Aroideæ*.) *Dry seed*.—Grains spherical or oval, sometimes slightly angular, due to pressure; three-fifths to as broad as long; frequently with a small central cavity. Length 8 to  $10\mu$ . The grains approach the centric-spherical (type 1).
- Ceratophyllum submersum* Linn. (*Ceratophyllaceæ*.) *Dry cotyledons*.—Grains rounded to oval; two-thirds to as broad as long; slightly compressed; no lamellæ; a longitudinal slit is seen on the narrow side, and a delicate one is sometimes visible in the wide aspect; also simple radial fissures. Length  $23\mu$ , width  $20\mu$ . The grains approach the centric-spherical (type 1).
- Nelumbium speciosum* Willd. (*Nymphaeaceæ*.) *Dry cotyledons*.—Grains oval or elliptical, sometimes one margin more curved than the others, or even plano-convex, many of them with 3 or 4 angles; the wide ones slightly compressed; no lamellæ, or single and indistinct; a marked slit is seen on the narrow side, less marked on the broad aspect. Length  $20\mu$ . The grains decrease in size toward the surface of the cotyledons. Plumule contains oil and starch. The grains small, rounded, somewhat irregular. Size 5 to  $6\mu$ . (Syn. *Nelumbo nucifera* Gærtn.)

- Nelumbium luteum* Willd. (*Nelumbo lutea* Pers.) *Dry seed*.—Grains as in the preceding, though on the whole somewhat broader. Length  $20\mu$ .
- Cistus vulgaris* Spach. var. *C. creticus* Linn. (*Cistaceæ*) *Dry seed*.—Grains oval, elliptical, oblong, blunt-triangular, and at times almost reniform; two-thirds to almost as broad as long; the broader ones compressed; no lamellæ; usually a longitudinal slit is seen in the narrow aspect. Length  $21\mu$ .
- Helianthemum ægypticum* Mill. (*Cistaceæ*) *Dry seed*.—Grains oval, very often reniform or rounded-triangular; one-half to two-thirds or seldom almost as broad as long; slightly compressed; no lamellæ; with an oblong cavity, or a longitudinal slit on the narrow side. Length  $18\mu$ .
- Lechia thymifolia* Michx. (*Cistaceæ*) *Dry seed*.—Grains rounded-oval to elliptical, often broadly triangular or almost reniform, two-fifths to almost as broad as long; the broader ones slightly compressed; no lamellæ; usually with a longitudinal slit which appears narrower, but more marked when seen from the narrow side. Length  $12\mu$ , rarely  $20\mu$ .
- Bixa orellana* Linn. (*Bixaceæ*) *Dry unripe seeds*.—Grains rounded-oval to elliptical, sometimes blunt-triangular and almost reniform; the long ones two-fifths to one-half as broad as long, and the shorter ones as broad as long; the broader ones compressed about two-thirds of their width; few delicate or no lamellæ; with a long slit-like cavity which is more or less distinct in both aspects; or with a narrow slit and short transverse fissures seen only from the narrow side. Length 30 and  $36\mu$ .
- Mangifera* species (probably *M. indica*) Linn. (*Anacardiaceæ*) *Dry cotyledons*.—Grains oval to oblong, sometimes blunt-triangular, rarely almost reniform; two-fifths to as broad as long; the broad ones compressed; no lamellæ; often with a slit-like cavity which is usually more distinct in the narrow aspect, and from which frequently very delicate cross-fissures radiate. Length  $30\mu$ . Some doublets among them.
- Anacardium occidentale* Linn. (*Anacardiaceæ*) *Dry cotyledons*.—Grains spherical or ellipsoidal, often on one margin curved or even plano-convex; two-thirds to as broad as long; the widest ones slightly compressed; no lamellæ; the spherical grains have a small central cavity, and the oval ones an indistinct longitudinal slit. Length  $10\mu$ . Among them compound grains of 2 to 3 part-grains.
- Lotus edulis* Linn.; *Krokeria edulis*. (*Leguminosæ*) *Dry cotyledons*.—Grains spherical-oval or reniform; three-fifths to almost as broad as long; the broad ones compressed, the narrower ones slightly compressed, few delicate, sometimes indistinct lamellæ; a delicate longitudinal slit with very indistinct radial fissures in the broad aspect; the slit as well as the radial fissures are more marked in the narrow aspect. Length  $45\mu$ .
- Caragana altagana* Poir. (*Leguminosæ*) *Dry cotyledons*.—Grains rounded or oval; three-fourths to equally as broad as long; slightly compressed; occasionally with a longitudinal slit on the narrow side. Length  $8\mu$ .
- Caragana arborescens* Lam. (*Leguminosæ*) *Dry cotyledons*.—Grains rounded or oval; three-fifths to almost as broad as long; slightly compressed; with indistinct cavity seen on the broad aspect; on the narrow side a distinct longitudinal slit. Size 8 to  $10\mu$ .
- Cicer arietinum* Linn. (*Leguminosæ*) *Fresh and dry cotyledons*.—Grains almost spherical to elliptical; twice as long as thick; with few, very indistinct lamellæ and an oval or oblong hilum; dry, with a longitudinal slit and delicate radial fissures. Length  $30\mu$ , thickness  $23\mu$ . Among them compound grains of few equal-sized part-grains (see type 14).
- Pisum sativum* Linn. (*Leguminosæ*) *Dry cotyledons*.—Grains oval to almost reniform, and blunt-triangular; half to almost as broad as long; the broad ones compressed. Lamellæ distinct, usually 5 to 7; an indistinct cavity with delicate radial fissures is seen on the broad side; a marked longitudinal slit on the narrow side. Length  $65\mu$ . A few doublets are found among them.
- Ervum lens* Linn. (*Leguminosæ*) *Fresh and dry cotyledons*.—Grains oval to triangular with rounded corners; half to almost as thick as long; with 2 to 3 lamellæ; and ovate or oblong hilum; dry, with delicate radial fissures in the broad aspect, and with a distinct slit in the narrow aspect, but rarely with cross-fissures. Length  $40\mu$ , width  $30\mu$ . According to Payen, the length is  $67\mu$ . Doublets with equal parts are often found among them (type 14).

- Ervum agrigentinum* Guss. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded-oval to oblong, occasionally conical or reniform, frequently irregular and obtuse-angled; half to as thick as long; the wider ones slightly compressed; lamellæ quite distinct (2 to 5); a longitudinal slit with numerous radial fissures. Length  $50\mu$ , breadth  $35\mu$ . Among them some doublets.
- Vicia calcarata* Desf. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to oblong, occasionally reniform or triangular, frequently irregular; three-fifths to as long as broad, the wider ones compressed to about half; lamellæ indistinct, if any, sometimes more distinct in the narrow aspect; lengthwise slit hardly visible in the broad aspect, but distinct and sometimes with several cross-fissures on the narrow aspect. Length 35 and  $45\mu$ .
- V. sativa* Linn. *Dry cotyledons*.—Grains as in the preceding, one-half to equally as broad as long. Length  $32\mu$ .
- Faba vulgaris* Still.; *Vicia faba* Linn. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded-oval to oval and reniform; half to as broad as long; compressed to about one-half or more; occasionally 1 or 2 indistinct lamellæ; fine, irregular, especially radial fissures are seen in the broad aspect; distinct longitudinal slit from which fissures sometimes radiate is seen on the narrow side. Length 45 to  $50\mu$ ; width  $34\mu$ . According to Payen, the grains attain to  $75\mu$ .
- Lathyrus sativus* Linn. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded, oval, reniform, frequently irregular; half to as long as broad; the broader ones compressed to about two-fifths; sometimes with delicate lamellæ, longitudinal slit and fissures, indistinct or fine from the broad aspect, well-marked in the narrow side. Length  $56\mu$ , width  $34\mu$ .
- Lathyrus nissolia* Linn. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to oval, frequently reniform or obtuse blunt-triangular or somewhat irregular; half to as broad as long; the broad ones compressed to about two-thirds; lamellæ rare, very fine; longitudinal slit marked in the narrow aspect; in the broad view fine or indistinct radial fissures. Length  $35\mu$ , breadth  $26\mu$ , thickness  $22\mu$ .
- Lathyrus aphaca* Linn. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to oval, frequently angular with rounded corners, or somewhat reniform; three-fifths to as broad as long; the broader ones compressed to about half their width; lamellæ very fine, or rare; longitudinal slit well marked in the narrow aspect, with almost invisible radial fissures on the broad aspect. Length  $37\mu$ .
- Orobus niger* Linn. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to oval, frequently somewhat angular; three-fifths to as broad as long; the wider ones compressed to about half their width; lamellæ rare, or indistinct; longitudinal slit marked in the narrow aspect, but indistinct from the broad aspect. Length about  $29\mu$ .
- Orobus lathyroides* Linn. *Dry cotyledons*.—Grains as above, frequently rounded-triangular, or reniform. Length about  $27\mu$ .
- Onobrychis caput-galli* Lam. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded or oval, occasionally somewhat angular; three-fourths to as broad as long; slightly compressed; no lamellæ; with distinct longitudinal slit seen in the narrow aspect. Length  $15\mu$ .
- Onobrychis sativa* Lam. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded or ovoid; three-fourths to just as broad as long; slightly compressed; longitudinal slit on the narrow side. Length about  $11\mu$ .
- Canavalia obtusifolia* DC. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to elliptical, occasionally triangular or reniform, or somewhat irregular; half to as broad as long; the wider ones compressed to about one-half, and the narrow ones very slightly compressed; lamellæ indistinct or rare; longitudinal slit and radial fissures fine on the broad side, marked on the narrow side. Length about 30 to  $35\mu$ , breadth  $24\mu$ .
- Phascolus multiflorus* Lam. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to oval, often triangular; three-fifths to as broad as long; the broad ones slightly compressed; no lamellæ; occasionally with radial fissures; longitudinal slit distinct on the narrow aspect, indistinct from the wide aspect, rarely with a transverse slit. Length about  $35\mu$ .
- Phascolus vulgaris* Linn. *Dry cotyledons*.—Grains as above; usually with marked fissures on the broad side and a distinct slit on the narrow side. Size  $40\mu$ . According to Payen,  $63\mu$ .
- Phascolus aureus* Hamilt. *Dry cotyledons*.—Grains as in *Ph. multiflorus*, but somewhat larger; sometimes with indistinct lamellæ; fissures less marked. Size  $55\mu$ .
- Phaseolus saponaceus*. *Dry cotyledons*.—Grains as in *Phaseolus multiflorus*, but larger; 3 to 6 lamellæ within the margin usually visible, very delicate, and crowded; longitudinal slit and fissures marked. Length 50 to  $56\mu$ .

- Vigna glabra* Savi. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to elliptical, sometimes almost reniform; half to as broad as long; the broad ones slightly compressed; numerous fine lamellæ; longitudinal slit moderately fine on the broad aspect and with radial fissures; slit mostly without fissures and more marked on the narrow side. Length about 40 to 48 $\mu$ .
- Dolichos monachalis* Brot. (*Leguminosæ*.) *Dry cotyledons*.—Grains oval, almost reniform, or rounded-triangular; half to almost as broad as long; the wide ones slightly compressed; no lamellæ; with longitudinal slit, and frequently with radial fissures. Length about 27 $\mu$ , width about 20 $\mu$ . Doublets are occasionally found.
- Lablab vulgaris* Savi. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded or oblong, often reniform; half to as broad as long; the wider ones compressed to about one-half; no lamellæ; a longitudinal and sometimes a transverse slit, besides numerous fissures running irregularly when seen on the broad aspect; in the narrow aspect the fissures usually radiate from the two ends of the longitudinal slit. Length 25 to 29 $\mu$ .
- Drepanocarpus lunatus* Meyer. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded to oval; often triangular and somewhat reniform or irregular; three-fifths to as broad as long; the broad ones slightly compressed; without lamellæ; occasionally numerous irregular fissures, more often a longitudinal slit from which few or many fissures radiate. Length about 45 $\mu$ .

#### TYPE 4. GRAINS SIMPLE, CENTRIC, SPINDLE-SHAPED.

Grains linear or lanceolate, either narrowed toward the ends (spindle-shaped) or with ends of equal width, or even somewhat broadened and blunt (rod-shaped); almost circular in cross-section. Hilum and lamellæ invisible. This type occurs only in the latex of native *Euphorbiaceæ*, and probably is but a transition to type 5.

- Euphorbia lathyris* Linn. (*Euphorbiaceæ*.) *Fresh latex from the stem*.—Grains spindle-shaped or rod-shaped, often somewhat irregular; circular in cross-section; 4 to 8 times as long as wide; frequently broadened at the ends, as if swollen; no lamellæ; with a long depression in the linear axis, and with numerous fine, short, transverse fissures. Length 55 $\mu$ , width 10 $\mu$ . The fissures are visible if the starch-grain be examined in the unchanged latex or in water.
- Euphorbia palustris* Linn. (*Euphorbiaceæ*.) *Fresh latex from the stem*.—Grains rod-shaped, or cylindrical spindle-shaped, thickened toward the middle; the ends are frequently rounded into small heads. Length 17 to 35 $\mu$ , width 3 to 6 $\mu$ .
- Euphorbia virgata* W.K. (*Euphorbiaceæ*.) *Fresh latex*.—Grains as in the preceding, but not much thickened toward the middle. Length 32 $\mu$ , width 5 $\mu$ .
- Euphorbia dulcis* Linn. (*Euphorbiaceæ*.) *Fresh latex from the stem*.—Grains rod-shaped, ends rounded, 6 to 10 times as long as wide. Length about 17 to 28 $\mu$ , width 2.05 to 3.05 $\mu$ .
- Euphorbia procerca* Biebrst. (*Euphorbiaceæ*.) Grains as in the preceding. Length 32 $\mu$ , width 4 $\mu$ .
- Euphorbia epithymoides* Linn.; *Euphorbia fragifera* Jan.—Grains as in the above, 7 to 12 times as long as thick. Length about 42 $\mu$ , thickness 3 to 5 $\mu$ .
- Euphorbia eyparissias* Linn. (*Euphorbiaceæ*.) *Fresh latex from the stems*.—Grains rod-shaped, or cylindrical spindle-shaped, ends round or blunt, usually broadened, rarely narrowed; 6 to 10 times longer than thick.
- Euphorbia nicæensis* All.; *Euphorbia glareosa* Biebrst. (*Euphorbiaceæ*.) *Fresh latex from the stem*.—Grains rod-shaped or cylindrical spindle-shaped; usually with blunt, equally broad, capitate ends, so that the whole grain appears almost bone-shaped; slightly terete or compressed at the widened portion. Length 38 $\mu$ , width at some places 11 $\mu$ .

#### TYPE 5. GRAINS SIMPLE, CENTRIC, BONE-SHAPED.

Grains elongated, compressed; linear spindle-shaped in the narrow aspect; at first spindle-shaped in the broad aspect, afterwards moderately broad toward the middle, and with very much broadened spatulate, sometimes lobate, split ends. Hilum invisible; lamellæ usually indistinct. This type is found only in the latex of arborescent *Euphorbiaceæ*. The middle part may be almost wanting in the short grains; and the width of the ends may even exceed the entire length of the grain. In the longer grains lateral lobes along the plane of the widest growth may also appear. The two ends sometimes develop unequally.

*Euphorbia nereifolia* Linn. (*Euphorbiaceæ*.) *Fresh latex*.—Grains in the broad aspect bone-shaped, with rounded, broadened, and usually lobate ends; two-ninths to one-third as broad as long about the middle, and half as broad as long at the ends. In the narrow aspect, rod-shaped or narrow-spindle-shaped, frequently curved; one-fifth to one-seventh as thick as long; usually without lamellæ; with a channel-like cavity along the median line from which short transverse fissures radiate. Length about  $50\mu$ . Some forms with lobate processes in the middle of the grain may also occur, in which case they are two-thirds as broad as long. The fissures are already present in the grain in the latex and do not alter in water.

TYPE 6. GRAINS SIMPLE, ECCENTRIC, INVERTED, CONE-SHAPED.

Lamellæ more numerous and coarser on one side, and fewer and finer on the diametrically opposite side. Grains more or less conical, almost circular on cross-section. The eccentric hilum is toward the slender end. The lamellæ, which completely encircle the grain, are interspersed at the thickened side with incomplete lamellæ, but rarely in great numbers. On drying, fissures radiating from the central lamellæ are formed which are usually turned toward the distal end, and have a funnel-like arrangement. This type usually has no well-marked characteristics, and through the grain with equal ends easily merges into types 7 and 9; and again through these grains to those which are slightly compressed at the distal end into type 8. This type (6) may possibly occur more often than would appear from the specimens described, of which the potato is the best example. At a first glance some grains seem to belong to this type, while in reality they are flattened grains belonging to type 8.

*Saururus cernuus* Linn. (*Piperaceæ*.) *Root-stock*.—According to Leon Soubeiran (*Journ. Pharm.*, 1854, xxv, 100) the grains are rounded or oval; size 10 to  $40\mu$ ; the majority with distinct centric lamellæ and a hilum. The drawing by Soubeiran represents the hilum as being very eccentric, and situated at the narrower end, so that the grains apparently belong to this type.

*Ipomæa purga* Schlecht. *Jalap*. (*Convolvulaceæ*.) *Dry tubers*.—Grains spherical or egg-shaped; three-fourths to as long as broad; 4 to 7 lamellæ, rarely distinct; frequently with single radial fissures in the interior; eccentricity of hilum usually one-fourth to one-seventh. Size about  $35\mu$ . According to Leon Soubeiran (*Journ. Pharm.*, 1854, xxv), the starch-grains in the root of *Batatas jalappa* Choisy are 30 to 70 and  $80\mu$  in size; many are somewhat round or oval; some indistinctly triangular; others elliptical, but at the distal end cut off at right angles to the longitudinal axis; with distinct lamellæ and eccentric hilum; in place of the hilum radiating fissures are frequently found. Nägeli queries: Can this be understood as a true jalap?

*Solanum tuberosum* Linn. (*Solanaceæ*.) *Fresh and dry tubers*.—Grains more or less egg-shaped, sometimes irregular, usually two-thirds to four-fifths as wide as long; lamellæ distinct, the majority of them complete; hilum, as a rule, at the narrow end, occasionally at the broad end and one-fourth to one-sixth eccentric; dry starch—usually with a small cavity in the central lamellæ, and occasionally a few very short fissures, usually in the direction of the longitudinal axis. Length 70 to  $90\mu$ . According to Payen, the grains in the large Rohan potato attain a length of  $185\mu$ , and in some other kinds of potatoes  $140\mu$ . Among them are semi-compound grains comprising 2, rarely 4, part-grains, and also compound grains of 2 to 3 part-grains.

*Niphæa oblonga* Lindl.; *Achimenes alba* Hort. (*Gesneraceæ*.) *Dry scales from the root-stocks*.—Grains oval to lanceolate; in transverse section usually almost circular; with numerous, delicate lamellæ, of which only a few of the innermost are complete; hilum at the smaller end, one-fifth to one-twelfth eccentric; the end opposite the hilum is occasionally broadened into a somewhat compressed knife-like edge. Length  $36\mu$ .

*Dicentra formosa* Walp; *Diclytra formosa* DC. (*Fumariaceæ*.) *Dry root-stocks*.—Grains oval, pear-shaped, 3 to 4 angles; usually more or less irregular; half to as broad as long; the broader ones slightly compressed; lamellæ very fine or invisible; hilum at the thin end, about one-eighth eccentric; in place of the hilum there is usually a small cavity from which several fissures radiate, forming a funnel-like groove. Length about  $26\mu$ , breadth  $20\mu$ . Some grains are obtusely triangular, inasmuch as one angle is formed by an outer system of lamellæ. Length  $26\mu$ , breadth  $20\mu$ .

- Oreodaphne exaltata* Nees; *Laurus exaltatus* Sieb. Pl. martin. 106. (*Lauraceæ*.) *Dry cotyledons*.—Grains rounded-oval to elliptical, frequently egg-shaped, frequently somewhat irregular or unsymmetrical; half to as broad as long, the broader ones slightly compressed; no lamellæ; hilum about one-seventh eccentric, usually at the narrow end, rarely at the broad end, and occasionally with a longitudinal slit; sometimes with several funnel-shaped fissures radiating from the center of the lamellæ. Length about  $21\mu$ . The two ends of the grain are very often alike in width.
- Laurus nobilis* Linn. (*Lauraceæ*.) *Dry cotyledons*.—Grains rounded, egg-shaped, frequently somewhat irregular, or angular owing to pressure; no lamellæ; hilum slightly eccentric, frequently at the narrow end; in place of the hilum there is usually a cavity with more or less numerous short radial fissures, frequently with one marked transverse fissure which divides the grain almost in half. Length  $23\mu$ , thickness  $19\mu$ . Among them some compound grains of few, equal components. (See type 14.)
- Mucuna pruriens* DC. (*Leguminosæ*.) *Dry cotyledons*.—Grains oval or elliptical, usually more or less irregular, the smaller ones almost round; usually half, the longest ones only two-fifths, as broad as long; generally circular in transverse section; the broader ones slightly compressed; no lamellæ; in the place of the one-seventh eccentric hilum, a small cavity is found from which delicate fissures pass out in the form of a funnel-like groove. Length 17 to  $21\mu$ . The center of these is found at the tapering end; the posterior end being more or less broadened and compressed, sometimes appearing to be cut off, leaving a doubt whether this is a transition type going over into type 8 or whether it is a separated-grain.
- Mucuna urens* DC. (*Leguminosæ*.) *Dry cotyledons*.—Grains egg-shaped to rounded-oval, one-half to almost as broad as long, circular in cross-section; no lamellæ; at the narrow end a small cavity instead of the one-half eccentric hilum, from this cavity two fissures diverge toward the distal end; and also usually one or more distinct transverse fissures, and sometimes also a few short ones, extend towards the hilum end. Length about 16 to  $28\mu$ . Among them some doublets, triplets, and some separated-grains.

#### TYPE 7. GRAINS SIMPLE, ECCENTRIC, CONE-SHAPED.

Lamellæ heaviest and most numerous on one side, and thinnest and least numerous on the diametrically opposite side. Grains more or less conical, almost circular in cross-section. The eccentric hilum toward the broad end. Only a few of the innermost lamellæ, as a rule, are complete; the outer ones at the thickened radius seem to be incomplete. On drying, radial fissures are formed which diverge from the center of the lamellæ, chiefly toward the distal end, among which one is often noted as coinciding with the median line. Perfect specimens of this type occur very rarely. Generally grains are present in which the distal end (distal to hilum) is tapering, but more or less broadened, approaching the wedge-shaped type (type 8), and sometimes, though rarely, this same end is condensed, or of equal thickness, so as to approach the inverted conical type (type 6) or the rod-shaped type (type 9).

- Schænus mucronatus* Linn.; *Cyperus ægyptiacus* Gloxin. (*Cyperaceæ*.) *Dry creeping root-stocks*.—Grains spherical and blunt-triangular to elliptical; frequently conical, or somewhat curved with beak-shaped distal end; two-fifths to as thick as broad; no lamellæ; instead of the one-fourth to one-fifth eccentric hilum a small cavity is found from which extend single, short fissures. Length  $21\mu$ . Among them, isolated doublets, of unequal halves arising from the cutting off of the tapering distal end.
- Cyperus esculentus* Linn. (*Cyperaceæ*.) *Dry root-stocks*.—Grains usually conical, rarely rounded or oval; slightly curved at the thin end and frequently with two beak-shaped processes; one-half to almost as broad as long; the broader ones laterally compressed to about two-thirds; no lamellæ; hilum at the broad end, one-third to one-fourth eccentric; occasionally a small or a slit-like cavity. Length about 12 to  $14\mu$ .
- Commelina hirsuta* R. Br. (*Commelinaceæ*.) *Dry tubers*.—Grains pear-shaped, or elliptical, or elongated-conical, rarely triangular, frequently somewhat irregular; usually half as broad as long; lamellæ numerous and distinct; mostly thickened at the hilum end, the distal end usually pointed; and in the triangular grains broadened and squared. Instead of the hilum, a small cavity with radiating fissures; eccentricity about one-tenth. Length about  $42\mu$ . Some of the grains approach the wedge-shaped (type 8) and the rod-shaped type (type 9).

- Triglochin barrelieri* Lois. (*Naiadaceæ*.) *Dry root-stocks*.—Grains usually oval-lanceolate or conical, rarely rounded or blunt-triangular, sometimes curved, frequently irregular; one-fourth to as broad as long; the broader ones slightly compressed; lamellæ rare and indistinct; usually a longitudinal slit, from which single fissures sometimes diverge laterally. Length about  $38\mu$ . The hilum is sometimes at the broad end. On the whole, the majority of the grains show no distinct type. Among them compound grains of few, unequal parts (see type 15). Very rich in starch.
- Scilla peruviana* Linn. (*Liliaceæ*.) *Dry bulbs*.—Grains rounded or oval pear-shaped; one-half to almost as broad as long; lamellæ delicate, the innermost ones complete, the outermost ones unilateral; hilum end thickened, the distal end narrow and obtuse, at times cut off, rarely broadened and squared; instead of a hilum, frequently a small cavity from which single short fissures radiate; eccentricity of hilum one-half to one-fourth. Length about  $16\mu$ , width  $36\mu$ . Among them some compound grains of few equal or unequal parts.
- Ornithogalum umbellatum* Linn. (*Liliaceæ*.) *Dry bulbs*.—Grains oval-rounded, rounded pear-shaped, and oval; no lamellæ; thickened at the hilum end; frequently a small cavity and sometimes single, with fine fissures; eccentricity about one-third. Length about  $24\mu$ . Among them a few doublets.
- Paris quadrifolia* Linn. (*Liliaceæ*.) *Dry root-stocks*.—Grains rounded, elliptical, oval pear-shaped, or reniform, half to almost as broad as long; the broad end slightly compressed, thickened at the hilum end; a small cavity or longitudinal slit; eccentricity about one-quarter. Length about  $13\mu$ , width  $10\mu$ . Rich in starch.
- Trillium rhomboideum* Michx. (*Liliaceæ*.) *Dry root-stocks*.—Grains rounded, rounded triangular to oblong, and pear-shaped; frequently somewhat irregular; one-half to about as broad as long, the broad ones slightly compressed; thickened at the hilum end; a small cavity, or more often a longitudinal slit, instead of the hilum; eccentricity about one-quarter. Length about  $11\mu$ , width  $9\mu$ . Rich in starch.
- Billbergia amæna* Lindl. (*Bromeliaceæ*.) *Dry root-stocks*.—Grains rounded-oval to elongated pear-shaped, one-half to two-thirds as broad as long, the broad ones slightly compressed; thickened at the hilum end; the hilum has a small cavity from which several marked fissures radiate; frequently also one short transverse fissure; eccentricity one-third and one-fourth. Length about  $21\mu$ . Among them compound grains of few usually equal-sized components (see type 14); also some semi-compound grains of two small "part-grains."
- Zostera nana* Roth. (*Naiadaceæ*.) *Dry root-stocks*.—Grains conical, or from one aspect a flattened oval, and from the other aspect cone-shaped (the distal end broadened and squared); usually almost twice as long as broad; lamellæ indistinct, or none; hilum at the broad end, eccentricity about one-sixth; usually a small cavity from which several short fissures pass out. Length about  $32\mu$ .
- Richardsonia scabra* Linn. (*Rubiaceæ*.) *Dry roots*.—Grains spherical to oval and conical; frequently 1.5 times, rarely twice as long as broad; almost circular in cross-section; at times with a few indistinct lamellæ; hilum at the broad end; rarely more eccentric than about one-sixth; occasionally a small cavity. Length about  $21\mu$ . Also compound grains of few, mostly equal components. (See type 15.)
- Vinca minor* Linn. (*Apocynaceæ*.) *Dry stolons*.—Grains rounded to oblong and conical; about one to three times as long as broad; occasionally broadly triangular, about 1.5 times as broad as long; frequently more or less irregular; sometimes with one or two protruding pointed angles; hilum at the broad end; the distal end rarely broadened into a knife-like edge; occasionally with a small cavity. Length about  $13\mu$ . Also some doublets of unequal components. Similar simple starch-grains occur also in the roots of *Vinca minor*, besides a few larger ones (about  $16\mu$ ), which are somewhat broader (at most twice as long as broad) and also rather thicker, and always with blunt ends. Among these are found many compound grains of few almost equal components.
- Symphytum bulbosum* Schimp. (*Boraginaceæ*.) *Dry tubers*.—Grains round to egg-shaped and oval-conical; 1.5 times, rarely twice, as long as broad; lamella none or very delicate; thickened at the hilum end, or with the two ends of similar thickness, or the distal end less dense and knife-like; usually with a small cavity and radial fissures; eccentricity about one-half or more. Length  $32$  to  $44\mu$ , thickness  $24$  to  $30\mu$ .

- Symphytum tuberosum* Linn. (*Boraginaceæ*.) *Dry root-stocks*.—Grains similar to the preceding, somewhat smaller and less well-developed, spherical to oval; 1.33 to 1.5 times as long as broad; no lamellæ; hilum end rarely distinctly broadened; a small cavity and occasionally short, delicate, radial fissures; eccentricity about one-half. Length about  $18\mu$ , thickness about  $13\mu$ . Also compound grains of few, usually equal components. (See type 14.)
- Nægelia zebrina* Regel. (*Rhamnaceæ*.) *Fresh scales of the root-stocks*.—Grains usually conical, sometimes oval-cylindrical, usually twice as long as broad; circular in cross-section; the broad ones slightly compressed; thickened at the hilum end, distal end narrowed, sometimes broadened, obliquely cut-off, or square-shaped; lamellæ and hilum distinct; eccentricity about one-sixth. Length about  $45\mu$ , width about  $33\mu$ . Very often the grain is thickened from the hilum end to about the middle, and from there on it narrows down to a cone, thus representing the appearance of a spindle with the two parts unequal; they approach the rod-form (type 9).
- Gloxinia speciosa* Lodd. (*Gesneraceæ*.) *Fresh tubers*.—Grains oval, conical, or oblong, about three times as long as broad; frequently with a lateral appendage (composed of a special external system of lamellæ); hilum and lamellæ usually distinct, but delicate; thickened at the hilum end; sometimes the two ends are equally thick, the distal end rarely broader and compressed; eccentricity about one-fourth. Length about  $48\mu$ , width  $22\mu$ . Some of the grains approach the rod-form (type 9).
- Gloxinia hirsuta* Lindl. (*Gesneraceæ*.) *Fresh tubers*.—Grains similar to the preceding, oval or conical; lamellæ not always distinct; hilum usually at the broad end; frequently single, radial fissures. Length about  $42\mu$ . Also some doublets and semi-compound grains with 2 to 3 components.
- Orobanche* sp. (*Scrophulariaceæ* or *Orobanchaceæ*.) *Dry root-stocks*.—Grains spherical to oval, and truncated-conical; 1.5 times, rarely twice, as long as broad; lamellæ indistinct; thickened at the hilum end, rarely broader and thinner at the distal end; a cavity, and marked more or less numerous radial fissures; eccentricity about one-third. Length about  $46\mu$ , width about  $38\mu$ . Also some compound grains of few usually equal-sized components. (See type 14.)
- Orobanche procera* Koeh. (*Scrophulariaceæ* or *Orobanchaceæ*.) *Dry root-stocks*.—Grains rounded-oval to conical and oblong; 1 to 2.5 times as broad as long; lamellæ none or delicate; thickened at the hilum end; distal end sometimes broadened with a knife-like edge; a small cavity, and frequently very short radial fissures; eccentricity about one-fourth to one-fifth. Length about  $28\mu$ , width  $20\mu$ . Some of the grains approach the wedge-shaped type (type 8) and others the rod-shaped type (type 9). Some doublets and triplets are present.
- Lathræa squamaria* Linn. (*Scrophulariaceæ* or *Orobanchaceæ*.) *Dry scales of the root-stocks*.—Grains oval, conical, elliptical, frequently somewhat irregular; almost circular in transverse section; rarely compressed; as a rule, twice as long as broad; occasionally broader than long; lamellæ distinct, the innermost ones complete and rather unsystematically arranged, the outer ones unilateral and crowded; usually thickened at the hilum end, distal end frequently drawn into a conical point or broadened into a knife-like form; radial fissures, especially in the direction of the "verdickungshalbmesser;" eccentricity one-fifth and one-eighth. Length about  $125\mu$ .
- Cyclamen hederifolium* Ait. (*Primulaceæ*.) *Dry bulbs*.—Grains triangular, oval, lanceolate, usually conical, often irregular or slightly curved; one-fourth to as broad as long; the broader ones compressed to about one-half, but the smaller not compressed; lamellæ none, or indistinct; thickened at the hilum end, distal end narrow, sometimes drawn into a point, sometimes broadened and knife-like; often a cavity, from which short, delicate fissures rarely pass out; eccentricity about one-tenth. Length about  $36\mu$ , width about  $24\mu$ . Triangular grains frequently occur, with two sets of lamellæ at right angles to one another, of which the outer set forms the upper angle of the triangle and the inner one the base; the hilum and the greatest thickness are found in one angle of the base.
- Dodecatheon meadia* Linn. (*Primulaceæ*.) *Dry roots*.—Grains triangular, oval, oblong, conical, elliptical, or spindle-shaped with the two unequal parts; frequently more or less irregular; 2 to 4 times, rarely equally, as long as broad, in the latter case compressed to about one-half; usually without lamellæ and hilum. Length about  $26\mu$ . The grains probably belong to this class, as their structure seems to resemble that of the cyclamens. Some doublets are found among them.

- Carum bulbocastanum* Koch.; *Bunium bulbocastanum* Linn. (*Umbelliferae*.) *Dry tubers*.—Grains spherical-oval or elliptical, frequently thicker at one end, very often reniform or triangular, the majority more or less irregular, some with one or more mammary processes; no lamellæ; rarely with a cavity, and short, radial fissures at the thick end, instead of a hilum, frequently with a longitudinal slit; eccentricity about one-fourth. In one sample from Zweibrücken the grains are about  $26\mu$  in length, and mostly one-half to two-thirds as thick; in one sample from Zermatt the grains are smaller, about  $18\mu$ , and mostly two-fifths to two-thirds as thick as long. Among them compound grains of few, usually unequal components. (See type 15.)
- Adoxa moschatellina* Linn. (*Caprifoliaceae*.) *Dry root-stocks*.—Grains spherical to oval, lamellæ rare and indistinct; occasionally a small cavity with several short, delicate radial fissures, usually at the broad end; eccentricity about one-third. Length  $31\mu$ . Among them some compound grains of 2 to 3 equal or unequal components.
- Umbilicus pendulinus* DC. (*Crassulaceae*.) *Dry root-stocks*.—Grains rounded, oval, conical, 3 to 4 angles; usually irregular; three-fifths to as broad as long; the broader ones compressed to about three-fourths; lamellæ indistinct, only the innermost ones complete; thickened at the hilum end; radial and sometimes also irregular fissures; eccentricity one-fourth to one-fifth. Length about  $50\mu$ . The young grains are conical; the older ones are frequently distorted and knife-like at the distal end.
- Cephalotus follicularis* R. Br. (*Cephalotaceae*.) *Dry root-stocks*.—Grains rounded, and rounded-triangular to oval, and oval-conical; no lamellæ; at times a delicate longitudinal slit, occasionally with a small cavity instead of the hilum; thicker at the hilum end; distal end narrowed, sometimes spread out into a knife-like edge; eccentricity about one-third to one-fifth. Length about 18 to  $21\mu$ .
- Cocculus palmatus* DC.; *Menispermum palmatum* Lam. (*Menispermaceae*.) *Dry roots*.—Grains oval, oblong, conical; rarely 3 to 4 angles; rounded and sometimes lobate; usually irregular; half to as broad as long; the broader ones slightly compressed; with 3 to 8 usually complete lamellæ; cavity instead of the hilum, usually with marked radial fissures at the broad end; eccentricity one-half to one-fourth. Length  $90\mu$ . According to Payen, they are sometimes as large as  $180\mu$ . Among them doublets and triplets of equal or unequal sized components. The grains are usually stained yellow in the cavity and fissures. The coloring disappears in water.
- Ranunculus garganicus* Ten. (*Ranunculaceae*.) *Dry, thickened roots*.—Grains spherical to shortened-conical; two-thirds to as broad as long; in the spherical ones a small, usually central cavity instead of the hilum; in the other grains the cavity is about one-eighth eccentric. Length about  $14\mu$ , width about  $10\mu$ . Starch plentiful.
- Ranunculus bulbosus* Linn. (*Ranunculaceae*.) *Dry tubers*.—Grains rounded, oval, conical, frequently irregular; half to almost as broad as long; no lamellæ; a small cavity instead of the hilum at the wide end; eccentricity about one-fourth. Length about  $12\mu$ . Some compound grains of few, usually unequal components. (See type 15.) Starch plentiful, filling the cells.
- Anemone ranunculoides* Linn. (*Ranunculaceae*.) *Dry root-stocks*.—Grains usually oblong or pear-shaped; generally three-fifths to three-fourths as broad as long; no lamellæ; at the wide end a small cavity from which a few delicate fissures sometimes radiate, or a longitudinal slit; eccentricity about one-third to one-fourth. Length about  $13\mu$ . Among them compound grains of few, equal or unequal components. (See type 15.) Starch plentiful, entirely filling the cells.
- Aconitum anthora* Linn. (*Ranunculaceae*.) *Dry napiform roots*.—Grains mostly pear-shaped, rarely oval, or rounded-reniform, or rounded-triangular, frequently somewhat irregular; no lamellæ; broadened at the hilum end, distal end in the triangular grains is broadened and squared; narrowed in the other forms; instead of the hilum, a small cavity from which several fissures radiate; eccentricity about one-third to one-fifth. Length about  $38\mu$ . Compound grains of few, equal or unequal parts. (See type 15.)
- Pæonia officinalis* Retz. (*Ranunculaceae*.) *Fresh root-stocks*.—Grains spherical to oblong and conical; sometimes somewhat irregular, 1 to 2 times as long as broad; lamellæ few (2 to 4), delicate, rarely distinct; hilum also indistinct, eccentricity two-ninths. Length about  $28\mu$ , width about  $19\mu$ . Many compound grains of few, equal and unequal components. (See type 15.)

- Corydalis cava* Schweigg., Koert.; *Corydalis bulbosa* Pers. (*Fumariaceæ*.) *Fresh and dry tuberous root-stocks*.—Grains rounded to oblong, often conical to reniform-triangular; generally somewhat irregular; two-fifths to as broad as long; the broader ones slightly compressed; no lamellæ; hilum at the broad end, one-fourth to one-fifth eccentric; in the triangular forms the distal margin is squared, so that the grain may be as much as 3 times as long as broad. In the dry grains either a longitudinal slit or a large or small cavity with single radial fissures. Length about 17 to 21 $\mu$ . Many grains approach the wedge-shaped type (type 8). Among them some compound grains of 2 to 4 usually unequal components.
- Cardamine granulosa* All. (*Brassicaceæ*.) *Dry root-stocks*.—Grains rounded, oval, conical, frequently more or less irregular; the broader ones slightly compressed; usually homogeneous; rarely with a small cavity at the broad end, and with a delicate longitudinal slit; eccentricity about one-third and one-fourth. Length about 14 $\mu$ . Some grains appear to approach type 8. Among them some doublets and triplets.
- Dentaria digitata* Lam. (*Brassicaceæ*.) *Fresh and dry scales of the root-stock*.—Grains oval or pear-shaped, almost circular in transverse section; two-fifths to almost as broad as long; lamellæ delicate (5 to 7), only the innermost ones complete; hilum at the broad end, one-fourth to one-sixth eccentric; the distal end frequently elongated, bluntly cut and squared. In the dry grains a small cavity, and occasionally a few delicate fissures. Length about 32 $\mu$ , width about 25 $\mu$ . Among them some doublets. In the young scales the starch-grains are about 16 $\mu$  long, and usually 2 to 3 times as long as thick, and homogeneous.
- Dentaria polyphylla* W. K. (*Brassicaceæ*.) *Dry scales of the root-stocks*.—Grains rounded-oval and blunt-triangular to oblong and elongated-conical; two-fifths to almost as broad as long, the broader ones slightly compressed; lamellæ distinct, only the innermost ones complete; usually at the broad end, instead of the hilum, a small cavity is found from which frequently a few short fissures radiate; eccentricity about one-fourth and one-seventh. Frequently the distal end is broader, but somewhat thinner, but at times thicker than the more dense hilum end; very often it is broadened by a lateral layer of lamellæ, the deposit forming a right or an obtuse angle with the axis of the grain. Length about 60 $\mu$ , width about 40 $\mu$ . Some grains approach the cuneiform and some the rod-shaped type (type 9).
- Nymphaea alba* Linn. (*Nymphaeaceæ*.) *Fresh root-stock and roots*.—Grains ovoid, oval and conical; twice as long as thick; hilum at the thick end, one-fourth eccentric. Length about 14 $\mu$ , thickness about 10 $\mu$ . Among these are compound grains of few, equal or unequal components. (See type 15.)
- Stellaria bulbosa* Wulfen. (*Caryophyllaceæ*.) *Dry tubers*.—Grains spherical, oval, or conical; 1 to 2 times as long as thick; at the thick end, instead of a hilum, a small cavity is found, one-fourth eccentric; the distal end narrowed, or as broad or somewhat broader than the hilum end, and compressed. Length about 10 $\mu$ , with compound grains of few usually equal components. (See type 14.)
- Althæa officinalis* Linn. (*Malvaceæ*.) *Dry roots*.—Grains rounded, oval, oblong, conical, frequently somewhat curved or reniform or rounded-triangular, frequently somewhat irregular; two-fifths to as broad as long; or the broader ones slightly compressed; no lamellæ; rarely only a cavity at the thicker end instead of the hilum, eccentricity about one-fourth; more often an irregular, longitudinal slit. Length about 21 $\mu$ , width 31 $\mu$ . Among them few doublets and triplets.
- Euphorbia dulcis* Jacq. (*Euphorbiaceæ*.) *Dry root-stocks*.—Grains rounded, rounded-triangular, oval, mostly conical, some over twice as long as thick, circular in transverse section; others twice as broad as long; compressed and squared at the distal end; eccentric; several radial fissures or a longitudinal slit present. Length about 13 $\mu$ . Among them are found some doublets and triplets. Starch plentiful.
- Geranium phœum* Linn., var. *lividum* L'Herit. (*Geraniaceæ*.) *Dry root-stocks*.—Grains rounded-triangular, oval, oblong, or conical, more or less irregular; two-fifths to almost as broad as long; the narrow ones circular in transverse section, the broad ones slightly compressed, thickened at the hilum end, the distal end somewhat broader but compressed; a small cavity is found from which a delicate longitudinal slit or radial fissures diverge; eccentricity about one-eighth. Length about 18 $\mu$ , width 13 $\mu$ .

- Geranium sylvaticum* Linn. (*Geraniaceæ*.) *Dry root-stocks and roots*.—Grains as in the preceding; most of them conical; many also triangular, about twice as broad as long; flattened at the distal margin; hilum end always thickened. Length about  $21\mu$ . Transition into wedge-shaped type (type 8).
- Oxalis acetosella* Linn. (*Oxalidaceæ*.) *Dry scales of the root-stocks*.—Grains rounded-triangular, oval, conical, oblong, frequently more or less irregular; 0.33 to 1.5 times as broad as long; the broader ones compressed to about half the width; hilum end usually thickened, sometimes both hilum and distal ends of equal thickness; a small cavity with delicate radial fissures or a longitudinal slit is present. Length about  $22\mu$ . Transition into the wedge-shaped type (type 8), and to the rod-shaped type (type 9). Some doublets and triplets.
- Orobus albus* Linn. (*Leguminosæ*.) *Dry thickened roots*.—Grains oval or conical, usually 1.5 times, rarely twice, as long as thick; at the thicker end instead of a hilum a small cavity is present, sometimes with single radial fissures; eccentricity about one-seventh. Length about  $16\mu$ . Among these are found compound grains, of few usually unequal components. (See type 15.)
- Apios tuberosa* Moench. (*Leguminosæ*.) *Fresh tubers*.—Grains elliptical, oblong, usually somewhat irregular; 1.33 to 2.5 times as long as thick, circular or slightly compressed in transverse section; lamellæ indistinct; hilum often visible, small, mostly at the thick end, one-third to one-seventh eccentric. Length about  $30\mu$ . Among them are found compound grains of 2 to 4 components.
- Encephalartos spiralis* Lehm. (*Cycadaceæ*.) *Dry embryo*.—Grains rounded-oval or conical, 1 to about 1.5 times as long as thick; with single lamellæ; at the thicker end, instead of the hilum, a small cavity is present, from which delicate, short fissures radiate; eccentricity about one-third and one-fourth. Length 30 to  $35\mu$ , width 25 to  $30\mu$ . Among these are found compound grains of few usually equal components. (See type 14.) All forms of transition into the simple type may be found among the separated grains.
- Viscum album* Linn. (*Loranthaceæ*.) *Fresh seed and embryo*.—Grains rounded to rounded-oval, usually without lamellæ; hilum often distinct, about one-third to one-fourth eccentric. Length about  $24\mu$ . Also compound grains of 2 to 4 components. The starch-grains in the embryo are like those in the seed, though smaller.
- Loranthus europæus* Linn. (*Loranthaceæ*.) *Dry seed and embryo*.—Grains rounded, oval-elliptical, or conical; frequently slightly curved or irregular; no lamellæ; hilum at the thicker end, one-third to one-fourth eccentric; instead of the hilum, a small cavity with several radial fissures may be present, rarely a longitudinal slit. Length about  $18\mu$ , width  $12\mu$ . There also are present numerous compound grains of 2 to 4 and more components. Starch plentiful in the seed; also in the embryo, though the grains are smaller.
- Psittacanthus vellozianus* Mart. (*Loranthaceæ*.) *Dry embryo*.—Grains oval, or conical, 1.5 times to almost twice as long as thick; rarely with distinct lamellæ, the outermost ones complete; instead of a hilum, a small cavity is frequently present, from which sometimes rather short, delicate fissures radiate; eccentricity about one-fourth; the hilum end usually thicker, frequently distinctly narrower, in which case the distal end also becomes somewhat narrower. Length 35 to  $40\mu$ , thickness about  $30\mu$ . The starch is a transition form to the inverted conical type (type 6) and the rod-shaped type (type 9).
- Carolinae princeps* Linn. (*Malvaceæ*.) *Dry cotyledons*.—Grains spherical, spherical-oval, or shortened-conical; lamellæ indistinct; at the thicker end, instead of the hilum, a small cavity is found; eccentricity about one-half to one-third. Length about  $25\mu$ . There are also present compound grains of few usually unequal components. (See type 15.)
- Sterculiaceæ*. *Dry seeds*.—These starch-grains greatly resemble those in the cotyledons of the *Leguminosæ* (*Phaseolus*, *Vicia*, etc.). They are oval, reniform, rounded-triangular, the broader ones slightly compressed, a fissure coinciding with the largest diameter is found in the flat grains, and on the circular ones a cylindrical canal. These at first on this account were classified by Nägeli among the centric-oval type (type 3). Triangular forms also occur with fissures radiating from an eccentric point, as well as conical ones in which this point is at the thickened end. It seemed to Nägeli therefore more likely that these starch-grains are of an eccentric structure and that they belonged partly to the conical type (type 7) and partly to the wedge-shaped type (type 8). Investigation upon the fresh grains Nägeli states must decide this point.

- Abroma angustum* Linn. fil. (*Sterculiaceæ*.) *Dry seed*.—Grains rounded-oval to oblong; sometimes curved, or reniform, or triangular, rarely conical, often irregular; two-fifths to as broad as long; the broader ones compressed; the narrower ones terete; no lamellæ; frequently from the narrow aspect a longitudinal slit is distinctly visible, and sometimes a small cavity with radial fissures is found at the thickened end; eccentricity one-third and one-fourth. Length  $30\mu$ , width  $20\mu$ . Compound grains of few components are present. Starch and oil present.
- Waltheria indica* Linn. (*Stereuliaceæ*.) *Dry seed*.—Grains rounded-oval to elongated-oval, frequently curved, reniform, or triangular, or even irregularly triangular or quadrangular, and a few tetrahedrons; two-fifths to almost as broad as long; the broader ones slightly compressed, and usually with a longitudinal slit, the narrower ones terete and with a channel-like furrow, also sometimes with several transverse or radial fissures; lamellæ rare, or very delicate. Length about 30 to  $35\mu$ , width  $25\mu$ . Some compound grains of few components are present.
- Melochia pyramidata* Linn. (*Sterculiaceæ*.) *Dry seed*.—Grains rounded to elongated-oval, sometimes reniform or rounded triangular; the broader ones compressed to about half their width; lamellæ delicate, or none; from the narrow aspect a longitudinal slit is usually very distinct, sometimes transverse fissures are also present. Length about 30 to  $36\mu$ , width about  $15\mu$ . Some doublets and triplets.
- Riedlea eorehorifolia* DC.; *Melochia corechorifolia* Linn. (*Sterculiaceæ*.) *Dry seed*.—Grains rounded-oval to oblong; frequently curved, reniform, or triangular, very often somewhat irregular; two-fifths to as broad as long, the broader ones slightly compressed; the smaller ones terete; no lamellæ; from the narrow aspect usually a clearly defined longitudinal slit, and occasionally many transverse fissures. Length about  $31\mu$ , width about  $19\mu$ .
- Hermannia althæifolia* Linn. (*Stereuliaceæ*.) *Dry seed*.—Grains rounded to oblong, sometimes conical or rounded-triangular, the longer ones one-third to one-half, the shorter ones half to as broad as long; the broader ones compressed to over half their width, the smaller ones not compressed; no lamellæ; with a longitudinal slit somewhat more marked from the narrow aspect. Length about  $20\mu$ , rarely  $28\mu$ .
- Hermannia nemorosa* Eckl. (*Stereuliaceæ*.) *Dry seed*.—Grains rounded to elongated-oval, at times somewhat conical or triangular; half to as broad as long; the broader ones slightly compressed; no lamellæ; a longitudinal slit is usually observed. Length about  $18\mu$ .
- Melhania didyma* Eckl., Zeyh. (*Sterculiaceæ*.) *Dry seed*.—Grains rounded-oval to elongated-oval, frequently curved, reniform, triangular, rarely conical, often somewhat irregular; two-thirds to as broad as long, the broader ones slightly compressed, the narrower ones terete; no lamellæ; from the narrow aspect usually a clearly defined longitudinal slit, and occasionally a single transverse fissure, are observed. Length about 30 to  $35\mu$ , width 20 to  $25\mu$ .
- Melhania erthroxyton* R. Br. (*Sterculiaceæ*.) Grains as in the preceding.
- Eriolena* species. (*Sterculiaceæ*.) *Dry seed*.—Grains rounded-oval, oval, rarely elongated-oval, many reniform or triangular, few conical, one-half or rarely two-fifths to as broad as long, the broader ones slightly compressed; lamellæ very delicate, or none; usually a longitudinal slit, and sometimes single transverse fissures are present; at the thickened end a small cavity from which radial fissures diverge; one-half to one-third eccentric. Length about  $33\mu$ , width about  $27\mu$ .
- Visenia tomentosa* R. P. (*Sterculiaceæ*.) *Dry seed*.—Grains rounded to oblong, some curved, or reniform, or triangular; one-half or rarely two-fifths to as broad as long; the broader ones slightly compressed; no lamellæ; usually with a longitudinal slit, sometimes with transverse fissures. Length about  $28\mu$ , width  $20\mu$ .
- Æsculus hippocastanum* Linn. (*Sapindaceæ*.) *Fresh and dry cotyledons*.—Grains usually conical, occasionally oval or triangular or angular; frequently irregular obtuse angular forms; half to as broad as long; lamellæ and hilum indistinct, or delicate; thickened at the hilum end, narrowed at the distal end, having a circular transverse section, or broadened and thinned and angular (the width may thus exceed the length of the grain); in dry grains there is present a small cavity and 1 to 4 radial fissures, especially in the direction of the long axis; eccentricity about one-seventh. Length  $29\mu$ , rarely  $36\mu$ . There are present a few semi-compound and compound grains, each with 2 to 4 components.

- Amyris sylvatica* Jacq. (*Burseraceæ*.) *Dry cotyledons*.—Grains rounded, oval, conical, two-thirds to almost as thick as long; no lamellæ; at the thick end, instead of the hilum, a small cavity with single fissures is observed; eccentricity one-third and one-fourth. Length about  $17\mu$ , width  $13\mu$ . There are also present compound grains of 2 to 4 (rarely more) equal or unequal components, and numerous separated grains (4 to 12) with 1 curved surface and 1 to 3 pressure facets; with a small cavity and radial fissures.
- Amyris* species. (*Burseraceæ*.) *Dry cotyledons*.—Grains rounded, oval, ellipsoid, shortened-conical, occasionally somewhat angular; half to as broad as long; the larger grains have a small cavity and single radial fissures. The cavity central, if one-half eccentric, then it is at the thicker end. Length about  $15\mu$ , width about  $12\mu$ . Among these are compound grains of 2 to 4 or more equal or unequal components with one curved surface and 1 to 3 pressure facets; a small central cavity and single radial fissures are present. Cells are entirely filled with starch; little or no oil.
- Peqanum harmala* Linn. (*Rutaceæ*.) *Dry unripe seeds*.—Grains rounded, oval, shortened-conical, frequently more or less irregular; no lamellæ; instead of the hilum, sometimes a small cavity, occasionally with short radial fissures, usually near the thick, rarely at the thinner end. Length about  $14\mu$ . Compound grains of 2 to 5 equal or unequal components. Starch plentiful in unripe seeds; ripe ones contain oil, but no starch.
- Memecylon capense* Eckl. (*Melastomaceæ*.) *Dry cotyledons*.—Grains conical, oval, frequently unsymmetrical, and oblique or curved; one-half to almost as thick as long; no lamellæ; a longitudinal slit with single transverse fissures; or a small cavity with radial fissures; the cavity is at the thick end, and eccentric about one-half to one-fourth. Length about 16 to  $20\mu$ , thickness about  $15\mu$ . There are also present compound grains of few usually equal components. (See type 14.)
- Memecylon amplexicaule* Roxb. (*Melastomaceæ*.) *Dry cotyledons*.—Grains as in the preceding, but less conical and more symmetrical. Also similar compound grains.
- Syzygium guineense* DC. (*Myrtaceæ*.) *Dry cotyledons*.—Grains oval-spherical to elongated-oblong, occasionally somewhat irregular; two-fifths to almost as thick as long, the broader ones slightly compressed; lamellæ numerous, delicate, sometimes not distinct, the innermost ones complete, the outer ones unilateral; instead of the hilum, there is a small cavity from which single fissures radiate, chiefly in the direction of the long radius; hilum end usually thickened, the distal end sometimes narrower and thinner, and sometimes wider, also either tapering or of equal thickness. Length about 34 to  $42\mu$ , thickness about  $25\mu$ .
- Carophyllus aromaticus* Linn. (*Myrtaceæ*.) *Dry cotyledons*.—Grains oblong or elongated-conical; usually two-fifths to three-fifths as broad as long; the broadest ones slightly compressed; lamellæ delicate; only the innermost ones complete; a small cavity is usually present instead of a hilum, and frequently a lengthwise slit with single oblique fissures; thickened at the hilum end, distal end narrowed and at the same time pointed or rounded, or rarely broadened and compressed into a knife-like edge, and besides often cut off obliquely; eccentricity about one-fifth to one-sixth. Length about  $36\mu$ .
- Jambosa vulgaris* DC. (*Myrtaceæ*.) *Dry cotyledons*.—Grains usually conical, and circular in transverse section; the longer ones about half as thick as long; the shorter ones thicker and sometimes rounded-triangular; usually without lamellæ, more rarely with few indistinct lamellæ, only the innermost ones being complete; instead of a hilum, a cavity is observed with a longitudinal slit, and sometimes with radial fissures; the cavity is at the thicker end, about one-sixth eccentric. Length about 30 to  $34\mu$ .
- Amphicarpa monoica* Nutt. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded-oval or conical; half to as broad as long; the broader ones sometimes slightly compressed; instead of a hilum, a small cavity with single, delicate, short fissures is usually observed; thickened at the hilum end; distal end tapering and conical, rarely broadened and squared. Length about  $44\mu$ . Semicompound grains of 2 to 5 part-grains and compound ones of few usually equal components.

## TYPE 8. GRAINS SIMPLE, ECCENTRIC, CUNEIFORM, OR FLATTENED.

Lamellæ coarsest and most numerous on one side, and finest and fewest on the diametrically opposite side. Grains usually broader at the distal end, at the anterior end narrowed and compressed; either equally thick throughout, or more frequently thicker at the anterior end, and at the distal

end flattened, angular, and squared. Hilum usually toward the narrow, thick end. As a rule, only a few of the innermost lamellæ are complete; all the outer ones appearing incomplete. On drying, the grain usually develops a cleft, coinciding with its greatest plane, which is visible from the narrow aspect. From the broad aspect several fissures are seen radiating from the hilum chiefly toward the distal margin; instead of the latter, one longitudinal or one transverse slit, or both combined, occasionally are observed. Pure types of the grain rarely occur, they are usually found mingled with others, mostly with the cone-shaped type (type 7), less frequently with the rod-shaped type (type 9), and the inverted cone-shaped type (type 6).

*Erythronium dens-canis* Linn. (*Liliacæ*.) *Dry bulb scales*.—Grains wedge-shaped from the broad aspect, rounded circumference, 0.6 to 1.5 times as broad as long; cone-shaped from the narrow aspect; hilum end is thickened, often somewhat protruding, the distal end is usually curved; lamellæ none, or very delicate; instead of the hilum a small cavity may be observed from which several delicate fissures emerge; eccentricity about one-ninth. Length about 34 $\mu$ .

*Tulipa gesneriana* Linn. (*Liliacæ*.) *Fresh bulb scales*.—Grains rounded, cuneiform, almost as broad as long; lamellæ and hilum about three-fifths as thick as broad, and are rarely distinct; eccentricity about one-ninth. Length about 30 $\mu$ , width about 27 $\mu$ . According to Raspail the size may reach 50 $\mu$ .

*Tulipa sylvestris* Linn. (*Liliacæ*.) *Fresh bulb scales*.—Grains rounded-triangular or oval-cuneiform, and oval from the narrow aspect, elongated-conical, usually one-half to two-thirds as broad as long; compressed to about one-fourth or more of their width, hilum end often somewhat protruding and thickened, lamellæ and hilum delicate or indistinct, eccentricity about one-tenth. Length about 50 $\mu$ . The smaller grains are usually oblong, and formed like little oval rods.

*Fritillaria meleagris* Linn. (*Liliacæ*.) *Scales of dry bulbs*.—Grains oval or rounded-triangular, occasionally somewhat protruding at the hilum end; the broader ones compressed to about one-half; the distal end of equal thickness or somewhat thicker than the hilum end; lamellæ delicate; occasionally a cavity, rarely with several short very delicate radial fissures, is found instead of the hilum; eccentricity about one-sixth. Length 21 $\mu$ .

*Lilium candidum* Linn. *Bulbs de Lis*. (*Liliacæ*.) *Bulbs*.—According to Payen (Ann. Sc. Nat., 1838, II, p. 17; pl. 4, fig. 5), the grains are oval, or oval-triangular; almost twice as long as broad; lamellæ delicate; hilum, which is sometimes double, at the narrow end; eccentricity about one-tenth. Length about 115 $\mu$ . The fully developed grains are irregular and often rough, with fissures radiating from the hilum.

*Lilium bulbiferum* Linn. (*Liliacæ*.) *Bulbs*.—According to Schleiden (Grundzuge, 3d ed., auf. 183, fig. 7), the grains are mussel-shell-shaped and broader than long; from the small aspect they are oval; lamellæ and hilum distinct; eccentricity one-fifth to one-sixth.

*Muscari botryoides* Mill. (*Liliacæ*.) *Scales of dry bulbs*.—Grains rounded, mostly with triangular, rarely rhomboid or irregular, outlines; 0.75 to 1.33 as broad as long; compressed, and from the narrow aspect, conical in form; thinner toward the distal end, or at times of equal thickness; lamellæ usually distinct, but delicate; sometimes a small cavity instead of a hilum; eccentricity about one-sixth; from the narrow aspect, a longitudinal slit is usually present. Length about 30 $\mu$ .

*Hyacinthus orientalis* Linn. (*Liliacæ*.) *Scales of fresh bulbs*.—Grains oval, cuneiform, triangular, more or less irregular, frequently with protruding angles; the distal end, which is thinned and squared, is as broad as, or broader, than the hilum end; lamellæ more or less distinct; hilum at the thicker end; eccentricity about one-fifth. Length about 45 $\mu$ . Some semi-compound (see type 11) and compound grains arranged in 1 to 2 rows (see type 13). Simple grains were almost exclusively found by Payen (Ann. Sc. Nat., 1858, II, p. 22).

*Scilla autumnalis* Linn. (*Liliacæ*.) *Scales of dry bulbs*.—Grains rounded, oval, and triangular; instead of the hilum a small cavity with single short fissures may frequently be observed at the thicker end; about one-fourth eccentric. Length about 24 $\mu$ . These are transition forms to the cone-shape (type 7).

*Dioscorea batatas* Desne. (*Dioscoreacæ*.) *Fresh tubers*.—Grains rounded, rounded pear-shaped, usually with 3 to 4 and 5 rounded angles, frequently somewhat irregular; 0.5 to 1.5 times as broad as long; compressed to about one-half to one-third of their width; from the narrow aspect

cone-shaped; thinner towards the distal end; lamellæ delicate or indistinct; hilum frequently invisible, approaching the thicker end; eccentricity one-sixth to one-eighth. In large tubers (20 cm. long by 2 cm. thick) the grains are larger and relatively more compressed, and have rather more distinct lamellæ. Length about  $75\mu$ , width  $45\mu$ , thickness about  $25\mu$ , average about  $55\mu$ . In small tubers (14 mm. in length, and of equal thickness) the starch-grains are smaller, relatively thicker, and with less distinct lamellæ. In the narrow (2.5 by 4 mm.) part of the root which bears these small tubers, grains are almost without lamellæ, more rounded, and their size is about 18 to  $24\mu$ .

*Dioscorea sativa* Linn. (*Dioscoreaceæ*.) *Root-stocks*.—According to Leon Soubeiran (Journ. Pharm., 1854, xxv, 181) the smallest grains are usually spherical or oval, the larger ones pear-shaped or elongated, the largest ones indistinctly triangular; lamellæ and hilum none. Length 40 to  $50\mu$ , diameter 10 to  $20\mu$ . According to Raspail, the size is up to  $60\mu$ .

*Dioscorea alata* Linn. (*Dioscoreaceæ*.) *Root-stocks*.—According to Payen (Verhandl. der Paris Academy, 1847, July 26), the grains are irregular, spherical, and without lamellæ; 2 to 13 adhering to each other.

*Galanthus nivalis* Linn. (*Amaryllidaceæ*.) *Fresh bulb scales*.—Grains almost circular and rounded-triangular to oval, frequently broader than long; thickened and narrowed at the hilum end; distal end broad and thinned; lamellæ rather distinct, the inner ones complete, the outer ones unilateral; hilum one-half to one-fourth eccentric, sometimes with a few short radial fissures. Length about  $28\mu$ . Among these are found some isolated, semi-compound grains. At the position of the hilum several part-grains are found lying beside one another, causing the proximal end to increase in width; also some compound grains with few, unequal components (see type 15), and some with 2 to 5 equal components, mostly arranged in one row.

*Leucoium vernum* Linn. (*Amaryllidaceæ*.) *Fresh scales of the bulbs*.—Grains rounded, triangular with rounded angles, usually broader than long to twice as broad, rarely somewhat longer than broad; strongly compressed; hilum almost in the middle of the thicker margin, from which point a large mammary process projects; the distal margin is less thick and squared. Lamellæ delicate, often indistinct; eccentricity one-third to one-ninth. Length about 25 to  $30\mu$ , width 40 to  $50\mu$ .

*Phaius grandiflorus* Lour.; *Bletia tankervilleæ* R. Br. (*Orchidaceæ*.) *Pseudo-tubers*.—According to Leon Soubeiran (Journ. Pharm., 1854, xxv, 181), grains generally oval or triangular, some much elongated, others with rugged protuberances; lamellæ distinct; no hilum. Width 40 to  $50\mu$ , length 100 to  $200\mu$ . Judging from the outline sketches, the grains must be much flattened. According to Schleiden (Grundzuge, 3, auf 1, p. 183, fig. 8), the grains are oval or rounded-conical, almost twice as long as broad; lamellæ distinct, frequently with an outer lateral group of lamellæ; hilum at the narrow end, one-sixth to one-tenth eccentric.

*Zingiber officinale* Rase. (*Zingiberaceæ*.) *Dry root-stocks*.—Grains rounded-cuneiform, oval, 4 and 5 angular, often somewhat irregular; half to as broad as long, the narrower ones compressed to about one-half or one-third, and the broader ones to about one-fourth or one-fifth of their width. The hilum end narrowed, triangular, or with a protuberance, which is sometimes turned laterally; lamellæ invisible; hilum frequently indistinct; instead of the hilum, there is often a small cavity with 1 or 2 very short fissures; eccentricity one-eighth to one-eleventh. Length about  $45\mu$ , width about 30 to  $40\mu$ , thickness 7 to  $9\mu$ .

*Curcuma zedoaria* Salisb. (*Zingiberaceæ*.) *Dry tubers*.—Grains oval, elongated, more or less triangular, occasionally irregular, two-fifths to two-thirds as broad as long; compressed to about one-half or one-third of their width (one-third to one-sixth as thick as long); from the narrow aspect of equal thickness throughout the entire length, with rounded ends; from the broad aspect the distal end is narrowed-triangular or protruding, at times turned laterally, frequently with a pointed wart-like protuberance; lamellæ numerous, delicate, and incomplete; instead of the hilum a small cavity is rarely present; 0.04 and 0.03 eccentric. Length about  $70\mu$ , thickness about  $12\mu$ .

*Curcuma leucorrhiza* Roxb. (*Tikhur-flour, partly East India arrowroot; Travancora starch.*) (*Zingiberaceæ*.) *Root-stocks*.—According to Walpers (Bot. Zeit., 1851, 337), Schleiden (Grundzuge, 3d ed., I, p. 185, fig. 11), Berg (Pharmacognosie, 481), and Leon Soubeiran (Journ. Pharm., 1854, xxv, 178), the grains are oval, elliptical, ovoid, elongated-oval, or almost spatulate or elongated-triangular; towards the base suddenly narrowed, short-pointed, or drawn out

into a rather elongated blunt point; strongly compressed to about one-fourth of their width; lamellæ none, or numerous and delicate; occasionally a small transverse fissure in the hilum; hilum about one-seventeenth eccentric. The grains are as large as, and occasionally larger than, those of potato starch. According to Soubeiran, width  $20\mu$ , length 60 to  $70\mu$ , and thickness  $10\mu$ .

Starch-grains of Tikhur-Mehl flour are about  $72\mu$  long and  $38\mu$  broad, thus they are 1 to 3 times as long as broad; 6 to  $8\mu$  thick; sometimes from the broad aspect they are oblique or irregular, and broadened toward the distal rounded end; while at the proximal end they are usually narrow and triangular; this end may occasionally be either almost blunt or the angle may protrude; even the place where the hilum is located may occasionally be more prominent and wart-shaped; in the long, narrow aspect the grains are rod-shaped, and of either almost equal thickness throughout or slightly thicker in the middle, with rounded or blunt ends; the hilum is rarely indicated by a small cavity about one twenty-fourth eccentric; lamellæ delicate and numerous.

*Curcuma longa* Linn. (*Zingiberacæ.*)—According to Munter, grains as in preceding.

*Curcuma angustifolia* Roxb. (*Zingiberacæ.*) *Root-stocks.*—According to Leon Soubeiran (*Journ. Pharm.*, 1854, xxv, 178), the grains are triangular, with rather blunt, not flattened, angles; lamellæ and hilum visible, but indistinct, grains very unequal in size, from 5 to  $30\mu$ , small ones numerous, many split and torn. Soubeiran concludes from this that Travancora starch can not be derived from *Curcuma angustifolia*.

*Hedychium flavescens* Carey. (*Zingiberacæ.*) *Root-stocks.*—According to Fritzsche (*Poggendorff's Annal.*, 1834, xxxii, 142, taf. II, 40-49), the grains are oval to lanceolate, narrow at the hilum end, or drawn out to a point; one-third to one-half as long as broad; very strongly compressed; lamellæ numerous, delicate; hilum invisible. Also semi-compound grains are present, with 2 to 4 part-grains, which at the hilum end are completely separated by fissures, but at the distal end are surrounded with the common lamellæ of the grain.

*Maranta arundinacea* Linn. (*Jamaica West Indian arrowroot, partly East Indian arrowroot; Maranta starch.*) (*Marantacæ.*) *Dry stolon.*—Grains rounded to oval, rarely oblong, usually more or less irregular; often triangular; two-fifths to twice as broad as long; the oblong and oval grains are about circular in transverse section; many are pressed in until they are about one-half as thick as broad; thicker at the hilum end; from the broad aspect the distal end is either narrower or broader than the hilum end (to about twice as broad as the length of the entire grain), the distal end is always thin; lamellæ delicate; instead of the hilum a small cavity is frequently found, either with a rather delicate, short, transverse fissure, or with 3 to 4 short radial fissures, or rarely with a longitudinal slit; eccentricity one-half to one-sixth. Length 40 to  $50\mu$ , width about  $40\mu$ . According to Soubeiran, the length is 60 to  $70\mu$ . Occasionally a smaller part-grain rests upon a grain, as if the grain had been cut off at one corner.

*Jamaica arrowroot* sp.—Related to the foregoing starch is a specimen which was sent to Nägeli from England as a variety of potato starch, but which, however, was found to be a kind of Jamaica arrowroot. The grains are oval, elongated-conical, triangular to quadrangular, usually more or less irregular; two-fifths to twice as long as broad; the hilum end thickened, the distal end broadened; compressed, and almost obliquely angular; lamellæ quite distinct; delicate, the outer ones frequently forming a special lateral group; the grain is usually solid, instead of the hilum there is occasionally observed either a short longitudinal or transverse slit or very short radial fissures, which form a triangle, or an oblique-angled cross or more rarely a right-angled cross; eccentricity about one-fifth. Length about  $50\mu$ . This starch in some respects acts like potato starch, or as a variety derived from it, swelling up and passing into solution when cooked, dissolving if subjected to moist heat, and soluble when treated with saliva. Maranta starch is easily distinguished, however, from potato starch, with which it is often adulterated, by means of the smaller, often obliquely triangular grains (scarcely two-thirds as large as potato starch-grains); by the many wedge-shaped, compressed forms with thickened hilum end and thinned angular distal margin (in the potato starch the hilum is usually found at the thinner end); by the more delicate, less distinct, narrower lamellæ and the short slits or radial fissures which develop when the grain is dry (of which, as a rule, the potato starch shows no indication).

- Canna*. (*Cannacæ*.) *The root-stock*.—The starch-grains are distinguished by the marked eccentricity of the hilum, by the unilateral, incomplete, usually very distinct, coarse lamellæ, and by their more or less mussel-like form, which is broadened and flattened, and at the hilum end somewhat protruding. In different parts of the same root-stock, and even in the same tissue, the grains present great variations in shape. In the following notes Nägeli communicates the results of his researches upon a series of species of *Canna* without regard to the question whether the variations are of specific or of merely individual value.
- Canna pedunculata* Sims. *Fresh root-stock*.—Grains oval to transverse-oval; at the hilum end usually narrowed (rounded cuneiform) or even slightly protruding; sometimes with lobate projections; half to twice as broad as long; the broader ones compressed to about one-third of their width; lamellæ indistinct; hilum often indistinct, and found either in the protruding blunt end, or in a small projecting wart-like structure; neither fissures nor cavity are observed in the dry grain. Length about  $60\mu$ . There are also compound grains with components arranged in a single row. (See type 13.)
- Canna picta* Hort. *Fresh root-stock*.—Grains oval to transverse-oval, triangular, usually protruding at the hilum end; compressed to about one-half to one-third of their width, lamellæ are numerous and distinct. Length about  $112\mu$ . In many grains there is an internal system of lamellæ, the longitudinal axis of which deviates from that of the outer system of lamellæ, mostly by about  $90^\circ$  or somewhat less; occasionally about 2 to 3 small part-grains lie beside one another at the hilum end.
- Canna linkii* Bouché. *Fresh root-stock*.—Grains rounded, or transverse-oval, once to almost twice as broad as long; compressed to one-half, rarely one-fourth, of their width; lamellæ numerous and distinct, the lateral ones incomplete; hilum in the slightly elongated, blunt hilum end; eccentricity one-sixth to one-tenth. Length about  $70\mu$ , thickness 15 to  $25\mu$ . Also a small number of semi-compound grains are present, which, at the hilum end, have 2 to 3 hila lying beside one another; and also scattered compound grains consisting of a few components.
- Canna vitatta* Hort. *Fresh root-stock*.—Grains similar to those of *Canna linkii*; usually rounded or transverse-oval, 1 to 1.5 times as broad as long; distal margin usually rounded; proximal margin rather straight, on both sides of which are two blunt often somewhat prominent angles; in the center is a wart-like protuberance in which the hilum is found. Length about  $75\mu$ .
- Canna altensteinii* Bouché. *Fresh root-stock*.—Grains similar to those of *Canna linkii*; 0.66 to 1.5 times as broad as long; lamellæ nearly always indistinct; upon drying often a short, somewhat curved, transverse slit appears in the hilum. Length about  $70\mu$ .
- Canna variegata* Hort. *Fresh root-stock*.—Grains as in *Canna linkii*; at the hilum end mostly slightly projecting; 0.66 to 1.5 times as broad as long; lamellæ numerous, quite distinct. Length  $70\mu$ .
- Canna floribunda* Hort. *Fresh root-stock*.—Grains similar to the preceding; mostly rounded. Length about  $95\mu$ .
- Canna albiflora* Hort. *Fresh root-stock*.—Grains similar to those of *Canna variegata*; mostly more or less rounded; somewhat narrowed and projecting at the proximal blunt end; 0.33 to 1.5 times as broad as long; compressed to about one-third of the width.
- Canna ramosa* Hort. *Fresh root-stock*.—Grains as in the preceding species; frequently somewhat irregular; 0.66 to 1.5 times as broad as long; eccentricity about one twenty-second. Length about  $105\mu$ . Many grains with an inner system of lamellæ, the longitudinal axis of which deviates about  $90^\circ$  from that of the external system. There are also several semi-compound grains with 2 to 3 small part-grains lying next to each other at the hilum end; and also some compound grains.
- Canna elegans* Hort. *Fresh root-stock*.—Grains triangular with blunt or rounded corners, sometimes mussel-shell-shaped, with protruding hilum end; 0.75 to 1.5 times as broad as long; compressed to one-third of their width; lamellæ few; hilum one-sixth and one-tenth eccentric. Length about  $40\mu$ . Some semi-compound grains are present with 2 to 4 part-grains lying next to each other at the hilum end; and also compound grains consisting of 2 to 5 almost equal components.
- Canna spectabilis* Hort. *Fresh root-stock*.—Grains rounded, oval, sometimes rounded cuneiform; more or less protruding at the hilum end; three-fifths to just as broad as long; compressed to about one-third, occasionally to one-fourth of their width; lamellæ numerous, distinct,

- incomplete; hilum in the protruding blunt end, one-eighth to one-thirteenth eccentric; no fissures in the dry grains. Length about  $120\mu$ , width 20 to  $30\mu$ . Some grains with projections on the surface; others with 2 groups of lamellæ, the longitudinal axis of which changes from  $0^\circ$  to  $180^\circ$ . Semi-compound grains are present with 2 to 3 hila lying next to each other at the hilum end; also some compound grains.
- Canna limbata* Rose. *Fresh root-stock*.—Grains rounded, oval, oblong, and conical; frequently irregular obliquely angular; half to as broad as long; the broad ones compressed to about one-half their width; lamellæ and hilum frequently indistinct. Length about  $95\mu$ . Some grains with an inner group of lamellæ. Some semi-compound grains with 2 or more hila lying behind one another; and also compound grains are present.
- Canna lanuginosa* Bosc. *Fresh root-stocks*.—Grains oval; very seldom elliptical, or 3, 4, or 5 angular, with blunt angles; one-half to almost as broad as long; slightly compressed; lamellæ numerous, complete; hilum in narrow, often wart-like protruding upper end; eccentricity one-thirtieth to one-fortieth. Length about  $100\mu$ . Compound and semi-compound grains are present. The latter with 2 to 3 small components at the proximal end, rarely in the center (in this case the grains are small, about 4 times as long and almost as thick as broad).
- Canna coccinea* Ait. *Fresh root-stock*.—Grains similar to preceding; one-half to fully as broad as long; the broader portion compressed to about half the width; the smaller are terete; no fissures after desiccation. Length  $70\mu$ . Several semi-compound grains with 2 to 3 hila lying beside one another are present; and also compound grains with components arranged in one row (see type 13). The African arrowroot (of *Canna coccinea*, according to Berg, Pharmacognosie, 483) grains are sheath-shaped, ovoid, or elongated, provided with distinct lamellæ, and hila located below one another. Frequently two grains grow together, which often reach twice the length of the potato starch-grain.
- Canna laguncensis* Lindl. *Fresh root-stock*.—Grains oval, rounded, oblong, more or less pear-shaped or almost violin-shaped, chiefly irregular, and often somewhat curved or oblique; mostly about one-half as broad as long; the smaller ones terete, the broader ones compressed to about one-half or more of their width; lamellæ very numerous, incomplete; hilum frequently indistinct, located at the narrow, sometimes projecting, wart-like end, about one-fortieth to one-seventh eccentric. In many grains an inner lanceolate or spindle-shaped ladder-like group of lamellæ extend from the hilum towards the distal end, to more than one-half to two-thirds the length of the grain. In the symmetrical grains it has a median and in the oblique and curved grains a lateral position. Semi-compound grains with two or more part-grains lying in a row are present; in addition there are compound grains with part-grains in one row (see type 13), and also some which consist of one large and one or two small components.
- Canna indica* var. *aureo-vittata* Hort. *Fresh root-stocks*.—Grains oval or oblong; rarely triangular or spindle-shaped; one-third to two-thirds as broad as long; the broadest ones compressed to half their width; lamellæ distinct, almost all of them incomplete; hilum end narrowed, or broad and rounded; hilum about one-twelfth, rarely one-twentieth, eccentric; occasionally a few short fissures radiate from the hilum. Length about  $130\mu$ . Semi-compound and compound grains, both consisting of 2 to 3 components, are rarely present.
- Canna cubensis* Hort. *Fresh root-stock*.—Grains as above, sometimes the proximal (hilum) end protruding and blunt. Length about  $80\mu$ . There are some grains which have a special internal system of lamellæ.
- Canna heliconiæfolia* Hort. berol.—Grains distinguished for their very irregular shape; strongly compressed; with lamellæ. Length  $65\mu$ . Many semi-compound grains are present having a row of components along one margin.
- Canna edulis* Ker.—According to Fritsche (Poggendorf's Annal., 1834, xxxii, 141; Taf. ii, 37-39), the grains are much flattened, almost as broad as long; lamellæ distinct; several semi-compound grains with 2 or more components. According to Leon Soubeiran (Journ. Phar., 1854, xxv, 179), some of the grains are very small, spherical or oval; others pear-shaped, circular, rounded or imperfectly triangular; the larger ones strongly compressed; lamellæ and hilum distinct. Size 40 to 80 or  $90\mu$ . According to the drawings, the grains are twice as long as broad, and have numerous tubercular prominences and depressions on their margins. The hilum is very eccentric, and is found at the narrow end.

- Canna glauca* Linn.—According to Berg (Pharmacognosie, 481) the grains are very irregular; usually disk-shaped, or unequally curved on both surfaces; ovoid, quadrangular, cuneiform, crescent-shaped, or reniform. They are as large as grains of the potato starch; the hilum lies at a point which in the reniform grains is emarginate, in the quadrangular grains often in the middle, and surrounded by numerous concentric lamellæ.
- Canna gigantea* Desf.—According to Payen (Ann. Sc. Nat., 1838, II, 16), the grains are pear-, flask-, and retort-shaped, and attain a length of  $175\mu$ ; compressed to three-fourths or two-thirds of their width; hilum at the narrow end; lamellæ delicate. (See also p. 229.)
- Canna discolor* Lindl.—According to Payen (Ann. Sc. Nat., 1838, 16), the grains are rounded-shield-shape, more or less elongated-conical; hilum at the narrow, often somewhat protruding, end, or sometimes between two more or less marked projections; lamellæ numerous. Length about  $150\mu$ .
- Hydrophyllum virginicum*. (*Hydrophyllaceæ*.) *Dry root-stocks and roots*.—Grains rounded-oval to elongated-oval (elliptical), more or less triangular; half as broad as long; two-ninths to two-fifths as thick as long, hilum end narrow and thick; distal end broad, thin, and squared; lamellæ none, or indistinct; instead of a hilum, a small cavity is usually present; eccentricity of hilum one-sixth. Length  $28\mu$ , width  $20\mu$ . Among these grains are some compound forms consisting of 2 to 5 equal or unequal components.
- Scheeria mexicana* Seem. (*Gesneraceæ*.) *Fresh scales of the root-stock*.—Grains oval, broadly-conical, rounded-triangular, rarely oblong; half to as broad as long; the broader ones compressed to about half their width; from the narrow aspect they are conical; lamellæ usually distinct; the hilum end is narrow and thick; the distal end is broad and squared; eccentricity of hilum about one-ninth. Length about  $40\mu$ , width about  $33\mu$ .
- Seiadocalyx warszewiczii* Regel. (*Gesneraceæ*.) *Fresh scales of the root-stocks*.—Grains rounded, rounded-triangular, or oval; 0.6 to 1.25 times as broad as long; the broader ones compressed to about one-third of their width; from the narrow aspect they are oblong, with both ends equal; lamellæ distinct. Length about  $50\mu$ , width about  $38\mu$ . Isolated semi-compound grains with several hila, and occasionally doublets, are also present.
- Achimenes hirsuta* DC.; *Locheria hirsuta* Regel. (*Gesneraceæ*.) *Fresh scales of the root-stock*.—Grains oval, elongated-triangular, conical; one-half to three-fourths as broad as long; the broader ones compressed; the narrower ones terete; from the narrow aspect both ends are either alike or the hilum end may taper; lamellæ delicate. Length about  $50\mu$ . Transitional forms to the rod-shape (type 9).
- Isoloma vestitum* Benth. (*Gesneraceæ*.) *Fresh scales of the root-stocks*.—Grains oval, broadly-conical, 3 to 4 angles with rounded corners; frequently somewhat irregular; 0.66 to 1.5 times as broad as long; the broader ones compressed to about one-third or more of their width; lamellæ usually indistinct. Size about  $28\mu$ . Some isolated doublets are also present.
- Tudæa regclii* Heer. (*Gesneraceæ*.) *Fresh scales of the root-stocks*.—Grains oval, usually rounded-triangular or mussel-shell-shaped; 0.66 to 1.5 times as broad as long; compressed to about one-half or more of their width; from the narrow aspect the proximal and distal ends of almost equal thickness; lamellæ usually distinct; hilum about one-seventh eccentric. Among them are found semi-compound or wholly compound grains, consisting of 2 to 3 equal or unequal components.
- Tudæa picta* Desne. (*Gesneraceæ*.) *Fresh scales of the root-stocks*.—Grains rounded, oval, triangular, often somewhat irregular and angular; the distal end usually broad, occasionally somewhat oblique; half to as broad as long; the broader ones compressed to about half their width; from the small longitudinal aspect cone-shaped, with thickened hilum end; lamellæ numerous, delicate, only the innermost ones complete; hilum about one-fifth to one-ninth eccentric; occasionally several short fissures radiate from the hilum. Length about  $50\mu$ , width  $35\mu$ . Some semi-compound grains with two hila are also present.
- Trevirania longiflora* Regel. (*Gesneraceæ*.) *Fresh scales of the root-stocks*.—Grains oval, conical, rounded, 3 and 4 angles; half to as broad as long; the broader ones compressed to one-half, rarely one-third, of their width, the narrower ones not at all or slightly compressed; lamellæ indistinct; hilum also often indistinct, located at the thicker end (in the broad, compressed grains both ends are frequently of almost equal thickness). Length about  $48\mu$ , width  $35\mu$ . Transitional forms to the cone-shape (type 7).

- Saxifraga granulata* Linn. (*Saxifragaceæ*.) *Dry scales of bulbils*.—Grains rounded, rounded-triangular to oval, with narrow proximal and broad distal ends; 0.6 to 1.33 as broad as long; the broad ones compressed to half their width; from the narrow aspect mostly cone-shaped with thickened hilum end; frequently with many distinct lamellæ; instead of the hilum there is a small cavity from which in the broad aspect several radial fissures proceed chiefly towards the distal end; in the narrow aspect a longitudinal slit is found. Length about  $28\mu$ , width about  $25\mu$ .
- Ranunculus aconitifolius* Linn. (*Ranunculaceæ*.) *Dry roots*.—Grains rounded, rounded-triangular, mostly rounded or reniform, usually twice as long as broad; compressed to about half the short, flattened diameter; one side (in the kidney-shaped grains the concave) thickened, the opposite one thinned, knife-like; hilum approaching the thickened margin; often in place of the hilum there is a small cavity, from which in the broad aspect proceeds several radial fissures or sometimes one transverse fissure (parallel to the proximal margin); in the small aspect there is often a distinct longitudinal slit; eccentricity of hilum about one-fourth to one-fifth. Length about  $42\mu$ . Some roots are poor, others rich in starch.
- Ficaria ranunculoides* Moench.; *Ranunculus ficaria* Linn. (*Ranunculaceæ*.) *Fresh and dry thickened roots*.—Grains rounded, rounded-triangular, and broadly-conical, and occasionally somewhat irregular; at the proximal end usually narrowed, and at the distal end broadened; almost as long as broad; one-half to two-thirds as thick as broad; from the narrow aspect of equal thickness throughout or tapering towards the proximal end; lamellæ delicate or invisible; hilum one-fourth to one-sixth eccentric, indistinct in fresh grains, usually indicated by a small cavity in dry grains. Length about  $33\mu$ , width about  $30\mu$ . Some semi-compound grains (see type 11); also a few doublets and triplets.
- Oxalis pentaphylla* Sims. (*Oxalidaceæ*.) *Dry scales of the small bulbs on the root-stock*.—Grains triangular with blunt angles, mussel-shell-shaped, with 4, 5, or 6 angles, rarely oval; two-thirds to almost as broad as long; lamellæ usually visible, but very delicate, only the innermost ones distinct; hilum often indistinct, one-ninth to one-twelfth eccentric. Length about  $60\mu$ , width about  $58\mu$ .
- Oxalis lasiandra* Zucc. (*Oxalidaceæ*.) *Dry scales of the small bulb*.—Grains rounded or rounded-oval, frequently 3, 4, or 5 angles; sometimes rather irregular; 0.66 to 1.66 as broad as long; compressed to about one-half or more of their breadth; from the broad aspect the hilum end projects into an oblique angle, or into a wart-like outgrowth; from the narrow longitudinal aspect both ends alike, or the proximal end thicker than the distal end; lamellæ delicate or indistinct; frequently instead of the one-eighth eccentric hilum a small cavity is observed with two short, delicate fissures, which usually resemble a curved cross-fissure. Size about  $28\mu$ .
- Oxalis crenata* Jacq. (*Oxalidaceæ*.) *Bulbs*.—According to Payen (*Ann. Sc. Nat.*, 1838, II, 17, pl. 6, fig. 3), the grains are cylindrical or somewhat conical in shape; over twice as long as broad; lamellæ distinct; hilum about one-third eccentric. Size about  $100\mu$ . A few semi-compound grains with double hila, and isolated doublets, are present. From the description and drawings this type can not be distinguished with certainty. Probably the compressed grains belong here (otherwise they hold a medium position between the inverted-cone-shaped and rod-shaped types).
- Rhizophora mangle* Linn. (*Rhizophoraceæ*.) *Dry radicle*.—Grains oval, conical, triangular, quadrangular, or of irregular form; half to as broad as long; the broader ones compressed to one-half or over; in the broad aspect, the proximal end is occasionally narrowed, the distal end broadened; on the narrow aspect, often conical with thickened proximal end and tapering distal end; in the narrow aspect a longitudinal slit is frequently present; sometimes there is a small cavity instead of the hilum, about one-fourth eccentric. Length to 24 to  $28\mu$ , width to  $20\mu$ . The structure of most grains is uncertain. Some belong here on account of their cuneiform appearance; others seem to approach the conical type (type 7).
- Globba marantina* Linn. (*Zingiberaceæ*.) *Dry seed*.—Grains rounded-oval to oblong, narrowed chiefly towards one end which projects in a small papilla, frequently unsymmetrical or arched, besides many irregular and angular forms, mostly 3, 4, and 5 angles; 0.33 to 1.33 as broad as long; the broader ones compressed to about one-third and one-fifth their width (to 4 and 5 times as long as thick); from the narrow aspect, the grains are elongated conical; without

lamellæ, hilum, and fissures. Length to  $30\mu$ , width to  $25\mu$ , thickness to 7 and  $8\mu$ . The structure of these grains is doubtful. No hilum was plainly discovered after the grain had been roasted to a yellowish color, boiled in water, and treated with sulphuric acid. Several times, however, Nägeli thought he detected a small cavity near the narrow end. He states that if the grains belong here, the hilum must have a very eccentric position.

*Canna gigantea* Desf.; *Canna indica*, Linn. (*Cannaceæ*.) *Dry seed*.—Grains scale-like, rounded-oval to elongated, usually more or less irregular, often with crenate margin; one-third to as broad as long; very strongly compressed; usually homogeneous, without lamellæ, cavity, or fissures, but occasionally with one or more refractive bands in the median line. Length about 18 to  $21\mu$ , thickness 1.5 to  $2\mu$ . The fresh grains of *Canna indica* are like those just described, somewhat undulating at the margins, entirely homogeneous. Length about  $21\mu$ , thickness 3 to  $4\mu$ . Nothing can be seen of a hilum in the fresh or dried starch-grains. After slight roasting, a small rounded cavity or gas-bubble very near one end is occasionally observed. This is probably the position of the hilum, the eccentricity of which is one-sixth and over. Nevertheless this description is not wholly safe, since sometimes in grains more thoroughly roasted two, three, or four such hollow spaces may be observed. (See also p. 227.)

*Maranta ramosissima* Wall. (*Marantaceæ*.) *Dry seed*.—Grains rounded-oval to oblong, frequently somewhat broader towards one end, mostly more or less irregular and tuberculated; two-thirds to almost as broad as long; the broader ones compressed to about one-third of their width (about 3 or 4 times as long as thick), from the narrow aspect frequently with a delicate longitudinal slit; almost homogeneous. Length about  $16\mu$ , breadth about  $10\mu$ , thickness 3 to  $4\mu$ . Nägeli states that he places these grains here, in which neither lamellæ nor hilum are visible, on account of their analogy with the canna. Among these are found a few distinctly compound grains with numerous coalesced components, similar to those of *Maranta* sp. (see type 12). It is uncertain whether the indented forms are not accidentally compound; from the narrow longitudinal aspect many of these same forms are also slightly torulose.

#### TYPE 9. GRAINS SIMPLE, ECCENTRIC, ROD-SHAPED.

Lamellæ coarser and more numerous on one side, finer and less numerous on the diametrically opposite one. Grains mostly elongated, terete, or somewhat compressed; at both ends of almost equal breadth and thickness. On drying, fissures are observed radiating from the hilum, chiefly in the direction of the longitudinal axis. In the terete grains two longitudinal slits are noticeable, sometimes crossing each other at right angles; in those which are compressed there is a slit which coincides with the greatest plane. There are many transition forms to the conical (type 7), and to the inverted-conical (type 6).

*Angiopteris*. (*Marattiaceæ*.) *Stems of leaves (fronds)*.—According to Harting (Rech. sur. Panat. l'Organogenis et l'Histiogenia du genre Angiopteris, plate VII, 8, 9), the grains are oval to cylindrical, the longer ones 4 times as long as broad; distinct, incomplete, lamellæ; hilum very eccentric; the hilum end occasionally narrowed, but more frequently somewhat broader than the distal end. Length about  $70\mu$ .

*Hemrocallis fulva* Linn. (*Liliaceæ*.) *Fresh root-stocks*.—Grains usually elongated-oval, 1.5 to 2.5 times as long as broad; the hilum end somewhat smaller, and almost circular in transverse section, the distal end rather broader and compressed; no lamellæ; hilum about one-sixth to one-eighth eccentric. Length about  $25\mu$ . These forms resemble the cuneiform type.

*Tamus communis* Linn. (*Dioscoreaceæ*.) *Dry root-stock*.—Grains of very varied and very irregular shape, rounded to elongated-conical, and rod-shape; about 6 times as long as thick; the broader ones somewhat compressed; lamellæ usually distinct, delicate, and numerous; hilum often invisible, can sometimes be recognized by means of a small cavity; hilum end frequently thickened; the distal end of equal width or even (in the oval and rounded-triangular grains) broadened and squared. Length about  $52\mu$ . This type is not well pronounced. There are many transitional forms to the cuneiform type (type 8). Among these are grains with an internal ladder-like system of coarse lamellæ. Furthermore, there are semi-compound grains with two inner large components, and compound grains of two to four components. Moreover, the grains in the course of solution exhibit very diverse and peculiar forms.

- Vallisneria spiralis* Linn. (*Hydrocharitaceæ*.) *Dry root-stocks and runners*.—Grains oval, elongated, conical, more rarely triangular with rounded corners, or pear-shaped; mostly 1.5 to twice as long as thick, lamellæ frequently distinct, all incomplete; instead of the hilum a small cavity with short radial fissures is observed; hilum end rarely thinner, sometimes of equal thickness, usually thicker than the distal end; the latter is sometimes broadened, thinned and squared; eccentricity about one-seventh. Length about 35 $\mu$ .
- Iris sambucina* Linn. (*Iridaceæ*.) *Fresh root-stock*.—Grains are elongated or oval, frequently 3 to 4 angles, with protruding rounded angles; a little longer than 3 times as long as broad; slightly compressed; one-half to two-thirds as thick as broad; lamellæ delicate, rarely visible; sometimes several (2 to 5) radiating fissures emerging from the hilum. Length about 27 $\mu$ , width about 16 $\mu$ . The type of these grains, like those of the other species of *Iris*, is not clearly defined. Transition forms to the other eccentric types are numerous.
- Iris florentina* Linn. (*Iridaceæ*.) *Dry root-stock*.—Grains spherically-oval, oval, rarely oblong; often narrowed at one end; circular in transverse section; a little longer than twice as long as thick; frequently several (3 to 5) distinct lamellæ on the side of the long radius; frequently instead of the hilum a very small cavity is observed, from which a few radial fissures rarely emerge; about one-fourth to one-sixth eccentric. Length about 25 $\mu$ , thickness about 19 $\mu$ . Transitional forms to the other eccentric types. Several doublets are present.
- Iris pallida* Lam. (*Iridaceæ*.) *Fresh root-stock*.—Grains spherical, rounded-triangular, oval, or conical, usually slightly compressed in transverse section; a little longer to scarcely twice as long as broad; lamellæ delicate and rarely visible; usually instead of the hilum a small cavity with 2 to 5 radiating fissures is observed. Length about 19 $\mu$ , width about 13 $\mu$ . Transitional forms to the other eccentric types. Several doublets are present.
- Himantoglossum hircinum* Rich. (*Orchidaceæ*.) *Dry bulbs*.—Grains oval, rod-shaped, conical, rarely rounded or rounded-triangular; most of them circular in transverse section; the broadest ones slightly compressed (about half as thick as long); lamellæ rather numerous, distinct and incomplete; sometimes instead of the hilum a small cavity is observed from which several very short and delicate fissures radiate; about one-eighth eccentric; both ends usually of equal thickness, occasionally the hilum end is thicker or thinner; the distal end often shortened, and if broader than the proximal end, it is slightly compressed. Length about 35 $\mu$ . Some grains have an almost central hilum; others are semi-compound, and with two components. Schacht (*Microseon*, 2 Aufl., pag. 48, fig. 2, *f* and *g*) shows drawings of two starch-grains from the bulbs of *Himantoglossum*, unlike any that Nägeli saw; most likely, Nägeli states, they are in process of solution.
- Hedychium gardnerianum* Wall. (*Zingiberaceæ*.) *Root-stocks*.—According to the description and drawings of Bischoff (*Bot. Zeit.*, 1844, 388) the grains are 2 to 4 times as long as broad; often distorted or sharply indented; both ends alike or thickened at one end, either claviform or capitate; plainly striated or repeatedly constricted. Length about 50 $\mu$ . All the grains that are illustrated are probably about to pass into solution. This view is supported by the illustrations, which Fritzehe (*Poggendorff's Annal.*, xxxii, Taf. ii, figs. 50 to 53) gives of the grains from the root-stocks of *Hedychium hirsutum* in process of solution.
- Alpinia galanga* Swartz. (*Zingiberaceæ*.) *Dry root-stocks*.—Grains lanceolate, conical, or rod-shaped, frequently somewhat irregular; mostly 2.5 to 4 times as long, occasionally a little longer than broad; the narrower ones terete, the broader ones slightly compressed; lamellæ delicate or indistinct, only the innermost ones complete; usually instead of the hilum, a small cavity is observed; one-seventh to one-seventeenth eccentric; the hilum end often thicker; occasionally the distal end broadened and also thinned and squared. Length to 55 $\mu$ , width to 25 $\mu$ . Several grains show traces of changes by moist heat. Among these isolated doublets are rarely found.
- Costus speciosus* Smith. (*Zingiberaceæ*.) *Root-stock*.—According to Fritzehe (*Poggendorff's Annal.*, 1834, xxxii, Taf. ii, fig. 32), the grains are almost cylindrical, about 4 times as long as thick; with distinct lamellæ; hilum about one-eighth eccentric.
- Costus spiralis* Rose.; *Costus comosus* Rose. (*Zingiberaceæ*.)—According to H. Crüger (*Bot. Zeit.*, 1854, Taf. ii, figs. 20 and 23), the grains are as above, but somewhat narrower at the hilum end.

- Musa paradisiaca* Linn. (*Musaceæ*.) *Fruit*.—According to H. Crüger (Bot. Zeit., 1854, Taf. II, fig. 1), the grains are oval and elongated (in the interior of the fruit) to linear (in the rind of the fruit); 1.5 to 10 times as long as broad; with distinct lamellæ; hilum apparently at the thicker end, one-half to almost one-eleventh eccentric.
- Caladium sequinum* Vent.; *Dieffenbachia seguine* Schott. (*Aroideæ*.) *Root-stock*.—According to Schleiden (Grundzuge, 3. Aufl., 1 fig., 2d on p. 184) and H. Crüger (Bot. Zeit., 1854, pl. II, figs. 8 and 15) the grains are rod-shaped; 2 to 10 times as long as thick; frequently with a lateral wart-like or lobe-like process formed by an outer system of lamellæ which is superimposed at right angles to the longitudinal axis; with distinct lamellæ; hilum end sometimes slightly thicker, sometimes slightly thinner, than the distal end; eccentricity about one-sixth.
- Philodendron grandifolium* Schott. (*Aroidæ*.) *Root-stock*.—According to H. Crüger (Bot. Zeit., 1854, Taf. 11, figs. 7 and 13), the grains are elongated to lanceolate, or rounded-triangular (and then probably compressed); sometimes bilobate; 3 to 4 times as long as broad; with distinct lamellæ; hilum at the broader end, about one-sixth or more eccentric.
- Achimenes tubiflora* Nicholson; *Dolichodeira tubiflora* Hanst.; *Gloxinia tubiflora* Hook. (*Gesneriaceæ*.) *Fresh bulbs*.—Grains rod-shaped, the smaller ones oval or conical; many with one (rarely with two) large or small lateral wart-like appendage, which is found occasionally at the proximal end, sometimes in the middle, but chiefly at the distal end; about 5 times as long as thick; terete; lamellæ numerous, distinct, almost all incomplete; instead of the hilum a small cavity with one small transverse fissure, or with two short fissures directed towards the distal end; about one-eighteenth eccentric; two ends usually alike in thickness, the hilum end rarely somewhat thicker (in the small grains, as a rule, the hilum lies in the thicker end). Length about  $66\mu$ , thickness without the appendage about  $16\mu$ , but with the appendage almost double the size.
- Guthnickia atrosanguinea* Regel. (*Gesneriaceæ*.) *Fresh scales of the root-stock*.—Grains rounded-oval to oval-cuneiform, and cylindrical-oval; about 2.5 times as long as broad; circular in transverse section; the broadest end slightly compressed; lamellæ distinct; frequently an external lateral system of lamellæ; both ends of nearly equal thickness; the hilum end occasionally somewhat thicker or thinner than the distal end. Length about  $35\mu$ , width about  $22\mu$ . Among these are some semi-compound and some completely compound grains of two components.
- Plectopoma fimbriatum* Hanst. (*Gesneriaceæ*.) *Fresh scales of the root-stock*.—Grains usually oval, less frequently triangular-oval; the broader ones slightly compressed, the narrower terete; both ends of almost equal thickness. Length about  $28\mu$ .
- Seemannia ternifolia* Regel. (*Gesneriaceæ*.) *Fresh scales of the root-stocks*.—Grains mostly elongated-oval or cylindrical-oval; more rarely conical or somewhat irregular; usually 2 to 3 times as long as broad; terete; the broadest ones slightly compressed; lamellæ usually distinct; often a lateral wart-like process consisting of a separate system of lamellæ (as in *Dolichodeira*); the two ends are usually of nearly equal thickness; although the hilum end may be either thicker or thinner than the distal end, which latter is sometimes broadened into a blunt, knife-like edge. Length about  $50\mu$ , thickness about  $30\mu$ .
- Dentaria enneaphyllos* Linn. (*Brassicaceæ*; *Cruciferae*.) *Dry scales of the root-stock*.—Grains oval, conical, oblong, oval-triangular, frequently somewhat irregular; 1.5 to 3 times as long as broad; the broader ones compressed to about half their width, the narrower terete; without lamellæ; instead of the hilum a small cavity is often observed from which a delicate fissure may radiate; the hilum end frequently thicker, sometimes of equal thickness, rarely thinner than the distal end. Length about  $42\mu$ , width about  $25\mu$ .
- Krameria triandra* R. P. (*Polygalaceæ*.) *Dry roots*.—Grains mostly rod-shaped or oval, sometimes conical or club-shaped, frequently arched or even irregular; also with single knob or lobe-like processes; terete; about 5 times as long as thick; the broader ones slightly compressed; about 3 times as long as broad, and 5 times as long as thick; lamellæ rare and indistinct; occasionally instead of the hilum a small cavity is observed from which short fissures occasionally radiate; about one-eighth eccentric; hilum end either narrower or broader than the distal end. Length about  $42\mu$ . Among these are compound grains of few and mostly equal-sized components (see type 14). Also rounded simple grains are often found, and indeed sometimes almost exclusively, while in some parts of the tissues the oblong grains and in still other parts the compound grains appear in greater numbers. Many of the apparently round, simple grains on closer investigation proved to be separated-grains.

- Colocasia odora* Brongn. (*Aroideæ*.) *Dry seed*.—Grains rounded to oval-conical, mostly somewhat irregular; half to just as broad as long; the narrower forms not compressed; the broader ones compressed to about half their width; no lamellæ; instead of the hilum there appears frequently a small round cavity from which a single fissure may emerge, more often a slit-like cavity, which from the broad aspect appears delicate and from the narrow aspect more clearly defined; hilum end in the small forms is broader than the distal end; eccentricity about one-third and one-fourth. Length about 22 to 26 $\mu$ , width about 20 $\mu$ . The structure is not very clearly defined, frequently approaching the conical type (type 7) and the cuneiform or compressed type (type 8). Doublets and triplets are also found.
- Mammea americana* Linn. (*Guttiferæ*.) *Dry cotyledons*.—Grains mostly oval or elongated-oval, rarely almost rounded or conical; usually twice as long as broad; circular in transverse section; the broadest and at the same time somewhat irregular forms are seldom slightly compressed; no lamellæ; instead of the hilum a small cavity is usually observed, generally with a cross-fissure and several radial fissures; ordinarily the grains are of almost equal width throughout their entire length; now and then narrowed at either the proximal or distal end, or even at both ends; eccentricity mostly one-third to one-fourth (seldom one-fifth and one-sixth). Length about 55 $\mu$ , thickness about 35 $\mu$ . Rich in starch; poor in oil.

#### TYPE 10. GRAINS SIMPLE, STRUCTURE OBSCURE.

Nägeli places here all the species of starch, the structure of which, mostly on account of their diminutive size, is either not fully developed or not identified, and hence remains doubtful as to which of the foregoing types they belong. The grains, as a rule, are undoubtedly simple. Now and then some separated-grains which are no longer recognized as such may occur. Lamellæ, hilum, cavities, fissures, and clefts are seldom mentioned, because they have not often been demonstrable.

- Vaucheria tuberosa* A. Braun. (*Algæ*.) *Dry tuberous swollen ends of root-like organs*.—Grains (all simple) oval to elongated, lanceolate, mostly somewhat unequal laterally, or elliptical and constricted in the middle, reniform, frequently somewhat irregular; 1.5 to 3.5 times as long as broad; the broadest ones compressed to about half the width; sometimes from the narrow aspect a delicate longitudinal slit is observed. Length about 13 $\mu$ , width about 7 $\mu$ , thickness 2.5 to 3.5 $\mu$ .
- Equisetum hyemale* Linn. (*Equisetaceæ*.) *Young tubers*.—According to Leon Soubeiran (*Journ. Pharm.*, 1854, xxv, 182) the smallest grains are spherical, the larger ones elongated, and the largest ones pear-shaped; there are many very irregular forms with dentate projections on the circumference; lamellæ and hilum rare and indistinct. Length 50 to 60 $\mu$ , width 10 to 30 $\mu$ .
- Olfersia undulata* Presl. (*Filicinæ*.) *Dry root-stock*.—Grains rounded or rounded-triangular, occasionally oval, not at all or very slightly compressed, many with a small or a large cavity. Size about 10 $\mu$ .
- Polypodium vulgare* Linn.; *Radix polypodii*. (*Proteaceæ*.) *Dry root-stocks*.—Grains rounded; rounded-triangular, oval, frequently somewhat irregular; compressed to about half or more of their width; many are thickened at the more convex margin or at one angle of the triangle, and at the opposite broad margin thinned and squared; without lamellæ, hilum, or cavity (even after roasting and boiling, the hilum is not visible). Size about 9 to 11 $\mu$ . Many of the grains agree closely in their form with those of the cuneiform type (type 8).
- Polypodium distans* Kaulf. (*Proteaceæ*.) *Dry root-stock*.—Grains rounded or rounded-triangular, rarely oval; compressed to about one-third of their width; elongated from the narrow aspect. Size about 14 $\mu$ .
- Adiantum capillus-veneris* Linn. (*Filicinæ*.) *Dry root-stocks*.—Grains rounded to oblong, mostly oval, sometimes somewhat irregular; narrow ones little or scarcely compressed, the broader ones to about half their width; from the broad aspect indistinct fissures are occasionally observed; from the small aspect the grains are elongated-oval, and generally have a distinct longitudinal slit. Length about 30 $\mu$ , width about 20 $\mu$ . In spite of the size of these grains, it is doubtful to which type they belong. In form and median cleft they resemble the centric-oval and centric-lanceolate types.
- Asplenium marinum* Linn. (*Filicinæ*.) *Dry root-stock*.—Grains rounded or oval, often somewhat irregular; the broad ones compressed to about half their width; the majority have a large cavity. Size about 11 $\mu$ . Isolated doublets are also present.

- Scolopendrium officinarum* Swartz. (*Filicineæ*.) *Fresh root-stock*.—Grains circular to oval, sometimes rather irregular; compressed to about half their width. Size  $9\mu$ .
- Diplazium plantagineum* Swartz. (*Filicineæ*.) *Dry root-stock*.—Grains rounded or oval, most of them only slightly compressed, the broad ones rarely to about half their width. Size about  $15\mu$ .
- Polystichum thelypteris* Roth. (*Filicineæ*.) *Dry root-stock*.—Grains rounded to oval, sometimes rather irregular, rarely somewhat angular; the broad ones compressed to about one-third and one-fourth of their width; from the narrow aspect elliptically oblong and elongated lanceolate; both ends of equal thickness; sometimes with one longitudinal cleft. Size about  $17\mu$ . These grains, like those from the root-stocks of several other *Filicineæ*, resemble very closely those of the centric-lenticular type.
- Aspidium filix-mas* Swartz.; *Radix filicis*. (*Filicineæ*.) *Dry root-stocks*.—Grains rounded to oblong, rounded-triangular, reniform, often somewhat irregular; the broad ones compressed to about one-half and one-third of their width; from the narrow aspect sometimes with a longitudinal cleft. Size about  $19\mu$ . A very few compound grains consisting of 2 to 3 components are also observed.
- Cystopteris fragilis* Bernh. (*Filicineæ*.) *Dry root-stocks*.—Grains rounded, oval, pear-shaped, frequently irregular and somewhat angular; the broad ones compressed to about half their width; somewhat thicker at one end, and thinned and squared at the opposite end. Size about  $9\mu$ .
- Cystopteris bulbifera* Bernh. (*Filicineæ*.) *Dry tuber-like bulbils on the fronds*.—Grains rounded or rounded-oval, rarely somewhat angular, as the result of pressure; compressed to about half their width; either equally thick throughout or thicker on one side. Size about  $7\mu$ .
- Ophioglossum vulgatum* Linn. (*Filicineæ*.) *Dry root-stocks and roots*.—Grains rounded or oval, frequently somewhat irregular; the broad ones compressed to about half their width; from the narrow aspect elongate or oval; often a longitudinal slit. Size about  $12\mu$ . Starch and oil present in the root-stocks; starch only in the roots.
- Botrychium Lunaria* Swartz. (*Filicineæ*.) *Dry root-stock and root*.—Grains rounded, the larger ones somewhat hollow and compressed. Size about  $7\mu$ . No oil in the root-stock.
- Molinia cærulea* Moench. (*Graminaceæ*.) *Dry roots*.—Grains oval, oblong, conical, rarely rounded-oval; broad ones distinctly compressed. Size about  $8\mu$ .
- Carex arenaria* Linn. (*Cyperaceæ*.) *Dry root-stocks*.—Grains rounded, oval, rounded pear-shaped, sometimes slightly compressed; rarely with a small cavity. Size about  $12\mu$ . Among these are observed compound grains of few, unequal components (see type 15). Starch quite plentiful.
- Carex hirta* Linn. *Dry*.—Grains similar to those in the preceding species, rounded to oval, rounded pear-shaped; the broadest ones compressed at about half their width. Size about  $12\mu$ . Among these are observed compound grains of few, unequal components. (See type 15.) Starch plentiful.
- Carex intermedia* Good.; *Carex distichia* Huds. *Dry*.—Grains rounded to oval; broad ones slightly compressed, the larger ones with a cavity. Size about  $8\mu$ . Starch not plentiful.
- Carex atrata* Linn. *Dry*.—Grains rounded. Size  $1.5$  to  $3\mu$ . Some of them are probably separated-grains. Starch not plentiful, found in rather thick-walled cells.
- Carex bicolor* All. *Dry*.—Grains rounded to oval, rounded pear-shaped. Size about  $5\mu$ . Among these grains are observed some compound grains of few mostly equal components. (See type 14.)
- Cyperus polystachyos* Rottb. (*Cyperaceæ*.) *Dry root-stocks*.—Grains spherical to oval and oval pear-shaped. Size about  $8\mu$ . Among these grains are observed some doublets and triplets. Starch not plentiful.
- Abilgaardia monostachya* Vahl. (*Cyperaceæ*.) *Dry root-stocks*.—Grains rounded to oval, and oval pear-shaped; a small cavity in the larger ones. Size about  $9\mu$ . Among these grains are found compound grains of few equal and unequal components. (See type 14.)
- Scirpus triquetus* Linn. (*Cyperaceæ*.) *Dry root-stock*.—Grains rounded, frequently triangular with rounded corners, oval, conical, and frequently somewhat irregular; the broad ones compressed to one-third their width; many with a small cavity. Size about  $18\mu$ . In some conical grains the cavity is at the thicker hilum end, and in some triangular forms the distal end is thinned and squared, so that the type seems to belong now to the eccentric-conical, now to the cuneiform. In most of the grains no hilum is visible. Starch plentiful.

- Scirpus pungens* Vahl.; *Scirpus rothii* Hoppe. (*Cyperaceæ.*) *Dry root-stock.*—Grains similar to the preceding; usually round, and frequently with 1 or 2 small wart-like or angular processes; the broad ones compressed to one-third or more of their width. Size about  $11\mu$ .
- Eriophorum capitatum* Host.; *Eriophorum scheuchzeri* Hoppe. (*Cyperaceæ.*) *Dry root-stock.*—Grains rounded pear-shaped, oval, and triangular. Size about  $7\mu$ . Some doublets and triplets are also observed.
- Triglochin maritimum* Linn. (*Naiadaceæ.*) *Dry root-stock.*—Grains spherical, oval, rounded pear-shaped, frequently with a small cavity. Size about  $8\mu$ . Starch quite plentiful.
- Scheuchzeria palustris* Linn. (*Naiadaceæ.*) *Dry root-stock.*—Grains rounded or oval, frequently somewhat irregular. Size about  $4\mu$ . Starch quite plentiful.
- Alisma plantago* Linn. (*Alismaceæ.*) *Fresh root-stock.*—Grains rounded, rounded-oval, sometimes rather irregular. Size about  $7\mu$ . Among these are large separated-grains with 1 to 3 pressure facets. Size about 2 to  $5\mu$ . Starch and oil plentiful in the upper part of the root-stock; no starch in the lower part.
- Butomus umbellatus* Linn. (*Alismaceæ.*) *Dry root-stock.*—Grains rounded and triangular with rounded corners to oblong and cuneiform, frequently more or less irregular; compressed to one-third or more of their width; frequently from the broad aspect a cavity is observed, and from the narrow aspect a longitudinal slit. Size about  $12\mu$ . Starch quite plentiful.
- Juncus bulbosus* Linn.; *Juncus compressus* Jacq. (*Juncaceæ.*) *Dry root and root-stocks.*—Grains usually rounded or slightly angular, rarely oval or rounded pear-shaped; compressed to half their width; many have a small cavity. Size about  $7\mu$ . Not much starch in the root-stock; more in the roots.
- Juncus balticus* Dethard. (*Juncaceæ.*) *Dry root-stock.*—Grains rounded to oval and pear-shaped. Size about  $7\mu$ . Among these grains are compound grains of 2 to 5 equal or unequal components. Starch not very plentiful.
- Luzula spadicæa* DC. (*Juncaceæ.*) *Dry root-stock.*—Grains rounded or oval, frequently somewhat angular. Size about  $8\mu$ . Among these are observed compound grains of few usually equal components (see type 14). Starch not plentiful.
- Tofieldia calyculata* Whalenb. (*Liliaceæ.*) *Dry root-stock.*—Grains rounded to oval, rounded pear-shaped; many slightly compressed; in the larger ones a small cavity is frequently observed. Size about  $6\mu$ .
- Veratrum album* Linn. (*Liliaceæ.*) *Dry root-stock.*—Grains rounded or, when crowded in the cell, somewhat angular; usually there is a large or a small central cavity from which single fissures sometimes radiate. Size about 13 to  $15\mu$ . Among these are found compound grains of 2 to 4 usually equal components. Possibly the greater part of the starch is found in the compound grains. In the root fibers there are grains of equal to somewhat larger size, and having the form of compound grains of type 15.
- Bulbocodium vernum* Linn. (*Liliaceæ.*) *Dry corn.*—Grains spherical, oval, or rounded pear-shaped. Size 6 to  $8\mu$ . Among these are compound grains of few usually unequal components. (See type 15.)
- Gagea stenopetala* Rehb. (*Liliaceæ.*) *Scales of dry bulbs.*—Grains spherical, nearly all of them flattened on one side; instead of a hilum, usually a small cavity is observed from which radial fissures emerge; hilum usually either near the middle of the flattened side, or almost central in the completely spherical forms; frequently a curved slit is observed running parallel to the flattened margin, the curved portion of which is directed towards the squared edge. Size 17 to  $26\mu$ . The grains look like separated-grains, resembling a segment of a circle.
- Gagea lutea* Schult. (*Liliaceæ.*) *Scales of dry bulbs.*—Grains spherical or oval-spherical, frequently somewhat angular or irregular; a small almost central cavity and radial fissures; lamellæ none, or very delicate, and concentric. Size about  $22\mu$ . Some compound grains of few unequal components are also present. (See type 15.)
- Narthecium ossifragum* Huds. (*Liliaceæ.*) *Dry root-stock.*—Grains rounded to oval and pear-shaped, frequently somewhat irregular. Size about  $17\mu$ . Compound grains with few, usually unequal components are also present. (See type 15.)
- Smilax china* Linn.; *Radix china.* (*Liliaceæ.*) *Dry root-stocks.*—Grains more or less angular, as a result of pressure, frequently polyhedral with sharp angles and edges, also with flat surfaces; isodiametric or longer in one diameter; instead of a hilum a small central cavity is observed

from which a few fissures radiate; lamellæ rarely distinct. Size 40 to 50 $\mu$ . The grains fill the cells. Originally the grains are almost spherical, but become angular as a result of pressure. In some grains the surface is flattened only here and there. Among these grains are some compound grains of few equal components. (See type 14.)

- Conostylis involucrata* Endl. (*Hæmodoraceæ*.) *Dry roots*.—Grains rounded, angular with rounded corners, or sharply polyhedral; sometimes they have a small central cavity from which single fissures rarely radiate. Size about 17 $\mu$ . Grains completely fill the cell and become angular as a result of pressure.
- Galanthus plicatus* Bierbst. (*Amaryllidaceæ*.) *Scales of dry bulbs*.—Grains rounded, oval, reniform, frequently somewhat irregular; compressed to one-half or over of their width; from the narrow aspect a longitudinal slit is frequently observed. Size about 42 $\mu$ . Hilum is usually central.
- Sternbergia lutea* Ker. (*Amaryllidaceæ*.) *Scales of dry bulbs*.—Grains rounded, oval, reniform, rounded-conical, often somewhat irregular and with blunt angles; compressed to about half their width; instead of the hilum there is a small central or about one-fourth eccentric cavity, from which radiates some very distinct usually cruciform fissures; from the narrow aspect a longitudinal cleft is observed; sometimes isolated lamellæ are present. Length about 63 $\mu$ , width to 40 $\mu$ . Among these are found equal or unequally divided doublets. In fresh bulbs from the Botanical Garden in Zurich (the starch above described is from a plant at Cephalonia) the grains are smaller (about 38 $\mu$  in size), rounded-reniform, and rounded-triangular to oblong, frequently more or less irregular; they have a small central or slightly eccentric cavity and very short fissures.
- Narcissus poeticus* Linn. (*Amaryllidaceæ*.) *Scales from fresh bulbs*.—Grains rounded, with triangular, rhomboidal, and quadrangular outlines; oval, reniform, or oval-cuneiform, frequently more or less irregular; two-fifths to as broad as long; the broad ones compressed to half or more of their width; lamellæ and hilum are distinct in only a few of the larger grains. Size about 21 $\mu$ . Some of the grains undoubtedly belong to the eccentric, cuneiform type (type 8); they are broader than long; the hilum is one-sixth eccentric. Among the grains above mentioned there are a few compound grains of 2 to 4 components.
- Cælogyne fimbriata* Lindl. (*Orchidaceæ*.) *Fresh pseudo-tubers*.—Grains usually spherical, without lamellæ; frequently with a small cavity from which a few (1 to 4) short or long fissures radiate. Size about 27 $\mu$ .
- Orchis mascula* Linn. (*Orchidaceæ*.) *Fresh tubers*.—Grains spherical, rounded-triangular, or rounded-conical; the broad ones slightly compressed; hilum rarely visible, located at the broader end, two-fifths to two-sevenths eccentric; lamellæ none, or unilateral, at the narrow end. Size about 23 $\mu$ . Among the above are some compound grains of 2 to 5 components, and also isolated semi-compound ones.
- Orchis globosa* Linn. *Dry*.—Grains are spherical, and have a small central cavity. Size about 14 $\mu$ . Also some compound grains of few components are observed. Starch quite plentiful.
- Orchis militaris* Linn. *Fresh not fully mature tubers*.—Grains spherical. Size about 12 $\mu$ . Doublets are also rarely observed.
- Orchis latifolia* Linn.—According to Payen (Ann. Sc. Nat., 1838, II, pl. 6, fig. 19) the grains are spherical, oval, or conical; two-thirds to twice as long as broad; lamellæ distinct; hilum at the thicker end, one-fourth eccentric. Size about 45 $\mu$ . Among the above are some semi-compound grains of two inclosed components.
- Platanthera bifolia* Rich. (*Orchidaceæ*.) *Tubers*.—According to Payen (Ann. Sc. Nat., 1838, II, pl. 6, fig. 18) the grains are almost spherical, oval, conical, broadly triangular; one-half to almost twice as long as broad; lamellæ distinct; hilum about one-fourth eccentric, in the elongated forms located at the thick end. Size about 45 $\mu$ . The broad grains appear to increase in width at the distal end by means of two prominent angles bounding the less dense and squared edge.
- Calla palustris* Linn. (*Aroideæ*.) *Dry root-stock*.—Grains spherical or oval; the larger ones occasionally with a small cavity or cleft. Size about 6 to 8 $\mu$ . Among the above are some doublets, usually of halves.
- Acorus calamus* Linn. (*Aroideæ*.) *Dry root-stock*.—Grains rounded, angular with rounded corners, oval or oval-conical; one-half to almost as thick as long. Length about 14 $\mu$ , thickness about 9 $\mu$ . Among the above are some compound grains of 2 to 3 equal or unequal components.

- Dorstenia brasiliensis* Linn.; *Radix contrajervæ* Moreen. (*Artocarpaceæ*.) *Dry root-stock*.—Grains spherical, usually more or less angular; the larger ones have a small central cavity. Size 6 to 7 $\mu$ . The cells are filled with starch. Some of the grains are simple; the majority, however, are the separated-grains of compound ones (see type 16).
- Parietaria diffusa* Mert. and Koch. (*Urticaceæ*.) *Dry root-stock*.—Grains spherical or rounded-oval; the larger ones hollow; Size about 7 $\mu$ . Among the above are some compound grains of few, equal components (see type 14). Generally poor in starch.
- Polygonaceæ*. *Underground parts*.—Since the seeds of *Polygonaceæ* contain centric starch-grains, it was desirable to examine the structure of the grains from other parts of the plant. Although a number of roots and root-stocks were examined, the question could not be definitely decided. In the analogous structure and in the frequent occurrence of a similar median slit, the grains resemble those in the seeds of *Leguminosæ*. They belong, however, to another type, as is particularly illustrated by the compound grains. Very often small particles are cut off from simple grains; and the large components sometimes show a distinctly eccentric, spherical hilum. Many grains have a swollen appearance.
- Polygonum viviparum* Linn. (*Polygonaceæ*.) *Dry root-stock*.—Grains rounded, rounded-triangular, oval, oblong, elliptical, pear-shaped, or reniform, frequently irregular, occasionally somewhat curved, and often with protruding corners or wart-like process; broad ones compressed; from the narrow aspect they have a central cavity or a distinct longitudinal slit. Size about 14 $\mu$ . Among the above are some doublets.
- Polygonum bistorta* Linn.; *Radix bistortæ*. (*Polygonaceæ*.) *Dry root-stock*.—Grains rounded, rounded-triangular; oval, reniform, pear-shaped, rod-shaped or irregular, occasionally curved, frequently with papillary protuberances on the circumference; the broad ones compressed; one-quarter to as broad as long; many have a longitudinal slit or a large central cavity. Length about 17 $\mu$ , width 11 $\mu$ . Among the above are some doublets consisting of frequently elongated, equal halves. The simple and the compound grains resemble those of *Polygonum viviparum*.
- Polygonum alpinum* All. (*Polygonaceæ*.) *Dry rootlets of young shoots*.—Grains rounded to oblong, elliptical, sometimes reniform or irregular; the broad ones compressed; the larger ones have a cavity. Size about 9 $\mu$ . Little starch.
- Polygonum aviculare* Linn. (*Polygonaceæ*.) *Dry root*.—Grains spherical or rounded-oval; the larger ones with a cavity. Size about 7 $\mu$ .
- Polygonum convolvulus* Linn. (*Polygonaceæ*.) *Dry root*.—Grains spherical to oval. Size about 8 $\mu$ . Poor in starch.
- Rumex obtusifolius*; *Radix lapathi acuti*. (*Polygonaceæ*.) *Dry root*.—Grains oval, rounded-triangular, elliptical, lanceolate or lanceolate spindle-shaped; one margin frequently more convex than the other, or even somewhat curved; one-third to as broad as long; not at all or slightly compressed; some grains have a longitudinal slit. Length about 16 to 20 $\mu$ , width 11 $\mu$ . Lamellæ and hilum invisible; the latter is probably toward the thicker end.
- Rumex sanguineus* Linn. (*Polygonaceæ*.) *Dry root*.—Grains rounded to lanceolate with either blunt or pointed ends; frequently with one strongly convex and one straight or slightly concave margin; sometimes triangular and irregular; 5 to 6 times as long as broad; the broad ones slightly compressed; from the narrow aspect there is a distinct longitudinal slit. Size 20 to 24 $\mu$ . Among the above some doublets are observed.
- Rumex crispus* Linn. (*Polygonaceæ*.) *Dry root*.—Grains oval, oblong, elliptical, pear-shaped, very often triangular or somewhat reniform with elevated ridges, often irregular, with either blunt or pointed angles; the broad ones distinctly compressed; from the narrow aspect the majority have a longitudinal slit. Size about 20 to 32 $\mu$ .
- Rumex acetosa* Linn. (*Polygonaceæ*.) *Dry root-stock*.—Grains spherical to oblong and conical; the larger ones with a cavity nearly the same shape as the grains. Size about 10 $\mu$ . Among the above are compound grains of few, usually unequal, components (see type 15).
- Rumex maritimus* Linn. (*Polygonaceæ*.) *Dry root*.—Grains rounded, oval, oblong, conical, the broad ones slightly compressed; some grains have a narrow slit-like cavity. Among the above are some compound grains of few equal or unequal components (see type 15).
- Rumex arifolius* All. (*Polygonaceæ*.) *Dry root-stock*.—Grains spherical or rounded-oval, sometimes slightly angular; the larger ones have a central cavity. Size about 9 $\mu$ . Also some compound grains of few equal or unequal components (see type 14).

- Aristolochia clematitis* Lam. (*Aristolochiaceæ*.) *Fresh root-stock*.—Grains usually spherical; also some lamellæ; hilum usually indistinct, about one-third eccentric. Size about  $11\mu$ . Also some compound grains of 2 to 4 equal components. Size about  $10\mu$ .
- Asarum europæum* Linn. (*Aristolochiaceæ*.) *Dry stolons*.—Grains spherical or rounded-oval. Size about  $6\mu$ . Also some compound grains of 2 to 4, rarely 6, equal components. Size about  $11\mu$ . Moderate amount of starch; oil plentiful.
- Plantago maritima* Linn. (*Plantaginaceæ*.) *Dry root-stock*.—Grains spherical to oval; rarely with a small cavity. Size about  $11\mu$ . Also some compound grains of few usually unequal components (see type 15).
- Plantago media* Linn. (*Plantaginaceæ*.) *Fresh root-stock*.—Grains rounded. Size about  $7\mu$ . Also some compound and separated-grains are observed (see type 15).
- Valeriana officinalis* Linn. (*Valerianaceæ*.) *Fresh root-stock and stock*.—Grains spherical; occasionally with a central hilum. Size about  $9\mu$ . Also there are some compound grains of few equal components (see type 15).
- Valeriana salinca* All. (*Valerianaceæ*.) *Dry root-stock*.—Grains spherical or rounded-oval. Size 4 to  $6\mu$ . Among the above are compound grains of few usually equal components (see type 14). Starch quite plentiful.
- Valeriana tuberosa* Linn. (*Valerianaceæ*.) *Dry root-stock*.—Grains spherical; large or small central cavity, frequently with several radiating fissures. Size about  $15\mu$ . Also a small number of compound grains of few almost equal components (see type 14).
- Dahlia variabilis* Desf. (*Compositæ*.) *Fresh tuberous roots*.—Grains circular or rounded-oval; the large ones compressed to one-fourth and more of their width; they are homogeneous. Size about  $40\mu$ . Judging from the shape, this starch seems to belong to the centric-lenticular type.
- Cinchona* sp.; *Cortex china* Huanaco. (*Rubiaceæ*.) *Dry bark*.—Grains rounded, irregular, very often with curved surfaces. Size about  $13\mu$ . Most of the grains look as if they had been affected by moist heat. Starch scarce.
- Swertia perennis* Linn. (*Gentianaceæ*.) *Dry root-stock*.—Grains rounded, frequently spherical, rarely with a small central cavity. Size 6 to  $8\mu$ . Among the above are compound grains of few nearly equal components (see type 14).
- Omphalodes verna* Moench. (*Boraginaceæ*.) *Dry root-stock*.—Grains rounded, rarely with a small cavity. Size 5 to  $7\mu$ . Also compound grains of few nearly equal components (see type 14). Starch scarce.
- Convolvulus soldanella* Linn. (*Convolvulaceæ*.) *Dry stolons*.—Grains spherical, rarely oval; few with single lamellæ; a small cavity is observed instead of the hilum, central to one-half eccentric. Size about  $17\mu$ . Among the above are some compound grains of few equal or unequal components (see type 17).
- Convolvulus lineatus* Linn. (*Convolvulaceæ*.) *Dry root-stock*.—Simple and compound grains (see type 14) as in the preceding species. Size of the simple grains about  $14\mu$ .
- Convolvulus imperati* Vahl.; *Batatas littoralis* Choisy. (*Convolvulaceæ*.) *Dry stolons*.—Grains spherical or oval-spherical; rarely with single delicate lamellæ. Size about  $19\mu$ . Among the above are some compound grains of few usually equal components (see type 14).
- Ipomœa turpethum* R. Br. (*Convolvulaceæ*.) *Roots*.—According to Leon Soubeiran (*Journ. Pharm.*, 1854, xxv, 91), the grains are oval or indistinctly triangular; without hilum or lamellæ; rarely with either straight or radial fissures; several grains are somewhat tuberculated and apparently compound. Size 20 to 30 or  $40\mu$ .
- Polemonium reptans* Linn. (*Polemoniaceæ*.) *Dry root-stock*.—Grains rounded to oval, about twice as long as broad; the broad ones slightly compressed; some grains have a small cavity. Size about  $8\mu$ . Among the above are also some compound grains of few nearly equal components (see type 14).
- Atropa belladonna* Linn. (*Solanaceæ*.) *Dry root*.—Grains rounded, oval, oblong, elongated-conical, frequently somewhat irregular; two-fifths to as broad as long; occasionally with indistinct and few lamellæ; mostly at the narrow aspect instead of the hilum a small cavity is observed; eccentricity one-half to one-sixth. Length about 14 to  $18\mu$ , width  $13\mu$ . Among the above are some compound grains of few equal components (see type 14).
- Scrophularia nodosa* Linn. (*Scrophulariaceæ*.) *Dry root-stock*.—Grains rounded to oblong and conical. Size about  $9\mu$ . Compound grains of few equal or unequal components (see type 14).

- Gratiola officinalis* Linn. (*Scrophulariaceæ*.) *Dry root-stock*.—Grains rounded to oval. Size about  $6\mu$ . Also compound grains with a few equal or unequal components are observed (see type 14).
- Veronica austriaca* Linn. (*Scrophulariaceæ*.) *Dry root*.—Grains spherical to oval; the large ones with a small central cavity. Size 8 to  $10\mu$ . Also some compound grains of few equal or unequal components (see type 14).
- Wulfenia carinthiaca* Jacq. (*Scrophulariaceæ*.) *Dry root-stock*.—Grains rounded to oval. Size 5 to  $7\mu$ . Also some compound grains of few equal or unequal components (see type 14).
- Pedicularis barrelieri* Rechl. (*Scrophulariaceæ*.) *Dry root*.—Grains spherical or rounded-oval; the larger ones with a small central cavity. Size about  $9\mu$ . With these are found compound grains of few almost equal components (see type 14). Starch quite plentiful.
- Pedicularis rosea* Wulf. (*Scrophulariaceæ*.) *Dry root*.—Grains as in the preceding. Size about  $7\mu$ . With these are found compound grains (see type 14). Starch quite plentiful in some parts of the plant.
- Pedicularis acaulis* Scop. (*Scrophulariaceæ*.) *Dry root*.—Grains spherical to oval; the larger ones with a small central cavity. Size about  $8\mu$ . Occasionally the size is about  $11\mu$ ; the grains then have a swollen appearance and a large cavity. Among the above are compound grains of few mostly equal components (see type 14).
- Primula calycina* Dub. (*Primulaceæ*.) *Dry root*.—Grains spherical to elliptical; the larger ones have a small cavity at the thicker end instead of a hilum, one-half and one-third eccentric. Size about  $15\mu$ . These grains appear to belong to the eccentric-conical type. Among the above are some compound grains of few almost equal components (see type 14). Starch plentiful. The starch in the root-stock is less plentiful, and is contained in thick-walled cells. The grains are smaller (about  $7\mu$ ), rounded to oval; the larger ones have a small cavity.
- Primula officinalis* Jacq. (*Primulaceæ*.) *Dry root-stock*.—Grains rounded to oval, the larger with a small cavity. Size about  $7\mu$ . Among the above are some compound grains of 2 to 4 components, and also separated-grains. Starch not plentiful; cells thick-walled.
- Soldanella alpina* Linn. (*Primulaceæ*.) *Dry root-stock*.—Grains spherical, oval, frequently somewhat irregular, occasionally with a small cavity. Size about  $9\mu$ . Also some compound grains of few components of equal size (see type 14). Starch plentiful in thick-walled, porous cells.
- Dry roots of the same plant*: Grains spherical or oval-spherical; with a small central cavity, frequently with several short radial fissures. Size about  $13\mu$ . Also some compound grains (see type 14).
- Glaux maritima* Linn. (*Primulaceæ*.) *Dry ereeping stems*.—Grains spherical or spherical-oval; frequently with a small cavity from which short fissures may radiate. Size about  $12\mu$ . Also, there are some compound grains composed of a few mostly equal components, as in type 14. Starch plentiful; cell-walls not noticeably thick.
- Lysimachia vulgaris*. (*Primulaceæ*.) *Dry root-stock*.—Grains spherical or rounded-oval; frequently with a central cavity and a few short radial fissures. Size about  $15\mu$ . Among these are some compound grains of few equal or unequal components (see type 14).
- Pyrola rotundifolia* Linn. (*Ericaceæ*.) *Dry root-stock*.—Grains rounded to rounded-oval, occasionally somewhat angular or irregular; many with a small cavity. Size 7 to  $9\mu$ . There are some compound grains of few nearly equal components (see type 14).
- Astrantia major* Linn. (*Umbelliferaæ*.) *Dry root-stock*.—Grains rounded or rounded-oval, often angular or irregular, many with a small cavity. Size about  $7\mu$ . There are several compound grains and many separated-grains.
- Bupleurum longifolium* Linn. (*Umbelliferaæ*.) *Dry root-stock*.—Grains rounded or oval, often irregular. Size about  $7\mu$ . There are some compound grains of few components. Starch not plentiful.
- Meum athamanticum* Jacq.; *Radix mei*. (*Umbelliferaæ*.) *Dry root*.—Grains rounded to lanceolate, occasionally somewhat angular or irregular; the broader ones compressed to one-half and over of their width. Size about  $8\mu$ . The small grains are rounded and isodiametric; the large ones tabulate or rod-shaped. There were no indications of compound forms as in the roots of other *Umbelliferaæ*. Some of the apparently simple grains may be separated-grains.
- Gaya simplex* Gaud. (*Malvaceæ*.) *Dry root-stock*.—Grains rounded to oval, often angular or irregular; many with a small cavity. Size 6 to  $8\mu$ . There are some compound grains and many separated-grains.

- Levisticum officinale* Koch. (*Umbelliferae*.) *Dry root*.—Grains spherical, occasionally somewhat angular; no lamellæ; with a small central cavity. Size 12 to 18 $\mu$ . There are separated-grains, as in type 14.
- Archangelica officinalis* Koffm. (*Umbelliferae*.) *Dry root*.—Grains rounded. Size about 8 $\mu$ . Very little starch.
- Imperatoria ostruthium* Linn. (*Umbelliferae*.) *Dry root-stock*.—Grains oval, conical, oblong, occasionally somewhat curved, rarely slightly constricted in the middle; one-third to one-half as thick as long; occasionally almost as thick as long; without fissures. Size about 14 $\mu$ , thickness about 6 $\mu$ . Hilum most likely toward the narrow aspect.
- Pastinaca sativa* Linn. (*Umbelliferae*.) *Dry root*.—According to Payen (*Ann. Sc. Nat.*, 1838, II, p. 28; pl. 4, fig L), the grains are rounded, and compressed to about half their width. Size 7.5 $\mu$ .
- Cornus suecica* Linn. (*Cornaceae*.) *Dry root-stock*.—Grains spherical to oval; the larger ones with a small cavity. Size 7 to 9 $\mu$ . There are compound grains of few almost equal components. (See type 14.)
- Sedum fabaria* Koch. (*Crassulaceae*.) *Dry root-stock*.—Grains rounded, rounded-triangular, oval, conical, spindle-shaped, lanceolate, at times somewhat curved and more or less irregular; some with 1 or even 2 and 3 prominent solid angles and papillary processes; one-third to as broad as long; the broad ones slightly compressed; no lamellæ; many with a longitudinal slit. Length about 17 $\mu$ , width 12 $\mu$ . Among the above are some compound grains of few components which arise through division of the hilum, as well as from the segmentation of the solid angles or the processes.
- Mittella pentandra* Linn.; *Drummondia mittelloides* DC. (*Saxifragaceae*.) *Dry root-stock*.—Grains oval, conical, rod-shaped, spindle-shaped, sometimes triangular, frequently somewhat curved, mostly more or less irregular; one-fourth to almost as broad as long; the broad ones compressed; some with a cavity or a slit. Size about 14 $\mu$ . Doublets with unequal halves. Starch quite plentiful.
- Mittella diphylla* Linn. (*Saxifragaceae*.) *Dry root-stock*.—Grains rounded, oval, oblong, conical, rounded-triangular, and reniform; frequently more or less irregular; two-fifths to as broad as long; the broad ones compressed to about one-third of their width. Size about 15 $\mu$ . Starch quite plentiful.
- Ranunculus pyrenæus* Linn. (*Ranunculaceae*.) *Dry root*.—Grains rounded, rounded-triangular, reniform, oval, or shortened-conical; frequently more or less irregular, compressed; from the narrow aspect a longitudinal slit is frequently observed; no lamellæ. Size about 14 $\mu$ .
- Ficaria ranunculoides* Moench. var. from Algiers; *Ranunculus ficaria* Linn. (*Ranunculaceae*.) *Dry thickened roots*.—Grains rounded, triangular with rounded corners, and quadrangular, oval, conical, frequently more or less irregular; the broad ones compressed to one-half and more of their width; from the narrow aspect a longitudinal slit is observed. Size 24 $\mu$ . Among the above are some doublets. The grains seem to belong to the eccentric-conical (type 7), or cuneiform type (type 8). The grains of indigenous plants show a distinct eccentric cuneiform or compressed structure. (See type 8.)
- Podyphyllum peltatum* Linn. (*Berberidaceae*.) *Dry root-stock*.—Grains spherical to oval. Size about 5 $\mu$ . Some compound grains of many components and separated-grains. (See type 16.)
- Sanguinaria canadensis* Linn. (*Papaveraceae*.) *Dry root-stock*.—Grains rounded, oval, conical, or frequently irregular; occasionally with a cavity. Size about 16 $\mu$ . There are some compound grains of few unequal components (see type 15). The starch undoubtedly belongs to an eccentric type.
- Papaver orientale* Linn. (*Papaveraceae*.) *Fresh root-stock*.—Grains rounded to oblong; one-half to as thick as long. Size 8 $\mu$ .
- Dentaria bulbifera* Linn. (*Brassicaceae*; *Cruciferae*.) *Dry scales of the small axillary bulbs*.—Grains oval, conical, or rounded-triangular; broad ones compressed; some with a longitudinal slit. Size about 14 $\mu$ . The grains appear to belong to the eccentric-conical type. Cells entirely filled with starch-grains.
- Cochlearia armoracia* Linn. (*Brassicaceae*; *Cruciferae*.) *Fresh root*.—Grains spherical to oblong; one-third to just as broad as long; occasionally slightly compressed; many with a rounded and elongated or a slit-like cavity. Size 11 to 14 $\mu$ .

- Parnassia palustris* Linn. (*Saxifragaceæ*.) *Dry root-stock*.—Grains rounded to elongated-oval, and conical; frequently somewhat irregular; with a cavity, a slit or some fissures. Size about  $18\mu$ . Type undetermined. The hilum is eccentric, and often appears to be at the narrow aspect.
- Viola palustris* Linn. (*Violacææ*.) *Dry root-stock*.—Grains spherical or rounded-oval. Size about  $8\mu$ . There are some compound grains of few equal or unequal components. (See type 15.)
- Cereus variabilis* Pfeiff.; *Cereus quadrangularis* Hort. (*Cactaceæ*.) *Fresh pith of the stem*.—Grains rounded or oval, rarely conical; usually irregular and with either protruding rounded or blunt angles; occasionally projecting on one side into a sharp angle; half to as broad as long; broad ones slightly compressed; one-third to as thick as long; lamellæ distinct, mostly more or less irregular, spiral-like, crooked, or noncontinuous in the radius of greatest thickness (in the latter case two to five systems of lamellæ may arise); hilum more or less eccentric, in some instances about one-fiftieth. Length about  $100\mu$ , thickness  $60\mu$ . Among the above are some semi-compound grains as in type 11 and compound ones of few unequal components, type 15. Many much smaller, simple grains and numerous compound and separated-grains were found in the pith of 1.5 inch sprouts, while the separated-grains rarely occurred in older stems. Eccentric structure could occasionally be noticed in the separated-grains. These may possibly have developed from those mentioned above with irregular lamellæ, as well as from the semi-compound and compound grains of the mature pith. The starch-grains in the base of the sprouts were decidedly larger than those at the summit. Those in the parenchyma of the cortex, which on the whole are very scarce, resemble those in the pith.
- Cereus martianus* Zuccar. (*Cactaceæ*.) *Fresh pith of the stem*.—Grains rounded, occasionally somewhat irregular or polyhedral as a result of pressure, sometimes just as thick as broad, sometimes compressed into a lenticular form to about two-fifths of their width; few lamellæ; hilum central or toward one margin. Size about  $35\mu$ . The surface of many of the grains is reticulated, probably a form of decomposition. Among the above are some semi-compound grains with 2 and 3 hila, and also some compound forms. Some cells in the parenchyma of the pith are filled with starch.
- Cereus erinaceus* Haw.; *Echinocactus erinaceus*. (*Cactaceæ*.) *Stems*.—According to Payen (*Ann. Sc. Nat.*, 1838, II, p. 18), the grains are usually rounded and irregular; lamellæ concentric and with sinuate radial fissures (*fentes sinuæ*). Size about  $75\mu$ . These grains were observed in an old exotic stem, and were very scarce. In the pith of a cultivated plant also examined by Payen (*loc. cit.*, p. 27; plate 4, fig. E) he found spherical or somewhat irregular grains; lamellæ and hilum indistinct. Size about  $12\mu$ . There are some doublets.
- Cereus peruvianus* Haw.; *Cactus peruvianus*. (*Cactaceæ*.)—According to Payen (*loc. cit.*, p. 24; pl. 6, fig. 21), the grains are usually spherical or ellipsoidal, sometimes irregular; with a few distinct lamellæ; hilum frequently visible, about one-fifth eccentric. Size about  $30\mu$ . Among these are many compound grains of 2 to 4 components. They frequently have lamellæ and an eccentric hilum.
- Cereus flagelliformis* Mill.; *Cactus flagelliformis*. (*Cactaceæ*.)—According to Payen (*loc. cit.*, p. 26, pl. 4, fig. D), the grains are irregular; many with upper surface sinuate; compressed to about one-third the width. Size about  $15\mu$ . Among these are indistinctly compound grains consisting of few components.
- Cereus serpentinus* Lagase.; *Cactus serpentinus*. (*Cactaceæ*.)—According to Payen (*loc. cit.*, p. 28, pl. 4, fig. M), the grains are round, sometimes uneven; lamellæ and hilum usually invisible. Size about  $7.5\mu$ ; also some doublets.
- Cereus monstrosus* DC.; *Cactus monstrosus*. (*Cactaceæ*.)—According to Payen (*loc. cit.*, p. 28; pl. 4, fig. N), very few grains are rounded; hilum and lamellæ mostly invisible. Size about  $6\mu$ .
- Mamillaria discolor* Haw. (*Cactaceæ*.)—According to Payen (*loc. cit.*, p. 27, pl. 4, fig. J), the grains are rounded, sometimes uneven; hilum and lamellæ invisible. Size  $8\mu$ . Some doublets are found among the simple grains.
- Rhipsalis funalis* Salm. (*Cactaceæ*.) *Fresh pith and parenchyma of the cortex*.—Grains rounded or rounded-oval, frequently somewhat angular or irregular; slightly compressed, or almost to the middle of their width; occasionally with small delicate, concentric lamellæ; from the narrow aspect frequently a longitudinal slit is observed. Size about  $22\mu$ , among these are observed some compound grains of small 2 to 4 components. Starch originated in the chlorophyll grains.

- Opuntia brasiliensis* Haw.; *Cactus brasiliensis*. (*Cactaceæ*.) *Stems*.—According to Payen (Ann. Sc. Nat., 1838, p. 25, pl. 4, fig. B), the grains are irregular, with elevations and depressions; lamellæ and hilum indistinct. Size about 20 $\mu$ ; some grains end in a lateral curved hook, probably a partly decomposed form. Among these are some compound grains of few components.
- Opuntia curassavica* Mill. (*Cactaceæ*.)—According to Payen (*loc. cit.*, p. 27; pl. 4, fig. H), the grains are rounded or oblong, somewhat sinuate; lamellæ and hilum invisible. Size about 10 $\mu$ . Some doublets and triplets are also observed.
- Opuntia tuna* Mill.; *Cactus opuntia tuna*. (*Cactaceæ*.)—According to Payen (*loc. cit.*, p. 27, pl. 4, fig. F), the grains are spherical, sometimes rather irregular; lamellæ and hilum invisible. Size about 10 $\mu$ . Some doublets are also observed.
- Opuntia ficus indica* Mill.—According to Payen (*loc. cit.*, p. 27, pl. 4, fig. G), the grains are similar to the preceding, only somewhat smaller and less numerous.
- Pereskia grandiflora* Haw.; *Cactus pereskia grandiflora*. (*Cactaceæ*.) *Pith*.—According to Payen (*loc. cit.*, p. 25, pl. 4, fig. A), the grains are rounded, usually irregular and angular; with a few distinct lamellæ and more or less eccentric hilum. Size about 22.5 $\mu$ . Many separated-grains are also observed.
- Portulaca megalantha* Steud. (*Portulacaceæ*.) *Dry root*.—Grains spherical, or rounded-oval; occasionally with a central cavity. Size about 14 $\mu$ . Also some compound grains of few equal components are observed (see type 14).
- Ullucus tuberosa* Lozano. (*Chenopodiaceæ*.) *Tubers*.—According to Leon Soubeiran (Journ. Pharm., 1854, xxv, p. 99), the smaller grains are oval or spherical, larger ones elongated and slightly curved, some indistinctly triangular; the oblong grains have distinct lamellæ and hilum. Size 20 to 50 and 60 $\mu$ .
- Saponaria officinalis* Linn. (*Caryophyllaceæ*.) *Dry root*.—Grains rounded or acute-angled; the latter evidently separated-grains. Size 7 to 8 $\mu$ . Poor in starch.
- Althæa rosea* Cav. (*Malvaceæ*.) *Fresh root*.—Grains rounded or oval, rarely oblong; not at all or slightly compressed. Size 7 to 9 $\mu$ . Poor in starch.
- Adansonia digitata* Linn. (*Malvaceæ*.) *Dry fruit pulp*.—Grains rounded-oval to elliptical; curved on one side and almost straight on the other; two-thirds to as broad as long; the broad ones compressed; lamellæ none, or very delicate; usually with a slit-like cavity from which delicate transverse fissures proceed; cavity is very distinct from the narrow aspect and very indistinct from the broad aspect. Size about 18 $\mu$ . The starch resembles the centric oval type.
- Androsæum officinale* All. (*Hypericaceæ*.) *Fresh root*.—Grains rounded to oval, often irregular; compressed to one-fourth their width; frequently sunken or with a cavity in the middle. Size 14 to 18 $\mu$ .
- Hypericum clodes* Linn. (*Hypericaceæ*.) *Dry creeping root-stock*.—Grains rounded to oval, usually slightly compressed; from the narrow aspect a longitudinal slit is usually observed. Size about 13 $\mu$ . Among these are found compound grains of few equal or unequal components (see type 14).
- Byrsonima crassifolia* DC. (*Malpighiaceæ*.)—Grains spherical or oval; once to almost twice as long as thick, lamellæ and hilum none; rarely a cavity in place of the hilum, about one-fourth eccentric; the two ends apparently alike in thickness. Length about 18 $\mu$ , thickness about 13 $\mu$ . Among these are found compound grains of few, mostly equal components (see type 14).
- Euphorbia cyparissias* Linn. (*Euphorbiaceæ*.) *Fresh root-stock*.—Grains rounded, oval, conical. Size about 10 $\mu$ . Separated-grains with 1 to 4 pressure facets. Size 2 to 8 $\mu$ . Only a few perfect compound grains were seen within the cells. They consist of 2 to about 6 and 8 chiefly unequal components.
- Croton eluteria* Swartz. *Cascarilla bark*. (*Euphorbiaceæ*.) *Dry bark*.—Grains rounded or oval, occasionally somewhat angular or irregular; many are hollow. Size about 12 $\mu$ . Poor in starch.
- Galipea officinalis* Hancock; *Cortex angusturæ verus*. (*Rutaceæ*.) *Dry bark*.—Grains spherical or angular with rounded angles; many with a cavity which is frequently large. Size about 12 $\mu$ . Poor in starch.

- Dictamnus albus* Linn. (*Rutaceæ*.) *Dry root*.—Grains rounded or angular. Size about  $8\mu$ . Many of them apparently separated-grains. Whole compound grains are seldom observed, and when present they consist of 2 to 8 almost equal components. Poor in starch.
- Guaiacum officinale* Linn.; *Cortex guaiaci*. (*Zygophyllaceæ*.) *Dry bark*.—Grains rounded, triangular with rounded angles, conical or elongated-oval, occasionally somewhat irregular; many with a small central cavity. Size about  $10\mu$ . Poor in starch.
- Muriophyllum verticillatum* Linn. (*Haloragacææ*.) *Base of dry rooted stems*.—Grains spherical or rounded-oval; the larger ones with a cavity. Size about  $10\mu$ . Among these, compound grains of few, nearly equal components are observed (see type 15).
- Trapa natans* Linn. (*Onagracææ*.) *Dry rooted stolons*.—Grains rounded or rounded-oval; the larger ones slightly compressed with a longitudinal slit from the narrow aspect. Size about  $12\mu$ . The starch resembles the eccentric-lenticular type. Doublets and triplets are also present.
- Punica granatum* Linn.; *Cortex radiceis granati*. (*Lythraceæ*.) *Dry cortex of the roots*.—Grains rounded to elongated-oval, sometimes triangular and often slightly irregular. Size about  $9\mu$ . Poor in starch.
- Potentilla tormentilla* Linn.; *Tormentilla erecta* Linn.; *Radix tormentillæ*. (*Rosacææ*.) *Dry root-stock*.—Grains rounded to oblong, frequently triangular with rounded angles, usually irregular; more or less compressed; from the narrow aspect a longitudinal slit is observed; occasionally somewhat thickened on the convex border, and thinned and squared on the opposite one. Size about  $17\mu$ . The grains seem to be affected by slight heat; from the broad as well as from the narrow aspect the margin is frequently bent with knob-like protuberances. The cells are packed with starch.
- Spiræa filipendula* Linn. (*Rosacææ*.) *Dry root-stock*.—Grains rounded-triangular, oval, conical, spindle-shaped; often more or less irregular; some with a cavity. Size about  $12\mu$ . Also compound grains of few equal or unequal components are observed. (See type 15.)
- Ononis spinosa*; *Radix ononidis*. (*Leguminosææ*.) *Dry root*.—Grains rounded, often somewhat angular, rarely oval; many with a smaller or somewhat larger central cavity. Size  $7\mu$ . Also compound grains of few components are observed. Some of the apparently simple ones may be separated-grains.
- Trifolium alpinum* Linn. (*Leguminosææ*.) *Dry root-stock*.—Grains rounded to oblong, conical, and elongated spindle-shaped; three or more times as long as thick. Length about  $10\mu$ .
- Trifolium montanum* Linn. (*Leguminosææ*.) *Dry root-stock*.—Grains oval, conical, spindle-shaped; frequently linear. Four or more times as long as thick. Length  $11\mu$ .
- Trifolium badium* Schreb. (*Leguminosææ*.) *Dry root-stock*.—Grains rounded, oval, conical; about twice as long as broad; the broad ones compressed, some with a small cavity. Length about  $10\mu$ . Doublets and triplets are also observed. All three species of *Trifolium* are poor in starch.
- Glycyrrhiza*. (*Leguminosææ*.) *Dry root*.—Grains usually spherical; sometimes elongated oval, or ovate-conical, frequently irregular; a few grains have a small central cavity. Size about  $10\mu$ . Also some compound grains of 2 to 4 components, and some separated-grains are observed. Starch is found in the pith, cortex, and wood.
- Phaca alpina* Jacq. (*Papilionacææ*.) *Dry root*.—Grains rounded to oval. Size about  $7\mu$ . Some compound grains of 2 to 4 components are also observed. Size  $9\mu$ . Poor in starch.
- Astragalus incanus* Linn. (*Leguminosææ*.) *Dry root-stock*.—Grains rounded or oval. Size about  $13\mu$ . Also compound grains of few mostly equal components are observed (see type 14). Not much starch in the pith cells, the walls of which are very thick, lamellate, and gelatinous.
- Chara*. (*Characææ*.)—The spores contain two kinds of grains: (1) large ones rounded and compressed (see type 2); (2) smaller grains without lamellæ or hilum. In shape the latter correspond to the starch-grains in the seeds of *Leguminosææ* and thus seem to approach type 3. They differ, however, in being thinned and knife-like at the concave border. This is always observed in grains at a certain stage of growth, and may later more or less completely disappear. Nägeli regards the large and small grains as being distinct, inasmuch as when viewed on end the former are always spindle-shaped or elliptical, never conical or pear-shaped. A small gas-bubble is noted in many of the small, usually homogeneous grains, after they have been subjected to dry heat ( $210^{\circ}$  C.) and examined in alcohol. This bubble indicates the position of the hilum, and corresponds to the middle of the curved slit which before treatment was visible. Eccentricity amounts therefore from one-half to one-third and slightly over.

- Chara fatida* A. Braun. *Fresh spores*.—Grains usually triangular, rarely quadrangular, oval, reniform or irregular; compressed to one-half or more of their width; one border, straight or concave, and thinned into a more or less knife-like edge; the other strongly convex and thickened, so that when viewed on end the grain appears conical or pear-shaped; without lamellæ or hilum; from the broad aspect a delicate curved slit is observed, from the center of which several short fissures may radiate. Size about 20 $\mu$ .
- Chara hispida* Linn. *Dry spores*.—Grains rounded; oval, frequently reniform or triangular with rounded angles; usually less dense at the straight or concave border; the broad ones compressed to half their width; from the narrow aspect a distinct slit, and from the broad longitudinal aspect a delicate slit is observed; the latter, in the reniform and triangular grains, is curved toward the convex margin. Size 16 to 25 $\mu$ .
- Chara aspera* Willd. *Dry spores*.—Grains usually triangular or reniform, sometimes oval or irregular; scarcely half to almost as broad as long; shortened to two-fifths of their length, one side of the margin usually strongly convex and thickened, the other, slightly convex, straight, or concave and thinned to a knife-like edge; occasionally from the broad aspect with a very delicate curved and somewhat eccentric slit; from the narrow aspect a somewhat more marked, straight median slit. Size about 16 $\mu$ .
- Chara alopecuroides* Willd. *Dry spores*.—Grains reniform, or 3, rarely 4 to 5, angles; usually almost as broad as long; compressed to one-half or over their width; thicker on one edge than on the other. Size about 22 $\mu$ . Among these there are smaller grains, which have one thickened convex and one sharpened more concave edge, as in *Chara aspera*.

- The following species of *Chara* have grains similar to those already described above, namely: *Chara bauceri* A. Braun, size about 25 $\mu$ ; *Chara barbata* Meyen, size about 20 $\mu$ ; *Chara fragilis* Desv., size about 17 $\mu$ ; *Chara contraria* A. Braun., size about 20 $\mu$ ; *Chara gymnophylla* A. Braun, size about 21 $\mu$ ; *Chara coronata* Zig., size about 18 $\mu$ . These were all examined in the dry state.
- Isoëtes lacustris* Linn. (*Isëtaceæ*.) *Dry gymnosporos*.—Grains spherical, rarely somewhat irregular. Size about 8 $\mu$ . Starch not plentiful, embedded among oil-bodies and plastids.
- Pinus sylvestris* Linn. (*Coniferæ*.) *Dry pollen*.—Grains oval to pear-shaped. Size about 4 $\mu$ . Poor in starch and rich in oil.
- Avena pubescens* Linn. (*Graminaceæ*.) *Fresh pollen*.—Grains rounded, oval, pear-shaped. Size about 4.5 $\mu$ . Starch plentiful.
- Bromus mollis* Linn. (*Graminaceæ*.) *Fresh pollen*.—Grains as in preceding. Size about 6 $\mu$ . Starch plentiful.
- Alpinia nutans* Rose.; *Globba nutans*. (*Zingiberaceæ*.) *Pollen*.—According to Payen (*Ann. Sc. Nat.*, 1838, p. 26, pl. 5, fig. 4), the grains are oblong; sometimes curved, about 3 times as long as broad. Size about 15 $\mu$ . Starch plentiful in the large pollen-grains; lacking in the smaller ones.
- Naias major* Roth. (*Naiadaceæ*.) *Pollen*.—According to Payen (*loc. cit.*, p. 28, pl. 5, figs. 5 and 6) and Fritsche (*Ueber den Pollen*, Taf. III, fig. 5), the grains are oval to almost cylindrical, more or less curved, and about 2 to 3 times as long as broad. Size about 7.5 $\mu$ . Starch plentiful.
- Ruppia maritima* Linn. (*Naiadaceæ*.) *Pollen*.—According to Payen (*loc. cit.*, p. 27, pl. 3, fig. 25), the grains are rounded or oval, more or less irregular, occasionally cylindrical with rounded ends, more or less crooked. Size about 11 $\mu$ . Starch plentiful.
- Syringa vulgaris* Linn. (*Oleaceæ*.) *Fresh pollen*.—Grains rounded. Size hardly 3 $\mu$ . Starch less plentiful than oil.
- Veronica chamædrys* Linn. (*Scrophulariaceæ*.) *Fresh pollen*.—Grains rounded, oval, pear-shaped. Size about 6 $\mu$ . Starch plentiful.
- Ranunculus bulbosus* Linn. (*Ranunculaceæ*.) *Fresh pollen*.—Grains rounded. Size at most 3 $\mu$ .
- Viola cornuta* Linn. (*Violaceæ*.) *Fresh pollen*.—Grains rounded, oval, pear-shaped. Size about 4 $\mu$ . Starch plentiful.
- Geranium molle* Linn. (*Geraniaceæ*.) *Fresh pollen*.—Grains more or less rounded. Size about 3 $\mu$ . Starch quite plentiful.
- Geranium pratense* Linn. (*Geraniaceæ*.) *Fresh pollen*.—Grains rounded, oval, pear-shaped. Size about 5 $\mu$ . Similar grains are found in the cell-lumen and in the cell-tissues.

- Encephalartos spiralis* Lehm. (*Cycadaceæ*.) *Dry seed*.—Grains rounded to oval, sometimes triangular to reniform, frequently more or less irregular; the broadest ones compressed to about one-third the width; without lamellæ and hilum; a longitudinal slit which is quite distinct from the narrow aspect, and rarely visible or less distinct from the broad one; Size 15 to 20 $\mu$ . Among the above are some compound grains of few components. Many of the grains are affected by heat. The starch-grains in the embryo belong to the eccentric-conical type.
- Brachypodium pinnatum* Beauv.; *Festuca pinnata* Moench.; *Bromus rupestris* Host. (*Graminaceæ*.) *Dry seed*.—Grains rounded to oblong, frequently conical, triangular, or reniform, usually more or less irregular; 1 to 4 times as long as broad; the broad ones compressed; some grains are hollow; from the narrow aspect a longitudinal slit is observed in the compressed forms. Length about 11 $\mu$ , width 8 $\mu$ . Among the above some compound grains of 2 to 3 components and some separated-grains are observed. Starch as in *Bromus*.
- Boissiera bromoides* Hochst. (*Graminaceæ*.) *Dry seed*.—Grains rounded and rounded-triangular to conical and oblong; the broad ones compressed. Size about 7 $\mu$ . Starch as in *Bromus*.
- Boissiera bromoides* Hochst., var. *pappophorum pumilio* Trin. (*Graminaceæ*.) *Dry seed*.—Grains oval to elongate-lanceolate; one-third to two-thirds as thick as long. Length about 6 $\mu$ , thickness about 2.5 $\mu$ .
- Bromus*. (*Graminaceæ*.) *Seed*.—The grains are simple, usually without lamellæ or hilum. Structure rarely distinct, if so always centric, but sometimes more spherical, sometimes more rounded-lenticular, sometimes oval or lanceolate, or even almost terete and compressed. Individual distinctly compound grains consisting of 2 to 3 components rarely occur. In many species, however, there are forms in varying numbers which are somewhat constricted in the middle and may be doublets.
- Bromus madritensis* Linn. *Dry seed*.—Grains spherical or rounded-oval, occasionally somewhat irregular; many slightly compressed, lamellæ concentric, delicate and numerous; instead of the hilum a small cavity is observed, which is either rarely spherical and occasionally elongated, or frequently flattened; from this cavity a few or numerous fissures radiate; from the narrow aspect a distinct median cleft is very often observed.
- Bromus polystachus* DC. *Unripe seeds*.—Grains simple, as in the preceding.
- Bromus maximus* Desf. *Dry seed*.—Grains as in *Bromus madritensis*, usually somewhat smaller in diameter.
- Bromus gussonii* Parl. *Dry seed*.—Grains rounded-oval, rounded-reniform; triangular, very frequently somewhat contracted in the middle and thus quadrangular; slightly compressed, up to about half their width; lamellæ invisible, or concentric and delicate; from the narrow aspect a longitudinal slit is frequently observed, from the broad aspect occasionally with some radial fissures. Size about 35 $\mu$ . The grains resemble those of the *Hordeaceæ*, differing, however, in their more angular (never circular) forms as well as in the lack of small grains. Grains of almost the same size are only found in one cell.
- Bromus rigidus* Roth. *Dry seed*.—Grains rounded, reniform, 3 to 4 angles; occasionally somewhat irregular, and frequently somewhat contracted in the middle; slightly compressed up to about half their width; lamellæ concentric and delicate, or indistinct; cavity often slit-like, from which a few short radial fissures may emerge. Size about 33 to 40 $\mu$ . Starch as in *Bromus gussonii*. Large starch-grains of seeds from botanical gardens in Berlin (1855) are 24 to 29 $\mu$ , and those from the Paris gardens 20 to 24 $\mu$ . Otherwise they agree with those above described.
- Bromus wolgensis* Jacq. *Dry seed*.—Grains rounded, or rounded-oval, more rarely oval, rounded pear-shaped, or triangular; two-thirds to as broad as long; not at all or slightly compressed with single distinct, concentric lamellæ; instead of the central hilum either a rounded or oblong cavity, or rarely a longitudinal slit is observed. Size about 31 $\mu$ . Also some compound grains of 2 to 4 components are present.
- Bromus squarrosus* Linn. *Dry seed*.—Grains rounded to oval, occasionally somewhat irregular; half to as broad as long; the broad ones compressed; occasionally with a central cavity. Length about 11 $\mu$ .
- Bromus arvensis* Linn. *Dry seed*.—Grains rounded to oval, slightly compressed; many with a cavity. Length about 17 $\mu$ .

- Bromus rubens* Linn. *Dry seed*.—Grains rounded, rounded-reniform, oval, frequently somewhat angular, the broad ones compressed to about half their width. Length about 13 to 17 $\mu$ .
- Bromus erectus* Huds. *Dry seed*.—Grains rounded, rounded-reniform, oval, shortened-conical, usually irregular; the broad ones compressed to about half their width; from the narrow aspect a longitudinal slit is frequently observed. Length about 13 $\mu$ .
- Bromus sterilis* Linn. *Dry seed*.—Grains rounded and rounded-triangular to oval, frequently somewhat angular or irregular; usually compressed to about one-half; with a central cavity or median slit from which fissures occasionally radiate. Size about 36 $\mu$ .
- Bromus tectorum* Linn. *Dry seed*.—Grains rounded to oval, sometimes triangular and quadrangular with rounded angles, frequently somewhat irregular; usually strongly compressed; from the narrow aspect a longitudinal slit is observed. Length about 23 $\mu$ .
- Bromus adoensis* Hochst. *Dry seed*.—Grains rounded, triangular to quadrangular with rounded angles, oval, shortened-conical; usually more or less irregular; the broad ones compressed to about one-half and over of their width. Length about 18 $\mu$ .
- Bromus ciliatus* Linn. *Dry seed*.—Grains rounded to oval, the broad ones compressed to half their width; a central cavity is occasionally observed. Length about 14 $\mu$ .
- Bromus brachystachys* Hornung. *Dry seed*.—Grains rounded, oval, shortened-conical; occasionally somewhat irregular; the broad ones compressed to about half their width; instead of a central hilum, a cavity is present which is rounded from the broad aspect and cleft-like from the narrow aspect. Length about 14 $\mu$ .
- Bromus caucasicus* Fisch. *Dry seed*.—Grains rounded, oval, shortened-conical, usually irregular; the broad ones compressed to about one-third of their width. Size about 12 $\mu$ .
- Bromus laxus* Hornem. *Dry seed*.—Grains rounded to oval-oblong, very often irregular and angular, and sometimes constricted in the middle; the broad ones compressed. Length about 14 $\mu$ .
- Bromus aleutensis* Trin. *Dry seed*.—Grains rounded-oval, oval-oblong, elongated-triangular; sometimes reniform or quadrangular, frequently somewhat irregular; half to almost as broad as long; the broad ones compressed to half their width. Length about 17 to 20 $\mu$ , width about 14 $\mu$ . Among the above some compound grains of 2 to 3 equal components are observed.
- Bromus longiflorus* Willd. *Dry seed*.—Grains rounded to oval-oblong, often somewhat irregular and angular and frequently constricted in the middle; the broad ones compressed to a little more than half their width. Length 10 to 12 $\mu$ . A central cavity is sometimes seen in each half of the constricted grains, demonstrating that they are doublets without a visible line of division.
- Bromus purgans* Linn. *Dry seed*.—Grains rounded, rounded-triangular to oblong, ovoid, and conical; occasionally constricted in the middle; the broad ones compressed to one-third of their width; from the narrow aspect a longitudinal slit is frequently observed. Length about 12 $\mu$ .
- Bromus brizæformis* F. & M. *Dry seed*.—Grains oval, oblong, conical; occasionally slightly compressed. Length about 6 $\mu$ .
- Bromus lanccolatus* Roth. *Dry seed*.—Grains oblong, rarely rounded. Length about 6 $\mu$ .
- Bromus velutinus* Schrad., var. *hordeaceus* Gmel.; *Bromus secalinus* var. *hordeaceus*. *Dry seed*.—Grains rounded-oval to oblong; one-half to three-fourths as broad as long, the broad ones slightly compressed, some have a central cavity. Length 10 to 11 $\mu$ .
- Bromus patulus* Mert. & Koch. *Dry seed*.—Grains rounded-oval to oblong, sometimes slightly irregular, two-fifths to four-fifths as broad as long; the broad ones slightly compressed; many with a central cavity. Length about 12 $\mu$ .
- Bromus schraderi* Kunth; *Ceratochloa pendula* Schrad. *Dry seed*.—Grains rounded, reniform, oval, oblong, frequently irregular; compressed, many with a central cavity. Length about 20 $\mu$ .
- Bromus unioloides* Willd.; *Ceratochloa unioloides* DC. *Dry seed*.—Grains as in the preceding species, the broad ones compressed to one-half and more of their width; many with a longitudinal slit-like cavity.
- Bromus commutatus* Schrad. *Dry seed*.—Grains rounded to oblong; frequently somewhat angular or irregular; two-fifths to almost as broad as long; the broad ones rather strongly compressed. Length about 13 $\mu$ .
- Bromus inermis* Poll. *Dry seed*.—Grains rounded-oval to oblong; broad ones compressed to half their width. Length about 10 $\mu$ .

- Bromus canadensis* Michx. *Dry seed*.—Grains rounded, oval, oval spindle-shaped, conical; the broad ones compressed to half their width. Length about  $8\mu$ .
- Bromus pendulinus* Schrad. *Dry seed*.—Grains rounded-oval to oblong and conical; sometimes constricted in the middle; frequently irregular; compressed to about one-half and one-third their width. Length about  $21\mu$ .
- Bromus arduennensis* Kunth. *Dry seed*.—Grains rounded to oblong, occasionally triangular and quadrangular with blunt angles; the broad ones compressed to about one-third their width; from the narrow aspect the central cavity is slit-like. Length about  $12\mu$ .
- Bromus asper* Murr. *Dry seed*.—Grains rounded-oval to elongated rod-shaped and conical; usually twice as long as broad. Length 5 to  $7\mu$ .
- Bromus secalinus* Linn. *Dry seed*.—Grains oval to elongated-lanceolate; one-third to two-thirds as broad as long. Length 6 to  $7\mu$ .
- Bromus diandrus* Curt. Hort. berol., 1855. *Dry seed*.—Grains rounded-oval and rounded-triangular to rod-shaped and elongated-conical; the broad ones compressed to one-third their width; narrow ones about 3 times as long as broad and almost terete; many somewhat irregular, and several more or less constricted. Length about  $14\mu$ .
- Bromus mollis* Linn. *Dry seed*.—Grains rarely rounded-oval, usually oblong or lanceolate. Length about 4 to  $5\mu$ , width 1.3 to  $1.8\mu$ .
- Bromus divaricatus* Rohde. *Dry seed*.—Grains rounded-oval to lanceolate, frequently irregular and angular, often triangular; one-fourth to almost as broad as long; the broad ones strongly compressed. Length about  $15\mu$ .
- Bromus vestitus* Nees. *Dry seed*.—Grains oval to lanceolate and lanceolate-linear; occasionally slightly constricted in the middle; 1.33 to 3 and 4 times as long as broad; the broad ones compressed to half their width. Length about  $10\mu$ .
- Bromus confertus* Biebrst. *Dry seed*.—Grains minute, rounded or oblong. Length  $2.5\mu$ , width hardly  $1.4\mu$ . If the starch-grains are freed from the cells they frequently hang together in globular masses, but these are unquestionably not compound grains. At least not any are ever noted within the uninjured cell.
- Hordeaceæ* (*Triticum*, *Agropyrum*, *Secale*, *Elymus*, *Hordeum*, *Aegilops*, *Braconnotia*). *Seeds*.—Besides the large grains which are related to the centric lenticular type, there are small grains in great numbers observed in the inner tissues, while only the small grains are found in the outer cells. They are spherical, rounded-angular, or even almost polyhedral, and are more or less markedly compressed. Size about  $10\mu$ . The grains are simple, although separated-grains are also found among them. Whole doublets and triplets are rarely observed.
- Cyperaceæ*.—The dry seeds contain starch-grains which are rounded or oval, sometimes compressed into a lenticular form; many of them are angular or polyhedral and resemble separated-grains; the latter are, however, undoubtedly simple grains which are merely changed in form as a result of pressure. Plastids which resemble compound starch-grains and which become yellow or golden yellow when treated with iodine are often observed.
- Cyperus flavescens* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains rounded or oval, frequently rounded-angular. Size 8 to  $11\mu$ .
- Cyperus strigosus* Linn. (*Cyperaceæ*.) *Dry*.—Grains rounded, oval, oval-conical, frequently somewhat irregular and angular. Size 8 to  $10\mu$ . Compound plastids are also observed.
- Mariscus jacquini* Humb., Kunth. (*Cyperaceæ*.) *Dry seed*.—Grains rounded or polygonal, occasionally irregular, strongly compressed. Size about  $12\mu$ , thickness 1 to  $2\mu$ .
- Mariscus umbellatus* Vahl. (*Cyperaceæ*.) *Dry seed*.—Grains as in the preceding.
- Kyllingia odorata* Vahl. (*Cyperaceæ*.) *Dry seed*.—Grains rounded or rounded-oval, angular or polygonal, compressed to half or more of their width. Size about  $7\mu$ , thickness about  $3\mu$ . Plastids are lacking.
- Heleocharis palustris* R. Br. (*Cyperaceæ*.) *Dry seed*.—Grains rounded, usually angular. Size about  $8\mu$ .
- Heleocharis ovata* R. Br. (*Cyperaceæ*.) *Dry seed*.—Grains rounded or rounded-angular; and compressed to half their width. Size about 8 to  $10\mu$ .
- Scirpus mucronatus* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains polyhedral, slightly compressed. Size about  $8\mu$ .
- Scirpus maritimus* Linn. (*Cyperaceæ*.) *Dry*.—Grains spherical or slightly angular. Size about  $5\mu$ .

- Isolepis setacea* R. Br.; *Scirpus setaceus* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains rounded-angular, many irregular, and some also polygonal; compressed into lenticular form, occasionally with large reticulated markings. Size about 9 $\mu$ . Plastids scarce and irregular.
- Isolepis supina* R. Br.; *Scirpus supinus* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains polygonal, compressed, with square reticulations on the surface. Size about 10 $\mu$ . Starch-grains are crowded in the cells. Plastids none or very few.
- Isolepis eckloniana* Schrad.; *Isolepis verruculosa* Steud. (*Cyperaceæ*.) *Dry seed*.—Grains rounded-angular or polygonal, usually irregular; compressed to one-third of their width. Size about 10 $\mu$ . Plastids few and irregular.
- Isolepis holoschanus* Roem. and Schult.; *Scirpus holoschanus* Linn. (*Cyperaceæ*.)—Grains as in the preceding.
- Fimbristylis dichotoma* Vahl. (*Cyperaceæ*.) *Dry seed*.—Grains rounded, oval, usually angular, often strongly compressed. Size about 10 $\mu$ . Plastids of irregular form are also present.
- Fimbristylis annua* Roem. and Schult. (*Cyperaceæ*.) *Dry seed*.—Grains rounded or rounded-angular, and compressed. Size about 9 $\mu$ .
- Fimbristylis brizoides* Smith.; *Fimbristylis laxa* Vahl. (*Cyperaceæ*.) *Dry seed*.—Grains rounded or rounded-oval, frequently somewhat angular, rarely almost polyhedral; slightly or not at all compressed. Size 9 to 12 $\mu$ .
- Eriophorum alpinum* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains rounded, oval, frequently angular. Size about 6 $\mu$ . Oil plentiful; plastids numerous.
- Eriophorum vaginatum* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains as in the preceding species.
- Rhynchospora fusca* Roem. and Schult. (*Cyperaceæ*.) *Dry seed*.—Grains rounded, usually angular, and often strongly compressed. Size about 15 $\mu$ . Some doublets and triplets are also observed. Plastids are irregular.
- Cladium mariscus* R. Br. (*Cyperaceæ*.) *Dry seed*.—Grains rounded or angular. Size about 9 $\mu$ . Some compound plastids are also observed.
- Chaetospira nigricans* Kunth.; *Schænus nigricans* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains rounded. Size about 5 $\mu$ . Some oil and plastids are also observed.
- Blysmus compressus* Panz.; *Schænus compressus* Pers. (*Cyperaceæ*.) *Dry seed*.—Grains spherical; rounded-triangular, oval, shortened-conical; occasionally somewhat angular, frequently slightly compressed. Size about 9 $\mu$ .
- Scleria triglomerata* Michx.; *Cladium triglomerata* Nees. (*Cyperaceæ*.) *Dry seed*.—Grains rounded, rarely elongated-oval, frequently somewhat angular, the broad ones occasionally compressed. Size about 6 to 10 $\mu$ . There is a great amount of oil and considerable starch in seeds, which are not fully ripe.
- Scleria bracteata* Cav. (*Cyperaceæ*.) *Dry seed*.—Grains as in preceding, but somewhat less numerous; size not more than 7 $\mu$ . Seeds not fully developed.
- Scleria microcarpa* Nees. (*Cyperaceæ*.) *Dry seed*.—Grains rounded, rarely oval, usually more or less angular, the broad ones slightly compressed. Size 8 $\mu$ . Seeds are well developed, and contain much oil and starch, as well as numerous almost polyhedral plastids.
- Scleria ophryoscleria*, species from Brazil. (*Cyperaceæ*.) *Ripe seed*.—Grains similar to the preceding. Size about 7 $\mu$ . Starch wanting in the embryo.
- Scleria hispidula* Hochst. (*Cyperaceæ*.) *Dry seed*.—Grains rounded. Size almost 5 $\mu$ . Comparatively little oil and starch in unripe seeds.
- Carex pulicaris* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains rounded-angular to polyhedral; frequently compressed; many have a central cavity. Size about 16 $\mu$ .
- Carex arenaria* Linn. (*Cyperaceæ*.) *Dry seed*.—Grains angular with rounded angles or polyhedral; the large ones compressed. Size about 7 $\mu$ .
- Carex mazina* Scop. (*Cyperaceæ*.) *Dry seed*.—Grains rounded-angular; the large ones have a central cavity. Size about 6 $\mu$ . Oil rather plentiful.
- Elyna spicata* Schrad. (*Cyperaceæ*.) *Dry seed*.—Grains spherical or oval, frequently angular. Size about 6 $\mu$ . Starch-grains and irregular plastids are crowded in the cells.
- Kobresia caricina* Willd.; *Elyna caricina* Mert. and Koch. (*Cyperaceæ*.) *Dry seed*.—Grains spherical, rarely oval or shortened-conical, occasionally somewhat angular. Size about 6 $\mu$ .
- Flagellaria indica* Linn. (*Flagellariaceæ*.) *Dry seed*.—Grains rounded or usually polyhedral as a result of pressure. Size about 5 $\mu$ . Starch-grains fill the cells.

- Lilium bulbiferum* Linn. (*Liliaceæ*.) *Fresh endosperm or unripe seeds*.—Grains rounded, rounded-triangular, oval, elliptical, shortened-conical; two-thirds (rarely one-half) to as broad as long. Length about  $10\mu$ , width  $7\mu$ . Starch quite plentiful, little oil, and much protoplasm are found in the thick-walled cells.
- Sparganium natans* Linn. (*Typhaceæ*.) *Dry seed endosperm*.—Grains rounded, oval-elliptical, shortened-conical, frequently somewhat irregular; three-fifths to as broad as long, the broad ones slightly compressed, many with a cavity. Size 7 to  $8\mu$ . Among the above are some doublets and triplets with parts almost equal.
- Quercus pedunculata* Willd. (*Cupuliferæ*.) *Dry cotyledons*.—Grains rounded or oval, sometimes irregularly angular with blunt angles; frequently slightly compressed; two-thirds to three-fourths as thick as broad; the hilum and very delicate lamellæ are rarely distinct; eccentricity about one-fourth. Size about  $29\mu$ . Compound grains of few mostly unequal components. (Type 15.)
- Quercus cerris* Linn. (*Cupuliferæ*.) *Dry cotyledons*.—Grains rounded, oval, shortened-conical, frequently more or less irregular; lamellæ indistinct; instead of the hilum a small cavity toward the thicker end is frequently observed; eccentricity usually one-half, rarely one-third and one-fourth; frequently one or two slits with delicate, radiating fissures are found. Size about  $26\mu$ . Isolated compound grains of few components also observed.
- Quercus ilicifolia* Wangh. (*Cupuliferæ*.) *Dry cotyledons*.—Grains as in both of the preceding species; the greatest number are oval. Size 17 to  $18\mu$ .
- Castanea vesca* Gart. (*Cupuliferæ*.) *Fresh and dry cotyledons*.—Grains rounded, triangular and quadrangular with rounded angles, oval, shortened-conical, frequently irregular; half to as broad as long; the broad ones slightly compressed; lamellæ are invisible, or very delicate; hilum in the fresh grains frequently indistinct. After desiccation, instead of the hilum, either a small cavity or occasionally a delicate triangular slit is observed, which is either central or toward the thicker margin. Size about  $20\mu$ , rarely  $27\mu$ . Many grains are triangular, thickened at the end in which the hilum is located, and thinned and squared at the opposite end. These belong to the cuneiform type (type 8). Among the above are some semi-compound grains, also some doublets and triplets, the components of which are usually unequal. Size of the separated-grains is about  $10\mu$ .
- Fagus sylvatica* Linn. var. *pendula*. (*Cupuliferæ*.) *Dry cotyledons*.—Grains spherical, the larger ones occasionally somewhat irregular; many have a small central cavity. Size about  $6\mu$ .
- Forestiera acuminata* Poir.; *Borya acuminata* Willd. (*Olcaceæ*.) *Dry cotyledons*.—Grains rounded or oval, frequently angular or irregular. Size about  $7\mu$ . Rather rich in starch, besides much oil. Only oil is found in the endosperm.
- Cinnamomum ceylanicum* Nees. (*Lauraceæ*.) *Dry seed endosperm*.—Grains spherical to oval with single radial fissures. Size 15 to  $18\mu$ . Also some compound grains of 2 to 4 equal components are observed. Oil and starch appear to be present in equal quantities.
- Apollonias canariensis* Nees. (*Lauraceæ*.) *Dry cotyledons*.—Grains rounded, conical, oblong and elongated spindle-shaped, frequently somewhat irregular. Size about  $7\mu$ .
- Agathophyllum aromaticum* Willd. (*Lauraceæ*.) *Dry cotyledons*.—Grains spherical, rarely somewhat angular, frequently with a small central cavity. Size about  $13\mu$ . Some compound grains of 2 to 4 equal components are also present.
- Hernandia* sp. (*Lauraceæ*.) *Dry cotyledons*.—Grains rounded or angular. Size about  $5\mu$ , rarely more. Some starch and much oil; seeds probably not entirely ripe.
- Plumbaginaceæ*. *Seed endosperm*.—The starch-grains (only those in ripe, dry seeds were examined) entirely fill the cells. They are rarely rounded, usually either bluntly angular, or more often polyhedral with sharp edges and angles, thus completely resembling separated-grains. No compound grains were noticed even within cells, so that in all probability they are all simple and have become flattened as result of pressure; even the small ones ( $2\mu$ ) sometimes show an angular structure. In all likelihood the grains belong to the centric-spherical or oval type.
- Armeria formosa* Hort. (*Plumbaginaceæ*.) *Dry seed endosperm*.—Grains polyhedral, usually with sharp edges and angles, frequently irregular; two-fifths to as broad as long; frequently with a rounded or oblong cavity from which sometimes radial fissures emerge. Size 15 to  $18\mu$ .

- Armeria alpina* Willd., var. *angustifolia*. (*Plumbaginaceæ*.) *Dry seed*.—Grains rounded or oval, bluntly angular or sharply polyhedral; two-fifths to as thick as long; usually with a rounded or oblong cavity and frequently with radial fissures. Size about 8 to 21 $\mu$ .
- Statice limonium* Linn. (*Plumbaginaceæ*.) *Dry seed endosperm*.—Grains rounded or oval, blunt-angular, or polyhedral, rarely with sharp edges and angles; frequently with a cavity or slit. Size about 3 to 18 $\mu$ .
- Statice elata* Fisch. (*Plumbaginaceæ*.) *Dry seed*.—Grains rounded to oblong, angular, or sharply polyhedral; one-third to as thick as long; either a rounded or an oblong cavity, or even a longitudinal slit, is frequently observed, from which fissures occasionally radiate. Many grains are slightly shrunken. Size about 27 to 32 $\mu$ .
- Goniolimon eximium* Boiss. (*Plumbaginaceæ*.) *Dry seed endosperm*.—Grains isodiametric to twice as long as thick, polyhedral with sharp angles and edges; frequently they have a central cavity; without either fissures or lamellæ. Size about 28 $\mu$ . Cells are thin-walled and filled with starch.
- Plumbago micrantha* Ledeb. (*Plumbaginaceæ*.) *Dry seed endosperm*.—Grains sharply polyhedral, with a small or a large cavity. Size about 8 to 10 $\mu$ .
- Campanula* sp. (*Campanulaceæ*.) *Fresh unripe seed endosperm*.—Grains rounded-oval or rounded-angular. Size about 7 $\mu$ . Also some separated-grains are present. Seeds very young, and contain considerable starch.
- Menodora* sp. (*Oleaceæ*.) *Dry seed tegment*.—Grains rounded, rounded-angular, or polyhedral; with a large or a small cavity. Isolated compound grains are also present.
- Erycibe paniculata* Roxb. (*Leguminosæ*.) *Dry cotyledons*.—Grains spherical or rounded-oval, occasionally somewhat angular. Size about 7 $\mu$ .
- Eutoea viscida* Benth. (*Euphorbiaceæ*.) *Dry seeds*.—Grains spherical; a small central cavity is found in the larger ones. Size about 6 to 8 $\mu$ . A great deal of oil is found, and most of the seeds contain only oil. Starch in small quantities is probably present in the seed, which is not fully ripe.
- Phacelia congesta* Hook. (*Hydrophyllaceæ*.) *Dry seed endosperm*.—Grains as in the preceding species.
- Digitalis lutea* Linn. (*Scrophulariaceæ*.) *Fresh, unripe fruit, placenta, and funiculus*.—Grains rounded, or oval, and frequently angular. Size about 10 $\mu$ . Also compound grains of 2 to 8 equal components are observed. Starch plentiful; wanting in the ovules and seeds.
- Verbascum schraderi* Mey. (*Scrophulariaceæ*.) *Fresh placenta of unripe fruit*.—Grains angular, here probably mostly separated-grains. Size about 6 to 8 $\mu$ . Starch very plentiful.
- Verbascum schraderi* Mey. (*Scrophulariaceæ*.) *Fresh, unripe seed-coats*.—Grains rounded or oval, usually angular. Size about 5 $\mu$ . Starch rather plentiful.
- Thunbergia fragrans* Roxb. (*Acanthaceæ*.) *Dry seeds*.—Grains spherical, oval, or somewhat irregular. Size about 10 $\mu$ . Many seem to be compound. Some show colorless appendages which are not stained after treatment with iodine. The grains are poor in starch, but rich in oil; the former may disappear at maturity.
- Delphinium ajacis* Linn. (*Ranunculaceæ*.) *Fresh unripe seed coats*.—Grains rounded, or angular with round angles. Size about 8 $\mu$ . Starch rather plentiful.
- Chelidonium majus* Linn. (*Papaveraceæ*.) *Fresh seed coat*.—Grains rounded. Size about 7 to 9 $\mu$ . They are stained brown when treated with iodine.
- Chelidonium majus* Linn. (*Papaveraceæ*.) *Fresh unripe seed coat*.—Grains rounded. Size 5 to 6 $\mu$ . They are stained brown or violet when treated with iodine.
- Brassica napus* Linn. (*Brassicaceæ; Cruciferae*.) *Fresh unripe seeds*.—Grains (in the embryo) rounded or oval, frequently somewhat angular; some are evidently separated-grains. Size about 5 $\mu$ . Compound and separated-grains predominate in the seed coats (see type 14). Starch is plentiful in the perisperm, especially rich in the embryo, and mostly found in the cells of the seed-coats with the exception of the outermost layer, the walls of which soon thicken. The perisperm later entirely disappears, the seed-coats and the embryo lose their starch, since in the former the membranes are thickened and in the latter starch is replaced by oil. As the green seeds turn yellow the solution of the starch takes place; in the brownish-yellow seeds it has entirely disappeared. In the earliest stage the embryo contains merely oil; when it has entirely replaced the perisperm and thus completely filled up the cavity within the seed coats both oil and starch are found, while in the latest stages oil alone is again observed.

- Cucumis sativis* Linn. (*Cucurbitaceæ*.) *Fresh unripe cotyledons*.—Grains rounded, rarely angular. Size about 5 to 6 $\mu$ . Starch rather plentiful.
- Vatica robusta* Steud.; *Shorea robusta* Roxb. (*Dipterocarpaceæ*.) *Dry cotyledons*.—Grains oval, elliptical, shortened-conical, rarely rounded-oval to oblong; occasionally somewhat angular, two-fifths to two-thirds as broad as long; the larger ones frequently have a cavity or a slit. Length about 11 $\mu$ , thickness about 8 $\mu$ . These grains may belong to the eccentric-conical type (type 7).
- Thea bohea* Linn. (*Camelliaceæ*.) *Dry cotyledons*.—Grains spherical or rounded-oval, rarely oval; instead of a hilum there is a small central cavity with radial fissures; the cavity is usually central, rarely one-half to two-thirds eccentric. Size about 16 to 19 $\mu$ . The starch probably belongs to the eccentric-conical type. Some compound grains of few unequal components are also observed as in type 14.
- Calophyllum lanceolatum* Blume. (*Guttiferæ*.) *Dry cotyledons*.—Grains spherical or slightly angular; majority with a small central cavity. Size about 10 to 12 $\mu$ . Some compound grains of 2 to 3 equal components are also observed.
- Calophyllum taamahaca* Willd. (*Guttiferæ*.) *Dry cotyledons*.—Grains mostly rounded. Size about 6 $\mu$ . Isolated larger, oval ones. Size about 13 $\mu$  or more. Starch appears to be in the process of solution; oil very plentiful.
- Acer laurinum* Hook. (*Lapindaceæ*.) *Dry cotyledons*.—Grains rounded or oval. Size 8 to 10 $\mu$ . Many are distinctly separated-grains with one curve and 1 to 3 pressure facets, others appear to be simple. Many of them are probably in the process of solution, so that the starch may disappear at maturity of the seed; oil plentiful.
- Acer pseudoplatanus* Linn. (*Lapindaceæ*.) *Fresh, unripe, still very green cotyledons*.—Grains rounded, rarely oval, usually more or less angular. Size about 5 to 7 $\mu$ . Starch rather plentiful.
- Banisteria* sp. (*Malpighiaceæ*.) *Dry cotyledons*.—Grains rounded to almost polyhedral. Size about 5 $\mu$ . The starch-grains lie singly or very few in the cells, which are filled with plastids of rounded, angular shape.
- Aleurites moluccana* Willd. (*Euphorbiaceæ*.) *Dry seeds*.—Grains spherical, slightly angular, or polyhedral; a small central cavity is found in the larger ones. Size about 7 $\mu$ .
- Aleurites* sp. (*Euphorbiaceæ*.) *Dry cotyledons*.—Grains spherical to oval, sometimes slightly irregular or angular. Size about 11 $\mu$ . Starch not very plentiful, and embedded between plastids. The albumen adhering to the seed coats was not examined.
- Ochna lucida* Lam. (*Ochnaceæ*.) *Dry cotyledons*.—Grains rounded, oval, elliptical, many somewhat angular; half to as thick as long; the larger ones have a small cavity. Length about 5 to 6 $\mu$ , thickness about 4 $\mu$ . The seeds are probably not fully developed. Protoplasm and rounded or angular plastids were found along with the starch. Fatty oil seems to be wanting.
- Ochna squarrosa* Linn. (*Ochnaceæ*.) *Dry cotyledons*.—Grains rounded, oval, frequently angular; the broad ones slightly compressed; the larger ones have a small central cavity. Size about 7 $\mu$ . Some oil is present.
- Ammannia latifolia* Linn. (*Lythraceæ*.) *Dry seeds*.—Grains spherical, or almost so, with small central cavity from which single fissures occasionally radiate. Size about 16 $\mu$ . Some doublets are also observed. Besides the oil the seeds contain varying amounts of starch, or sometimes none.
- Ammannia vesicatoria* Roxb.; *Ammannia baccifera* Linn. (*Lythraceæ*.) *Dry seeds*.—Grains rounded or oval, occasionally somewhat angular or irregular; the broad ones slightly or not at all compressed; with a small, almost central, cavity, and some single radial fissures. Size about 10 to 13 $\mu$ . Some doublets and triplets are found among the simple grains. The seeds of both species contain much oil. Starch, which occurs in varying quantities, is probably found in special cells. On pressing the seeds the oil comes out first, and later the starch.
- Galega biloba* Sweet. (*Leguminosæ*.) *Dry cotyledons*.—Grains rounded or oval, sometimes irregular. Size about 4 to 5 $\mu$ . Starch is not very plentiful, in addition much protoplasm and little oil is present.
- Arachis hypogæa* Linn. (*Leguminosæ*.) *Dry cotyledons*.—Grains spherical, with a small central cavity. Size about 12 $\mu$ . Much oil is also found.

*Guilandina bonduc* Linn. (*Leguminosæ*.) *Dry cotyledons*.—Grains spherical, many with a small central cavity; in the larger ones single, delicate, short fissures are occasionally observed. Size about  $7\mu$ .

*Acacia melanoxyylon* R. Br.; *Acacia latifolia* Desf. (*Leguminosæ*.) *Dry cotyledons*.—Grains spherical or rounded-oval. Size about  $4\mu$ . Much oil and protoplasm are also found.

## TYPE 11. GRAINS SEMI-COMPOUND.

Several components are enveloped completely or merely upon one side by a common substance which is not pierced by partitions. This common substance, which belongs to the originally simple grain as well as the components, may or may not have lamellæ. The components usually arise by the splitting of the hilum, and develop into the type of the simple grains. More or less delicate lines of division are found between the components, which extend to the common lamellæ. Sometimes such partitions are wanting, and should there also be no lamellæ then the semi-compound structure is indicated only by the presence of several hila lying in a homogeneous mass.

*Chara stelligera* Bauer. (*Algæ*.) *Dry star-shaped bodies*.—Grains rounded, oval, irregularly blunt-angular with 1 to 5 angles; usually isodiametric; sometimes elongated to twice as long as thick and occasionally compressed to about two-thirds of their width; or slightly cuneiform; with 2 to 25 or even 40 hila which usually have the appearance of small cavities, and are sometimes embedded in an apparently homogeneous mass, and also appear singly or in a group surrounded by distinct lamellæ. Lines of division between the components usually absent. Size about 70 to  $85\mu$ . Among the inclosed grains a central one frequently is found which far exceeds the others in size. This one is surrounded by distinct lamellæ, is almost spherical in shape, and contains one or more hila near its center. Several short radial fissures are usually found in the interior of these larger grains. Single parts of many grains are cut off by delicate lines. This structure forms a transition to the true compound type, occurring among the semi-compound ones (see type 15).

*Marsilea pubescens* Tenore. (*Marsilaceæ*.) *Dry gymnospires*.—Grains oval or oblong, sometimes slightly irregular; 2 to 4 times as long as broad; the broad ones compressed to three-fifths of their width; with 2, rarely 3 and 4, hila or components placed in one row; components appear oval from the broad aspect, and lanceolate or linear-lanceolate from the narrow aspect; the axes and the largest planes of which coincide with those of the whole grain; single fissures occasionally are observed which may be between the components, or may pass through the centers of the grains, and thus either coincide with the longitudinal axis or even cut it at right angles; with distinct lamellæ in the common surrounding substance, and in the outer substance of the components. Length about  $175\mu$ , breadth  $52\mu$ , thickness  $33\mu$ . Among the above some simple centric-oval types are found.

*Hyacinthus orientalis* Linn. (*Liliaceæ*.) *Fresh scales of the bulbs*.—Grains rounded to oblong, usually irregular, with more or less numerous protruding angles; two-fifths to as broad as long; very often slightly compressed and cuneiform, the shorter border thickened, the opposite longer one with a sharp edge; lamellæ are occasionally observed which are chiefly found toward the sharp edge; with 2 to 7 hila arranged in a single row along the thickened border; incomplete fissures are frequently seen between the hila; small components are sometimes found in the angles or at the sharp-edged margin. Length about  $45\mu$ , breadth about  $35\mu$ . Among the above are found some simple, eccentric, cuneiform grains, and also compound grains with components arranged in 1 or 2 rows. In the young bulbs which are still inclosed within the scales of the older bulbs the grains are very small; in fact, they frequently show a Brownian movement. They are spherical, frequently somewhat irregular in shape, and without lamellæ; a broad, somewhat sharpened edge can be noticed in some of the grains, and occasionally one of the corners is cut off. Size about 10 to  $15\mu$ . Among the above many compound grains of 2 to 8 components with delicate lines of division are observed. Last year's bulbs which have borne this year's leaves and buds contain at the base of the inner scales similar but larger compound grains. Separated-grains are wanting. The simple grains have developed still more irregularly, and by splitting of the hilum have changed into semi-compound ones, and by the breaking away of corners into compound grains of unequal components. Semi-compound grains were found almost exclusively in the scales

at the top of these bulbs. Complex compound grains and separated-grains of the same do not occur; they are either not formed at all or they fall to pieces and the separated-grains then look like the simple ones. Fissures usually radiating from the hilum are formed in grains in bulbs which have been allowed to dry over the winter, and the divisions between the components become wider. The grains in the outer, decayed scales of the bulbs are undergoing disintegration, which usually begins where the partitions between the components touch or nearly touch the surface.

*Canna lanuginosa* Rosc.; *Canna lagunensis* Lindl., etc. (*Cannaceæ*.) *Fresh root-stock*.—Grains simple, usually more or less mussel-shell-shaped; hilum very eccentric, lying in the upper, narrow, protruding end. In this species as well as in others the hilum is frequently split in two; 2, rarely 3 or 4, hila lying next to each other. In the longitudinal axis of the grains of some species a single and here and there a double row of inclosed components is formed. In the symmetrical grains this row is usually in the median line, while in the unsymmetrical ones, the grains being usually more or less curved, it is found toward the concave border. The number of inclosed grains varies from 2 to 12; either by transverse or longitudinal fission of these grains individual ones arise, in which lamellæ become visible as they increase in size.

*Ficaria ranunculoides* Moench.; *Ranunculus ficaria* Linn. (*Ranunculaceæ*.) *Dry thickened roots*.—Grains rounded-cuneiform, rounded-triangular, frequently unequally quadrangular, sometimes pentagonal, frequently somewhat irregular; about twice as broad as long; compressed to half their width; in the triangular grains one of the rounded angles and in the quadrangular ones the narrow side is thickened; the latter with a row of 2 to 6 or more hila at right angles to the longitudinal axis; eccentricity one-fourth and one-sixth; the distal margin broad, with a thinned knife-like edge, which terminates mostly on either side in rounded or somewhat pointed angles; lamellæ none or indistinct; instead of the hila, small cavities, frequently with single delicate, short, radiating fissures are found; delicate separating fissures between the components are rare. Size about  $35\mu$ . Among the above some doublets and triplets and simple eccentric-cuneiform grains are observed.

*Cereus variabilis* Pfeiff. (*Cactaceæ*.) *Fresh pith of the stem*.—Grains rounded or oval, usually of irregular form and with lamellæ; with 2 to 6 and 9 larger or smaller inclosed components, the latter when larger have lamellæ, and are usually divided from one another by partitions; lamellæ eccentric; occasionally some of the larger components are also semi-compound. Size about  $80\mu$ . Among these are simple grains of uncertain structure.

#### TYPE 12. GRAINS COMPOUND, WITH FUSED COMPONENTS.

The components are not surrounded by a common substance, nor separated from one another by lines of cleavage. At first glance the grains of this type bear a resemblance to certain semi-compound grains, but differ from them in not possessing a common surrounding substance. They also differ from the ordinary compound grains in the lack of lines of cleavage between the components; and for this reason they do not, as a rule, split into separated-grains. In *Commelina* and other species, compound grains arise by grains which were originally separate, and pressing upon one another as the result of further growth, so that at first grains in one cell of the same generation, and later those of older ones, fuse with one another, and at the same time the clefts between them disappear. The whole cell lumen is then filled with a uniform, reticulated, parenchymatous mass, in which the hollow space represents the soft, internal matter of the components, while the net-like framework corresponds to the coalesced dense external substance.

*Commelina cælestis* Willd. (*Commelinaceæ*.)—Compound grains polyhedral, due to pressure; 1 to 2.5 times as long as thick, of parenchymatous structure consisting of 2 to more than 200 components, the latter usually fused with one another, rarely separated by delicate clefts, each with a large angular cavity. Separated-grains (which occasionally can be set free by pressing) rounded-angular or polyhedral. Size of the compound grains about 40 to  $55\mu$ , thickness about  $30\mu$ . Size of the components 1.5 to 6 and  $8\mu$ . Some isolated simple spherical grains. Size about  $9\mu$ . In semi-ripe seeds the compound grains are spherical to oblong (about  $44\mu$ ), consisting of apparently solid, equally dense components separated by delicate lines or distinct, narrow clefts. In young seeds, where the endosperm has just begun to

develop, the cells forming a 3 to 4 layered covering for the embryo-sacs are completely filled with small starch-grains. The latter are usually spherical, some distinctly compound, and some only granular or almost homogeneous. Some diminutive separated or simple grains also occur.

*Commelina nudicaulis* Burm. (*Commelinaceæ*.) *Dry ripe seeds*.—Grains as in *Commelina cælestis* Willd. In half-ripe seeds the compound grains are spherical-oval, elliptical or oblong. Size 28 to 34 $\mu$ . The components of the same are solid, and consist of a uniformly dense substance; they are separated by lines or by distinct clefts which contain water; in this stage the compound grains easily fall apart, thus giving rise to numerous obtuse-angular or polyhedral separated-grains.

*Tinnatia fugax* Scheidw. (*Commelinaceæ*.) *Dry endosperm*.—Starch as in *Commelina*. Compound grains about once to twice as long as thick, consisting of 2 to more than 1700 components. Size about 50 $\mu$ , thickness about 36 $\mu$ . On pressing the grain, rounded or polyhedral separated-grains are set free, often still hanging together in irregular masses, and are usually 2 to 3 $\mu$ , rarely 4 and 5 $\mu$ , in size. Some cells seem to be filled with a homogeneous mass.

*Cyanotis cristata* Don.; *Tradescantia virginica* Linn.; *Heteractia pulchella* Kze. (*Commelinaceæ*.) *Dry endosperm*.—Grains as in *Commelina*.

*Zingiber officinale* Rosc.; *Amomum zingiber* Linn. (*Zingiberaceæ*.) *Dry seed endosperm*.—The cells are densely filled with components which usually represent a uniform mass, showing no indication of the compound grain. Through the complete union of the components this mass appears either reticular parenchymatous by complete fusion of the components, since only the hollow spaces lying in the apparently homogeneous substance are visible, or granular, in which case the components may be separated from one another. The compound grains, rounded or oval grains within the cell, can rarely be seen or set free. Size of components 1 to 5 $\mu$ . The smaller components are solid, the larger ones are hollow.

*Amomum cardamomum* Linn.; *Amomum javanicum*. (*Zingiberaceæ*.) *Dry seed endosperm*.—The cells usually filled with a uniform parenchymatous network which often falls out of the sectioned cells in a single mass, and in which either indistinct or no divisions corresponding to compound grains may be noticed. This mass frequently has a granular appearance and easily falls apart by means of pressure into separated-grains, of which the smaller ones are frequently rounded and the larger ones polyhedral, both having a large or a small cavity. Size of the separated-grains 1.5 to 5.5 $\mu$ .

*Amomum granum-paradisi* Afzel; *Elettaria cardamomum* White; *Cardamomum minus*. (*Zingiberaceæ*.)—Grains as in preceding.

*Hedychiium gardnerianum* Wall. (*Zingiberaceæ*.) *Dry seed endosperm*.—Compound grains, spherical, oval, rarely somewhat angular; slightly granular; containing about 8000 components. Size about 21 $\mu$ . Separated grains rounded. Size 0.7 to 2 $\mu$ . These grains are transition forms to ordinary compound grains of many components.

*Costus* sp. (*Zingiberaceæ*.) *Dry seed endosperm*.—The separated-grains, which are 1 to 3 and 4 $\mu$  in size, are packed in the cells as a dense uniform mass in which the structure of the compound grains can rarely be recognized. This mass sometimes appears granular, but more often has a reticular parenchymatous appearance, the meshes of which are frequently arranged in parallel rows. The endosperm cells are distinguished by numerous knob-like processes.

*Thalia dealbata* Fras. (*Zingiberaceæ*.) *Dry seed endosperm*.—Compound grains, polyhedral as a result of pressure, with sharp angles and edges; frequently more or less irregular; about twice as long as broad; with 2 to 12 rounded or angular, more or less distinct, cavities lying in a homogeneous mass, giving to the grain a parenchymatous appearance; lines of divisions are seldom noted between the cavities. Size of the compound grains about 20 to 25 $\mu$ . The cavities in the outer denser part of the endosperm are small (1 to 2 $\mu$ ) and rounded; in the inner part they are large (about 8 $\mu$ ) and angular.

*Maranta* sp. (*Marantaceæ*.) *Dry seed endosperm*.—Compound grains consisting of 2 to 6, rarely 8, almost equally large components, but usually of irregular structure and irregularly disposed. The components are more or less fused, with indistinct or no lines of cleavage, each component containing a rather large but frequently very indistinct cavity. Size of the compound grains 9 to 13 $\mu$ , and of the components 2 to 5 $\mu$ . These grains seem to come between those of *Thalia*

and those constituting type 14. Some simple toruloid or indented grains also occur similar to those in *Maranta ramosissima* (p. 229), but smaller (about 11 to 14 $\mu$ ), and only slightly compressed.

*Heliconia* sp. (*Musaceæ*.) *Dry seed endosperm*.—Grains rounded-oval to oblong, sometimes curved, often irregular, frequently somewhat toruloid; one-fourth to almost as broad as long; the broad ones slightly compressed. Some appear to be simple grains; in the majority of them several (2 to 12 or more) cavities are noted. Length about 17 $\mu$ , width about 10 $\mu$ . These grains stand between those of the *Maranta* species and *Thalia dealbata*.

*Piper*. (*Piperaceæ*.) *Seed endosperm*.—Besides the ordinary compound grains with numerous components, there are some whose components are completely fused, as in type 16.

#### TYPE 13. GRAINS COMPOUND, IN ONE OR TWO ROWS.

Components 3 to 11; form a compound grain; arranged in 1 or 2 rows separated by clefts, and at maturity falling away as separated-grains. These compound grains arise in two ways, either by the repeated splitting of the hilum of an originally simple grain, or by the fusion of a number of originally separate grains lying next to each other in an elongated chloroplast (as in *Chara*). In the latter case they may for some time partially or entirely retain their green color.

*Chara hispida* Linn. (*Algæ*.) *Fresh cells of the internodes*.—Compound grains oblong or rod-shaped; one-fifth to one-half as broad as long; usually slightly compressed; consisting of 2 to 8 almost equal components. Length about 34 $\mu$ , width about 9 $\mu$ . Components in two rows with overlapping ends; two-fifths to two-thirds as broad as long; without lamellæ. Length 8 to 16 $\mu$ . The starch-grains which originate in the chloroplast are originally green and later become colorless.

*Hyacinthus orientalis* Linn. (*Liliaceæ*.) *Fresh scales of the bulbs*.—Compound grains oval to lanceolate; one-half to one-third as broad as long; consisting of 2 to 8 unequal components. Length about 35 $\mu$ . Components are arranged in one or two rows; lamellæ rare, delicate, usually with a small central hilum; also simple cuneiform and semi-compound grains are found.

*Canna lagunensis* Lindl. (*Cannaceæ*.) *Fresh root-stock*.—Compound grains, elongated to linear, usually more or less curved; 3 to 8 times as long as broad; consisting of 3 to 11 usually unequal components arranged in 1 and sometimes in 2 rows. Length about 7 $\mu$ . Components with distinct or indistinct concentric lamellæ, and either an almost central hilum or occasionally with several hila, which are undergoing longitudinal transverse fission. Size 3 to 18 $\mu$ . Among these are simple, eccentric compressed grains and semi-compound grains, as well as all the intermediate transitional stages between these two.

*Canna pedunculata* Sims. (*Cannaceæ*.) *Fresh root-stock*.—Compound grains oblong or lanceolate, usually irregular, frequently curved; 2 to 6 usually unequally divided grains often arranged in one row. Size of smallest components are 2 to 3 and of the largest 30 $\mu$ ; in the latter the lamellæ are sometimes distinct and the hilum very eccentric. Also simple, eccentrically compressed grains are found.

*Canna lanuginosa* Bosc. (*Cannaceæ*.) *Fresh root-stock*.—Compound grains oblong or linear-lanceolate, usually irregular, frequently curved, occasionally compressed; 2 to 8 equal or unequal components arranged in one row. Among these simple and semi-compound grains are found.

*Canna coccinea* Ait. (*Cannaceæ*.) *Fresh root-stock*.—Compound grains oblong or linear-lanceolate, usually irregular and frequently curved; 2 to 6 to 8 equal or unequal components arranged either in one or rarely in two rows. Simple grains and all transitions to the semi-compound ones are found.

*Fagopyrum esculentum*; *Polygonum fagopyrum* Linn. (*Graminaceæ*.) *Fresh and dry seeds*.—Compound grains rounded, elongated, rod-shaped, sometimes curved or bent; frequently irregular; angular or lobulate; 1 to 8 times as long as broad; the narrow ones terete, the broad ones compressed; consisting of 2 to 15 components which are arranged in one or two rows, rarely in more rows, or even in a simple layer. Length about 38 $\mu$ , width about 20 $\mu$ . Components are homogeneous, or each have a central hilum. Size 4 to 10 $\mu$ . Simple centric spherical grains are also observed.

## TYPE 14. GRAINS COMPOUND, OF FEW COMPONENTS OF EQUAL SIZE.

Two to ten or more components of about equal size, united into one compound grain, separated by fissures, and at complete maturity breaking away as separated-grains with one curved surface and one or more pressure facets. The compound grains originate by the division of the hilum and by repeated division of the same, and pass over into the compound type with many components, as in type 16. These occur rarely alone, but are frequently mingled either with simple grains or with compound ones belonging to type 15 which are formed by their angles or edges being cut off.

*Cycas circinalis* Linn. (*Cycadaceæ*.) *Pith*.—According to Payen (Ann. Sc. Nat., 1838, II, p. 18 pl., 6, figs. 4, 5), the compound grains consist of 2 to 8 and 10 equal and regular components. Separated-grains with one curved surface and one to seven pressure facets; with indistinct lamellæ; hilum about one-fourth eccentric. Size about 45 $\mu$ . Commercial sago changes slightly in moist heat. Size of the starch-grains about 70 $\mu$ . Starch-grains in the base of the petiole similar to those in the pith of the stem.

*Coix lacryma* Linn. (*Graminaceæ*.) *Dry roots*.—Compound grains, rounded or oval; consisting of 2 to 4 and 8 almost equal components. Size about 11 $\mu$ . Separated-grains 2.5 to 5 $\mu$ . Also some simple spherical grains are observed. Size about 7 $\mu$ . Starch quite plentiful in the cortex of the root.

*Panicum arenarium* Brot.; *Panicum repens* Linn. (*Graminaceæ*.) *Dry root-stock*.—Compound grains, consisting of 2 to 4 equal, rarely unequal, components (only a few that have fallen to pieces are present). Size about 20 to 26 $\mu$ . Separated-grains rounded or oval; homogeneous or with a small cavity instead of a hilum; about one-fourth eccentric. Size about 13 $\mu$ .

*Oplismenus colonus* Humb. and Kunth.; *Panicum colonus* Linn. (*Graminaceæ*.) *Dry root-stock*.—Separated-grains rounded or angular with rounded angles, usually compressed. Size about 6 $\mu$ . Some are distinctly separated-grains with one curved surface and 1 to 6 pressure facets. The compound grains, of which none are any longer present, may have consisted of 2 to 10 components. Poor in starch; starch entirely wanting in the roots.

*Vilfa pungens*; *Sporobolus pungens* Kunth. (*Graminaceæ*.) *Dry stolons*.—Grains compound, consisting of 2 to 10 and 12 almost equally large components. Size about 14 $\mu$ . Separated-grains 3 to 8 $\mu$ ; the larger ones have a small cavity, occasionally with single delicate radial fissures. Cell-walls very thick, and with numerous indentations.

*Cynodon dactylon* Pers. (*Graminaceæ*.) *Dry stolons*.—Grains compound, consisting of 2 to 7 and 9 equal, rarely unequally large components. Size about 12 to 16 $\mu$ . Separated-grains nearly rounded, 3 to 8 $\mu$ ; the larger ones hollow.

*Andropogon muricatus* Retz.; *Anatherum muricatus* Beauv.; *Radix iwarancusæ*. (*Graminaceæ*.) *Dry roots*.—Grains compound or 2 to 4 equally triangular or tetrahedral components. Size about 25 $\mu$ . Separated-grains conical, with blunt, slightly protruding ends; 0.75 to 1.33 times as broad as long; lamellæ none; toward the narrow aspect instead of a hilum a small cavity is occasionally found with 1 to 2 radiating fissures; eccentricity about one-fourth. Length 7 to 13 $\mu$ , width 8 to 11 $\mu$ . Also a few simple, spherical grains are observed.

The grains described by Schleiden (Grundzuge, 3 Aufl. I, 185, fig. 15) had been changed by heat. According to Berg (Pharmacognosie), *Radix iwarancusæ* contains no starch. In numerous specimens obtained from various sources, Nägeli always found starch plentiful in the pith, but none in the cortex.

*Scirpus maritimus* Linn. (*Cyperaceæ*.) *Dry root-stock*.—Compound grains spherical or oval, consisting of 2 to 10 usually equal and regularly disposed components. Size about 18 $\mu$ . Separated grains mostly have a small central cavity from which, in the larger grains, several fissures radiate. Size 5 to 14 $\mu$ . Also some simple spherical grains, with small central cavity and single radial fissures. Size about 15 $\mu$ .

*Carex maxima* Scop. (*Cyperaceæ*.) *Dry root-stock*.—Compound grains rounded, oval, usually more or less irregular; consisting of 2 to 10 and 14 usually equal components. Size about 9 $\mu$ . Size of the separated-grains 1.5 to 4 $\mu$ . Also some simple rounded and oval grains are found.

*Carex bicolor* All. (*Cyperaceæ*.) *Dry root-stock*.—Compound grains rounded or oval, frequently somewhat irregular, consisting of 2 to 8 and 10 usually almost equal components. Size about 7 $\mu$ . Size of the separated-grains 1 to 3 $\mu$ . Simple grains of incomplete formation are also found.

- Willdenowia teres* Thumb. (*Restionaceæ*.) *Dry roots*.—Separated-grains with one curved and 1 to 5 pressure facets, occasionally with a central cavity. Size about  $10\mu$ . Only a few compound grains of 2 to 4 components are still present. The greater number have fallen apart. Judging from these separated-grains they must have consisted of at least 8 components. Some simple spherical and rounded-oval grains are also found.
- Restio incurvatus* Thumb. (*Restionaceæ*.) *Dry root-stock*.—Grains compound spherical or oval, consisting of 2 to 10 and 20 unequal or chiefly equal components. Size about  $14\mu$ . Separated-grains 2 to  $8\mu$ ; the larger ones have a small cavity.
- Luzula spadicca* DC. (*Juncaceæ*.) *Dry root-stock*.—Compound grains consisting of 2 to 8 and 12 usually almost equal components. Size about  $13\mu$ . Separated-grains 2 to  $6\mu$ .
- Colchicum autumnale* Linn. (*Colchicaceæ*.) *Fresh tubers*.—Grains compound, of 2 to 4 usually equal components, usually arranged in a tetrahedron, rarely 3 in a row. Size about  $28\mu$ . Separated-grains without lamellæ; with distinct hilum; occasionally, instead of the hilum, a small cavity from which 2 fissures extend toward the edges. Size 3.8 to  $18\mu$ . Also a few simple spherical or flattened spherical grains are found.
- Colchicum variegatum* Linn.; *Radix hermodactyli* (*Colchicaceæ*.) *Dry tubers*.—Compound grains, spherical, oval, or oblong; 2 to 8 usually equal components. Size about  $60\mu$ . Separated-grains without lamellæ, and with distinct fissures radiating toward the corners. Size about  $30\mu$ . Some simple spherical and spherically-oval grains are found.
- Monochoria plantaginea* Kunth, Pl. Ind. or ed. Hohenacker. (*Pontederiaceæ*.) *Dry root-stock*.—Compound grains, rounded or oval; consisting of 2 to 10 and 16 usually almost equal components. Size about  $12\mu$ . Separated-grains 2 to  $8\mu$ ; the larger ones have a central cavity.
- Smilax* sp. (*Liliaceæ*.) *Dry sarsaparilla root*.—Compound grains spherical, rarely oval or oblong; consisting of 2 to 8 usually equal components, which are either arranged as 3 in a triangle (rarely in a row), 4 in a tetrahedron (rarely in a square), or 6 in a hexahedron. Size about  $23\mu$ . Separated-grains without lamellæ; instead of a hilum usually a small almost central cavity is observed with frequently 2, rarely 4, radial fissures. Also simple, spherical grains with small central cavity and radial fissures are rarely observed. Size  $16\mu$ .
- Smilax china* Linn.; *Radix china*. (*Liliaceæ*.) *Dry root-stock*.—Compound grains more or less angular as a result of pressure, frequently polyhedral, consisting of 2 to 4, rarely 6, equal components. Size about  $60\mu$ . Separated-grains polyhedral, rarely with a few distinct lamellæ; instead of a hilum a small central cavity is found, from which several fissures usually radiate. Size 15 to  $30\mu$ . Also simple grains with incomplete formation.
- Tacca pinnatifida* Forst. (*Taccaceæ*.) *Tahiti arrowroot*. *Root-stock*.—According to Walpers (Bot. Zeit., 1851, p. 333), the compound grains probably consist of 2 to 6 components. Separated-grains with 1 to 4 pressure facets; indistinct lamellæ; hilum eccentric, with one well-marked transverse fissure, as in *Maranta arundinacea*. Size as in the latter or somewhat smaller. According to Leon Soubeiran (Journ. Phar., 1854, xxv, 180) the grains are spherical, often cut off vertically to the longitudinal axis, elliptical, occasionally somewhat pear-shaped; some have stellate fissures in the place of the eccentric hilum. Size varies from 30 to  $40\mu$ , some grains, however, are not over  $10\mu$ . (Judging from the illustration the larger grains appear to be simple grains, not separated ones.)
- Meristostigma silenoides* Dietr. (*Iridaceæ*.) *Dry bulbs*.—Grains compound, consisting of 2 to 10 and 12 usually equal-sized components which are regularly arranged. Size about  $42\mu$ . The larger separated-grains have a small cavity from which single short fissures may radiate; one-half eccentric in oval grains. Size 2 to  $18\mu$ . Some simple spherical or oval grains are also observed. Size about  $20\mu$ .
- Gladiolus communis* Linn. (*Iridaceæ*.) *Dry bulbs*.—Compound grains spherical or oval; 2 to 6 and 8 equal or somewhat unequal components which are usually regularly arranged. Size 7 to  $28\mu$ . Separated-grains, size 2.5 to  $18\mu$ . The larger ones with a small cavity from which strongly marked fissures radiate toward the edges.
- Trichonema bulbocodium* Ker. (*Iridaceæ*.) *Dry bulbs*.—Compound grains consisting of 2 to 10 and 12 usually equal components, which are regularly arranged. Size  $28\mu$ . Separated-grains, size 2 to  $14\mu$ ; the larger ones have a small cavity, instead of a hilum, from which occasionally single short radial fissures radiate toward the edges. Some simple spherical grains are also present. Size about  $16\mu$ .

- Crocus sativus* All. (*Iridaceæ*.) *Dry bulbs*.—Compound grains, of 2 to 8 and 10 usually equal components, which are regularly arranged. Size about  $38\mu$ . Separated-grains, size 2 to  $18\mu$ , the larger ones have a small cavity, instead of a hilum, from which long, delicate fissures radiate toward the edges.
- Anigosaanthus rufa* Labil. (*Hæmodoraceæ*.) *Dry root-stocks*.—Compound grains, of 2 to 4 and 6 usually equal components. Size about  $18\mu$ . Separated-grains of 3 to  $10\mu$ ; frequently with a small cavity.
- Billbergia amæna* Lindl. (*Bromeliaceæ*.) *Dry root-stocks*.—Compound grains of 2 to 4 usually equal, sometimes unequal, components. Separated-grains, size 5 to  $15\mu$ ; instead of a hilum a small cavity is observed from which short, distinct fissures radiate; in the larger oval separated-grains the hilum is about one-third eccentric. Some simple eccentric-conical grains are also found.
- Maranta indica* Rose. (*Cannaceæ*.) *West India arrowroot*.—According to Schleiden (Grundzuge, 3 Aufl., 1, p. 185, fig. 13), the compound grains consist of 2 to 4 almost equal components. He also finds similar grains in other species of *Maranta*, and Münter (Bot. Zeit., 1845, p. 203) records the same for *Maranta bicolor* Ker. (*Calathea bicolor* Steud.). According to Walpers (Bot. Zeit., 1851, p. 334), the grains of *Maranta indica* are either oval, drum-shaped, drum-shaped with inclined sides, or almost pear-shaped, as well as with 2 to 3 glandular-comnate divisions. These grains, Nägeli states, should not be confused with those of any other kind of arrowroot, as they must surely come from a plant different from those described by Schleiden and Münter.
- Sparganium ramosum* Huds. (*Typhaceæ*.) *Dry root-stock*.—Grains consisting of 2 to 6 and 12 usually equal components. Size about  $17\mu$ . Separated-grains usually with a small cavity; one-third eccentric in some oval forms. Size 3 to  $10\mu$ . Also isolated simple, oval grains, hilum about one-fourth eccentric, are found. Size about  $13\mu$ .
- Aponogeton*. (*Alismaceæ*.) *Tubers*.—According to Schleiden (Grundzuge, 3 Aufl., 1, p. 185) the compound grains consist of 2 to 4 almost equal-size components.
- Parietaria diffusa* Mert and Koch. (*Urticaceæ*.) *Dry root-stocks*.—Grains consisting of 2 to 4 almost equal components. Size of the separated-grains 3 to  $6\mu$ . Some simple grains, of undeveloped structure, are also found.
- Rheum undulatum* Linn. (*Polygonaceæ*.) *Fresh root*.—Compound grains rounded or angular with rounded regular or irregular angles; 2 to 8 equal or unequal components which are often irregularly arranged. Size about  $25\mu$ . Separated-grains obtuse-angled to sharply polyhedral; lamellæ rarely visible, very few (1 to 4), and delicate; hilum usually distinct, central, or slightly eccentric. Size 4 to  $20\mu$ . Simple spherical grains rare.
- Rheum* sp. (*Polygonaceæ*.) *Rhubarb root*.—Compound grains consisting of 2 to 5 components. Separated-grains with obtuse or acute angles; lamellæ none; instead of a hilum a small cavity is found which is central or slightly eccentric, from which 2 to 5 short radial fissures pass out. Size 3 to  $17\mu$ . Some simple, almost round grains are also found.
- Rumex arifolius* All. (*Polygonaceæ*.) *Dry root-stock*.—Compound grains rounded to elliptical; 2 to 8 equal or unequal components which are often regularly arranged. Size about  $12\mu$ . Separated-grains 2 to  $6\mu$ ; a small cavity is found in the larger ones. Also simple grains of undeveloped structure.
- Rumex tuberosus* Linn. (*Polygonaceæ*.) *Dry root-stock and thickened roots*.—Compound grains consisting of 2 to 6, rarely 10, equal or unequal components, sometimes several small components adhere to 1 or 2 larger ones. Size about  $26\mu$ . Separated-grains with a small cavity from which single fissures radiate toward the edges; eccentricity about one-half. Size 3 to  $14\mu$  and  $17\mu$ . Swollen, hollow separated-grains, size  $22\mu$ . The grains in the thickened roots are generally somewhat larger than those in the root-stock.
- Bærhavia repens* Linn. (*Nyctaginaceæ*.) *Dry root*.—Compound grains spherical to oblong and tabular; 2 to 10 and 14 usually equal components, which are regularly arranged. Size about  $30\mu$ . Separated-grains usually isodiametric, with a small cavity from which long, delicate fissures radiate toward the corners; eccentricity about one-half. Size 3 to  $20\mu$ .
- Cinnamomum ceylanicum* Nees.; *Cortex cinnamomi interior*. (*Lauraceæ*.) *Cortex*.—Compound grains consisting of 2 to 4 almost equal components. Size about  $14\mu$ . Numerous spherical and angular grains with rounded corners, the former being simple and the latter separated-grains. Size about  $9\mu$ . A small central cavity is found in the large grains.

- Aristolochia serpentaria* Linn. (*Aristolochiaceæ*.) *Pith of dry root-stock*.—Compound grains spherical or oval-spherical, 2 to 8 usually equal components, arranged regularly in triangles, tetrahedrons, hexahedrons, or octahedrons. Size about  $18\mu$ . Separated-grains without lamellæ, hilum, and fissures. Size 3 to  $12\mu$ . Simple spherical homogeneous grains are also present. Size about  $12\mu$ .
- Aristolochia pistolochia* Linn. (*Aristolochiaceæ*.) *Dry roots*.—Compound grains spherical, rarely elliptical, consisting of 2 to 8 and 12 usually equal components which are regularly arranged. Size about  $18\mu$ . Separated-grains 3 to  $11\mu$ ; the larger ones have a cavity. Some large separated-grains swollen and apparently hollow; and some simple, rounded ones are also observed.
- Valeriana officinalis* Linn. (*Valerianaceæ*.) *Fresh root-stock and roots*.—Compound grains consisting of 2 to 4 equal components which are arranged regularly in triangles or tetrahedrons. Separated-grains sometimes with a distinct central hilum. Size about  $8\mu$ . Also simple grains of incomplete formation are observed.
- Valeriana tuberosa* Linn. (*Valerianaceæ*.) *Dry root-stock*.—Grains consisting of 2 to 4, rarely 6, almost equal components. Size  $22\mu$ . Separated-grains with a large or a small cavity from which fissures radiate toward the corners. Size 4 to  $15\mu$ . Also some simple grains with structure undeveloped.
- Valeriana salinca* All. (*Valerianaceæ*.) *Dry root-stock*.—Compound grains spherical to oval and elliptical, with unequal sides, consisting of 2 to 6 and 10 equal or somewhat unequal components. Size about  $11\mu$ . Separated-grains 2 to  $5\mu$ , the larger ones with a small cavity. Also simple grains with imperfect structure.
- Succisa pratensis* Moench. (*Dipsacaceæ*.) *Dry root and root-stock*.—Compound grains spherical to oval and elliptical; consisting of 2 to 10 and 16 components which may be equal, though more frequently unequal. Size about  $20\mu$ . Separated-grains have a small or large cavity, from which short, delicate, radial fissures very often pass out, usually almost central, rarely one-half eccentric. Size 2.5 to 10 and  $14\mu$ .
- Vinca minor* Linn. (*Apocynaceæ*.) *Dry root*.—Grains consisting of 2 to 4 and 6 almost equal, rarely unequal, components. Size about  $25\mu$ . Separated-grains somewhat oblique; instead of a hilum usually a small central cavity is observed from which delicate fissures radiate. Size about  $12\mu$ . Simple, usually eccentric-conical grains are also found.
- Swertia pcreunis* Linn. (*Gentianaceæ*.) *Dry root-stock*.—Grains consisting of 2 to 4 and 6 usually almost equal components. Size about  $12\mu$ . Separated-grains rarely have a small cavity. Size 3 to  $7\mu$ . Simple grains of incomplete formation are also observed.
- Teucrium hyrcanicum* Linn. (*Labiataceæ*.) *Fresh unripe pericarp*.—Grains rounded, usually angular; size about  $8\mu$ ; majority probably separated-grains. Some compound grains of 2 to 6 components are also found.
- Omphalodes verna* Moench. (*Boraginaceæ*.) *Dry root-stock*.—Compound grains rounded or oval, consisting of 2 to 4 and 6 equal or rarely unequal components. Size about  $9\mu$ . Separated-grains have occasionally a small cavity. Size 2 to  $5\mu$ . Simple grains of incomplete formation are also observed. Starch not very plentiful.
- Symphytum tuberosum* Linn. (*Boraginaceæ*.) *Dry root-stock*.—Grains consisting of 2 to 4 usually equal components. Separated-grains have a small central cavity from which sometimes several short, delicate radial fissures emerge. Size about  $12\mu$ . Simple, usually eccentric-conical, grains are also found.
- Convolvulus soldanella* Linn. (*Convolvulaceæ*.) *Dry stolons*.—Compound grains rounded or oval, consisting of 2 to 6 and 8 equal or unequal components. Size about  $24\mu$ . Separated-grains, size 3 to 14 and  $18\mu$ ; instead of the hilum, the larger ones have a small cavity from which fissures radiate towards the corners; oval forms with eccentric hilum, almost one-half, are rarely present. Simple grains of incomplete formation are also found.
- Convolvulus lincatus* Linn. (*Convolvulaceæ*.) *Dry root-stock*.—Starch as in the preceding. Grains usually smaller, consisting of 2 to 10 components. Size about  $20\mu$ . Size of the separated-grains 2 to  $13\mu$ . Simple grains of imperfect formation are also observed.
- Convolvulus imperati* Vahl.; *Batatas littoralis* Choix. (*Convolvulaceæ*.) *Dry stolons*.—Compound grains rounded or oval; consisting of 2 to 4 and 6 usually equal components. Size about  $28\mu$ .

Separated-grains with a small cavity from which fissures radiate toward the margin; in the oval forms the eccentricity is one-half. Size 5 to 16 $\mu$ . Simple grains of incomplete formation are also found.

- Batatas edulis* Choix. (*Convolvulaceæ*.) *Tubers*.—According to Payen (Ann. Sc. Nat., 1838, pl. 6, figs. 15, 17), the compound grains consist of 2 to 6 and possibly 10 apparently almost equal components. Separated-grains, size about 45 $\mu$ , broadly-conical, semi-spherical, or polyhedral, with 1 curved surface, and 1 to 7 pressure facets; lamellæ distinct; hilum about one-fifth to one-sixth eccentric, at the narrow arched end. According to Crüger (Bot. Zeit., 1854, taf. II, fig. 4) some of the separated-grains are thickened and club-shaped at the hilum end and thinned at the distal end, where the pressure facets are located. According to Leon Soubeiran (Journ. Pharm., 1854, xxv, 92), the grains vary from 10 to 20 $\mu$  and 40 to 50 $\mu$ ; the smallest ones are spherical or oval; those of the middle size are almost regularly polyhedral, and the largest ones oval and elliptical. (Judging from the description, Nägeli writes, it is quite possible that some simple forms occur among the compound grains.)
- Nolana prostrata* Linn. (*Nolanaceæ*.) *Dry root*.—Compound grains of 2 to 8 and 10 almost equal components. Size about 16 $\mu$ . The separated-grains 3 to 11 $\mu$ , usually with a central cavity. No simple grains are observed.
- Polemonium reptans* Linn. (*Polemoniaceæ*.) *Dry root-stock*.—Compound grains of 2 to 8 almost equal, rarely unequal, components. Size about 10 $\mu$ . Size of the separated-grains 2 to 6 $\mu$ . Among these are simple grains of imperfect formation.
- Physalis alkekengi* Linn. (*Solanaceæ*.) *Dry stolons*.—Compound grains rounded or oval, consisting of 2 to 8 and 12 almost equal, rarely unequal, components. Size about 18 $\mu$ . Separated-grains, size 3 to 9 $\mu$ ; the larger ones have a small cavity from which several delicate, short radial fissures rarely emerge, cavity central, rarely (in oval forms) about one-half eccentric. Starch plentiful. Simple grains are not present. Similar grains were found in the roots; the compound grains, however, had nearly all fallen apart into separated-grains. Spherical simple forms may perhaps occur in the roots.
- Solanum nigrum* Linn. (*Solanaceæ*.) *Dry root*.—Compound grains; rounded or oval, consisting of 2 to 8 and 12 almost equal components. Size about 20 $\mu$ . Separated-grains, size 4 to 11 $\mu$ ; the larger ones have a small cavity. Starch here and there is quite plentiful.
- Atropa belladonna* Linn. (*Solanaceæ*.) *Dry roots*.—Compound grains; nearly all have fallen apart; few remaining ones consisting of 2 to 4 components. Separated-grains somewhat elongated; in the direction of the longitudinal axis about twice as long as broad; with 1 to 3 pressure facets at the broad end; lamellæ invisible or indistinct; instead of the hilum, usually a small cavity is observed which is rarely central, usually nearer the narrow end, two-fifths to one-fourth eccentric. Simple grains of imperfect formation are also found.
- Scrophularia nodosa* Linn. (*Scrophulariaceæ*.) *Dry root-stock*.—Compound grains rounded to elliptical, frequently irregular, consisting of 2 to 4 and 8 almost equal or unequal components. Size about 12 $\mu$ . Separated-grains solid, size 2 to 6 $\mu$ . Also compound grains of imperfect formation are observed.
- Gratiola officinalis* Linn. (*Scrophulariaceæ*.) *Dry root-stock*.—Compound grains rounded to elliptical, frequently irregular, consisting of 2 to 4 and 8 almost equal or unequal components. Size about 9 $\mu$ . Separated-grains are solid, size 1.5 to 5 $\mu$ . Simple grains of incomplete formation. Starch rather scarce.
- Veronica austriaca* Linn. (*Scrophulariaceæ*.) *Dry roots*.—Compound grains (many of them fallen apart) rounded or oval, consisting of 2 to 4 and 8 equal or unequal components. Size about 13 $\mu$ . Separated-grains, size 2 to 9 $\mu$ ; the larger ones have a small central cavity; also simple grains of incomplete formation are present.
- Wulfenia carinthiaca* Jacq. (*Scrophulariaceæ*.) *Dry root-stock*.—Compound grains rounded to oval, frequently irregular, consisting of 2 to 4 and 6 equal or unequal components. Size about 9 $\mu$ . Separated-grains are large and solid, size 1.5 to 6 $\mu$ . Simple grains of incomplete formation are also found. Starch rather scarce.
- Pedicularis barrelierii* Rehb. (*Scrophulariaceæ*.) *Dry root*.—Compound grains rounded to oval, consisting of 2 to 4 and 7 almost equal components. Size about 14 $\mu$ . Separated-grains, size 2 to 6 $\mu$ ; the larger ones have a small central cavity. Simple grains of incomplete formation are also found.

- Pedicularis rosea* Wulf. (*Scrophulariaceæ*.) *Dry root*.—Compound grains (most of which have fallen to pieces) consist of 2 to 5 almost equal components. Separated-grains, size 2 to 5 $\mu$ ; the larger ones have a central cavity. Simple grains of incomplete formation are also found.
- Pedicularis acaulis* Scop. (*Scrophulariaceæ*.) *Dry root*.—Compound grains consisting of 2 to 6 usually almost equal components. Size about 14 $\mu$ . Separated-grains, size 2 to 6 $\mu$ ; the larger ones have a small cavity. Occasionally 10 $\mu$  in size, then swollen up owing to a large cavity. Simple grains of imperfect formation are also observed.
- Orobanche* sp. (*Scrophulariaceæ* or *Orobanchaceæ*.) *Dry root-stock*.—Compound grains consisting of 2 to 4 usually equal components. Size about 50 $\mu$ . Separated-grains, lamellæ none or not distinct; instead of the hilum a central or one-half eccentric cavity is observed from which well-marked fissures radiate to the margins. Size about 30 $\mu$ . Also simple, usually eccentric-spherical grains are observed.
- Primula calycina* Duby.; *Primula glaucescens* Moretti. (*Primulaceæ*.) *Dry roots*.—Compound grains of 2 to 4 usually equal components. Separated-grains, size 5 to 10 $\mu$ ; instead of a hilum a small central or one-half eccentric cavity is observed. Simple grains of incomplete formation are also found.
- Soldanella alpina* Linn. (*Primulaceæ*.) *Dry root-stock*.—Compound grains of 2 to 4 almost equal, rarely somewhat unequal, components. Separated-grains, size about 27 $\mu$ ; the larger ones have a small cavity. Simple grains of incomplete formation are also found. The grains in the root are somewhat larger. Size of the separated-grains is about 10 $\mu$ ; they have a small cavity with isolated short fissures. Simple grains are also present.
- Glaux maritima* Linn. (*Primulaceæ*.) *Dry creeping stems*.—Compound grains of 2 to 4 and 8 usually equal, sometimes unequal, components. Size about 15 $\mu$ . Separated-grains, size 4 to 10 $\mu$ ; the larger ones have a small cavity from which fissures occasionally radiate. Simple grains of incomplete formation are also observed.
- Lysimachia vulgaris* Linn. (*Primulaceæ*.) *Dry root-stock*.—Compound grains of 2 to 8 and 10 equal or unequal components. Size about 21 $\mu$ . Separated-grains, size 4 to 17 $\mu$ ; the larger ones have a small cavity from which fissures radiate toward the corners. Simple grains of incomplete formation are also observed. Starch plentiful; cell-walls are not perceptibly thickened.
- Pyrola rotundifolia* Linn. (*Ericaceæ*.) *Dry root-stocks*.—Compound grains of 2 to 4 and 6 usually equal components. Size about 15 $\mu$ . Separated-grains, size 3 to 8 $\mu$ ; the larger ones have a small cavity. Simple grains of incomplete formation are also observed.
- Hydrocotyle vulgaris* Linn. (*Umbelliferaæ*.) *Dry rooted caulicles*.—Compound grains of 2 to 4 and 10 usually equal, rarely unequal, components. Size 18 to 24 $\mu$ . Separated-grains with 1 to 5 pressure facets; instead of a hilum, a small cavity is observed from which fissures radiate toward the corners. Size 4 to 17 $\mu$ ; also isolated simple, spherical or ovate grains are found.
- Apium graveolens* Linn., var. cult. (*Umbelliferaæ*.) *Fresh root*.—Separated-grains rounded or angular with rounded corners, with 1 curved surface and 1 to 5 pressure facets; neither hilum nor lamellæ. Size about 12 $\mu$ . The compound grains have fallen apart; only a few may be distinguished within the cells, and these are oval or spherical and consist of 2 to 8 components.
- Petroselinum sativum* Hoffm.; *Radix petroselini*. (*Umbelliferaæ*.) *Dry root*.—Compound grains spherical to elongated-oval, consisting of 2 to 10 and 15, rarely 30, components. Size about 25 $\mu$ . Separated-grains, size 2 to 10 $\mu$ ; the majority have one curved surface, but several are bounded merely by pressure facets; the larger ones have a small central cavity, and several fissures turned toward the corners.
- Pimpinella saxifraga* Linn.; *Radix pimpinella alba*. (*Umbelliferaæ*.) *Dry roots*.—Grains rounded or angular with rounded angles, frequently with one curved surface and 1 to 4 distinct pressure facets; solid and homogeneous. Size about 9 $\mu$ . The majority of these grains are undoubtedly separated-grains. However, simple grains also occur, which can be recognized by their spherical shape. Only a few compound grains are present.
- Levisticum officinale* Koch. (*Umbelliferaæ*.) *Dry root*.—Separated-grains with one curved surface and 1 to 4 pressure facets; instead of a hilum, usually a small central cavity is present from which isolated fissures diverge. Size about 12 to 18 $\mu$ . Grains are crowded in the cells; only a very few compound grains were noted. Some simple grains of incomplete formation are also found.

- Ostericum palustre* Bess. (*Umbelliferae*.) *Dry root-stock*.—Compound grains of 2 to 4 and 8 usually almost equal components. Size about  $14\mu$ . Separated-grains, size 3 to  $8\mu$ ; the larger ones have a small cavity. Some spherical and oval grains, size  $11\mu$ , are also observed.
- Peucedanum cervaria* Lap. (*Umbelliferae*.) *Dry root-stock*.—Compound grains consisting of 2 to 4, rarely 6, usually almost equal components. Size about  $20\mu$ . Separated-grains, size 3 to  $12\mu$ ; the larger ones have a small cavity.
- Cornus suecica* Linn. (*Cornaceae*.) *Dry root-stock*.—Compound grains consisting of 2 to 8 and 12 usually almost equal components. Size about  $13\mu$ . Separated-grains, size 4 to  $8\mu$ ; the larger ones have a small cavity. Also simple grains of incomplete formation are also observed.
- Ranunculus thora* Linn. (*Ranunculaceae*.) *Dry roots*.—Compound grains consisting of 2 to 6 and 8 equal or unequal components. Size about  $21\mu$ . Separated-grains, size 4 to  $15\mu$ ; the larger ones have a small central cavity and several short fissures; one-half eccentric in oval forms. No simple grains are present.
- Ranunculus flammula* Linn. (*Ranunculaceae*.) *Dry roots*.—Compound grains consisting of 2 to 8 and 10 components; the majority of them have fallen apart. Separated-grains with one curved surface and 1 to 6 pressure facets. Size about  $10\mu$ ; the larger ones frequently have a small cavity. Starch plentiful.
- Ranunculus rutafolius* Linn. (*Ranunculaceae*.) *Dry roots*.—Compound grains of 2 to 8 components, the majority fallen apart. Separated-grains, size 2 to  $7\mu$ ; the larger ones have a small cavity. Some apparently simple rounded grains are also observed. Not very rich in starch.
- Chelidonium majus* Linn. (*Papaveraceae*.) *Fresh root*.—Separated-grains with 1 curved surface and 1 to 7 pressure facets. Size 2 to  $10\mu$ . A few compound grains, consisting of 2 to 10 almost equal components are present.
- Corydalis solida* Smith. (*Fumariaceae*.) *Dry tuberous root-stock*.—Compound grains of 2 to 4 and 6, rarely 8, components which are mostly equal. Size about  $32\mu$ . Separated-grains with a small central cavity, and several radial fissures running toward the corners. Size 4 to  $18\mu$ . No simple grains are found.
- Corydalis fabacea* Pers. (*Fumariaceae*.) *Dry root-stock*.—Grains as in the preceding. Size of separated-grains 14 to  $17\mu$ .
- Corydalis pumila* Host. (*Fumariaceae*.) *Dry root-stock*.—Grains as in the two preceding. Size of the separated-grains 16 to  $20\mu$ .
- Drosera rotundifolia* Linn. (*Droseraceae*.) *Dry roots*.—Compound grains rounded or oval, mostly of somewhat irregular shape, consisting of 2 to 5 and 8 components of different sizes, which generally are somewhat irregularly arranged. Size about  $11\mu$ . Separated-grains, size 2 to  $8\mu$ ; a cavity is frequently observed. Starch quite plentiful.
- Viola cucullata* Ait. (*Violaceae*.) *Dry root-stock*.—Compound grains of 2 to 4 and 6 equal or unequal components; size  $18\mu$ . Separated-grains, size 4 to  $13\mu$ ; instead of a hilum a small almost central cavity is observed from which single fissures radiate; in oval forms the cavity is about one-half eccentric.
- Viola palustris* Linn. (*Violaceae*.) *Dry root-stock*.—Compound grains of 2 to 6 and 9 equal or unequal components. Size about  $12\mu$ . Size of the separated-grains 2 to  $6\mu$ . Simple grains of incomplete formation are also present.
- Viola pinnata* Linn. (*Violaceae*.) *Dry roots and root-stock*.—Compound grains rounded or oval, consisting of 2 to 8 and 12 equal or unequal components. Size about  $14\mu$ . Separated-grains, size 2 to  $8\mu$ ; the larger ones have a cavity.
- Portulaca megalantha* Steud. (*Portulacaceae*.) *Dry roots*.—Compound grains consisting of 2 to 4 equal components. Separated-grains 5 to  $11\mu$ ; frequently with a central cavity. Also simple grains of incomplete formation are found.
- Stellaria bulbosa* Wulfen. (*Caryophyllaceae*.) *Dry tubers*.—Compound grains of 2 to 4 almost equal components, the majority have fallen apart. Separated-grains, size 3 to  $8\mu$ ; occasionally a small cavity is observed, instead of a hilum, which may be about one-half eccentric. Also simple, eccentric-spherical grains are present.
- Melochia pyramidata* Linn. (*Sterculiaceae*.) *Dry roots*.—Compound grains of 2 to 4 and 8 almost equal components, the majority fallen apart. Separated-grains, size 3 to  $8\mu$ . Also simple, spherical, rounded-oval grains are found.

- Triumfetta schimperi* Hochst. (*Tiliaceae*.) *Dry root*.—Compound grains rounded or oval; consisting of 2 to 4 and 8 equal or unequal components, the majority fallen apart. Size about  $15\mu$ . Separated-grains, size 3 to  $9\mu$ ; the larger ones have a small cavity or a slit. Also some simple rounded grains are present.
- Canella alba* Murray; *Cortex canellæ albæ interior*. (*Canellaceae*.) *Dry cortex*.—Compound grains of 2 to 4 and 5 mostly equal components. Size about  $9\mu$ . Size of separated-grains  $6\mu$ ; also almost rounded simple grains, size  $7\mu$ , are observed.
- Hypericum elodes* Linn. (*Hypericaceae*.) *Dry creeping root-stock*.—Compound grains almost round or oval, consisting of 2 to 4 and 6 equal or unequal components. Size about  $19\mu$ . Separated-grains, size 3 to  $13\mu$ ; most of them have a small cavity or a delicate cleft from which solitary short delicate fissures may radiate. Simple grains of incomplete formation are also found.
- Byrsonima crassifolia* DC. (*Malpighiaceae*.) *Cortex*.—Compound grains of 2 to 4 almost equal components. Size about  $25\mu$ . Separated-grains, size about  $13\mu$ ; homogeneous, almost solid. Simple eccentric grains of incomplete formation are also observed.
- Krameria triandra* R. P.; *Ratanhiawurzel krameriæ*. (*Polygalaceae*.) *Dry roots*.—Compound grains of 2 to 4, rarely 6, almost equal components which are regularly arranged; rarely small components adhere to a large one either upon one side or upon the two opposite sides, elongated curved doublets are mostly found. Size about  $35\mu$ . Separated-grains rounded to oval; occasionally slightly compressed; usually homogeneous; rarely with indistinct lamellæ; usually solid; rarely, instead of the hilum, a small cavity is located at the narrow arched end, about one-fourth eccentric, from which short radial fissures occasionally proceed. Simple eccentrically elongated grains are also found.
- Manihot utilissima* Pohl.; *Jatropha manihot* Linn. (*Euphorbiaceae*.) *Tapioca, cassava starch, Brazilian arrowroot*. *From the root*.—Separated-grains semi-spherical to oval, occasionally 3 to 4, more rarely 5, angles with one curved surface and 1 to 5 and 7 pressure facets; the larger ones have one or several delicate lamellæ; instead of the hilum, usually a small cavity about one-half eccentric is observed, frequently with single, short, radial fissures. Size  $2.5$  to  $25\mu$ . Compound grains have all disappeared; they evidently consisted of 2 to 8 partly equal or partly unequal components (several small components adhere to a single large one). Among the starch-grains above described are mingled simple grains which are oval, strongly compressed, and have delicate lamellæ and eccentric hilum. Size  $50$  and  $60\mu$ . These resemble the grains of *Curcuma zedoaria*. They probably belong to *Curcuma* starch (*Tikmehl*).
- Other species of tapioca which are somewhat changed by means of heat: Separated-grains, size  $17$  to  $50\mu$ ; lamellæ rarely distinct; with a central or slightly eccentric, often large, cavity, from which two fissures pass out and embrace a conical substance or a dense, almost round, granule within the cavity. Only a few compound grains of 2 to 4 almost equal components are still present. Also a few simple grains, spherical or oval-spherical, with concentric lamellæ, or with a central cavity, are observed.
- Pachysandra procumbens* Michx. (*Euphorbiaceae*.) *Dry root-stock*.—Compound grains of 2 to 4 almost equal components. Separated-grains, size 3 to  $11\mu$ ; the larger ones have a small cavity. Some simple grains, spherical and rounded-oval, size about  $12\mu$ . This specimen is poor in starch.
- Epilobium hirsutum* Linn. (*Onagraceae*.) *Dry stolons*.—Compound grains of 2 to 4 and 6 components which are frequently equal. Size about  $21\mu$ . Separated-grains, size 4 to  $13\mu$ ; instead of the hilum, a small central or about one-half eccentric cavity is observed, from which delicate fissures radiate to the corners.
- Myriophyllum verticillatum* Linn. (*Haloragaceae*.) *Base of dry rooted stems*.—Compound grains of 2 to 4 almost equal components; size about  $15\mu$ . Separated-grains, size 4 to  $9\mu$ ; mostly with a cavity. Also simple grains of incomplete formation are observed.
- Pyrus malus* Linn. (*Rosaceae*.) *Fresh pulp of the fruit*.—Compound grains of 2 to 4 usually equal components. Size about  $16\mu$ . Components with indistinct, small, almost central hilum. Size 4 to  $11\mu$ . Also a few simple spherical grains are present.
- Comarum palustre* Linn. (*Rosaceae*.) *Dry root-stock*.—Compound grains of 2 to 4 and 6 components which are usually almost equal. Size about  $10\mu$ . Separated-grains, size 2 to  $6\mu$ ; the larger ones generally have a small cavity. Also simple grains, spherical or rounded-oval, are observed; size about  $8\mu$ .

- Potentilla aurea* Linn. (*Rosaceæ*.) *Dry root-stock*.—Separated-grains, size 2 to  $5\mu$ ; the smaller ones rounded, the larger ones more or less polyhedral, and frequently with a small cavity. Most of the compound grains have fallen apart; those still present are composed of 2 to 8 almost equal components. Some more complex ones seem to have been present. Simple rounded grains are also found.
- Alchemilla alpina* Linn. (*Rosaceæ*.) *Dry root-stock*.—Compound grains, almost round to elongated-oval, frequently somewhat irregular, consisting of 2 to 10 and 20 components which are almost equal. Size about  $14\mu$ . Separated-grains, size 2 to 4 and  $6\mu$ ; occasionally with a small cavity. Some simple spherical and oval grains are also present.
- Geum urbanum* Linn.; *Radix caryophyllacæ*. (*Rosaceæ*.) *Dry root-stock*.—Compound grains almost rounded or oval, rarely elongated-lanceolate, consisting of 2 to 8, rarely more than 8, components which are equal and regularly arranged (a few are in one row). Sizes about  $10\mu$ . Separated-grains almost round or angular with rounded corners; size 1.5 to  $5\mu$ . Some simple spherical grains are also observed, many of which appear at first sight to be simple prove by closer observation to be divided by delicate lines.
- Geum montanum* Linn. (*Rosaceæ*.) *Dry root*.—Compound grains of 2 to 8 different-sized components. Size of separated-grains, 2 to  $6\mu$ . Simple almost round or oval grains are also present.
- Crotolaria incana* Linn. (*Leguminosæ*.) *Dry root*.—Compound grains of 2 to 4 and 6 components which are generally equal. Size about  $14\mu$ . Separated-grains, size 2 to  $7\mu$ ; many with a small cavity. Also simple, almost round grains are found. Starch rather scarce.
- Astragalus incanus* Linn. (*Leguminosæ*.) *Dry root-stock (rhizome)*.—Compound grains of 2 to 4 almost equal components. Size about  $17\mu$ . Size of the separated-grains 4 to  $10\mu$ . Simple grains of incomplete formation are also observed.
- Lathyrus pratensis* Linn. (*Leguminosæ*.) *Dry roots*.—Compound grains consisting of 2 to 8 components of different sizes. Size about  $16\mu$ . Separated-grains, size 2 to  $9\mu$ ; occasionally with a small cavity. Also solitary simple, almost round grains are present. Starch plentiful. In the root-stock of the same plant the starch-grains are rather scarce. Size of the compound grains  $10\mu$ ; of the separated-grains 1.7 to  $6\mu$ . Also simple round grains are present.
- Lathyrus palustris* Linn. (*Leguminosæ*.) *Dry root-stock*.—Compound grains of 2 to 10 and 15 components of different sizes. Most of the compound grains have fallen apart. Size about  $24\mu$ . Separated-grains, size 3 to  $14\mu$ ; instead of the hilum a small cavity is usually observed, which in the oval forms is one-half and one-third eccentric. Starch plentiful, filling up the cells of the cortex.
- Zornia angustifolia* Smith. (*Leguminosæ*.) *Dry root-stock*.—Compound grains of 2 to 10 and more components of different sizes; size about  $14\mu$ . Size of the separated-grains, 2 to  $8\mu$ . Some simple grains, almost round or oval, are also found.
- Alysicarpus ferrugineus* Steud. (*Leguminosæ*.) *Dry root-stock*.—Compound grains of 2 to 10 and  $15\mu$ , almost equal components. Size about  $16\mu$ . Size of the separated-grains 2 to  $7\mu$ . A few simple rounded and oval grains are also present. Starch rather plentiful.
- Desmanthus virgatus* Willd. (*Leguminosæ*.) *Dry root*.—Compound grains of 2 to 4 and 6 components. The majority have fallen apart. Size about  $18\mu$ . Size of the separated-grains 3 to  $10\mu$ . Also simple spherical and oval grains are also found. Starch not plentiful.
- Encephalartos spiralis* Lehm. (*Cycadaceæ*.) *Dry embryo*.—Compound grains of 2 to 4, rarely more (up to 8), components which are usually equal and regularly arranged. Separated-grains with 1 to 3 pressure facets, which are semi-spherical and also frequently somewhat oblique, or they may be oval and often cut off obliquely at one end; instead of the hilum a small cavity is observed from which short delicate fissures radiate, one-half to one-third eccentric; usually one or two circular lamellæ around the hilum, as well as several incomplete lamellæ toward the distal end. Size of the separated-grains, 5 to 25 and  $30\mu$ . Simple eccentric-conical grains are also present.
- Ophirus athiopicus* Rupr.; *Ophirus papillosus* Hochst. (*Graminaceæ*.) *Dry endosperm*.—Compound grains when lying loose in the cell are almost round or oval; when crowded they are angular or even polyhedral. They consist of 2 to 10 rarely 20 components. Size about  $15\mu$ . Separated-grains, size 2 to  $8\mu$ ; usually polyhedral, sometimes with 1 curved surface and 1 to 6

- pressure facets; the larger ones have a small cavity and frequently several radial fissures. Simple spherical and spherical-oval grains are also observed; size  $13\mu$ . These apparently belong to the centric-spherical type. Some of the polyhedral grains may also be simple forms.
- Rottbælla arundinacæ* Hochst. (*Graminacæ*.) *Dry endosperm*.—Separated-grains with one curved surface and 1 to 4 pressure facets; instead of the hilum a small cavity is usually observed from which single fissures sometimes radiate. Size 3 to  $8\mu$ . Compound grains are less freely observed; they probably consist of not more than 8 to 10 components. Simple centric-spherical grains are also present.
- Rottbælla campestris* Nutt. (*Graminacæ*.) *Dry endosperm*.—Compound grains spherical or oval, consisting of 2 to 20 equal parts (few seen free). Separated-grains, size 2 to  $10\mu$ ; some with one curved surface and 1 to 6 pressure facets; others completely polyhedral; the larger ones usually with a small cavity and often with single radiating fissures. Also simple grains, spherical or spherically-oval; size about  $12\mu$ ; the larger ones with a small central cavity and several radial fissures, belonging to the centric-spherical type (type 1). Many of the polyhedral grains may also be simple.
- Lucea colorata* Hochst. (*Graminacæ*.) *Dry endosperm*.—Separated-grains with 1 curved surface and 1 to 5 pressure facets, hollow. Size 3 to  $11\mu$ . Compound grains of not many components are very scarce; also simple hollow grains almost round, rarely oval, and somewhat compressed. Size about  $13\mu$ . The simple and the separated-grains (the former about one-half to one-third as numerous as the latter) look exactly alike; the central cavity in both is large, uneven, angular, jagged, or extends into short radial fissures.
- Wachendorfia hirsuta* Thumb. (*Hæmodoracæ*.) *Dry endosperm*.—Compound grains (most of which have fallen apart) consist of 2 to 12 and more components, which are almost equal, and regularly or irregularly arranged. Separated-grains, size 3 to  $20\mu$ ; rounded-angular or polyhedral, with large or small cavity which is frequently very conspicuous. The starch gives the impression of having been affected by heat.
- Hæmodorum* (a species from Swan River). (*Hæmodoracæ*.) *Dry endosperm*.—Compound grains almost round to oblong and pear-shaped, frequently somewhat irregular, consisting of 2 to 10 almost equal components (frequently arranged 4 in a square, and sometimes in a row), rarely falling apart; lines of division sometimes indistinct or entirely wanting, so that the cavities of the components are seen, as in type 12. Size about  $28\mu$ . Single components, size about  $13\mu$ ; almost oval and have rather large, often slit-like cavity. Simple grains, size about  $16\mu$ ; oval, and slightly compressed with a slit-like cavity. It is rather uncertain whether these belong to the eccentric or to the oval type. A constant analogy which the compound grains of few components holds with those of the seeds of *Leguminosæ* is in support for the latter type.
- Barbaenia rogieri* Hort. (*Amaryllidacæ*.) *Dry endosperm*.—Separated-grains more or less polyhedral, frequently with sharp angles and edges; isodiametric or twice as long as broad; with a central almost round or elongated cavity from which radial fissures diverge. Size 3 to  $18\mu$ , width about  $14\mu$ . Few compound grains are less seldom present; they consist of 2 to 8 components.
- Hohenbergia strobilacea* Schult. fil. (*Bromeliacæ*.) *Dry endosperm*.—Compound grains spherical or oval, sometimes somewhat irregular as a result of pressure, consisting of 2 to 5 almost equal components which are arranged either irregularly or regularly (3 in a triangle or 4 in a square or tetrahedron). Size about  $15\mu$ . Separated-grains almost isodiametric; they frequently have a small central cavity, without fissures. Also simple centric-spherical grains are also found.
- Billbergia zebrina* Lindl. (*Bromeliacæ*.) *Dry endosperm*.—Compound grains spherical-oval, rarely irregular; consisting of 2 to  $6\mu$ , rarely more, almost equal components which are regularly arranged. Size about  $21\mu$ . Separated-grains with rather sharp angles and margins; with a small central cavity from which single fissures occasionally proceed. Size 4 to  $10\mu$ . Simple centric-spherical grains are also present.
- Pitcairnia albucafolia* Schrad.; *Pitcairnia punicea* Lindl. (*Bromeliacæ*.) *Dry endosperm*.—Separated-grains with 1 to 4 pressure facets with rather sharp angles and margins; usually with a large cavity from which single radial fissures occasionally proceed. Size 4 to 12 and  $16\mu$ . Compound grains few, consisting of 2 to 6 components. Simple centric-spherical grains are also present.

- Dyckia remotiflora* Otto. (*Bromeliaceæ*.) *Dry endosperm*.—Compound grains spherical to oval, usually of almost regular shape, about twice as long as broad, consisting of 2 to 10 and 12 nearly equal components which are often regularly arranged. Length about  $24\mu$ , width about  $19\mu$ . Separated-grains with acute angles and margins, with a cavity at the hilum which is somewhat nearer the pressure facets and from which 1 to 3 fissures radiate toward the angles. Size about  $14\mu$ . Isolated spherical or oval simple grains are also observed.
- Pistia texensis* Klotsch. (*Aroidæ*.) *Dry endosperm*.—Compound grains spherical, oval, rarely shortened-conical; consisting of 2 to 12 and 20 almost equal components. Size about 11 to  $14\mu$ . Separated-grains, size 2 to  $6\mu$ ; the larger ones with a small central cavity.
- Pistia stratiotes* Linn. (*Aroidæ*.) *Dry endosperm*.—Separated-grains occasionally with a small central cavity; size 2 to  $8\mu$ . Compound grains few, consisting of 2 to 8 components. The starch-grains of both species of *Pistia* form a wall-like layer in the endosperm cells. The lumen is filled with a substance which takes a golden yellow stain when treated with iodine.
- Aponogeton distachyum* Thumb. (*Alismaceæ*.) *Dry endosperm*.—Separated-grains with one curved surface and 1 to 3 pressure facets; usually with a small cavity. Size 2.5 to  $11\mu$ . Compound grains (only a few intact ones present); size about  $18\mu$ ; consisting of 2 to 5, rarely 10, components. According to Payen (*Ann. Sc. Nat.*, 1838, II, 25, pl. 6, fig. 23), the size of the separated-grains is  $22.5\mu$ ; with eccentric hilum and occasionally with several fissures radiating toward the distal end.
- Corylus avellana* Linn. (*Corylaceæ*.) *Fresh immature cotyledons*.—Compound grains consisting of 2 to 8 almost equal components, which are irregularly arranged; small and falling apart easily. After the compound grains have fallen to pieces the separated-grains which are angular with rounded angles increase considerably in size about  $5\mu$ . Simple almost round or oval grains are also found. Size about  $6\mu$ .
- Nectandra rodigi* Schomburgh; *Fructus bebeeru*. (*Thymelaceæ*.) *Dry cotyledons*.—Compound grains of 2 to 4, rarely more, almost equal components which are arranged regularly. Separated-grains isodiametric to twice as long as broad; instead of the hilum a small cavity is observed from which 2 short fissures occasionally radiate toward the proximal end (the ones with pressure facets), eccentricity about one-third. Size 8 to  $22\mu$ . A few rounded or shortened-conical simple grains are also present; the hilum is toward the thicker end. Therefore they belong to the eccentric-conical type (type 7).
- Laurus nobilis* Linn. (*Lauraceæ*.) *Dry cotyledons*.—Compound grains almost round to elongated-oval, consisting of 2 to 6 almost equal components. Separated-grains with a slightly eccentric cavity from which several short fissures diverge; size about  $15\mu$ . Simple eccentric inverted-conical grains are also present.
- Scabiosa atropurpurea* Linn. (*Dipsacaceæ*.) *Fresh immature endosperm*.—Grains rounded or rounded-angular; size about  $4\mu$ . The greater number of these are probably separated-grains; the smaller number are simple. Some larger compound grains consisting of 2 to 8 almost equal components are also observed. The starch forms a lining to the wall of the cells, which have transparent, colorless contents.
- Atropa belladonna* Linn. (*Solanaceæ*.) *Fresh immature seed-coats*.—Grains spherical-oval and rounded-angular; size about 6 to  $8\mu$ . Many of them are separated-grains. Compound grains consisting of 2 to 8 components are also present.
- Ægiceras majus* Gart. (*Myosinaceæ*.) *Dry cotyledons*.—Compound grains consisting of 2 to 4, rarely 8, usually equal components which are arranged regularly. Separated-grains, size 4 to  $18\mu$ ; with a small cavity from which fissures radiate toward the circumference of the pressure facets. Starch very plentiful.
- Lucuma rivicoa* Gart. fil. (*Sapotaceæ*.) *Dry cotyledons*.—Compound grains consisting of 2 to 4, rarely 8, equal or unequal components. Size about  $28\mu$ . Separated-grains with 1 curved surface and 1 to 4 pressure facets. Size 4 to  $15\mu$ ; the larger ones have a small cavity and single radial fissures; eccentricity about one-half, rarely one-third. Isolated simple, rounded or shortened-conical grains are also present. Length about  $20\mu$ ; with a cavity toward the thicker end, from which 2 fissures radiate toward the narrowed end. Starch belongs to the eccentric-conical type (type 7).

- Lucuma* sp. (perhaps *Lucuma cainito* A. DC.) (*Sapotaceæ*.) *Dry cotyledons*.—Compound grains consisting of 2 to 4, occasionally more, components which are usually equal. Size about  $20\mu$ . Separated-grains, with 1 to 3 and 4 pressure facets; size 3 to  $12\mu$ ; the larger ones have a small cavity with single fissures radiating toward the angles; eccentricity about one-half.
- Lucuma* sp. (*Sapotaceæ*.) *Dry cotyledons*.—Compound grains rounded to oblong, occasionally somewhat irregular, 2.5 times as long as broad, consisting of 2 to 12 and more almost equal components. Length about  $23\mu$ , thickness about  $18\mu$ . Separated-grains with one curved surface and several pressure facets; with one large or small cavity; size 2 to  $10\mu$ . Some simple grains are also found.
- Myristica moschata* Thumb. (*Myristicaceæ*.) *Dry endosperm*.—Compound grains rounded to elongated-oval, occasionally somewhat compressed, consisting of 2 to 10, rarely more, equal components which are usually arranged regularly. Size about  $27\mu$ . Separated-grains, size 4 to  $15\mu$ ; with a small central cavity from which single short fissures occasionally proceed.
- Myristica salicifolia* Willd. (*Myristicaceæ*.) *Dry seed*.—Compound grains almost round to oblong, consisting of 2 to 10 and 16 usually equal components which are arranged quite regularly. Size about  $34\mu$ . Separated-grains frequently with a small central cavity from which short fissures radiate; size 5 to  $18\mu$ .
- Pæonia*. (*Ranunculaceæ*.) *Fresh unripe endosperm of various species*.—Compound grains spherical or oval, consisting of 2 to 4 and 6, rarely 8, components which are usually equal. Size about  $16\mu$ . Separated-grains size 3 to  $12\mu$ . Isolated spherical or oval simple grains are also present. Seeds have sprouted and are either still green or have become red, and are entirely filled with starch. At complete maturity they contain no trace of starch, but a great deal of oil.
- Corydalis lutea* DC. (*Fumariaceæ*.) *Fresh placenta of immature fruit*.—Separated-grains rounded-angular; size about  $5\mu$ . Compound grains consisting of 2 to 8 components are also found. Starch quite plentiful.
- Brassica napus* Linn. (*Brassicaceæ* or *Crucifereæ*.) *Fresh endosperm, cotyledons, and seed-coats of immature unripe seeds*.—Separated-grains with 1 curved surface and 1 to 4 pressure facets; size 8 to  $12\mu$ . The few compound grains which have not fallen apart consist of 2 to 4 almost equal components. Simple grains of incomplete formation are also observed.
- Carolinea princeps* Linn. (*Malvaceæ*.) *Dry cotyledons*.—Compound grains consisting of 2 to 6 components which sometimes are equal and regularly or often unequal and irregularly arranged; 1 large and 1 to 4 small components. Separated-grains with 1 curved surface and 1 to 4 pressure facets; with a small central cavity and single radial fissures. Size about 15 to  $20\mu$ ; also simple eccentric-conical grains are also observed.
- Heritiera littoralis* Ait. (*Sterculiaceæ*.) *Dry cotyledons*.—Compound grains spherical elongated-lanceolate, frequently somewhat irregular, 3 times as long as broad, consisting of 2 to 12 or more components which are almost equal and usually arranged regularly (rarely in a simple row). Size about  $23\mu$ . Separated-grains with a central cavity from which short fissures radiate. Size 4 to  $10\mu$ . Some simple spherical grains are also observed.
- Theobroma cacao* Linn. (*Sterculiaceæ*.) *Dry cotyledons*.—Separated-grains rounded-angular, frequently with 1 curved surface and 1 to 3 pressure facets. Size about  $8\mu$ . Compound grains consisting of 2 to 4, rarely more, almost equal components. The cells are filled with oil in which the starch-grains are scattered.
- Thea bohea* Linn. (*Camelliaceæ*.) *Dry cotyledons*.—Compound grains consisting of 2 to 4, rarely 8 components, which are usually equal and regularly arranged (of which 3 are usually arranged lineally, 3 and 4 in one plane). Size about 20 to  $25\mu$ . Separated-grains, size 4 to 9 and  $12\mu$ ; instead of a hilum a small cavity is observed from which fissures usually radiate toward the surface of the pressure facets. Simple grains of incomplete formation are also found.
- Triphasia aurantiola* Lour.; *Limonia trifoliata* Linn. (*Rutaceæ*.) *Dry cotyledons*.—Grains consisting of 2 to 4 and 8 components of different sizes. Separated-grains usually with 1 to 3 pressure facets and a small cavity; size 3 to  $8\mu$ . A few simple rounded and rounded-oval grains, size about  $9\mu$ , are also present. The seeds are quite conspicuous and seem to be mature. The cells are filled with starch, and have little or no oil.
- Trichilia micrantha* Benth. (*Meliaceæ*.) *Dry cotyledons*.—Compound grains rounded to oblong, frequently irregular, consisting of 2 to 8 and 12 components which are usually equal. Size about 15 to  $20\mu$ . Separated-grains, size 3 to  $11\mu$ ; the larger ones have a small central cavity.

- Trichilia* sp. (*Meliaceæ*). *Dry*.—Compound grains consisting of 2 to 8, rarely more, equal components which are generally arranged regularly. Size about  $18\mu$ . Separated-grains, size 4 to  $12\mu$ ; a small cavity and single indistinct, short radial fissures are observed.
- Guarua trichilioides* Linn. (*Meliaceæ*). *Dry cotyledons*.—Compound grains rounded or oval, consisting of 2 to 12 and 20 mostly equal components. Size about  $15\mu$ . Separated-grains usually with 1 curved surface and 1 to 5 pressure facets; the larger ones with a small cavity. Size 2 to  $8\mu$ .
- Erythroxyton columbinum* Mart. (*Linaceæ*). *Dry cotyledons*.—Compound grains rounded or oval, as thick as broad or somewhat compressed, consisting of 2 to 12 and 20 components which are usually almost equal. Size about 20 to  $25\mu$ . Separated-grains, size 3 to  $11\mu$ ; with a small central cavity and single radiating fissures. The starch-grains in the scarce perisperm of the same plant are like those in the cotyledons, although the average compound ones consist of only 2 to 8 and 10 components, and are somewhat larger. The diameter of the separated-grains is about 14 and  $17\mu$ , and the cavity at the hilum is frequently one-half and one-third eccentric.
- Erythroxyton* (species from Brazil). (*Linaceæ*). *Dry seed*.—In the large cotyledons the grains are compound, consisting of 2 to 8 components; the size of the separated-grains is about  $14\mu$ . Separated-grains in the scarce perisperm are about  $20\mu$ .
- Erythroxyton nitidum* Mart. (*Linaceæ*). *Dry seed*.—The grains are similar to those in *Erythroxyton columbinum*. The compound grains in the large cotyledons consist of 2 to 10 and 16 components. Size  $15\mu$ . Size of the separated-grains about 6 to  $7\mu$ . Perisperm almost entirely wanting.
- Erythroxyton mucronatum* Benth. (*Linaceæ*). *Dry seed*.—Grains as in *E. columbinum*. Size of the separated-grains in the large cotyledons about  $8\mu$ , and in the very scant perisperm about  $9\mu$ .
- Erythroxyton rufum* Cav. (*Linaceæ*). *Dry seed*.—Grains as in *Erythroxyton columbinum*. The size of the separated-grains in the small cotyledons is about  $10\mu$ , in the plentiful perisperm about  $15\mu$ . Compound grains in the latter consist of 2 to 8 and 12 components.
- Erythroxyton obtusum* DC. (*Linaceæ*). *Dry seed*.—Grains as in *Erythroxyton columbinum*. Size of the separated-grains in the abundant perisperm is about  $15\mu$ . Some simple spherical grains, size about  $15\mu$ , are also found.
- Cupania tomentosa* Swartz. (*Sapindaceæ*). *Dry cotyledons*.—Compound grains consisting of 2 to 8 and 12 usually equal components which are arranged regularly. Size about  $12\mu$ . Size of the separated-grains 2 to  $7\mu$ .
- Protium pubescens*; *Icica pubescens* Benth. (*Burseraceæ*). *Dry cotyledons*.—Separated-grains angular with rounded angles, or oval with 1 curved surface and 1 to 5 pressure facets; instead of a hilum a small cavity is observed, and in the larger ones also single radial fissures. Size 2 to  $9\mu$ . Compound grains (the most of which have fallen apart) consist of 2 to about 10 usually equal components. Simple grains, rounded, oval, and low cone-shaped, size about  $10\mu$ , are also present; instead of the hilum a small cavity is found from which single fissures radiate, about one-half eccentric. These grains probably belong to the eccentric-conical type.
- Alangium decapetalum* Lam. (*Cornaceæ*). *Dry endosperm*.—Separated-grains with 1 curved surface and 1 to 4 pressure facets; usually with a small central cavity; size 2.5 to  $8\mu$ . Compound grains are no longer present; probably they consisted of 2 to 8 equal components.
- Alangium hexapetalum* Lam. (*Cornaceæ*). *Dry endosperm*.—Grains polyhedral, with a small central cavity; size 3 to  $9\mu$ . They lie closely packed in the cells, which they completely fill. They are probably separated-grains. Nägeli could not clearly distinguish any compound ones.
- Memecylon capense* Eckl. Zeyh. (*Melastomaceæ*). *Dry cotyledons*.—Compound grains consisting of 2 to 4 and more mostly equal, rarely unequal, components. Separated-grains, size 4 to  $14\mu$ , are usually oblique and unsymmetrical; they have a small cavity and single radial fissures. Also simple eccentric-conical grains are present.
- Memecylon amplexicaule* Roxb. (*Melastomaceæ*). *Dry cotyledons*.—Grains as in the preceding species, but the separated-grains are more symmetrical. Simple grains are also present.
- Pyrus malus* Linn.; *Pyrus communis* Linn. (*Rosaceæ*). *Fresh unripe cotyledons*.—Grains rounded and angular with rounded corners; size about  $5\mu$ . These are separated-grains, since in the still younger stages only small compound grains occur, consisting of 2 to 6 and 8 almost equal components.

*Cicer*, *Pisum*, *Ervum*, *Orobus*, etc. (*Leguminosæ*.) *Cotyledons*.—Among the simple centric-oval grains are found compound grains which consist of 2 to 4 usually equal components which are more or less regularly arranged. Separated-grains oval, rarely rounded, with 1 curved surface and 1 to 3 small pressure facets; lamellæ none, or few and delicate; with an oblong compressed hilum; after drying, a distinct median slit is mostly visible from the narrow aspect, and radial fissures from the broad aspect. Size of the compound grains of *Pisum sativum* Linn. about 75 $\mu$ . Size of the separated-grains about 56 $\mu$ . Size of the separated-grains in *Cicer arictinum* Linn. about 19 $\mu$ .

*Amphicarpæa monoica* Nutt. (*Leguminosæ*.) *Dry cotyledons*.—Compound grains consisting of 2 to 6 components which are usually almost equal. Size about 14 $\mu$ . Separated-grains; size 3 to 10 $\mu$ ; the large and oval forms have instead of a hilum a small cavity about one-half eccentric. Also simple eccentric-conical grains are found.

#### TYPE 15. GRAINS COMPOUND, COMPONENTS FEW AND UNEQUAL.

From 2 to 10 unequal components united into a compound grain; divided by fissures and at maturity falling apart into separated-grains which have one curved surface and one or more flattened pressure facets. These compound grains for the most part arise from the cutting off of angles and edges. For this reason they frequently consist of several small components piled upon one large one, or even of two or more large ones which have small ones at their line of union. They rarely occur alone, but usually are mingled with simple grains or compound grains of equal components, the latter being formed by division of the hilum and thus are related to type 14. They may have their origin by a number of simple grains in one chloroplast uniting into a compound one, the latter frequently, partially or entirely, retaining its green color.

*Chara fatida* A. Braum. (*Algæ*.) *Fresh nodes of the stem (basal nodes of the whorled branches)*.—Compound grains spherical and oval to rod-shaped; about 4 times as long as thick; consisting of 2 to 12, rarely 20, unequal components; green at first, colorless later. Length about 34 $\mu$ , thickness about 23 $\mu$ . Separated-grains polyhedral, usually with 1 curved surface and 1 to 4 pressure facets; 1 to 2 times as long as broad; no lamellæ; usually without a hilum. Size 5 to 17 $\mu$ . Starch-grains arise within the chloroplasts.

*Pteridophyta*. *Green parts*.—Compound grains spherical, arising within the chloroplasts and consisting sometimes of 2 to 5, sometimes 2 to 10 or more, components which are more or less equal, and usually lie in one plane, and are green to colorless. Occasionally with irregular accretions of fatty oil. Some simple grains are also present.

*Opuntia coccinellifera* Mill. (*Cactaceæ*.) *Green parts*.—The simple grains are somewhat disk-shaped; size about 16 $\mu$ . The compound ones, size about 24 $\mu$ , consist of 2 to 5 components; the size of a component is 3 to 12 $\mu$ .

*Begonia* sp. (*Begoniaceæ*.) *Leaves*.—Compound grains consisting of 1 to 3, rarely 16, components; size 24 $\mu$ ; part-grains 3 to 12 $\mu$ .

*Nerium oleander* Linn. (*Apocynaceæ*.) *Young leaves*.—Simple grains almost rounded; size usually about 6 $\mu$ . Compound grains, of 2 to 4 and 5 components; size about 9 $\mu$ .

*Nephrolepis exaltata* Schott. (*Polypodiaceæ*.) *Base of the frond*.—Compound grains of 2 to 8 and more components; size 31 $\mu$ . Size of the components 3 to 16 $\mu$ .

*Chara stelligera* Bauer. (*Algæ*.) *Dry stellate bodies*.—These compound grains, which approach the semi-compound type, are distinguished by having 1 to 30 to 70, and occasionally 100, small components adhering to one large rounded one. These contain one or usually several hila (or as a result of drying, small hollow spaces are found instead of the hila), and thus they become semi-compound, frequently showing distinct concentric lamellæ (around the mathematical center), and short radial fissures in the interior. The smaller components have a small central cavity, but neither lamellæ nor fissures. They are found either at one or the two opposite sides of the large component, and when 2 to 5 are joined they lie in one row, thus forming a border. When present in larger numbers, they lie in one flat plane and form a sort of shell which covers one-sixth to two-thirds of the circumference of the large component. This shell can often be loosened by means of pressure as well as by the expansion of the larger component, which in the center has from two to several lamellæ. Size of the complete compound grain about 70 to 85 $\mu$ ; of the large components 55 and 70 $\mu$ ; and of the small adhering components 5 to 13 and 20 $\mu$ .

- Isoëtes lacustris* Linn. (*Isoëtaceæ*.) *Fresh stems*.—Compound grains, very irregular, with protruding angles and short lobes; 1 to 3 times as long as broad; consisting of 2 to about 10 unequal, irregularly arranged components. Length about  $33\mu$ , thickness about  $20\mu$ . Separated-grains irregular, usually with sharp corners; homogeneous or with a small central hilum; occasionally with short, radial fissures. Size about  $17\mu$ . Simple grains of almost round or irregular shape are also present. The starch-grains in the youngest part of the stem are also irregular, one margin being thickened and the opposite one broadened, thinned, and angular. They are clearly either simple grains, or very delicate lines may be recognized which indicate division. In somewhat older parts of the stem almost all of the grains show more or less distinct lines of separation, and therefore appear to be compound, while the oldest tissues contain compound grains, as well as numerous separated-grains, and also simple rounded forms (perhaps separated-grains which after having fallen apart have become rounded by further growth). The fissures of the innermost components are first indicated in the later stages.
- Mariscus elatus* Vahl. (*Cyperaceæ*.) *Dry root-stock*.—Compound grains consisting of 2 to 4 and 6 usually unequal components. Size about  $11\mu$ . Separated-grains, size 2 to  $6\mu$ ; frequently with a small cavity. Simple, spherical, oval, and low-conical grains are also found.
- Cyperus phymatodes* Mhlbrg.; *Cyperus repens* Ell. (*Cyperaceæ*.) *Dry root-stock*.—Compound grains consisting of 2 to 4 mostly unequal components. Size about  $14\mu$ . Separated-grains, size 2 to  $8\mu$ ; usually with a small cavity. Simple spherical, oval, and pear-shaped grains (resembling those of *Cyperus esculentus*) are also present.
- Abildgaardia monostachya* Vahl. (*Cyperaceæ*.) *Dry root-stock*.—Compound grains consist of 2 to 4 and 6 equal and unequal components. Size about  $10\mu$ . Separated-grains, size 2 to  $6\mu$ ; occasionally with a small cavity. Simple grains of incomplete formation.
- Carex arcnaria* Linn. (*Cyperaceæ*.) *Dry root-stock*.—Compound grains consisting of 1 large almost round or rounded-oval and 1 to 2 small components. Size of the former  $8\mu$ , of the latter, 1.3 to  $2\mu$ . Also simple grains of incomplete formation are present.
- Carex hirta* Linn. (*Cyperaceæ*.) *Dry root-stock*.—Grains as in the preceding.
- Triglochin barrelieri* Lois. (*Naiadaceæ*.) *Dry root-stock*.—Compound grains consisting of 2 to 4 mostly unequal, rarely equal, components. Separated-grains, size 4 to  $28\mu$ ; the larger ones have a longitudinal axis from which sometimes single, lateral fissures proceed. Simple grains of incomplete formation.
- Veratrum album* Linn. (*Liliaceæ*.) *Dry roots*.—Compound grains of 2 to 4 and 5, occasionally equal, more frequently unequal, components. Size about  $25\mu$ . Separated-grains almost rounded or oval, with 1 to 3 pressure facets. Size 4 to  $18\mu$ . The larger ones have a small cavity from which two short fissures diverge; about one-third eccentric. Simple spherical or spherical-oval grains are observed which have instead of the hilum a small central, or about one-half eccentric, cavity. Size about  $18\mu$ . The starch-grains in the root-stocks are similar to the above, but smaller and usually simple.
- Bulbocodium vernum* Linn. (*Liliaceæ*.) *Dry tubers*.—Compound grains of 2, rarely 3 and 4, components, which are occasionally equal, more frequently unequal. Size about  $8\mu$ . Size of the separated-grains 1.5 to  $6\mu$ . Simple grains of incomplete formation are also present.
- Gagea lutea* Schult. (*Liliaceæ*.) *Dry bulb*.—Compound grains, of 1 large and 1 to 3 and 4 small components. Separated-grains, size 4 to  $30\mu$ ; the larger ones almost round, with a small central cavity and radial fissures. Also simple grains of rudimentary formation are found.
- Scilla maritima* Linn. (*Liliaceæ*.) *Dry bulbs*.—Compound grains almost round to oblong, usually irregular, consisting of 2 to 15 and 20 components which are usually either unequal, or occasionally equal, and arranged irregularly. Size about  $21\mu$ . Separated-grains, size 2 to  $11\mu$ ; frequently with a small cavity. Simple, round oval, and pear-shaped grains are also present. Size about  $15\mu$ .
- Narthecium ossifragum* Huds. (*Liliaceæ*.) *Dry root-stock*.—Compound grains consisting of 2 to 4 and 8 components which are usually unequal, or sometimes equal, and arranged irregularly. Size about  $10\mu$ . Size of the separated-grains, 2 to  $6\mu$ . Simple grains of rudimentary structure are also found.

- Galanthus nivalis* Linn. (*Amaryllidaceæ*.) *Fresh bulb*.—Compound grains consisting mostly of 1 large and 1 to 8 small components. Size of the former about  $25\mu$ , of the latter 2 to  $5\mu$ . Simple eccentric-cuneiform grains are also observed. Small components (part-grains) are cut off from the angles and the distal margin of these cuneiform grains; these components when very numerous usually form one or two transverse rows; very rarely do they show an irregular arrangement.
- Canna logunensis* Lindl.; *Canna pedunculata* Sims; *Canna* sp.; *Canna warszewiczii*. (*Cannaceæ*.) *Fresh root-stock*.—Compound grains consisting of one large and several (2 to 10) small components. The latter are usually cut away from the distal end, rarely from the lateral surfaces. Length of the large component about  $117\mu$ , width about  $75\mu$ . Diameter of the small component 3 to  $5\mu$ . Simple eccentrically compressed grains are also found.
- Arum maculatum* Linn. (*Aroideæ*.) *Fresh root-stock*.—Compound grains round or oval, usually obtuse-angular and irregular; consisting of 2 to 10 components which are sometimes equal but usually unequal, and often irregularly arranged. Size about  $23\mu$ . Separated-grains with one curved surface and several pressure facets, usually with sharp corners; lamellæ none; rarely with a distinct hilum, size of the more polyhedral separated-grains about  $9\mu$ , and of the semi-spherical or oval ones about  $15\mu$ . A few simple spherical grains are also present.
- Arum ternatum* Thumb. (*Aroideæ*.) *Fresh root-stock*.—Compound grains consisting of 2 to 10 components which are mostly unequal, and often irregularly arranged. Separated-grains usually without lamellæ, though occasionally a few delicate ones are present; hilum is generally visible and often with 2 to 3 short, radial fissures. Size of the separated-grains 5 to  $24\mu$ . Also a few simple spherical grains are found.
- Typha minima* Hoppe. (*Typhaceæ*.) *Dry root-stock*.—Compound grains consisting of 2 to 12 and 20 components which are occasionally equal, though more frequently unequal. Size about  $15\mu$ . Size of the separated-grains 1.6 to  $8\mu$ . Isolated simple round grains are also present; size about  $9\mu$ .
- Sagus rumphii* Willd. (*Palmaceæ*) and other palms. *Sago from the pith of the stem*.—According to Schleiden (*Grundzuge*, 3 Aufl., 1, 186), the grains are compound by means of several small ones adhering to one large one.
- The sago flour which has not been changed by heat consists principally of separated-grains; length  $65\mu$ , width about  $50\mu$ ; about 0.66 to 2.5 times as long as broad; the narrow ones terete, the wider ones slightly compressed; instead of the hilum, a small cavity is observed, mostly with a cross-fissure, occasionally with radial fissures; about one-fourth to one-sixth eccentric; lamellæ numerous, but delicate and indistinct. The larger separated-grains have 1 to 3 pressure facets at the distal end, since these small components have been cut off at this end. Only a few compound grains are present; they consist of 1 large and 1 to 3 small components. A few simple grains also occur, resembling the larger separated-grains but without pressure facets; length about  $70\mu$ , width about  $55\mu$ ; rounded, conical, oblong, or triangular, broader at the distal end; two-thirds to almost 3 times as long as broad; the broader ones slightly compressed; from the narrow longitudinal aspect they are sometimes thicker, sometimes thinner, towards the distal end. All eccentric forms are represented among the simple and the separated-grains, namely, the inverted-conical, the conical, and the flattened forms; but generally they are without pronounced features; the greatest number, however, belong to the eccentric rod-shaped type (type 9).
- Peperomia monostachya* R. P. (*Piperaceæ*.) *Dry root-stock*.—Grains compound, of 2, 3, rarely 4, mostly unequal components. Size about  $21\mu$ . Separated-grains, size 5 to  $15\mu$ ; the larger ones have a small central cavity from which several radial fissures proceed.
- Polygonum aviculare* Linn. (*Polygonaceæ*.) *Dry root*.—Compound grains consisting of 2 to 4 and 6 components which are occasionally equal, more frequently unequal, most of which have fallen apart. Size 10 to  $13\mu$ . Size of the separated-grains about  $7\mu$ ; usually with a small or large cavity. Also simple grains of incomplete formation are present.
- Polygonum convolvulus* Linn. (*Polygonaceæ*.) *Dry root*.—Compound grains consisting of two to four components of different sizes. Size about  $11\mu$ . Separated-grains, size about  $6\mu$ . Also simple grains of incomplete formation are present.
- Rumex acetosa* Linn. (*Polygonaceæ*.) *Dry root-stock*.—Compound grains round to elongated-oval and conical, consisting of 2 to 4, rarely 6, components which are occasionally equal, though more frequently unequal. Size about  $12\mu$ . Separated-grains, size 2 to  $8\mu$ ; the larger ones have a cavity. Simple grains of incomplete formation are also observed.

- Rumex maritimus* Linn. (*Polygonaceæ*.) *Dry roots*.—Compound grains consisting of 2 to 4 equal or unequal components; the majority have fallen apart. Size about  $14\mu$ . Separated-grains isodiametric or oval; instead of the hilum a small cavity is observed from which fissures frequently radiate toward the angles; occasionally with a large cavity, eccentricity about one-half. Size about  $10\mu$ . Simple grains of incomplete formation.
- Aristolochia longa* Linn. (*Aristolochiaceæ*.) *Dry root*.—Compound grains consisting of 2 to 8 components which are occasionally equal and regularly arranged, though frequently unequal and of irregular arrangement. Size about 20 to  $25\mu$ . Separated-grains, size about  $16\mu$ ; 0.5 to 1.5 times as broad as long; lamellæ none, or indistinct; instead of a hilum a small cavity from which two fissures diverge towards the distal end, one-half to one-fourth eccentric; very often 1 to 4 small components are attached to a large one. If two large components are united, they have usually a depressed form and very frequently even an oblique form, and one or several small components are then found adhering at the line of junction.
- Plantago maritima* Linn. (*Plantaginaceæ*.) *Dry root-stock*.—Compound grains round, rarely oval, consisting of 2 to 4 and 6 usually unequal components. Size about 10 to  $14\mu$ . Separated-grains, size 3 to  $11\mu$ ; rarely with a small cavity. Simple grains with complete formation are also found.
- Plantago media* Linn. *Fresh root-stock*.—Separated-grains with one curved surface and 1 to 3 pressure facets; size 2 to  $7\mu$ ; nearly all the compound grains have fallen apart. Simple grains of incomplete formation are also observed.
- Diodea dasycephala* Cham. (*Rubiaceæ*.) *Dry root*.—Compound grains consisting of 2 to 12 components which are occasionally equal, but more frequently unequal, in size. (Often 1 to 7 components are attached at one end of a large grain, or 1 to 6 are firmly fixed at the line of union of two large components.) Size about  $24\mu$ . Separated-grains, size 3 to  $14\mu$ ; usually with a small cavity from which in the larger ones delicate radial fissures diverge.
- Richardsonia scabra* Linn. (*Rubiaceæ*.) *Dry root*.—Grains consisting of 2 to 4, rarely 6, mostly unequal components (rarely 3 to 4 small components attached to one large one). Size about 21 to  $28\mu$ . Separated-grains, size 2 to  $21\mu$ ; the larger ones are oval; and rarely a small cavity instead of a hilum, eccentricity about one-half. Also simple eccentric-conical grains are present.
- Cephalis ippecacuanha* Rich. (*Rubiaceæ*.) *Gray variety; dry root*.—Compound grains rounded, elongated-oval, consisting of 2 to 6 components, about one-half of the grains having them equal and regularly arranged (many in tetrahedrons), and the other half containing unequal and irregularly arranged components. In the latter case, 1 to 4 small components frequently adhere to one end of a large one, rarely with 1 to 3 small ones attached at the line of junction between two large components. Size about 14 to  $16\mu$ . Separated-grains isodiametric or oval, with one curved surface and 1 to 4 pressure facets; lamellæ none; solid or with a small cavity instead of a hilum, eccentricity about one-half.
- Ippecacuanha root, brown species, variety 1*.—Compound grains rounded or elongated-oval, consisting of 2 to 6 and 10 components which are either equal and regularly arranged or unequal and irregularly arranged (in the latter case 1 to 4 small components are attached to one large one, and sometimes 1 to 3 small ones at the junction between the two large components). Size about 15 to  $20\mu$ . Separated-grains isodiametric or oval, with one curved surface and 1 to 5 pressure facets; homogeneous, or with a few very indistinct lamellæ; solid, or instead of a hilum a small cavity is observed from which very short radial fissures occasionally diverge; eccentricity about one-third.
- Ippecacuanha root, brown species, variety 2*.—Compound grains spherical or round-oval, rarely oval, consisting of 2 to 12, seldom up to 30, components which are usually equal and regularly arranged (quadrangular, tetrahedrons, hexahedrons, and octahedrons). Size about  $23\mu$ . Separated-grains isodiametric, usually with 1 curved surface and 1 to 6 pressure facets; lamellæ none; generally with a small central cavity and several short radial fissures. Size 4 to  $12\mu$ .
- Carum bulbocastanum* Koch.; *Bunium bulbocastanum* Linn. (*Umbelliferae*.) *Dry tubers*.—Compound grains consisting of 2 to 4 and 6 components which are usually unequal, though rarely equal. Size about  $28\mu$ . Separated-grains, size 3 to  $20\mu$ ; instead of the hilum a small cavity is found in the larger ones from which fissures radiate, eccentricity about one-half and one-third.

Also simple eccentric-conical grains are present. In the two examples which were examined the one from Zweibrücken contained compound grains, size about  $28\mu$ , and separated-grains, size 5 to  $20\mu$ ; on the one from Zermatt the size of the compound grains was about  $18\mu$ , and of the separated-grains 3 to  $13\mu$ .

- Charophyllum bulbosum* Linn. (*Umbelliferae*.) *Fresh tubers*.—Separated-grains, size 2 to 12 and  $18\mu$ ; frequently more or less irregular; almost twice as long as broad; with 1 to 2, rarely 3 to 4, pressure facets; hilum about one-third eccentric. Most of the compound grains have fallen apart; the majority of them consisted of few (2 to 5) unequal components. Numerous simple grains are found which are round, low-conical shaped, rarely cuneiform, and frequently irregular. Size 12 to  $18\mu$ ; hilum about one-fourth eccentric. Starch plentiful, entirely filling the cells.
- Drimys winteri* Forster.; *Cortex winteranus interior*. (*Magnoliaceae*.) *Dry bark*.—Compound grains spherical or oval, consisting of 2 to 5 and 8 usually unequal components. Size about  $20\mu$ . Separated-grains (very numerous), size 2 to  $14\mu$ ; more or less angular, usually with sharp edges; without a cavity. Isolated simple spherical or oval grains are also present.
- Anemone ranunculoides* Linn. (*Ranunculaceae*.) *Dry root-stock*.—Compound grains consisting of 2 to 10 and 16 equal or unequal components. Size about  $22\mu$ . Separated-grains, size 2.5 to  $8\mu$ ; the larger ones have small cavity, and sometimes also delicate radial fissures. Simple eccentric-conical grains are also observed.
- Ranunculus bulbosus* Linn. (*Ranunculaceae*.) *Dry tubers*.—Compound grains consisting of 2 to 4 and more components which are sometimes equal, more often unequal; size about  $14\mu$ . Separated-grains, size 3 to  $9\mu$ ; the larger ones have a small cavity, and occasionally also single delicate radial fissures. Simple eccentric-conical grains are also present.
- Helleborus viridis* Linn. (*Ranunculaceae*.) *Fresh root-stock*.—Compound grains consisting of 2 to 8 equal or unequal components; most of them have fallen apart. Separated-grains with 1 curved surface and 1 to 6 pressure facets; no lamellæ; rarely with a distinct hilum. Size 3 to  $14\mu$ ; the more polyhedral forms, about  $10\mu$ . Simple spherical grains are found; size about  $14\mu$ .
- Helleborus dumetorum* Waldst. Kit. (*Ranunculaceae*.) *Dry root-stock*.—Compound grains consisting of 2 to 10 sometimes equal, more frequently unequal, components. Separated-grains, size 2 to  $8\mu$ ; the larger ones with a small cavity.
- Aconitum anthora* Linn. (*Ranunculaceae*.) *Dry napiform root*.—Compound grains consisting of 2 to 8 equal or unequal components; most of them fallen apart. Separated-grains, size 2 to  $10\mu$ ; isodiametric; with one curved surface and 1 to 6 pressure facets; the larger ones with a small central cavity, and also several short radial fissures. Isolated separated-grains are oval; size  $17\mu$ ; cavity at the hilum somewhat eccentric. Simple eccentric-conical grains are also present.
- Aconitum napellus* Linn. (*Ranunculaceae*.) *Dry tuberous root*.—Compound grains consisting of 2 to 8 equal or unequal components. Separated-grains without or with very delicate lamellæ; they have a small, slightly eccentric cavity, instead of a hilum. Size of the more polyhedral separated-grains about  $12\mu$ , of the oval about  $20\mu$ .
- Pæonia officinalis* Retz. (*Ranunculaceae*.) *Fresh root-stock*.—Compound grains consisting of 2 to 4 equal or unequal components. Separated-grains, lamellæ none, or delicate and few (2 to 3); hilum not often visible; size about  $18\mu$ . Simple eccentric-conical grains are also observed.
- Sanguinaria canadensis* Linn. (*Papaveraceae*.) *Dry root-stock*.—Compound grains consisting of 2 to 4 and 6 sometimes equal, more often unequal, components. Separated-grains, size 4 to  $11\mu$ ; the larger ones with a small cavity. Simple grains of incomplete formation are also found.
- Nymphaea alba* Linn. (*Nymphaeaceae*.) *Fresh root and root-stock*.—Separated-grains isodiametric or oval with one curved surface and 1 to 5 pressure facets; lamellæ none; hilum frequently distinct, in the oval forms about one-third eccentric. Size 2.5 to  $12\mu$ . Few intact compound grains, consisting of 2 to 8 equal or unequal components. Simple eccentric-conical grains are also found.
- Bryonia dioica* Jacq. (*Cucurbitaceae*.) *Dry root*.—Compound grains rounded or oval, occasionally slightly compressed; consisting of 2 to 8 components which are rarely almost equal, but more frequently unequal (in the former case the components are arranged in a layer, in the center of which one or two supernumerary ones are superimposed; in the latter case, 1 to 6 small components are attached to the end of one larger one). Size about  $30\mu$ . Separated-grains, without lamellæ, cavity or fissures; in the largest ones eccentric lamellæ are very rarely visible; size 3 to  $24\mu$ .

- Cereus variabilis* Pfeiff. (*Cactaceae*.) *Fresh pith of the stem*.—Compound grains rounded or oval, usually irregular, consisting of 2 to 12 and 20 components which are almost always unequal. Size 40 to 60 $\mu$ . Separated-grains, size 4 to 30 and 50 $\mu$ ; the larger ones with eccentric and frequently irregular lamellæ. Occasionally semi-compound forms with 2 or more inclosed components are observed. Simple grains, as in type 10 of incomplete formation are also present.
- Maha borealis* Wallmann. (*Malvaceae*.) *Dry root*.—Compound grains consisting of 2 to 4 and 8 components which are sometimes equal, though more often unequal; size about 13 $\mu$ . Separated-grains, size 3 to 9 $\mu$ ; the larger ones have a small cavity, and occasionally also several very short, radial fissures. Simple grains spherical and rounded-oval are observed. Starch not very plentiful.
- Gossypium indicum* Linn. (*Malvaceae*.) *Dry root*.—Compound grains consisting of 2 to 10, rarely to more than 20, components which are equal, though more frequently unequal; size about 24 $\mu$ . Separated-grains, size 3 to 16 $\mu$ ; the larger ones have a small cavity, and occasionally also single short radial fissures.
- Circæa lutetiana* Linn. (*Onagraceae*.) *Dry stolons*.—Compound grains round to oblong, frequently irregular, consisting of 2 to 12 components, which are either equal or unequal, and usually irregularly arranged; size about 24 $\mu$ . Separated-grains, size 3 to 10 $\mu$ ; the larger ones have a small cavity instead of the hilum; eccentricity about one-half.
- Spiræa filipendula* Linn. (*Rosaceae*.) *Dry roots*.—Compound grains rounded to elongated spindle-shaped, most of them more or less irregular, consisting of 2 to 8 and 12 equal or unequal components; size about 16 $\mu$ , separated-grains; size 2 to 12 $\mu$ ; the larger ones with a small cavity. Simple grains of incomplete formation are also present.
- Orobus albus* Linn. (*Leguminosae*.) *Dry thickened roots*.—Compound grains consisting of 2 to 4 and 6 mostly unequal components; size about 16 $\mu$ . Separated-grains, size 3 to 12 $\mu$ ; isodiametric or oval; frequently they have a small cavity instead of a hilum, which in the larger oval forms is about one-third eccentric. Simple eccentric-conical grains are also present.
- Ruppia maritima* Linn. (*Naiadaceae*.) *Dry seeds*.—Compound grains consisting of 2 to 3, rarely 4 to 5, components which are occasionally equal though usually unequal (in the latter case, 1 to 2 small components are attached to a large one). Separated-grains have central, frequently irregular, cavity from which radial or irregular fissures diverge; size about 21 $\mu$ . Simple grains, probably eccentric-oval, are also present.
- Quercus pedunculata* Ekrh. (*Cupuliferae*.) *Fresh cotyledons*.—Compound grains consisting of 2 to 3 and 4 components which are sometimes regular, though more often irregular, components. Separated-grains isodiametric or oval; occasionally with a distinct central hilum; size about 16 $\mu$ . Simple grains of incomplete formation are also present.
- Castanospermum australe* Cunn. (*Cupuliferae*.) *Dry cotyledons*.—Compound grains spherical or oval, consisting of 2 to 8, rarely more, usually unequal components. Size about 20 to 25 $\mu$ . Separated-grains; size 3 to 15 $\mu$ ; with one curved surface and 1 to 5 pressure facets; the larger ones have a small cavity, and single fissures extending to the surface of the pressure facets.

## TYPE 16. GRAINS COMPOUND, MANY COMPONENTS.

From 20 to many thousand components united into a compound grain separated by slits (sometimes almost invisible), and at full maturity falling away into separated-grains, most of which are outlined on all sides by flattened pressure facets. These compound grains probably arise from repeated division, and usually consist of almost equal or exactly equal components. Occasionally they are not associated with other types, but often are found either with isolated grains of few and equal components (which belong to type 14), or very rarely with a few simple grains. Sometimes transitions between types 16 and 14 occur, making it doubtful to which group such grains belong. As the components increase in number there is usually a decrease in their size and in the width of the separating fissures, the two, however, do not always occur in like proportion. If the separating lines decrease in width more rapidly than the components, the grain appears netted or delicately reticulated, but if the components decrease in size until they have become about 1 $\mu$  in diameter, the whole grain appears granulated, and if they are smaller than 1 $\mu$  it looks homogeneous. In both cases the fact that the starch-grains are compound can be recognized only after they have fallen apart. Occasionally at certain stages in the development of an organ, for example in ripening seeds,

only separated-grains are found since the compound grains had fallen apart already within the cells or during the preparation. In such cases some doubt may exist as to whether the polyhedral forms are simple grains changed by pressure, or whether they are separated-grains. Such cases must be decided by the life-history, or if that is lacking, by their analogy to related genera and species. Good examples may be found in the seeds of *Chenopodiaceæ*, *Amarantaceæ*, and *Caryophyllaceæ*.

- Arundo donax* Linn. (*Graminaceæ*.) *Fresh root-stock*.—Compound grains rounded, granular, containing about 200 components; size  $11\mu$ . Separated-grains rounded or rounded-angular, rarely polyhedral; the larger ones have a small central cavity; size 0.7 to  $6\mu$ .
- Crocus vernus* All. (*Iridaceæ*.) *Fresh bulbs*.—Compound grains spherical, elongated-oval, consisting of 2 to 20 equal components; size about  $20\mu$ . Separated-grains usually polyhedral with acute angles; without lamellæ or hilum; size 2 to  $9\mu$ .
- Cypripedium calceolus* Linn. (*Orchidaceæ*.) *Dry root-stock*.—Compound grains spherical or oval, consisting of 10 to 20 and 40 components; size about  $10\mu$ . Separated-grains, size 1.5 to  $5\mu$ ; usually rounded-angular, compressed to about one-half their width.
- Dorstenia brasiliensis* Linn.; *Radix contrajervæ*. (*Artocarpaceæ*.) *Dry root-stock*.—Compound grains spherical or oval-spherical, consisting of 2 to about 50 equal components; size about  $14\mu$ . Separated-grains, size 2.5 to 6 and  $7\mu$ ; rounded-angular or polyhedral; the larger ones have a small central cavity. Simple grains of incomplete formation are also present.
- Dorstenia contrajerva* Linn. (*Artocarpaceæ*.) *Dry root-stock*.—Compound grains spherical or oval, consisting of 2 to 40 and 60 equal or unequal components; size about 12 to  $14\mu$ . Separated-grains; size 1.8 to  $7\mu$ ; rounded-angular or polyhedral; the larger ones hollow. Simple spherical grains are also found.
- Chiococca racemosa* Linn. (*Cainea root*). (*Rubiaceæ*.) *Dry root-stock*.—Compound grains rounded to sometimes rather irregular, granular or reticulate, consisting of about 70 components; size about  $18\mu$ . Separated-grains size 2 to  $6\mu$ ; rounded or polyhedral; the larger ones hollow. Isolated compound grains have an appearance similar to a delicate parenchyma, which is due to the fusing of the components and the cavities being separated from one another by homogeneous walls, similar to the starch-grains found in type 12.
- Chrysophyllum glycyphlæum* Casar.; *Cortex monesiæ*. (*Sapotaceæ*.) *Dry bark*.—Compound grains spherical or oval, consisting of 2 to about 60 components; size about  $10\mu$ . Size of the separated grains 2 to  $5\mu$  (not easily separated). Simple spherical or oval grains are also present.
- Podophyllum peltatum* Linn. (*Berberidaceæ*.) *Dry root-stock*.—Compound grains reticulated or merely granular, consisting of 3 to 20 and more components; size about  $10\mu$ . Separated-grains, size 2 to  $4\mu$ ; rounded-angular. Simple grains of incomplete formation also present. It is difficult to decide whether many of the grains are simple or separated grains.
- Epimedium alpinum* Linn. (*Berberidaceæ*.) *Dry root-stock*.—Compound grains spherical or oval, sometimes slightly angular and irregular, reticulated or granular, consisting of 3 to 500 and more components; size about  $15\mu$ . Separated-grains size 1.5 to  $7\mu$ ; the smaller ones rounded, larger ones polyhedral.
- Epimedium macranthum* Lindl. (*Berberidaceæ*.) *Dry root-stock*.—Compound grains spherical, oval, conical, rarely somewhat angular as a result of pressure, granular, consisting of 12 to more than 2000 components; size about  $17\mu$ . Separated-grains, size 1 to  $2\mu$ ; round or rounded-angular.
- Ayenia pusilla* Linn. (*Stereuliaceæ*.) *Dry roots*.—Compound grains rounded or oval, consisting of 2 to about 40 usually equal components; size about  $17\mu$ . Separated-grains frequently have a small cavity; size 2 to  $8\mu$ . Starch not plentiful.
- Tribulus terrestris* Linn. (*Zygophyllaceæ*.) *Dry root*.—Separated-grains, size 3 to  $8\mu$ ; almost polyhedral, sometimes with a small cavity. Only a few intact compound grains are present, consisting of 2 to 12 or more components, which are usually equal. Starch rather scarce.
- Oxalis stricta* Linn. (*Oxalidaceæ*.) *Dry tap-root*.—Compound grains round or oval, consisting of 2 to 20 equal or unequal components; size about  $15\mu$ . Separated-grains, size 3 to  $9\mu$ ; the larger ones have a small cavity instead of a hilum, which is central or about one-half eccentric.
- Orobos tuberosus* Linn. (*Leguminosæ*.) *Dry tubers of the root-stock*.—Compound grains round or oval; consisting of 4 to 20 equal or unequal components; size about  $13\mu$ . Separated-grains, size 1.5 to  $7\mu$ ; the larger ones have instead of a hilum a small central or about one-half eccentric cavity.

- Leersia oryzoides* Swartz. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, frequently somewhat angular as a result of pressure, sometimes almost polyhedral, consisting of about 6000 or more components; size about 25 and 30 $\mu$ . Separated-grains rounded-angular, or polyhedral; the larger ones with a small cavity; size 2 to 7 $\mu$ .
- Oryza sativa* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, consisting of 4 to 100 or more almost equal components; size about 25 $\mu$ . Separated-grains polyhedral; frequently with a rather large cavity; size 3.5 to 8 $\mu$ .
- Hydrophyrum esculentum* Linn.; *Zizania aquatica* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains, polyhedral as a result of pressure; with numerous components; size 25 and 28 $\mu$ . Separated-grains rounded-angular; size 1.5 to 4 $\mu$ .
- Zizania clavulosa* Michx. (*Graminaceæ*) probably belongs to this type. The compound grains consist of numerous components; they are isodiametric to oblong, and polyhedral as a result of pressure; size about 30 $\mu$ . Separated-grains; size 2 to 5 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral and hollow.
- Ehrharta panicæ* Smith. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, occasionally somewhat irregular, homogeneous, or reticulate and granular, consisting of more than 3000 components. Size about 40 and 45 $\mu$ . Separated-grains polyhedral, usually with sharp borders and angles; frequently hollow; size 2 to 6 $\mu$ .
- Microstena stipoides* R. Br. (*Graminaceæ*.) *Dry endosperm*.—Compound grains almost round or oval, frequently angular as a result of pressure, numerous components; about 20 $\mu$ . Separated-grains, size 2 to 5 and 7 $\mu$ ; the smaller ones are rounded-angular, the larger ones polyhedral, and have a cavity.
- Cornucopia cucullatum* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded to elongated-lanceolate, sometimes slightly irregular; two-fifths to as thick as long; reticular-granulated; size about 54 $\mu$ . Separated-grains rounded-angular or polyhedral, usually with a cavity; size 1.5 to 6 $\mu$ .
- Phalaris canariensis* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical to oblong, rarely angular, half to as thick as long, reticulate-granulated, rarely almost homogeneous, consisting of 4 to 2000 components; length about 36 $\mu$ , thickness about 25 $\mu$ . Separated-grains rounded-angular or polyhedral; size 2 to 5 $\mu$ .
- Phalaris bulbosa* Cav.; *Phalaris caerulea* Desf. (*Graminaceæ*.) *Dry endosperm*.—Starch as in the preceding. Compound grains consisting of 3000 and more components; size about 48 $\mu$ .
- Anthoxanthum amarum* Brot. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulate-granulated; size about 25 $\mu$ . Separated-grains rounded-angular; size 1 to 4 $\mu$ .
- Hierochloa borealis* Roem. and Schult. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, sometimes slightly irregular; consisting of 1000 or more components; size about 30 to 40 $\mu$ . Separated-grains with rather sharp borders and angles; size 2 to 7 $\mu$ .
- Holcus lanatus* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, homogeneous or distinctly granular, consisting of 1000 or more components; size about 14 $\mu$ . Size of the separated-grains 1 to 2 $\mu$ .
- Bechmannia eruciformis* Hochst. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded-oval to oblong, finely granular; size about 28 to 35 $\mu$ . Separated-grains round or polyhedral; size 1 to 3 and 4 $\mu$ .
- Lygeum spartum* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, reticulated-granular; size about 24 $\mu$ . Separated-grains rounded angular or polyhedral; size 1 to 4 $\mu$ .
- Milium effusum* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular; size about 24 $\mu$ . Separated-grains rounded to polyhedral, frequently with a small cavity; size 1.5 to 5 $\mu$ .
- Milium vernale* Biebrst. *Dry endosperm*.—Compound grains spherical or oval; homogeneous, or granulated by delicate reticulations; size about 14 $\mu$ . Separated-grains rounded-angular; size 1 to 3 $\mu$ .
- Panicum commelinæfolium* Rudge. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, frequently more or less angular as the result of pressure, granular; size about 12 to 15 $\mu$ . Separated-grains rounded; size 1 to 2.5 $\mu$ . Starches of the seeds from Brazil and Guatemala agree perfectly with those above described.

- Urochloa depressa* Steud. (*Graminaceæ*.) *Dry endosperm*.—Compound grains isodiametric to oblong, more or less polyhedral as the result of pressure, reticulated-granular or granular; size about  $20\mu$ . Separated-grains round or rounded-angular; size  $0.7$  to  $2.5\mu$ .
- Arundinella nepalensis* Trin. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical, or oval, or low cone-shaped (not angular); two-fifths to as thick as long; reticulated-granular or almost homogeneous; consisting of 4 to 1000 or more components; size about  $30\mu$ . Separated-grains size 2 to  $6\mu$ ; the larger ones polyhedral, and have a cavity.
- Dichelaehne vulgaris* Trin. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded, elliptical, oblong, conical; delicately granular; consisting of 1000 or more components; size about  $18\mu$ . Separated-grains rounded; size  $0.5$  to  $2\mu$ .
- Uraehne parviflora* Trin.; *Piptatherum multiflorum* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, consisting of 6 to 1000 or more equal components; size about  $18\mu$ . Separated-grains rounded-angular, the larger ones polyhedral, usually with a central cavity; size 2 to  $7\mu$ .
- Stipa papposa* Nees. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular, the larger ones polyhedral, and have a cavity; size 2 to 6 and  $7\mu$ . Of compound grains only fragments and a few complete ones remain.
- Stipa gigantea* Lagase. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular, the larger ones with a small cavity; size 1 to  $4\mu$ . Only a few compound grains are present. Simple centric-lenticular grains are also found.
- Stipa pennata* Linn. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular or polyhedral; size 2 to  $4\mu$ . Compound grains are only observed within the cells.
- Lasiagrostis calamagrostis*; *Stipa calamagrostis* Willd. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, frequently slightly angular, as a result of pressure; reticulated-granular; falling apart easily; size about  $15\mu$ . Separated-grains usually polyhedral, frequently have a cavity; size 2 to 9 and  $11\mu$ . Grains which are probably simple are found among the above; size 9 to 11 and  $13\mu$ ; with central cavity and occasionally single radial fissures.
- Aristida hystrix* Linn. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains, size  $1.5$  to  $6\mu$ ; the smaller ones rounded, larger ones polyhedral, and have a cavity. Compound grains embedded in protoplasm are only observed within the cells of thin sections of the tissue; they are rounded or rounded-angular; size about 15 to  $20\mu$ .
- Aristida amplissima* Trin.; *Aristida stipiformis* Lam. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, and have a cavity; size 2 to  $10\mu$ . A few free compound grains which in thin sections can be distinctly seen within the protoplasm, which latter takes a yellow color when treated with iodine. Grains spherical, oval, or rounded-angular; reticulated; size about  $28\mu$ .
- Aristida plumosa* Linn. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral; the larger ones have a cavity; size 2 to  $8\mu$ . Not many distinct compound grains; round, oval, or elliptical; reticulate, or reticulated-granular; size about  $26\mu$ .
- Aristida funiculata* Trin.; *Aristida kotschyi* Hochst. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains, size 1 to  $5\mu$ ; smaller ones rounded, larger ones polyhedral, and have a cavity. Compound grains few, round or oval.
- Nardus stricta* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval (not angular), three-fifths to as thick as long, and consisting of 4 to 300 components; size about  $18\mu$ . Separated-grains rounded-angular, the larger ones polyhedral, and have a cavity; size  $1.5$  to  $5\mu$ .
- Ægopogon cenehroides* Willd. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, the larger ones have a cavity; size  $2.5$  to  $7\mu$ . A few compound grains are free and some are indistinctly observed within the cells; round or oval and more or less angular as a result of pressure; size about  $20\mu$ .
- Ægopogon multisctus* Trin. (*Graminaceæ*.) *Dry endosperm*.—Starch as in the preceding. Size of the separated-grains 2 to  $6\mu$ .
- Lyeurus phalaroides* Humb. Bonpl. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, and have a cavity; size 2 to  $10\mu$ . Compound grains are observed indistinctly within the cells, and also a few free grains; isodiametric or oval, more or less polyhedral due to pressure; size about  $25\mu$ .

- Phippsia algida* R. Br. (*Graminaceæ*). *Dry endosperm*.—Compound grains round or oval, more or less angular, as a result of pressure; reticulate or reticulated-angular. Size about 20 to 25 $\mu$ . Separated-grains rounded-angular or polyhedral, the larger ones with a cavity; size 1.5 to 5 $\mu$ .
- Colcanthus subtilis* Seidel; *Schmidtea utriculosa* Sternb. (*Graminaceæ*). *Dry endosperm*.—Compound grains spherical to elongated-oval, more or less angular as a result of pressure; homogeneous or reticulated-granular; size about 24 $\mu$ . Separated-grains rounded-angular or polyhedral, the larger ones with a cavity; size 2 to 5 $\mu$ .
- Alopecurus geniculatus* Linn. (*Graminaceæ*). *Dry endosperm*.—Compound grains spherical or oval; delicately granular, or reticulated-granular; size about 25 $\mu$ . Separated-grains rounded-angular, rarely polyhedral; size 1.5 to 4 $\mu$ .
- Alopecurus pratensis* Linn. (*Graminaceæ*). *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular; size about 26 $\mu$ , rarely 33 $\mu$ . Separated-grains rounded-angular, or polyhedral; the larger ones with a cavity; size 1.5 to 5 $\mu$ .
- Alopecurus alpinus* Smith. (*Graminaceæ*). *Dry endosperm*.—Starch as in the preceding species.
- Alopecurus utriculatus* Schrad. (*Graminaceæ*). *Dry endosperm*.—Starch as in *Alopecurus pratensis*. Size of the separated-grains about 7 $\mu$ , of compound grains about 33 $\mu$ .
- Phleum asperum* Vill. (*Graminaceæ*). *Dry endosperm*.—Compound grains almost rounded or oval, sometimes slightly irregular, three-fifths to as thick as long, consisting of 2 to about 60 equal components; size about 38 $\mu$ . Separated-grains polyhedral, usually with sharp borders and angles, frequently with a cavity and single fissures; size 6 to 16 $\mu$ .
- Phleum temue* Schrad.; *Achnodonton bellardi* Beauv. (*Graminaceæ*). *Dry endosperm*.—Compound grains spherical or oval, frequently slightly angular and irregular, consisting of 4 to about 60 components; size about 40 $\mu$ . Separated-grains polyhedral, usually with sharp borders and angles, frequently with a cavity from which single radial fissures proceed; size 4 to 14 $\mu$ .
- Crypsis schænoïdes* Lam. (*Graminaceæ*). *Dry endosperm*.—Compound grains almost round elongated-oval, half to as thick as long, reticulated-granular or almost homogeneous; size about 30 $\mu$ . Separated-grains polyhedral, frequently with sharp margins and angles, the larger ones have a cavity; size 2.5 to 7 $\mu$ .
- Vilfa coromandelina* Beauv.; *Sporobolus coromandelina* Kunth. (*Graminaceæ*). *Dry endosperm*.—Compound grains spherical or oval, three-fifths to as thick as long, reticulated-granular, consisting of 6 to 1000 and more components; size about 19 $\mu$ . Separated grains rounded-angular, or polyhedral; the larger ones usually with a small cavity; size 2 to 5 $\mu$ .
- Agrostis verticillata* Vill. (*Graminaceæ*). *Dry endosperm*.—Compound grains round or oval reticulated-granular; size about 20 $\mu$ . Separated-grains rounded to polyhedral; size 2 to 7 $\mu$ .
- Triachyrum longifolium* Hochst. (*Graminaceæ*). *Dry endosperm*.—Compound grains, isodiametric or oval, more or less polyhedral as a result of pressure, consisting of numerous components, most of them fallen apart; size about 21 $\mu$ . Separated-grains polyhedral, the majority with a large or small cavity; size 3 to 10 $\mu$ .
- Triachyrum cordofanum* Hochst. (*Graminaceæ*). *Dry endosperm*.—Separated-grains, size 1.5 to 5 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral, and have a cavity. Only a few intact compound grains are observed; these are reticulated and about 14 $\mu$  in size.
- Colpodium steveni* Trin. (*Graminaceæ*). *Dry endosperm*.—Compound grains round, oval, and low-cone-shaped, slightly angular or almost polyhedral, consisting of 400 and more components; size about 25 $\mu$ . Separated-grains 2.5 to 6 $\mu$ , polyhedral, the larger ones frequently with a small cavity.
- Apera spica-venti* Beauv.; *Agrostis* sp. Linn. (*Graminaceæ*). *Dry endosperm*.—Compound grains round to oblong, two-fifths to as thick as long, granular or almost homogeneous; length about 25 $\mu$ , thickness about 15 $\mu$ . Separated-grains round or rounded-angular; size 0.7 to 2 $\mu$ .
- Muehlenbergia willdenowii*. (*Graminaceæ*). *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular (most of them have fallen apart); size about 24 $\mu$ . Separated-grains, polyhedral; the larger ones have a cavity; size about 7 $\mu$ .
- Cinna racemosa* Kunth.; *Muehlenbergia glomerata* Trin. (*Graminaceæ*). *Dry endosperm*.—Separated-grains polyhedral, the larger ones have a cavity; size about 17 $\mu$ . Among the above there are a few spherical or oval compound grains which have not fallen apart; size about 22 $\mu$ .

- Cinna arundinacea* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, frequently somewhat angular, consisting of numerous components, most of them fallen apart; size about 20 $\mu$ . Separated-grains rounded-angular to polyhedral; the larger ones have a cavity; size 2 to 5 and 7 $\mu$ .
- Echinopogon ovatus* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round, oval, or low cone-shaped, more or less angular as a result of pressure, delicately reticulated; size about 20 $\mu$ . Separated-grains rounded-angular to polyhedral; size 1 to 4 $\mu$ .
- Lagurus ovatus* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, homogeneous or finely granulated, consisting of 10 to over 6000 components; size about 20 $\mu$ . Separated-grains round or somewhat angular; size 1 to 3 $\mu$ .
- Polypogon monspeliensis* Desf. (*Graminaceæ*.) *Dry endosperm*.—Compound grains oval, rarely round, one-half to almost as thick as long; finely granulated, frequently almost homogeneous, consisting of 30 to 1000 components; size about 18 $\mu$ , angular. Separated-grains are also present; size 1.5 to 4 $\mu$ .
- Chaturus fasciculatus* Link. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains round or rounded-angular; size 1 to 3 $\mu$ . Compound grains have all fallen apart.
- Gastridium australe* Beauv., var. *muticum* Spreng. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, usually with sharp angles and borders, with a cavity; size 3 to 12 $\mu$ . Compound grains nearly all have fallen apart; those remaining are more or less angular or even exactly polyhedral as a result of pressure; size about 23 $\mu$  or more.
- Perotis latifolia* Ait. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains, size 2 to 8 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral, usually with a larger or a small cavity. The cells are crowded with separated-grains, while no compound grains could be positively distinguished.
- Chamagrostis minima* Borekh.; *Sturmia minima* Hoppe. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, three-fifths to as long as broad, usually finely reticulated, consisting of 8 to 500 components; size about 33 $\mu$ . Separated-grains usually polyhedral, without a cavity; size 2 to 8 and 9 $\mu$ .
- Calamagrostis sylvatica* DC. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded or oval consisting of 2 to more than 300 components, which when few in number are often unequal; size about 48 $\mu$ . Separated-grains usually polyhedral; the larger ones with a central cavity; size 2 to 9 $\mu$ .
- Calamagrostis willdenowii* Trin.; *Deyeuxia retrofracta* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, consisting of numerous components; size about 16 $\mu$ . Separated-grains angular or rounded-angular; size 1.5 to 4 $\mu$ .
- Ammophila arenaria* Link.; *Calamagrostis arenaria* Roth. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, two-fifths to as thick as long, reticulated-granular; size about 50 $\mu$ . Separated-grains rounded-angular or polyhedral; size 1 to 4 $\mu$ .
- Arundo mauritanica* Desf. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains size 2.5 to 8 $\mu$ , polyhedral, the larger ones usually with a small angular cavity. A few intact compound grains are present, rounded-oval, frequently angular, or even polyhedral, consisting of numerous components.
- Phragmites communis* Trin. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded or rounded-angular; the larger ones rarely with a distinct cavity; size 0.7 to 2.5 $\mu$ . Only a few compound grains are present; small, rounded, granular.
- Gynerium cinereum* Humb. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, frequently slightly angular, reticulated-granular; size about 23 $\mu$ . Separated-grains, size 2.5 to 8 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral with sharp borders and angles, frequently with a small cavity.
- Gynerium argenteum* Nees. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains size 1 to 4 $\mu$ , rounded-angular to polyhedral. Only a few compound grains could be positively distinguished. Besides the starch, much oil and protoplasm are found in the cells.
- Pappophorum schimperianum* Hochst., var. *persicum*. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval (not at all angular), consisting of 2 to over 100 components; size about 25 and 30 $\mu$ . Separated-grains polyhedral, usually with a small central cavity, and sometimes also with single delicate radial fissures; size 3 to 10, rarely 12 $\mu$ . The isolated simple grains, size about 16 $\mu$ ; the smaller ones spherical or rounded-oval, the larger ones circular or rounded-oval and compressed to about half their width. The starch-grains which are embedded in protoplasm are not closely packed in the cells.

- Pappophorum macrostachyum* Nees. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains size 3 to 8 and 10 $\mu$ ; polyhedral or with 1 curved surface and 1 to 5 pressure facets; usually with a large or a small cavity. Nägeli observed only a few compound grains, and also spherical or oval simple grains.
- Pappophorum nigricans* R. Br. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, consisting of 2 to 10 or more equal components; size about 30 and 36 $\mu$ . Separated-grains size 5 to 20 and 25 $\mu$ ; usually with one curved surface and 1 to 5 pressure facets; rarely entirely polyhedral (only outlined by pressure facets); with a central cavity and radial fissures. Simple centric-spherical grains are also present. The compound grains of *Pappophorum nigricans* properly belong to type 14. They are placed here because of their relationship to the other two species.
- Ptiloncilema plumosum* Steud.; *Eutriana abyssinica* R. Br. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, frequently with a small cavity and also single delicate radial fissures; size 3 to 12 $\mu$ . The majority of the compound grains have fallen apart, the few remaining ones are polyhedral as the result of pressure.
- Echinaria capitata* Desf. (*Graminaceæ*.) *Dry endosperm*.—Compound grains (few intact ones still present), spherical or oval, frequently somewhat angular; size about 34 $\mu$ . Separated-grains polyhedral with sharp borders and angles; size 4 to 18 $\mu$ .
- Ctenium elegans* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains solid or with a central angular cavity, frequently with 1 curved surface and 1 to 5 pressure facets, many of them entirely polyhedral. The majority of the compound grains have fallen apart, those remaining are rounded or oval, consisting of 2 to about 20 components; size about 12 $\mu$ . Simple grains few, spherical, or rounded-oval, solid or with a small central cavity; size about 10 $\mu$ .
- Ctenium chapadense* Trin. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains, size about 11 $\mu$ ; frequently polyhedral (only outlined by pressure facets); many of them have one curved surface and 3 to 5 pressure facets; the larger ones have an angular cavity from which short radial fissures proceed. Very few compound grains are present.
- Microchloa setacea* R. Br. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, the majority with a large or a small cavity; size 3 to 10 $\mu$ . Only a few free compound grains are present; oval or almost polyhedral; size about 20 to 25 $\mu$ .
- Chloris petraea* Thumb.; *Eustachys petraea* Desv. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, the larger ones with a central cavity; size 2 to 9 $\mu$ . All the compound grains have fallen apart.
- Chloris submutica* Humb. Bonp.; *Eustachys submutica* R. and S. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated; size about 24 $\mu$ . Separated-grains rounded-angular to polyhedral; rarely with a small cavity in the larger ones; size 2 to 8 $\mu$ .
- Ctenopsis pectinella* Tavis.; *Festuca Nop. Del.* (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded or rounded-angular; size 0.8 to 3 $\mu$ , a few compound grains are still found, granular; size about 14 $\mu$ .
- Eleusine coracana* Gart. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, only outlined by pressure facets or with one curved surface and several pressure facets, with sharp margins and angles; the larger ones have a small central cavity from which several fissures frequently radiate toward the angles; size 4 to 13 $\mu$ . The greater number of the compound grains have fallen apart; the remaining ones are spherical or oval, about 30 $\mu$  in length, and consist of 2 to 50 and 100 equal components, which when few in number (4 to 10) show a regular arrangement.
- Dactyloctenium aegyptiacum* Willd. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval (not at all angular), granular or reticulated-granular; size about 15 to 21 $\mu$ . Separated-grains rounded-angular to polyhedral; the larger ones with a small cavity; size 1.5 to 5.5 $\mu$ .
- Cynodon dactylon* Pers.; *C. linearis* Willd. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, usually with a small or large angular cavity; size 2.5 to 8 $\mu$ . A few free compound grains are observed, oval or nearly polyhedral; size about 20 $\mu$ .
- Chondrosium* sp. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular to polyhedral; size 2 to 5 $\mu$ . Only a few compound grains are observed.
- Spartina cynosuroides* Willd. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, several with a small cavity. Only a few intact compound grains still present, consisting of 2 to 12 components.

- Eutriana oligostachya* Kunth.; *Atheropogon oligostachya* Nutt. (*Graminaceæ.*) *Dry endosperm.*—Separated-grains polyhedral, the larger ones with an angular cavity; size 2 to 8 $\mu$ . Only a few compound grains are observed, consisting of 2 to over 300 components, spherical, oval, or, as the result of pressure, angular; size about 20 $\mu$ .
- Melanocenchrys royleana* Nees.; *Pommereulla royleana* Steud. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical or oval, frequently angular by reason of pressure, consisting of 2 to 200 or more components; size about 20 to 25 $\mu$ . Most of the separated-grains are polyhedral, many with 1 curved surface and several pressure facets; the larger ones with a small central cavity, also occasionally with single radial fissures; size 3 to 12 $\mu$ .
- Corynephorus canescens* Beauv.; *Aira canescens* Linn. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical or oval, granular or homogeneous; size about 13 $\mu$ . Separated-grains rounded; size 0.7 to 1.5 $\mu$ .
- Deschampia juncea* Beauv.; *Aiva juncea* Vill. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical or oval, frequently angular as a result of pressure, reticulated-granular or granular; size about 24 $\mu$ . Separated-grains size 2 to 7 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral, and frequently with a small cavity.
- Deschampia cæspitosa* Beauv.; *Aiva cæspitosa* Linn. (*Graminaceæ.*) *Dry endosperm.*—Starch as in the preceding. Size of compound grains about 30 $\mu$ , of separated-grains 2 to 8 $\mu$ .
- Deschampia pulchella* Trin., var. *tenovei*; *Aiva pulchella* Willd., var. *tenorei* Guss. (*Graminaceæ.*) *Dry endosperm.*—Separated-grains rounded-angular; size 2 to 6 $\mu$ .
- Airopsis globosa* Desv.; *Aiva globosa* Thore. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical, oval, pyriform, occasionally slightly angular by reason of pressure; more or less distinctly granular, or often homogeneous; size about 12 $\mu$ . Separated-grains round or rounded angular; size 1 to 3 $\mu$ .
- Airopsis agrostidea* DC.; *Aiva agrostidea* Loise. (*Graminaceæ.*) *Dry endosperm.*—Starch as in the preceding species.
- Trisetum argenteum* Reen and Schult. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical or oval, occasionally angular through pressure; delicately reticulated or granular-reticular to homogeneous; consisting of 3 to 100 components; size about 14 $\mu$ . Separated-grains round, more or less angular; size 1.5 to 5 $\mu$ .
- Trisetum neglectum* Willd. (*Graminaceæ.*) *Dry endosperm.*—Starch as in the preceding species. Size of the compound grains 13 $\mu$ , of separated-grains 1.5 to 4 $\mu$ .
- Avena orientalis* Schreb.; *Avena hirsuta* Roth; *Avena brevis* Roth. (*Graminaceæ.*) *Fresh and dry endosperm.*—Compound grains almost round to oblong, frequently somewhat angular through pressure; consisting of 2 to 300 equal or somewhat unequal components; size about 5 $\mu$ . Separated-grains polyhedral with rather sharp margins; if fresh they have a small central indistinct hilum, when dry, they frequently have a small cavity instead of the hilum; size 7 to 12 $\mu$ . Simple spherical grains are found among the above described.
- Gaudinia fragilis* Beauv.; *Avena fragilis* Linn. (*Graminaceæ.*) *Dry endosperm.*—Separated-grains rounded-angular to polyhedral; the larger grains with a small central cavity; size 2 to 6, rarely 1 to 8 $\mu$ . Compound grains few, spherical or oval, consisting of 5 to 200 almost equal components; size about 21 $\mu$ .
- Arrhenatherum elatius* Mert. & Koch.; *Avena elatius* Linn. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical, oval, or pyriform; consisting of 4 to 400 or more components; size about 30 $\mu$ . Separated-grains polyhedral; size 2 to 10 $\mu$ .
- Eriachne ampla* Nees; *Airopsis ampla* Nees. (*Graminaceæ.*) *Dry endosperm.*—Separated-grains, size 2 to 6 and 7 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral with a central cavity. A few free compound grains observed; round or oval, frequently angular; size about 21 $\mu$ .
- Eriachne microphylla* Nees. *Dry endosperm.*—Compound grains rounded or oval, more or less angular by pressure, granulated; size about 15 $\mu$ . Separated-grains 1 to 4 $\mu$ ; rounded or rounded-angular.
- Tristachya barbata* Nees. (*Graminaceæ.*) *Dry endosperm.*—Separated-grains polyhedral; the larger ones occasionally with a small central cavity; size 1.5 to 5 and 6 $\mu$ . A few free compound grains are observed, only perceived indistinctly within the cells; rounded or oval, frequently angular by reason of pressure; size about 20 $\mu$ . Isolated simple spherical grains and many separated-grains with one curved surface and one or several pressure facets are also present.

- Danthonia provincialis* DC. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, sometimes irregular; reticulated-granular; size about  $38\mu$ . Separated-grains usually polyhedral; the larger ones with a small cavity; size 2 to  $7\mu$ .
- Danthonia kæstlini* Hochst. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains, size 1.5 to 5 and  $6\mu$ ; the smaller ones round, the larger ones polyhedral and with a central cavity. Few compound grains observed; round or oval, frequently angular; size about  $20\mu$ .
- Uralcypis aristulata* Nutt. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded angular to polyhedral: size 2 to 8 and  $10\mu$ ; the larger ones with a small central cavity and often with single radial fissures. Only a few compound grains of polyhedral form can positively be distinguished.
- Triodia decumbens* Beauv.; *Danthonia decumbens* DC. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded-angular or polyhedral; size about 20 and  $25\mu$ . Separated-grains polyhedral; the large ones have a small cavity, also frequently radial fissures; size 2.5 to  $8\mu$ .
- Poa nemoralis* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval (not at all angular), consisting of about 1000 components; size about 30 to  $36\mu$ . Separated-grains usually polyhedral, frequently more or less distinctly compressed, without a cavity; size 2 to  $8\mu$ .
- Eragrostis abyssinica* Link. (*Graminaceæ*.) *Dry endosperm*.—The compound grains have become polyhedral as the result of pressure, mostly with acute margins and angles; consisting of 20 to 500 and more components; size  $33\mu$ . Separated-grains polyhedral with acute margins and angles, rarely with a small central cavity; size 2 to  $12\mu$ .
- Brizopyrum siculum* Link.; *Festuca unioloides* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Compound grains almost rounded-oval, oblong, frequently irregular, homogeneous or finely granulated; size about  $16\mu$ . Separated-grains round or rounded-angular; size about 1 to 2 or rarely  $3\mu$ .
- Brizopyrum acutiflorum* Nees. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, and have a cavity; size 2 to  $7\mu$ . Only very few compound grains are observed.
- Briza triloba* Nees.; *Calotheca triloba* Beauv.; *Chascolytrum triloba* Nees. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval-spherical, frequently somewhat angular, finely reticulated or reticulated-granular, sometimes almost homogeneous, consisting of 4 to 400 components; size about  $16\mu$ . Separated-grains round or angular; size 1.5 to  $4\mu$ .
- Briza geniculata* Thumb. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded or angular; size 2 to  $7\mu$ ; nearly all the compound grains have fallen apart.
- Glyceria nervata* Trin.; *Glyceria michauxii* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, usually with acute margins and angles; occasionally with pressure facets; the larger ones with a small central cavity; size 2 to 7 and  $9\mu$ . Compound grains few, round or oval; size about  $30\mu$ .
- Glyceria distans* Mert. & Koch.; *Festuca thalassica* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular or with distinct lines of separation; size about  $28\mu$ . Separated-grains 2 to  $7\mu$ ; the majority are polyhedral, the larger ones have a cavity.
- Glyceria maritima* Mert. and Koch.; *Festuca thalassica* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded or oval; finely reticulated or with distinct separating lines; size about  $25\mu$ . Separated-grains usually polyhedral, the larger ones have a cavity; size 2 to  $7\mu$ .
- Catabrosa aquatica* Beauv.; *Glyceria aquatica* Presl. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round to oblong and high cone-shaped, reticulated-granular; size about  $28\mu$ . Separated-grains, size 1.5 to  $4\mu$ ; the smaller ones round, the larger ones angular.
- Lophochlæna californica* Nees. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains size 1.7 to 5 and  $6\mu$ ; the smaller ones somewhat rounded-angular, larger ones polyhedral and with a cavity. Compound grains distinctly observed within the cells, but only a few are found free (since they fall apart as they emerge from the cells). They are rounded or oval, more or less angular as the result of pressure; size about  $19\mu$ .
- Lophochlæna obtusiflora* Trin. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral; usually with a conspicuous more or less angular cavity; many also have radial fissures. No free compound grains are observed; polyhedral when found in the cells; size about 16 to  $21\mu$ .
- Melica ciliata* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains polyhedral by means of pressure, sometimes with acute, sometimes rounded angles and margins, consisting of 4 to over 500 components; size about  $40\mu$ . Separated-grains polyhedral, usually with acute margin and angles; the larger ones frequently with a small central cavity; size 2 to  $15\mu$ .

- Molinia carulea* Moench. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular or polyhedral, with comparatively large cavity; size 2 to 5 $\mu$ . Compound grains have nearly all fallen apart.
- Kæleria laxa* Link. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical, oval or elliptical (not at all angular), three-fifths to as thick as long, granulated, consisting of 5 to over 2500 components; size about 15 $\mu$ . Separated-grains round or slightly angular; size 1 to 3 $\mu$ .
- Schismus marginatus* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, frequently somewhat irregular, reticulated-granular; size about 21 $\mu$ . Separated-grains angular, mostly polyhedral, frequently have a cavity; size 2 to 6 $\mu$ .
- Sesleria elongata* Host. (*Graminaceæ*.) *Dry endosperm*.—Compound grains, spherical or oval, frequently irregular, reticulated-granular; size 24 to 28 $\mu$ . Separated-grains, rounded-angular, or mostly acute-polyhedral, very frequently have a cavity; size 2 to 6 and 7 $\mu$ .
- Cynosurus echinatus* Linn.; *Chrysurus echinatus* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical to oblong and conical, frequently irregular, two-fifths to just as thick as long, granular or homogeneous, consisting of 10 to over 13,000 almost equal components; length about 42 to 51 $\mu$ , thickness about 30 to 36 $\mu$ . Separated-grains, round or rounded-angular; size 0.7 to 2, rarely 3 $\mu$ .
- Lamarkia aurea* Moench. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded-oval to oblong, half to almost as thick as long, homogeneous or finely granular, consisting of 30 to over 8000 components; length about 14 $\mu$ . Separated-grains, rounded; size 0.5 to 1.5 $\mu$ .
- Harpachne schimperii* Hochst. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains size 1.7 to 8 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral, and usually with a central cavity which is rounded-angular, or provided with single radial fissures. The compound grains are indistinctly observed within the cells, and only a few are found free.
- Ectrosia leporina* R. Br. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains polyhedral; size 2 to 10 $\mu$ ; the larger ones have an angular cavity, also occasionally with several radial fissures. The compound grains are only indistinctly observed within the cells; more or less polyhedral; size about 24 $\mu$ . A few free forms are also found.
- Elytrophorus articulatus* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, by means of pressure they become more or less polyhedral, consisting of over 300 components; size about 25 to 30 $\mu$ . Separated-grains polyhedral, with a small round or a large angular cavity, occasionally with several radial fissures; size 2 to 10 $\mu$ .
- Festuca fascicularis* Lam.; *Diplachne fascicularis* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains, rounded-oval, elliptical, oblong, conical; most of them by reason of pressure become angular or even polyhedral; reticulated or almost homogeneous. Size about 18 to 22 $\mu$ . Separated-grains usually acute-polyhedral; the larger ones with a central cavity. Size 2 to 8 and 9 $\mu$ . The grains above described came from seeds of cultivated specimens; in other seeds (of North American plants) the sizes of the compound grains were about 30 to 40 $\mu$  and of the separated-grains 2.5 to 7 $\mu$ .
- Festuca flavescens* Bellard; *Festuca varia* Haenk; *Festuca* var. *flavescens*. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains, size 2 to 8 $\mu$ ; the smaller ones rounded-angular, larger ones acute polyhedral, and frequently have a cavity. No free compound grains are observed. The rather thick-walled endosperm cells separate easily; they are filled with polyhedral parts-grains which frequently lie in groups.
- Festuca arundinacea* Schreb.; *Festuca elatior* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains, spherical or oval, reticulated, consisting of 2 to over 1000 components. Size about 35 $\mu$ . Separated-grains, size 2 to 8 $\mu$ ; the smaller ones rounded-angular, the larger ones acute-polyhedral; simple grains few, almost round or oval; size about 9 $\mu$ .
- Festuca diversifolia* Balansa. (*Graminaceæ*.) *Dry endosperm*.—Compound grains, round or oval, reticulated; size about 20 $\mu$ . Separated-grains polyhedral, the larger ones sometimes hollow; size 2 to 7 $\mu$ .
- Festuca elatior* Linn.; *Festuca pratensis* Huds. (*Graminaceæ*.) *Dry endosperm*.—Compound grains almost round or elliptical, reticulated-granular or with distinct dividing lines, consisting of 6 to over 2000 components. Size about 21 $\mu$ . Separated-grains, size 1.5 to 7 $\mu$ ; the larger ones polyhedral with a central cavity. In other specimens Nägeli found the size of compound grains to be 28 $\mu$ , and of the separated-grains 1 to 4 and 5 $\mu$ .

- Festuca sylvatica* Vill.; *Festuca calamaria* Smith. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated. Size about 25 $\mu$ . Separated-grains, size 1.5 to 7 $\mu$ ; the smaller ones rounded-angular, the larger ones acute polyhedral, and often have a cavity.
- Festuca spadicea* Linn. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular or frequently acute polyhedral, and with a central cavity. Size 2 to 6 and 7 $\mu$ . Compound grains few.
- Festuca spectabilis* Jan. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded to oblong, reticulated-granular, or with distinct lines of division. Size about 21 $\mu$ . Separated-grains, size 1.5 to 5 and 7 $\mu$ ; the larger ones acute polyhedral, with a central cavity.
- Festuca heterophylla* Haenke. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, reticulated-granular or homogeneous. Size about 25 $\mu$ . Separated-grains mostly polyhedral; size 2 to 6 $\mu$ .
- Festuca elegans* Boiss. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, reticulated-granular. Size about 17 $\mu$ . Separated-grains rounded-angular, or mostly acute-polyhedral; size 2 to 6 $\mu$ .
- Festuca fenas* Lagasc. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular. Size about 30 $\mu$ . Separated-grains rounded-angular or mostly acute-polyhedral. Size 2 to 6 $\mu$ .
- Festuca procumbens* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular. Size 36 $\mu$ . Separated-grains rounded angular or the majority polyhedral; with a central cavity.
- Festuca salzmanni* Boiss. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, frequently a result of pressure becoming somewhat angular, reticulated. Size about 30 $\mu$ . Separated-grains size 2 to 6 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral.
- Festuca pumila* Vill. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, reticulated (most of them have fallen apart). Size about 18 $\mu$ . Separated-grains 2 to 5 and 6 $\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral, and occasionally have a cavity.
- Festuca petræa* Guthn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round, oval, elliptical, frequently angular as result of pressure, reticulated. Separated-grains rounded-angular or polyhedral, sometimes have a cavity. Size 2 to 5 and 6 $\mu$ .
- Festuca lachenalii* Spenn.; *Festuca poa* Kunth. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, reticulated-granular. Size 36 $\mu$ . Separated-grains rounded-angular or polyhedral, usually with a central cavity. Size 1.5 to 6 $\mu$ .
- Festuca nutans* Willd. (*Graminaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular or polyhedral. Size 2 to 5 $\mu$ . Very few compound grains observed free, while in the cells they are distinctly perceived.
- Festuca urvilleana* Stend. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded, elliptical, elongated-oval, conical, finely reticulated. Size about 35 $\mu$ . Separated-grains rounded-angular or polyhedral. Size 1.5 to 5 $\mu$ .
- Festuca corealis* Mert. et Koch. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval; reticulated, reticulated-granular or homogeneous. Size about 28 $\mu$ . Separated-grains rounded-angular to acute polyhedral. Size 1.5 to 5 $\mu$ .
- Festuca gigantea* Vill.; *Bromus giganteus* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular or finely granular. Size about 24 to 30 $\mu$ . Separated-grains rounded-angular to acute polyhedral; many are hollow. Size 1.5 to 5 $\mu$ .
- Festuca rubra* Linn. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round, reticulated-granular. Size about 20 $\mu$ . Separated-grains rounded-angular or polyhedral; the larger ones are hollow. Size 1.5 to 5 $\mu$ .
- Festuca pseudo-eskia* Boiss. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, reticulate (most of them fallen apart). Size about 20 $\mu$ . Separated-grains, size 1.5 to 5 $\mu$ ; the smaller ones rounded-angular, larger ones polyhedral.
- Festuca vaginata* Wald. Kit. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, occasionally somewhat irregular, homogeneous, or reticulated-granular. Size about 25 $\mu$ . Separated-grains rounded angular to acute polyhedral. Size 1.5 to 5 $\mu$ .

- Festuca stuartina* Steud. (*Graminaceæ.*) *Dry endosperm.*—Compound grains round, oval, pyriform, frequently somewhat angular as result of pressure, reticulated. Size about  $15\mu$ . Separated-grains, size 1.5 to  $5\mu$ ; the smaller ones rounded-angular, the larger ones polyhedral, and have a cavity. Simple grains are also observed which are rounded, oval, or pyriform; the larger ones hollow. Size 6 to  $7\mu$ .
- Festuca bromoides* Linn. (*Graminaceæ.*) *Dry endosperm.*—Compound grains round to elongated-elliptical and conical, granular or reticulated-granular. Size about  $28\mu$ . Separated-grains, size 1.5 to  $5\mu$ ; the smaller ones rounded, the larger ones polyhedral, frequently with a central cavity.
- Festuca alopecurus* Schousb. (*Graminaceæ.*) *Dry endosperm.*—Compound grains almost round to elongated-oval and conical, occasionally by means of pressure, somewhat angular, reticulated. Size about  $17\mu$ . Separated-grains rounded-angular or polyhedral. Size 1.7 to  $5\mu$ .
- Festuca maritima* DC. (*Graminaceæ.*) *Dry endosperm.*—Compound grains round, pyriform, elliptical oblong, occasionally somewhat angular as result of pressure, reticulated. Size about  $40\mu$ . Separated-grains rounded-angular to polyhedral. Size 1.5 to  $5\mu$ .
- Festuca dumetorum* Linn; *Festuca rubra* var. Linn. (*Graminaceæ.*) *Dry endosperm.*—Compound grains usually oval or elliptical, frequently somewhat angular, granular or reticulated-granular. Size about  $31\mu$ . Separated-grains, size 1 to 4 and  $5\mu$ ; the smaller ones rounded, the larger ones polyhedral with a central cavity.
- Festuca abyssinica* Hochst. (*Graminaceæ.*) *Dry endosperm.*—Compound grains rounded, oval, elliptical, conical, frequently somewhat angular as result of pressure. Size about  $17\mu$ . Separated-grains rounded-angular or polyhedral; size 1.5 to  $4\mu$ . Simple grains (or separated-grains which as result of continued growth have become rounded off); rounded or oval are also present. Size about  $5\mu$ .
- Festuca uniglumis* Soland.; *Vulpia membranacea* Link. (*Graminaceæ.*) *Dry endosperm.*—Compound grains rounded or oval; reticulated-granular. Size about  $23\mu$ . Separated-grains round to polyhedral, the larger ones with a small cavity. Size 1 to  $4\mu$ .
- Festuca nigrescens* Lam.; *Festuca heterophylla* var. Haenke. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical or oval, homogeneous or granular. Size about  $16\mu$ . Separated-grains rounded to polyhedral; size 1 to  $4\mu$ .
- Festuca glauca* Lam.; *Festuca ovina* var. (*Graminaceæ.*) *Dry endosperm.*—Compound grains spherical, oval, or conical; half to as thick as long, homogeneous or granular, consisting of 20 to over 8000 components. Size about  $18\mu$ , rarely  $25\mu$ . Separated-grains rounded to polyhedral. Size 1 to 3 and  $4\mu$ .
- Festuca triflora* Desf. (*Graminaceæ.*) *Dry endosperm.*—Compound grains round or oval, reticulated or reticulated-granular. Size about  $17\mu$ . Separated-grains, size 1 to 3 and  $4\mu$ ; the smaller ones rounded, the larger ones polyhedral.
- Festuca myurus* Linn. (*Graminaceæ.*) *Dry endosperm.*—Compound grains rounded, oval, or elliptical, angular through pressure, reticulated or granular. Size about 15 to  $20\mu$ . Separated-grains, size 1 to  $3.5\mu$ ; the small ones rounded, the larger ones polyhedral.
- Festuca broteri* Boiss. and Reut. (*Graminaceæ.*) *Dry endosperm.*—Compound grains rounded, oval, elliptical, conical, finely reticulated. Size about  $14\mu$ . Separated-grains, size 1 to  $3\mu$ , rounded-angular.
- Festuca cynosuroides* Desf. (*Graminaceæ.*) *Dry endosperm.*—Compound grains round or elliptical, granular or almost homogeneous. Size about  $18\mu$ . Separated-grains rounded angular. Size 0.7 to  $3\mu$ .
- Festuca tenella* Willd. (*Graminaceæ.*) *Dry endosperm.*—Compound grains rounded-oval to elongated-elliptical, granular. Size about  $26\mu$ . Separated-grains rounded-angular; size 1 to  $3\mu$ .
- Festuca sabulicola* Dufour (*Graminaceæ.*) *Dry endosperm.*—Compound grains rounded to elongated-elliptical, reticulated-granular. Size about  $20\mu$ . Separated-grains rounded-angular; size 1 to  $3\mu$ .
- Festuca lolium* Balansa. (*Graminaceæ.*) *Dry endosperm.*—Compound grains round or oval, reticulated-granular or granular. Size about  $17\mu$ . Separated-grains rounded or rounded-angular. Size 0.7 to  $3\mu$ .
- Festuca rotiballoides* Kunth. (*Graminaceæ.*) *Dry endosperm.*—Compound grains rounded, oval, elliptical, occasionally somewhat angular by means of pressure, finely reticulated or almost homogeneous. Size about  $16\mu$ . Separated-grains rounded-angular; size 1 to  $3\mu$ .

- Festuca rigida* Kunth.; *Sclerochloa rigida* Panz. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round to oblong usually somewhat irregular, half to almost as thick as long, granular or almost homogeneous, consisting of over 2000 components. Size about 27 $\mu$ . Separated-grains almost round or rounded-angular; size 1 to 3 $\mu$ .
- Festuca divaricata* Desf. (*Graminaceæ*.) *Dry endosperm*.—Compound grains almost round or elliptical, granular. Size about 21 $\mu$ . Separated-grains rounded-angular; size 0.7 to 2 and 3 $\mu$ .
- Festuca tenuiflora* Schrad. (*Graminaceæ*.) *Dry endosperm*.—Compound grains almost round or oval, frequently somewhat irregular, homogeneous or finely granular. Size about 20 $\mu$ . Separated-grains rounded-angular; size 1 to 2, rarely 3 $\mu$ .
- Festuca delicatula* Lagasc.; *Vulpia delicatula* Link. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded-oval to oblong, occasionally somewhat irregular, two-fifths to almost as broad as long, granular or almost homogeneous, containing over 3000 components. Size about 16 to 22 $\mu$ . Separated-grains round or rounded angular. Size 0.7 to 2.5 $\mu$ .
- Festuca ciliata* Link. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded, elliptical, oblong, conical, frequently angular by means of pressure, finely reticulate or almost homogeneous. Size about 16 $\mu$ . Only a few separated-grains rounded or rounded-angular are present. Size 0.7 to 2.5 $\mu$ .
- Festuca geniculata* Willd. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round or oval, frequently angular, also granular. Size about 18 $\mu$ . Separated-grains rounded, size 0.8 to 2 $\mu$ .
- Festuca memphitica* Boiss. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical, oval, elliptical, homogeneous or finely granulated. Size about 15 $\mu$ . Separated-grains rounded, size 0.8 to 2 $\mu$ .
- Festuca macrophylla* Hochst. (*Graminaceæ*.) *Dry endosperm*.—Compound grains round to oblong, frequently angular as a result of pressure, reticulated-granular or granular. Separated-grains rounded. Size 0.7 to 2 $\mu$ .
- Bromus littoralis* Hort. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated-granular, finely granular or homogeneous. Size about 17 $\mu$ . Separated-grains rounded-angular or acute polyhedral, size 1.5 to 5 $\mu$ . This starch is related to the genus *Festuca*.
- Diarrhena americana* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, reticulated. Size about 24 $\mu$ . Separated-grains polyhedral with acute angles and margins; larger ones have a cavity. Size 2 to 6 $\mu$ .
- Lolium canadense* Michx. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, half to as thick as long; granular, consisting of 20 to over 4000 components. Length 40 to 45 $\mu$ ; thickness about 30 $\mu$ . Separated-grains rounded-angular to acute polyhedral. Size 1.5 to 6 $\mu$ .
- Lolium temulentum* Linn., var. *speciosum* Link. (*Graminaceæ*.) *Dry endosperm*.—Compound grains spherical, oval, or somewhat irregular, homogeneous, or reticulated-granular. Size about 42 $\mu$ . Separated-grains polyhedral; the larger ones have a small cavity. Size 1.5 to 6 $\mu$ .
- Psilurus nardoides* Trin. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded to oblong, frequently irregularly angular, half to almost as thick as long, granular to homogeneous, consisting of over 2000 components. Size about 15 $\mu$ . Separated-grains round or rounded-angular. Size 0.5 to 4 $\mu$ .
- Lepturus incurvatus* Trin.; *Ophiurus incurvatus* Beauv. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded-oval to oblong (not at all angular), two-thirds to almost as thick as long; slightly granular or almost homogeneous, consisting of 20 to 8000 components. Length about 33 $\mu$ ; thickness about 18 $\mu$ . Separated-grains round. Size 1 to 2 $\mu$ .
- Lepturus filiformis* Trin.; *Ophiurus filiformis* Roem and Schult. (*Graminaceæ*.) *Dry endosperm*.—Compound grains rounded-oval to oblong, usually more or less angular by means of pressure, homogeneous, rarely finely granular. Size about 16 $\mu$ . Separated-grains round. Size 0.5 to 2 $\mu$ .
- Centrolepis fascicularis* Labil.; *Desvauzia billardieri* R. Br. (*Centrolepideæ*.) *Dry endosperm*.—Compound grains spherical or oval, consisting of 2 to over 16 equal components. Size about 18 $\mu$ . Separated-grains semi-spherical with one or more pressure facets, or polyhedral with one curved surface, or exactly polyhedral (only bounded by pressure facets), usually with a large or small cavity and several radial fissures.
- Aphelia cyperoides* R. Br. (*Centrolepideæ*.) *Dry endosperm*.—Separated-grains angular or polyhedral, more or less irregular, usually compressed. Size 2 to 9 $\mu$ . Compound grains could not be positively distinguished.

- Restio ferruginosus* Link. (*Restiaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, many somewhat angular as a result of pressure, reticulate, containing about 1000 components. Size about 30 $\mu$ . Separated-grains polyhedral; size 2 to 7 $\mu$ ; the larger ones frequently have a small cavity and several delicate radial fissures.
- Anarthria prolifera* R. Br. (*Restiaceæ*.) *Dry endosperm*.—Separated-grains polyhedral, sometimes with a very small cavity and delicate radial fissures. Size 1.5 to 7 $\mu$ . Very few free compound grains are observed, though they are frequently distinctly perceived in the cells; almost round or oval, by means of pressure more or less polyhedral; size about 20 $\mu$ . The whole cell lumen sometimes appears to be cuneiform filled with separated-grains.
- Pæpalanthus caulescens* Kunth. (*Eriocaulaceæ*.) *Dry endosperm*.—Compound grains almost round to elongated-oval, frequently polyhedral, reticulated-granular, or almost homogeneous, consisting of about 1000 components. Size 15 to 22 $\mu$ . Separated-grains polyhedral, the larger ones with a central cavity. Size 1.5 to 8 $\mu$ .
- Pæpalanthus frigidus* Mart. (*Eriocaulaceæ*.) *Dry endosperm*.—Starch as in the preceding species. Compound grains containing over 1000 components. Size about 25 to 30 $\mu$ . Separated-grains, size 2 to 10 $\mu$ .
- Xyris operculata* Labill. (*Xyridaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, usually more or less polyhedral by reason of pressure, reticulate, containing over 300 components. Size about 30 $\mu$ . Separated-grains polyhedral; the larger ones sometimes have a small central cavity, and also single delicate radial fissures. Size 2.5 to 10 and 12 $\mu$ .
- Xyris semifuscata*. (*Xyridaceæ*.) *Dry endosperm*.—Starch as in the preceding. Compound grains spherical to elongated-conical; containing 400 or more components. Size about 35 $\mu$ . Size of the separated-grains 2 to 8 and 10 $\mu$ .
- Mayaca vandellii* Schott and Endl. (*Xyridaceæ*.) *Dry endosperm*.—Separated-grains, rounded-angular to acute polyhedral; many have a very small central cavity and single delicate radial fissures. Size 2 to 8 and 10 $\mu$ . The endosperm cells are entirely filled with these polyhedral grains; only indistinct groups may be recognized within these cells, which probably correspond to compound grains.
- Mayaca nichauxii* Schott and Endl. (*Xyridaceæ*.) *Dry endosperm*.—Starch as in the preceding. Separated-grains polyhedral, usually with a small or large cavity and radial fissures. Size 2.5 to 12 $\mu$ .
- Arum orientale* Biebrst. (*Aroideæ*.) *Dry endosperm*.—Compound grains rounded or oval, three-fifths to as thick as long, consisting of 4 to 1400 components. Length about 36 and 48 $\mu$ , thickness about 30 $\mu$ . Separated-grains polyhedral, usually with acute margins, edges and angles; the larger ones have a central cavity and a few short radial fissures. Size 2 to 11 $\mu$ .
- Zantedeschia athiopica* Spreng.; *Richardia athiopica* Kunth. (*Aroideæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval and conical, consisting of 2 to over 400 components. Size about 28 $\mu$ . Separated-grains polyhedral, frequently with a small cavity. Size 2 to 8 and 12 $\mu$ . Simple isolated rounded or elongated-oval grains are also present.
- Typha tenuifolia* Humb. Bonp. (*Typhaceæ*.) *Dry endosperm*.—Compound grains rounded to oblong-oval, frequently somewhat angular or irregular, reticulated-granular. Size about 15 $\mu$ . Separated-grains more or less polyhedral. Size 2 to 5 $\mu$ . Poor in starch.
- Piper nigrum* Linn. (*Piperaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, frequently polyhedral as result of pressure, reticulated-granular, containing over 4000 components. Size about 33 $\mu$ . Separated-grains rounded to polyhedral. Size 1 to 4 $\mu$ .
- Piper cubeba* Linn. fil. (*Piperaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, frequently by means of pressure polyhedral, reticulate, or with distinct lines of separation, containing over 600 components (most of them have fallen apart). Size about 32 $\mu$ . Separated-grains polyhedral, rarely with a small central cavity. Size 3 to 10 $\mu$ . The endosperm cells of *Piper nigrum* and *cubeba* are closely packed with starch. When making sections, a few compound grains fall out along with numerous separated-grains. The compound grains are a little larger and not so crowded in the innermost less compact tissues of the seed; they are packed in the more external dense tissue, the divisions between the compound grains frequently being indistinctly observed, while in the outermost cells the compound grains consisting of coalesced components form a continuous uniform mass having a reticulated or parenchymatous appearance (with dense septa and apparently hollow alveoli) as in *Amomum*, *Commelina*, etc. (See type 12.)

- Patomorphe sidaefolia* Miq.; *Hekeria sidaefolia* Kunth. (*Piperaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, occasionally by means of pressure somewhat angular, homogeneous or distinctly granular. Size about  $24\mu$ . Separated-grains rounded and rounded-angular. Size 1 to  $3\mu$ . In other seeds nearly all the compound grains have fallen apart. Size of the rounded and polyhedral separated-grains 1.5 to  $5\mu$ .
- Peperomia maculosa* Hook. (*Piperaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, occasionally somewhat angular as result of pressure, almost homogeneous to distinctly granular. Size about  $22\mu$ . Separated-grains rounded or rounded-angular. Size 0.7 to  $3\mu$ .
- Atriplex hortensis* Linn. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, half to almost as thick as long, finely granular or almost homogeneous, containing over 15,000 components. Length about  $45\mu$ ; thickness about  $25\mu$ . Separated-grains rounded. Size 0.5 to  $1.5\mu$ . The compound grains within the endosperm cells are frequently embedded in a finely granulated mass of separated-grains.
- Atriplex hastata* Linn., var. *calotheca* Rafn. (*Chenopodiaceæ*.) *Dry endosperm*.—The endosperm cells are filled with round separated-grains. Size 0.5 to  $1\mu$ .
- Axyris amarantoides* Linn. (*Chenopodiaceæ*.) *Dry endosperm*.—The endosperm cells are filled with a finely granular starchy mass of large separated-grains. Size 0.5 to  $1\mu$ .
- Aenida tuberculata* Moq. (*Chenopodiaceæ*.) *Dry endosperm*.—The endosperm cells are packed with round or rounded-angular separated-grains sometimes hanging together in short rows. Size 1 to  $3\mu$ .
- Spinacia glabra* Mill.; *Spinacia inermis* Moench. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded-oval, elliptical, conical, and lanceolate, one-fifth to almost as thick as long, sometimes polyhedral by means of pressure, also granular, rarely almost homogeneous, consisting of 20 to 30,000 components. Length about 60 and  $100\mu$ , thickness about 20 to  $41\mu$ . Separated-grains rounded. Size 0.5 to  $2.5\mu$ .
- Panderia pilosa* Fisch. and Mey. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded to oblong, occasionally somewhat angular, finely granulated or homogeneous. Size about 20 to  $24\mu$ . Separated-grains, round. Size 0.5 to  $1.5\mu$ .
- Blitum capitatum* Linn. (*Chenopodiaceæ*.) *Dry endosperm*.—Separated-grains round, size 0.7 to  $2\mu$ , rarely  $3\mu$ . Very few compound grains are present.
- Ambrina graccolens* Moq. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, occasionally somewhat angular, granular. Size about 23 to  $30\mu$ . Separated-grains rounded. Size 0.7 to  $2.5\mu$ .
- Beta orientalis* Heyn. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded, elliptical, oblong. Size about  $30\mu$ . Separated-grains rounded or somewhat angular. Size 1 to  $3\mu$ .
- Beta vulgaris* Linn. (*Chenopodiaceæ*.) *Dry endosperm*.—According to Payen (Ann. Sc. Nat., 1838, II, p. 28, plate 4), the separated-grains are rounded. Size about  $4\mu$ .
- Echinopsilon hyssopifolium* Moq. (*Chenopodiaceæ*.) *Dry endosperm*.—Separated-grains rounded. Size 1 to  $2\mu$ . No compound grains are observed.
- Kochia scoparia* Schrad. (*Chenopodiaceæ*.) *Dry endosperm*.—Separated-grains rounded. Size 0.5 to  $1\mu$ . No compound grains are present.
- Cycolopsis platyphylla* Moq. (*Chenopodiaceæ*.) *Dry endosperm*.—Separated-grains rounded. Size 0.5 to about  $1\mu$ . No compound grains are observed.
- Teloxys aristata* Moq.; *Chenopodium aristatum* Linn. (*Chenopodiaceæ*.) *Dry endosperm*.—The endosperm cells are entirely filled with almost rounded or rounded-angular separated-grains, frequently lying in rows. Size 1 to 2 and  $3\mu$ .
- Chenopodium quinoa* Willd. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded, oval, oblong, conical, one-third to almost as thick as long, not often slightly angular by means of pressure; sometimes pointed at one end, more rarely at both ends; finely granular or homogeneous; consisting of over 14,000 components. Length about  $40\mu$ , rarely  $54\mu$ ; thickness about  $23\mu$ , rarely  $28\mu$ . Separated-grains round. Size 0.7 to  $2\mu$ .
- Acroglochis chenopodioides* Schrad.; *Acroglochis persicarioides* Moq.; *Lecanocarpus cauliflorus* Nees. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded to elongated-oval, sometimes slightly angular, granulated or reticulated-granular. Size about  $36\mu$ . Separated-grains rounded; the largest ones rounded-angular. Size 0.8 to rarely  $3\mu$ .

- Hablitzia tamnoides* Biebrst. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-lanceolate, frequently somewhat angular; one-third to as thick as long, granular. Size about 30 $\mu$ . Separated-grains rounded. Size 0.7 to scarcely 3 $\mu$ .
- Basella alba* Linn.; *Basella ramosa* Jacq. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded or oval. Size about 25 $\mu$ . Separated-grains round to polyhedral. Size 2 to 6 $\mu$ . Starch found only in the endosperm.
- Schoberia corniculata* Meyer. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains spherical to lanceolate, one-seventh to as thick as long; with blunt or pointed ends; frequently by means of pressure more or less polyhedral, granular or homogeneous, consisting of over 10,000 components. Length about 50 $\mu$ , thickness about 14 $\mu$ . Separated-grains rounded. Size 0.7 to 2 $\mu$ .
- Schoberia salsh* Meyer. (*Chenopodiaceæ*.) *Dry endosperm*.—Separated-grains round. Size 1 to 2 $\mu$ .
- Monolepis chenopodioides* Moq. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains round, oval, oblong, two-fifths to almost as thick as long, rarely somewhat angular as result of pressure, finely granulated or homogeneous, consisting of over 12,000 components. Length about 38 to 45 $\mu$ , thickness about 25 $\mu$ . Separated-grains rounded. Size 0.5 to 1.5 and 2 $\mu$ .
- Corispermum hyssopifolium* Linn. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, sometimes oblong, two-fifths to almost as thick as long, rarely somewhat angular by means of pressure, granular or almost homogeneous, consisting of over 9000 components. Length about 25 to 31 $\mu$ , thickness about 18 $\mu$ . Separated-grains almost round. Size 0.5 to 1.5 and 2 $\mu$ .
- Corispermum marshallii* Steven. (*Chenopodiaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, granular, or almost homogeneous. Size about 20 $\mu$ . Separated-grains rounded. Size 0.7 to 1.5 $\mu$ .
- Achyranthes argentea* Lam. (*Amarantaceæ*.) *Dry endosperm*.—According to Leon Soubeiran (Journ. Pharm., 1854, xxv, 96), the endosperm cells, which are easily separated from one another, contain rounded or oval grains with one dark central spot (probably separated-grains). Size 5 $\mu$ .
- Achyranthes fruticosa* Lam. (*Amarantaceæ*.) *Dry endosperm*.—According to Leon Soubeiran (Journ. Pharm., 1854, xxv, 96), starch as in the preceding, except that the grains have fissures radiating from the center. In the embryo, single rounded, rather large starch-grains may also be present.
- Albersia blitum* Kunth; *Amaranthus blitum* Linn. (*Amarantaceæ*.) *Dry endosperm*.—The endosperm cells are filled with rounded separated-grains. Size 1 to 2 $\mu$ .
- Alternanthera paronychioides* St. Hil. (*Amarantaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, occasionally somewhat angular, granular or homogeneous. Size about 27 $\mu$ . Separated-grains rounded. Size 0.5 to 2 $\mu$ .
- Amarantus bullatus* Besser.; *Amarantus sanguineus* Linn.; *Amarantus frumentaceus* Roxb. (*Amarantaceæ*.) *Dry endosperm*.—The cells, which easily separated from one another, are filled with separated-grains. Size scarcely 1 $\mu$ . No compound grains were observed.
- Amblyogyne polygonoides* Rafn. (*Amarantaceæ*.) *Dry endosperm*.—Cells closely filled with separated-grains, frequently arranged in rows. Size scarcely 1 $\mu$ . No compound grains were observed.
- Celosia cristata* Linn. (*Amarantaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, finely granular. Size 16 $\mu$ . Separated-grains rounded or rounded-angular. Size 1 to 2.5 $\mu$ .
- Chamissoa albida* Mart. (*Amarantaceæ*.) *Dry endosperm*.—Compound grains mostly spherical, rarely oval, consisting of from 6 to over 200 almost equal components. Size 17 $\mu$ . Separated-grains nearly round to almost polyhedral, rarely with a small cavity. Size 1 to 4 $\mu$ .
- Desmochata patula* Roem & Schult.; *Achyranthus patula* Linn. fil. (*Amarantaceæ*.) *Dry endosperm*.—Compound grains spherical, oval, oblong (not at all angular), half to as thick as long, finely granular, consisting of over 6000 components. Size about 25 $\mu$ . Separated-grains round. Size 0.7 to 1.5 $\mu$ .
- Euxolus emarginatus* A. Br. and Bouché. (*Amarantaceæ*.) *Dry endosperm*.—Separated-grains rounded or rounded-angular. Size 1 to 3 $\mu$ . Compound grains no longer present.
- Euxolus caudatus* Moq. (*Amarantaceæ*.) *Dry endosperm*.—According to Leon Soubeiran (Journ. Pharm., 1854, xxv, 99), the starch-grains in the cells which are easily separated are oval or spherical, without lamellæ or hilum. Size 5 $\mu$ . (Probably homogeneous compound grains.)

- Hoplotheca floridana* Nutt.; *Frælichia floridana* Moq. (*Amarantaceæ.*) *Dry endosperm.*—Compound grains spherical, oval, oblong (not at all angular); two-fifths to as thick as long, finely granular. Length about  $23\mu$ ; thickness about  $16\mu$ . Separated-grains rounded. Size 0.7 to  $1.5\mu$ .
- Hoplotheca texana* A. Braum.; *Frælichia gracilis* Moq. (*Amarantaceæ.*) *Dry endosperm.*—Size of separated-grains hardly  $1\mu$ . No compound grains are present.
- Gomphrena decumbens* Jacq. (*Amarantaceæ.*) *Dry endosperm.*—Size of separated-grains hardly over  $1\mu$ . No compound grains are present.
- Iresine nervosa* Hort. (*Amarantaceæ.*) *Dry endosperm.*—Separated-grains round. Size 0.5 to  $1\mu$ . No compound grains are present.
- Polygonum majus* A. Braun. (*Amarantaceæ.*) *Dry endosperm.*—Separated-grains entirely filling the cells, arranged in rows. Size 1 to  $1.5\mu$ . No compound grains are visible.
- Pupalia prostrata* Mart. (*Amarantaceæ.*) *Dry endosperm.*—Compound grains spherical to elongated-oval, sometimes angular, two-fifths to as thick as long, finely granular or homogeneous, consisting of 25,000 components. Size  $30\mu$ , rarely  $40\mu$ . Separated-grains round. Size 0.5 to scarcely over  $1\mu$ .
- Scleropus amarantoides* Schrad. (*Amarantaceæ.*) *Dry endosperm.*—Separated-grains round or rounded-angular, closely packed in the cells, and frequently arranged in rows. Size 1 to  $3\mu$ .
- Teleianthera polygonoides* Moq., var. *brachiata*. (*Amarantaceæ.*) *Dry endosperm.*—Compound grains spherical or oval, finely granulated, size about  $30\mu$ . Separated-grains round, size 0.5 to about  $2\mu$ .
- Abronia arenaria* Horh. (*Nyctaginaceæ.*) *Dry endosperm.*—Compound grains rounded to elongated-oval (completely filling the cells); granulated or reticulated-granular, consisting of over 4000 components. Size about 20 and  $25\mu$ . Separated-grains rounded 1 to  $2\mu$ .
- Mirabilis jalappa* Linn. (*Nyctaginaceæ.*) *Dry endosperm.*—Separated-grains rounded or slightly angular, completely filling the cells. Size 0.7 to  $2\mu$ . Compound grains are not visible.
- Mirabilis longiflora* Linn. *Dry endosperm.*—Separated-grains rounded. Size 1 to  $2\mu$ . No compound grains are present.
- Oxybaphus cervantesii* Lagasc. (*Nyctaginaceæ.*) *Dry endosperm.*—Compound grains spherical, granular, consisting of 30 to over 1,000 equal components. Size about  $15\mu$ . Separated-grains round or rounded-angular. Size 1 to  $3\mu$ .
- Allionia ovata* Pursh.; *Calxyhymenia paniculata* Desf. (*Nyctaginaceæ.*) *Dry endosperm.*—Separated-grains rounded. Size 0.7 to  $1.5\mu$ . Few compound grains remain (partly in the cells, partly free), rounded or oval. Size about  $35\mu$ , finely granulated, containing over 24,000 components; most of the cells are packed merely with tiny separated-grains arranged in rows.
- Allionia nyctaginea* Michx. (*Nyctaginaceæ.*) *Fresh and dry endosperm.*—Separated-grains almost round to rounded-angular. Size 1 to 2 and  $2.5\mu$ . They completely fill the endosperm cells. No compound grains could be distinctly observed.
- Allionia incarnata* Linn. (*Nyctaginaceæ.*) *Dry endosperm.*—Separated-grains round. Size 0.7 to about  $2\mu$ ; cells are crowded with them; no compound grains occur.
- Bougainvillea spectabilis* Willd. (*Nyctaginaceæ.*) *Dry endosperm.*—Separated-grains rounded or somewhat angular. Size 0.7 to  $2\mu$ . No compound grains are present.
- Arvicennia tomentosa* Linn. (*Verbenaceæ.*) *Dry cotyledons.*—Compound grains mostly rounded, rarely oval or conical, frequently somewhat irregular and angular, consisting of 2 to over 20 components which are almost equal, rarely unequal. Size about 14 and  $18\mu$ . The large separated-grains have a small central cavity. Size 2 to  $8\mu$ . Starch plentiful.
- Nymphaea rubra* Roxb. (*Nymphaeaceæ.*) *Dry endosperm.*—Compound grains by means of pressure polyhedral, with rather sharp margins and angles, reticulate, consisting of 8 to over 200 components. Size about  $23\mu$ . Separated-grains round, rounded angular to acute polyhedral; the larger ones with a central cavity. Size 2 to  $10\mu$ .
- Nymphaea dentata* Th. et Schum. (*Nymphaeaceæ.*) *Dry endosperm.*—Starch as in the preceding species.
- Nymphaea cærulea* Savign. (*Nymphaeaceæ.*) *Dry endosperm.*—Starch as in *Nymphaea rubra*. Separated-grains mostly polyhedral, without a cavity. Size 2 to  $7\mu$ .
- Nuphar luteum* Smith. (*Nymphaeaceæ.*) *Dry endosperm.*—Compound grains spherical or rounded-oval, slightly angular as result of pressure, finely granulated, consisting of over 10,000 components. Size 25 to  $30\mu$ . Separated-grains rounded or rounded-angular. Size hardly 1 to  $3\mu$ .

- Barclaya oblonga* Wall. (*Nymphaeaceae*.) *Dry endosperm*.—Compound grains rounded or oval, usually polyhedral as a result of pressure, reticulate, consisting of 20 to over 800 components. Size about  $26\mu$ . Separated-grains 2 to  $8\mu$  in size; the smaller ones rounded-angular, the larger ones polyhedral and with a central cavity.
- Frankenia pulverulenta* Andr. (*Frankeniaceae*.) *Dry seeds*.—Separated-grains almost rounded, frequently clinging to each other like flakes of snow. Size 0.7 to  $2\mu$ . No compound grains could be positively distinguished. The starch is probably found in the endosperm.
- Mesembryanthemum pinnatifidum* Linn. fil. (*Ficoideae* or *Mesembryaceae*.) *Dry endosperm*.—Compound grains spherical to oblong, one-third to as thick as long, frequently angular or even polyhedral, as result of pressure, granular or homogeneous, containing over 5000 components. Length about  $36\mu$ ; thickness about  $20\mu$ . Separated-grains rounded-angular. Size 1 to  $3\mu$ .
- Tetragonia expansa* Ait. (*Ficoideae* or *Mesembryaceae*.) *Dry endosperm*.—Compound grains oval, elliptical, or conical, one-fourth to three-fourths as thick as long; polyhedral as result of pressure, reticulated-granular or almost homogeneous, consisting of over 4000 components. Length about  $60\mu$ , thickness about  $27\mu$ . Separated-grains rounded-angular to polyhedral. Size 1.5 to  $5\mu$ . Also isolated simple spherical grains, size about  $12\mu$ , are observed, as well as compound grains of few components, which increase in size as the grains are less compound (in doublets about  $10\mu$ ).
- Aizoon hispanicum* Linn. (*Ficoideae* or *Mesembryaceae*.) *Dry endosperm*.—Separated-grains rounded to polyhedral. Size 1 to 3 and  $4\mu$ . Completely filling the endosperm cells, in which groups of polyhedral grains which resemble compound grains may occasionally be seen. Size about  $16\mu$ .
- Aizoon canariense* Linn. (*Ficoideae* or *Mesembryaceae*.) *Dry endosperm*.—Separated-grains round or rounded-angular. Size 0.5 to hardly  $2\mu$ . Completely filling the endosperm cells. No compound grains were observed.
- Trianthema monogynum* Linn. (*Ficoideae* or *Mesembryaceae*.) *Dry endosperm*.—Compound grains, rounded. Size 10 to  $12\mu$ . Separated-grains rounded to polyhedral, the larger ones have a central cavity. Size 1 to  $5\mu$ .
- Portulaca megalantha* Steud.; *Portulaca grandiflora* Hook. (*Portulacaceae*.) *Dry endosperm*.—Separated-grains rounded-angular to acute-polyhedral. Size 1.5 to  $6\mu$ . A few small compound grains and broken fragments of larger ones are also present.
- Talinum patens* Willd. (*Portulacaceae*.) *Dry endosperm*.—Compound grains rounded or oval, two-thirds to as thick as long, reticulated-granular, consisting of 4 to over 600 components. Size about  $18\mu$ . Separated-grains rounded-angular to almost polyhedral. Size 1.5 to  $4\mu$ .
- Calandrinia compressa* Schrad. (*Portulacaceae*.) *Dry endosperm*.—Separated-grains rounded or rounded-angular. Size 1 to  $3\mu$ . No compound grains observed. The membrane of the endosperm cells is stained blue with iodine.
- Claytonia perfoliata* Don. (*Portulacaceae*.) *Dry endosperm*.—Compound grains oval or lanceolate, frequently narrowed at both ends and spindle-shaped, one-fifth to two-thirds as thick as long, somewhat polyhedral by means of pressure, granular or homogeneous, consisting of 100 to over 6000 components. Length about  $46\mu$ , thickness about  $16\mu$ . Separated-grains round. Size 1 to  $2\mu$ .
- Monocosmia corrigioloides* Fenzl. (*Portulacaceae*.) *Dry endosperm*.—Compound grains round to lanceolate, one-fourth to as thick as long, often angular by means of pressure and even polyhedral, granular or almost homogeneous, consisting of over 8000 components. Length about  $45\mu$ , thickness about  $20\mu$ . Separated-grains round or rounded-angular. Size 1 to  $3\mu$ .
- Montia minor* Gmel. (*Portulacaceae*.) *Dry endosperm*.—Separated-grains rounded or rounded-angular. Size 0.7 to almost  $3\mu$ . No compound grains are present.
- Mollugo cerviana* Seringe. (*Ficoideae* or *Mesembryaceae*.) *Dry endosperm*.—Separated-grains polyhedral, the larger ones compressed to about half their width, with a central cavity. Size 2 to  $8\mu$ . No compound grains could be distinctly observed.
- Pharnaceum verticillatum* Spreng.; *Mollugo verticillata* Linn. (*Ficoideae* or *Mesembryaceae*.) *Dry endosperm*.—Compound grains rounded or oval, three-fifths to just as thick as long, reticulated-granular, consisting of 4 to over 800 components. Size about  $20\mu$ . Separated-grains rounded-angular or polyhedral. Size 2 to  $4\mu$ .

- Adenogramma galioides* Frenzl. (*Ficoideæ* or *Mesembryaceæ*.) *Dry endosperm*.—Separated-grains polyhedral. Size 3 to 14 $\mu$ . Compound grains of 2 to 10 components, and fragments of more complex grains, are found, besides some simple grains, rounded or oval. Size about 15 $\mu$ .
- Corrigiola littoralis* Linn. (*Illecebraceæ*.) *Dry endosperm*.—Compound grains spherical, oval, low cone-shaped; three-fifths to as thick as long, granular, consisting of 10 to over 5000 components. Size about 30 $\mu$ . Separated-grains round to polyhedral. Size 1.5 to 4 and 5 $\mu$ .
- Herniaria glabra* Linn. (*Illecebraceæ*.) *Dry endosperm*.—Separated-grains polyhedral with acute sharp margins and angles (only outlined by pressure facets), the larger ones with a small central cavity. Size 2 to 11 $\mu$ . Compound grains occur; they appear to be polyhedral, and at all events contain many more than 100 components.
- Illecebrum verticillatum* Linn. (*Illecebraceæ*.) *Dry endosperm*.—Separated-grains polyhedral, usually irregular; very strongly compressed. Breadth 2 to 14 $\mu$ , thickness 1 to 2 $\mu$ . Only a few compound grains occur, which are flattened.
- Anychia dichotoma* Michx. (*Illecebraceæ*.) *Dry endosperm*.—Separated-grains rounded or angular. Size 1 to 4 $\mu$ . Few compound grains are present.
- Telephium imperati* Linn. (*Ficoideæ*.) *Dry endosperm*.—Separated-grains rounded-angular or frequently polyhedral. Size 1 to 4 $\mu$ . Compound grains crowded in the cells, polyhedral (a few of the compound grains are free), reticulated-granular, containing over 2000 components. Size about 28 $\mu$ .
- Polycarpæa teneriffæ* Lam. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains polyhedral with acute angles, mostly irregular and scale-like, very strongly compressed, homogeneous. Breadth 2 to 9 $\mu$ , thickness about 1 $\mu$ . No compound grains are found.
- Lepigonum medium* Wahlbg.; *Spergularia salina* Presl. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular, acute-polyhedral, frequently irregular and scale-like, very strongly compressed. Breadth 2.5 to 12 and 16 $\mu$ , thickness 1 $\mu$ . No compound grains.
- Spergula arvensis* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains oval-spherical to oblong; occasionally somewhat angular, and frequently irregular; one-half to almost as thick as long; granular to very nearly homogeneous, consisting of 20 to over 4000 components. Size about 28 $\mu$ . Separated-grains rounded or rounded-angular; usually rather strongly compressed. Breadth 1 to 3 $\mu$ , thickness 1 $\mu$ .
- Drymaria cordata* Willd. (*Filicineæ*.) *Dry endosperm*.—Separated-grains circular or rounded-angular, very strongly compressed, homogeneous. Breadth 3 to 20 $\mu$ , thickness 1 to 1.5 $\mu$ . No compound grains could be seen.
- Scleranthus perennis* Linn. (*Illecebraceæ*.) *Dry endosperm*.—Separated-grains rounded-angular or polyhedral, very strongly compressed. Breadth 2 to 15 and 20 $\mu$ , thickness 1 $\mu$  or a little more. No compound grains.
- Sagina apetala* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains round or rounded-angular. Size 1 to 2 $\mu$ . No compound grains present.
- Bufonia annua* DC. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, finely granular. Size about 16 $\mu$ . Separated-grains round. Size 0.5 to 1.5 $\mu$ .
- Arenaria holosteoides*; *Lepyrodiclis holosteoides* Fenzl. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular, or more often with 5 to 6 acute angles, most of them compressed to about half their width, the larger ones have a small central cavity and occasionally also some (1 to 3) short radial fissures. Size 2.5 to 11 $\mu$ . A few compound grains occur round or oval, consisting of 10 to 100 components. Size about 16 $\mu$ .
- Arenaria graminifolia* Schrad. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains oval, one-half to two-thirds as broad as long, compressed to one-third of their width, occasionally almost as thick as broad, consisting of over 100 components. Size about 40 $\mu$ . Separated-grains rounded to acute angular, occasionally irregular and scale-like, very strongly compressed. Size 3 to 13 $\mu$ , thickness hardly over 1 $\mu$ .
- Arenaria grandiflora* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains (completely filling the cells) angular, sometimes with a cavity. Size 3 to 15 $\mu$ . No compound grains are present.
- Arenaria globulosa* Labil. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded. Size 1 to 3 $\mu$ . Only a very few compound grains occur.

- Cerastium chloraefolium* Fisch and Mey. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular or acute-polyhedral, the larger ones compressed to about half their width. Size 1.5 to 6 $\mu$ . Only fragments of compound grains and a few intact compound grains are present.
- Dianthus atrorubens* All. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, occasionally through pressure slightly angular, granular or almost homogeneous. Size about 14 $\mu$ . Separated-grains rounded. Size 0.7 to 2 $\mu$ .
- Tunica saxifraga* Koch. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded or rounded-angular. Size 1.7 to 2 and 3 $\mu$ . No compound grains are present.
- Gypsophila altissima* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded to polyhedral. Size 1 to 4 and 5.5 $\mu$ . No compound grains are present.
- Saponaria persica* C. A. Mey. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, rarely slightly angular, granular, containing over 6000 components. Size about 22 $\mu$ . Separated-grains rounded. Size 0.7 to hardly 2 $\mu$ .
- Vaccaria vulgaris* Host.; *Saponaria vaccaria* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains spherical, oval, oblong, rarely somewhat angular, one-half to just as thick as long, granular, consisting of over 5000 components. Length about 25 and 35 $\mu$ , thickness about 25 $\mu$ . Separated-grains rounded. Size 1 to 2 $\mu$ .
- Silene conoidea* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular to polyhedral. Size 1.5 to 4 $\mu$ . Compound grains (only visible within the cells) rounded to oblong.
- Silene ambigua* Camb. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains almost rounded, size scarcely over 1 $\mu$ . No compound grains could be seen.
- Eudianthe cali-rosa* Fenzl.; *Lychnis cali-rosa* Desr. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains rounded or oval, granular. Size about 15 $\mu$ . Separated-grains round. Size 0.7 to scarcely 2 $\mu$ .
- Lychnis dioica* Linn.; *Lychnis vespertina* Sibth. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains rounded-oval to oblong, two-fifths to almost as thick as long, granular, containing 30 to over 3000 components. Size about 27 $\mu$ . Separated-grains rounded to angular. Size 1 to 3 and 4 $\mu$ .
- Agrostemma coronaria* Linn.; *Lychnis coronaria* Desr. (*Caryophyllaceæ*.) *Dry endosperm*.—Compound grains rounded to elongated-lanceolate, one-fourth to almost as thick as long, with either rounded surfaces or more rarely by means of pressure somewhat angular and sharp-edged, granular, consisting of over 5000 components. Size about 40 $\mu$ . Separated-grains rounded or rounded-angular. Size 1 to 3 $\mu$ .
- Cucubalus bacciferus* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded. Size 1 to scarcely 2 $\mu$ . No compound grains are observed.
- Drypis spinosa* Linn. (*Caryophyllaceæ*.) *Dry endosperm*.—Separated-grains rounded or slightly angular. Size 0.7 to 2 $\mu$ .
- Petiveria alliacea* Linn. (*Phytolaccaceæ*.) *Dry endosperm*.—Compound grains spherical to elongated-oval, occasionally somewhat angular or irregular, two-fifths to just as thick as long. Size about 36 $\mu$ . Separated-grains rounded or rounded-angular. Size 1.5 to 3 $\mu$ .
- Rivina purpurascens* Schrad. (*Phytolaccaceæ*.) *Dry endosperm*.—Compound grains spherical, granular. Size about 17 $\mu$ . Separated-grains rounded or rounded-angular. Size 1 to 3 $\mu$ .
- Limcum glomeratum* Eckl. Zeyh. (*Ficoideæ*.) *Dry endosperm*.—Compound grains oval, elongated-elliptical, lanceolate-conical, one-third to almost as thick as long, granular. Length about 30 $\mu$ , thickness about 15 $\mu$ . Separated-grains round or rounded-angular. Size 1 to 3 $\mu$ .
- Microtea maypurensis* G. Dou. (*Phytolaccaceæ*.) *Dry endosperm*.—Separated-grains rounded-angular. Size 1.5 to 4 $\mu$ . Compound grains nearly all fallen apart, only a few small rounded ones remaining. Size about 15 $\mu$ .
- Phytolacca esculenta* V. Houtte. (*Phytolaccaceæ*.) *Dry endosperm*.—Compound grains spherical or oval, two-fifths to equally as thick as long, reticulated-granular, consisting of 9000 components. Size about 65 $\mu$ . Separated-grains rounded to almost polyhedral. Size 1.5 to 5 $\mu$ .
- Pircuvia lathenia* Moq. (*Phytolaccaceæ*.) *Dry endosperm*.—Compound grains spherical-oval to elongated-oval, conical, or slightly irregular, reticulated-granular, consisting of over 20,000 components. Size about 77 $\mu$ . Separated-grains rounded to polyhedral. Size 1.5 to 5 $\mu$ .

- Reaumuria vermiculata*. (*Tamariscineæ*.) *Dry endosperm*.—Compound grains rounded, oval, elliptical, oblong, conical; one-half to as thick as long, sometimes by pressure polyhedral, granular or reticulate, consisting of over 7000 components. Length about  $31\mu$ , thickness about  $20\mu$ . The smaller separated-grains almost spherical, larger ones rounded-angular or polyhedral, compressed into the shape of a disk. Breadth 1 to  $5\mu$ , thickness 1 to  $1.5\mu$ .
- Moacurra* sp. (*Dichapclaceæ*.) *Dry cotyledons*.—Separated-grains rounded and rounded-angular to polyhedral; the larger ones have a small central cavity. Size 1 to  $5\mu$ . Compound grains (few distinct) rounded or oval, consisting of 2 to about 40 components. Size  $12\mu$ .
- Dipteryx odorata* Willd. (*Leguminosæ*.) *Dry cotyledons*.—Compound grains rounded, rarely oval, frequently somewhat irregular or angular, granulated or reticulated-granular, consisting of 4 to about 60 components. Size 10 to  $12\mu$ . Separated-grains few (since the compound grains do not fall apart easily), rounded-angular, the larger ones with a small cavity. Size 2 to  $4\mu$ . The starch-grains are embedded in the protoplasm and are rather crowded.
- Entada gigalobium* DC. (*Leguminosæ*.) *Dry cotyledons*.—Compound grains rounded, oval, low cone-shaped, frequently somewhat angular or irregular, granular, consisting of 10 to over 200 components. Size about  $12\mu$ . Few separated-grains (the compound ones not falling apart easily), rounded or rounded-angular. Size 1 to  $2.5\mu$ .

## TYPE 17. GRAINS COMPOUND, HOLLOW-SPHERICAL.

Grains hollow, at first homogeneous, afterwards formed by means of radial fissures into a globular shell-like layer, consisting of 12 to over 100 components, which rarely are entirely separated from each other. These grains are best known in *Zygnemaceæ* and *Desmidiaceæ*, but also occur in other low forms of *Algæ*. They have a globular or spheroidal-shape, and at first appear as a homogeneous ring which is afterwards broken by radial fissures. They are always embedded in chloroplasts and inclose protoplasm in their hollow spaces. They rarely fall apart into rounded or angular separated-grains.

- Closterium lanceolatum* Kutz. (*Algæ*.) *Fresh seed*.—Separated-grains disk-shaped or cuneiform, angular and usually irregular, strongly compressed. Size about  $7\mu$ . They fill a conspicuous median cylindrical space in the entire length of the cell and probably arise by the breaking up of the inner spheres which are no longer present. Other specimens show the spheres but no separated-grains.
- Spirogyra jugalis* Kutz. (*Algæ*.) *Fresh seed*.—Compound grains consisting of 14 to about 40 components. Size about  $11\mu$ .
- Spirogyra orthospira* var. *spiralis* Nag. (*Algæ*.) *Fresh seed*.—Compound grains consisting of 12 to about 30 components. Size about  $10\mu$ .
- Zygnema cruciatum* Ag. (*Algæ*.) *Fresh seed*.—Compound grains consisting of 40 to over 100 components. Size about  $17\mu$ .
- Mougeotia gracilis* Kutz. (*Sterculaceæ*.) *Fresh seed*.—Compound grains consisting of 16 to about 50 components. Size about  $12\mu$ .



## CHAPTER VI.

### METHODS USED IN THE STUDY OF STARCHES IN THIS RESEARCH.

From the data of the preceding chapters it is obvious that quite a number of very different types of methods and very different substances may be used to demonstrate not only the general properties of starch, but also to differentiate starches from different sources, different grains of the same kind of starch, and different parts of the same grain. Various conditions, such as the limitations of actual needs, of time and assistance, of the number of pages that could with reason be devoted to the report, of expense, etc., have of necessity restricted the number of methods and reagents that could possibly be used to advantage in a field of investigation which involved the study of over 300 starches. Hence, it was necessary to select only such as gave promise of positive results and which differ in certain essential respects; hence, optical, physico-chemical, chemical, and photographic methods were chosen, and also such reagents as upon chemical grounds might be assumed to act differently.

There seems to be no doubt that starches of different origin can to some extent be differentiated from one another by careful analyses of the various proximate constituents, both by the inorganic and organic constituents generally; and also by the characters of the starch-substance, which undoubtedly is not a uniform body. Such analytic work was out of question because of the enormous amount of time involved and of the necessarily very limited quantities of starch that could be expected from nearly all of our sources of supply, unless at very heavy and unreasonable expenditure. Many differences peculiar to genera or species could undoubtedly be brought out by such ordinary analytic methods. Nor did color reactions apart from those with iodine and certain aniline dyes, nor the physical and physico-chemical properties of starch-pastes and the pseudo-solutions and true solutions, nor the refractive index, seem to offer opportunities for differential study that were equal to those suggested by other procedures. Nor did digestion experiments seem at all promising. The exceedingly contradictory data bearing on the digestibility of *raw* starches led the author to perform a number of experiments with different starches under aseptic conditions, but with negative results, as already stated. Likewise with boiled starches, the many conflicting records of differences in the digestibility of boiled starches of different kinds have been clearly shown by Ford, Ford and Guthrie, and by the present research, to be owing to errors attending the investigations; and that, moreover, as shown in the preceding chapter, so far as the degree of digestibility is concerned, all starches from whatever source, if of equal degree of purity, may be regarded as being practically absolutely identical. Although our knowledge of the exact intermediate products of saccharification is yet in an exceedingly unsatisfactory state, the indications are that by the actions of different decomposing agents the corresponding products by these various agents may differ more or less, and also that even with a given enzyme or acid under given conditions they will be found, with better methods of preparation, to be different in starches from various sources. At present, however, such variations as have been observed in both intermediate and final products of the decomposition of starches have been traceable almost invariably to the decomposing agent, and but rarely to the particular form of starch. The fact that the decomposition products from different parts of the same grain differ is significant not only as to the existence of stereoisomeric forms, but also of corresponding peculiarities in the decomposition products.

Our methods of differentiating stereoisomers are almost entirely quantitative, that is, by differences in density, solubility, color, temperature of gelatinization, melting-point, degree of decomposability, digestibility, precipitability under given conditions, color reactions, intensity and rapidity of staining with aniline dyes, etc. By physical methods they may be distinguished by their intensity and direction of rotation of the ray of polarized light, by the peculiarities of the interference figure in polarized light, by the form of crystals, etc. Sometimes seemingly qualitative differences in the reactions may appear which may be more or less illusory, as, for instance, in the behavior of different parts of the starch-grain to different aniline dyes. This is well illustrated in experiments in which two dyes are used that have different affinities for different parts of the grain, as in the experiments of Denniston (page 56). Thus, when grains are exposed to a solution of gentian violet for 5 minutes and then treated with orange G for different periods varying from 1 minute to 3 hours, it was found that with exposure to the orange G for 1 minute the peripheral layer is stained a pale violet and the inner layers a dark violet. With exposure to the orange G for 5 minutes a peripheral orange layer is plainly differentiated which extends entirely around the violet inner portion of the grain. With exposure to the orange G for 60 to 100 minutes the layers inside still show a pale violet and the outer layer orange. This differentiation might be taken to mean a specific qualitative difference in the reactions of the inner and outer parts in the sense that only the outer part reacts to orange G; but if exposure to the orange G be longer the inner part also becomes orange except a few layers midway between the hilum and the distal end of the grain, which doubtless by longer exposure would also become orange. The inner part of the grain has a greater affinity for the gentian violet than for the orange, while the reverse is the case with the outer parts, the presence of the violet interfering for a time with the combination with the orange G; but with an increase in the orange G the inner part also becomes orange, showing therefore merely a difference in the intensity of the reactions of these two fundamental parts of the grain. Therefore, in attempts to differentiate stereoisomers by means of physico-chemical and chemical means we are to expect in the present state of our knowledge differences in degree rather than in quality, and that differences that many assumed to be qualitative may be found to be merely quantitative if properly investigated.

By processes of exclusion and selection a number of methods were finally adopted which for various reasons seemed especially desirable in view of the main objects and the conditions attending the prosecution of the research. Undoubtedly some of these methods might be replaced by others that are better, but an investigation of the relative values of different methods would have of itself proved a laborious inquiry. The following methods were employed:

#### HISTOLOGICAL METHOD.

As has been pointed out in preceding chapters, this method has been found to be of signal usefulness; and, in fact, up to very recent years it has been the sole reliance in attempts to determine the kind of starch. It was, however, perfectly obvious at the very inception of this research, and rendered clear as far back as the investigations of C. Nägeli in 1858, that this method, unless associated with others, could not be depended upon, and that it was liable to be absolutely misleading. Moreover, differences in form may not in the least imply differences in the starch-substance, as has been made evident in early chapters. Magnification ranging from 85 to 400, sometimes higher, was used, according to the size of the grains and incidental conditions. A sufficient amount of dried starch was placed on a slide and mounted in a very dilute Lugol's solution, care being taken not to add a larger quantity of iodine than is sufficient to accentuate the lamellæ. Since starches of different sources show wide differences in the intensity with which they become colored with iodine it was found convenient to have on hand a number of solutions ranging from 1 to 2 per cent down. By the aid of such ordinary microscopic technique there were

recorded the form and size of the grain; the position and form of the hilum, or the assumed point of origin of growth or center of organic structure; the form, number, and other characteristics of the lamellæ; the characteristics pertaining to the form of the grains, whether singly or in doublets, triplets, or aggregates, etc. Many of the minuter features of the grains that were observed will not for obvious reasons be seen in the photographic reproductions. In describing the grains the terms "proximal end" and "distal end" have been adopted, the former being the end nearer which the hilum is located. The "longitudinal axis" corresponds with an imaginary line, extending from the proximal end through the hilum to the distal end. In different starches and in different grains of the same kind of starch this may be the long or the short axis. The measurements of eccentricity of the hilum have reference to the distance of the hilum from the proximal end of the longitudinal axis.

#### IODINE REACTIONS.

The use of iodine not only served to bring out certain histological peculiarities, but also valuable data in the differentiation of different kinds of starch. The typical or ordinarily observed reaction of starch with iodine is an indigo-blue, but if an excess of iodine be avoided the reaction of the grains will be found to vary usually from a blue to a reddish-violet, including within these extremes all shades of violet from a purple to a reddish-violet according to the kind of starch. In fact, with few exceptions, starches are colored in the presence of minute quantities of iodine some shade of violet, varying with the kind of starch. Certain starch-grains yield with any quantity of iodine a red reaction. In studying this reaction we employed 0.125 and 0.25 per cent Lugol's solution. The starch was placed on a slide, and one or two or more drops of the iodine solution added, the whole covered with a cover-slip.

#### ACTIONS WITH ANILINE DYES.

A number of these agents have been found by various investigators to be of value in the differentiation of starches from different sources, of different grains of the same kind of starch, and of different parts of individual grains. Quite a number of aniline dyes have been used, and some experimenters have employed double or triple stains. The task of selecting from these, not to consider the very large number of agents of this kind that are available for such work, would of itself have been a rather large undertaking. There is also no doubt that the use of double or triple stains would bring out, at times at least, many points of much histological importance, but this would have involved the carrying out of the histological examinations in such detail as to be prohibitive in a research of this character. Safranin and gentian violet were selected, not because they are probably the best of these stains for differential purposes, but because they have been found very useful in starch examinations and as they yield single color reactions.

Aniline colors in solution, especially when in weak solution and exposed to light, are notably unstable, and in order to secure strictly comparable results a quantity of a relatively strong standard solution was prepared and kept in the dark, tightly corked. Each day a certain amount of a diluted solution, containing 0.05 per cent of aniline, was prepared from the standard solution, 5 c.c. of this was placed in a test-tube containing a small amount of starch, the preparation agitated, and a drop or two of mixture withdrawn from time to time, placed on a slide and covered, and the grains examined, first as to the time of the beginning of staining, and second as to the intensity and uniformity of coloration. In the color determinations the microscope is used with low power and open diaphragm.

#### REACTIONS IN POLARIZED LIGHT, WITHOUT AND WITH SELENITE.

Starches have been found to exhibit not only marked differences in the degrees with which they rotate polarized light, but also differences in the characteristics of the "interference figure," or "cross," as it is generally termed. Moreover, this method can be used

to determine the beginning of disorganization of the grain by heat, since with the very onset of gelatinization the polariscopical properties begin to disappear. In these examinations (excepting in determining the temperature of gelatinization) the grains were mounted in Canada balsam. The general characteristics, distinctness, shape, regularity, and position of the interference figure, and also the approximate degree of anisotropy or intensity of polarization were readily studied. By the aid of selenite it was determined whether the optical properties were negative or positive, and also the size, shape, and regularity of the quadrants, as well as the intensity and pureness of the blue and yellow colors. In spherical grains with centrally located hila, the two parts of the "cross" intersect at the hilum, or mathematical center, of the grain, so that the term quadrant has a proper application; but in the case of grains having eccentric hila the position of the point of intersection of the two parts of the cross, together with their curvatures, may destroy every semblance of quadrants according to the conventional definition of this word. This term has therefore been used in a very broad sense throughout our investigation to indicate the four parts of the grain that are defined by the two parts of the cross, in preference to the great multiplicity of terms that would be required to define these parts if great accuracy were attempted. Likewise, for convenience we have referred to the "lines" of the interference figure in preference to the "arms" of the cross.

All starches are "optically negative," hence no special references have been made in the text in this particular.

#### TEMPERATURE OF GELATINIZATION.

While the records of various investigators indicate that there are more or less marked differences in the temperatures of gelatinization of different kinds of starches, and even in case of different grains of the same starch, the figures applying to the same kind of starch are generally so at variance that not much value is to be attached to them. The sources of fallacy in such observations, unless the determinations are made with the greatest precautions, are well known to every biochemist. We therefore carried out this work with especial care. A long quadrangular water-bath was used, holding about 4 liters of water; one end was placed over the gas flame, and in the other end was inserted a thermometer which was calibrated in tenths centigrade, but which could readily be read in hundredths. A small quantity of starch with 10 c.c. of water was placed in a test-tube, into which was inserted through a perforated cork a thermometer similar to the one in the water-bath, and the test-tube immersed in a suspended wire basket in the part of the water-bath farthest from the flame. The temperature of the water was raised very slowly, and the water occasionally stirred, so that at no time did the two thermometers differ more than about 2°. As the temperature increased, specimens of the starch were examined at intervals, the tube being shaken, and a specimen being obtained by inserting the end of the pipette to the bottom of the tube, a fresh pipette being used to remove each specimen. Each specimen was placed on a slide, upon which was recorded both temperatures, and the slide was examined in the polarizing microscope. The temperature at which there is a disappearance of anisotropy of practically all of the grains was recorded as the temperature of the tube. The lower temperature recorded on the slide was that of the thermometer in the test-tube, and the higher temperature that of the water-bath. The actual temperature of gelatinization lies somewhere between the two, and for convenience, especially for purposes of comparison, the mean of the two was for obvious means taken as the "temperature of gelatinization." In the records in Part II all three temperatures are given in accordance with the foregoing.

#### ACTIONS OF SWELLING REAGENTS.

Quite a number of swelling or gelatinizing reagents, of very diverse chemical composition and exhibiting more or less individuality of action, have been used by various

experimenters in studies of the structural peculiarities of starch-grains or in the differentiation of different kinds of starch or for other incidental purposes. This method of differentiating starches seemed so promising that five such reagents were selected. For obvious reasons choice was made of those which differ widely in chemical composition and which yield sufficiently prompt and characteristic results. Those selected include chloral hydrate-iodine, pyrogallie acid, chromic acid, ferric chloride, and Purdy's solution.

The chloral hydrate-iodine solution was prepared by saturating a saturated solution of chloral hydrate with iodine. This solution, sooner or later, not only causes swelling and ultimate partial dissolution of the grains, but also, owing to the presence of iodine, yields important accompanying color reactions; and it is on the whole to be regarded as a very valuable reagent.

Chromic acid was used in the form of a 25 per cent solution, and it is the only one of the five reagents that causes within the periods of observation a complete disintegration of the grains. It gives rise to gas bubbles during the decomposition processes.

The pyrogallie acid solution was prepared by making a saturated solution and diluting this with 3 parts of water, adding oxalie acid in the proportion of 4 per cent to prevent oxidation.

The ferric chloride solution consisted of equal parts of a saturated solution and water. Purdy's solution was made of equal volumes of the standard solution and water.

The last reagent was usually found to be the least active of the five, and it is, so far as the effects on the grains are concerned, probably essentially an aqueous solution of potassium hydroxide, and therefore likely possesses no advantages, except perhaps in keeping qualities, over the simple aqueous solution. Oxygen or exposure to the air favors the actions of pyrogallie acid, but hinders those of chloral hydrate and ferric chloride. In the former case, the grains near the edge, or on the outside, of the cover-slip are decidedly more affected than those within, while with the latter the opposite is true.

There are some forms of commercial chloral hydrate that have very little action, which may be due to under-hydration or over-hydration. The crystals put up by Schering were used throughout this investigation. It is important that fresh solutions of the reagents be prepared at short intervals, as all tend to deteriorate, and it is well to let them stand over night before using (see page 311).

In using these reagents a small amount of starch is placed on a slide, several drops of the reagent added, a cover glass put on, and the progress of events examined under the microscope. In using a given reagent with a given kind of starch, it was found that there occurred a certain amount of variation in the effects from time to time, which are probably to be attributed chiefly to variations in temperature, so that these studies were made as far as possible under constant temperature conditions. The variations were unimportant. These agents give rise to gelatinization and swelling of the grain, and cause the existence of the outer and inner parts of the grains to become very conspicuous—the outer part becoming sae-like and inclosing a less dense or semifluid substance.

#### PREPARATION OF THE STARCHES.

The starches used in this research were for the most part prepared from the underground portions of the plants, that is, from tubers, rhizomes, bulbs, corms, etc. In a few instances the specimens were obtained from the stalk, as from *Dieffenbachia*; occasionally from both the stalk and fruit, as from *Musa*; occasionally from pseudo-tubers, as from *Orchis*; or from the fruit, as from *Castanea* and *Quercus*; or from the side-shoots, as from *Cycas*; or from the seeds, as in *Graminaceæ*. Unless otherwise stated it will be understood that the starch is from underground parts.

The starches were prepared by comminuting the specimen by the aid of an ordinary kitchen grater or nutmeg grater, or of very coarse sand-paper, or of a small drug-mill,

adopting one or the other in accordance with the quantity and physical characters of the specimen. Sufficient water was added and the mixture thoroughly stirred, then strained through several thicknesses of cheesecloth, and then centrifugalized. The supernatant fluid was then removed and fresh water added in its place, and the starch and other matters that had settled to the bottom thoroughly mixed with the water and again centrifugalized. This operation was repeated; or followed by repeated decantation when there was a tendency for much foreign matter to be thrown down with the starch during centrifugalization. By this simple process we were almost invariably able to prepare starches that were practically free from gross impurities, that is, as free as the nature of our investigations demanded. To have attempted further purification to the extent of practical demineralization would doubtless have proven of disadvantage without proportionate gain.

#### PHOTOMICROGRAPHIC RECORDS.

Verbal descriptions of the histological characteristics of starch-grains fail to convey adequate conceptions, and the same applies to the differences shown by different starches in their polariscopic properties. The notes included in the text have therefore been accompanied by photomicrographs, both of the grains lightly colored with iodine as seen in the microscope and also in water as seen in the polarizing microscope with crossed Nicol prisms. Absolute reliance can not be placed upon the photomicrographs in showing the differences in the degree of anisotropy of different starches, because the variations in the intensity of the light and in the characters of the negatives or prints may intensify or lessen the effect. The photographs showing the polarizing effects must therefore be checked by comparison with the descriptions in the text, and their usefulness is to be found chiefly in showing in each picture the characters of the interference figure, or cross, and differences in the intensity of polarization of different grains and different aspects of the same grains of that particular specimen. In making these photographs we used an ordinary Bausch and Lomb microscope with a one-fourth objective and a 2-inch eye-piece, which gave us a magnification on the field of projection of 300 diameters. Several photographic records are included which show the effects of reagents.

Details in reference to the values and other features of the methods employed in this research, and in connection with matters associated therewith, will be found in Chapter VII and in the Prefatory Notes in Part II.

#### CURVES OF THE REACTION-INTENSITIES OF DIFFERENT STARCHES.

It is exceedingly difficult to associate the different reaction-intensities of any given starch in such a way as to get a mental picture of their values, and such an association becomes much more difficult if one attempts to duplicate pictures in comparing two or more starches. Hence, the reaction-intensities in relation to each starch and to each genus have been set forth graphically in the forms of curves which not only give at once a strikingly clear presentation of the quantitative reaction peculiarities, but also permit of the readiest and most satisfactory comparisons. In the construction of the charts (see charts in Part II) the abscissas have been used to express the degree of polarization (P), the intensity of the iodine reaction (I), the intensity of the gentian violet reaction (G V), the intensity of the safranin reaction (S), the temperature of gelatinization (T), the time-reaction of chloral hydrate-iodine (CHI), the time-reaction of chromic acid (CA), the time-reaction of pyrogallie acid (PA), the time-reaction of ferric chloride (FC), and the time-reaction of Purdy's solution (PS). The letter or letters as above given in parentheses each lie at the head of a special column or ordinate, and indicate the agent, while those of the abscissas give the values of the reactions. The letters of the column under P indicate, respectively, very high, high, fair, low, and very low; and under I, G V, and S, very dark, dark, fair, light, and very light. The temperatures are in the centigrade scale; and the time-reactions with the

chemical reagents are expressed in minutes. A different time column had to be given to chromic acid (CA) because of its relatively much greater intensity of action, the abscissas having with this reagent five times the values of those of the other reagents. The abscissas therefore designate quantitative relationships, while each of the ordinates indicates a specific reaction. Having the data, by marking upon the chart on the proper ordinates the several values of the reactions of the different reagents, and then drawing lines in regular order from one mark to another, the curve of reaction-intensities was obtained. Rarely have the reaction-curves been carried beyond the 60-minute line, because very few of the time-reactions were observed beyond this period. In a number of cases at the end of the section on a family, as after Liliaceæ and Amaryllidaceæ, a group of curves is given which may be taken tentatively as representing generic curves, that is, each shows the composite reaction-curve of the starches of the genus, or the curve of a single representative of the genus.

#### CHARTS OF COMPARATIVE REACTION-INTENSITIES OF STARCHES WITH EACH AGENT.

Additional charts constructed on an entirely different plan from the foregoing will be found in Chapter VII, pages 314 to 333. Each chart shows *comparatively* the reaction-intensities of *all* of the starches studied by means of a *given* agent; hence, there are separate charts for polarization, iodine, gentian violet, safranin, temperature, chloral hydrate-iodine, chromic acid, pyrogallie acid, ferric chloride and Purdy's solution reactions. These charts have been plotted out on so simple a plan that no explanation is required in order that they may be understood.

#### NOTE IN REGARD TO PART II.

As stated in the Introduction, in the final arrangement of this report it was found necessary to carry the voluminous detailed accounts of the laboratory investigations, which logically belong here, into a separate volume.

## CHAPTER VII.

### DIFFERENTIATION AND SPECIFICITY OF STARCHES IN RELATION TO GENERA, SPECIES, ETC., AS DEMONSTRATED BY THE METHODS EMPLOYED IN THIS RESEARCH.

#### STARCH-SUBSTANCE A NON-UNIT SUBSTANCE.

One of the most important problems underlying the differentiation of starches from different sources is whether or not starch is a uniform substance; and, if not, whether important and diagnostic variations occur under various conditions of plant life; and, if so, the influences of these conditions. Frequent reference has been made to facts which indicate, or show, not only that starch is not a unit body, but also that it exists in a large number of forms, and that the starch of any given plant may be regarded as consisting of a mixture of stereoisomers, which differ not only in their intramolecular structure, but also in their intermolecular arrangements, and hence in their properties. Moreover, it has been shown by the literature quoted that the starch from any given plant is a heterogeneous collection of grains which vary in microscopical and molecular properties, and that the individual grains, except perhaps the embryonic, spherical, and seemingly amorphous grains, are likewise of non-uniform composition. In this research confirmatory evidence of the foregoing statement is abundantly supplied, especially in the reactions, as, for instance: In *Pisum*, in the starches from Eugenie and Thomas Laxton peas, in which the grains of the rosette type are gelatinized within 5 minutes by chloral hydrate-iodine, while those of the bean type require an hour or more; in *Æsculus*, *Brodiaea*, *Convallaria*, *Sprekelia*, and *Watsonia*, in which the small grains react more vigorously with chemical reagents than the large grains; in *Fritillaria* and *Hæmanthus*, in which the small grains react less vigorously than the large grains; in *Hyacinthus*, in which a reversibility of sensitivity is noted in relation to different agents, the small grains with regular outline being the most resistant of the three sizes to chloral hydrate-iodine, the medium-size grains with irregular outline being the most resistive to Purdy's solution, and the small grains the least reactive with iodine; in *Lachenalia*, in which the small grains are the least reactive with chloral hydrate-iodine, but the most reactive with pyrogallie acid, ferric chloride, and Purdy's solution; in *Convallaria*, in which the small grains are gelatinized at 61° and the large grains at 71.75°. Such instances could be considerably multiplied. In fact, every starch affords such illustrations.

The structure, both histological and molecular, of individual grains of a given starch is not uniform, and this applies not only to compound grains, but also to simple grains, excepting possibly very young grains, before, for instance, the formation of a hilum and the appearance of lamellation. The differences in the behavior of the inner and outer parts, or, according to general ideas, of the so-called amylose and cellulose, can be demonstrated with the greatest ease, and in ways to show that these parts represent different forms of starch-substance. As already repeatedly pointed out, the individualities of these two parts are markedly shown in their different behavior towards various reagents. As a rule, the outer part is the more resistive, but toward some reagents it is the less resistive. In relation to moist heat, when the grains are boiled in water the outer part is always the last to disappear, sometimes resisting boiling for many minutes, appearing in suspension in the form of empty capsules from which the less resistive inner starch has escaped in semi-liquid form and passed into the so-called solution.

That the capsular or outer part of the normal grain is the last part to be disorganized by heat can readily be proved by observation of grains undergoing gelatinization on the stage of the polarizing microscope between crossed Nicol prisms. Sometimes even a markedly higher temperature is required to gelatinize the outer than the inner part, as in *Iris*, in which

the proximal end and sides persist after the distal end and inner part have broken down. In the behavior of these two parts with iodine distinct differences are noted in both the intensity and the color of the reactions. After gelatinization by boiling, and the addition of iodine, if the grains are intact, they are usually colored a light to a deep indigo-blue or a bluish-violet. By further heating the capsules are ruptured and the semi-fluid contents, which are colored an indigo-blue, partially or completely escape. Unless a decided excess of iodine has been added, the capsules will be found to be colorless, sometimes containing more or less of the blue-reacting starch, usually at the proximal end of the grain. By the further addition of iodine the affinity of the blue-reacting starch for iodine is practically satisfied, so that now the capsules react and become colored, but never blue, generally some tone of blue-red, ranging from a bluish-violet to a reddish-violet, heliotrope, and old-rose, or a wine-color or rarely a yellow-brown or brownish-red. Similar peculiarities are recorded in the chloral hydrate-iodine reactions, in which of course the color reaction depends on the presence of iodine. In the least resistant starch to the reagent the reaction is blue, and as resistance is more and more marked in accordance with molecular peculiarities of the different forms of starch, there is noted a corresponding and characteristic difference in the color reaction, the most resistant yielding at first an old-rose or an ashes-of-rose, deepening into a wine-color or a reddish-brown coloration.

At times in a given grain the progress of the reaction, together with the different degrees of resistance of the component starch-forms, can be followed and determined by the differences and the changes in coloration, as, for instance, in the chloral hydrate-iodine reaction with *Mucuna*, *Lens*, *Lathyrus*, *Pisum*, and many other starches. Gelatinized starch is more sensitive to iodine than raw starch, and as far as known all boiled starches, and starches gelatinized by chemical reagents, if the reagents do not interfere with the iodine reactions, whatever their color reactions with iodine in a raw state, give a blue reaction with iodine, with the single exception of grains like those of *Alocasia putzeyssi*, which normally give a wine-color or other color of reaction which is at present ordinarily accepted as being peculiar to erythrodextrin and glycogen, but not to starch.

It would seem from this, with the exception noted, that gelatinization, by whatever means, by breaking up the intermolecular structure dissipates partially or completely certain of the properties which serve to distinguish one form of starch from another. The only part of the grain that is not thus reduced is the capsule, which represents but a small fraction of the original weight of the grain. If these capsules are collected, repeatedly washed by centrifugalization, boiled until completely disintegrated, and then tested with iodine, the color-reaction will be found to correspond with that recorded in the intact capsules. There is thus shown to be some marked molecular difference between the outer and inner parts of the grains which is very persistent. It is doubtless in this intermolecular disorganization of the starch-grain that is to be found the explanation of the identity in the degree of digestibility of different starches, whatever the source, as shown on page 190.

The so-called solution of starch is, as has been clearly pointed out in previous chapters, a heterogeneous mixture; and, moreover, it is evident that such solutions prepared from different starches are heterogeneous mixtures which differ from one another. It therefore follows that differences in starches are owing not merely to peculiarities of intermolecular arrangement, but also to peculiarities of intramolecular structure or molecular configuration.

A striking and significant differentiation of the inner and outer parts of the starch-grain is manifested in the reactions with the chemical reagents. Thus, in the reactions with chloral hydrate-iodine and ferric chloride the outer part of the grain in almost every starch is first to react and to be gelatinized, while with chromic acid, pyrogallie acid, and Purdy's solution the inner part is usually the more sensitive. Again, different parts of the surface of the grains and different internal parts may exhibit more or less marked differences in sensitivity. When secondary lamellæ exist, they are usually affected before the

primary lamellæ; grains with regular outline are usually more resistant than those with irregular outline, in the latter the reaction beginning at the protuberances or angles; the proximal end of the grains of most starches is more resistant, irrespective of the reagent, than the distal end, but in other starches the reverse; and in some grains more or less marked differences are observed in the behavior of different lamellæ or parts of lamellæ, one of two or more lamellæ, or some parts of lamellæ, being resistive for a relatively long time after the breaking down of other parts. Generally these lamellæ are centrally located, or they may be noted as regularly arranged concentric bands or groups with intermediate areas of gelatinized starch; and sometimes there will be a few resistant granules which may not be gelatinized after a relatively exceedingly prolonged action. In this connection must be considered the literature quoted in Chapter III, which indicates the existence of different kinds of starch in the starch-grains, and also of the formation of reversion products. The latter may have important significance in connection with the causes of the marked differences in different grains and parts of grains of a given starch.

It has long been recognized that the different lamellæ of the mature starch-grain are of less and less density from without inward. These peculiar variations are, it seems clear, not owing to an increase in the density of each additional lamella as it is deposited, but to a gradual transition of the molecular states of the inner or older lamellæ to a less dense condition. Such a change is explicable in the light of the ready transmutability of one stereoisomeric form into another owing to slight differences in attendant conditions, as has been pointed out in Chapter I, page 9. The mere separation of the previously deposited starch from direct contact with the plastid by the later deposited starch, age, and such other incidental conditions are of themselves doubtless sufficient to satisfactorily account for this transmutation. Likewise stereochemic differences in other parts, such as in primary and secondary lamellæ, protuberances, etc., in relation to other parts of the grains may be explained in the same way.

Differences in the forms and in the reactions of starch-grains obtained from different parts of a given plant, or parts of a given organ, have been recorded by a number of observers, and referred to in previous chapters. In the present investigation such differences have been shown incidentally in several instances, as in the case of the starches of the pith and cortex of the stalk of *Dieffenbachia*, and in the stalk and green fruit of *Musa*. Moreover, much data of this character have been obtained that are not included in the present memoir, but which will be published at some subsequent date. The differences in the reactions in these cases, while as a rule not marked, are strongly suggestive of differences in the starch-substances formed in the several starch-producing parts. That important and characteristic differences will be found by studies of the starches from different parts of the same plant, and from different parts of a given starch-forming organ, seems to be conclusive. That histological differences of this kind exist will be seen by consulting especially the data included in Chapter V, and it may be put down as an axiom that where different histological types exist stereochemical differences exist. In some instances secondary lamellation is of so peculiar a character as to be almost if not absolutely specific to the plant or plant-part; as, for instance, in the striking peculiarities exhibited by the starches of *Dieffenbachia* (plates 17, 18, and 19), *Gesneria* (plate 100), *Phaius* (plate 102).

Owing to the fact that starch is not a uniform substance, and as it appears in different plants and in different parts of the same plant in the form of a heterogeneous mixture which is likely somewhat variable in accordance with changes in internal and external conditions, it follows as a corollary that the characteristics of a given form of starch is the sum of the peculiarities of the individual components. Hence it will be found that while in every form of starch the different grains or even parts of the individual grains will show more or less marked differences in their forms and reactions, the sum of these peculiarities will be as characteristic of the form of starch as are similar sums characteristic of a species, genus, etc.

## METHODS EMPLOYED AND THEIR VALUES IN THE DIFFERENTIATION OF STARCHES.

Inasmuch as this investigation was undertaken to determine primarily the molecular and not the microscopical peculiarities of different starches; and since for reasons already set forth differences in histological peculiarities may of themselves be physical or physico-mechanical rather than molecular, although one may be most intimately associated with the other; and since differences in the various forms of starch can be brought out with certainty only by means that determine differences in intramolecular or intermolecular arrangement, such as the physical, physico-chemical, and chemical methods used in this investigation, the direction of the research has been concentrated along the latter lines.

## THE HISTOLOGICAL METHOD.

That the histological method has a certain very marked value is evident in the fact shown particularly by the investigations of the elder Nägeli of the definite relationships that frequently exist between the microscopical characters of the grains and species, genus, and family (p. 60). But often starches may be much alike, or even seemingly identical, yet be derived from unrelated species, genera, or families; and on the other hand the starches of different genera of the same family may be so entirely different as to suggest an absolute absence of generic relationships. Hence if one were to rely only on histological peculiarities he might be led far astray. Thus, for instance, the starches of the members of *Iridaceæ* often show such striking dissimilarities as to imply an absolute absence of generic relationship. On the other hand, the starches of *Arum*, *Dracunculus*, *Richardia*, *Colchieum*, *Glad-iolus*, *Freesia*, *Babiana*, *Trianosperma*, and *Cycas* show such similarities as to suggest very close relationship, notwithstanding that they belong not only to entirely different *families* but also even to different *orders* and *classes*. The families here represented are *Aroideæ*, *Colchi-caceæ*, *Iridaceæ*, *Cucurbitaceæ*, and *Cycadaceæ*; the orders are *Arales*, *Liliales*, *Campanuales* and *Cycadales*; the classes are *Monocotyledones*, *Dicotyledones*, and *Gymnosperma*. Were it not for incidental conditions, as for instance, alterations in food-supply and temperature and alterations in light and darkness, the presence in the cell of crystals and the contact of grains with each other which mechanically modify the forms of the starch-grains, the varying mechanical influences which affect the physical relations of the plastid to the growing grain, and numbers of conditions which may purely mechanically affect the form of the starch-grain during its development, the starch-grain would be conceived to be developed along intermolecular lines as definite as in the formation of spherocrystals generally. In other words, as Meyer puts it, every plastid has a "biology of its own," which, applied to the foregoing, means that did not mere incidental conditions, through purely mechanical or artificial ways, influence the form of the starch-grain, the form of the grain would be absolutely specific to the character of the mechanism that formed it; that is, the form of the grain would be as specific in relation to the plastid as are the forms of the teeth in relation to the osteoblasts which deposit the osseous tissue.

That the starch-grains are developed upon definite plans of intermolecular growth would seem to follow from the laws governing the growth of crystals generally, and since stereoisomeric forms of other substances are built up upon specific intermolecular plans by which one may be distinguished from another, and that the histological forms of the crystals may be modified by varying conditions attending crystallization, it follows, as a corollary, that different stereoisomeric forms of starch are likewise different in their intermolecular arrangements and that these peculiarities may be obscured by conditions which interfere with the development of the true histological form. Any given normal rectilinear crystal owes its form to the intermolecular arrangement of its constituent particles, yet the same substance with the same inherent intermolecular arrangement may appear in histological forms having little or absolutely no likeness to the normal crystal,

because, during crystalline growth, of pressure or other conditions which may give rise to morphological departures in various directions.

If, however, it should be found that definite relationships exist between the histological, polariscopical, and chemical reactions, the histological data would prove of indirect and coöperative value in determining the particular form or forms of starch-substances present, and in indicating inherent peculiarities of the protoplasmic processes concerned in starch-formation. Throughout this investigation only sufficient attention has been given to the gross histological characters of the different forms of starch to permit, if possible, the recognition of each starch; to permit of gross comparisons; and to show the likenesses of the starches of the members of a genus, and of closely related genera, and of the likenesses and unlikenesses of the various genera assigned to a given family, etc. As the research progressed it was found that closer examination often brought out features of importance, and it is to be regretted that throughout the work the microscopical studies were not carried out more in detail, especially with the aid of aniline dyes and other microscopical reagents. (See Prefatory Notes in Part II.)

#### THE POLARIZATION METHOD.

The behavior of starch-grains in polarized light was discovered by Biot in 1844, or likely some years earlier, since he and Persoz studied the polariscopical properties of dextrin in 1833. Starch-grains in whole or in fragments are doubly refractive or anisotropic, and hence rotate or polarize the plane of polarized light from one plane to another. The extent of this rotation is indicated, other things being equal, by the degree of illumination of the starch-grain when viewed between crossed Nicol prisms. The brightness, or "degree of polarization," or intensity of reaction, varies with the coefficient of the anisotropic substance and directly as the thickness of the layer of substance through which the light passes. Hence, when the grains are viewed on edge or end, usually the thicker diameter, they accordingly appear to have a higher degree of polarization than when viewed on the flat or through the narrower diameter. When grains overlap, the overlapped parts are brighter than the other parts; and if grains vary in thickness in any aspect, the thicker the part the higher usually the illumination. In all starches the grains vary in size; hence, other things being equal, the larger the grain (or, in other words, the thicker the grain) the higher the polarization. Hence in rating the relative degrees of polarization of different starches, the average effect is taken as the index for that starch.

The property of anisotropy or optical activity depends upon internal molecular structure or upon intermolecular arrangement, or both. In the case of substances which are anisotropic in solution as well as in solid form, the property is primarily inherent in the molecules and due to peculiarities of the internal structure of the molecules, to which may be added secondarily anisotropy due to external molecular arrangement. This is the case with such substances as sugar, which, whether in solution or in crystalline form, rotate the ray of polarized light. But if the substance is anisotropic only when in crystalline form as in sodium chlorate, the property is dependent upon the arrangements of the molecules in relation to one another; hence, if the structural arrangement be destroyed by solution, gelatinization, etc., they lose their property of anisotropy and are said to be optically inactive.

If the starch-grains, or fragments of grains, be placed on the stage of the polarizing microscope between crossed Nicol prisms, and gradually heated in water, or subjected to the action of a solvent or gelatinizing chemical reagent, it will be observed that the moment gelatinization begins at any part of the grain, the part of the grain that is affected loses, apparently entirely, its property of polarization because the intermolecular arrangement is destroyed. This seeming complete loss of optical activity has been utilized in this research in the determinations of the "temperature of gelatinization." But the optical activity of starch is not due solely to intermolecular arrangement of the particles in solid

form, as is evident in the fact that even a *true* solution of starch is active although to a relatively very minor degree.

It is questionable if much value is generally to be attached to variations in the degree of polarization of different starches in the recognition of differentiations of different forms of starch, because such differences are, as a rule, very closely related to the thickness of the grains and variations in the form; and in the absence of exact measurements may be entirely misleading. By means of the polarizing microscope the "interference figure" or "cross," and the color-effects of starch in the presence of the selenite plate, may be studied, and also with the latter certain structural peculiarities; but these means are useful only with reference to the grosser characteristics of starches, rather than with the recognition of different kinds of starch-substances, and on the whole, while having a certain individuality and distinct usefulness, seem to have a distinctly less value than other available methods.

#### THE IODINE METHOD.

Since the discovery of the starch-iodine reaction by Stromeyer this test has been the recognized standard without interruption to the present time; but certain popular misconceptions have become attached to the reaction, as for instance, that starch, whether in the form of grains or in solution, always gives a blue reaction with iodine, and that the blue reaction is diagnostic of starch. There are forms of cellulose, glycogen, and plant mucus that give a blue reaction with iodine, and normal starch-grains may give color reactions varying from an intense indigo-blue to purple, bluish-violet, violet, red-violet, old-rose, heliotrope, wine-color, etc. Similar differences in reaction may under appropriate conditions be observed in the starch-grains *in situ*.

Differences of this kind in relation to different starches have been beautifully demonstrated by Dubosc (page 171) by means of iodine vapor, corn starch becoming colored a blackish-violet, wheat starch a bluish-gray, sago starch a brownish-gray, and potato starch a yellowish-gray, the amount of yellow in the latter being proportional to the amount of foreign matter. With weak solutions of iodine, such as were used in this research, the grains almost invariably color a bluish-violet, violet, or reddish-violet; and when grains are subjected to moist heat to bring about complete gelatinization and the rupture of the capsules without their disintegration, the starch which has passed through the capsules and passed into solution gives with rare exceptions, as noted under red-reacting starches, a blue reaction, and likewise any starch that may be retained within the capsules; but the capsules themselves always, with the exceptions noted, are colored some tone of a blue-red, or a red or brown, etc., as stated on page 303. Hence we may speak of the intracapsular starch as a blue-reacting starch, and of that constituting the capsules as a violet-, or red-, etc., reacting starch.

The starch-grains from some sources become red on the addition of iodine, which has led to the assumption that the grains consist of erythro-dextrin instead of starch; and in the case of grains which yield a violet reaction, it has been taken for granted that the red element of the blue-red color is likewise due to erythro-dextrin, or that a specific individual substance which we may term amylo-dextrin is the reacting substance. As heretofore stated, there are not sufficient reasons for the assumption of the existence of the latter, since all of the so-called amylo-dextrin reactions can be accounted for upon the basis of a modified form of starch or of contamination of starch with erythro-dextrin.

The nature of the substance of starch-grains which give a red (not a violet) reaction with iodine is yet problematical. Apart from the color reaction, such grains behave like ordinary starches when subjected to the other processes used in this research. Thus the grains of *Alocasia putzeysi* color a wine-red in the presence of iodine, and if they are boiled in water until the capsules rupture, the solution and the capsules color red, but otherwise yield typical starch reactions. Starches which give a reddish-violet reaction, such as those of *Oryza sativa*, must not be confounded with grains which, like those of *Alocasia putzeysi*, give a red reaction.

## THE ANILINE METHOD.

The use of anilines has demonstrated some differences, as in the finer histological characters of different starches, and also in different grains of the same starch and in different parts of the same grain. The experiments of Denniston (Chapter II, page 56), in which it is shown that the outer or capsular part of the grain will permit the passage of the aniline to the intracapsular part without itself becoming colored, unless an excess of stain be present, is most significant of an important difference in the composition of the two parts. Moreover, the differences shown by starches from different sources in the rapidity with which they combine with a given aniline and different anilines suggest distinct differences in the nature of the starch-substance. Whether or not we regard the union of anilines with starch as being a phenomenon of adsorption or of chemical union in the conventional sense (both are chemical), it is certainly a manifestation of variations in starch properties which can not be accounted for on the basis of varying physical or physico-mechanical conditions, because, for instance, in one form of starch the reaction will be greater with one aniline than with another, while with another starch they will be of equal degree, and with another the reverse of what was found in the first place. Were it a matter, for instance, merely of varying porosity, such differences would not be observed. On the whole, much may be expected of the use of anilines in differentiating different starch-substances, especially in the individual grain.

## THE TEMPERATURE METHOD.

The temperature of gelatinization of starch-grains and the melting-point of crystalline substances generally are terms that have the same significance, that is, they express the temperature at which the intermolecular structure of the substance is broken down. Differences in melting-point have been an important means of differentiating isomers; similarly, differences in the temperature of gelatinization have served to differentiate starches from different sources, but the value of this method has been very low; partly because of the heterogeneity of starch and partly and chiefly because of improper methods of research. Hence, very variable and contradictory records have resulted. In fact, one of the latest experimenters states that the results obtained by the ordinary method of determining gelatinization temperatures were so conflicting as not to be worthy of publication.

The absence of homogeneity of the grains constituting any given form of starch can not be overcome, but a proper method will eliminate very important sources of fallacy. In the methods heretofore used, the determinations were based upon observations with the microscope, dependence being placed upon gross changes in the size and appearance of the grain, and in the reactions with iodine, the starch with water being placed in a test-tube or beaker, and this in turn in a water-bath which is slowly heated, etc. In the present research the reactions in the polarizing microscope were depended upon.

As already stated, when raw starch is heated in water, a certain temperature is reached at which the intermolecular arrangement of the starch molecules begins to undergo disorganization, with an attendant loss of optical activity; and the progress of gelatinization can be followed clearly until the last vestige of the grain has been disorganized. Gelatinization begins in some of the grains and in parts of a given grain before it appears in others, and it is likewise earlier complete in some grains and parts of grains than in others. In these determinations, the temperature of gelatinization means the lowest temperature at which disorganization in practically all of the grains was complete. With care the range of error is, with rare exceptions, less than a degree centigrade. Occasionally a starch is found in which rare grains resist gelatinization at temperatures even much higher than the others. In such cases these exceptional grains are disregarded. In many starches the outer part is distinctly more resistant than the inner. When the degree of polarization is low, especially in the very small grains, it may be found impossible to get accurate temperatures.

## THE CHEMICAL REAGENT METHOD.

While all of the chemical agents bring about a complete intermolecular disorganization of raw starch, gelatinizing the grains and causing the loss of optical activity, the different starches and different grains of the same starch and different parts of the same grain may show more or less marked differences in their reactions, or, in other words, in the manner in which this disorganization is brought about; moreover, each reagent exhibits certain peculiarities of action which are more or less distinctive. In some starches the small grains are less resistant than the large, and in others the reverse; and some parts of the grains may react very much sooner than others, as already stated. The processes with chromic acid are in certain respects different from those of pyrogallie acid and the other chemical reagents; and those of chloral hydrate-iodine and ferric chloride differ from those of chromic acid, pyrogallie acid, and Purdy's solution, as is instanced by those of the former being conspicuous by their concentration in the outer part of the grain, and those of the latter in the inner part. With chromic acid the grain is broken down into a gelatinous capsule and a semi-liquid contents, the capsule rupturing, the contents escaping and passing into solution, and then the capsule disappearing. With no other of the reagents was this observed, and there are other differences which are more or less marked and characteristic.

Individualities in the reactions with chloral hydrate-iodine were observed in some starches, as, for instance, in *Triticum*, in which the grains become deeply colored, almost black, and the intermolecular structure disorganized, but with relatively little swelling. Great variations were recorded in the different starches in regard to the extent of swelling and in the changes in the forms of the grains. With some starches the alterations are so slight that the forms of the normal grains are retained after the completion of gelatinization, while in others the swelling and distortion may be so great that the gelatinized grains bear no resemblance to the originals.

Moreover, the sequence of changes, and also the various characters of the processes, vary in the starches of different kinds, in different grains of the same starch, and also to some extent with different reagents. In some starches the reaction with a given agent begins typically at the proximal end, or at the distal end, or at protuberances or angles, or at disseminated spots. Such peculiarities are not without significance because they are common to closely related starches, as, for instance, those of members of a given genus. These differences are not explicable upon the basis of mere physical or physico-mechanical conditions, because if they were the region of beginning reaction and the sequence of reactions in any given kind of grain would be the same with all of the reagents. The very fact of the selectivity of parts by each reagent, and the differences in the sequence of the events shown by the different reagents in many cases, are unmistakable indications of peculiarities due to specific relationships between each reagent and the specific stereo-chemic form of starch, each reagent demonstrating by its peculiar behavior the lack of homogeneity of the starch-grain itself, of different grains of the same starch, and of different starches as regards the form or forms of the starch-substance present. Of course, it is to be expected that when any two or more agents exhibit common tendencies, there will be corresponding propensities for the same or similar lines or sequences of reaction to be followed; for instance, if chloral hydrate-iodine and ferric chloride attack the outer part of the grain in preference to the inner, and if chromic acid, pyrogallie acid, and Purdy's solution would likewise exhibit a greater affinity for the inner part there will be corresponding tendencies in each group for similar lines or sequences of reaction.

Reactions are recorded in a number of instances which at first sight may appear to be aberrant or wide and irreconcilable departures from a given prototype that is exhibited by closely related genera, recognized groups of genera, or members of a given genus, etc. Thus, the reaction-curves of all the representatives of the *Graminaceæ* (*Zea*, *Andropogon*, *Panicum*, *Oryza*, *Triticum*, *Secale*, *Hordeum*, *Avena*, and *Arrhenatherum*) closely correspond

in type excepting in the ferric chloride reactions of *Triticum*, *Secale* and *Hordeum*, the chloral hydrate-iodine reactions of *Triticum* and *Hordeum*, and the Purdy's solution reaction of *Triticum*. In a word, out of ten reactions in each starch all are in accord with the Gramineæ type excepting those noted in members of the tribe *Hordeæ*, in which there occur aberrant reactions as noted. Among individual members of a genus such deviations are instanced in *Maranta musaica*, in which the reactions are in accord with the *Maranta* type, except in the temperature and ferric chloride reactions, the former being exceptionally high and the latter correspondingly low; and in *Zingiber officinale*, in which the temperature reaction is comparatively exceedingly low and the ferric chloride and Purdy's solution reactions very high. Such departures are at times observed in sub-generic groups such as in *Pisum* and *Brodiaea*. Thus, if we take the type of reaction-curve of the genera constituting the group formed by *Vicia*, *Phaseolus*, *Dolichos*, *Mucuna*, *Lens*, and *Lathyrus* as a basis of comparison (to which we will refer as the bean type), it will be found in studying the records of *Pisum* that the reaction-curves of the members of group 2, in which the starch-grains are histologically of the bean type, correspond quite closely with the bean type of reaction-curve, and that on the other hand, the curves of group 1, in which the grains are of a distinctly different type, show radical departures, especially in the chloral hydrate-iodine, pyrogallie acid, ferric chloride, and Purdy's solution reactions, exhibiting far greater sensitivity in the chloral hydrate-iodine, ferric chloride, and Purdy's solution and strikingly less reactivity in the pyrogallie acid reactions. Likewise in *Brodiaea* is found a very high sensitivity of group 1 to pyrogallie acid as compared with groups 2 and 3 (see chart No. 198, page 615, Part II). Such aberrant reactions may for the moment suggest unreliability of reaction-curves in expressing species and generic specificities, but this is met by frequently recorded corresponding aberrations from the typical that have been noted by the botanist and zoologist, and by the pharmacologist and other specialists engaged in biological investigation, general and medical. In fact, these discrepant phenomena may be merely seemingly and not actually aberrant. As already repeatedly pointed out, slight stereochemic differences may cause more or less marked variations in properties.

Bearing upon an explanation of such aberrant reactions is a *peculiar* relationship that has been recorded in these investigations between the *reactivity of a given starch* and the *concentration of the reagent*. A form of starch which reacts with extreme slowness with a reagent of a standard strength may react with great rapidity if the reagent be somewhat diluted or concentrated. In other words, a definite relationship exists between the molecular constitution of the particular form of starch and the concentration of the reagent, which relationship determines the rapidity of interaction, other things being equal. Thus, in *Triticum* the molecule of starch has been so modified in its properties in relation to chloral hydrate-iodine that it is feebly reactive with the standard strength of solution, but very reactive with a slightly modified strength of solution, or, in other words, with a solution of proper molecular concentration and relationship. Kabsch (p. 92) found with some haloid salts that swelling may or may not occur depending upon the strength of solution. A comparable condition was long ago observed in mixtures of variable proportions of gasoline vapor and air, which in order to be explosive or to yield the maximum explosive effect must be in percentages within definite limits; *i.e.*, 1 volume of gasoline vapor to 16 of air, and 1 to 1, are not explosive, but all intermediate mixtures are, the greatest effect being obtained when the mixture is in the proportion of about 1 to 9. In other words, certain molecular conditions are requisite not only for reaction but also for different intensities of reaction. It is probable that these seemingly aberrant reactions will be found to be of considerable importance in the final analyses and comparisons of the peculiarities of any given starch and its relatives, in bringing to light obscure molecular variations and their causes, and especially in the differentiation of the stereoisomers.

## GENERAL CONSIDERATIONS.

The methods used in this research in the differentiation of the starches of different plants and plant parts, and especially in indicating different forms of the starch-substance, are exceedingly crude, and, as a consequence, the range of error of experiment is in comparison with chemical procedures generally very large. The differentiation of isomers at the present time depends, as previously stated, upon methods which, in a very large measure, are crude and quantitative in nature; as, for instance, differences in density, solubility, decomposability, color and color reactions, digestibility, toxicity, physiological properties, temperatures of gelatinization and melting-point, optical reactions, crystalline forms, etc. The closer the relationship of isomers the greater usually the difficulty in their differentiation; hence while little or no difficulty may be experienced in differentiating the isomers constituting a group of polysaccharoses, or disaccharoses, or monosaccharoses, the differentiation of corresponding substances, or stereoisomers, such as different forms of starch, glycogen, hemoglobin, etc., may be attended with great obstacles.

In the case of many isomerides which are optically active and crystallize in rectilinear forms, as, for instance, the hemoglobins, accurate differential and diagnostic data can be obtained, but in case of starch and starch-like substances the conditions are obviously quite different. Were it not that the starch-grain during its growth is subjected to various mechanical and changeable conditions which affect its form, and that different plant parts may not give rise to the same form of grains because of different forms of plastids or other conditions, and that the grains vary in form at different stages of development, the starch-grains of any given plant would in all probability be of a single histological type and as specific in relation to the plant as the offspring to its parents—in other words, its form would be an index of the peculiar biology of the plastid (were there a single form of plastid), and as this plastid differed from the plastid of another plant the product would likewise differ in its microscopical properties; but having different forms of plastids in the same plant, and variable conditions, the forms of the starch-grains are correspondingly variable.

These differences in conditions give rise not only to the presence of a variety of forms of starch-grains, but also to variations in the proportions of the different forms in any given starch. It therefore follows that if a number of preparations of starches were obtained from as many plants of the same species, no two need be exactly alike, either in the relative proportions of different types of grains or in the characters of the most conspicuous type of grains present. Moreover, owing to this heterogeneous and variable mixture of shapes and sizes, no two fields seen on the stage of the microscope are ever identical, and sometimes they are so different as to suggest that the fields represent starches from different species.

In comparing the plates with the text such discrepancies may be apparent in a seeming lack of absolute agreement of text with picture. Therefore in the descriptions of the grains the impressions given are those obtained by a general examination of what might be expressed as the exhibit of the starch as a whole. Similarly, variations in the position, form, and other characters of the hilum, variations in the lamellæ, variations in fissuration, and variations in the number and character of aggregates and compound grains, etc., are likewise to be expected. Moreover, no two persons would in the least likelihood be apt to give the same description of any given kind of starch, as is instanced in the records in various parts of this work, as in *Maranta arundinacea* (page 224 and Part II, S13), and also in the various popular works treating of starch-grains. It is therefore obvious that the gross histological descriptions of starch-grains can not be laid down on hard-and-fast lines as in the case of ordinary geometric forms.

Again, in the reactions the personal factor plays a very important part on the records. The intensities of the color reactions are based upon an arbitrary standard of the experi-

menter, and the time-reactions of the chemical reagents may vary enormously, according to the standard adopted. At the outstart of the investigation the determination of the intensity of the iodine reactions was attempted by means of a calibrated color-scheme, but this was found valueless because of the differences in color, as, for instance, a tint of violet could not properly be matched with one of blue. If all starches reacted in tints of the same color, the method would have been easy enough. Then again arose the trouble of different grains of the same starch reacting with different degrees of intensity; as a consequence, an arbitrary standard was fixed which expresses the degrees very light, light, fair, dark, and very dark, the average degree of coloration being taken as the index.

In recording the times of the reactions there are opportunities for considerable errors, owing chiefly to the heterogeneous mixture of grains, some grains reacting immediately, others perhaps not for minutes, and others in turn not for an hour or hours. In some starches, with a given reagent 90 per cent of the grains will be broken down in 4 or 5 minutes and the remaining 10 per cent within 60 minutes; and in another starch the reaction may go on quite regularly, so that at the end of an hour it likewise is complete. In both instances one hour is required for complete gelatinization, but obviously the reaction was much more intense on the whole in the first than in the last. In most starches there is a small percentage of grains (usually from about 0.25 to 1 per cent) that are very distinctly more resistant than the others; hence it is usually necessary to disregard these grains in recording the time the reaction has reached its termination. Therefore, in most of the records the time of completion of the reaction means that all, or practically all, of the grains have been disorganized.

Another source of error is the presence of impurities within and without the grain. The former can not be eliminated without introducing sources of fallacy greater than the impurities themselves; and the latter, as in the case of most starches that must be prepared from dry seeds, sometimes offer difficulties that are not easy or which are impossible to meet without injury to the chemical peculiarities of the starch-grain itself.

It must therefore be obvious, with such gross methods and such heterogeneous material to investigate, and where, as in the present preliminary study, usually but a single experiment is made with each starch with each reagent, that the results must be regarded as being of a grossly quantitative or qualitative character, and absolutely tentative until such investigations can be carried out under conditions which will yield figures that can safely be adopted as fixed standards or constants. In fact, it is probable that their greatest value lies in indicating what important results are to be expected by research carried out along strictly scientific lines.

With so many contributory factors to fallacious results, the reader, as was the author, may be skeptical as to what value, if any, is to be attached to results obtained under such conditions. He, like the author, is dependent upon the minds and hands of others (see Introduction, page 14), and like the author he can make his own deductions from the records, which, if they have intrinsic value, must not only speak for themselves but speak plainly and satisfactorily. That they have a high intrinsic value will be apparent to any one who will compare the records of horticultural forms and varieties of a given species, of closely related species, of distantly related species, of closely related genera, etc. Such remarkable relationships, like or unlike, in correspondence generally with botanical peculiarities, could not occur by chance, and if in some instances there appear to be records which are departures from what was to be expected, such differences will doubtless be found to have their explanation in unrecognized errors or misinterpreted results of experiment, or in errors of botanical classification, which latter unfortunately are only too numerous, as is evident in the continual shifting that has been and is going on in systematic botany. In fact, the general accord of the results of this investigation with those of the systematic botanist is not short of astonishing.

## DIFFERENCES IN THE REACTION-INTENSITIES OF VARIOUS STARCHES.\*

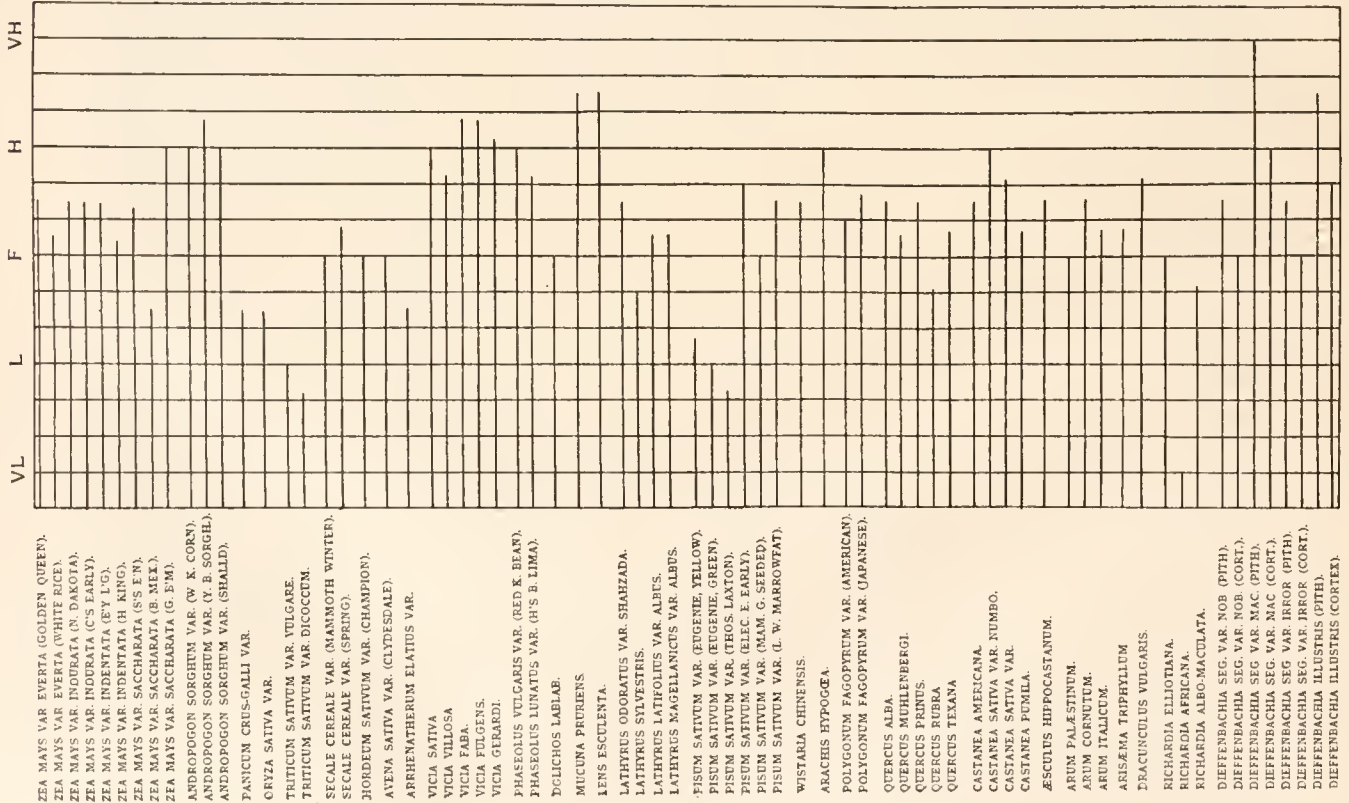
The reaction-intensities in the case of each starch with each reagent vary within wide limits, especially in the case of the reactions with heat and chemical reagents. For purposes of comparison these differences are expressed more satisfactorily in diagrammatic form than in words and figures in the form of text. Charts A to J will be found extremely instructive in showing: (1) the range of reaction-intensity of each reagent; (2) the close correspondence in the case of horticultural forms, varieties of a given species, the members of a genus, closely related genera, etc.; and (3) the independence in the intensities of the different reactions of a given kind of starch, for instance, when, in a given starch, the temperature of gelatinization is high but the chemical reactions low, or vice versa, or when the reaction with one chemical reagent may be high while with others they are low, etc. These charts do not require any detailed statement as to what they show, but as an indicator it might be desirable to suggest to the reader that the data of any arbitrarily selected chart be examined in a general way. For instance, Chart D, parts 1 to 4, at a glance show the wide range of the reaction, and the groupings of horticultural forms, varieties of a species, members of a genus, and closely related genera. In fact, by the temperature of gelatinization alone, had we more data, one might say that the starch may belong to this genus or not to that genus. Thus, upon the basis of the records and limitations of this research, a starch having a temperature of gelatinization in excess of 80° might be suspected to belong to *Amaryllidaceæ*, *Iridaceæ*, *Zingiberaceæ*, or *Marantaceæ*; but it would not be thought to belong to *Graminaceæ*, *Aroideæ*, *Liliaceæ*, or other families noted. While, therefore, little weight is to be attached to the indications of the temperature of gelatinization of itself as indicating the genus, yet by taking the sum of the mean reactions, the positive recognition of every form of starch seems not only possible but usually exceedingly easy.

In the case of each reagent, while the range of reaction extends from almost the lowest limit of the chart to or beyond the highest limit, it will be seen that the degree of variability of the different starches with each agent is quite different. Thus, in the polarization reactions the records range for the most part from fair to high, and in the iodine reactions generally from fair to dark. The variability in the reactions with the two anilines is somewhat more marked, and there is a decided tendency for the records of the two charts to run parallel, the gentian violet reactions being usually more marked than those with safranin. In fact, eliminating the probable errors of record due to the gross standards of determining the reaction, the differences in the results with these two agents might in most cases be regarded as being due to differences in concentration of a given reagent. In the temperatures of gelatinization there is, on the whole, a greater tendency to variation than in the polarization and iodine reactions but less than with the anilines, and markedly less than with the chemical reagents, excepting Purdy's solution, with which the reactions generally tend to be towards one extreme or the other, that is, very slow or quite rapid. There appears very frequently to be a manifest tendency for the grades of reaction with chloral hydrate-iodine, chromic acid, pyrogallic acid and ferric chloride to correspond, so that if reaction be moderate to slow with one it is apt to be the same with the others; but in this respect the reactions with Purdy's solution appear to stand distinctly apart. However, this is a very gross generalization, inasmuch as a starch that is very responsive to one reagent may be very resistive to another, and as there are so many exceptions to the generalization absolutely no rule can be formulated. In other words, the reaction-intensity with each reagent must be looked upon as an independent unit which may or may not have a quantitative correspondence with that of another reagent.

\* In cases where the temperatures of gelatinization exceeded the limits of the chart the line (ordinate) was drawn to the limit; and in the chemical reactions where the ordinate is drawn to the 60-minute abscissa the reaction is indicated as being complete or incomplete at this time, in almost all instances incomplete.

CHART A.—The Degrees of Polarization of Various Starches.

PART 1.



PART 2.

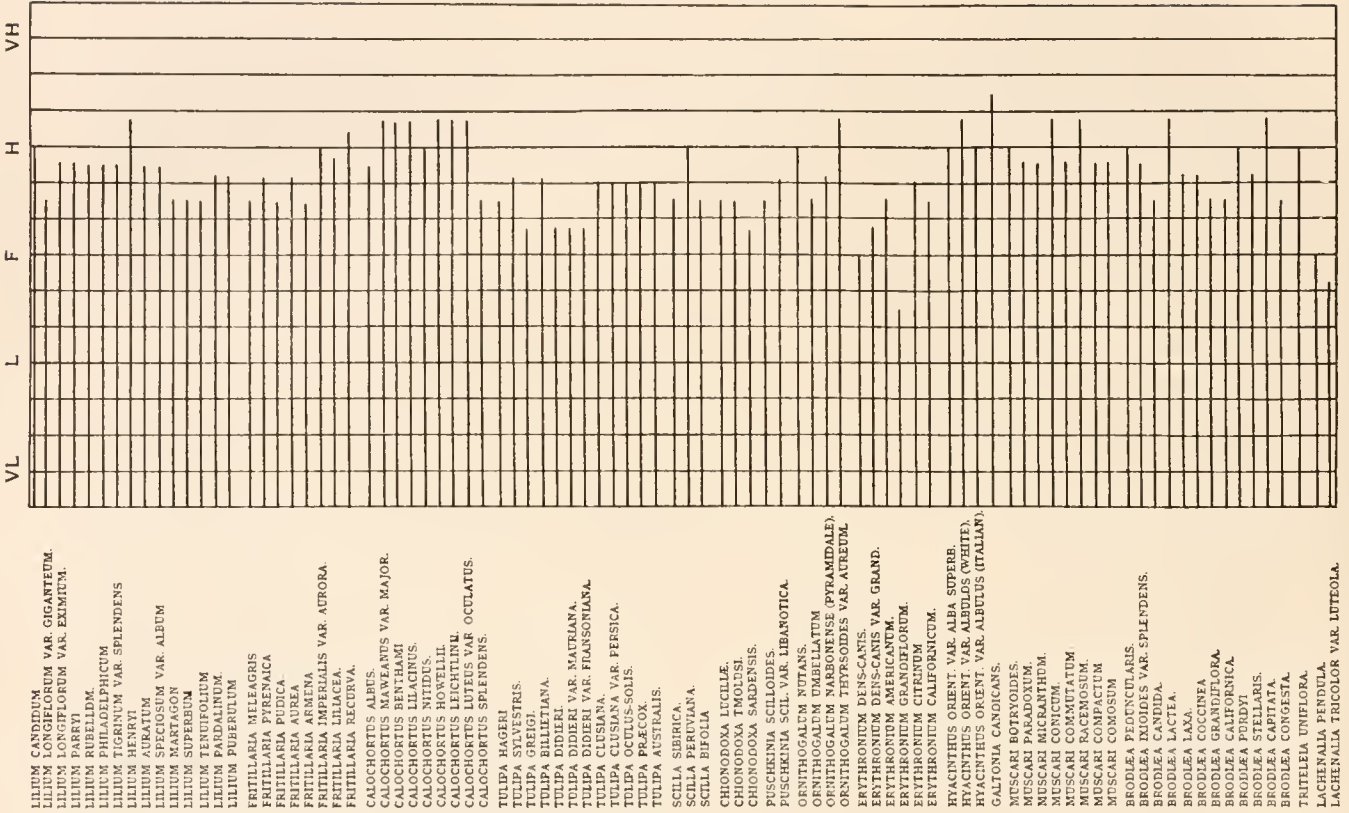


CHART A.—The Degrees of Polarization of Various Starches—continued.

PART 3.

	VL	L	F	H	VH
CORVALLARIA MAJALIS.					
TRILLIUM GRANDIFLORUM.					
TRILLIUM OVATUM					
TRILLIUM SESSILE VAR. CALIFORNICUM.					
COLCHICUM PARKINSONI.					
AMARYLLIS BELLADONA MAJOR.					
HIPPEASTRUM VITIATUM.					
HIPPEASTRUM EQUESTRE.					
HIPPEASTRUM ALLICUM VAR. ROBUSTUM.					
VALLOTA PURPUREA.					
CRINUM FIMBRIATUM.					
CRINUM AMERICANUM.					
ZEPHYRANTHES CANDIDA					
ZEPHYRANTHES ROSEA.					
SPREKELIA FORMOSISSIMA.					
HEMANTHUS KATHERINE.					
HYMENOCALLIS UNDPULATA					
HYMENOCALLIS CALATHIHA.					
LEUCODIUM VERNUM.					
LEUCODIUM ÆSTIVUM.					
GALANTHUS NIVALIS.					
GALANTHUS ELWESI.					
ALSTROEMERIA LIGTU.					
ALSTROEMERIA BRASILIENSIS.					
ALSTROEMERIA AURANTIACA (AUREA).					
STERNBERGIA LUTEA.					
NARCISSUS HORSEFIELDII.					
NARCISSUS MAXIMUS.					
NARCISSUS BULBOCODIUM					
NARCISSUS BULBO. VAR. CONSPECTA.					
NARCISSUS BULBO. VAR. MONOPHYLLUS.					
NARCISSUS INCOMPARABILIS.					
NARCISSUS ODORUS.					
NARCISSUS POETICUS.					
NARCISSUS BIFLORUS.					
NARCISSUS JONQUILLA.					
NARCISSUS JONQUILLA VAR. CAMPERNELLI RUG.					
NARCISSUS TAZETTA VAR. ORIENTALIS.					
TACCA PINNATIFIDA.					
IRIS FLORENTINA.					
IRIS PALLIDA SPECIOSA.					
IRIS PALLIDA VAR. CHANA.					
IRIS BISMARCKIANA.					
IRIS IBERICA.					
IRIS XIPHIDUM VAR. GRAND TRESORIER.					
IRIS XIPHIDUM VAR. WILHELMINE.					
IRIS XIPHIDUM VAR. LUSITANICA.					
IRIS TINGITANA.					
IRIS RETICULATA.					
IRIS HISTRIO.					
IRIS ALATA.					
IRIS CAUCASICA.					
MORÆA TRISTIS.					
HOMERIA COLLINA.					
TIGRIDA PAVONIA VAR. GRAND ALBA.					
TIGRIDA PAVONIA VAR. CONCHIFLORA.					
GLADIOLUS RIZANTINUS.					
GLADIOLUS PRINILINUS.					
GLADIOLUS PRINILINUS. BELSHO BRIDE.					
GLADIOLUS FLORIBUNDUS.					
WATSONIA HUMILIS.					
WATSONIA BIFIDIFOLIA VAR. O'BRIENI.					
WATSONIA MELJANA.					
TRITONIA CROCATA.					
TRITONIA CROCATA VAR. LILACINA.					
TRITONIA CROCATA VAR. ROSEA.					
TRITONIA CROCATA VAR. LUTEA.					
TRITONIA CROCATA VAR. PINKI.					
TRITONIA CROCATA VAR. FLORIDA.					
FREESIA REFRACTA VAR. ALBA.					
FREESIA REFRACTA VAR. LEICHTLINII.					
ANTHOLYZA CROCOSMOIDES.					
ANTHOLYZA PANICULATA.					
CROCUS SUSANUS (CLOTH-OF-GOLD)					
CROCUS VERSICOLOR (CLOTH-OF-SILVER)					
CROCUS VAR. (BARON VON BRUNOW).					
ROMULEA ROSEA VAR. SPECIOSA.					
CYPELLA HERBERTI.					

PART 4.

	VL	L	F	H	VH
MARICA GRACILIS.					
GELASINE AZUREA.					
SPARAXIS GRANDIFLORA ALBA.					
SPARAXIS VAR. (ALBERTINE).					
IKIA SPECIOSA.					
IKIA VAR. (MADAGASCAR).					
IKIA VAR. (YAMBA).					
BABIANA VAR. (VIOLACEA).					
BABIANA VAR. (ATTRACCIA).					
MUSA CAVENDISHII					
MUSA CAVENDISHII (GREEN FRUIT).					
MUSA SAPIENTUM.					
MUSA ENSETE.					
ZINGIBER OFFICINALE.					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 1.					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 2.					
ZINGIBER OFFIC. VAR. COCHIN.					
HEDYCHUM CORONARIUM.					
HEDYCHUM GARDNERIANUM.					
CURCUMA LONGA					
CURCUMA PETIOLATA.					
CANNA WARSEWICZII.					
CANNA ROSCOENII.					
CANNA EDULIS.					
CANNA EDULIS.					
CANNA VAR. (KONIGEN CHARLOTTE).					
CANNA VAR. (L. E. BALLY).					
CANNA VAR. (MRS. KATE GREY).					
CANNA VAR. (JEAN TISSOT).					
CANNA VAR. (J. D. EISELE).					
MARANTA ARUNDINACEA.					
MARANTA ARUNDINACEA VAR. NO. 1.					
MARANTA ARUNDINACEA VAR. NO. 2.					
MARANTA MASSANGEA.					
MARANTA LEUCONEURA.					
MARANTA MUSICA.					
CALATHEA LIETZEL.					
CALATHEA VITTATA.					
CALATHEA WOTIAMA.					
CALATHEA VANDENHECKEL.					
STROMANTHE SANGUINEA.					
NYPHÆA ALBA.					
NYPHÆA MARLIACEA VAR. ALBIDA.					
NYPHÆA MARLIACEA VAR. CARNEA.					
NYPHÆA GLAD-STONIANA.					
NYPHÆA ODORATA.					
NYPHÆA ODORATA VAR. ROSEA.					
NELUMBO NUCIFERA.					
NELUMBO LUTEA.					
ANEMONE APERNINA.					
ANEMONE FULGENS.					
ANEMONE BLANDA.					
ANEMONE JAPONICA.					
ACONITUM NAPELLUS.					
ACTÆA ALBA.					
ACTÆA SPICATA VAR. RUBRA.					
CIMICIFUGA RACEMOSA.					
ERANTHIS HYEMALIS.					
RANUNCULUS RUBROSUS.					
RANUNCULUS FICARIA.					
ADONIS AMPURENSIS.					
COCHLEARIA ARMORACIA.					
JATROPHA CURCASA.					
MANIHOT UTILISSIMA.					
CYCLAMEN REPANDIUM.					
CYCLAMEN CODM.					
SOLANUM TUBEROSUM.					
BATATAS EDULIS.					
GESNERIA TUBIFLORA.					
GLOXINIA VAR.					
TELIANUSPERMA FICIFOLIA.					
CYCAS REVOLUTA.					
CYCAS CIRCINALIS.					
DIOON EDULE.					
ZAMIA INTEGRIFOLIA.					

CHART B.—The Iodine Reactions of Various Starches.

PART 1.

	VL	L	F	D	VD
ZEA MAYS VAR. EVERTA (GOLDEN QUEEN).					
ZEA MAYS VAR. EVERTA (WHITE RICE).					
ZEA MAYS VAR. INDRATA (N. DAKOTA).					
ZEA MAYS VAR. INDRATA (S. EARLY).					
ZEA MAYS VAR. INDRATA (EY LG).					
ZEA MAYS VAR. INDRATA (H. KING).					
ZEA MAYS VAR. SACCHARATA (S. E. W.).					
ZEA MAYS VAR. SACCHARATA (B. MEK.).					
ZEA MAYS VAR. SACCHARATA (G. P. M.).					
ANDROPOGON SORGHUM VAR. (W. K. CORN).					
ANDROPOGON SORGHUM VAR. (Y. B. SORGH).					
ANDROPOGON SORGHUM VAR. (SHALLU).					
PANICUM CRUS-GALLI VAR.					
ORYZA SATIVA VAR.					
TRITICUM SATIVUM VAR. VULGARE.					
TRITICUM SATIVUM VAR. BIGOCUM.					
SECALE CEREALE VAR. (MAMMOTH WINTER).					
SECALE CEREALE VAR. (SPRING).					
HORDEUM SATIVUM VAR. (CHAMPION).					
AVENA SATIVA VAR. (CLYDESDALE).					
ARRHEMATHERON ELATIS VAR.					
VICIA SATIVA.					
VICIA VILLOSA.					
VICIA FABA.					
VICIA FULGENS.					
VICIA GERARDI.					
PHASEOLUS VULGARIS VAR. (RED E. BEAN).					
PHASEOLUS LUNATUS VAR. (H. S. B. LIMA).					
DOLICHOS LABLAB.					
MUCUNA PROLENS.					
LENS ESCULENTA.					
LATHYRUS ODORATUS VAR. SHAHZADA.					
LATHYRUS SYLVESTRIS.					
LATHYRUS LATIFOLIUS VAR. ALBUS.					
LATHYRUS MAGELLANICUS VAR. ALBUS.					
PISUM SATIVUM VAR. (EDGENE, YELLOW).					
PISUM SATIVUM VAR. (EUGENIE, GREEN).					
PISUM SATIVUM VAR. (THOS. LANTON).					
PISUM SATIVUM VAR. (ELEC. E. EARLY).					
PISUM SATIVUM VAR. (MAM. G. SEEDED).					
PISUM SATIVUM VAR. (L. W. MARROWEAT).					
WISTARIA CHINENSIS.					
ARACHIS HYPOGAEA.					
POLYGONUM FAGOPYRUM VAR. (AMERICAN).					
POLYGONUM FAGOPYRUM VAR. (JAPANESE).					
QUERCUS ALBA.					
QUERCUS MUEHLENBERGL.					
QUERCUS RUBRA.					
QUERCUS TEXANA.					
CASTANEA AMERICANA.					
CASTANEA SATIVA VAR. (MOMO).					
CASTANEA SATIVA VAR.					
CASTANEA FUMILA.					
ÆSCULUS HIPPOCASTANUM.					
ARUM PALESTINUM.					
ARUM CORINTHUM.					
ARUM ITALICUM.					
ARISEMA TRIPHYLLUM.					
DRACUNCULUS VULGARIS.					
RICHARDIA ELLIOTIANA.					
RICHARDIA AFRICANA.					
RICHARDIA ALBO-MACULATA.					
DIFFENBACHIA SEG. VAR. NOB. (PITH).					
DIFFENBACHIA SEG. VAR. NOB. (CORT.).					
DIFFENBACHIA SEG. VAR. MAC. (PITH).					
DIFFENBACHIA SEG. VAR. MAC. (CORT.).					
DIFFENBACHIA SEG. VAR. IROR. (PITH).					
DIFFENBACHIA SEG. VAR. IROR. (CORT.).					
DIFFENBACHIA ILLUSTRIS (PITH).					
DIFFENBACHIA ILLUSTRIS (CORTEX).					

PART 2.

	VL	L	F	D	VD
LILIUM CANDIDUM.					
LILIUM LONGIFLORUM VAR. GIGANTEUM.					
LILIUM LONGIFLORUM VAR. EXIMUM.					
LILIUM PARRY.					
LILIUM RUBELLUM.					
LILIUM PHILADELPHICUM.					
LILIUM SPECIOSUM VAR. SPLENDENS.					
LILIUM ABRATUM.					
LILIUM SPECIOSUM VAR. ALBUM.					
LILIUM MARTAGON.					
LILIUM SUPERBUM.					
LILIUM TENUIFOLIUM.					
LILIUM PARDALINUM.					
LILIUM PABERULUM.					
FRITILLARIA MELEAGRS.					
FRITILLARIA PYRENAICA.					
FRITILLARIA AUREA.					
FRITILLARIA BIFIDA.					
FRITILLARIA IMPERIALIS VAR. AUROEA.					
FRITILLARIA LILIACEA.					
FRITILLARIA RECURVA.					
CALOCHORTIS ALBUS.					
CALOCHORTIS MAWEANTIS VAR. MAJOR.					
CALOCHORTIS BENTHAMI.					
CALOCHORTIS LILACINUS.					
CALOCHORTIS NITIDUS.					
CALOCHORTIS HOWELLI.					
CALOCHORTIS LEICHTLINI.					
CALOCHORTIS ROTUNDUS VAR. OCELLATUS.					
CALOCHORTIS SPLENDENS.					
TULIPA HAGERI.					
TULIPA STEVENIS.					
TULIPA AUSTRALES.					
TULIPA BILLETIANA.					
TULIPA DIDIERI.					
TULIPA DIDIERI VAR. MAURIANA.					
TULIPA CUSIANA.					
TULIPA CUSIANA VAR. PFRSICA.					
TULIPA OCELLUS-SOLIS.					
TULIPA AUSTRALES.					
SCILLA SIBIRICA.					
SCILLA ITALIANA.					
SCILLA BIFOLIA.					
CHONODOKA LUCIFL.					
CHONODOKA LUCIFL. VAR. ALBA.					
CHONODOKA SARDENSIS.					
PSUCHENIA SCLOIDES.					
PSUCHENIA SCIL VAR. LIBANOTICA.					
PSYDOPALM VITANS.					
ORNTITHOGALUM DIMBELLITUM.					
ORNTITHOGALUM NARBONENSE (PYRAMIDALE).					
ORNTITHOGALUM THYRSOIDES VAR. AUREUM.					
ERYTHRONIUM DENSCANIS.					
ERYTHRONIUM DENSCANIS VAR. GRAND.					
ERYTHRONIUM AMERICANTUM.					
ERYTHRONIUM GRANDIFLORUM.					
ERYTHRONIUM CITRINUM.					
ERYTHRONIUM CALIFORMICUM.					
RYACINTHUS ORIENT. VAR. ALBA SUPERE.					
RYACINTHUS ORIENT. VAR. ALBULUS (WHITE).					
RYACINTHUS ORIENT. VAR. ALBULUS (ITALIAN).					
GALTONIA CANDICANS.					
MUSCARI BOTRYOIDES.					
MUSCARI PARADOXUM.					
MUSCARI MONTANUM.					
MUSCARI COMITATUM.					
MUSCARI RACEMOSUM.					
MUSCARI COMPACTUM.					
MUSCARI COMOSUM.					
BRODIAEA PEDUNCULARIS.					
BRODIAEA IXIODES VAR. SPLENDENS.					
BRODIAEA CANDIDA.					
BRODIAEA LACTEA.					
BRODIAEA LAXA.					
BRODIAEA COCCINEA.					
BRODIAEA ALBO-ROSEA.					
BRODIAEA CALIFORNICA.					
BRODIAEA PUEDEY.					
BRODIAEA STELLARIS.					
BRODIAEA CAPITATA.					
BRODIAEA CONGESTA.					
TRITILEA UNIFLORA.					
LACHENALIA PENOLA.					
LACHENALIA TRICOLOR VAR. LUTEOLA.					

CHART B.—The Iodine Reactions of Various Starches—continued.

PART 3.

	VL	L	F	D	VD
CONVALLARIA MAJALIS.					
TRILLIUM GRANDIFLORUM.					
TRILLIUM OVATUM.					
TRILLIUM SESILE VAR. CALIPORNICUM.					
COLCHICUM PARKINSONI.					
AMARILLIS BELLADORA MAJOR.					
HIPPEASTRUM VITTATUM.					
HIPPEASTRUM EQUESTRE.					
HIPPEASTRUM ALLUCUM VAR. ROBUSTUM.					
VALLOTA PURPUREA.					
CRINUM FIBRILLATUM.					
CRINUM AMERICANUM.					
ZEPHYRANTHES CANDIDA.					
ZEPHYRANTHES ROSEA.					
SPREKELIA FORMOSISSIMA.					
HÆMANTHUS KATHERINÆ.					
HYMENOCALLIS UNDECIMATA.					
HYMENOCALLIS CALATHINA.					
LEUCODIUM VERNUM.					
LEUCODIUM ÆSTIVUM.					
GALANTHUS NIVALIS.					
GALANTHUS ELWESI.					
ALSTREMERIA LIGULI.					
ALSTREMERIA BRASHLIENSIS.					
ALSTREMERIA AURANTIACA (AUFEA).					
STERNBERGIA LUTEA.					
NARCISSUS HORSFELDI.					
NARCISSUS MAXIMUS.					
NARCISSUS BULBOCOCHUM.					
NARCISSUS BULBO VAR. CONSPIDUA.					
NARCISSUS BULBO VAR. MONOPHYLLUS.					
NARCISSUS INCOMPARABILIS.					
NARCISSUS ODORUS.					
NARCISSUS POETICUS.					
NARCISSUS BUNGEI.					
NARCISSUS JONQUILLA.					
NARCISSUS JONQUILLA VAR. RUGULOSUS.					
NARCISSUS JONQUILLA VAR. CAMPANELLI RUG.					
NARCISSUS TAZETTA VAR. ORIENTALIS.					
TACCA PINNATIFIDA.					
IRIS FLORENTINA.					
IRIS PEGIVENSIS.					
IRIS PUMILA VAR. CYANEA.					
IRIS BISMARCKIANA.					
IRIS IBERICA.					
IRIS XIPHIDUM VAR. GRAND TRESORIER.					
IRIS XIPHIDUM VAR. WILHELMINE.					
IRIS XIPHIDUM VAR. LUSITANICA.					
IRIS TINGITANA.					
IRIS RETICULATA.					
IRIS HISTRIO.					
IRIS LUTICA.					
IRIS CAUCASICA.					
MOREA TRISTIS.					
HOMERIA COLLINA.					
TIGRIDIA PAVONIA VAR. GRAND ALBA.					
TIGRIDIA PAVONIA VAR. CONCHIFLORA.					
GLADIOLUS BYZANTINUS.					
GLADIOLUS GIBBULUS.					
GLADIOLUS CARDINALIS (BELSHG BRIDE).					
GLADIOLUS FLORIBUNDUS.					
WATSONIA HUMILIS.					
WATSONIA HEDIFOLIA VAR. O'BRIENI.					
WATSONIA MERIANA.					
TRITONIA CROCATA.					
TRITONIA CROCATA VAR. LILACINA.					
TRITONIA CROCATA VAR. ROSEA.					
TRITONIA SECURIGERA.					
TRITONIA POTTII.					
TRITONIA CROCOSMÆLORA.					
FREESIA REFRACTA VAR. ALBA.					
FREESIA REFRACTA VAR. LEICHTLINI.					
ANTHOLYZA CROCOSMIDES.					
ANTHOLYZA PANICULATA.					
CROCUS SUSIANDS (CLOTH-OF-GOLD).					
CROCUS VERSICOLOR (CLOTH-OF-SILVER).					
CROCUS VAR. (BARON VON BRUNDT).					
ROMULEA ROSEA VAR. SPECTIOSA.					
CYPELLA HERBERTI.					

PART 4.

	VL	L	F	D	VD
MARICA GRACILIS.					
GELASINE AZUREA.					
SPEAEGIS GRACIFLORA ALBA.					
SPEAEGIS VAR. (ALBERTINE).					
IXIA SPECTIOSA.					
IXIA VIRIDIFLORA.					
IXIA VAR. (EMMA).					
BABIANA VAR. (VIOLACEA).					
BABIANA VAR. (ATHRACTA).					
MUSA CAVEROSISSI (GREEN FRUIT).					
MUSA SAPIENTUM.					
MUSA ENSETE.					
ZINGIBER OFFICINALE.					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 1.					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 2.					
ZINGIBER OFFIC. VAR. COCHIN.					
EDYCHNUM COROMANDIUM.					
EDYCHNUM GARDNERIANUM.					
CURCUMA LONGA.					
CURCUMA PETIOLATA.					
CANNA WARSZEWICZII.					
CANNA ROSCOEANA.					
CANNA MDS.FOLIA.					
CANNA EDULIS.					
CANNA VAR. (KONIGEN CHARLOTTE).					
CANNA VAR. (PREMIER CARROT).					
CANNA VAR. (E. BALLY).					
CANNA VAR. (MRS. KATE GREY).					
CANNA VAR. (JEAN TISSOT).					
CANNA VAR. (J. D. EISELE).					
MARANTA ARUNDINACEA.					
MARANTA ARUNDINACEA VAR. NO. 1.					
MARANTA ARUNDINACEA VAR. NO. 2.					
MARANTA MASSANGENA.					
MARANTA LEUCONEURA.					
MARANTA MUSAUCA.					
CALATHEA LIETZEL.					
CALATHEA VITTATA.					
CALATHEA WIoTIANA.					
CALATHEA VANDENHECKEL.					
CALATHEA VANEGUINEA.					
STROMANTHE SANGUINEA.					
NYPHÆA ALBA.					
NYPHÆA MARLIACEA VAR. ALBIDA.					
NYPHÆA MARLIACEA VAR. CARNEA.					
NYPHÆA GLADSTONIATA.					
NYPHÆA ODORATA.					
NYPHÆA ODORATA VAR. ROSEA.					
NELUMBO NICEFERA.					
NELUMBO LUDEA.					
ANEMONE APENNINA.					
ANEMONE FULGENS.					
ANEMONE BLANDA.					
ANEMONE JAPONICA.					
ACONITUM NAPELLUS.					
ACTÆA ALBA.					
ACTÆA SPICATA VAR. RUBRA.					
CIMICIFUGA RACEMOSA.					
ERANTHIS HYEMALIS.					
RANUNCULUS BULBOSUS.					
RANUNCULUS FICARIA.					
ADONIS AMURENSIS.					
COCILEARIA ARMDRACIA.					
IATROPHA CURCASA.					
MANIHOT OTULISSIMA.					
CYCLAMEN REPANDUM.					
CYCLAMEN CDDUM.					
SOLANUM TUBEROSUM.					
BATATAS EDULIS.					
GESNERIA TUBIFLORA.					
GLOXINIA VAR.					
TRIANOSPERSMA FICTIFOLIA.					
CYCAS REVOLUTA.					
CYCAS CIRCONALIS.					
DIOON EDULE.					
ZAMIA INTEGRIFOLIA.					

CHART C.—The Gentian Violet Reactions of Various Starches.

PART 1.

	VL	L	F	D	VD
ZEA MAYS VAR. EVERTA (GOLDEN QUEEN).					
ZEA MAYS VAR. EVERTA (WHITE RICE).					
ZEA MAYS VAR. INDIURATA (N. DAKOTA).					
ZEA MAYS VAR. INDIURATA (S. EARLY).					
ZEA MAYS VAR. INDIURATA (EY. L'G).					
ZEA MAYS VAR. INDIURATA (H. KING).					
ZEA MAYS VAR. SACCHARATA (S. EY.).					
ZEA MAYS VAR. SACCHARATA (B. MEX.).					
ZEA MAYS VAR. SACCHARATA (G. B'N.).					
ANDROPOGON SORGHUM VAR. (W. K. CORN).					
ANDROPOGON SORGHUM VAR. (V. B. SORGH).					
ANDROPOGON SORGHUM VAR. (SHALLU).					
PANICUM CRUS-GALLI VAR.					
ORYZA SATIVA VAR.					
TRITICUM SATIVUM VAR. VULGARE.					
TRITICUM SATIVUM VAR. DICOCUM.					
SECALE CEREALE VAR. (MAMMOTH WINTER).					
SECALE CEREALE VAR. (SPRING).					
HORDEUM SATIVUM VAR. (CHAMPION).					
AVENA SATIVA VAR. (GLYDESDALE).					
ARRHENATHERUM ELATIS VAR.					
VICIA SATIVA.					
VICIA VILLOSA.					
VICIA FABA.					
VICIA FULGENS.					
VICIA GERARDI.					
PHASEOLUS VULGARIS VAR. (RED & BEAN).					
PHASEOLUS LUNATUS VAR. (H. S. B. LIMA).					
DOLICHOS LABELAB.					
MUCUNA PRURIENS.					
LENS ESCULENTA.					
LATHYRUS ODORATUS VAR. SHAHZADA.					
LATHYRUS SILVESTRIS.					
LATHYRUS LATIFOLIUS VAR. ALBUS.					
LATHYRUS MAGELLANICUS VAR. ALBUS.					
PISUM SATIVUM VAR. (EUGENIE YELLOW).					
PISUM SATIVUM VAR. (EUGENIE GREEN).					
PISUM SATIVUM VAR. (THOS. LAYTON).					
PISUM SATIVUM VAR. (ELEC. F. EARLY).					
PISUM SATIVUM VAR. (MAM. G. SEEDFO).					
PISUM SATIVUM VAR. (L. W. MARROWFAT).					
WISTARIA CHINENSIS.					
ARABIS HYPOGAEA.					
POLYGONUM FAGOPYRUM VAR. (AMERICAN).					
POLYGONUM FAGOPYRUM VAR. (JAPANESE).					
QUERCUS ALBA.					
QUERCUS MUEHLBERGI.					
QUERCUS PRINUS.					
QUERCUS ROBR.					
QUERCUS TEXANA.					
CASTANEA AMERICANA.					
CASTANEA SATIVA VAR. NOMB.					
CASTANEA SATIVA VAR.					
CASTANEA POMILA.					
ESCLUSUS HIPPOCASTANUM.					
ARUM PALMESTINUM.					
ARUM CORNUTUM.					
ARUM ITALICUM.					
ARISEMA TRIPHYLLIDM.					
DICUCUCULUS VULGARIS.					
RICHARDIA ELLIOTIANA.					
RICHARDIA AFRICANA.					
RICHARDIA ALBO-MACULATA.					
DIFFENBACHIA SEG. VAR. HOB. (PITH).					
DIFFENBACHIA SEG. VAR. MOB. (CORT).					
DIFFENBACHIA SEG. VAR. MAC. (PITH).					
DIFFENBACHIA SEG. VAR. MAC. (CORT).					
DIFFENBACHIA SEG. VAR. IRKOR. (PITH).					
DIFFENBACHIA SEG. VAR. IRKOR. (CORT).					
DIFFENBACHIA ILLUSTRIS (PITH).					
DIFFENBACHIA ILLUSTRIS (CORTEX).					

PART 2.

	VL	L	F	D	VD
LILIUM CANDIDUM.					
LILIUM CANDIDUM VAR. GIGANTEUM.					
LILIUM LONGIFLORUM VAR. EXIMUM.					
LILIUM PARVY.					
LILIUM RUBELLUM.					
LILIUM PHILADELPHICUM.					
LILIUM TIGRINUM VAR. SPLENDENS.					
LILIUM HENRYI.					
LILIUM AFRICANUM.					
LILIUM MARIAGON.					
LILIUM SUPERBUM.					
LILIUM TENUIFOLIUM.					
LILIUM PARDALINUM.					
LILIUM PUBERULUM.					
FRITILLARIA MELEGRENS.					
FRITILLARIA PYRENAICA.					
FRITILLARIA PUDEICA.					
FRITILLARIA AUREA.					
FRITILLARIA ARMENA.					
FRITILLARIA IMPERIALIS VAR. AURORA.					
FRITILLARIA IMPERIALIS VAR. ALBA.					
FRITILLARIA RECURVA.					
CALOCHORTUS ALBUS.					
CALOCHORTUS ALBUS VAR. MAJOR.					
CALOCHORTUS BENTHAMI.					
CALOCHORTUS LILACINUS.					
CALOCHORTUS NITIDUS.					
CALOCHORTUS HOWELLII.					
CALOCHORTUS LEICHTLINII.					
CALOCHORTUS LUTEUS VAR. OCCULTUS.					
CALOCHORTUS SPLENDENS.					
TULIPA HAGERI.					
TULIPA SILVESTRIS.					
TULIPA GREIGI.					
TULIPA DUDLEYANA.					
TULIPA DUDLEYI.					
TULIPA DIDIERI VAR. MAURIANA.					
TULIPA DIDIERI VAR. FRANSONIANA.					
TULIPA CLUSIANA.					
TULIPA CLUSIANA VAR. PERSICA.					
TULIPA OCLUS-SOLIS.					
TULIPA PLECOX.					
TULIPA AUSTRALIS.					
SCILLA SIBIRICA.					
SCILLA PERUVIANA.					
SCILLA BIFOLIA.					
CHIRONDOXA LUCILIE.					
CHIRONDOXA TMOLUSI.					
CHIRONDOXA SARDENSIS.					
PUSCHKINA SCILLOIDES.					
PUSCHKINA SCL. VAR. LIBANOTICA.					
ORNITHOGALUM MUTANS.					
ORNITHOGALUM MUTANS VAR. (PYRAMDALE).					
ORNITHOGALUM MABONENSE VAR. AUREUM.					
ORNITHOGALUM THYRSOIDES VAR. AUREUM.					
ERYTHRONIUM DENSCANIS.					
ERYTHRONIUM DENSCANIS VAR. GRANO.					
ERYTHRONIUM AMERICANUM.					
ERYTHRONIUM GRANDIFLORUM.					
ERYTHRONIUM CITRINUM.					
ERYTHRONIUM CALIFORNICUM.					
HYACINTHUS ORIENT. VAR. ALBA SUPERB.					
HYACINTHUS ORIENT. VAR. ALBULUS (WHITE).					
HYACINTHUS ORIENT. VAR. ALBULUS (ITALIAN).					
GALTONIA CANDICANS.					
MUSCARI BOTRYOIDES.					
MUSCARI PARADOXUM.					
MUSCARI MICRANTHUM.					
MUSCARI COMPACTUM.					
MUSCARI RACEMOSUM.					
MUSCARI COMPACTUM.					
MUSCARI COMOSUM.					
BRODIAEA PEOINGULARIS.					
BRODIAEA IXIODES VAR. SPLENDENS.					
BRODIAEA CANDIDA.					
BRODIAEA LACTEA.					
BRODIAEA LAXA.					
BRODIAEA COCCINEA.					
BRODIAEA GRACILOPEA.					
BRODIAEA PANAMICA.					
BRODIAEA PUREYI.					
BRODIAEA STELLARIS.					
BRODIAEA CAPITATA.					
BRODIAEA CONGESTA.					
TRITELEIA UNIFLORA.					
LACHENALIA PENUDIA.					
LACHENALIA TRICOLOR VAR. LUTEOLA.					

CHART C.—The Gentian Violet Reactions of Various Starches—continued.

PART 3.

	VL	L	F	D	VD
CONYVALARIA MAJALIS					
TRILLIUM GRANDIFLORUM					
TRILLIUM OVATUM					
TRILLIUM SESSILE VAR. CALIFORNICUM					
COLCHICUM PARKINSONI					
AMARYLLIS BELLADONNA MAJOR					
HIPPEASTRUM VITATUM					
HIPPEASTRUM IBERICUM					
HIPPEASTRUM AULICUM VAR. ROBUSTUM					
VALLOTA PURPUREA					
CRINUM FIMBRILATUM					
CRINUM AMERICANUM					
ZEPHYRANTHES CANDIDA					
ZEPHYRANTHES ROSEA					
SPREKELIA FORMOSISSIMA					
HELMANTHUS KATHERINÆ					
HYMENOCALLIS UNDOULATA					
HYMENOCALLIS CALATHINA					
LEUCIUM VERNUM					
LEUCIUM ÆSTIVUM					
GALANTHUS NIVALIS					
GALANTHUS ELWESI					
ALSTREMERIA LIGITA					
ALSTREMERIA BRASILIENSIS					
ALSTREMERIA AFRICANA (UREA)					
STERNBERGIA LUTEA					
NARCISSUS HORSEFIELDI					
NARCISSUS MAXIMUS					
NARCISSUS BULBOCODIUM					
NARCISSUS BULBO VAR. CONSPECTA					
NARCISSUS INCORPORABILIS					
NARCISSUS ODORIS					
NARCISSUS POETICUS					
NARCISSUS BIFLORUS					
NARCISSUS JONQUILLA					
NARCISSUS JONQUILLA VAR. RUCULOSUS					
NARCISSUS JONQUILLA VAR. CAMPERNELLI ROG.					
NARCISSUS TAZETTA VAR. ORIENTALIS					
TACCA PINNATIFIDA					
IRIS FLORENTINA					
IRIS PALLIDA SPECIOSA					
IRIS PUMILA VAR. CYANEA					
IRIS BISMARCKIANA					
IRIS IBERICA					
IRIS XIPHIDIUM VAR. GRAND TRESORIER					
IRIS XIPHIDIUM VAR. WILHELMINE					
IRIS XIPHIDIUM VAR. LUSITANICA					
IRIS RETICULATA					
IRIS HISTRIG					
IRIS ALATA					
IRIS CAUCASICA					
MOREA TRISTIS					
HOMERA COLLINA					
TIGRIDA PAVONIA VAR. GRAND. ALBA					
TIGRIDA PAVONIA VAR. CONCHIFLORA					
GLADIOLUS BYZANTINUS					
GLADIOLUS PRIMULINIS					
GLADIOLUS CARDINALIS (BLUSH'G BRIDE)					
GLADIOLUS FLORIBUNDUS					
WATSONIA HUMILIS					
WATSONIA IRIDIFOLIA VAR. O'BRIENI					
WATSONIA MERIANA					
TRITONIA CROCATA					
TRITONIA CROCATA VAR. LILACINA					
TRITONIA CROCATA VAR. ROSEA					
TRITONIA SECURIGERA					
TRITONIA POTTII					
TRITONIA CROCOSMÆFLORA					
FREESIA REFRACTA VAR. ALBA					
FREESIA REFRACTA VAR. LEICHTLINI					
ANTHOLIZA CROCOSMOIDES					
ANTHOLIZA PANICULATA					
CROCUS SUSIANUS (CLOTH-OF-GOLD)					
CROCUS VERSICOLOR (CLOTH-OF-SILVER)					
CROCUS VAR. (BARON VON BRUNOV)					
ROMULEA ROSEA VAR. SPECIOSA					
CYPELLA BERBERTI					

PART 4.

	VL	L	F	D	VD
MARICA GRACILIS					
GELASINE AZUREA					
SPARAXIS GRANDIFLORA ALBA					
SPARAXIS VAR. (ALBERTINE)					
IXIA SPECIOSA					
IXIA VIRIDIFLORA					
IXIA VAR. (EMMA)					
BABIANA VAR. (VIOLACEA)					
BABIANA VAR. (ATHRACTION)					
MUSA CAVENDISHII (GREEN FRUIT)					
MUSA SAPIENTUM					
MUSA ENSETE					
ZINGIBER OFFICINALE					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 1					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 2					
ZINGIBER OFFIC. VAR. COCHIN.					
HEDYCHUM CORONARIUM					
HEDYCHUM GARDNERIANUM					
CURCUMA LONGA					
CURCUMA PETIOLATA					
CANNA WARSEWICZII					
CANNA ROSCOEANA					
CANNA MUSÆFOLIA					
CANNA EDULIS					
CANNA VAR. (KONIGEN CHARLOTTE)					
CANNA VAR. (PHELIPPE CARNOT)					
CANNA VAR. (MRS. KATE GREY)					
CANNA VAR. (JEAN TISSOT)					
CANNA VAR. (J. D. EISELE)					
MARANTA ARUNDINACEA					
MARANTA ARUNDINACEA VAR. NO. 1					
MARANTA ARUNDINACEA VAR. NO. 2					
MARANTA MASSANGEANA					
MARANTA LEUCONEURA					
MARANTA MUSAICA					
CALATHEA LIETZEL					
CALATHEA VITTIATA					
CALATHEA WOTIANA					
CALATHEA VANDEHNECKEL					
STROMANTHE SANGUINEA					
NYMPHÆA ALBA					
NYMPHÆA MARLIACEA VAR. ALBIDA					
NYMPHÆA MARLIACEA VAR. CARNEA					
NYMPHÆA GLADSTONIANA					
NYMPHÆA ODORATA					
NYMPHÆA ODORATA VAR. ROSEA					
NELUMBO NUCIFERA					
NELUMBO LUTEA					
ANEMONE APEENNINA					
ANEMONE FULGENS					
ANEMONE BLANDA					
ANEMONE JAPONICA					
ACONITUM NAPELLUS					
ACTÆA ALBA					
ACTÆA SPICATA VAR. RUBRA					
CIMICIFUGA RACEMOSA					
ERANTHUS HYEMALIS					
RANDIOLUS BULBOSUS					
RANDIOLUS FICARIA					
ADONIS ANURENSIS					
COCHLEARIA ARMORACA					
IATROPHA CURCAS					
MANHOT UTILISSIMA					
CYCLAMEN REPANDITUM					
CYCLAMEN COMUM					
SOLANUM TUBEROSUM					
BATAIAS EDULIS					
GESNERIA TUBIFLORA					
GLOXINIA VAR.					
TRIANOSPHERIA FICIFOLIA					
CYCAS REVOLUTA					
CYCAS CIRCIHALIS					
DMOON EDULE					
ZAMIA INTEGRIFOLIA					



CHART D.—The Safranin Reactions of Various Starches—continued.

PART 3.

	VL	L	F	D	VD
CONVALLARIA MAJALIS.					
TRILLIUM GRANDIFLORUM.					
TRILLIUM BESSLEYI VAR. CALIFORNICUM.					
TRILLIUM BESSLEYI VAR. CALIFORNICUM.					
COLCHICUM PARKINSONI.					
AMARYLLIS BELLADONNA MAJOR.					
HIPPEASTRUM VITTATUM.					
HIPPEASTRUM FOLIOSUM.					
HIPPEASTRUM AULICUDA VAR. ROBUSTUM.					
VALLOTA PURPUREA.					
GRINUM FIMBRITATULUM.					
GRINUM AMERICANUM.					
ZEPHYRANTHES CANDIDA.					
ZEPHYRANTHES ROSA.					
SPEKELIA FORMOSISSIMA.					
HÆMANTHUS KATHERINE.					
HYMENOCALLIS UNDULATA.					
HYMENOCALLIS CALATHINA.					
LEUCODIUM VERNUM.					
LEUCODIUM ESTIVUM.					
GALANTHUS NIVALIS.					
GALANTHUS ELWESI.					
ALSTREMERIA LIGULI.					
ALSTREMERIA BRASILIENSIS.					
ALSTREMERIA AFRICANTLACA (AUREA).					
STERBERGIA LUTEA.					
NARCISSUS HORSFIELDII.					
NARCISSUS MAXIMUS.					
NARCISSUS BULBOCODIUM.					
NARCISSUS FULBO VAR. CONSPICUA.					
NARCISSUS FULBO VAR. MONOPHYLLUS.					
NARCISSUS GORRIUS.					
NARCISSUS POEFTICUS.					
NARCISSUS BIFLORUS.					
NARCISSUS JONQUILLA.					
NARCISSUS JONQUILLA VAR. RUGULOSUS.					
NARCISSUS JONQUILLA VAR. CAMPANELLI RUG.					
NARCISSUS TAZETTA VAR. ORIENTALIS.					
TACCA PINNATIFIDA.					
IRIS FLORENTINA.					
IRIS PALLIDA SPECIOSA.					
IRIS PUMILA VAR. CYANEA.					
IRIS BISMARCKIANA.					
IRIS IBERICA.					
IRIS XIPHUM VAR. GRAND TRESORIER.					
IRIS XIPHUM VAR. WILHELMINE.					
IRIS XIPHUM VAR. LUSHANICA.					
IRIS XIPHUM VAR. LUSHANICA.					
IRIS RETICULATA.					
IRIS HISTRIO.					
IRIS ALATA.					
IRIS CAUCASICA.					
MORÆA TRISTIS.					
HOMERA COLLINA.					
TIGRIDA PAVONIA VAR. GRAND ALBA.					
TIGRIDA PAVONIA VAR. CONCHIFLORA.					
GLADIOLUS BYZANTINUS.					
GLADIOLUS PRIMULINUS.					
GLADIOLUS CARDINALIS (BLUSH'G BRIDE).					
GLADIOLUS FLORIBUNDUS.					
WATSONIA HUMILIS.					
WATSONIA IRIDIIFOLIA VAR. OTRENTI.					
WATSONIA MERIANA.					
TRITONIA CROCATA.					
TRITONIA CROCATA VAR. LILACINA.					
TRITONIA CROCATA VAR. ROSEA.					
TRITONIA SECURIGERA.					
TRITONIA FORTSIL.					
TRITONIA CROCOSMEZELORA.					
FREESIA REFRACTA VAR. ALBA.					
FREESIA REFRACTA VAR. LEICHTLINI.					
ANTHOLYZA CROCOSMOIDES.					
ANTHOLYZA PANICULATA.					
CROCUS SDSANTOR (CLOTH-OF-GOLD).					
CROCUS VERSICOLOR (CLOTH-OF-SILVER).					
CROCUS VAR. ORAHOH (VON BREUNOW).					
ROMULEA ROSA VAR. SPECIOSA.					
CYPELLA HERBERTI.					

PART 4.

	VL	L	F	D	VD
MARICA GRACILIS.					
GELASIRE AZUREA.					
SPARAXIS GRANDIFLORA ALBA.					
SPARAXIS VAR. (ALBERTINE).					
IXIA SPECIOSA.					
IXIA VIRIDIFLORA.					
IXIA VAR. (EMMA).					
BABIANA VAR. (VIOLECEA).					
BABIANA VAR. (ATHRACTIO).					
MUSA CAVENTOSHII (GREEN FRUIT).					
MUSA CAVENTOSHII (GREEN FRUIT).					
MUSA ENSETI.					
ZINGIBER OFFICINALE.					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 1.					
ZINGIBER OFFIC. VAR. JAMAICA, NO. 2.					
ZINGIBER OFFIC. VAR. COCHIN.					
HEDYCHUM CORONARIUM.					
HEDYCHUM GARDNERIANUM.					
CURCUMA LONGA.					
CURCUMA PETIOLATA.					
CANNA WARSZEWICZII.					
CANNA ROSCOEANA.					
CANNA MUS-REFOLIA.					
CANNA EDLIS.					
CANNA VAR. (KONIGEN CHARLOTTE).					
CANNA VAR. (PRESIDENT CARRHOT).					
CANNA VAR. (L. E. HULLI).					
CANNA VAR. (JEAN TISSOT).					
CANNA VAR. (J. D. FISLE).					
MARANTA ARUNDINACEA.					
MARANTA ARUNDINACEA VAR. NO. 1.					
MARANTA ARUNDINACEA VAR. NO. 2.					
MARANTA MASSANGANA.					
MARANTA LENCONEURA.					
MARANTA MUSAICA.					
CALATHEA LIETZEL.					
CALATHEA VITATA.					
CALATHEA WOTIANA.					
CALATHEA VANDENHECKEL.					
STROMANTHE SANGUINEA.					
NYMPHÆA ALBA.					
NYMPHÆA ALBA VAR. ALBIDA.					
NYMPHÆA MARLIACEA VAR. CARNEA.					
NYMPHÆA GLADSTONIANA.					
NYMPHÆA ODORATA.					
NYMPHÆA ODORATA VAR. ROSEA.					
NELUMBO NUCIFERA.					
NELUMBO LUTEA.					
ANEMONE APERNINA.					
ANEMONE FULGENS.					
ANEMONE BLANDA.					
ANEMONE JAPONICA.					
ACONITUM NAPELLUS.					
ACTÆA ALBA.					
ACTÆA SPICATA VAR. RUBRA.					
CIMICIFUGA RACEMOSA.					
ERANTHIS HYEMALIS.					
RANUNCULUS BILBOUSUS.					
RANUNCULUS FICARIA.					
ADONIS AMURENSIS.					
COCHLEARIA ARMORACIA.					
JATROPHA CURCAS.					
MANIHOT UTILISSIMA.					
CYCLAMEN REPANDUM.					
CYCLAMEN COM.					
SOLANUM TUBEROSUM.					
BATATA EDULIS.					
GESNERIA TUBIFLORA.					
GLOXINIA VAR.					
TRIANOSPHERMA FICIFOLIA.					
CYCAS REVOLUTA.					
CYCAS CIRCNALIS.					
DIOON EDDLE.					
ZAMIA INTEGERSFOLIA.					

CHART E.—The Temperatures of Gelatinization of Various Starches.

PART 1.

PART 2.

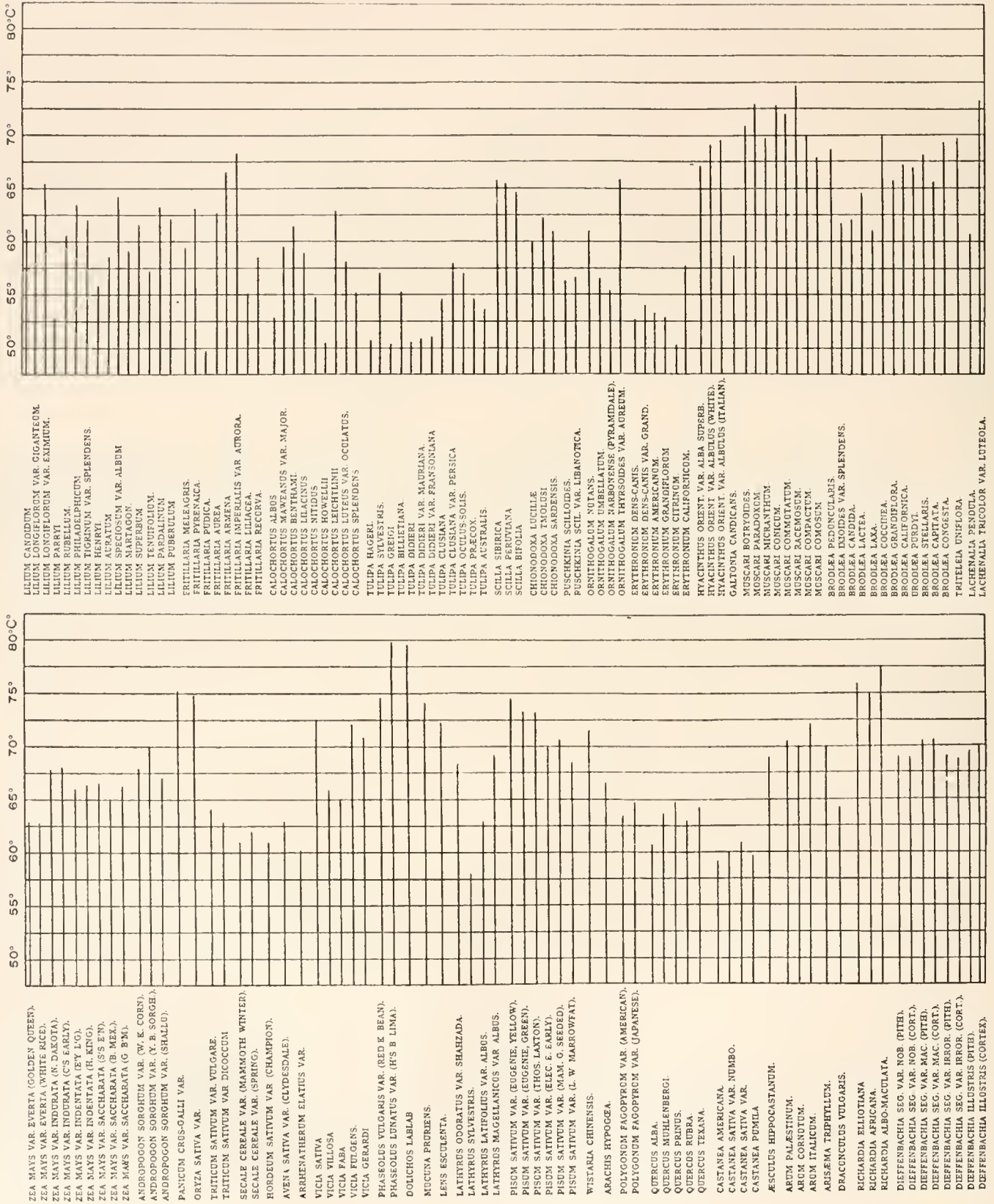
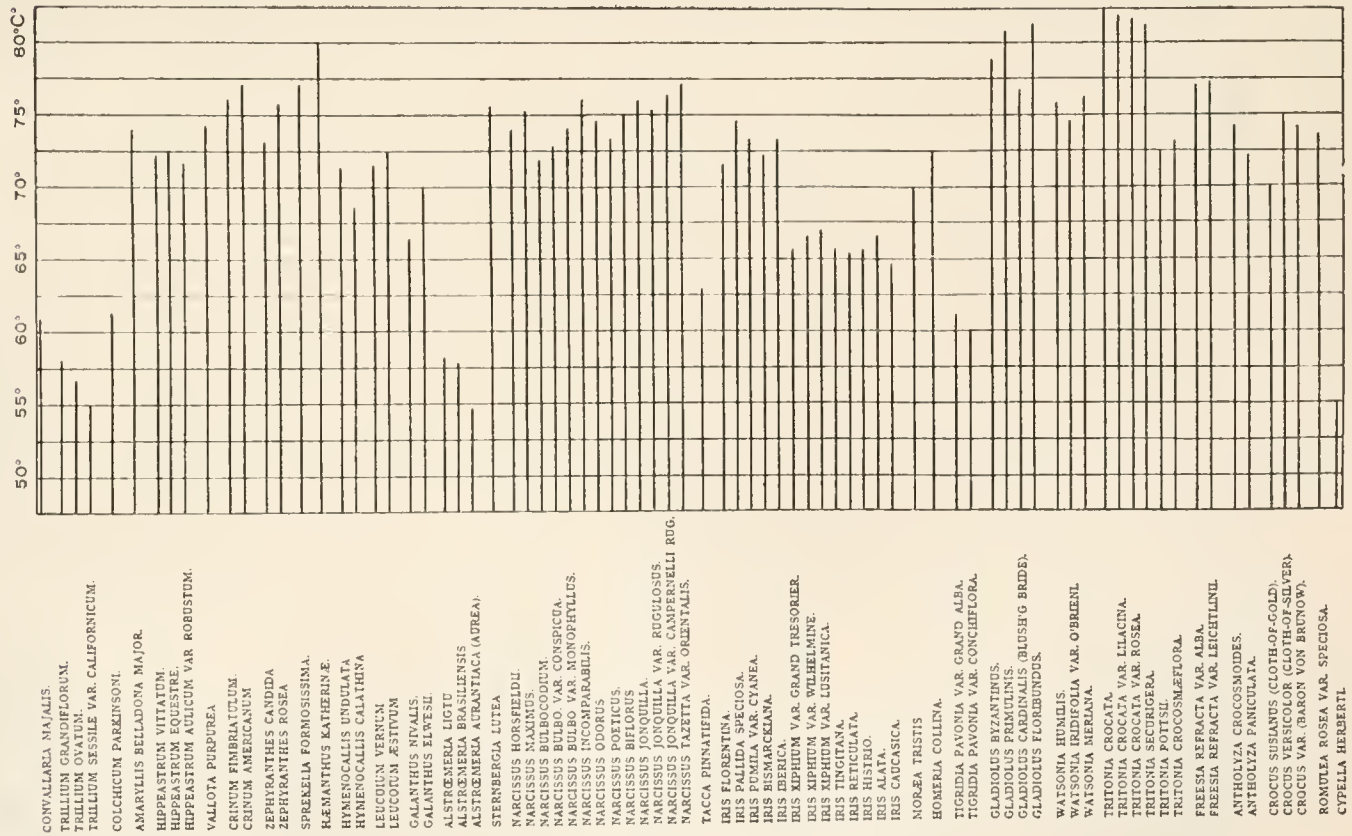


CHART E.—The Temperatures of Gelatinization of Various Starches—continued.

PART 3.



PART 4.

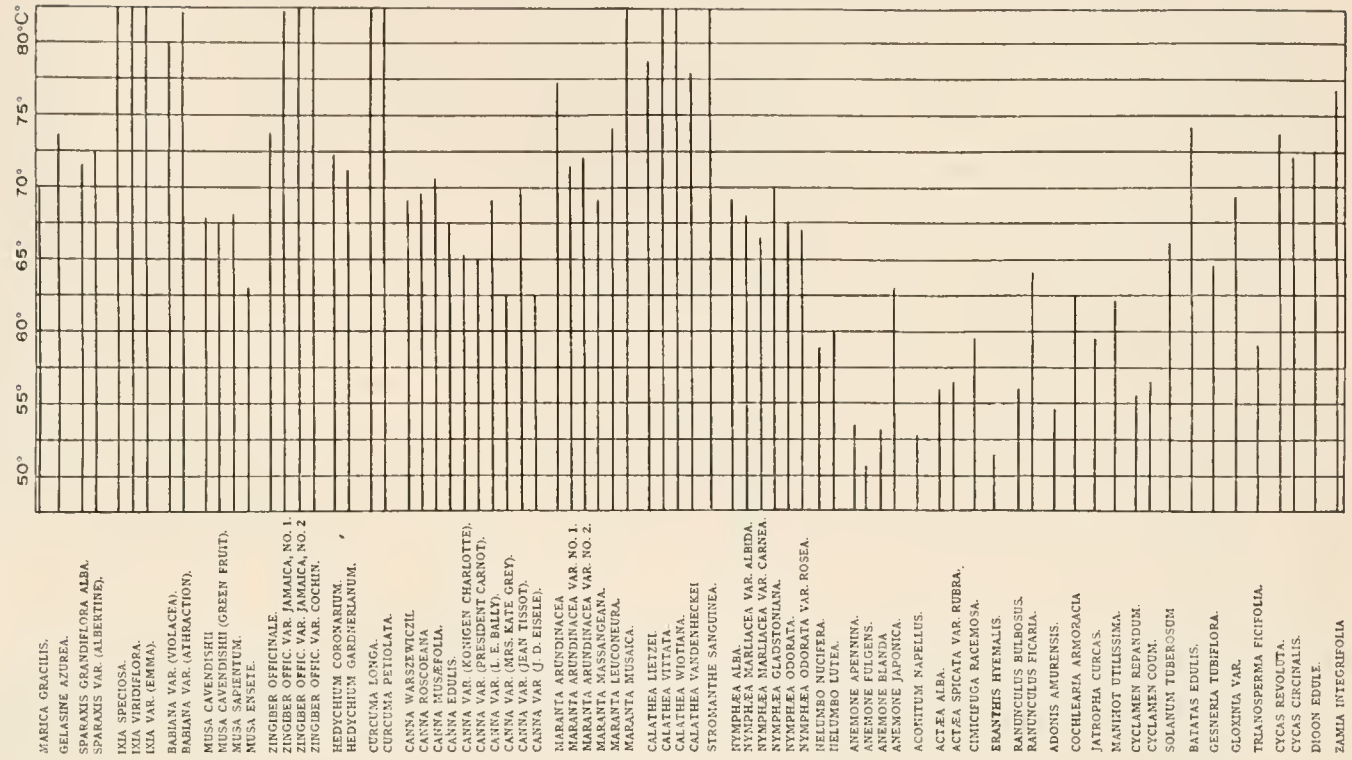
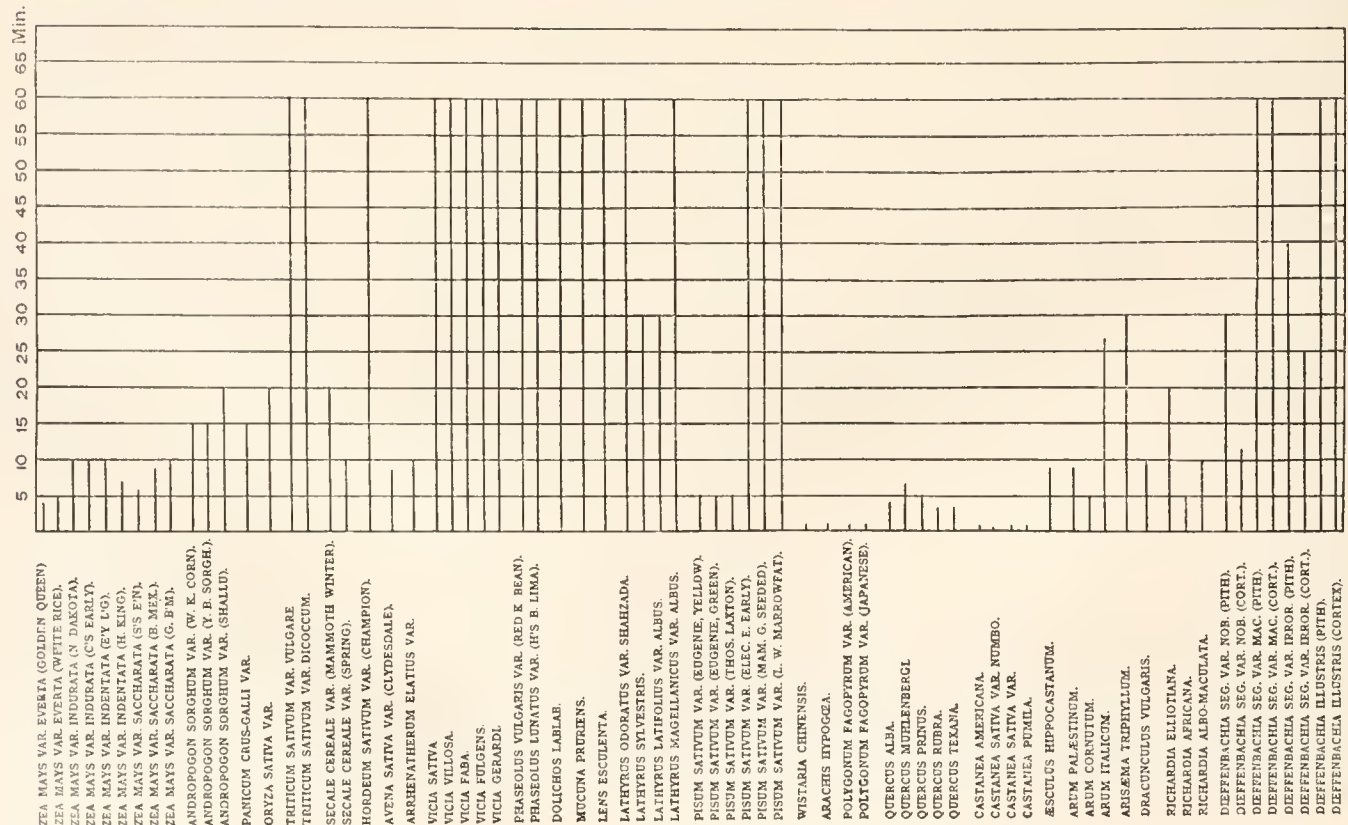


CHART F.—The Chloral Hydrate-Iodine Reactions of Various Starches.

PART 1.



PART 2.

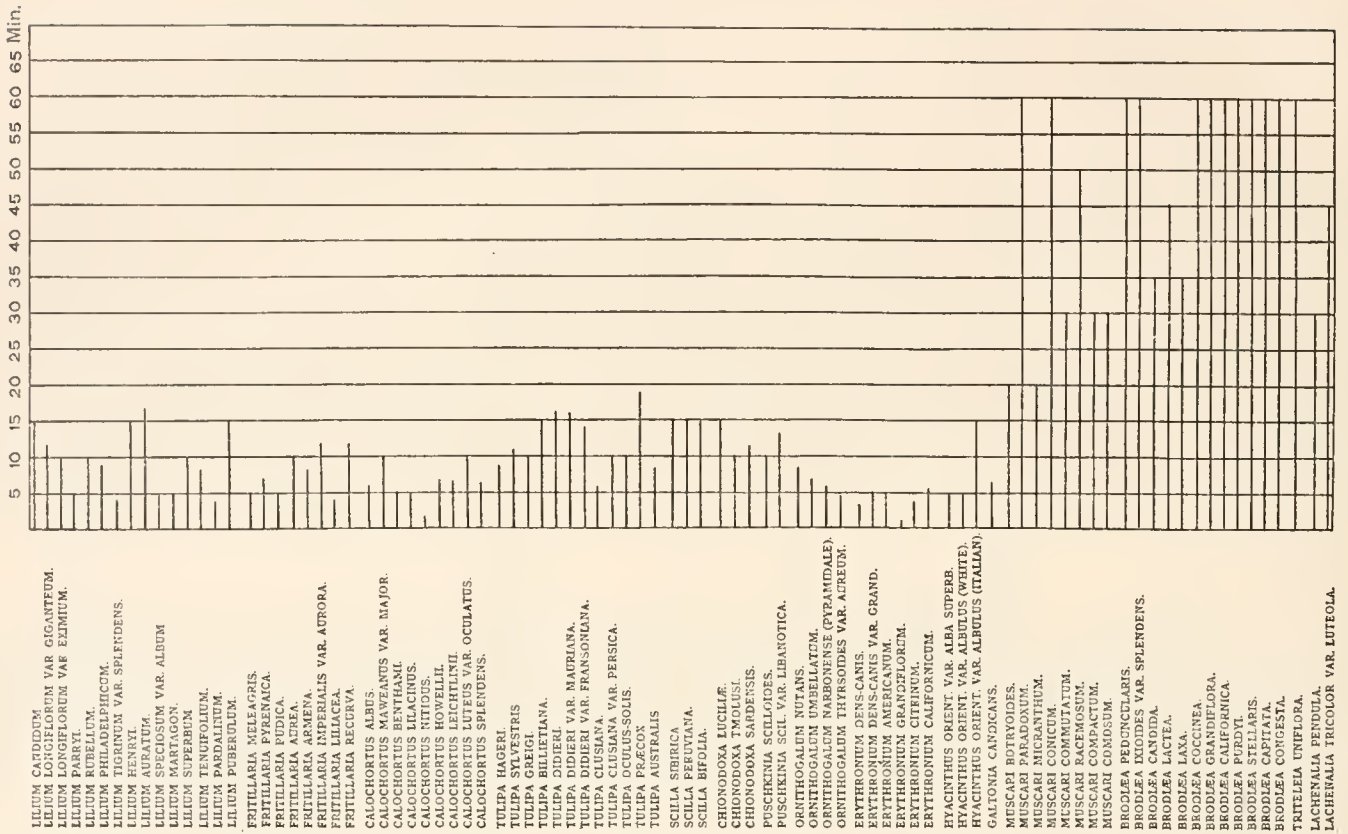
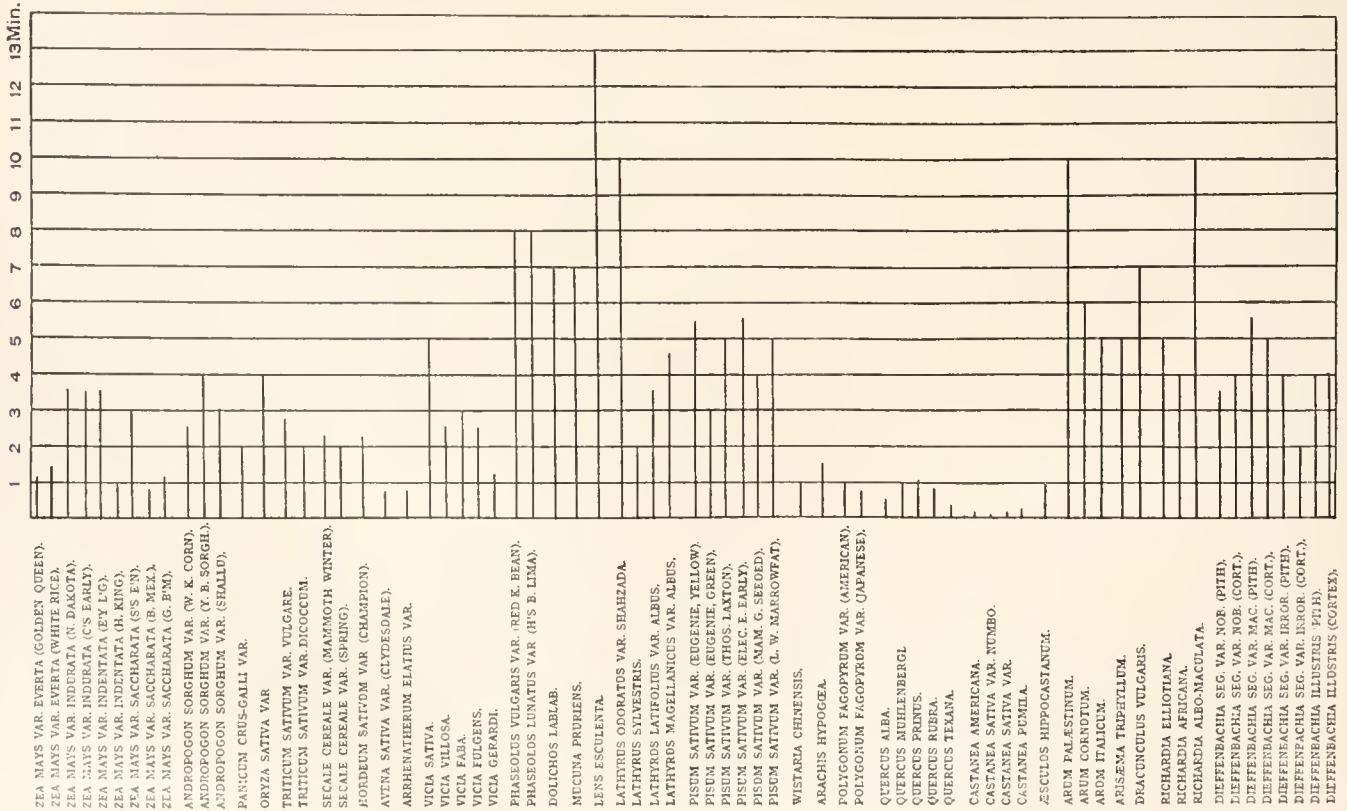




CHART G.—The Chromic Acid Reactions of Various Starches.

PART 1.



PART 2.

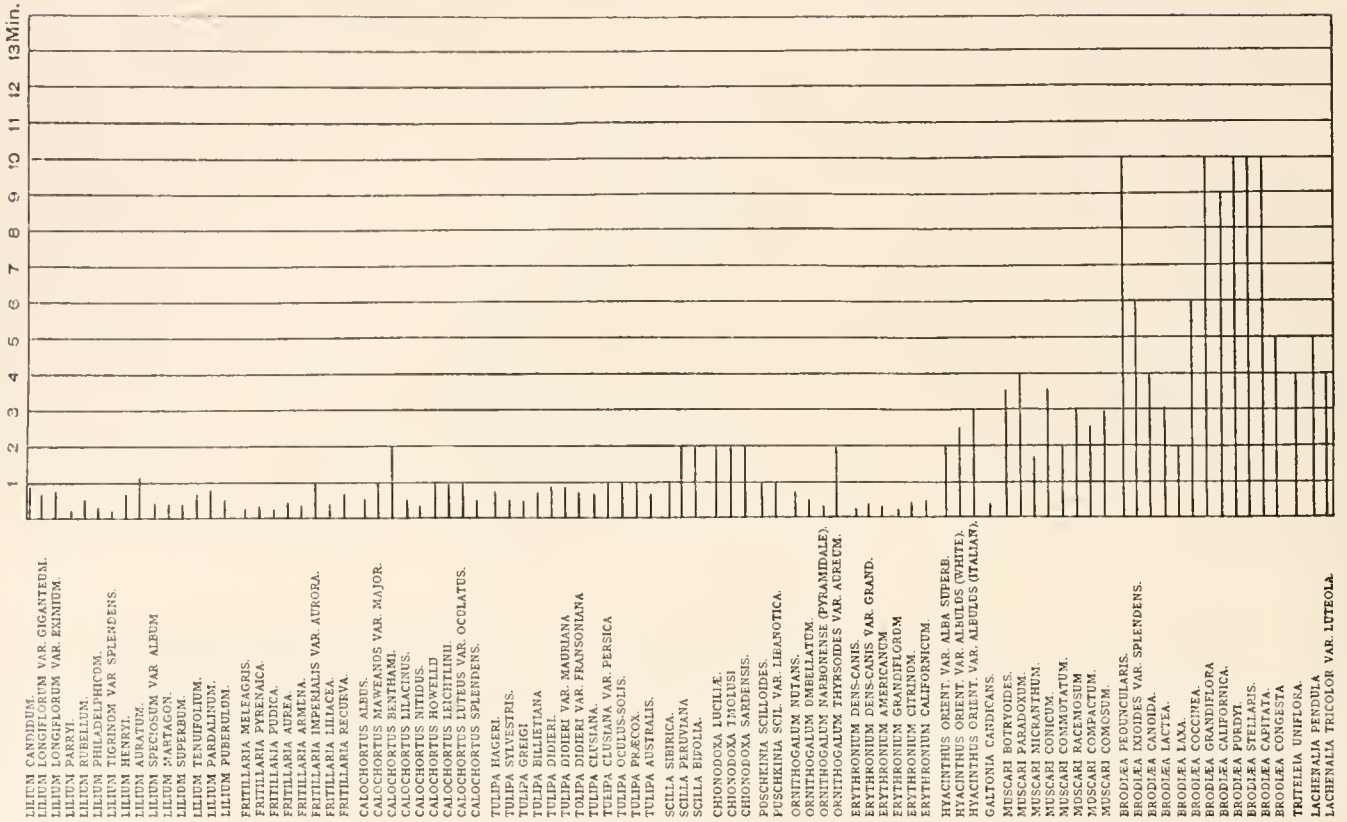
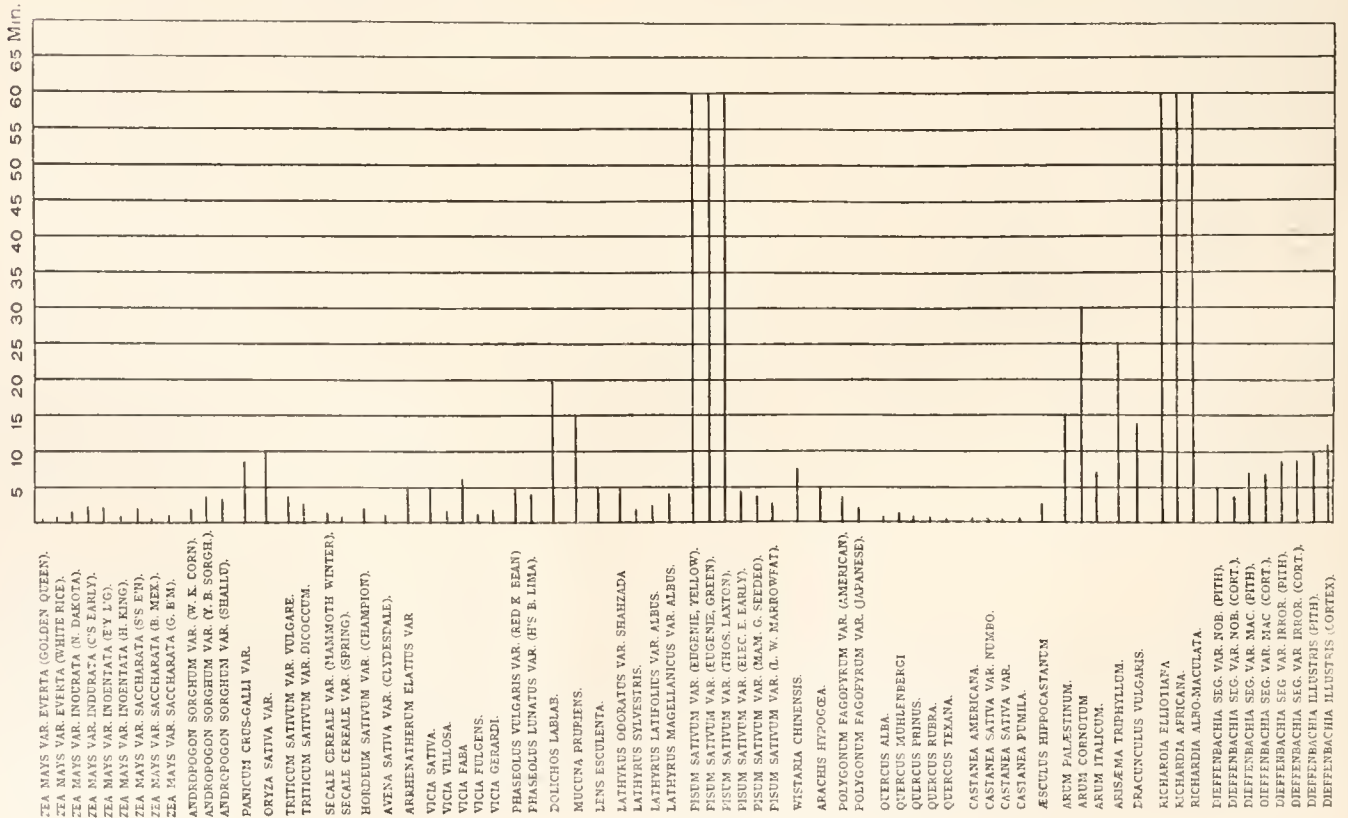




CHART H.—The Pyrogallic Acid Reactions of Various Starches.

PART 1.



PART 2.

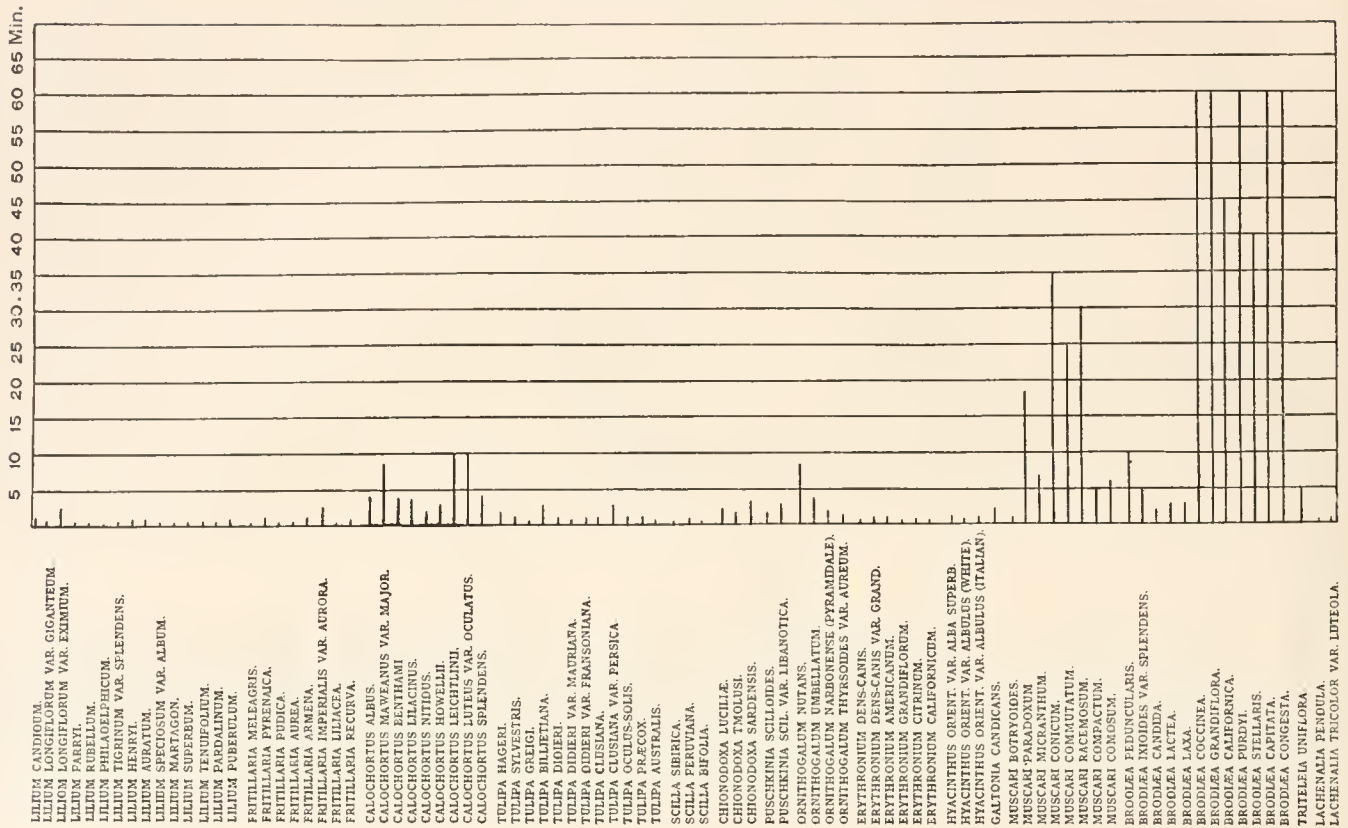
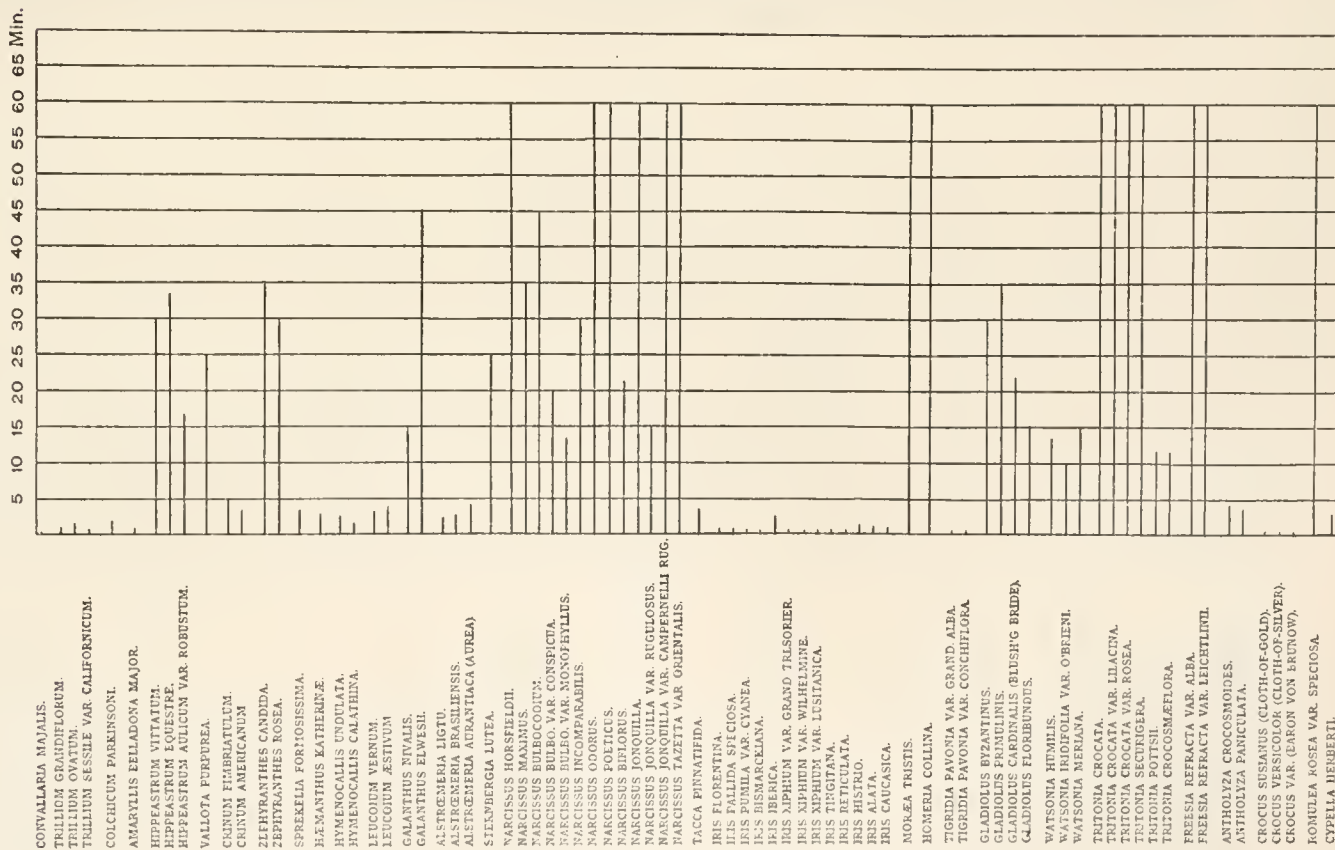


CHART H.—The Pyrogallic Acid Reactions of Various Starches—continued.

PART 3.



PART 4.

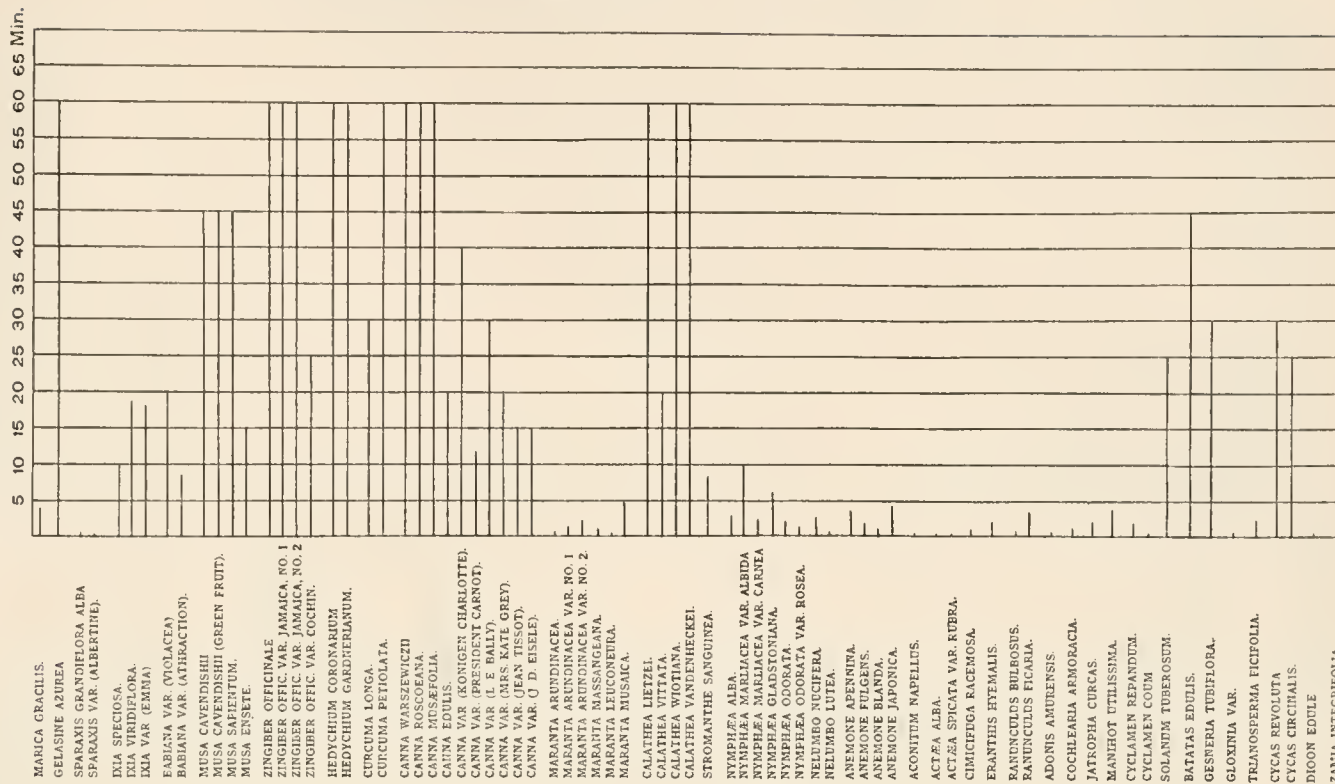
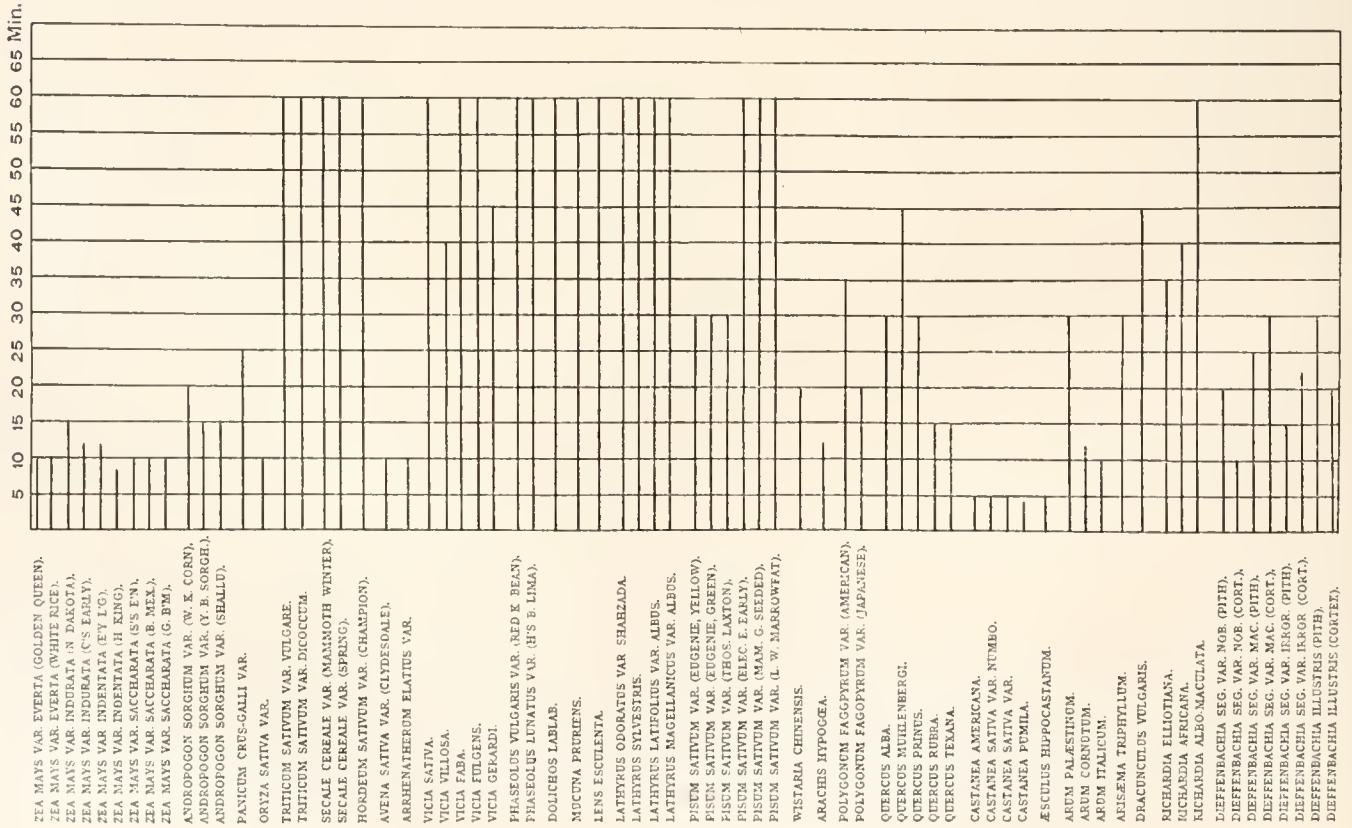
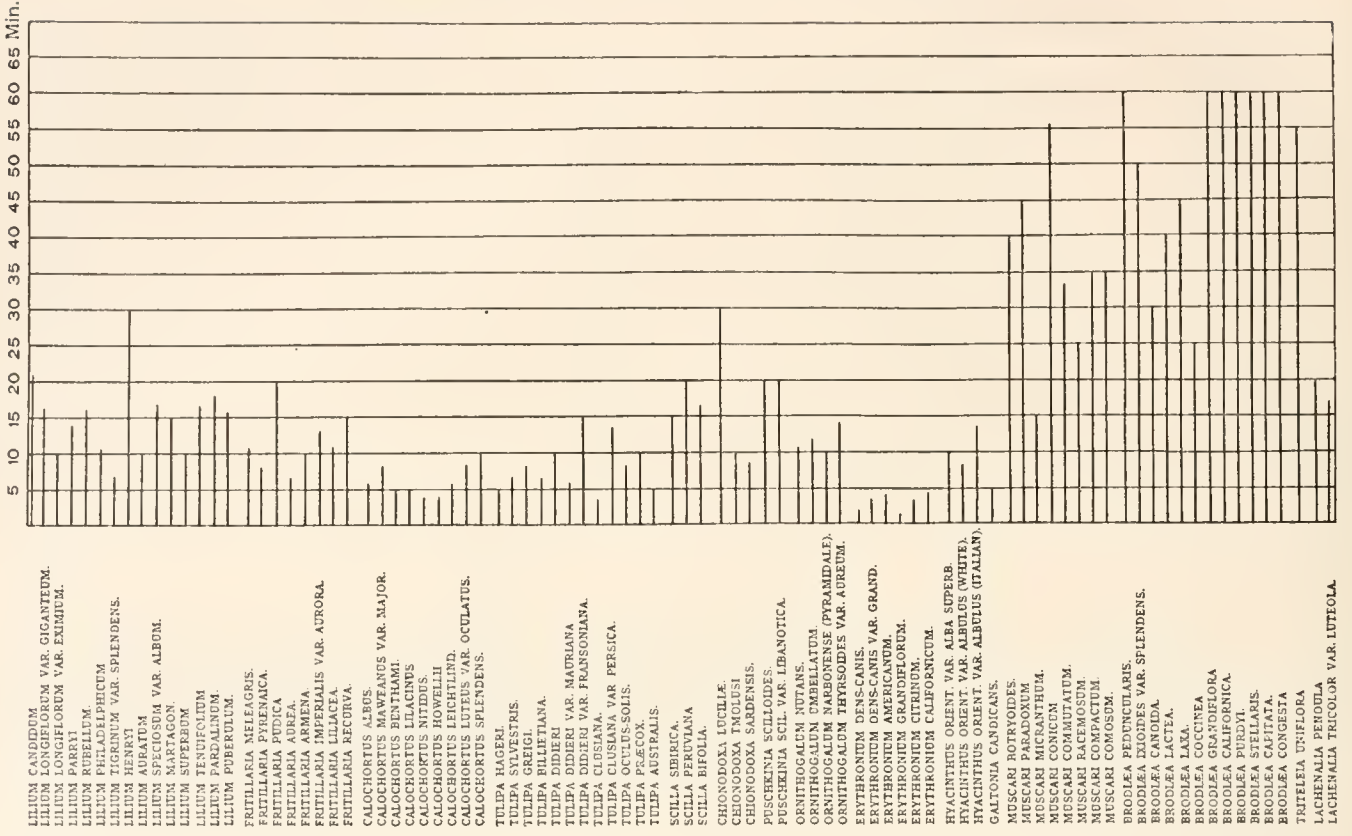


CHART I.—The Ferric Chloride Reactions of Various Starches.

PART I.



PART 2.









## THE MEAN TEMPERATURES OF GELATINIZATION OF VARIOUS STARCHES.

The importance that has been attached to differences in the temperatures of gelatinization of starches, together with the fact of the exceedingly contradictory records by different investigators, and the reliability of the method used in this research, together with the value of this means alone of differentiating starches, has suggested that a table stating the mean temperature records recorded in this investigation would be found of special convenience and value.

*The Mean Temperatures of Gelatinization of Various Starches.*

<i>Zea mays</i> var. <i>everta</i> (Golden Queen).....	63.25°	<i>Richardia</i> <i>albo-maculata</i> .....	77.50°
<i>Zea mays</i> var. <i>everta</i> (White Rice).....	63.25°	<i>Dieffenbachia</i> <i>seguine</i> var. <i>nobilis</i> (Pith).....	69.00°
<i>Zea mays</i> var. <i>indurata</i> (North Dakota).....	68.00°	<i>Dieffenbachia</i> <i>seguine</i> var. <i>nobilis</i> (Cortex).....	69.00°
<i>Zea mays</i> var. <i>indurata</i> (Compton's Early).....	68.50°	<i>Dieffenbachia</i> <i>seguine</i> var. <i>maculata</i> (Pith).....	70.50°
<i>Zea mays</i> var. <i>indentata</i> (Early Leaning).....	66.50°	<i>Dieffenbachia</i> <i>seguine</i> var. <i>maculata</i> (Cortex).....	70.60°
<i>Zea mays</i> var. <i>indentata</i> (Hickory King).....	66.75°	<i>Dieffenbachia</i> <i>seguine</i> var. <i>irrorata</i> (Pith).....	69.00°
<i>Zea mays</i> var. <i>saccharata</i> (Stowell's Evergreen).....	66.85°	<i>Dieffenbachia</i> <i>seguine</i> var. <i>irrorata</i> (Cortex).....	68.75°
<i>Zea mays</i> var. <i>saccharata</i> (Black Mexican).....	65.00°	<i>Dieffenbachia</i> <i>illustris</i> (Pith).....	69.50°
<i>Zea mays</i> var. <i>saccharata</i> (Golden Bantam).....	66.50°	<i>Dieffenbachia</i> <i>illustris</i> (Cortex).....	70.00°
<i>Andropogon sorghum</i> var. (White Kaffr Corn).....	68.00°	<i>Lilium</i> <i>candidum</i> .....	61.65°
<i>Andropogon sorghum</i> var. (Yellow Branching Sorghum).....	70.00°	<i>Lilium</i> <i>longiflorum</i> var. <i>giganteum</i> .....	62.55°
<i>Andropogon sorghum</i> var. (Shallu).....	66.90°	<i>Lilium</i> <i>longiflorum</i> var. <i>eximium</i> .....	65.60°
<i>Panicum crus-galli</i> var.....	75.25°	<i>Lilium</i> <i>parryi</i> .....	52.60°
<i>Oryza sativa</i> var.....	74.75°	<i>Lilium</i> <i>rubellum</i> .....	63.95°
<i>Triticum sativum</i> var. <i>vulgare</i> .....	64.00°	<i>Lilium</i> <i>philadelphicum</i> .....	63.90°
<i>Triticum sativum</i> var. <i>dicoccum</i> .....	62.90°	<i>Lilium</i> <i>tigrinum</i> var. <i>splendens</i> .....	62.05°
<i>Secale cereale</i> var. (Mammoth Winter).....	61.00°	<i>Lilium</i> <i>henryi</i> .....	56.00°
<i>Secale cereale</i> var. (Spring).....	62.00°	<i>Lilium</i> <i>auratum</i> .....	58.60°
<i>Hordeum sativum</i> var. (Champion).....	61.00°	<i>Lilium</i> <i>speciosum</i> var. <i>album</i> .....	61.35°
<i>Avena sativa</i> var. (Clydesdale).....	63.00°	<i>Lilium</i> <i>martagon</i> .....	59.10°
<i>Arrhenatherum clatius</i> var.....	60.25°	<i>Lilium</i> <i>superbum</i> .....	61.60°
<i>Vicia sativa</i> .....	72.50°	<i>Lilium</i> <i>tenuifolium</i> .....	57.30°
<i>Vicia villosa</i> .....	66.00°	<i>Lilium</i> <i>pardalinum</i> .....	63.30°
<i>Vicia faba</i> .....	65.00°	<i>Lilium</i> <i>puberulum</i> .....	62.15°
<i>Vicia fulgens</i> .....	72.00°	<i>Fritillaria</i> <i>meleagris</i> .....	59.50°
<i>Vicia gerardi</i> .....	71.00°	<i>Fritillaria</i> <i>pyrenaica</i> .....	63.15°
<i>Phaseolus vulgaris</i> var. (Red Kidney Bean).....	74.50°	<i>Fritillaria</i> <i>pudivca</i> .....	49.60°
<i>Phaseolus lunatus</i> var. (Henderson's Bush Lima).....	79.75°	<i>Fritillaria</i> <i>aurea</i> .....	62.75°
<i>Dolichos lablab</i> .....	79.50°	<i>Fritillaria</i> <i>armena</i> .....	66.60°
<i>Mucuna pruriens</i> .....	74.00°	<i>Fritillaria</i> <i>imperialis</i> var. <i>aurora</i> .....	68.40°
<i>Lens esculenta</i> .....	72.25°	<i>Fritillaria</i> <i>lilacea</i> .....	55.10°
<i>Lathyrus odoratus</i> var. <i>shahzada</i> .....	68.50°	<i>Fritillaria</i> <i>recurva</i> .....	58.70°
<i>Lathyrus sylvestris</i> .....	58.00°	<i>Calochortus</i> <i>albus</i> .....	53.00°
<i>Lathyrus latifolius</i> var. <i>albus</i> .....	63.00°	<i>Calochortus</i> <i>mawcanus</i> var. <i>major</i> .....	59.50°
<i>Lathyrus magellanicus</i> var. <i>albus</i> .....	69.00°	<i>Calochortus</i> <i>benthani</i> .....	61.50°
<i>Pisum sativum</i> var. (Eugenie, Yellow).....	73.50°	<i>Calochortus</i> <i>lilacinus</i> .....	59.00°
<i>Pisum sativum</i> var. (Eugenie, Green).....	74.50°	<i>Calochortus</i> <i>nitidus</i> .....	51.75°
<i>Pisum sativum</i> var. (Thomas Laxton).....	73.25°	<i>Calochortus</i> <i>howellii</i> .....	50.50°
<i>Pisum sativum</i> var. (Electric Extra Early).....	70.00°	<i>Calochortus</i> <i>leichtlinii</i> .....	63.00°
<i>Pisum sativum</i> var. (Mammoth Gray Seeded).....	70.50°	<i>Calochortus</i> <i>luteus</i> var. <i>oculatus</i> .....	58.10°
<i>Pisum sativum</i> var. (Large White Marrowfat).....	68.50°	<i>Calochortus</i> <i>splendens</i> .....	55.10°
<i>Wistaria chinensis</i> .....	71.50°	<i>Tulipa</i> <i>hageri</i> .....	56.90°
<i>Arachis hypogaea</i> .....	66.50°	<i>Tulipa</i> <i>sylvestris</i> .....	57.15°
<i>Polygonum fagopyrum</i> var. (American).....	63.50°	<i>Tulipa</i> <i>greigi</i> .....	50.30°
<i>Polygonum fagopyrum</i> var. (Japanese).....	64.75°	<i>Tulipa</i> <i>billietiana</i> .....	55.25°
<i>Quercus alba</i> .....	60.75°	<i>Tulipa</i> <i>didieri</i> .....	50.75°
<i>Quercus muhlenbergi</i> .....	63.75°	<i>Tulipa</i> <i>didieri</i> var. <i>mauriana</i> .....	50.95°
<i>Quercus prinus</i> .....	64.75°	<i>Tulipa</i> <i>didieri</i> var. <i>fransoniana</i> .....	54.85°
<i>Quercus rubra</i> .....	63.00°	<i>Tulipa</i> <i>elusiana</i> .....	57.10°
<i>Quercus texana</i> .....	64.25°	<i>Tulipa</i> <i>elusiana</i> var. <i>persica</i> .....	54.75°
<i>Castanea americana</i> .....	59.25°	<i>Tulipa</i> <i>oculus-solis</i> .....	53.75°
<i>Castanea sativa</i> var. <i>numbo</i> .....	60.00°	<i>Tulipa</i> <i>præcox</i> .....	55.80°
<i>Castanea sativa</i> var.....	61.00°	<i>Tulipa</i> <i>australis</i> .....	51.15°
<i>Castanea pumila</i> .....	59.75°	<i>Scilla</i> <i>sibirica</i> .....	66.00°
<i>Aesculus hippocastanum</i> .....	69.00°	<i>Scilla</i> <i>peruviana</i> .....	65.50°
<i>Arum palæstinum</i> .....	70.50°	<i>Scilla</i> <i>bifolia</i> .....	64.75°
<i>Arum cornutum</i> .....	70.00°	<i>Chionodoxa</i> <i>lucilike</i> .....	60.00°
<i>Arum italicum</i> .....	72.00°	<i>Chionodoxa</i> <i>tmolusi</i> .....	62.35°
<i>Arisæma triphyllum</i> .....	70.00°	<i>Chionodoxa</i> <i>sardensis</i> .....	61.10°
<i>Dracunculus vulgaris</i> .....	64.25°	<i>Puschkinia</i> <i>scilloides</i> .....	56.35°
<i>Richardia elliotiana</i> .....	76.00°	<i>Puschkinia</i> <i>scilloides</i> var. <i>libanotica</i> .....	56.70°
<i>Richardia africana</i> .....	75.00°	<i>Ornithogalum</i> <i>nutans</i> .....	61.25°
		<i>Ornithogalum</i> <i>umbellatum</i> .....	56.75°

*The Mean Temperatures of Gelatinization of Various Starches.—Continued.*

Ornithogalum narbonense (pyramidale).....	55.50°	Iris florentina.....	71.50°
Ornithogalum thyrsoides var. aureum.....	66.00°	Iris pallida speciosa.....	74.50°
Erythronium dens-canis.....	52.65°	Iris pumila var. cyanea.....	73.25°
Erythronium dens-canis var. grandiflorum.....	51.00°	Iris bismarekiana.....	72.00°
Erythronium americanum.....	53.45°	Iris iberica.....	73.10°
Erythronium grandiflorum.....	53.00°	Iris xiphium var. grand tresorier.....	65.50°
Erythronium citrinum.....	50.45°	Iris xiphium var. wilhelmine.....	66.50°
Erythronium californicum.....	57.90°	Iris xiphium var. lusitania.....	67.00°
Hyacinthus orientalis var. alba superbissima.....	67.00°	Iris tingiana.....	65.50°
Hyacinthus orientalis var. albulus (White).....	69.00°	Iris reticulata.....	65.25°
Hyacinthus orientalis var. albulus (Italian).....	69.50°	Iris histrio.....	65.50°
Galtonia candicans.....	58.60°	Iris alata.....	66.50°
Muscari botryoides.....	71.00°	Iris caucasica.....	64.50°
Muscari paradoxum.....	73.00°	Moræa tristis.....	70.00°
Muscari micranthum.....	69.75°	Homeria collina.....	72.50°
Muscari conicum.....	73.00°	Tigridia pavonia var. grandiflora alba.....	61.00°
Muscari commutatum.....	72.00°	Tigridia pavonia var. conchiflora.....	60.00°
Muscari racemosum.....	74.75°	Gladiolus byzantinus.....	78.50°
Muscari compactum.....	70.00°	Gladiolus primulinus.....	80.50°
Muscari comosum.....	68.00°	Gladiolus cardinalis (Blushing Bride).....	76.50°
Brodiaea peduncularis.....	68.50°	Gladiolus floribundus.....	81.00°
Brodiaea ixioides var. splendens.....	61.75°	Watsonia humilis.....	75.50°
Brodiaea candida.....	62.00°	Watsonia iridifolia var. o'brici.....	74.50°
Brodiaea lactea.....	64.50°	Watsonia meriana.....	76.25°
Brodiaea laxa.....	61.00°	Tritonia crocata.....	82.50°
Brodiaea coccinea.....	70.00°	Tritonia crocata var. filacina.....	81.75°
Brodiaea grandiflora.....	65.90°	Tritonia crocata var. rosea.....	81.50°
Brodiaea californica.....	67.30°	Tritonia securigera.....	81.00°
Brodiaea purdyi.....	67.00°	Tritonia pottsi.....	72.50°
Brodiaea stellaris.....	68.10°	Tritonia crocosmaeflora.....	73.00°
Brodiaea capitata.....	65.57°	Freesia refracta var. alba.....	76.75°
Brodiaea congesta.....	69.25°	Freesia refracta var. leichtlinii.....	77.25°
Triticleia uniflora.....	69.45°	Antholyza crocosmoides.....	74.00°
Lachenalia pendula.....	60.65°	Antholyza paniculata.....	72.00°
Lachenalia tricolor var. luteola.....	73.10°	Crocus susianus (Cloth-of-Gold).....	70.00°
Convallaria majalis.....	61.00°	Crocus versicolor (Cloth-of-Silver).....	75.00°
Trillium grandiflorum.....	58.00°	Crocus var. (Baron von Brunow).....	74.00°
Trillium ovatum.....	56.75°	Romulea rosea var. speciosa.....	73.50°
Trillium sessile var. californicum.....	55.00°	Cepylla herberti.....	55.00°
Cofehicum parkinsoni.....	61.25°	Marica gracilis.....	70.00°
Anaryllis belladonna major.....	74.00°	Gelasine azurea.....	73.50°
Hippeastrum vittatum.....	72.00°	Sparaxis grandiflora alba.....	71.50°
Hippeastrum equestre.....	72.50°	Sparaxis var. (Albertine).....	72.50°
Hippeastrum aulicum var. robustum.....	71.75°	Ixia speciosa.....	84.00°
Vallota purpurea.....	74.25°	Ixia viridiflora.....	83.00°
Crinum fimbriatum.....	76.00°	Ixia var. (Emma).....	84.50°
Crinum americanum.....	77.00°	Babiana var. (Violacea).....	80.00°
Zephyranthes candida.....	73.00°	Babiana var. (Anthraction).....	82.00°
Zephyranthes rosea.....	76.00°	Musa cavendishii.....	67.75°
Spreckelia formosissima.....	77.00°	Musa cavendishii (green fruit).....	67.50°
Hæmanthus katherinae.....	80.00°	Musa sapientum.....	68.00°
Hymenocallis undulata.....	71.50°	Musa ensete.....	63.00°
Hymenocallis calathina.....	68.50°	Zingiber officinale.....	73.85°
Leucoium vernum.....	71.50°	Zingiber officinale var. Jamaica, No. 1.....	82.25°
Leucoium æstivum.....	72.50°	Zingiber officinale var. Jamaica, No. 2.....	85.90°
Galanthus nivalis.....	66.50°	Zingiber officinale var. Cochinchina.....	90.00°
Galanthus elwesii.....	70.00°	Hedyehium coronarium.....	72.30°
Alstroemeria ligata.....	58.50°	Hedyehium gardnerianum.....	71.15°
Alstroemeria brasiliensis.....	57.75°	Curcuma longa.....	82.50°
Alstroemeria aurantiaca (aurea).....	54.75°	Curcuma petiolata.....	82.50°
Sternbergia lutea.....	75.50°	Canna warszewiczii.....	69.00°
Narcissus horsfieldii.....	74.00°	Canna roscoeana.....	69.50°
Narcissus maximus.....	75.25°	Canna muscifolia.....	70.50°
Narcissus bulbocodium.....	72.00°	Canna edulis.....	67.50°
Narcissus bulbocodium var. conspicua.....	72.75°	Canna var. (Königen Charlotte).....	65.25°
Narcissus bulbocodium var. monophyllus.....	74.00°	Canna var. (President Carnot).....	65.50°
Narcissus incomparabilis.....	76.25°	Canna var. (L. E. Bally).....	69.00°
Narcissus odoratus.....	74.50°	Canna var. (Mrs. Kate Grey).....	62.50°
Narcissus poeticus.....	73.25°	Canna var. (Jean Tissot).....	70.00°
Narcissus biflorus.....	75.00°	Canna var. (J. D. Eisele).....	62.50°
Narcissus jonquilla.....	76.00°	Maranta arundinacea.....	77.00°
Narcissus jonquilla rugulosus.....	75.25°	Maranta arundinacea var. No. 1.....	72.50°
Narcissus jonquilla campenelli rugulosus.....	76.50°	Maranta arundinacea var. No. 2.....	72.00°
Narcissus tazetta var. orientalis.....	77.00°	Maranta massangeana.....	69.00°
Tacca pinnatifida.....	63.00°	Maranta leuconeura.....	74.00°

*The Mean Temperatures of Gelatinization of Various Starches.*—Continued.

Maranta musaica.....	88.50°	Actæa spicata var. rubra.....	56.50°
Calathea lietzei.....	78.50°	Cimicifuga racemosa.....	59.50°
Calathea vittata.....	83.25°	Eranthis hyemalis.....	51.50°
Calathea wiotiana.....	85.50°	Ranunculus bulbosus.....	56.00°
Calathea vandenheckei.....	77.75°	Ranunculus ficaria.....	64.00°
Stromanthe sanguinea.....	82.75°	Adonis amurensis.....	54.50°
Nymphaea alba.....	69.00°	Cochlearia armoracia.....	62.50°
Nymphaea marliacea var. albida.....	68.00°	Jatropha curcas.....	59.50°
Nymphaea marliacea var. carnea.....	66.50°	Manihot utilisissima.....	62.00°
Nymphaea gladstoniana.....	70.00°	Cyclamen repandum.....	55.50°
Nymphaea odorata.....	67.50°	Cyclamen coum.....	56.50°
Nymphaea odorata var. rosea.....	67.00°	Solanum tuberosum.....	66.00°
Nelumbo nucifera.....	58.75°	Batatas edulis.....	74.00°
Nelumbo lutea.....	60.00°	Gesneria tubiflora.....	64.50°
Anemone apennina.....	53.50°	Gloxinia var.....	69.25°
Anemone fulgens.....	50.75°	Trianosperma ficifolia.....	59.00°
Anemone blanda.....	53.25°	Cycas revoluta.....	73.50°
Anemone japonica.....	63.00°	Cycas circinalis.....	72.00°
Aconitum napellus.....	52.75°	Dioon edule.....	72.50°
Actæa alba.....	56.00°	Zamia integrifolia.....	76.50°

Especial attention may be directed to the figures of the foregoing table for comparison with those recorded by other investigators, and also to certain features regarding the range of temperature of gelatinization of the starches as a whole. The records of Lippmann (page 175), from the time of their publication, over 50 years ago, have had a very wide publication and generally have been accepted as standards of reference, but the methods used by him and succeeding workers are crude and liable to lead to very fallacious results. Since Lippmann's studies, little has been published on the temperatures of gelatinization, notwithstanding that much literature has accumulated that treats of the properties of starch, and the inherent importance of the meanings of differences of such temperatures in relation to the starches of different plant sources. This seeming neglect is doubtless to be accounted for in the very variable results that must have been obtained by various experimenters who have felt that their records were unworthy of publication. Whymper (page 175), for instance, found with barley, maize, rye, potato, rice, wheat, and tapioca starches that the values differed in most cases from previous figures and varied with the maturity and size of the grains. Such discrepancies are illustrated in the case of *rice* starch, the temperatures by Lippmann, Lintner, and Dafert being 61.25°, 80°, and 73°, respectively; of *barley*, Lippmann recording 62.5°, and Lintner 80°; of *corn* starch, Lippmann giving 62.5° and Lintner 75°; and of *wheat* starch, Lippmann recording 67.5° and Lintner 75° to 80°. In the preceding table the temperatures given as determined by the method used in this research are for the same starches, *rice* 74.75°, *barley* 61°, *corn* 63.25° to 68.5°, and *wheat* 62.9° to 64°—the last two varying according to the variety.

The lowest temperatures recorded were among the *Liliaceæ* (among members of *Tulipeæ*—*Calochortus*, *Tulipa*, and *Erythronium*) in which in six instances the figures ranged between 49.60° and 50.95°; while the other extreme, where the temperature exceeded 85°, was noted among the *Zingiberaceæ* (*Zingiber*) and *Marantaceæ* (*Maranta* and *Calathea*), the highest being 90°—a range of over 40°, which is certainly remarkable. Of the total number of starches studied, 0.3 per cent had a temperature of gelatinization in the forties, 20 per cent in the fifties, 39 per cent in the sixties, 34 per cent in the seventies, and somewhat more than 6 per cent from eighty to ninety. Nearly 75 per cent had a temperature of gelatinization between 60° and 80°, and about 65 per cent between 60° and 75°.

## CHAPTER VIII.

### GENERAL APPLICATIONS OF THE RESULTS OF THIS RESEARCH.

#### SPECIFICITY AND CONSTANCY OF THE STEREOCHEMIC CHARACTERS OF STARCHES IN RELATION TO GENERA AND SPECIES.

Reference has so frequently been made to the relations of the reactions in both specificity and constancy to genera and species that it would be almost a matter of supererogation to do more at this point than to lay down the dictum that as starch-producing plants become modified in their botanical characters the properties of the complex synthesized substances, such as starch, are accordingly modified, and hence that every genus and species has a form of starch which is specific and constant in relation to that genus or species. Evidence in support of this is found throughout the work.

In the investigations on the crystallography of hemoglobins, referred to in the Preface, it was found that these substances exhibit differences which are in specific relationship to genera and species; and, moreover, that the results of a few preliminary studies with plant proteins and certain other groups of corresponding vital substances justified the announcement of the belief that the remarkable zoological distinctions shown by the hemoglobins would be found to be presented by other complex organic metabolites. Furthermore, it was made clear that not only does there exist these specific stereochemic differences, but also that there are definite gradations of these differences which correspond with the positions occupied by animals in relation to one another as is recognized by the systematic zoologist. Upon such a basis, one may assume upon logical grounds that corresponding gradations will be demonstrated to exist between starches and plants. It follows, as a corollary, that in the existence of individualities of corresponding stereoisomers in relation to genus and species we have *a strictly scientific basis for the classification of plants and animals.*

#### APPLICATIONS OF THE RESULTS OF THIS RESEARCH TO PHARMACOGNOSTICS, COMMERCE, AND TECHNICAL PURSUITS.

The grains of the starches of vegetable drugs vary considerably in size, shape, and other characteristics, as is instanced in the classification of starches by Kraemer (page 71). It is well known that differences merely in the histological characters of starches are often of value in suggesting or practically determining the plant source, but it is also clear, as has been frequently pointed out, that the histological method may be absolutely misleading. If, therefore, there be coupled with this method such others as have been employed in this research, especially in the examination of storage starches (as those of seeds, tubers, rhizomes, corms, etc.), the identification and the determination of substitution and adulteration of drugs that contain starch are usually rendered quite easy.

Commercial starches probably number about thirty, including among them particularly those of corn, rice, and white potato, together with the relatively many kinds that are marketed solely or chiefly as "arrowroot." The first three starches are, as is well known, used extensively in various technical industries and in the arts, and as articles of food, while the arrowroots are sold almost entirely as articles of diet, especially for the use of the sick. The recognition of corn, rice, and white potato starches, respectively, and the detection of adulterations, substitutions, and gross impurities is a matter of common

experience and usually of simple microscopic procedure. With the arrowroots one has not to deal with a single kind of starch but with a number of kinds from many different botanical sources. The commercial term "arrowroot" long since lost its original significance in so far as it applies to the derivation of the starch from *Maranta arundinacea* or its varieties and forms or closely allied species. The supposed especial dietetic value of this form of starch reported by Hughes in 1751 naturally brought arrowroot into prominence as a peculiarly valuable form of food, since which time a number of so-called, or false, arrowroots obtained from a most diverse variety of plants have been introduced into trade. In another part of this report (Part II, The Starches of *Marantaceæ*) it is pointed out that the arrowroots of commerce are derived from species, varieties, and agricultural forms which represent twelve families, seven orders, and three classes. It will be observed from the results of this research that the recognition of the source of the arrowroot, true or false, by means of the methods employed is a simple matter.

In many technical pursuits the employment of starch enters, to a more or less important degree, into various operations, particularly those of certain textile industries. Especial reference was made on pages 176 and 177 to the differences in the characters of starch-pastes and pseudo-solutions obtained from starches of different kinds, and to the notable variations in penetrability and stiffening strength exhibited by boiled starches from different plant sources when prepared under the same conditions. From the records of this investigation it is manifest that these properties, which are of such importance in the manufacture of many fabrics and in laundering, are variable to marked degrees in different starches, so that a given starch may be entirely satisfactory for a certain purpose whereas another may be very poor or almost if not entirely useless. Applications of the results of this investigation to such conditions are strikingly apparent. For instance, if a starch from a given species be found somewhat deficient in stiffening strength, such deficiency may be absent from the starch of another species, variety, form, or hybrid of the same genus. Moreover, as the starches of the parents are represented in the hybrid in modified form it would seem obvious that desirable properties are obtainable by intelligent experimentation along the lines carried out by the plant-breeder. Inasmuch, however, as a form of starch that is lacking in certain properties that may be essential in certain textile industries or in the arts may likely be rendered into a suitable state by simple and inexpensive procedures, as instanced in the method of Bellmas (page 176), the kind of starch will in all probability be found to be of insignificance in comparison with the cost of the raw material. Nevertheless, the principles underlying the natural production of special forms of starch by cultivation, selection, in-breeding, cross-breeding, and hybridization have a broad applicability in connection with many plant products which have a wide economic value, especially as regards medicinal substances, notwithstanding the very important production of the latter synthetically during very recent years.

#### APPLICATIONS OF PRINCIPLES TO PHARMACODYNAMICS.

The cultivation of medicinal plants, which as yet has been carried on to but a very limited extent, has shown that in the case of certain members of *Rubiaceæ* and *Erythroxylaceæ* cultivation has increased the yield of medicinal substances, whereas in other plants, especially those of *Solanaceæ*, the change from the wild to the cultivated state has had the opposite effect. The author is not familiar with any instance where an attempt has been made to *qualitatively* modify medicinal substances by any of the methods in use by the plant-breeder, but there are many known cases where certain toxic properties common to a genus are modified both quantitatively and qualitatively in the different members of the genus. To what extent the qualitative variations are to be attributed to different proportions of the active constituents or to stereoisomeric modifications is as yet wholly speculative. It would seem, however, inasmuch as the various gross characteristics of

plants are expressions of molecular peculiarities, that such peculiarities should be shown in individual substances as well as in the cellular aggregates that constitute the basic data of the systematic botanist; and, moreover, inasmuch as it has been found in these researches that the starches are modified in relationship to botanical peculiarities, and hemoglobins in relation to zoological peculiarities (and incidentally the same relationships in the case of glycogens and certain other complex synthetic metabolites), that there is adequate justification for assuming that corresponding modifications will be found to exist in alkaloids. It would therefore seem improbable that such complex organic substances as hyoscyamine, strychnine, and other alkaloids, which in each case have been obtained from different species, and in some instances from different genera and even different families and orders, exist in single forms, but rather that each substance exists in as many stereoisomeric forms as there are different botanical sources of origin. For instance, hyoscyamine has been obtained from *Hyoscyamus niger*, *Atropa belladonna*, *Datura stramonium*, *Scopolia carniolica*, *Duboisia myoporoides*, *Anisodus luridus*, and *Lactuca sativa*, and also from other species of some of the genera represented. Here are seven genera that belong to two families (*Solanaceæ* and *Compositæ*) that are so distantly related as to be assigned to different orders (*Polemoniales* and *Valerianales*). That stereochemic differences have not been recorded, or that subtle physiological differences may not have been observed in so virulent a poison, is by no means evidence in disproof of the hypothesis of different stereoisomeric forms. In fact, our knowledge of the chemical constitution of alkaloids is generally very meager, and is not only being added to but accepted statements are being modified or entirely discarded. A few years ago hyoscyamine and atropine were given different molecular formulae, but now they are recognized as stereoisomers; piturine ( $C_{12}H_{16}N_2$ ) from *Duboisia hopwoodii* was believed to be identical with nicotine ( $C_{10}H_{14}N_2$ ), but is now recognized as being a distinct individual; and the statement is frequently found that a given plant species contains poisons which resemble those of another of the same or of a related genus. Such instances might be considerably multiplied in order to show not only the superficiality of our knowledge of the chemistry of the toxic constituents of plants, but in justification of the hypothesis of the existence of such substances in stereoisomeric forms.

Moreover, as instanced in this memoir, and recorded in much subsequent investigation as yet unpublished, hybridization has the effect, in both plants and animals, of causing definitely distinctive differences in the offspring in both gross and molecular properties, so that, as regards especially the latter, the starch or hemoglobin, etc., can easily be distinguished from the corresponding substance of the parents. It would follow from the foregoing, as a corollary, that given medicinal substances may, by hybridization, be modified in their physiological properties because of stereochemic changes.

In the Introduction (page 8 *et seq.*) attention is directed to the extremely important fact that slight stereochemic differences may be manifested in more or less marked variations in physiological actions, as have been recorded in the nicotines, cocaines, hyoscines, hyoscyamines, and other organic substances; and, moreover, that under conditions similar to or identical with those which exist in plants there may occur a transmutation of one stereoisomeric form into another. In supplementation of what is there stated, it may be of value to refer to certain facts of much significance: *Atropa belladonna* yields both atropine and hyoscyamine, the former being the racemic form of the latter, while the latter is laevo-rotatory (dextro-hyoscyamine is an artificial base). It was a baffling problem for years why the proportion of these two substances obtained from given specimens of belladonna varied with differences in the laboratory methods used in their preparation, and it was finally discovered that there occurred a ready transmutation of the hyoscyamine (laevo-rotatory) into atropine (racemic) in the presence of fusion, a weak alcoholic solution of sodium hydroxide, ammonia, etc. Another instance of especial interest is recorded in the researches of Lanterer with the active principles of *Duboisia*. He found that the *old*

leaves of *D. myoporoides* yield hyoseyamine ( $C_{17}H_{23}NO_3$ ), but the *young* leaves scopolamine ( $C_{17}H_{21}NO_4$ ), alkaloids that are closely related chemically and physiologically. Whether or not the difference in the product of the old and new leaves is owing to a chemical transformation of the scopolamine into hyoseyamine, or to a change in the protoplasmic mechanism incident to development, or to some other obscure condition, is entirely speculative, yet this instance (coupled with the foregoing, and to which others might be added) is highly suggestive of the plasticity of such substances and of protoplasm, and hence of the exceedingly important results that may be expected in the modifications of stereoisomers by hybridization.

#### GENERAL APPLICATIONS OF THIS RESEARCH IN SYSTEMATIC BOTANY.

It follows, as a corollary from the foregoing data, that upon the basis of intramolecular and intermolecular peculiarities of starches, plants can be classified by means not only of the modifications that are observed in their metabolites, as are manifested in the gross macroscopic forms, such as the leaves, flowers, roots, sexual organs, etc., but also by means of the molecular characters of products which are passive, non-structural constituents of the plant. It will be observed that while the findings of this research are in general in correspondence with the recognized classifications of the systematic botanist obtained by means of macroscopic and microscopic methods, they also are in harmony with the shifting of species from one genus to another, and in the remodeling of classes, families, etc., that is continually going on; and that by the aid of the peculiarities of such metabolites as starch it is manifest that a logical, practically permanent, and scientific classification of plants can be established. For instance, *Galtonia candicans* was for years known as *Hyacinthus candicans* and referred to the genus *Hyacinthus*. It is clear from the reaction curve that its assignment to another genus is correct, and judging from the peculiarities of the reaction curves it stands between the hyacinths and the members of the tulip tribe. Many plants known years ago as species of *Amaryllis* have since been moved to other genera, as for instance (among plants represented in this research) *Hippeastrum equestre* and *H. aulicum*, *Sprekelia formosissima*, *Sternbergia lutea*, *Vallota purpurea*, and *Zephyranthes candida*, all of which have been or are recorded as being corresponding species of *Amaryllis*. It will be seen, by examining plates 51 to 54 and charts 241 to 246, inclusive, and 252, that while all of the starches have in common the same gross fundamental histological characters, the reaction curves show quite clearly that not one could be mistaken for a species of *Amaryllis*. Likewise, some of the Crinums have been designated *Amaryllis*, but it will be noted by an examination of charts 241 and 242 that the generic peculiarities of *Crinum* are quite distinct from those of *Amaryllis*. Likewise, *Tritonia* and *Montbretia* have been confused: *Tritonia pottsii* was named by Baker *Montbretia pottsii*; *T. crocosmaeflora* is a hybrid of *T. pottsii*  $\times$  pollen of *Crocosmia aurea*, also known as *T. aurea* Pappé. Comparing the forms of the grains of the six specimens of *Tritonia* studied in this research, it will be noted that the grains of *T. crocata* and *T. securigera* have in common certain features which distinguish them from *T. pottsii* and its hybrid, which in turn have some characteristics in common. An examination of the reaction-curves will show this same grouping very strikingly, the curves of the first and those of the last corresponding. The differences are so marked that it would seem that *T. pottsii* is misclassified. *T. securigera* has been known as *Gladiolus securiger*, but while the starch-grains have general characteristics in common with those of *Gladiolus* a comparison of the reaction-curves indicates clearly that its grouping as a *Tritonia* is correct.

Again, there has been a shifting of *Tritonias* and *Ixias*, and here too, while the grains belong to the same class so far as their shapes are concerned, the reaction-curves are distinctly unlike, so much so that there should not be any difficulty in distinguishing the members which strictly belong to one genus from those of the other. *Tritonias* and Free-

sias have also been confounded, and, in fact, among the earliest illustrations of *Freesia* are pictures of *Tritonia refracta* and *T. odorata*. While the starch-grains of these genera are so alike in shape that it would be difficult or impossible to distinguish one from the other with certainty, the reaction-curves differ distinctly, and those of *Freesias* bear much closer resemblances to *T. pottsii* and its hybrid than to *T. crocata* and *T. securigera*.

Finally, the systematic classification of plants is of an arbitrary character, as is evident in the large number of classifications that have been used from time to time, in the continual changes that are going on at the present time, and in the recognition that there is no system at present that is universally accepted or which is regarded as having more than a tentative value. In view of these facts, coupled with the specificities of starches which have been shown to exist in relation to species and genera, and the concordance of these specificities with the classification of the botanist whenever the assignment of the latter has been accepted as being absolutely or reasonably permanent, it would seem to follow, as a corollary, that where doubt exists as to classification that is based upon botanical characters, such doubt can be confirmed or set aside and revisions suggested by the reaction method employed in this investigation. It has been conclusively shown that all members of a genus have a certain type of reaction, and that each may be distinguished from the others by variations of the reaction-curves; that in the case of all genera of a given family the genera when closely related show striking resemblances in the types of their reaction-curves; and that the farther the members of a genus or of a family are separated, the greater the departures from the corresponding given types of curves that may be taken to be typical of the genus or family. If, therefore, in a given recognized genus we find among its members one, for instance, that has a reaction-curve that does not correspond with those of the others, it may be taken for granted that it either belongs to another genus or is a hybrid resulting from a cross with a member of another genus, or some aberrant form, etc.; and again, if the reaction-curves of different genera assigned to a family do not correspond in general characters, it may be likewise concluded that one or more of the genera are misclassified.

The systematic botanist, by the conventional methods of research, is continually finding evidence of misclassification, as is evident in the continual shifting of species and genera in the rebuilding of broken-up families, and in the breaking-up of families by assigning certain genera to other families or establishing entirely new families. Thus we find in very recent years that *Convallaria*, *Trillium*, and *Asparagus* have not, by some botanists, been included among the *Liliaceæ*, but have been set apart as a distinct family, *Convallariaceæ*; and *Aletris* has been transferred to *Hæmodoraceæ*. *Musaceæ*, *Zingiberaceæ*, *Cannaceæ*, and *Marantaceæ* are by some grouped in one family, but by others as separate families. We are therefore dealing not with a stable classification, but one that is most unstable. If therefore, as stated, we find in the reaction-curves evidence of misclassification, it may be taken for granted that we have the strongest kind of evidence to suggest modifications. For instance, it will be seen that reaction-curves of *Lilium*, *Fritillaria*, *Calochortus*, *Tulipa*, *Scilla*, *Chionodoxa*, *Ornithogalum*, *Puschkinia*, and *Erythronium* are all in accord with a given type; that in *Hyacinthus* and *Muscari* there is a marked modification of this type which is manifested chiefly in the much lower temperature of gelatinization and the low reactive intensity with Purdy's solution; that in *Galtonia* there is another kind of modification, and of a character which suggests that *Galtonia* stands, as it were, between *Hyacinthus* and *Muscari* on the one hand and the first group (which constitutes almost wholly the tulip tribe) on the other; that in *Brodiaea* and *Triteleia* there are types of curves which bear close resemblances to each other, but which differ distinctly from those of the other groups noted. According to this data, without going into further detail, all of which is in accord with the data of the botanist, it would seem that there is a logical basis for a separation of the *Liliaceæ* into a number of families, each of which can be distinguished by the sum of the botanical and

reaction peculiarities. Upon such a basis a family might be formed including *Brodiaea*, and certain other genera of the *Liliaceæ* that have a recognized very close relationship.

Similarly, it seems manifest that the family *Iridaceæ*, as now constituted, contains a number of genera which should be assigned to other families; as it is, it certainly seems, from the reaction-curves and forms of the starch-grains, to be a very heterogeneous group. If we take the reaction-curve of *Iris* as being the family type, it will be observed that, as in the case of *Liliaceæ*, the reaction-curves of some of the genera correspond, as for instance *Tigridia*, while those of others differ so markedly as to suggest misclassification. Thus, for instance, the curves of *Moraa* and *Homeria* differ so markedly from the *Iris* type as to suggest that the genera properly belong to another family. *Homeria* is stated to be closely allied to *Tigridia*, but there are certainly well-defined differences in their starches which do not confirm this view. Then, again, *Gladiolus*, *Watsonia*, *Tritonia*, and *Antholyza* have forms of starch-grains and reaction-curves which correspond, but which do not harmonize with the forms and curves of *Iris*; and *Marica* and *Gelasine* differ so from each other, and also from *Iris*, in the same respects that they could not be identified as members of the same family. In other words, it would seem that the family *Iridaceæ* contains a number of genera which strictly speaking could with much appropriateness be assigned to other families. These are but few of the many instances suggested by the results of this preliminary study, which indicate that by means of such methods as have been pursued in this research the classification of plants may be revolutionized and placed upon a lasting basis. (See Prefatory Notes in Part II.)

#### SUMMARY AND CONCLUSIONS.

(1) Every starch and every mature starch-grain is a mixture of different forms of the starch-substance.

(2) Owing to the heterogeneity of starches and the crudities of the methods employed in the differentiation of different starches the results of this investigation are to be regarded as being for the most part of a gross quantitative and qualitative character.

(3) The temperatures of gelatinization of starches are as specific in relationship to the chemical composition and constitution of starches as are the melting-points of various isomers in their distinction, and the method employed in this research is approximately exact.

(4) With each agent the starches exhibit a wide range of reaction-intensity, with a tendency to a close correspondence between varieties of a species, closely related species, and closely related genera, respectively.

(5) The characters of the modified forms of starch in relation to each genus and each species are specific and constant.

(6) In pharmacognosies, commerce, and technical pursuits the methods employed in this research must obviously have great value in the identification of vegetable drugs and starches, and in the detection of adulterations and substitutions, and in showing the way of providing starches and other plant products which have such special properties as may be demanded in certain textile industries, etc.

(7) It is probable that by hybridization and other procedures of the plant-breeder the properties of medicinal substances can be modified by the changes in stereoisomeric forms.

(8) Stereochemic peculiarities of starches, hemoglobins, and similar complex synthetic metabolites constitute *a strictly scientific basis for the classification of all forms of life.*

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NOTE.—At the ends of Chapters II, III and IV (pages 79, 160 and 195, respectively) there will be found summaries and conclusions relating to the subject-matter of those chapters.

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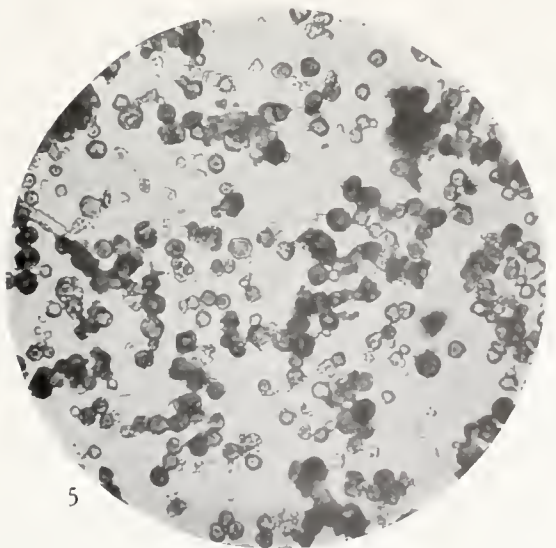
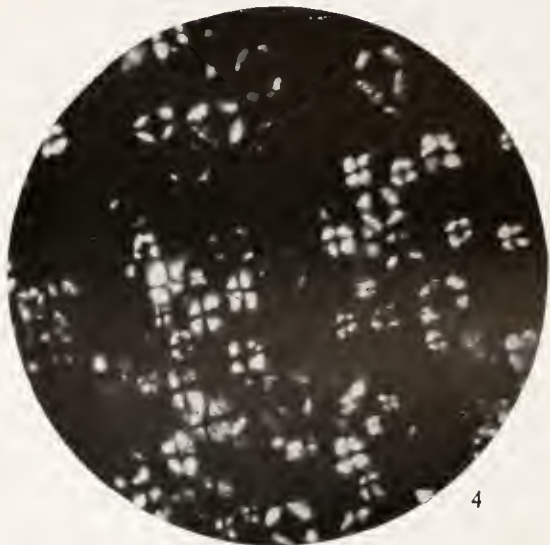
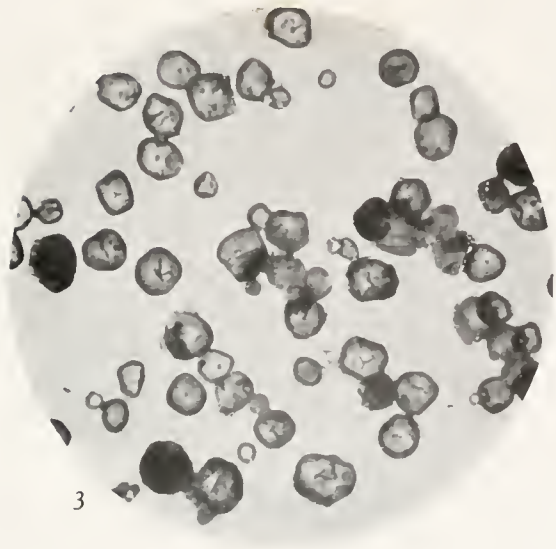
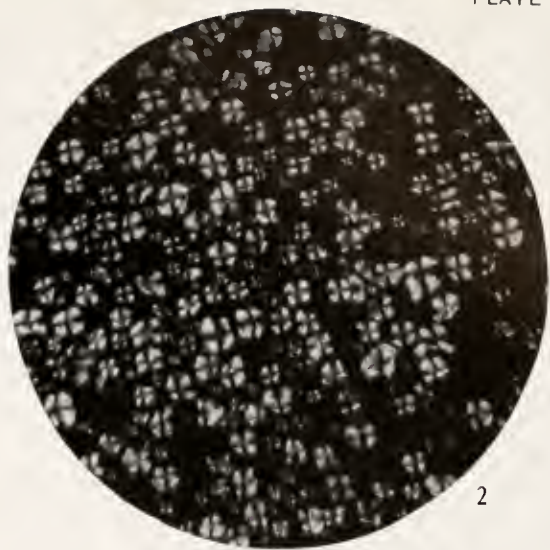
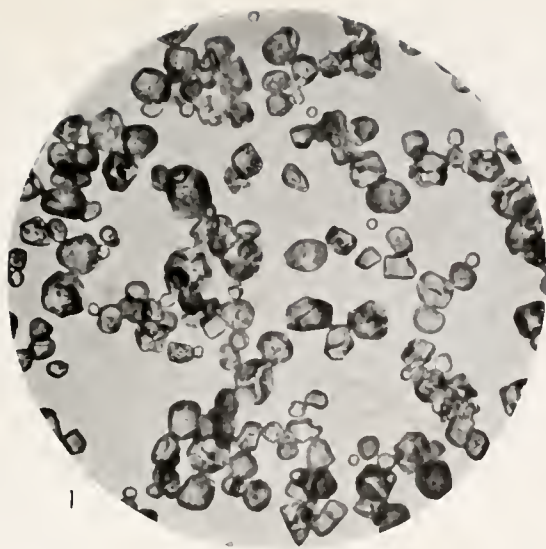
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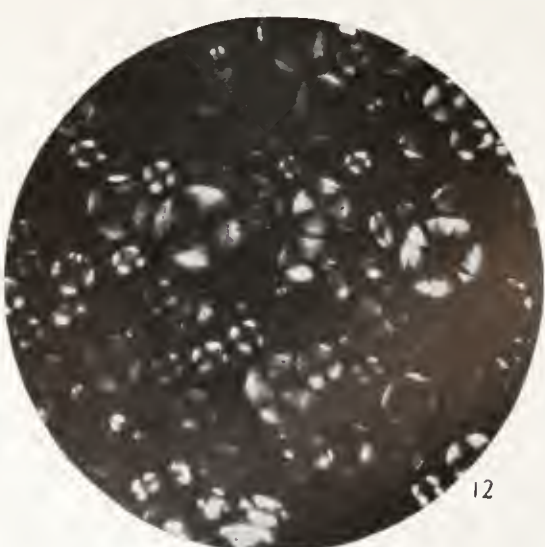
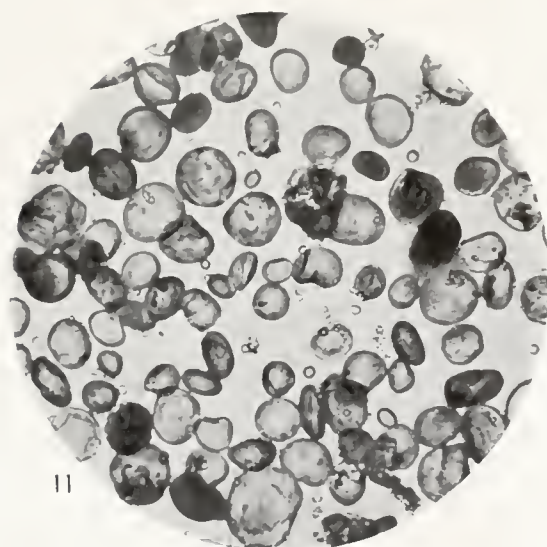
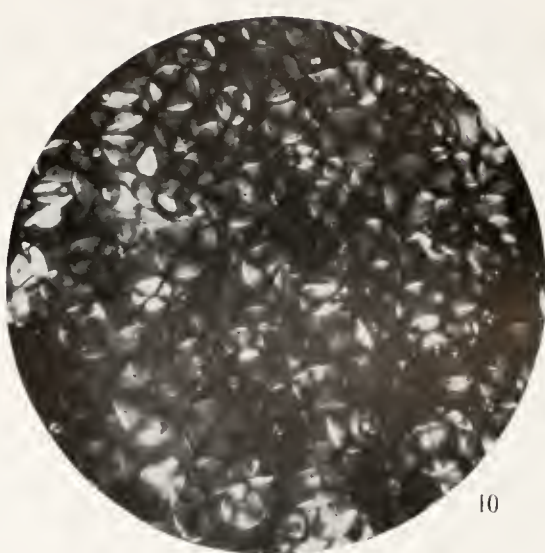
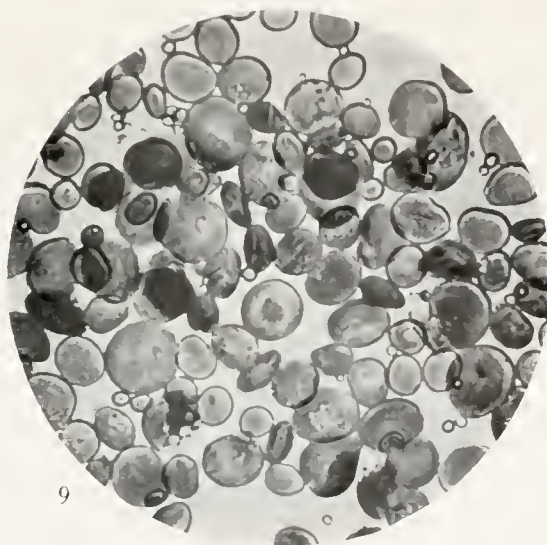
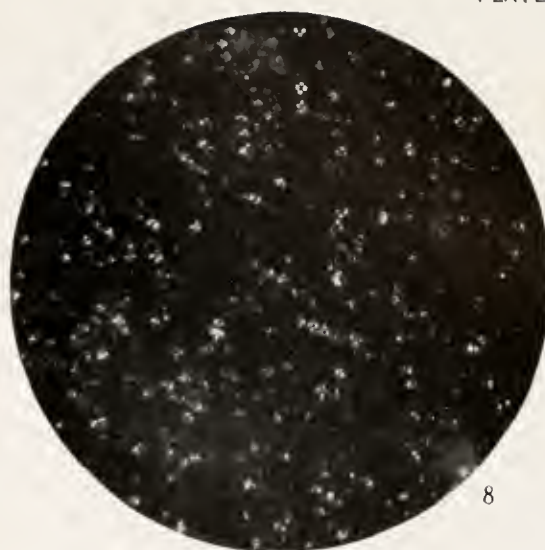
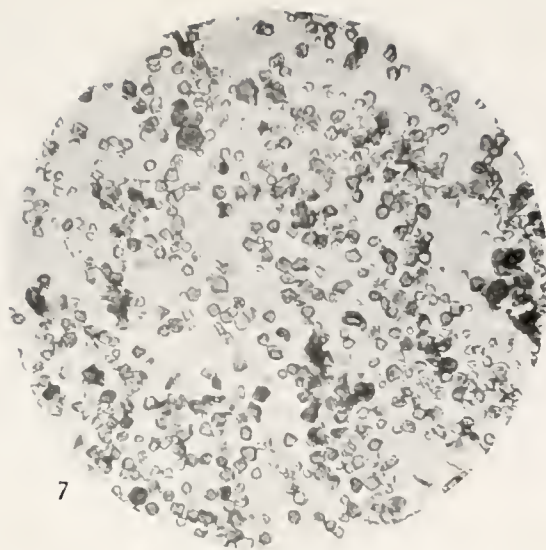
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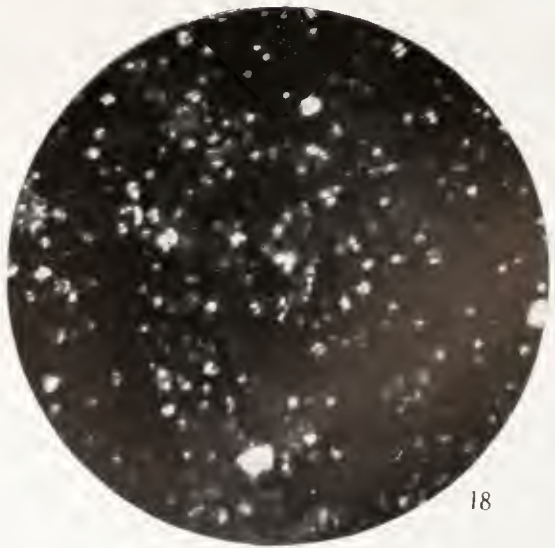
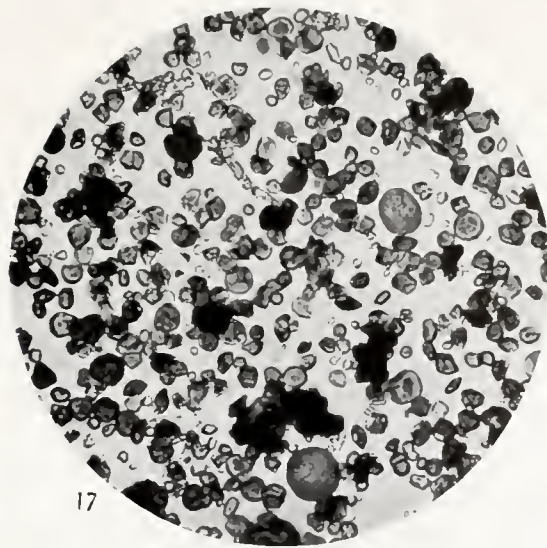
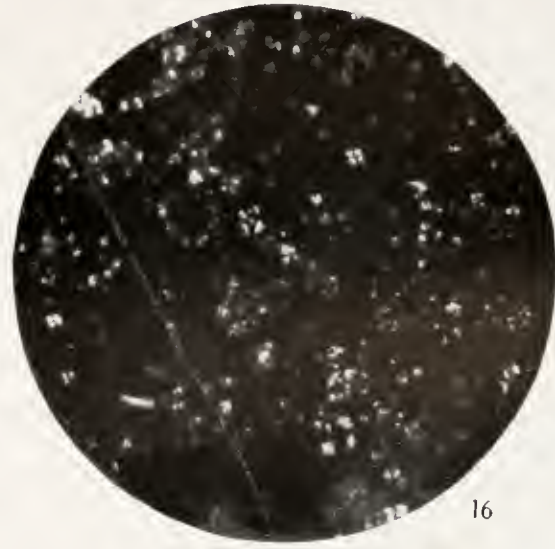
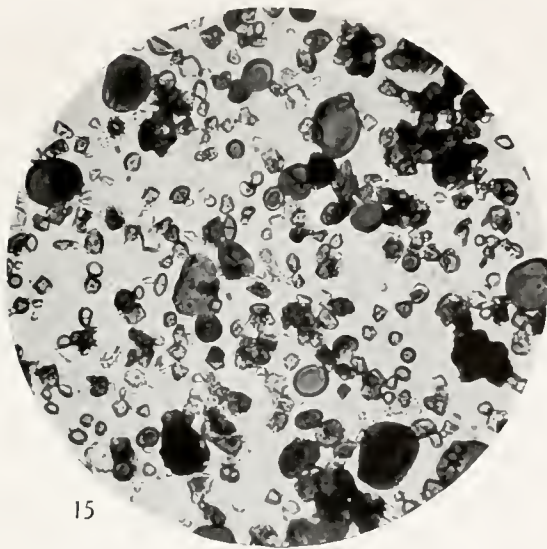
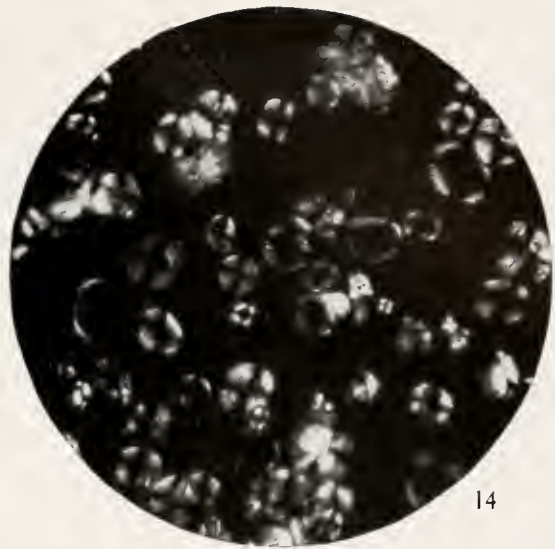
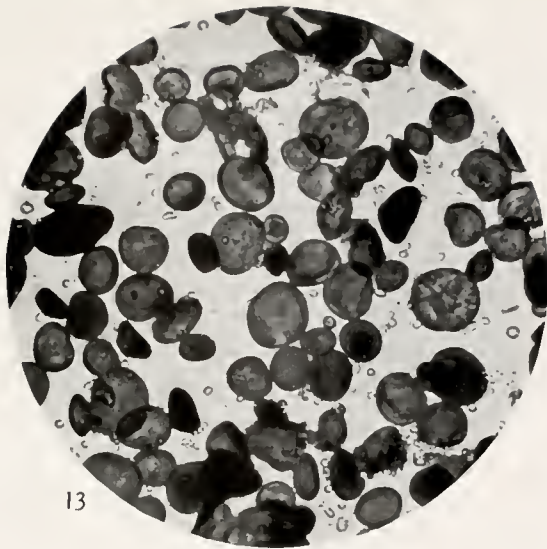
1 and 2. *Zea mays* var.  
3 and 4. *Andropogon sorghum* var.  
5 and 6. *Panicum crus-galli* var.





7 and 8. *Oryza sativa* var.  
9 and 10. *Triticum sativum* var.  
11 and 12. *Secale cereale* var.



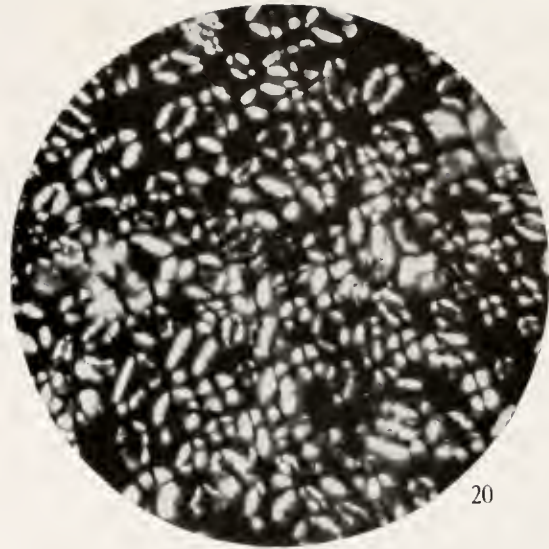


13 and 14. *Hordium sativum* var.  
15 and 16. *Arena sativa* var.  
17 and 18. *Arrhenatherum elatius* var.





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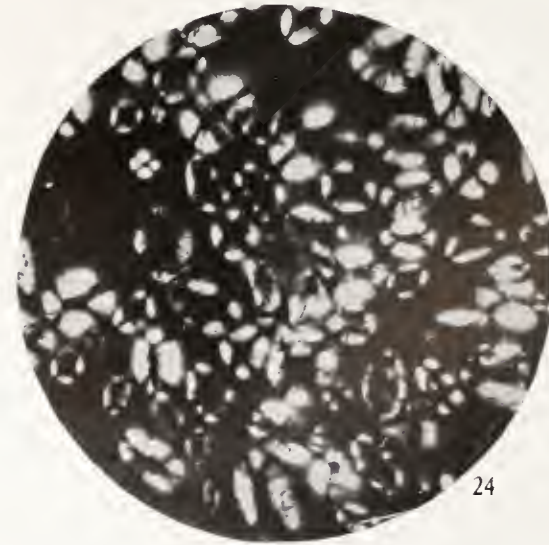
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19 and 20. *Vicia sativa*.  
21 and 22. *Vicia villosa*.  
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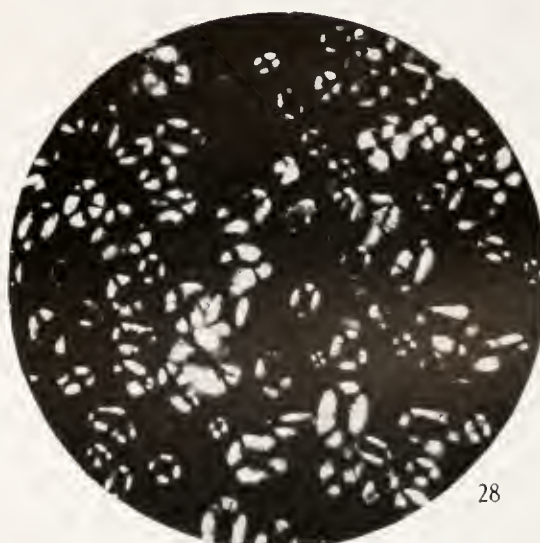
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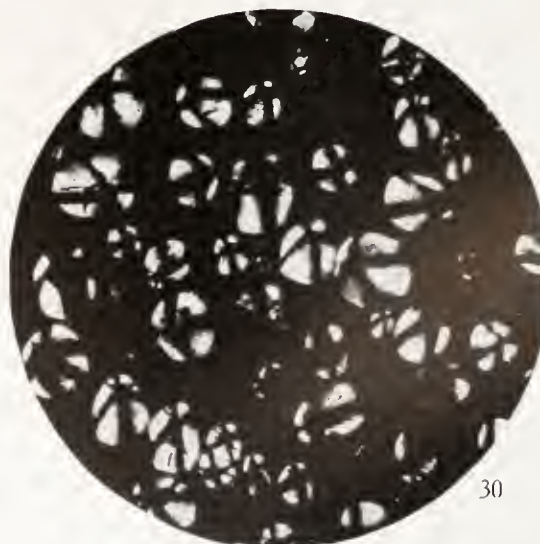
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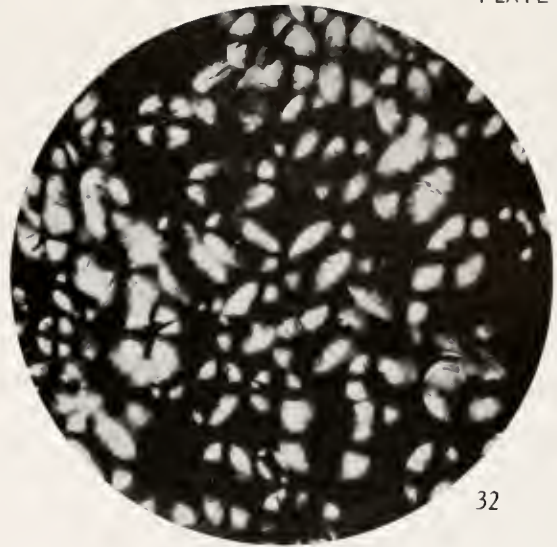
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25 and 26. *Vicia fulgens*.  
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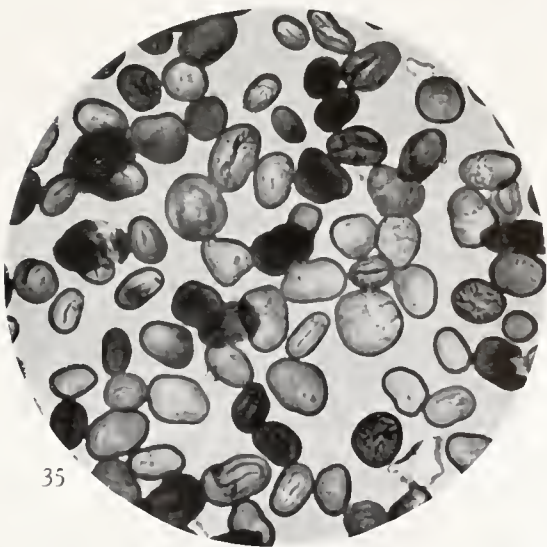
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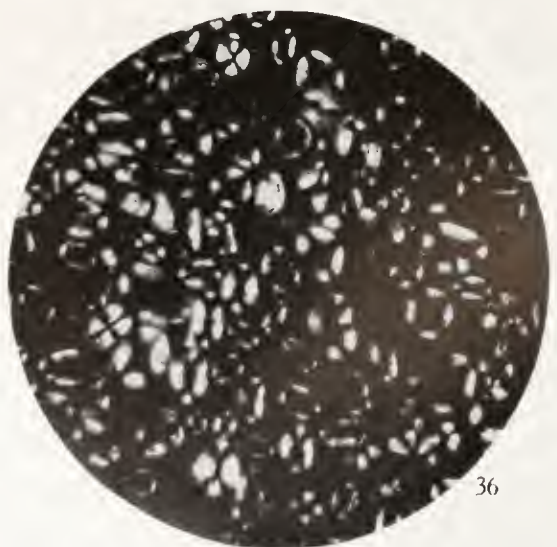
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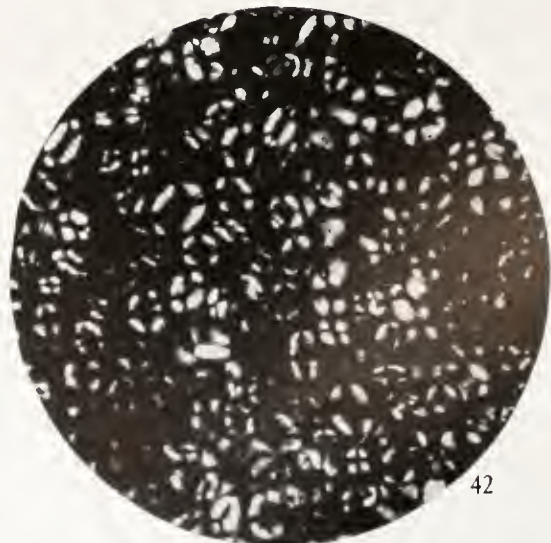
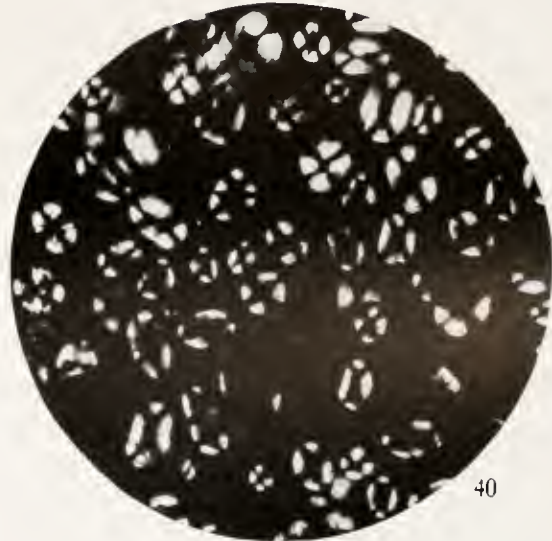
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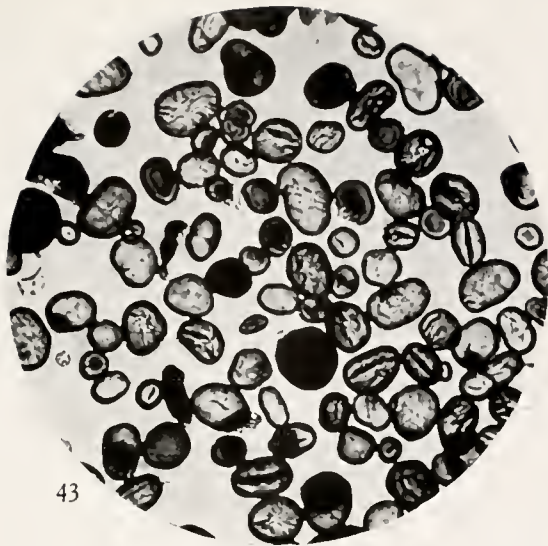
31 and 32. *Phascolus vulgaris* var.  
33 and 34. *Phascolus lunatus* var.  
35 and 36. *Dolichos lablab*.



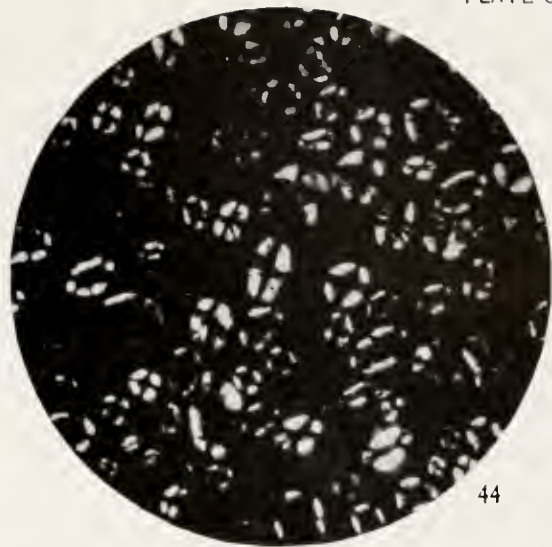


37 and 38. *Lens esculenta* var. *shahzada*.  
39 and 40. *Lathyrus odoratus* var. *shahzada*.  
41 and 42. *Lathyrus sylvestris*.





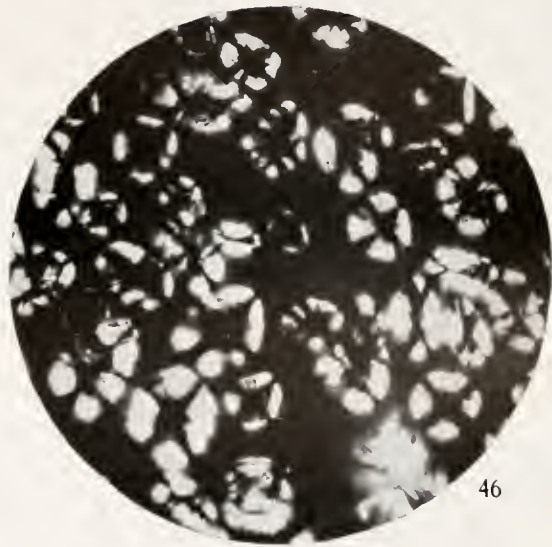
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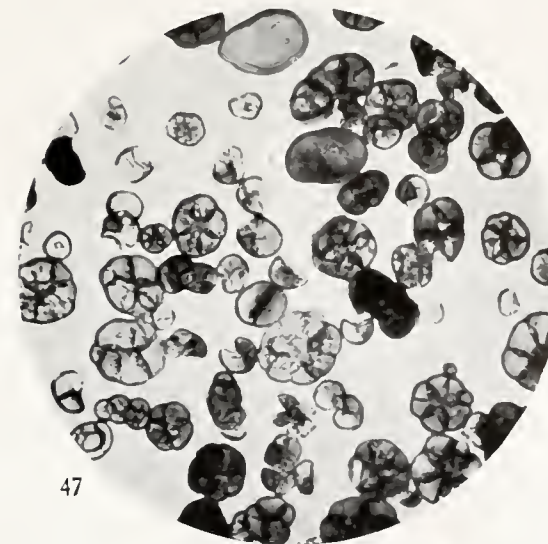
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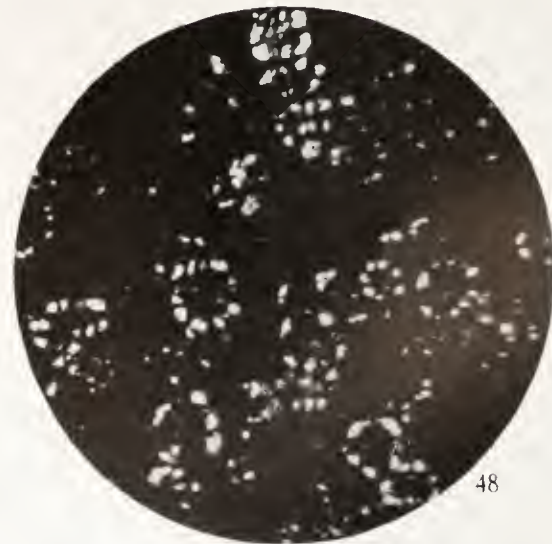
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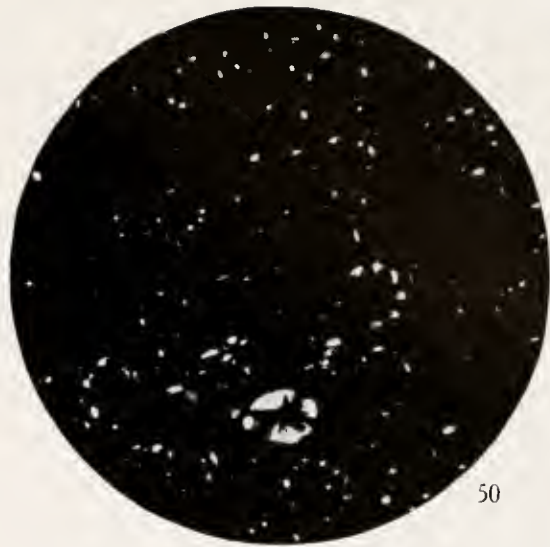
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43 and 44. *Lathyrus latifolius* var. *albus*.  
 45 and 46. *Lathyrus magellanicus* var. *albus*.  
 47 and 48. *Pisum sativum* var. (Eugenie).





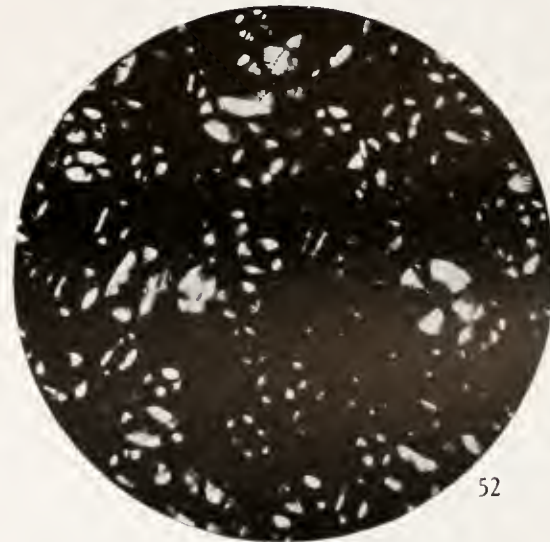
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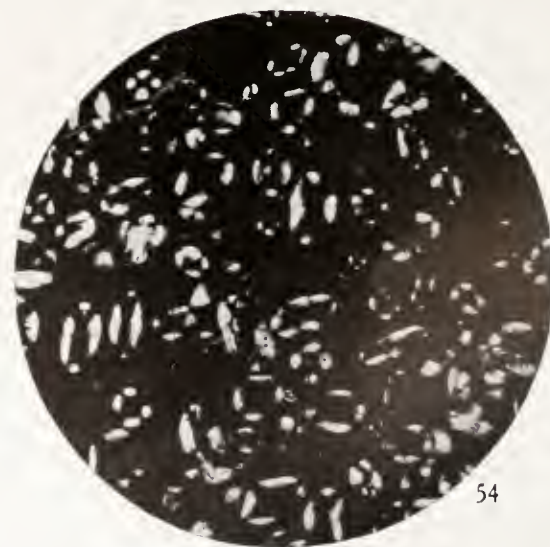
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49 and 50. *Pisum sativum* var. (Thomas Laxton).  
 51 and 52. *Pisum sativum* var. (Electric Extra Early).  
 53 and 54. *Pisum sativum* var. (Mammoth Grey Seeded).

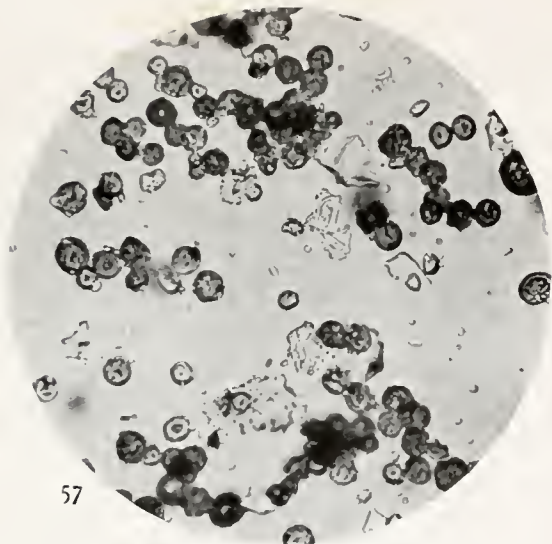




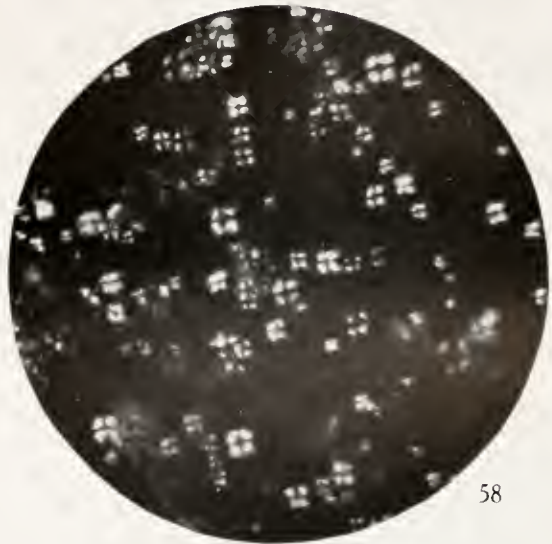
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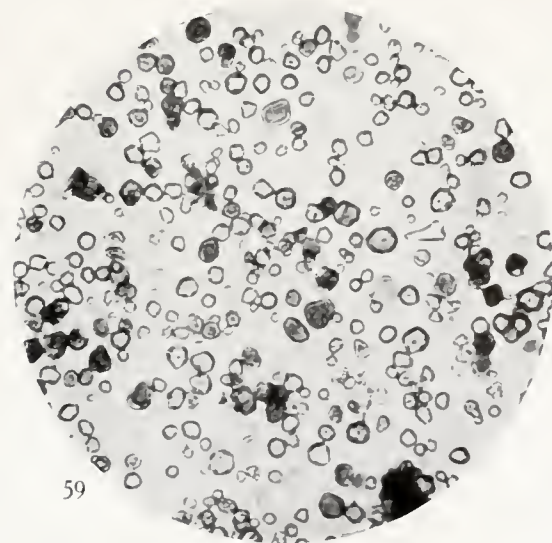
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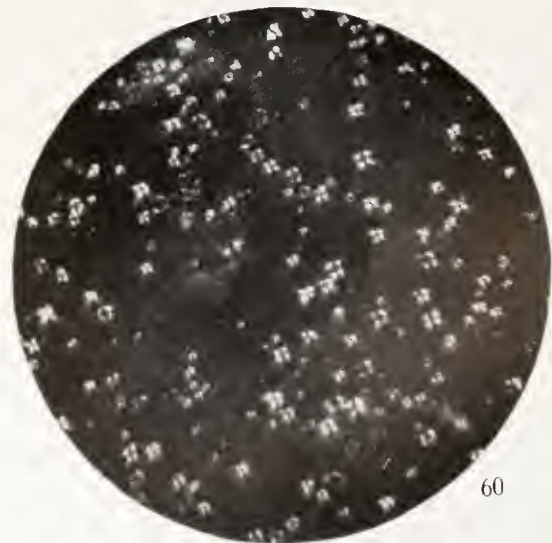
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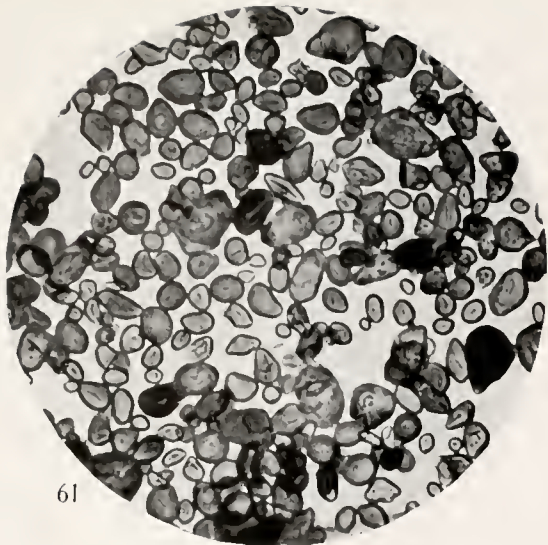
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55 and 56. *Pisum sativum* var. (Large White Marrowfat).  
57 and 58. *Arachis hypogaea* var.  
59 and 60. *Polygonum fagopyrum* var.

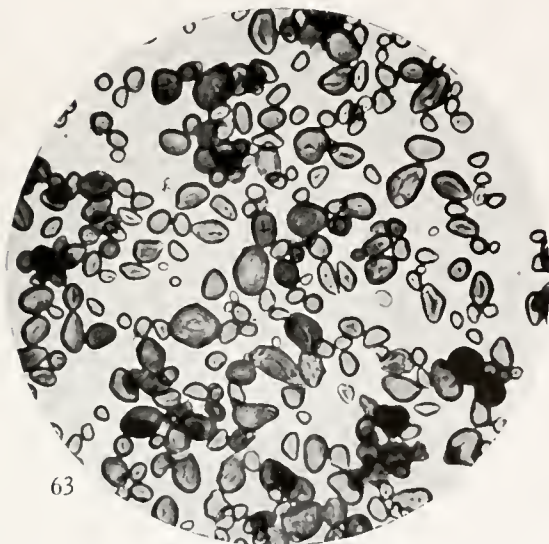




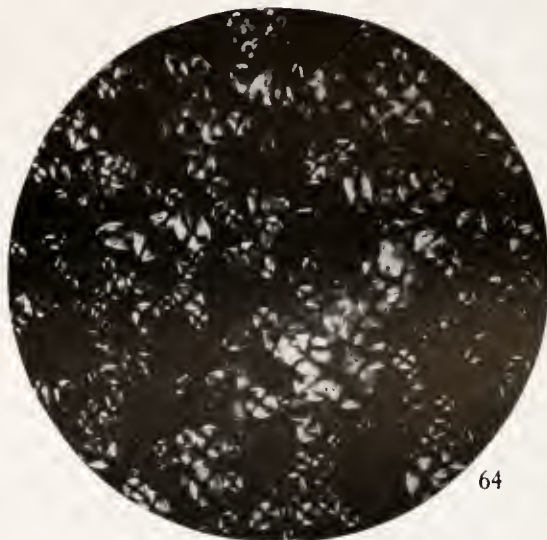
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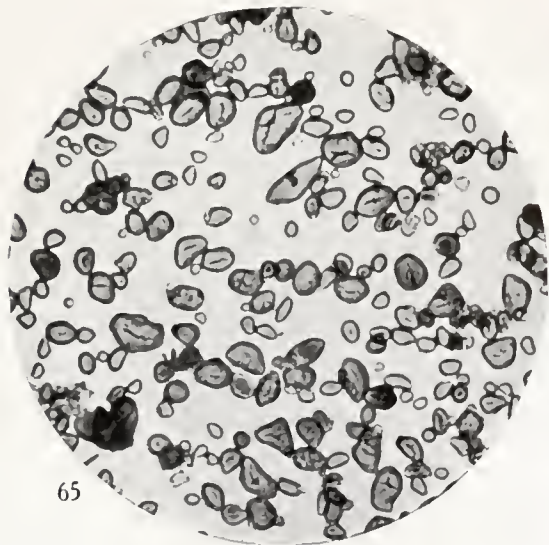
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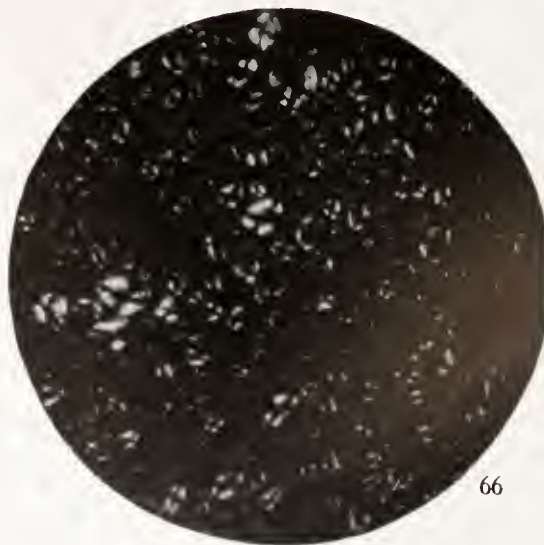
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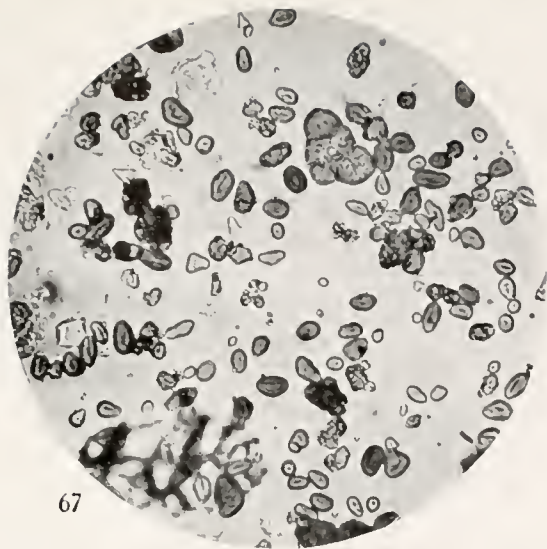
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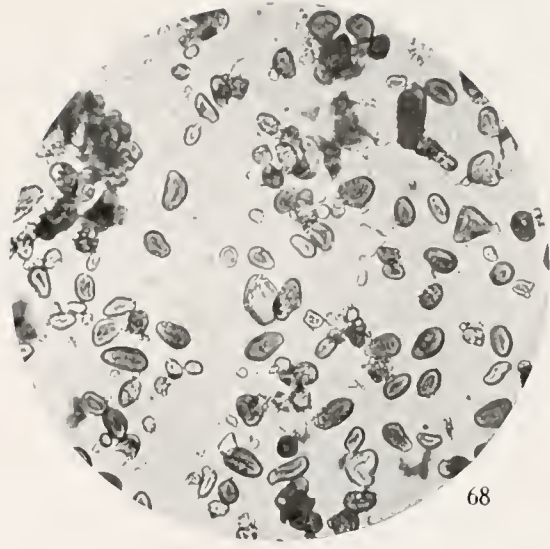
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61 and 62. *Quercus alba*.  
 63 and 64. *Quercus muhlenbergi*.  
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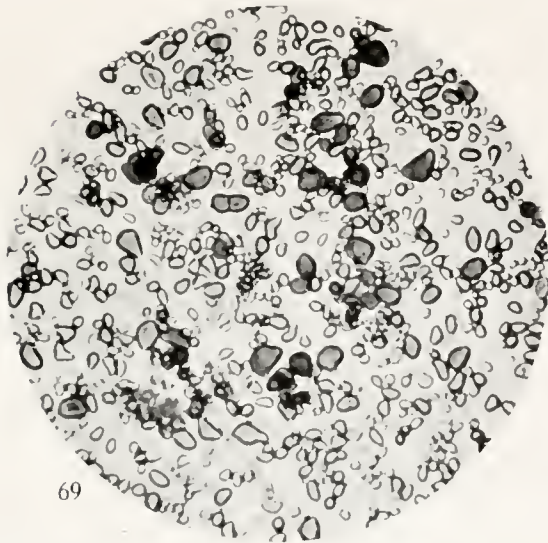




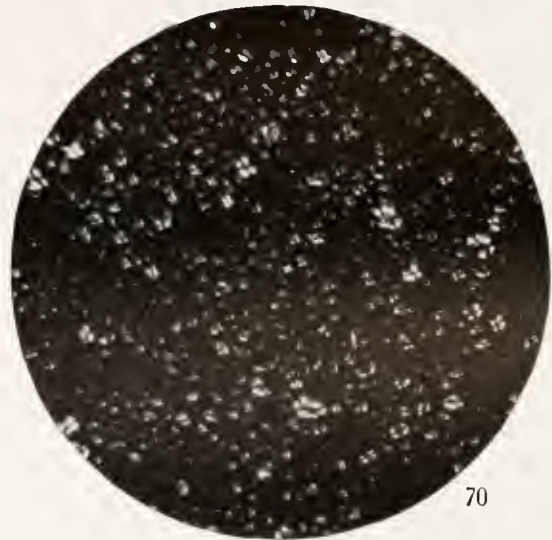
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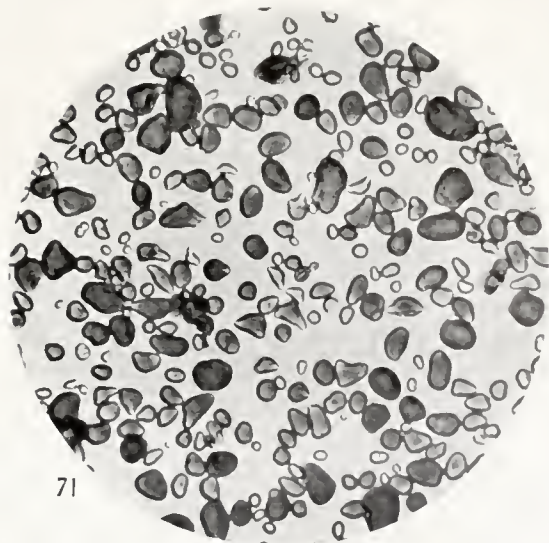
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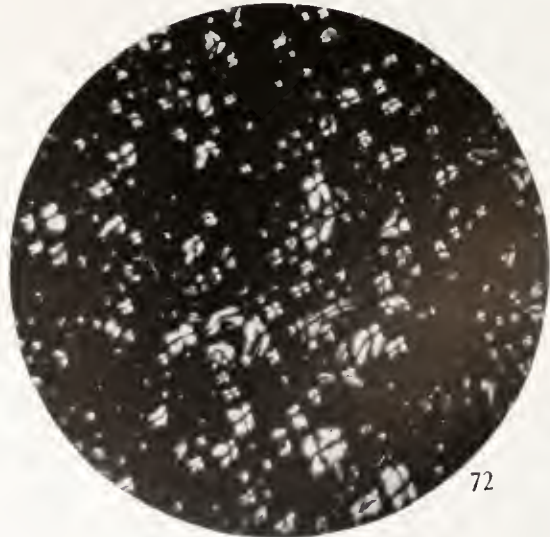
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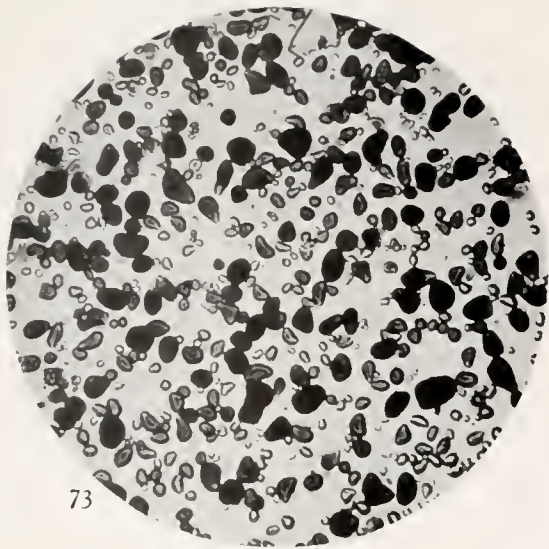
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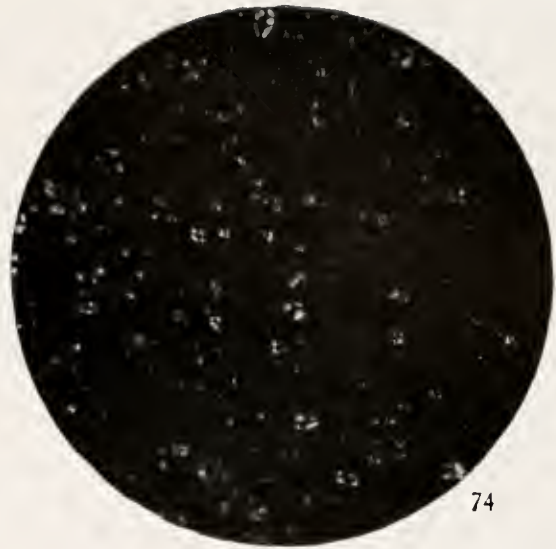
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67. *Quercus rubra*.  
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69 and 70. *Castanea americana*.  
71 and 72. *Castanea sativa* var. *numbo*.

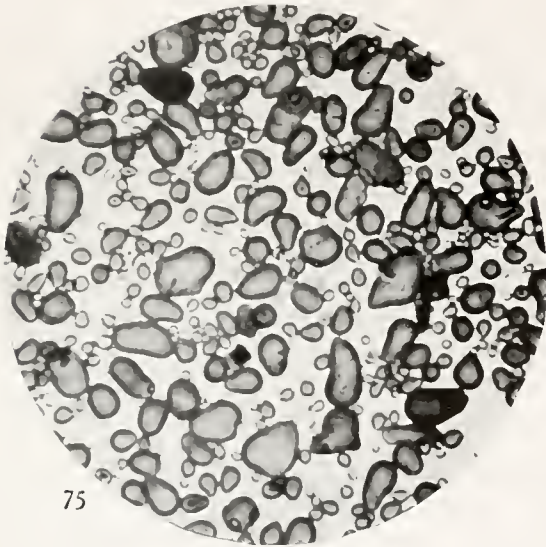




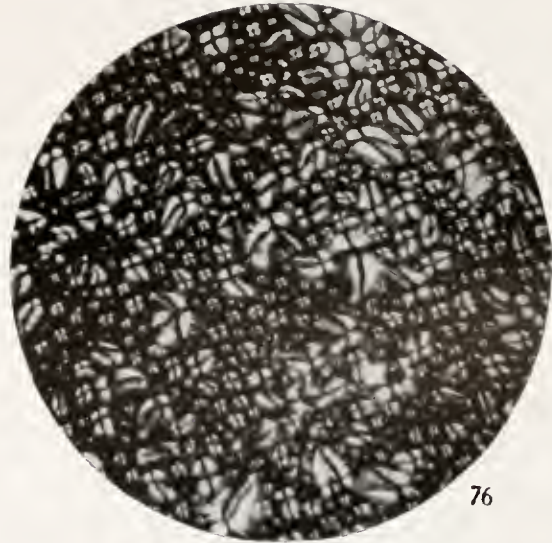
73



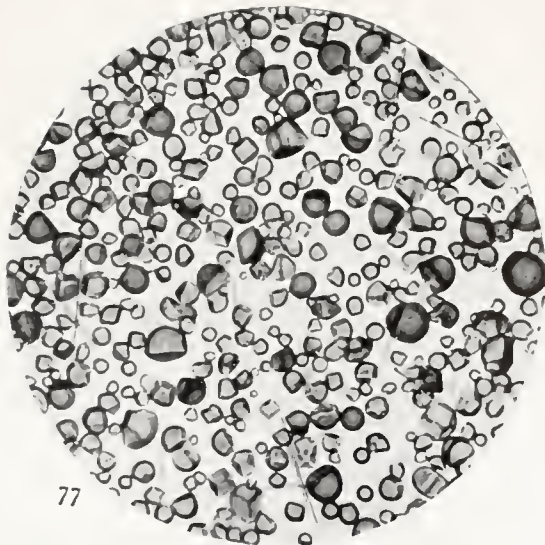
74



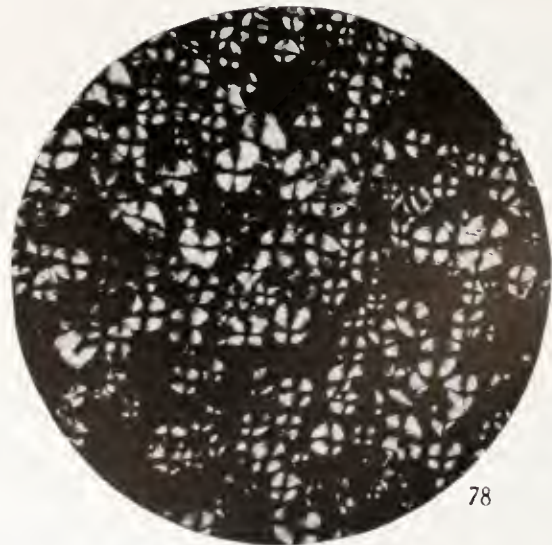
75



76



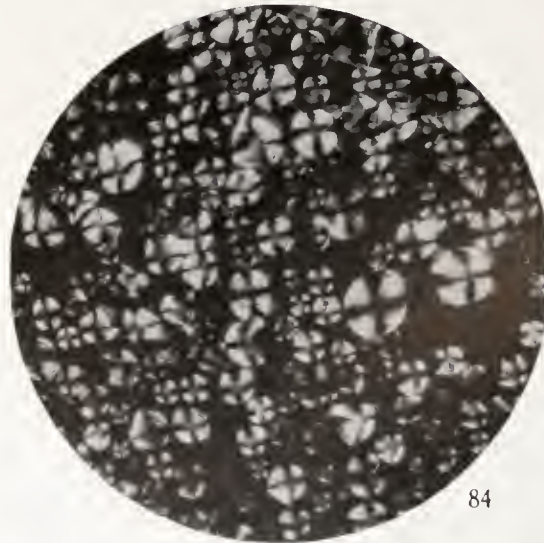
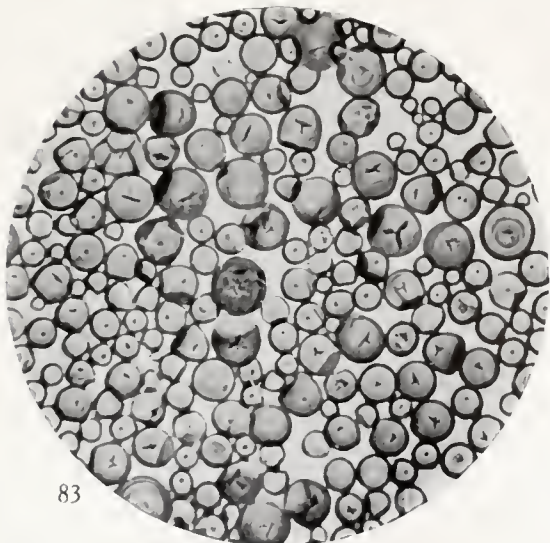
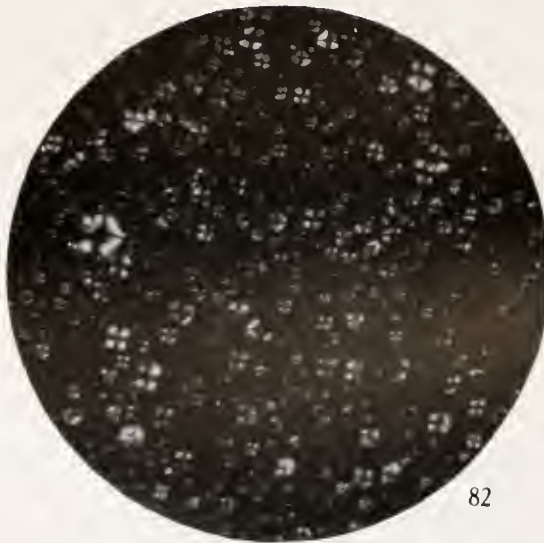
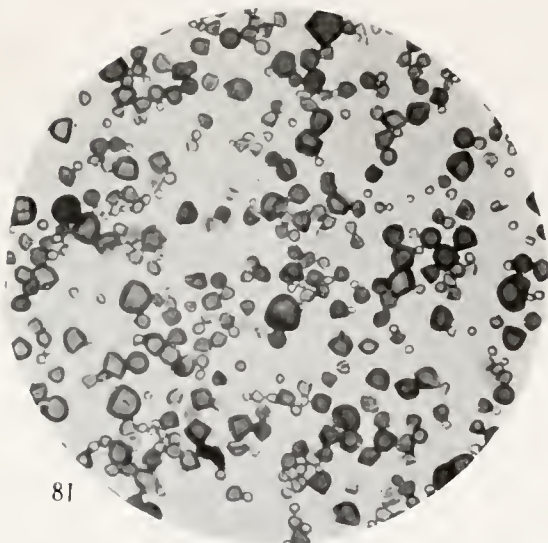
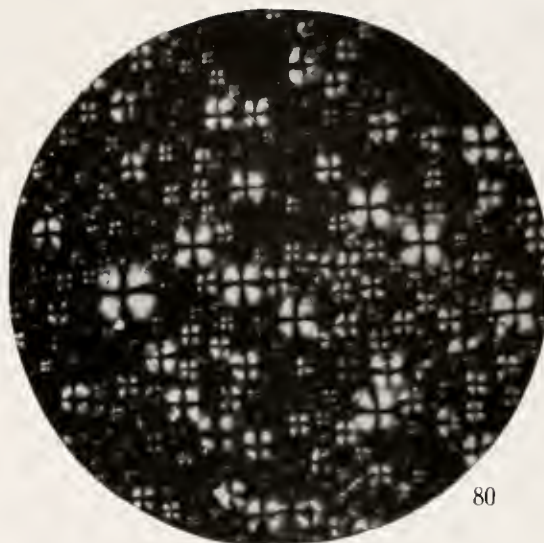
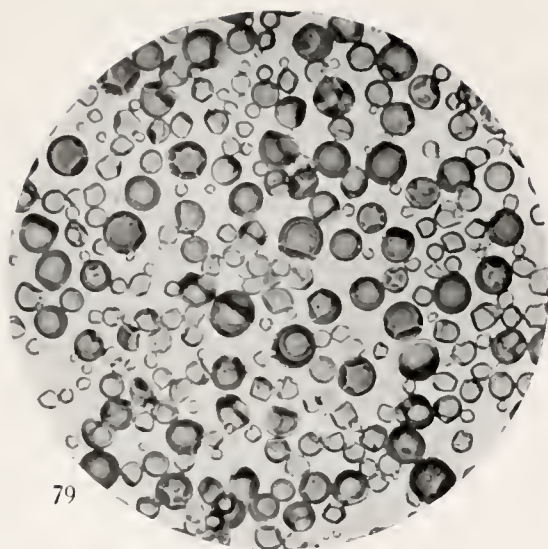
77



78

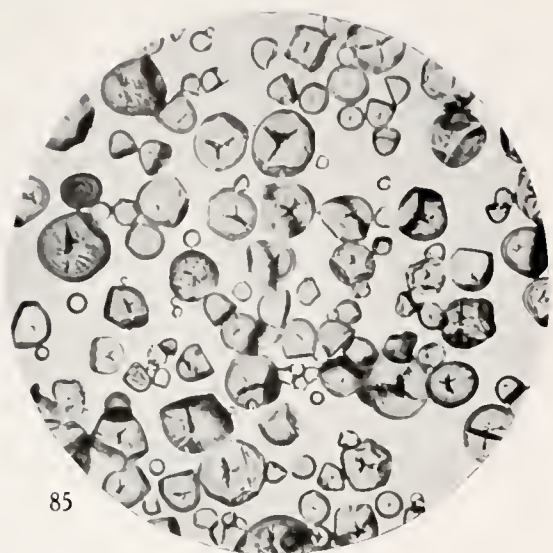
73 and 74. *Castanea pumila*.  
 75 and 76. *Esulus hippocastanum*.  
 77 and 78. *Arum palastinum*.





79 and 80. *Arum cornutum*.  
81 and 82. *Arum italicum*.  
83 and 84. *Arisaema triphyllum*.

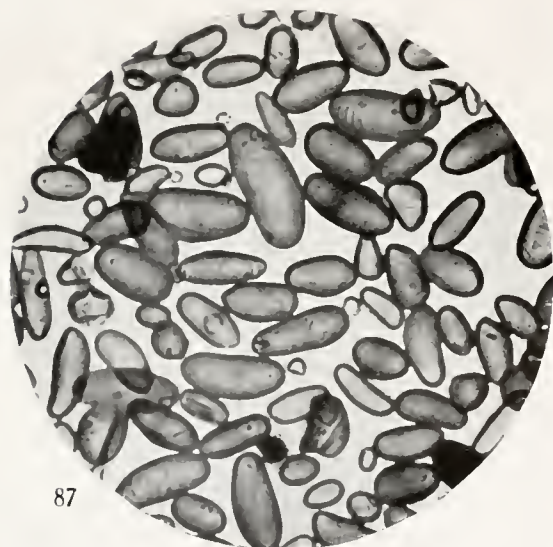




85



86



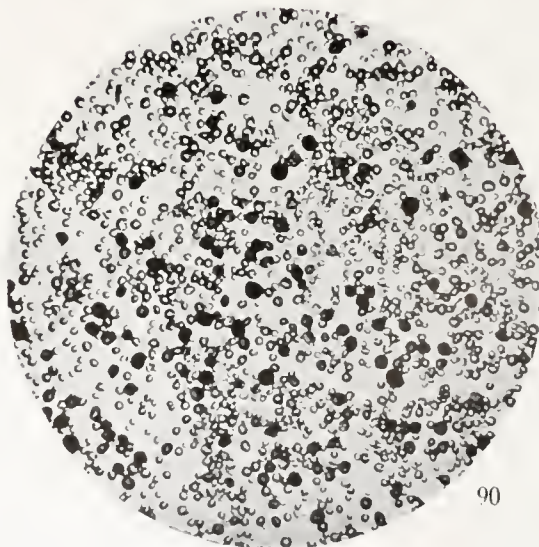
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88



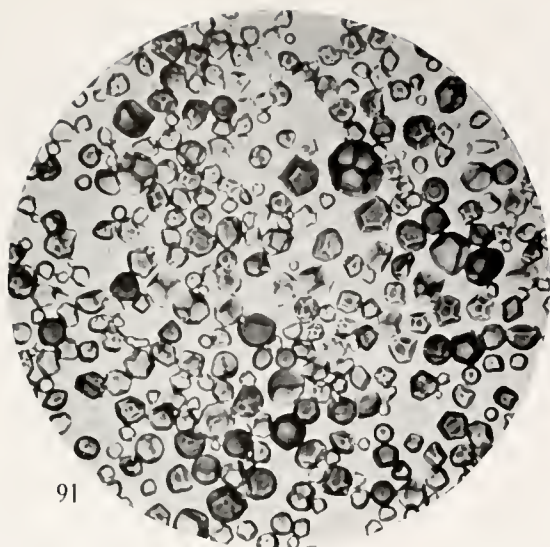
89



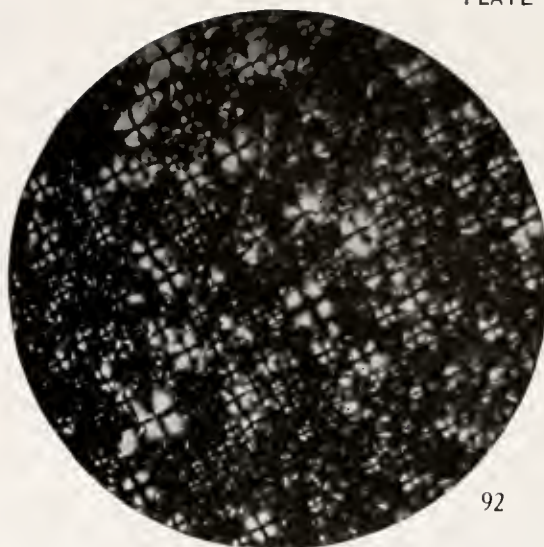
90

85 and 86. *Dracunculus vulgaris*.  
 87 and 88. *Peltandra undulata*.  
 89. *Alocasia pitzeysi*.  
 90. *Amorphophallus rivieri*.

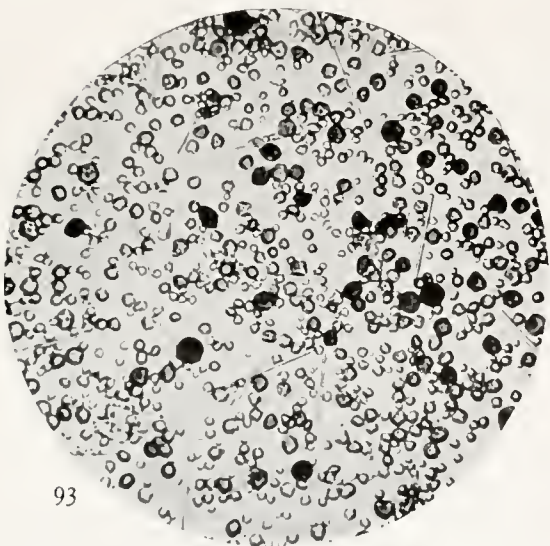




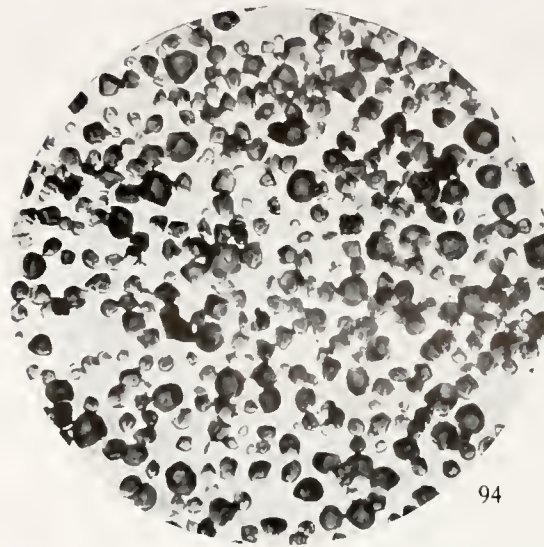
91



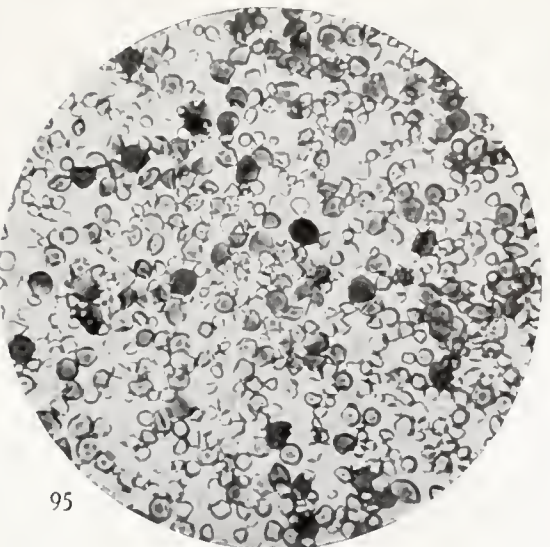
92



93



94



95



96

91 and 92. *Richardia elliotiana*.  
93. *Richardia africana*.  
94. *Richardia solfatarre*.  
95 and 96. *Richardia albo-maculata*.





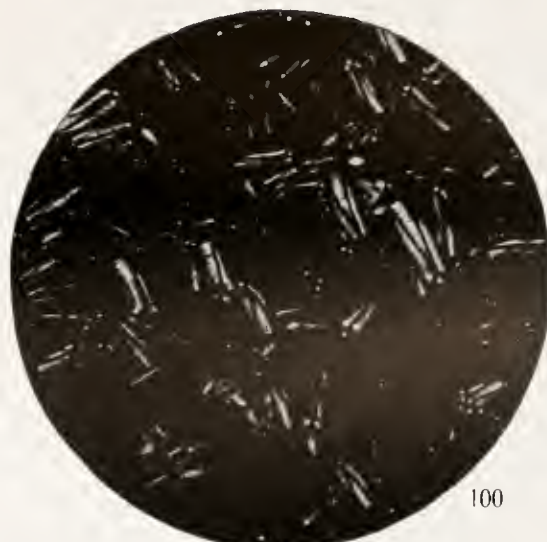
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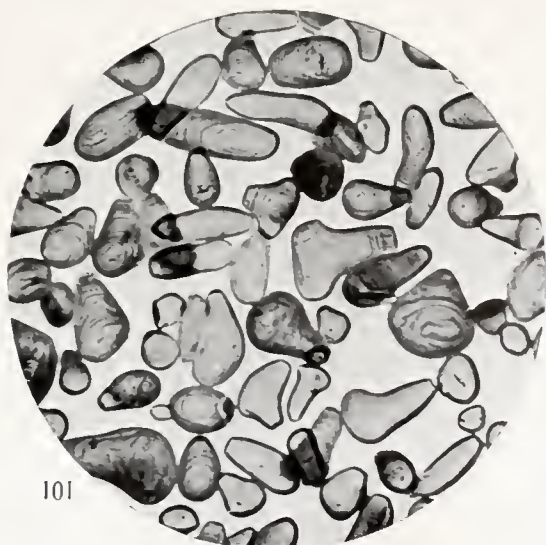
98



99



100



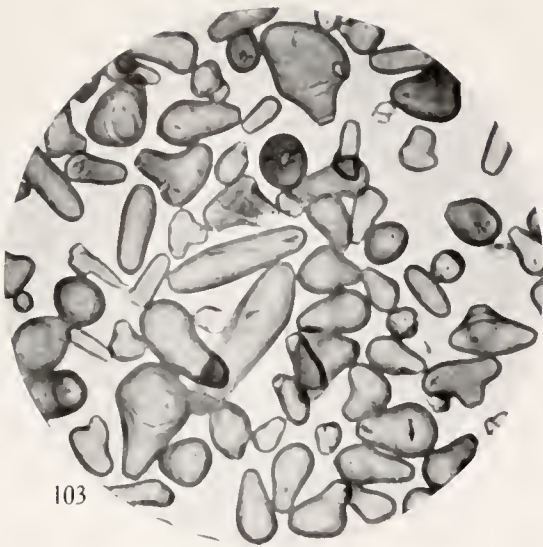
101



102

97 and 98. *Dieffenbachia seguine* var. *nobilis* (pith).  
 99 and 100. *Dieffenbachia seguine* var. *nobilis* (cortex).  
 101 and 102. *Dieffenbachia seguine* var. *maculata* (pith).





103



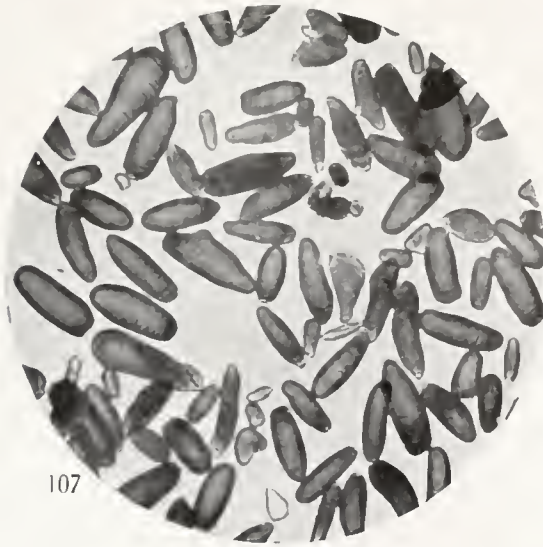
104



105



106



107



108

103 and 104. *Dicffenbachia sequine* var. *maculata* (cortex).  
 105 and 106. *Dicffenbachia sequine* var. *irrorata* (pith).  
 107 and 108. *Dicffenbachia sequine* var. *irrorata* (cortex).





109



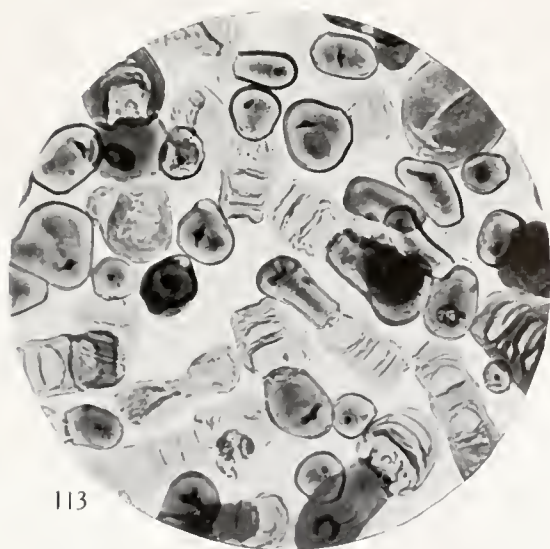
110



111



112



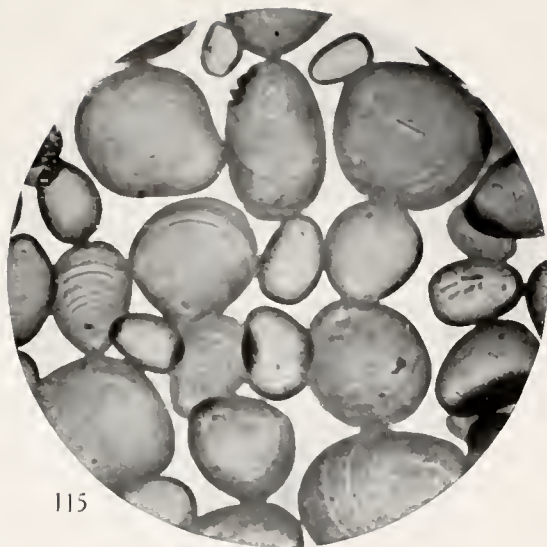
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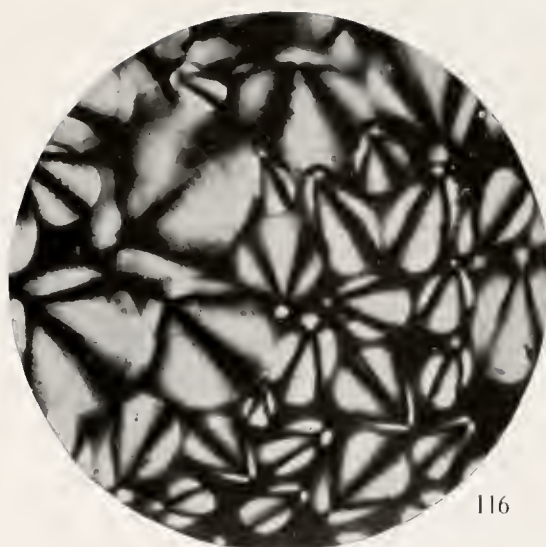
114

109 and 110. *Dieffenbachia illustris* (pith).  
 111 and 112. *Dieffenbachia illustris* (cortex).  
 113 and 114. Effect of chloral hydrate-iodine on the starch of *Dieffenbachia seguine* var. *maculata* (pith), partial reaction.

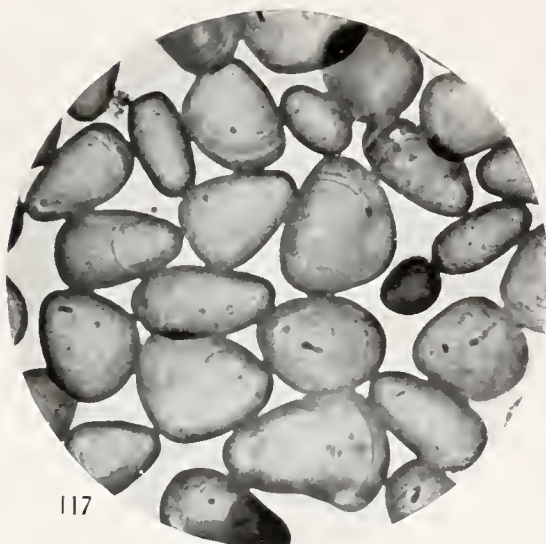




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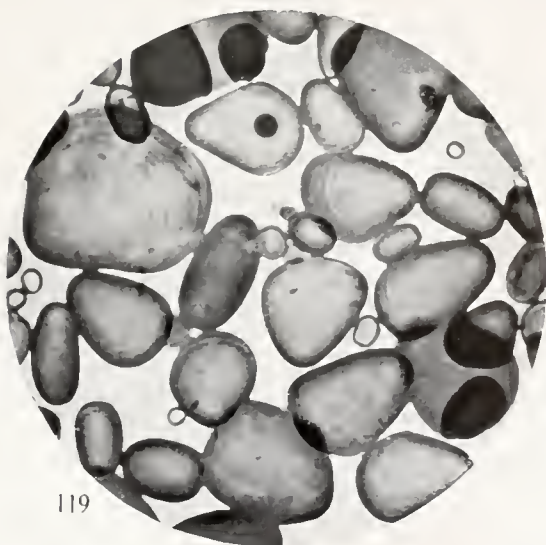
116



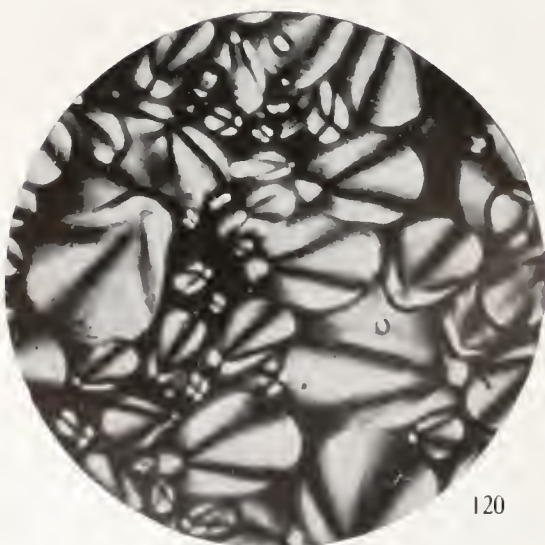
117



118



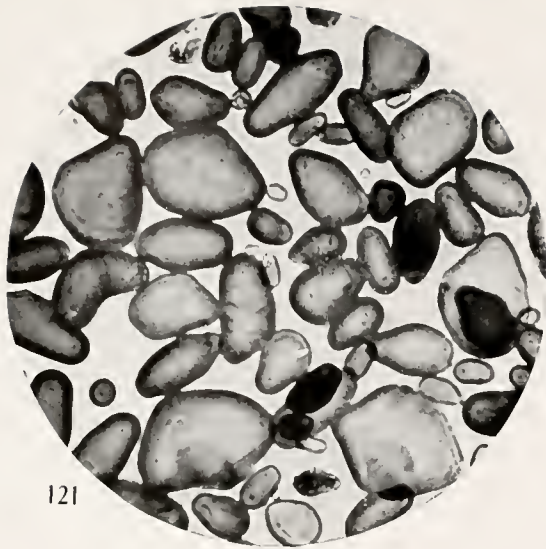
119



120

115 and 116. *Lilium candidum*.  
 117 and 118. *Lilium longiflorum* var. *giganteum*.  
 119 and 120. *Lilium longiflorum* var. *eximium*.





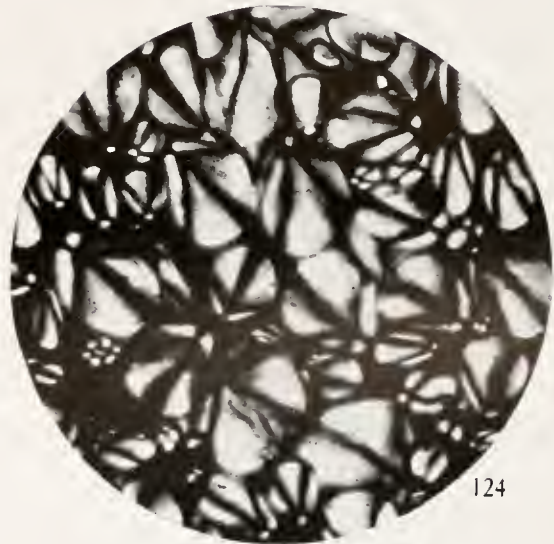
121



122



123



124



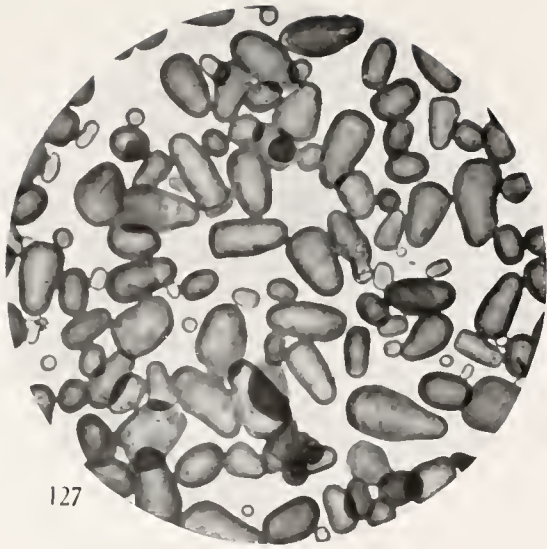
125



126

121 and 122. *Lilium parryi*.  
123 and 124. *Lilium rubellum*.  
125 and 126. *Lilium philadelphicum*.

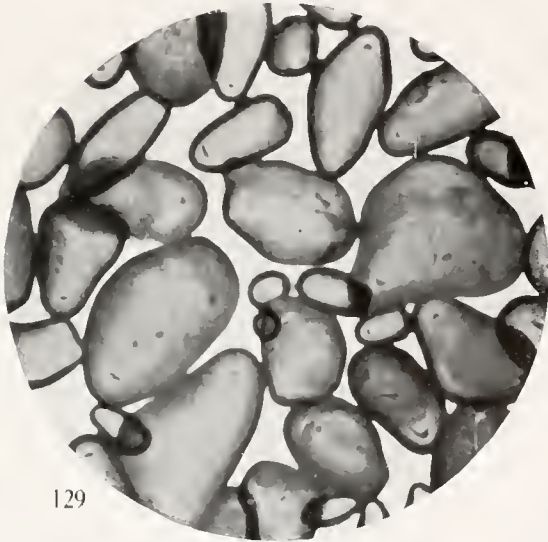




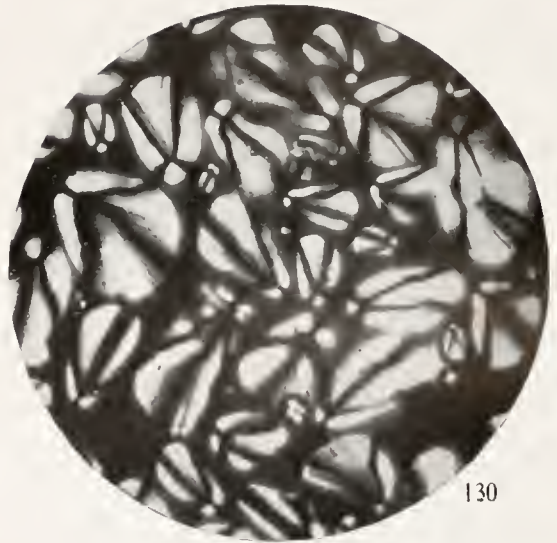
127



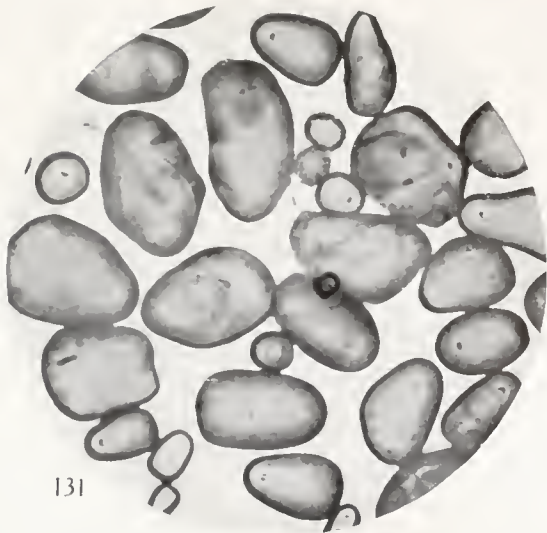
128



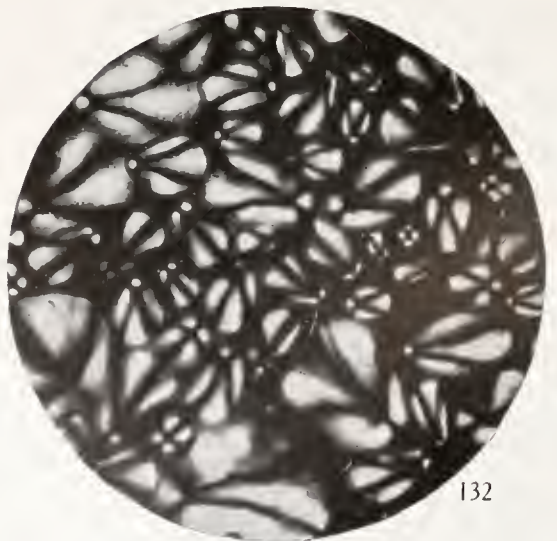
129



130



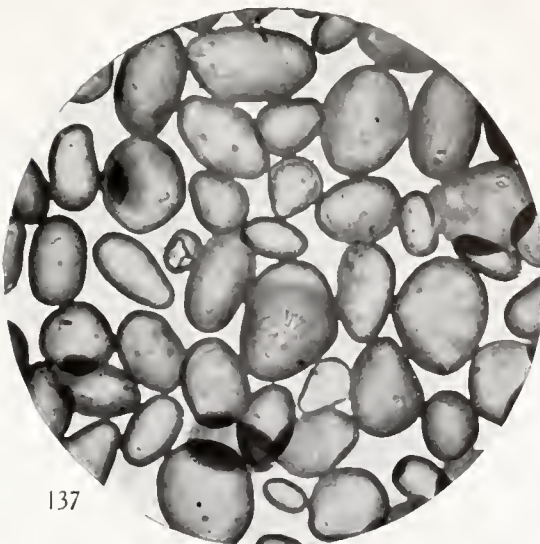
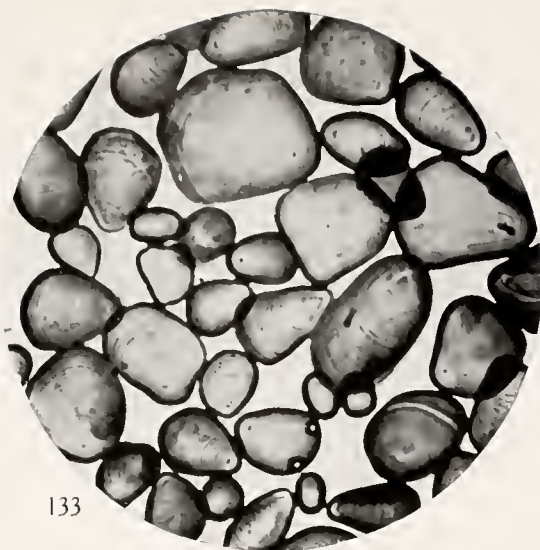
131



132

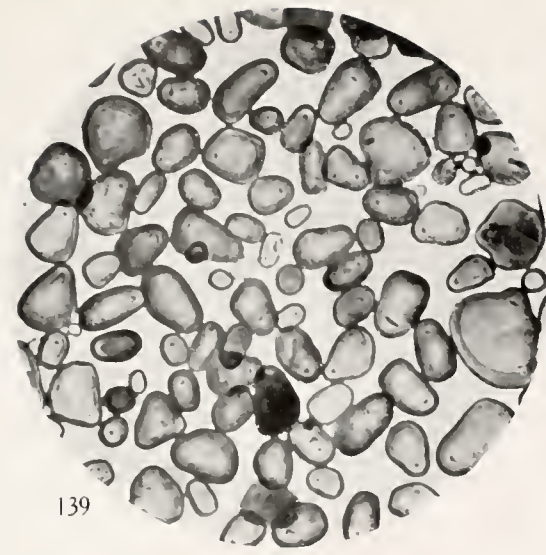
127 and 128. *Lilium tigrinum* var. *splendens*.  
129 and 130. *Lilium henryi*.  
131 and 132. *Lilium auratum*.





133 and 134. *Lilium speciosum* var. *album*.  
135 and 136. *Lilium martagon*.  
137 and 138. *Lilium superbum*.

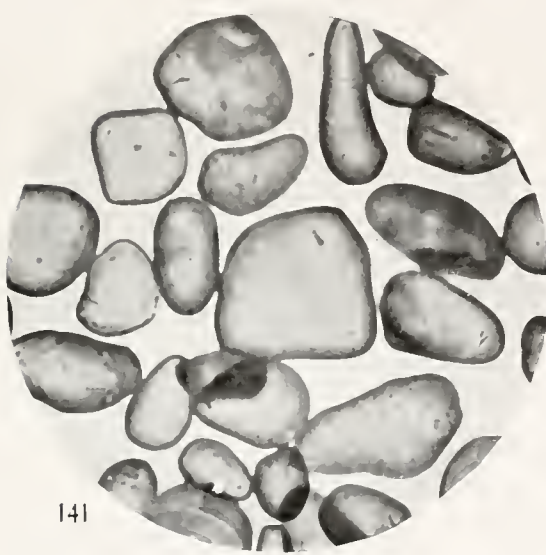




139



140



141



142



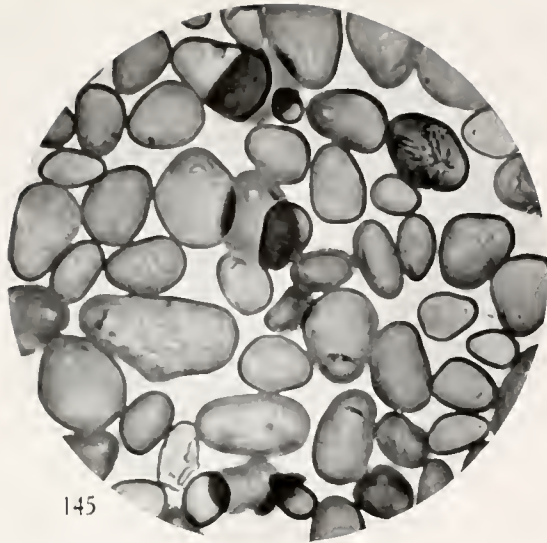
143



144

139 and 140. *Lilium tenuifolium*.  
141 and 142. *Lilium pardalinum*.  
143 and 144. *Lilium puberulum*.

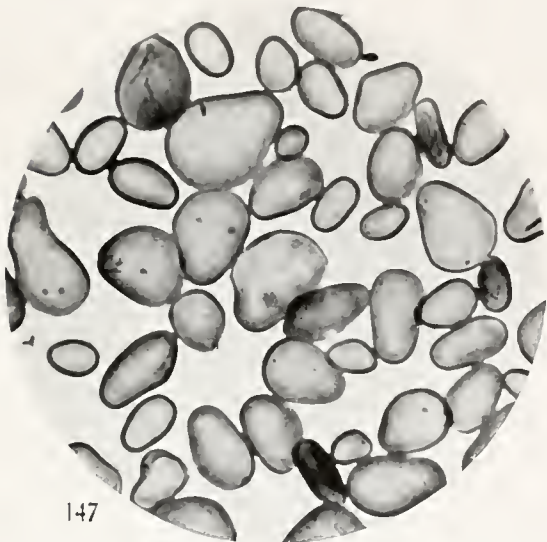




145



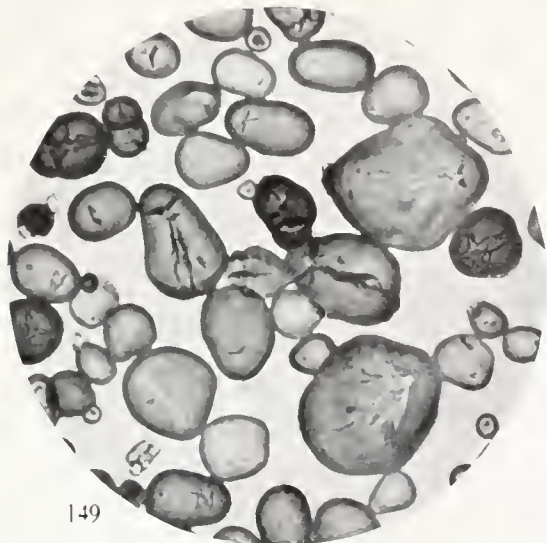
146



147



148



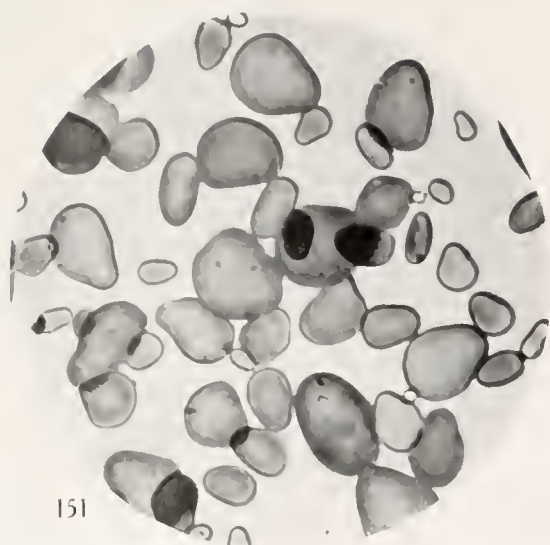
149



150

145 and 146. *Fritillaria melagrifolia*.  
147 and 148. *Fritillaria pyrenaica*.  
149 and 150. *Fritillaria pudica*.

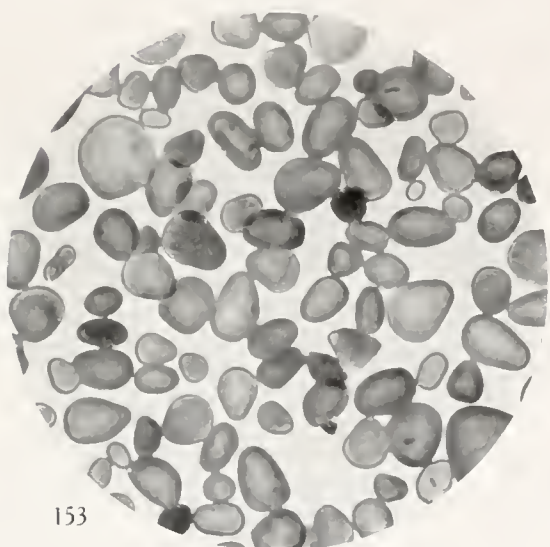




151



152



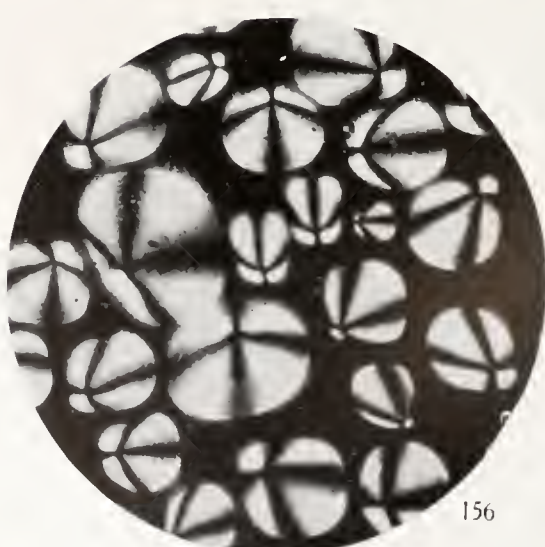
153



154



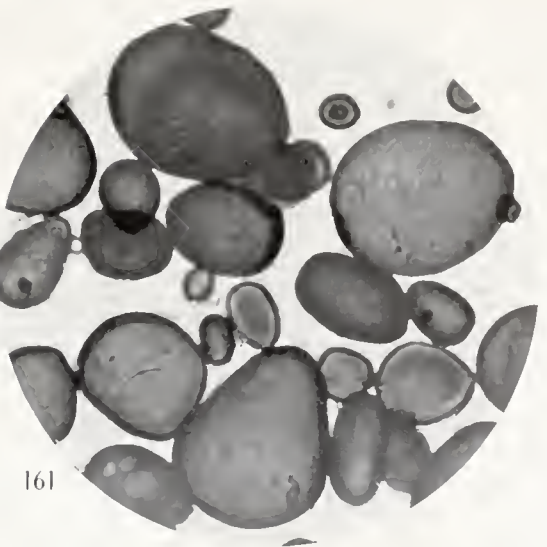
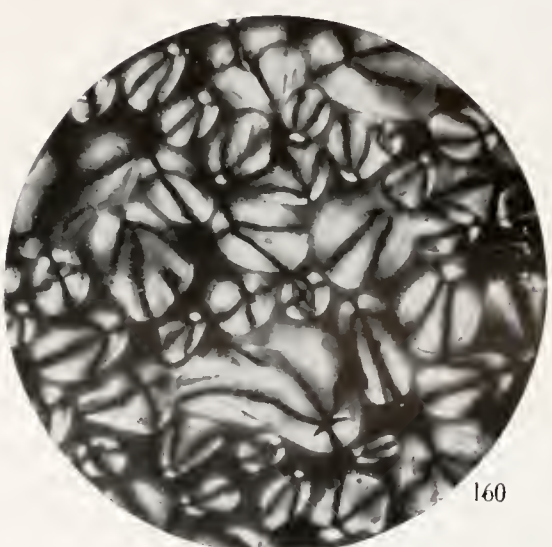
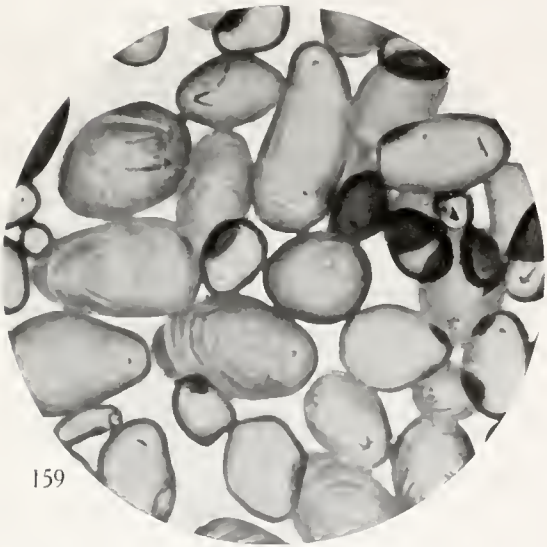
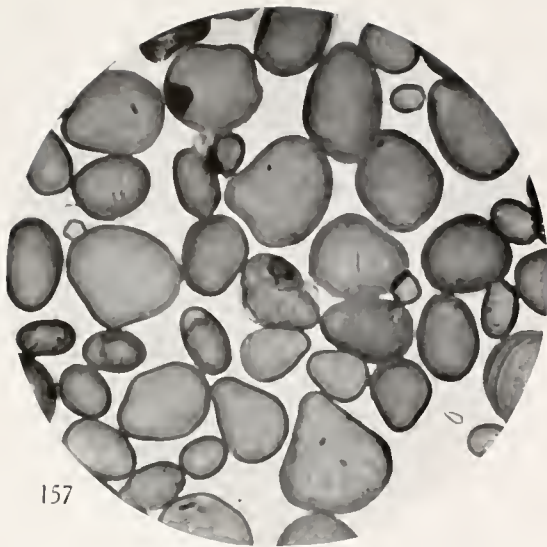
155



156

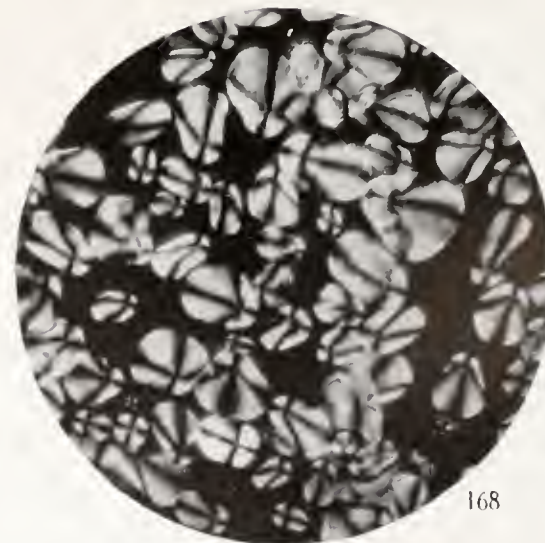
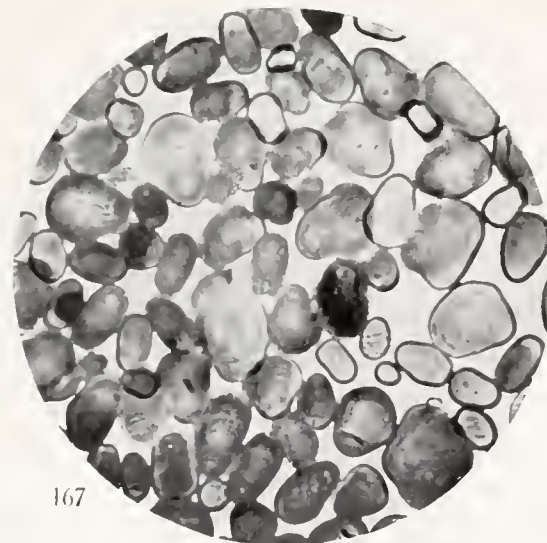
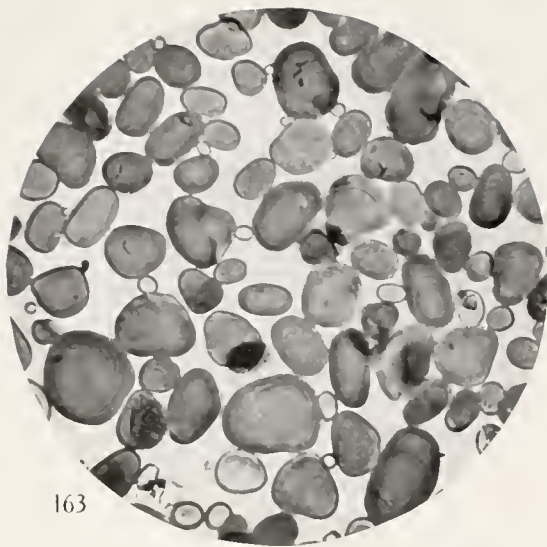
151 and 152, *Fritillaria aurea*,  
153 and 154, *Fritillaria armena*,  
155 and 156, *Fritillaria imperialis* var. *aurora*.





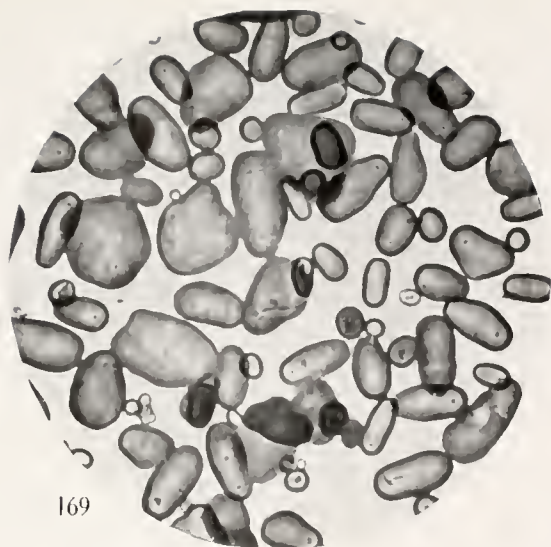
157 and 158. *Fritillaria liliacea*.  
159 and 160. *Fritillaria recurva*.  
161 and 162. *Fritillaria persica*.





163 and 164. *Calochortus albus*.  
165 and 166. *Calochortus maweanus* var. *major*.  
167 and 168. *Calochortus benthami*.

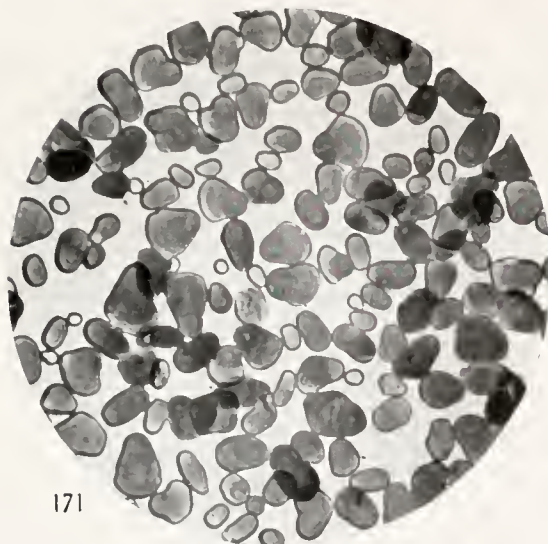




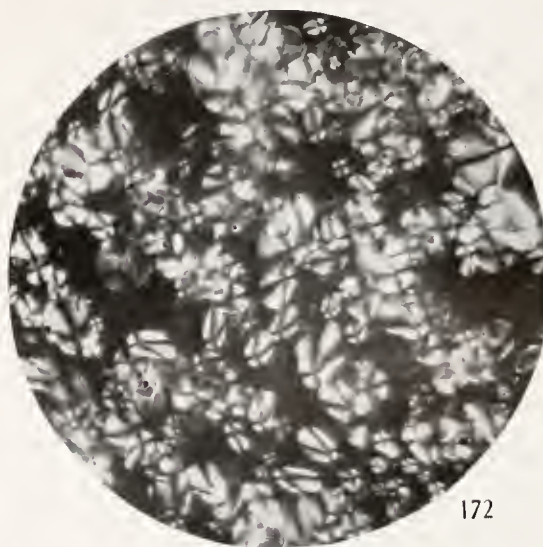
169



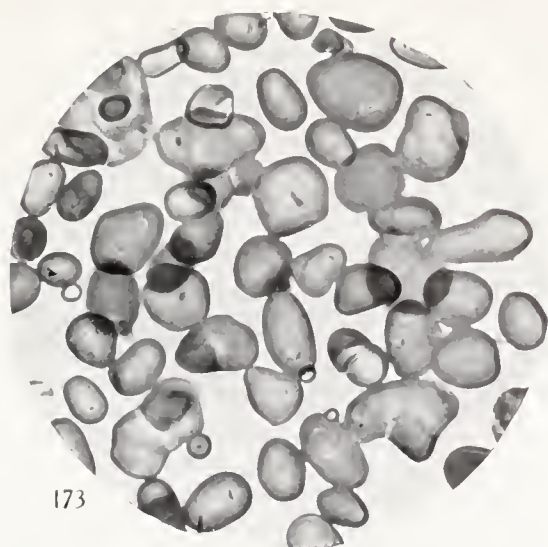
170



171



172



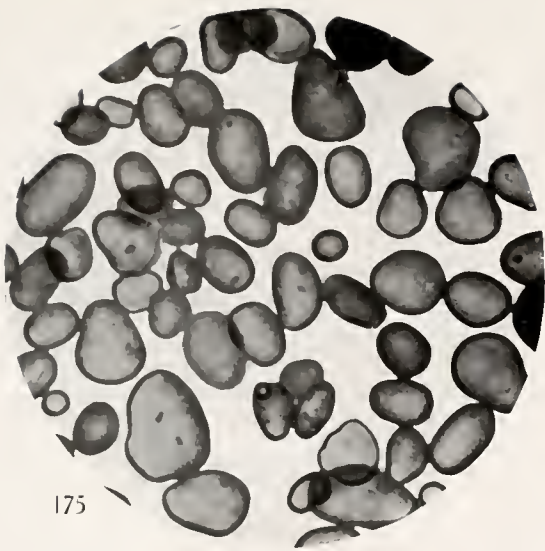
173



174

169 and 170. *Calochortus lilacinus*.  
171 and 172. *Calochortus nitidus*.  
173 and 174. *Calochortus howellii*.

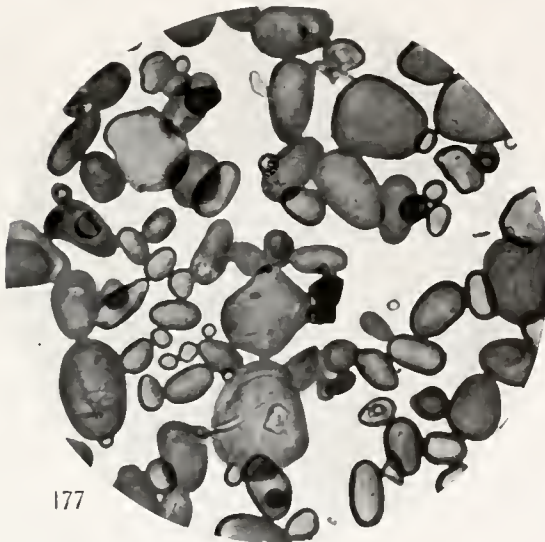




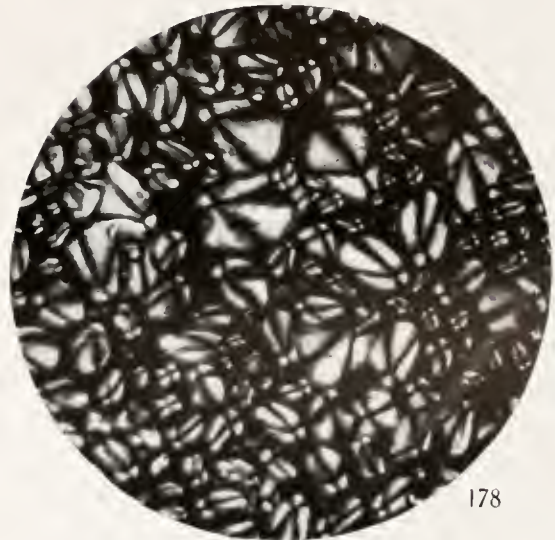
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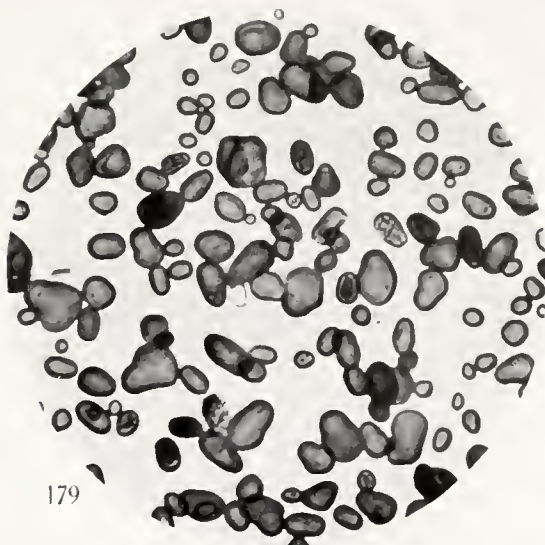
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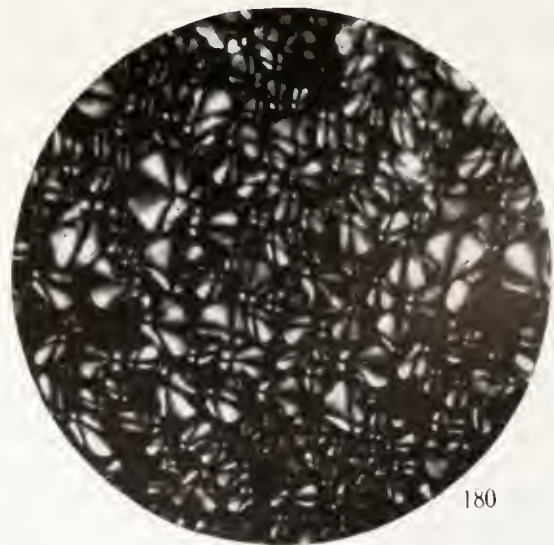
177



178



179



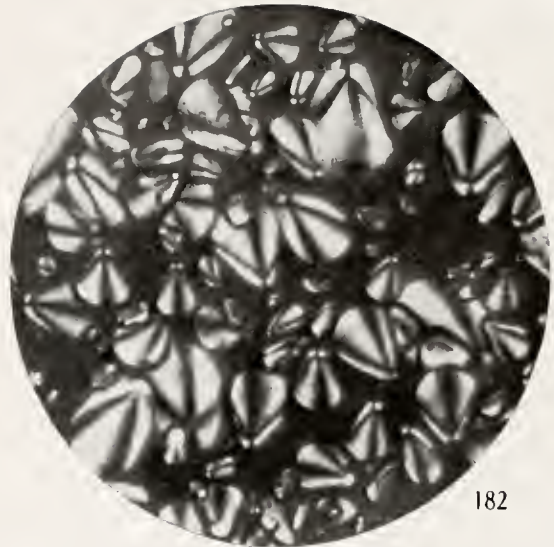
180

175 and 176. *Calochortus leichlinii*.  
177 and 178. *Calochortus luteus* var. *oculatus*.  
179 and 180. *Calochortus splendens*.

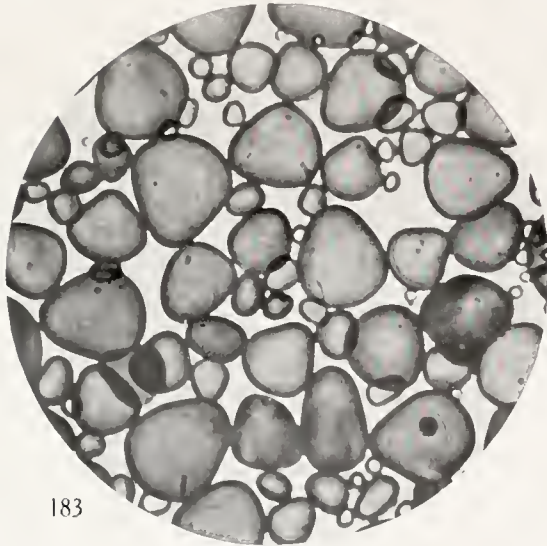




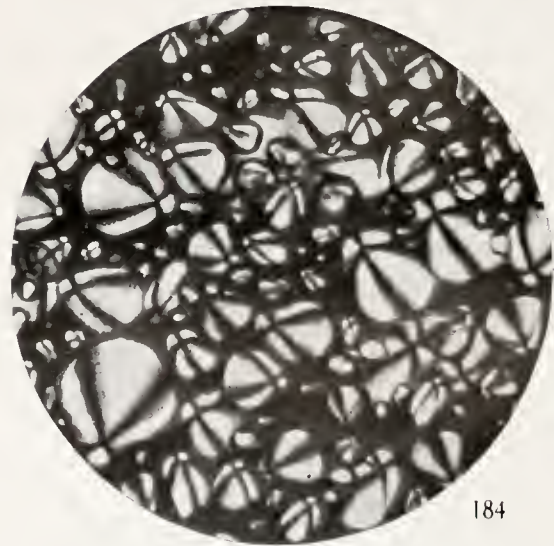
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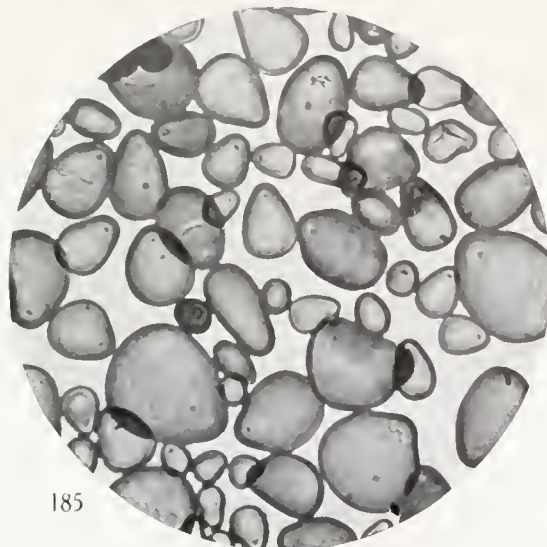
182



183



184



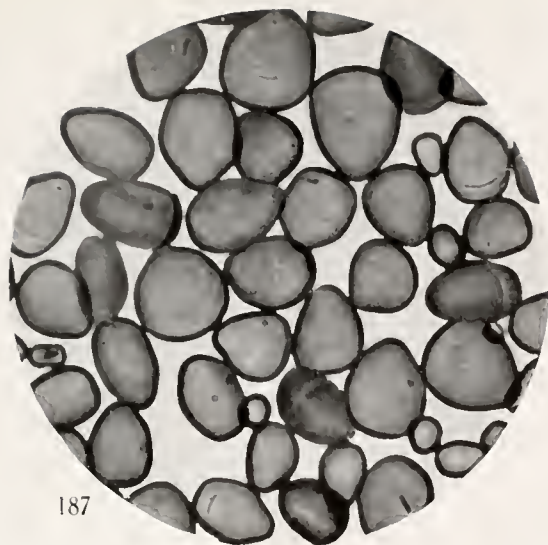
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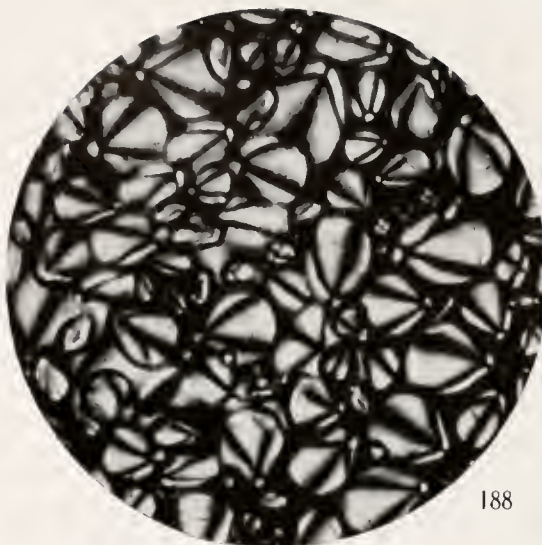
186

181 and 182. *Tulipa hageri*.  
183 and 184. *Tulipa sylvestris*  
185 and 186. *Tulipa greigi*.

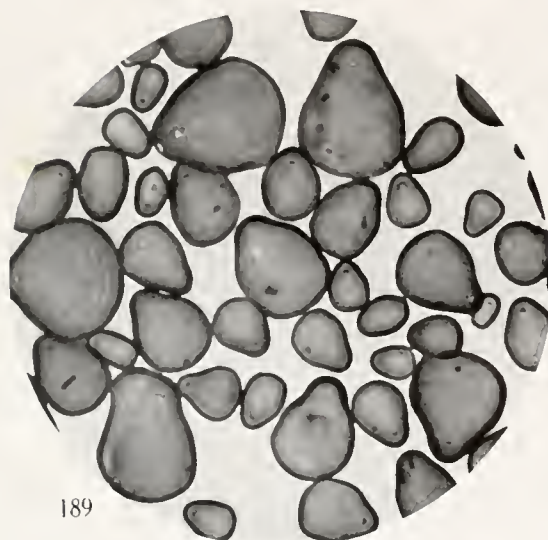




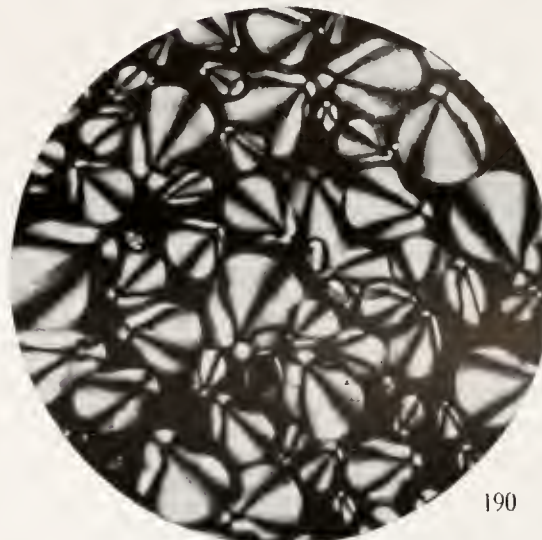
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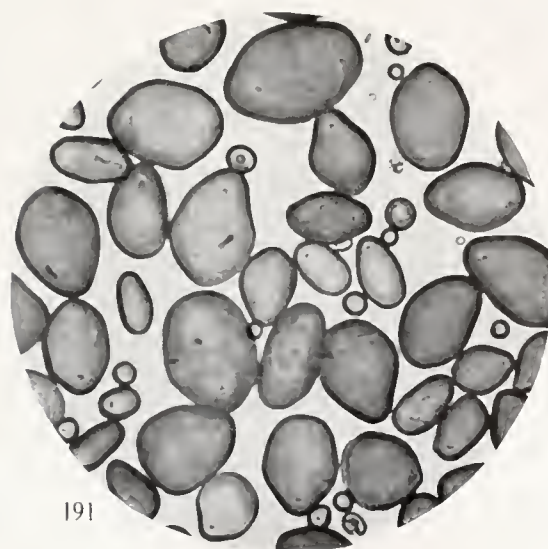
188



189



190



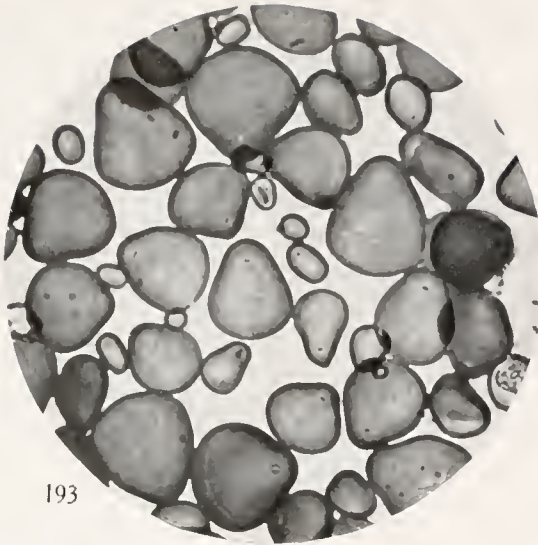
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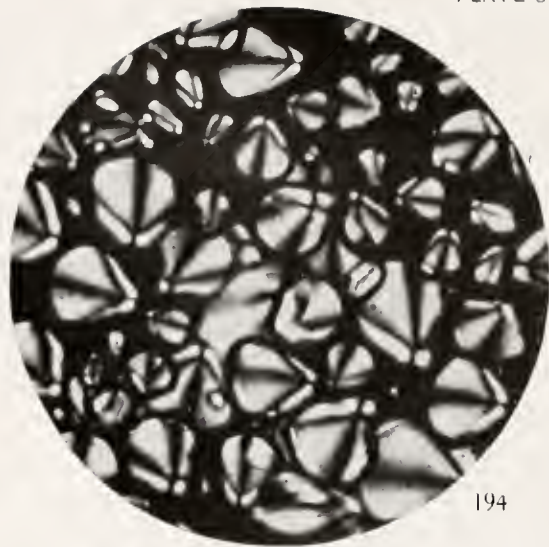
192

187 and 188. *Tulipa billictiana*.  
189 and 190. *Tulipa didieri*.  
191 and 192. *Tulipa didieri* var. *mauriana*.

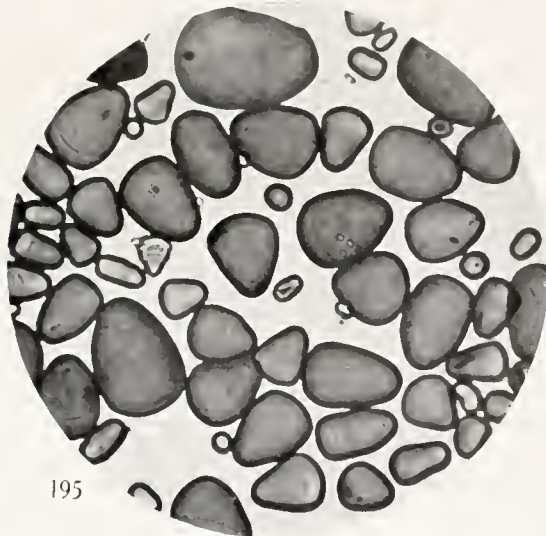




193



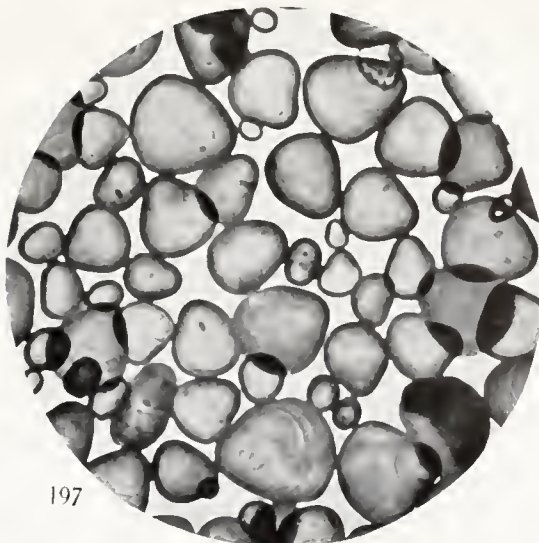
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195



196



197



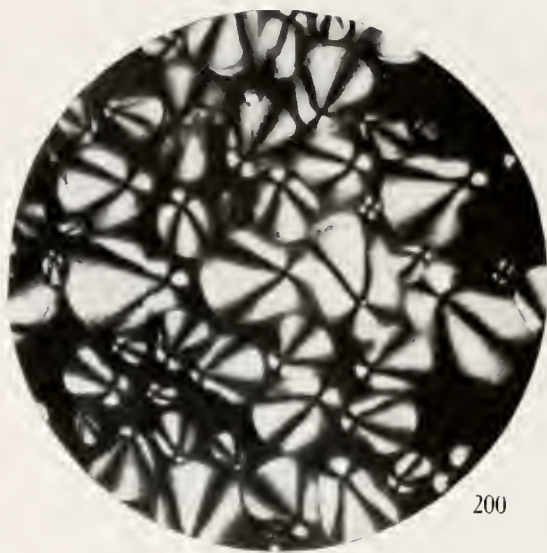
198

193 and 194. *Tulipa didieri* var. *fraisoniana*.  
 195 and 196. *Tulipa chusiana*.  
 197 and 198. *Tulipa chusiana* var. *persica*.

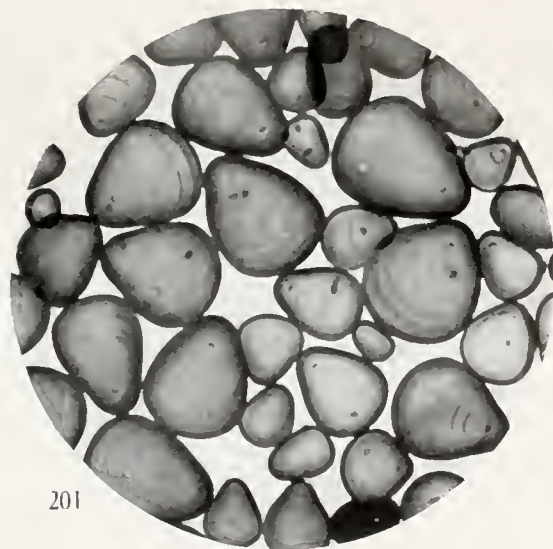




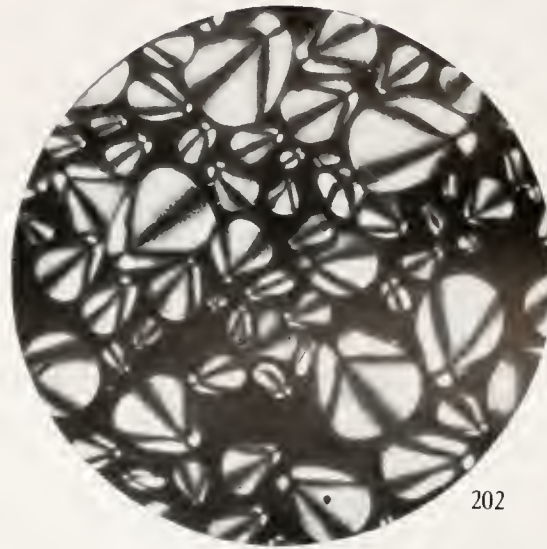
199



200



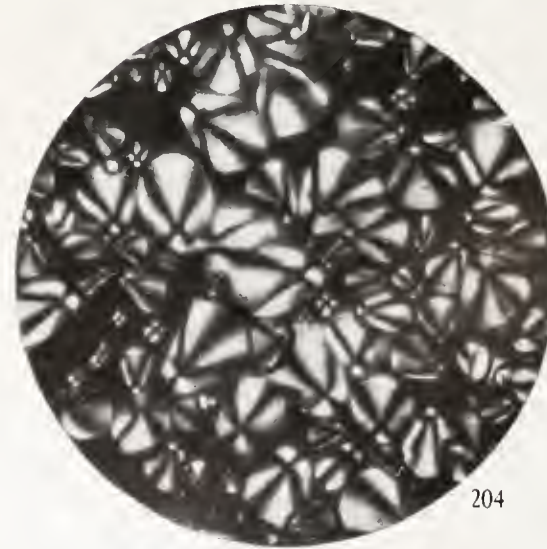
201



202



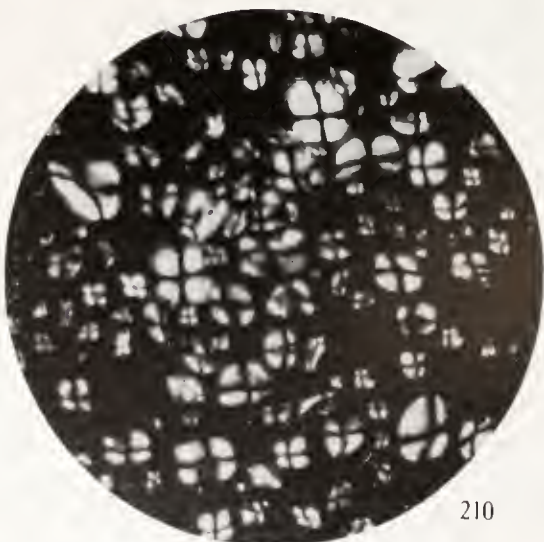
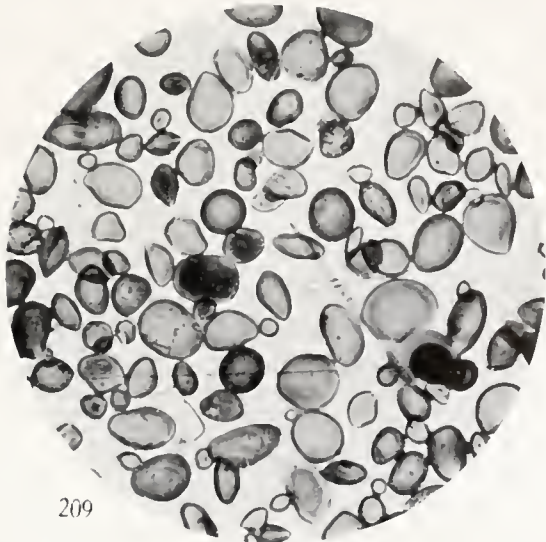
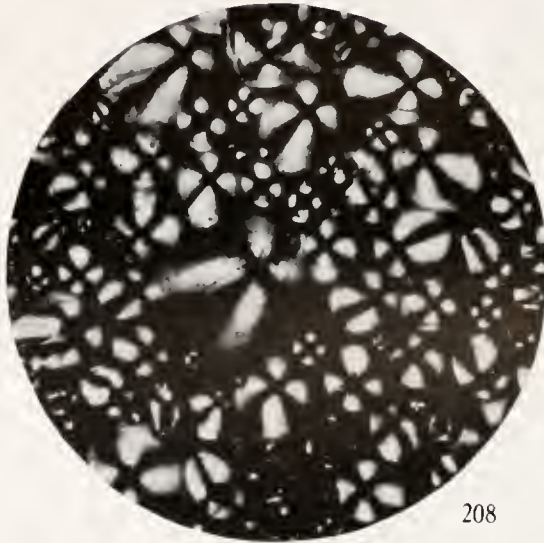
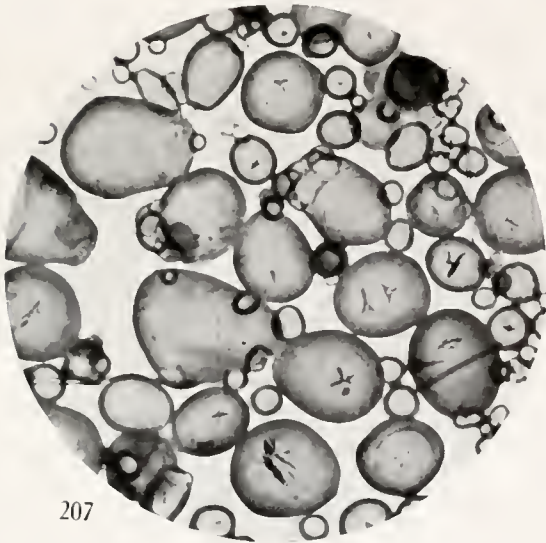
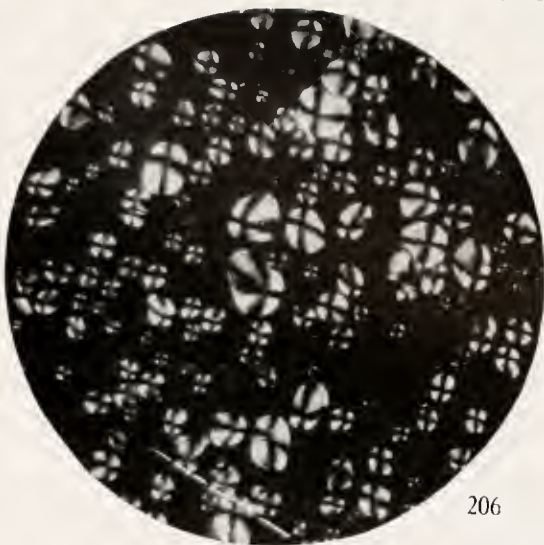
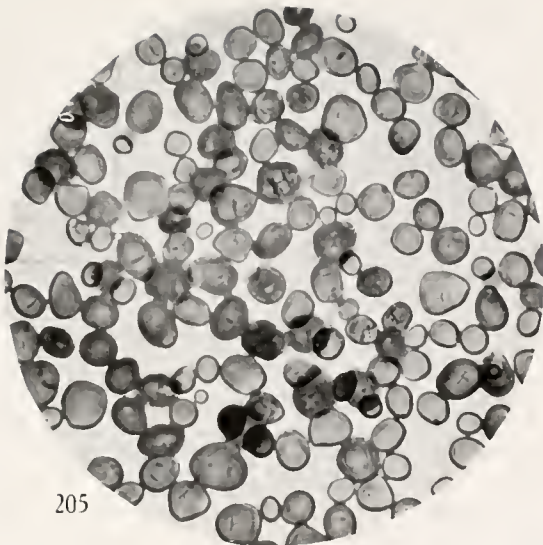
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204

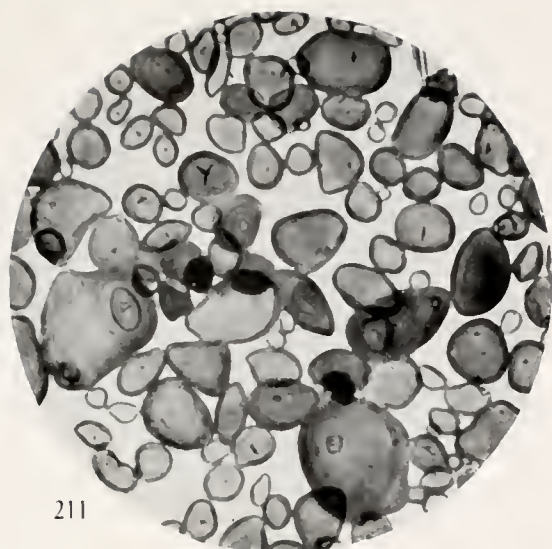
199 and 200. *Tulipa oculus-solis*.  
201 and 202. *Tulipa praeor*.  
203 and 204. *Tulipa australis*.



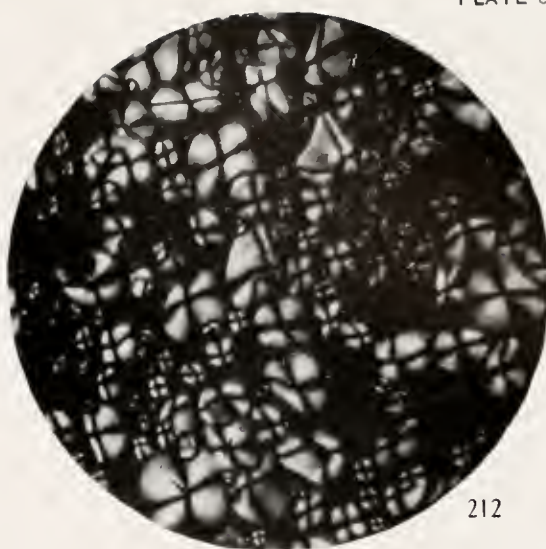


205 and 206, *Scilla sibirica*.  
207 and 208, *Scilla peruviana*.  
209 and 210, *Scilla bifolia*.

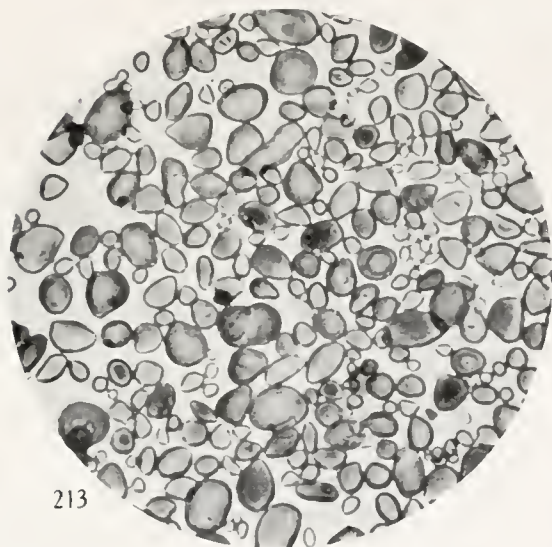




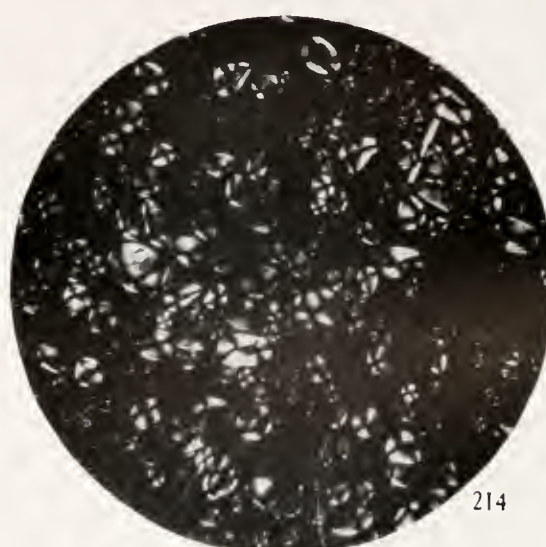
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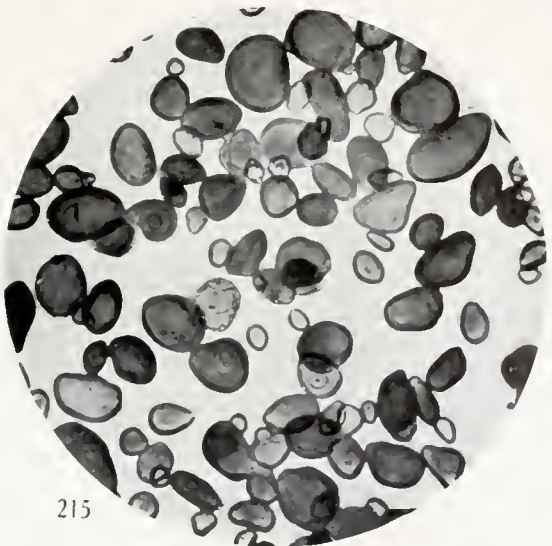
212



213



214



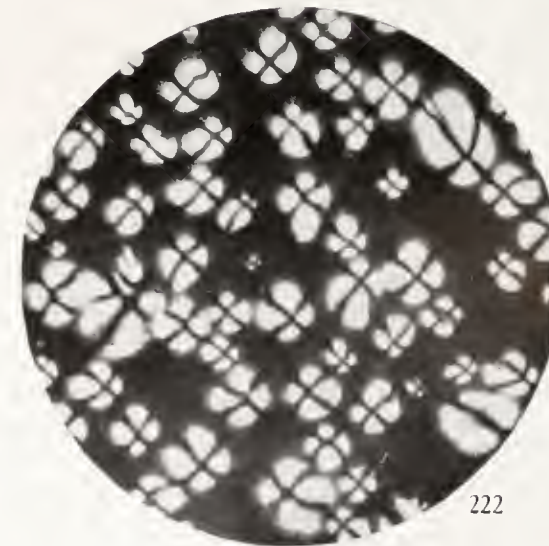
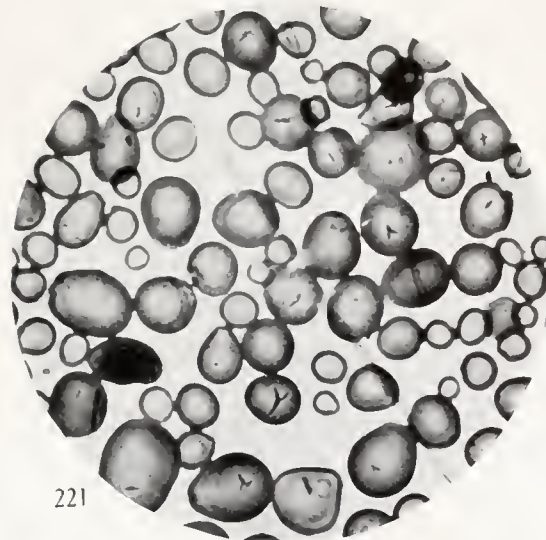
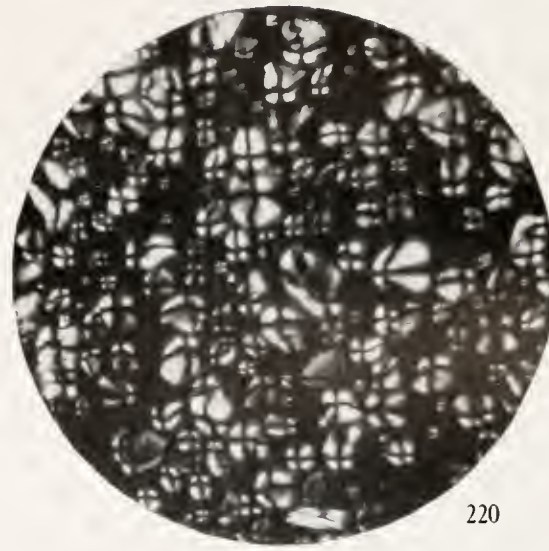
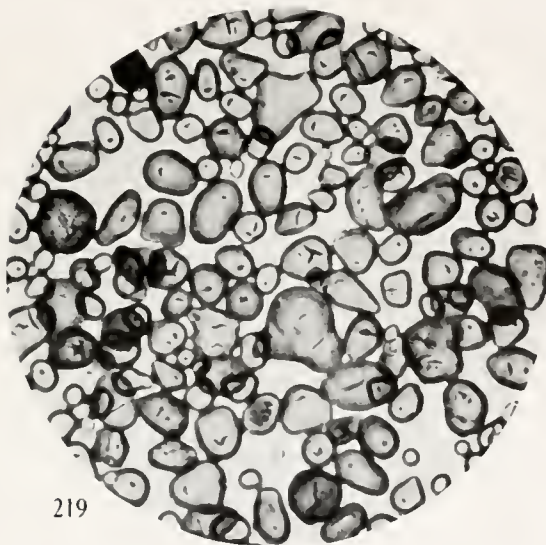
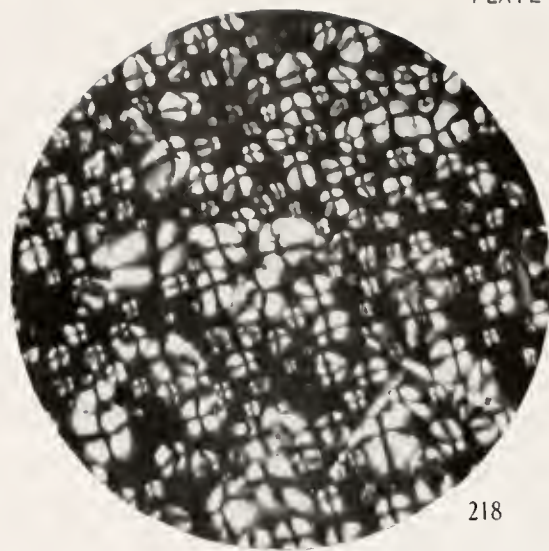
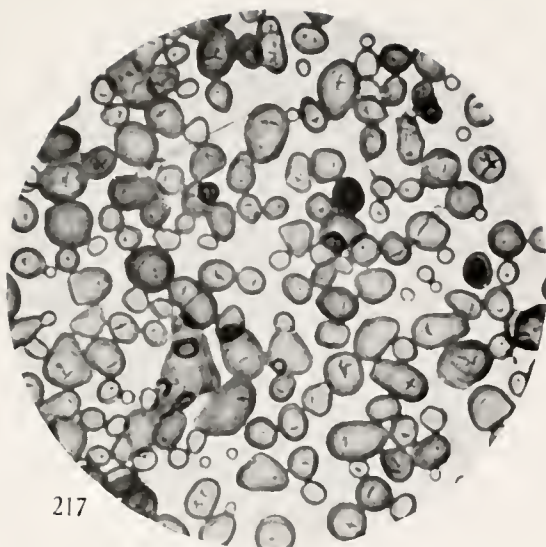
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216

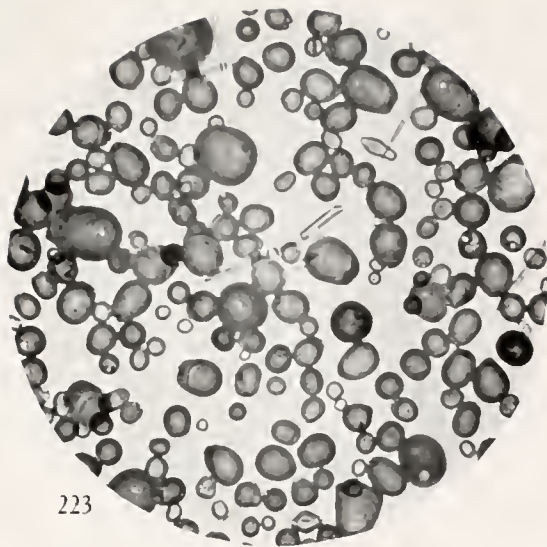
211 and 212. *Chionodoxa lucilia*.  
213 and 214. *Chionodoxa imolusi*.  
215 and 216. *Chionodoxa sardensis*.





217 and 218. *Puschkinia scilloides*,  
219 and 220. *Puschkinia scilloides* var. *libanotica*,  
221 and 222. *Ornithogalum nolanii*.

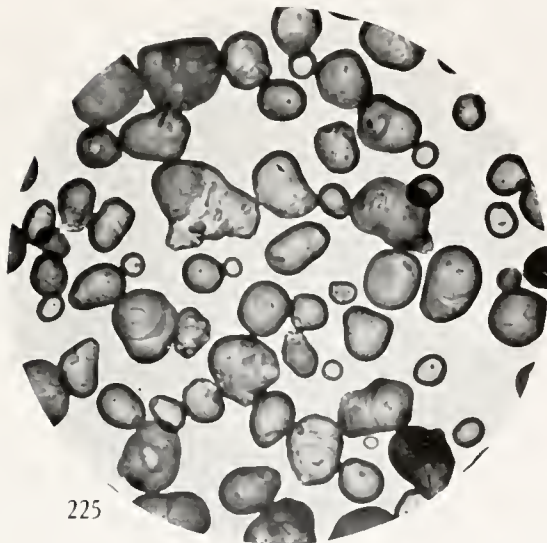




223



224



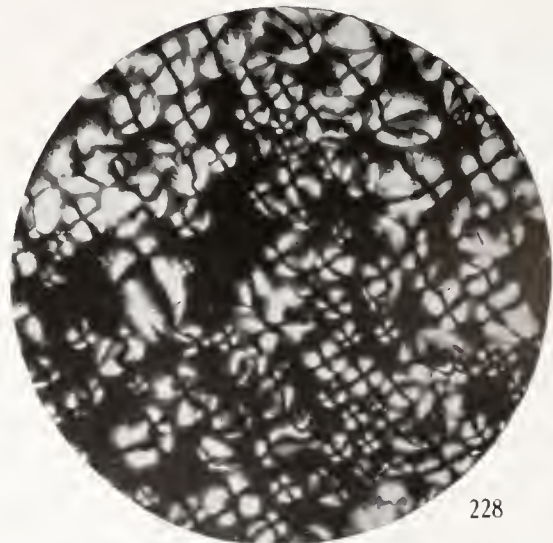
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226



227



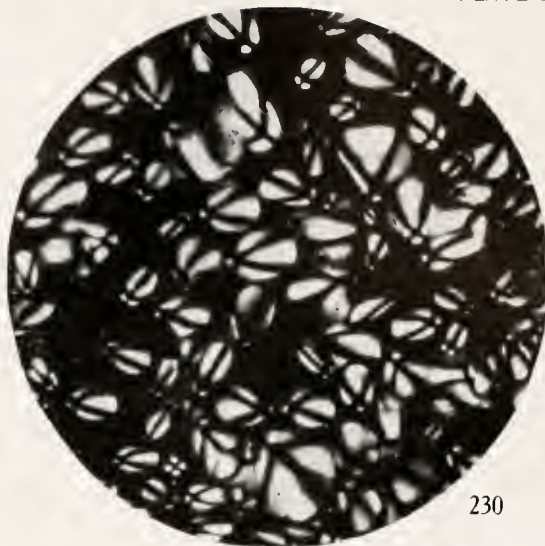
228

223 and 224. *Ornithogalum umbellatum*.  
 225 and 226. *Ornithogalum narbonense* (pyramidal).  
 227 and 228. *Ornithogalum thyrsoides* var. *aureum*.





229



230



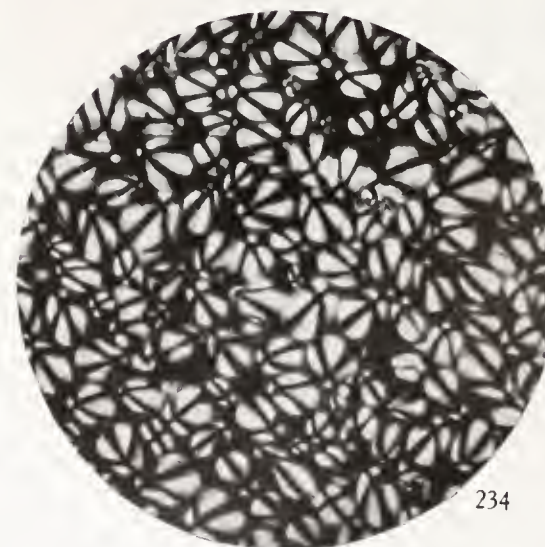
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232



233



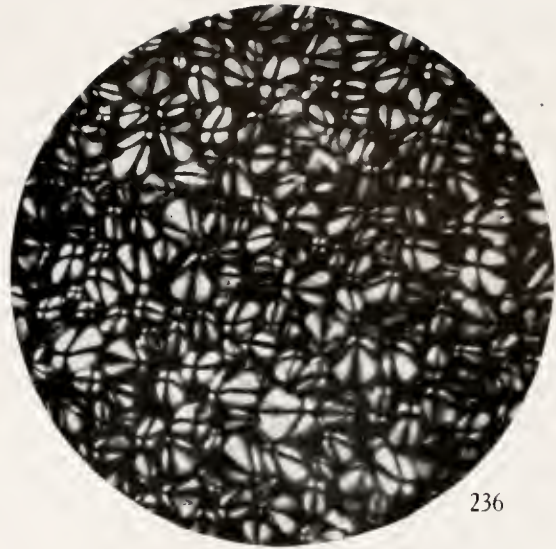
234

229 and 230. *Erythronium dens-canis*.  
231 and 232. *Erythronium dens-canis* var. *grandiflorum*.  
233 and 234. *Erythronium americanum*.





235



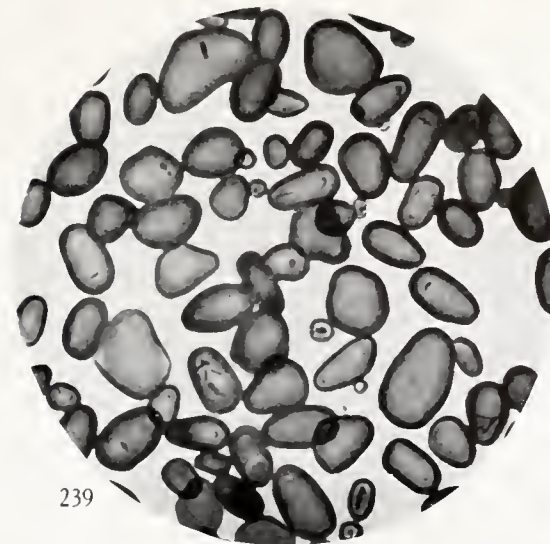
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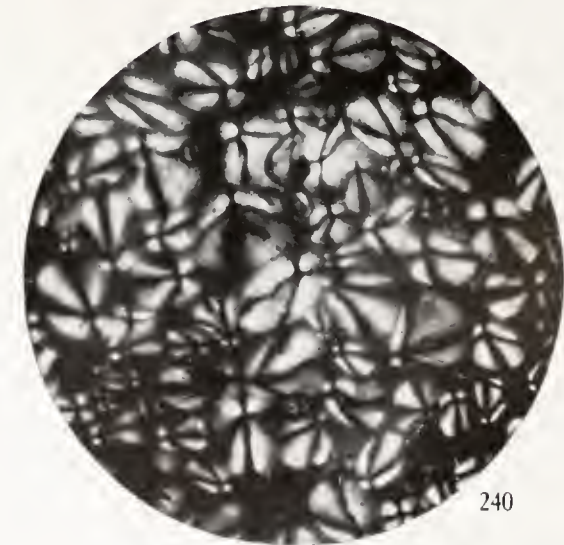
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238



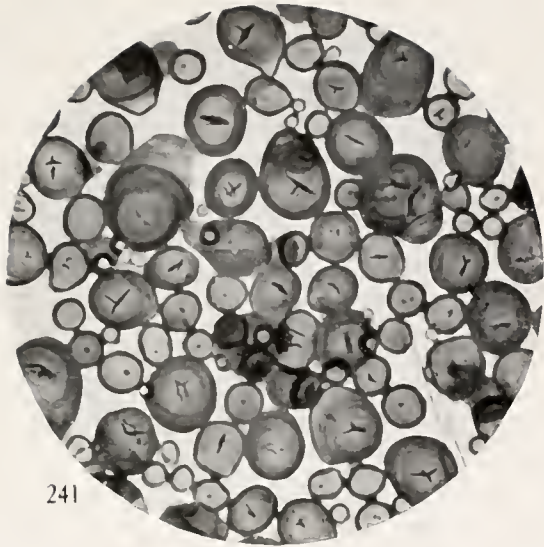
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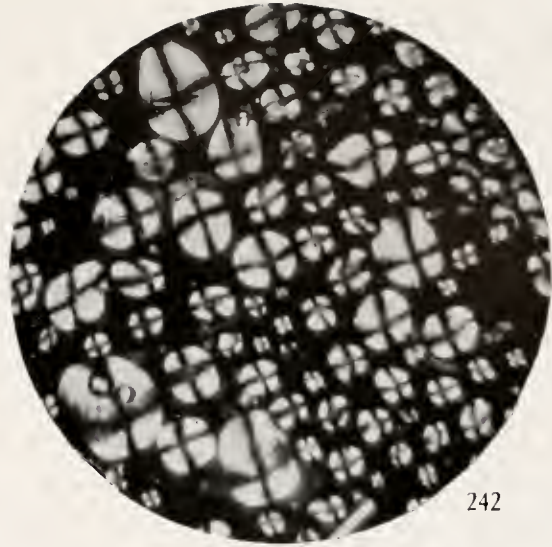
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235 and 236. *Erythronium grandiflorum*.  
237 and 238. *Erythronium citrinum*.  
239 and 240. *Erythronium californicum*.

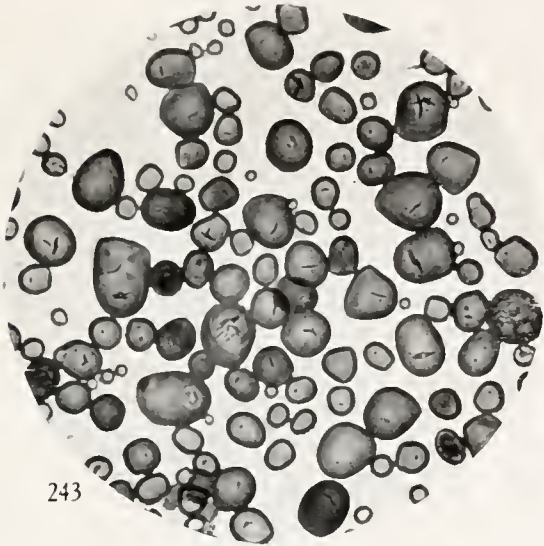




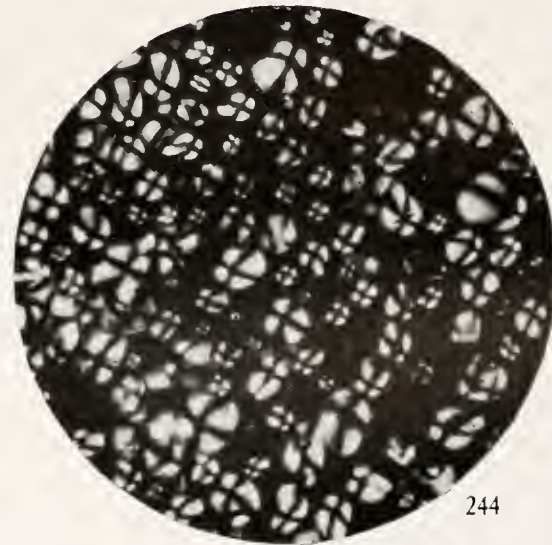
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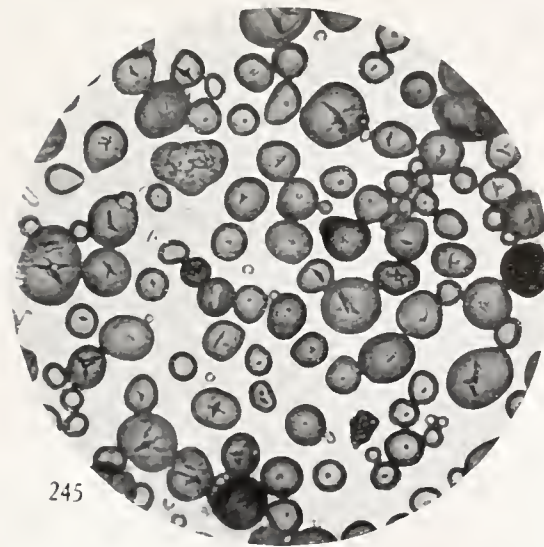
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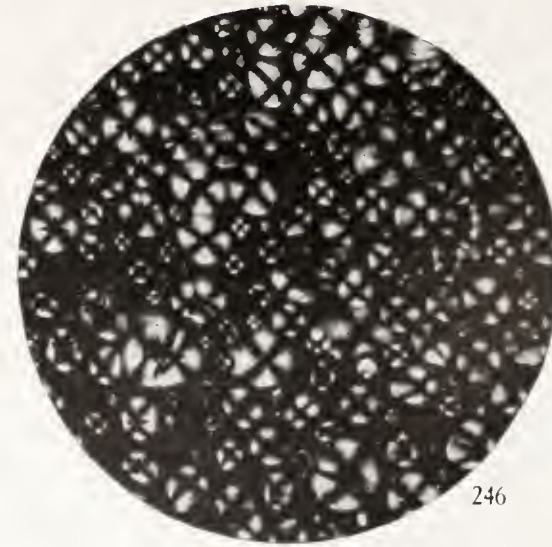
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244



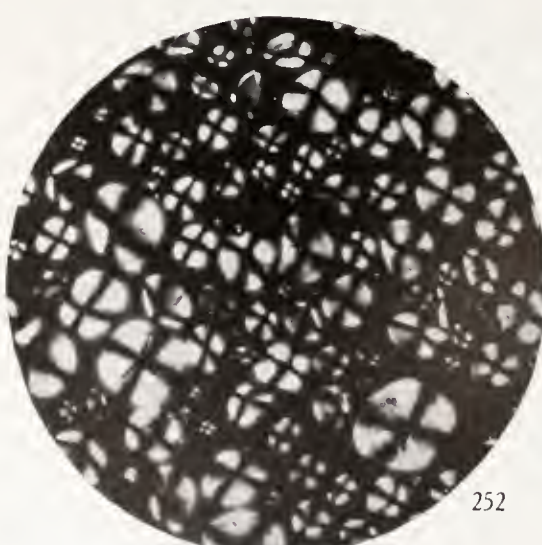
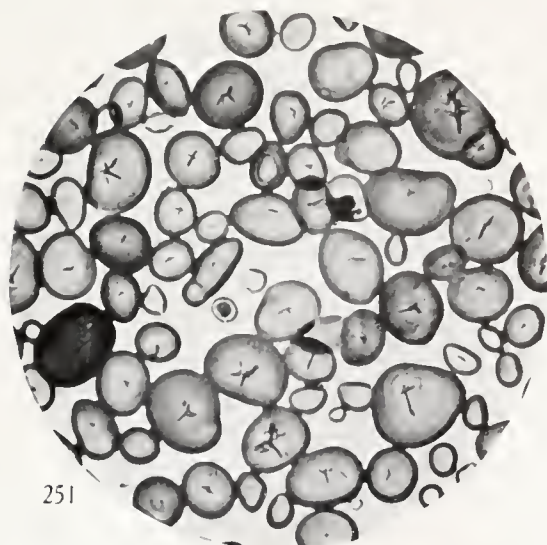
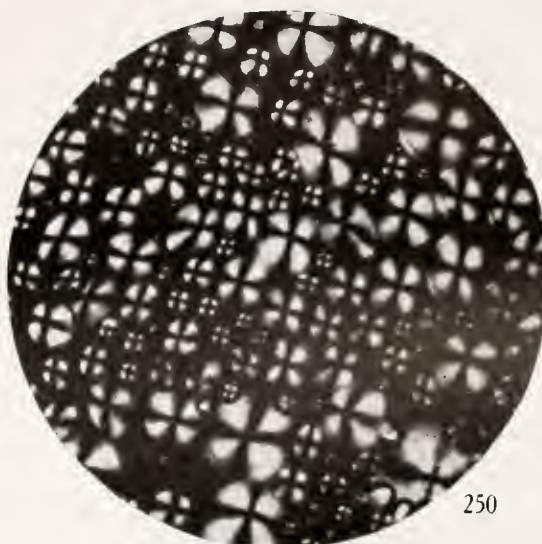
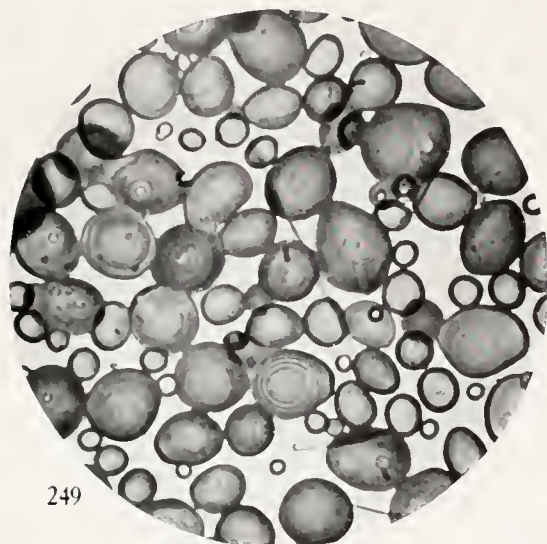
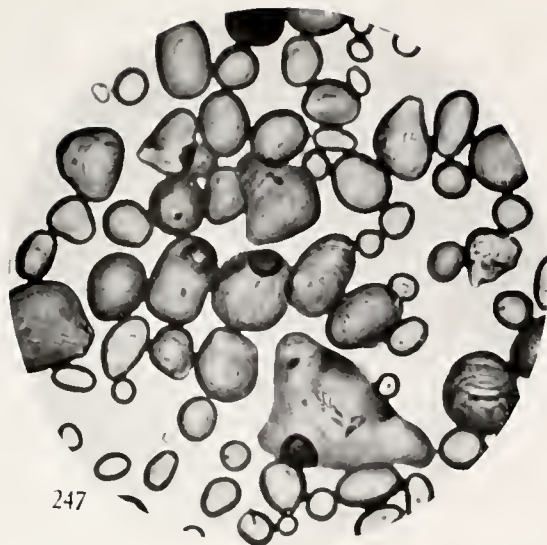
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246

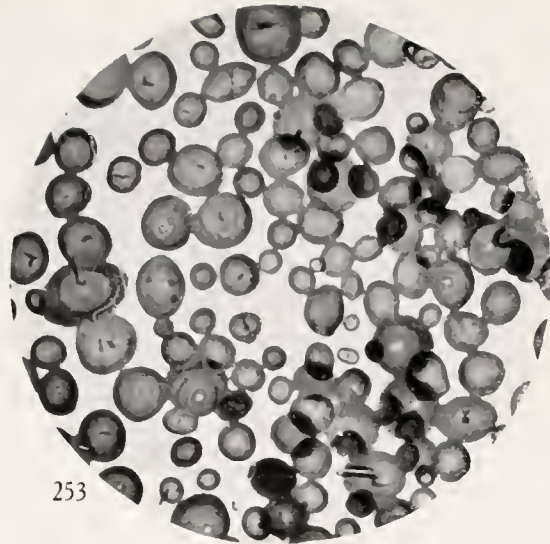
241 and 242. *Hyacinthus orientalis* var. *alba superbissima*.  
 243 and 244. *Hyacinthus orientalis* var. *albus* (white).  
 245 and 246. *Hyacinthus orientalis* var. *albus* (Italian)



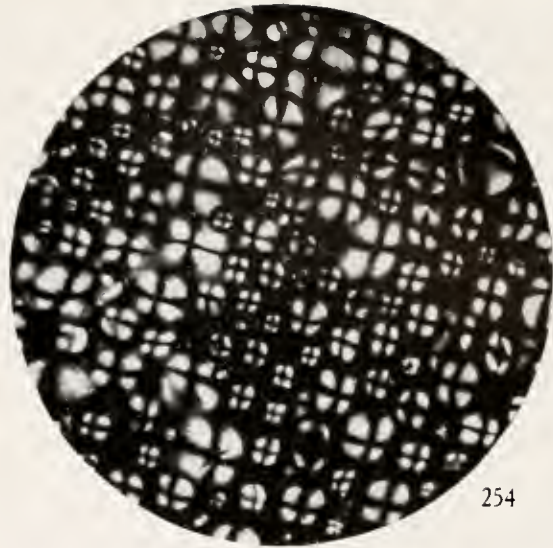


247 and 248. *Gallonia caudicans*.  
249 and 250. *Muscari botryoïdes*.  
251 and 252. *Muscari paradoxum*.

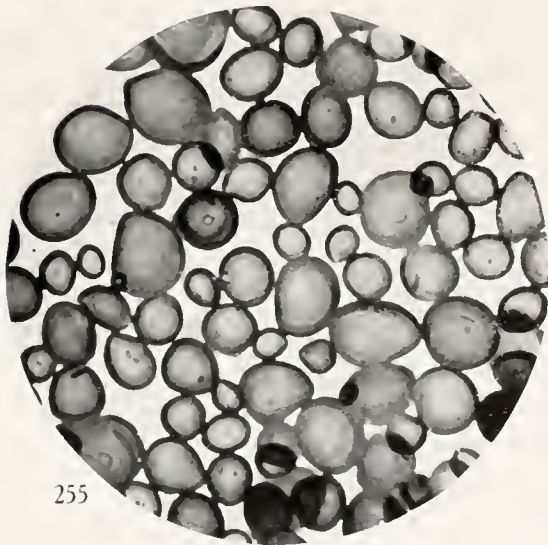




253



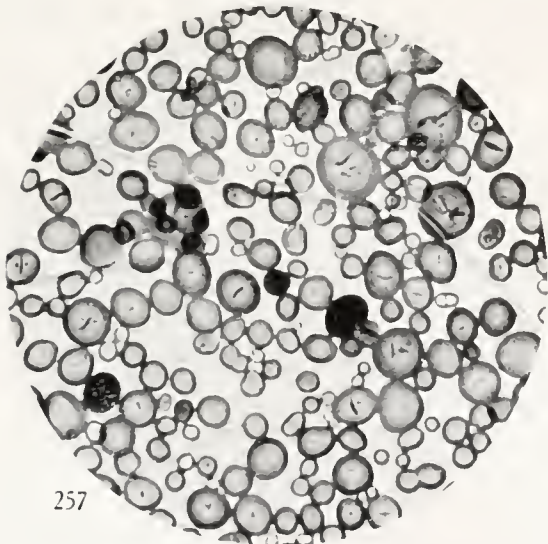
254



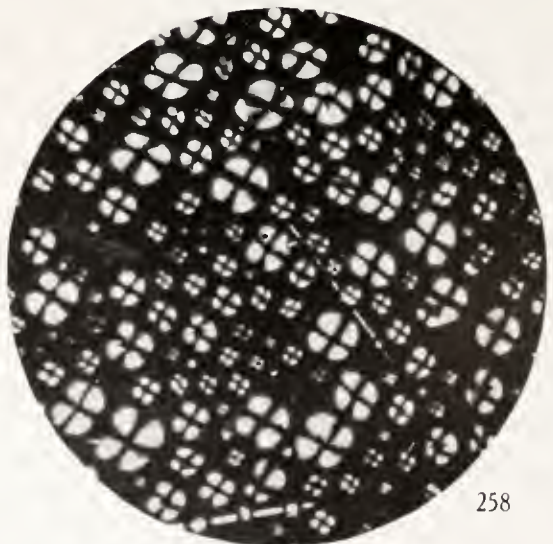
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256



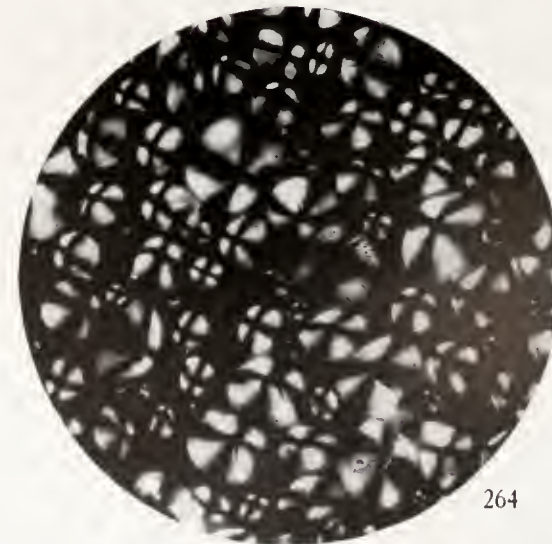
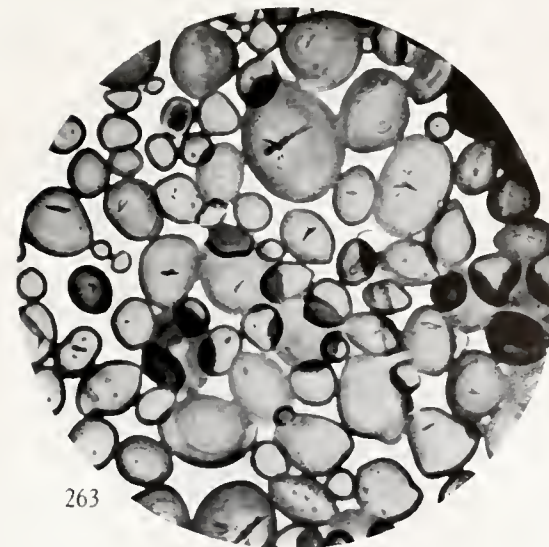
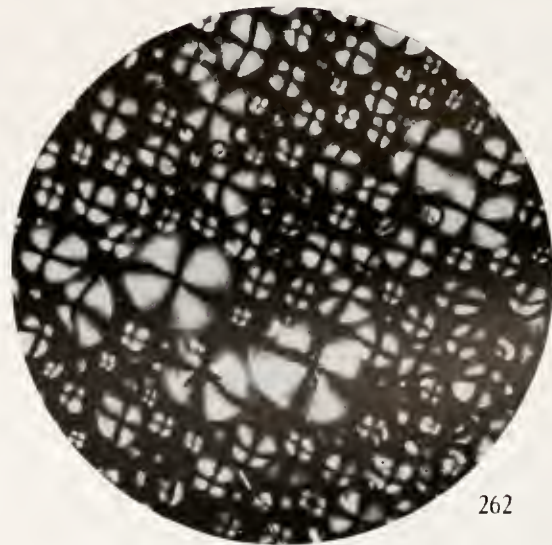
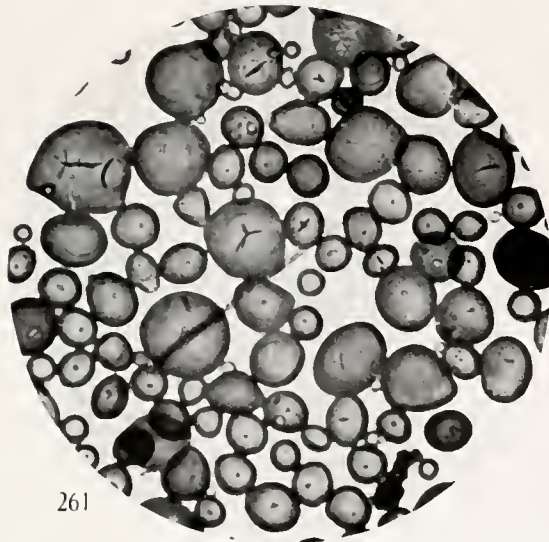
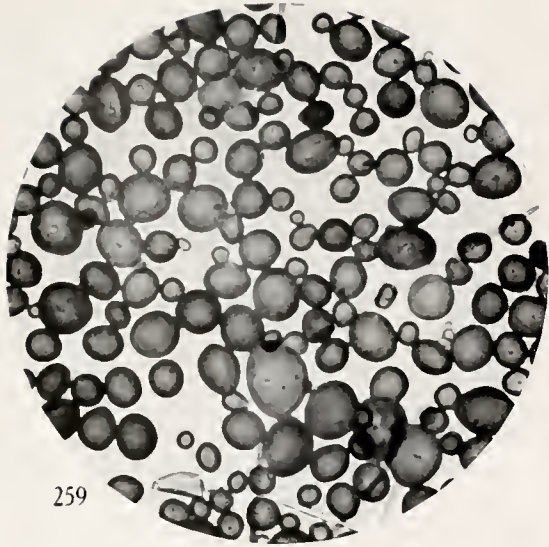
257



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253 and 254. *Muscari micranthum*.  
 255 and 256. *Muscari conicum*.  
 257 and 258. *Muscari commutatum*.





259 and 260. *Muscari racemosum*.  
261 and 262. *Muscari compactum*.  
263 and 264. *Muscari comosum*.

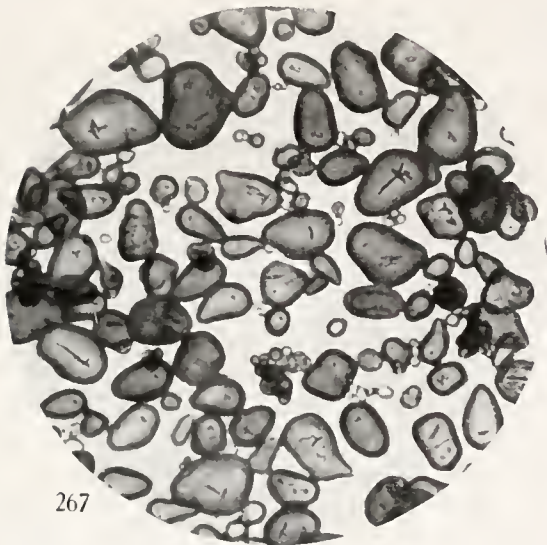




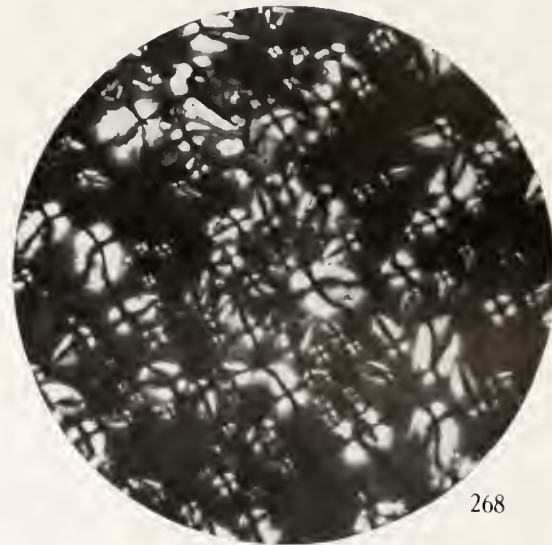
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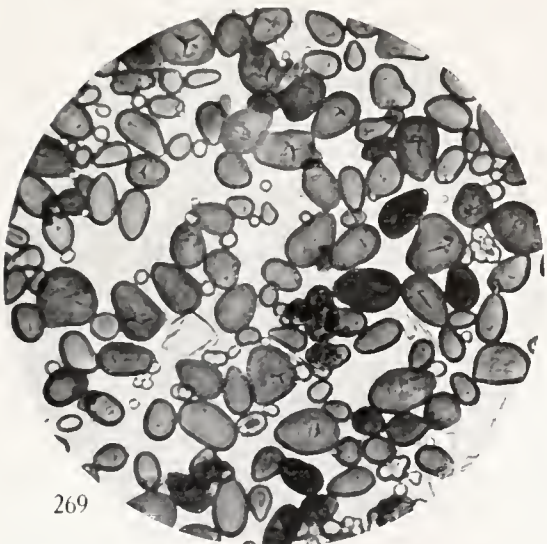
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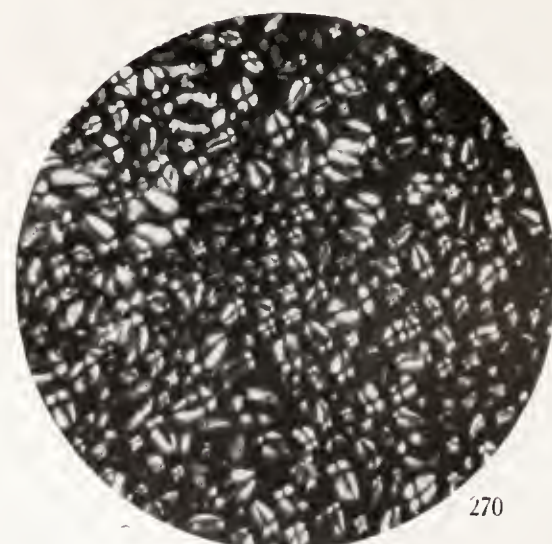
267



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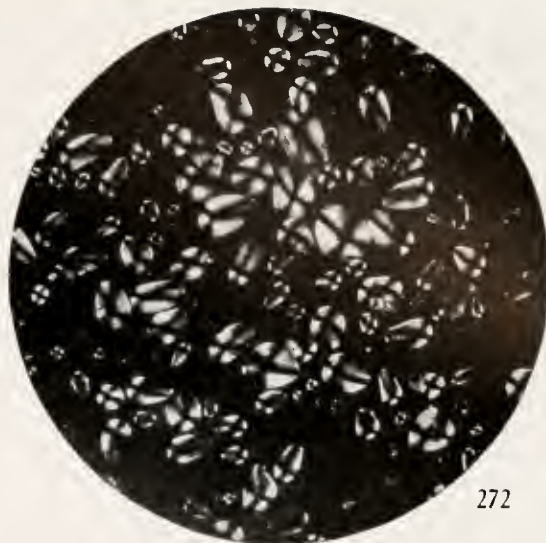
270

265 and 266. *Brodiaea peduncularis*.  
267 and 268. *Brodiaea irioides* var. *splendens*.  
269 and 270. *Brodiaea candida*.

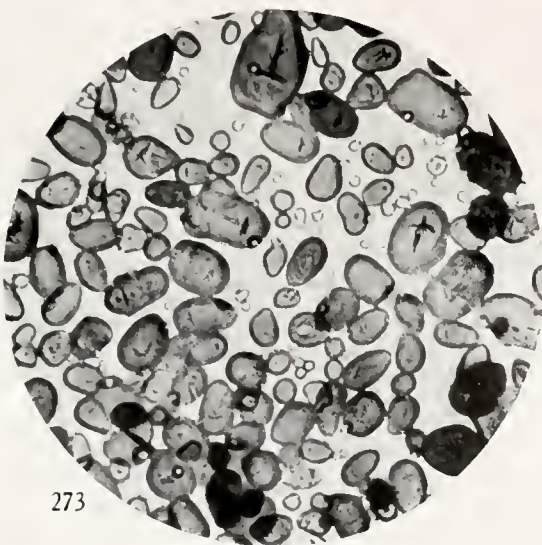




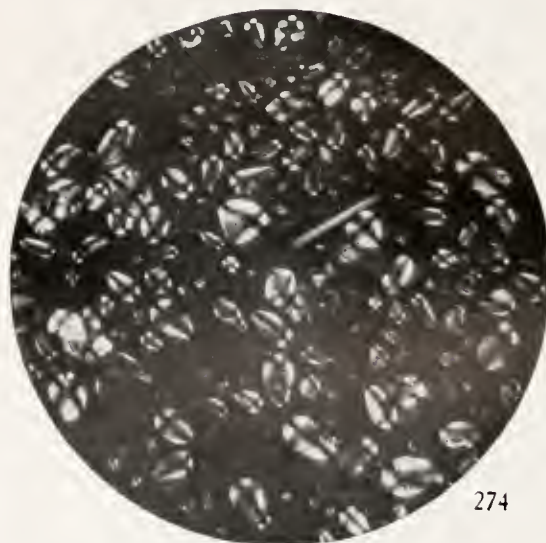
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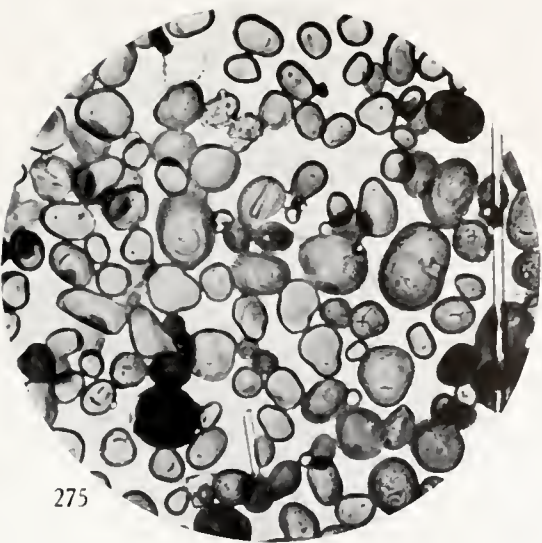
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273



274



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276

271 and 272. *Brodiaea lactea*.  
273 and 274. *Brodiaea laxa*.  
275 and 276. *Brodiaea coccinea*.

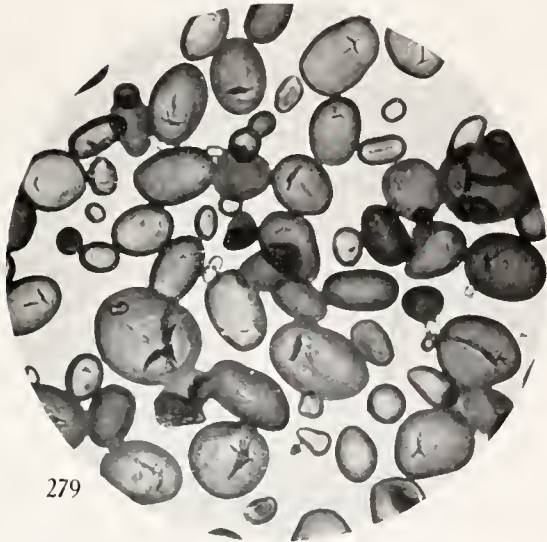




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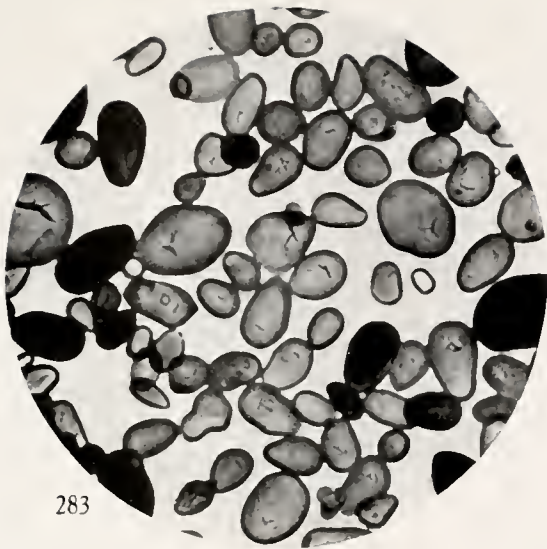
281



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277 and 278. *Brodiaea grandiflora*.  
279 and 280. *Brodiaea californica*.  
281 and 282. *Brodiaea purdyi*.

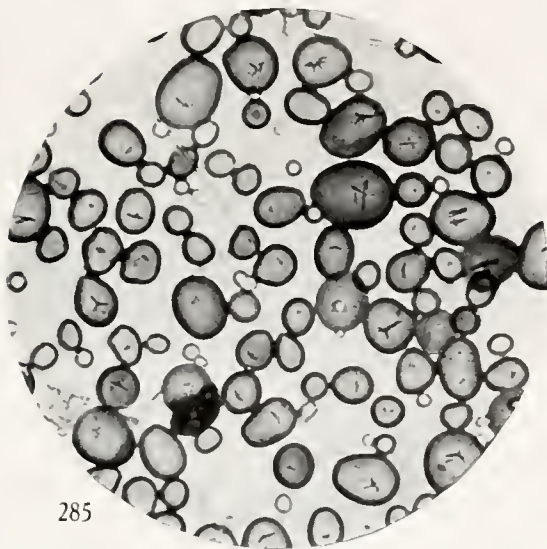




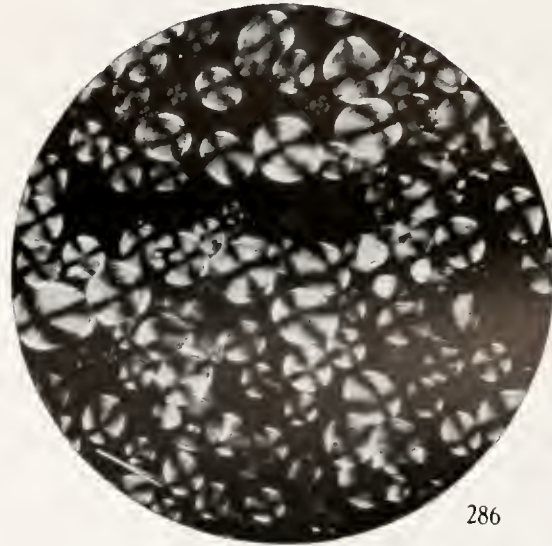
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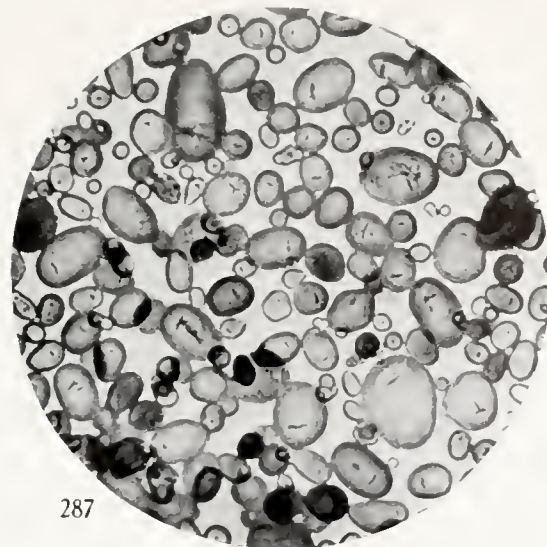
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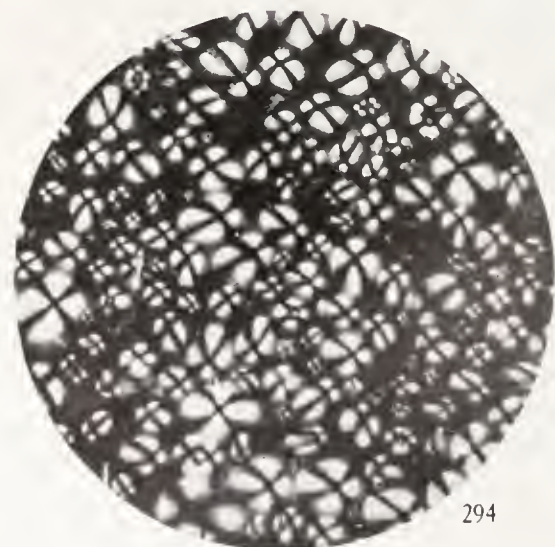
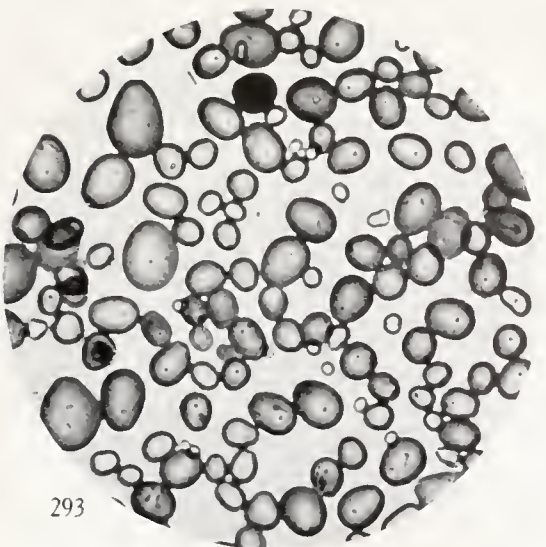
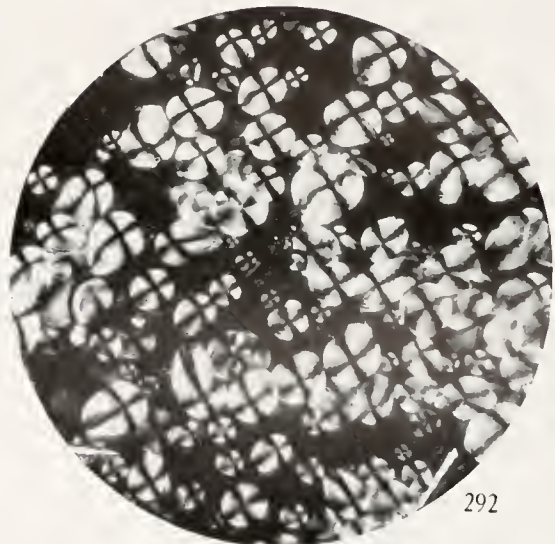
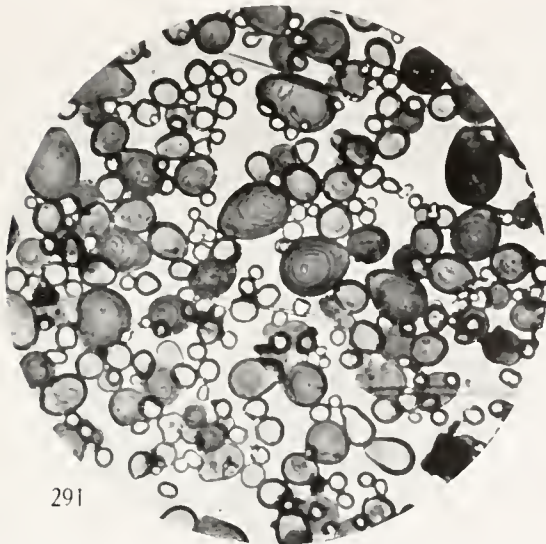
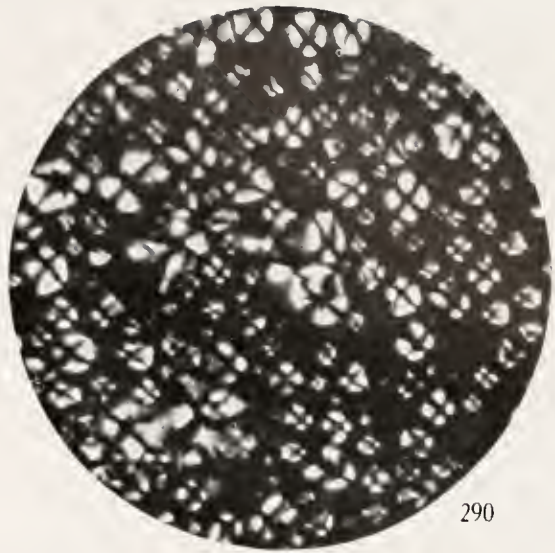
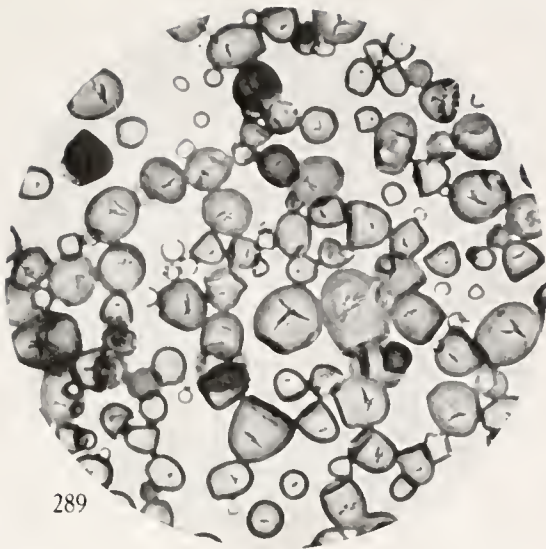
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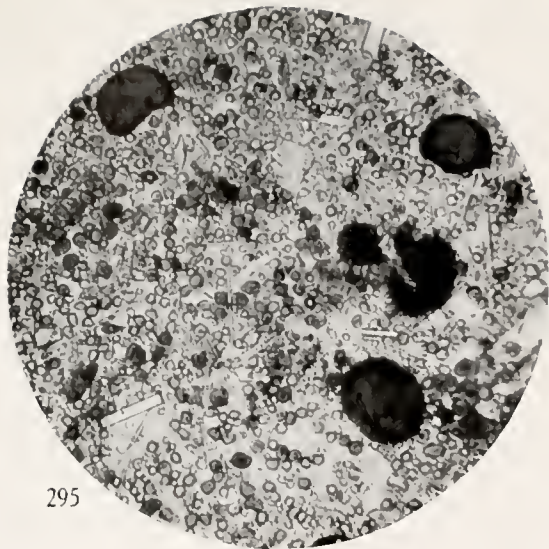
283 and 284. *Brodiaea stellaris*.  
285 and 286. *Brodiaea capitata*.  
287 and 288. *Brodiaea congesta*.





289 and 290. *Triteleia uniflora*.  
291 and 292. *Lachenalia pendula*.  
293 and 294. *Lachenalia tricolor* var. *luteola*.

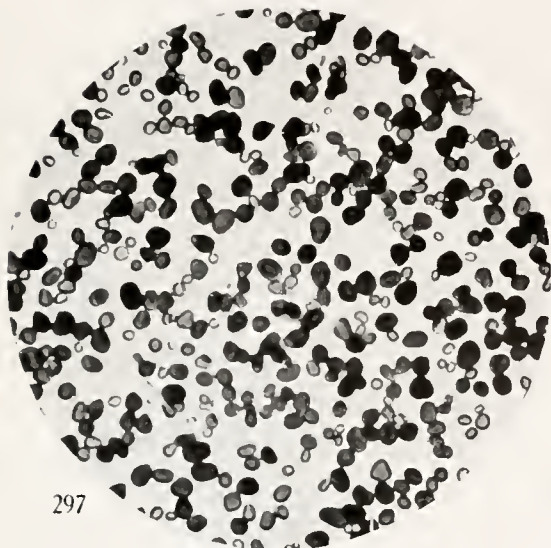




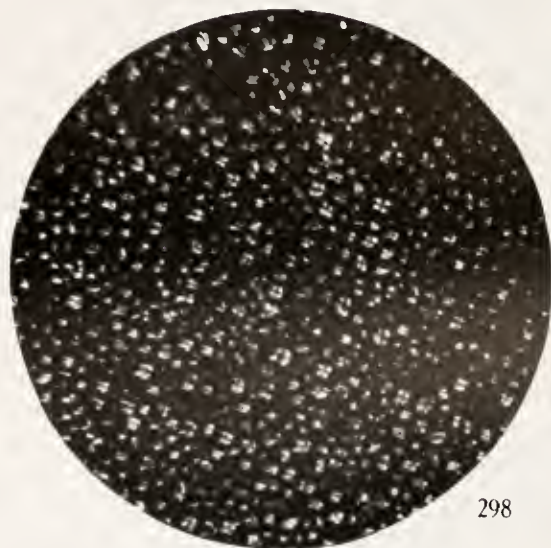
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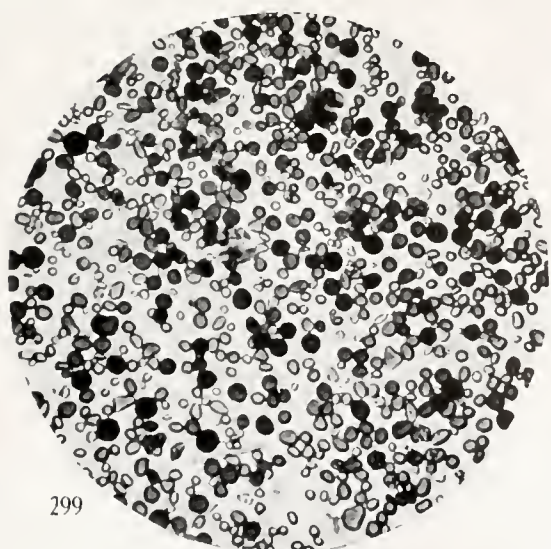
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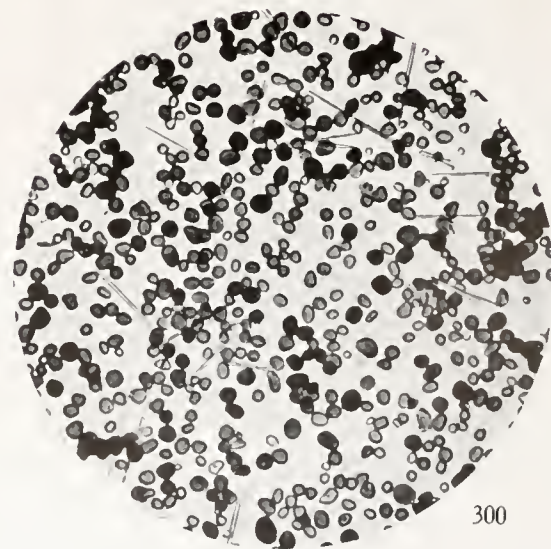
297



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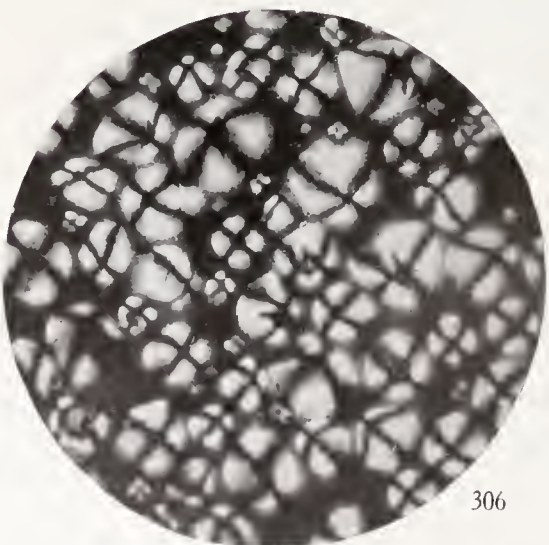
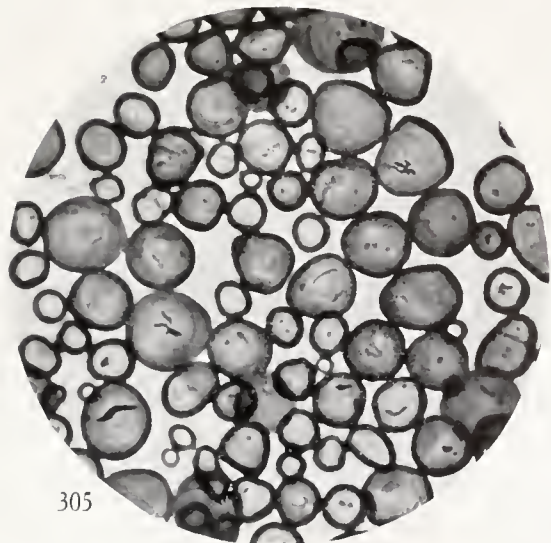
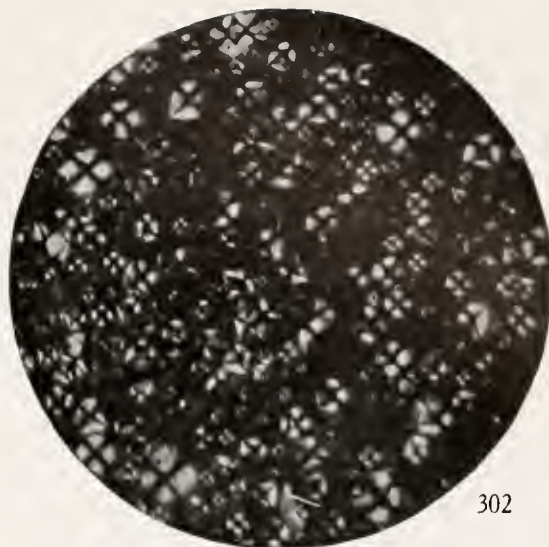
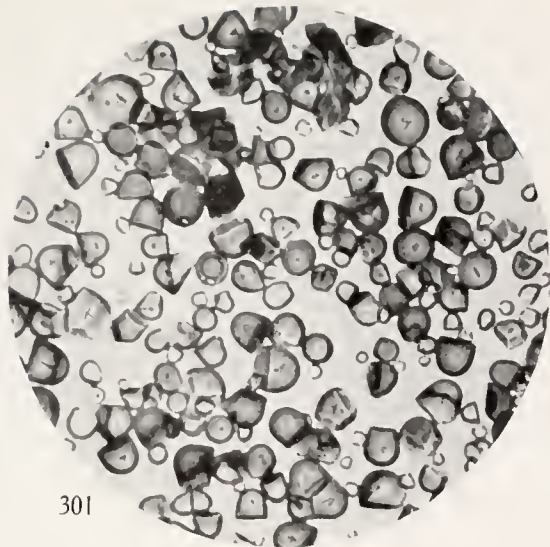
299



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295 and 296. *Convallaria majalis*.  
297 and 298. *Trillium grandiflorum*.  
299. *Trillium ovatum*.  
300. *Trillium sessile* var. *californicum*.



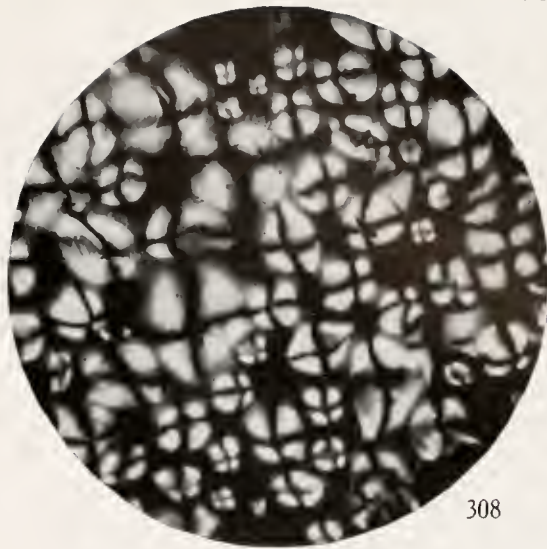


301 and 302. *Colchicum parkinsonii*.  
303 and 304. *Amaryllis belladonna major*.  
305 and 306. *Vallota purpurea*.

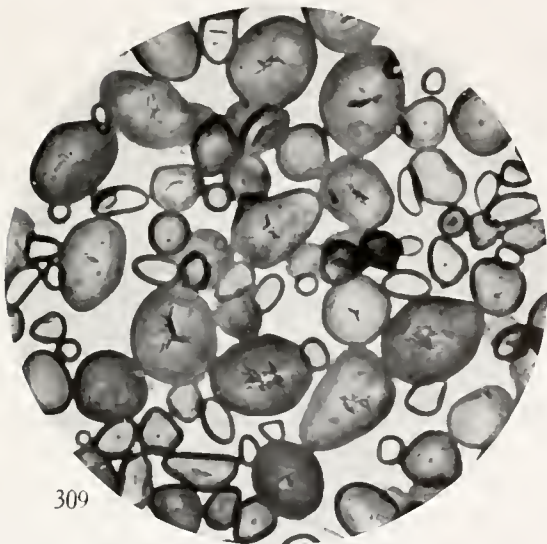




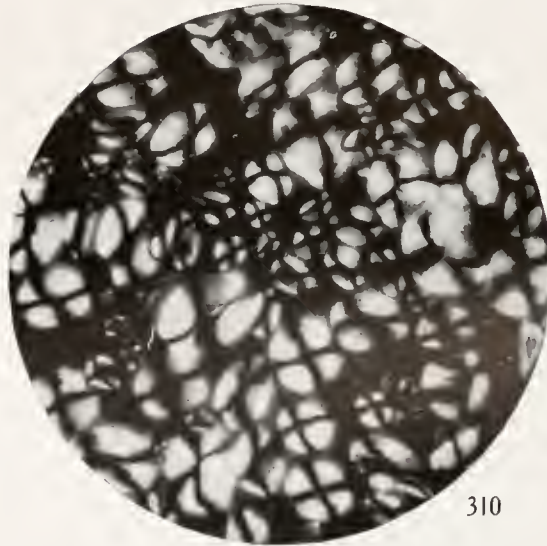
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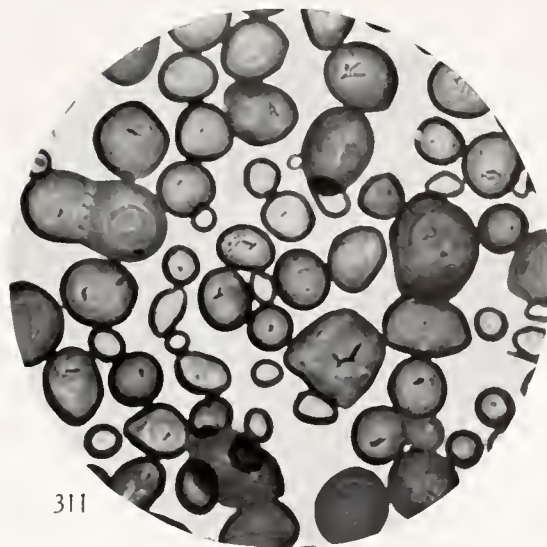
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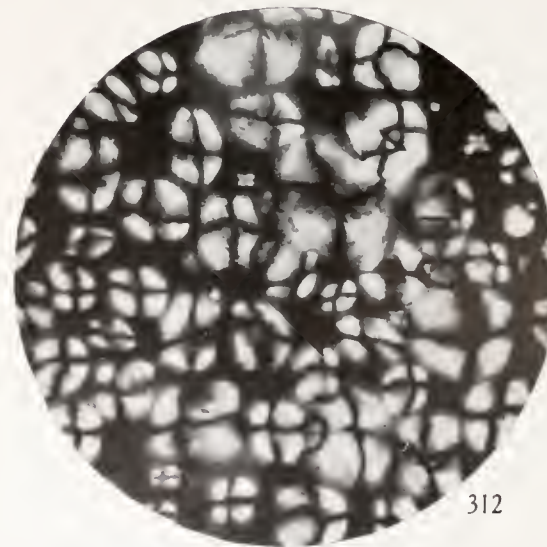
309



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307 and 308. *Hippeastrum vittatum*.  
309 and 310. *Hippeastrum equestre*.  
311 and 312. *Hippeastrum alicum* var. *robustum*.

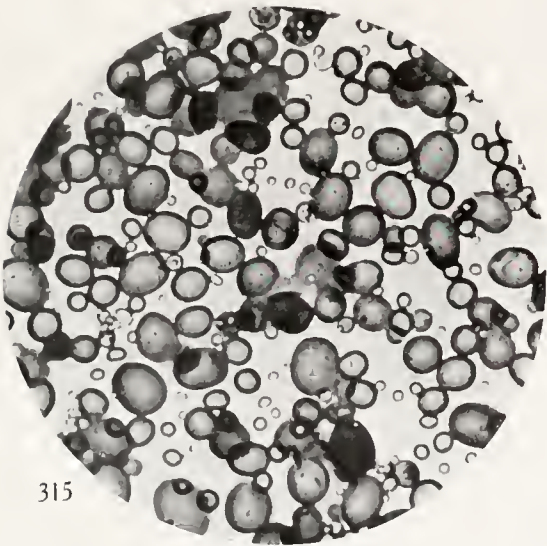




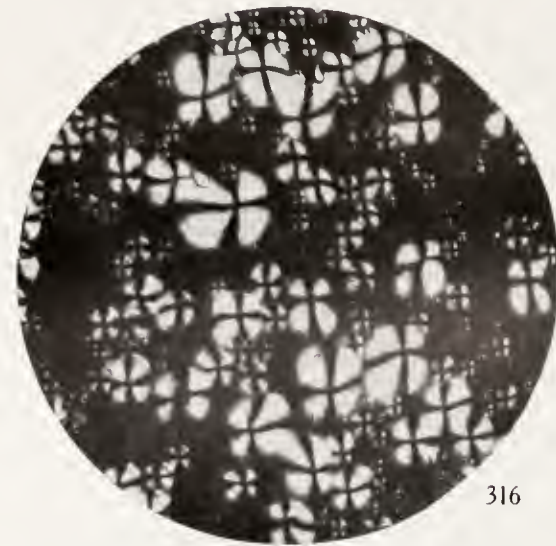
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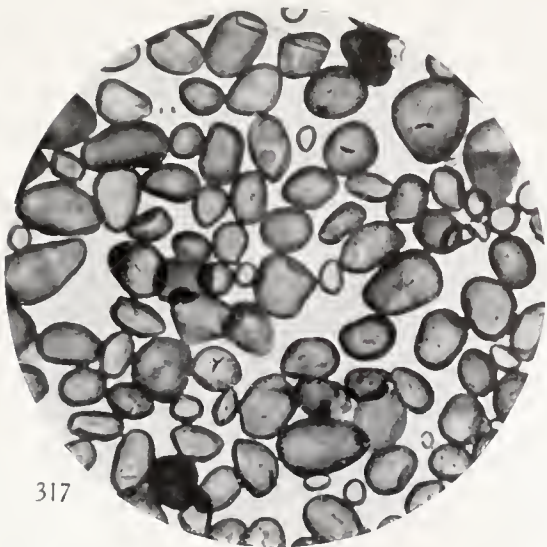
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315



316



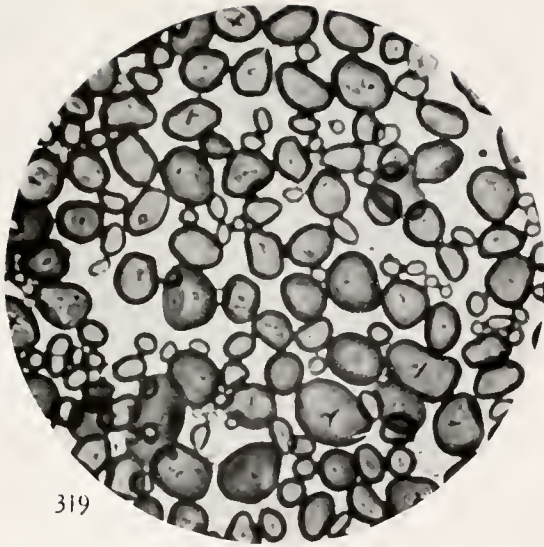
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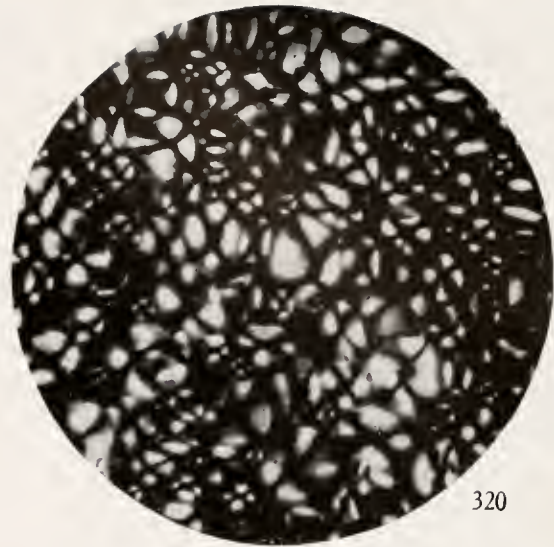
318

313 and 314. *Crinum fimbriatum*.  
315 and 316. *Crinum americanum*.  
317 and 318. *Sprekelia formosissima*.

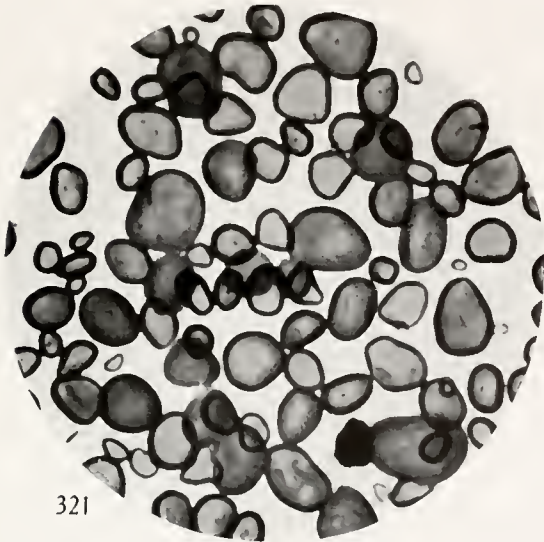




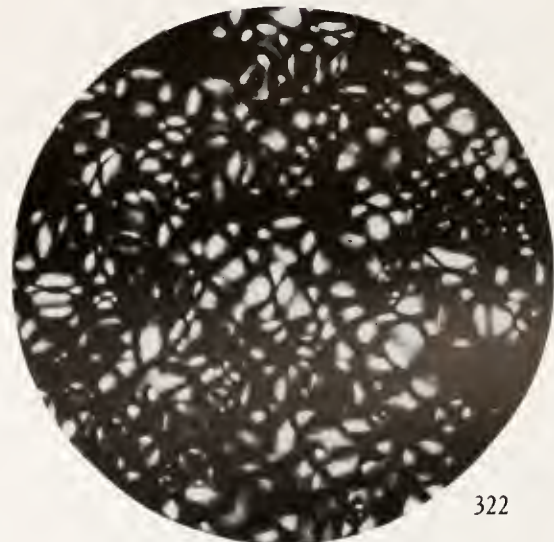
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320



321



322



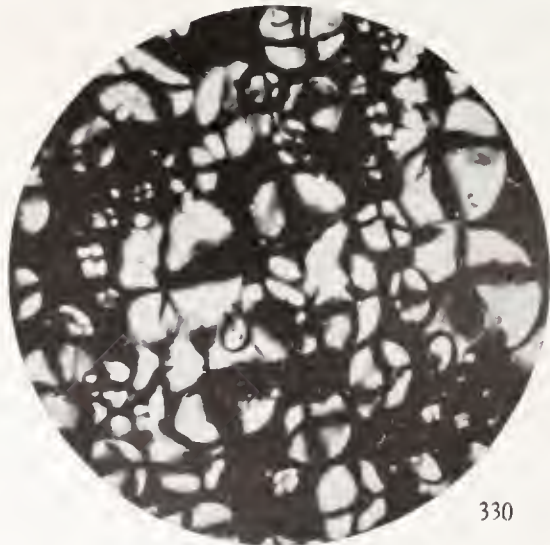
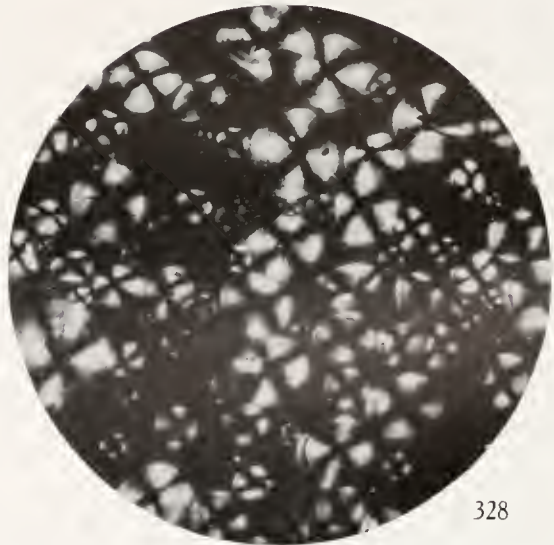
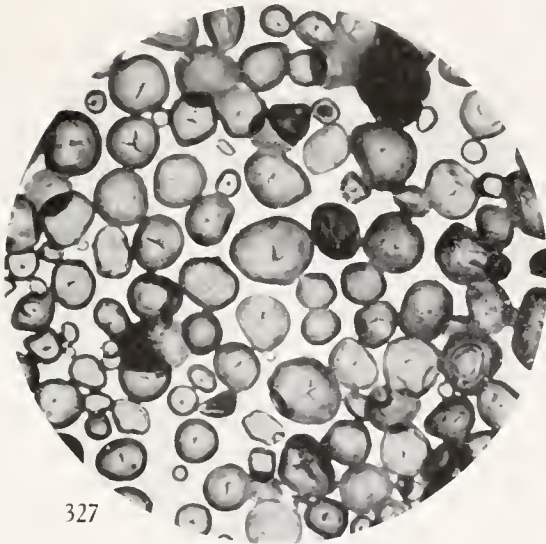
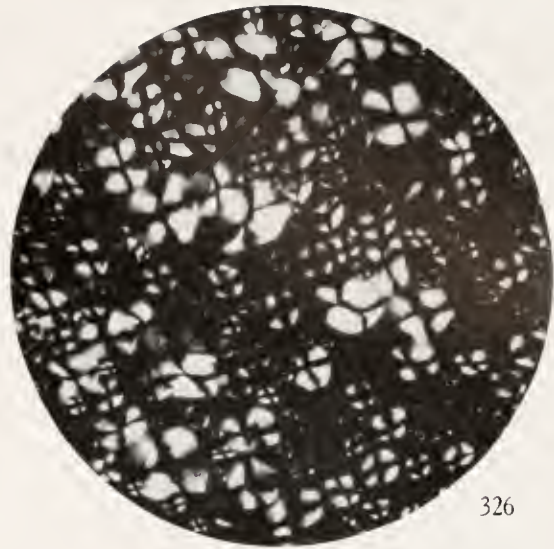
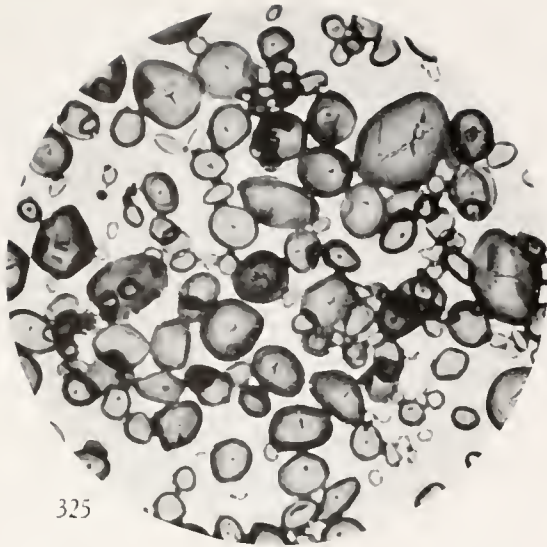
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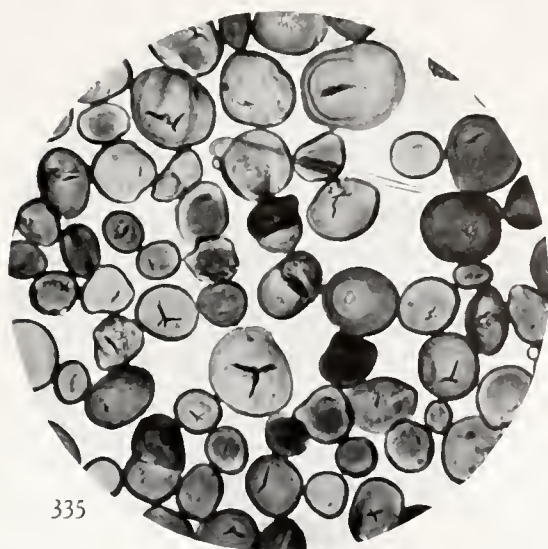
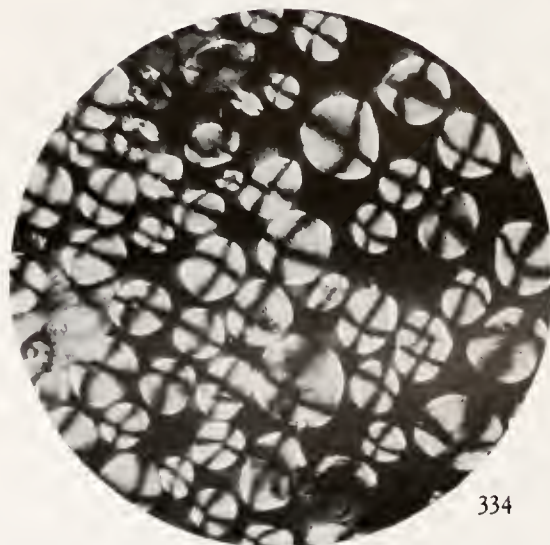
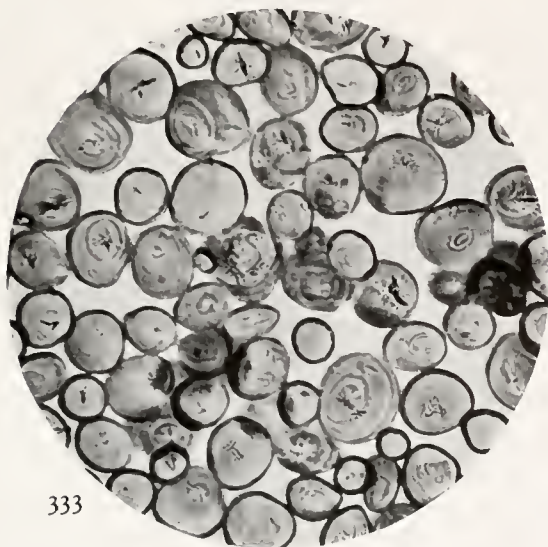
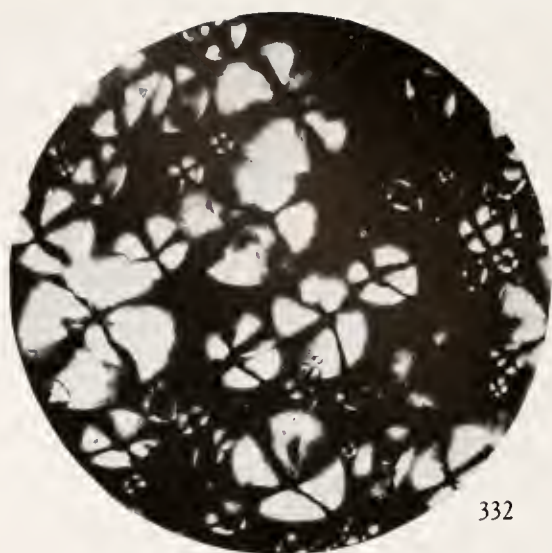
319 and 320. *Zephyranthes candida*.  
321 and 322. *Zephyranthes rosea*.  
323 and 324. *Hamanthus katherina*.





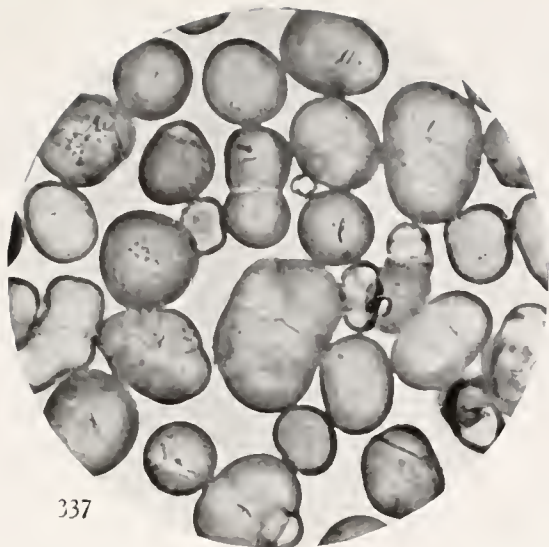
325 and 326. *Hymenocallis undulata*.  
327 and 328. *Hymenocallis calathina*.  
329 and 330. *Leucoium astivum*.





331 and 332. *Leucojum vernum*.  
333 and 334. *Galanthus nivalis*.  
335 and 336. *Galanthus clivicus*.

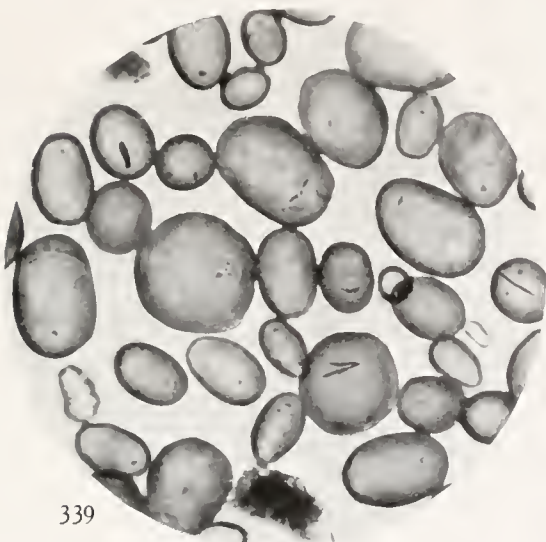




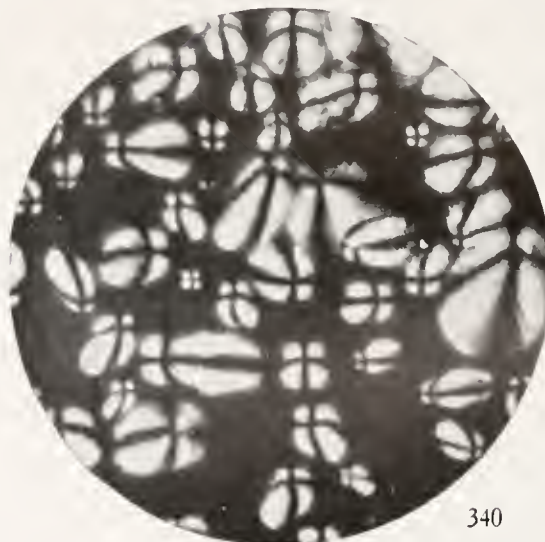
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338



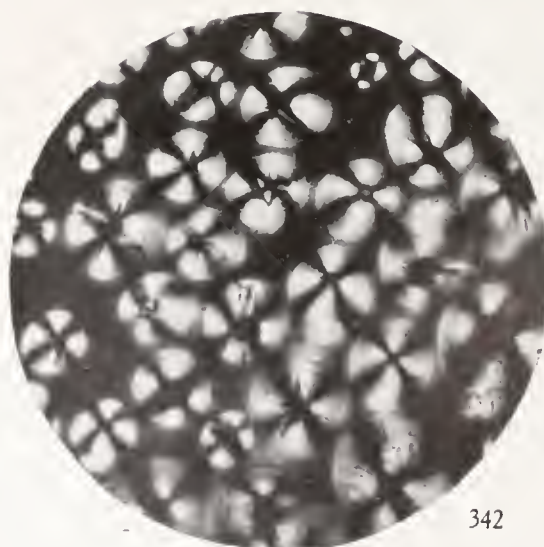
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340



341



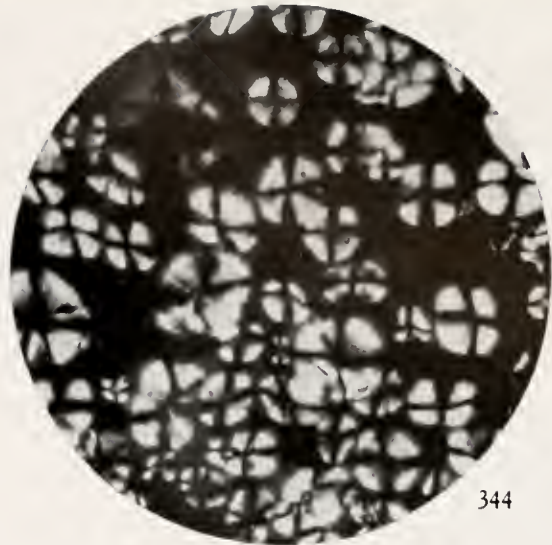
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337 and 338. *Alstrameria ligta*.  
339 and 340. *Alstrameria brasiliensis*.  
341 and 342. *Alstrameria aurantiaca* (*aurca*).

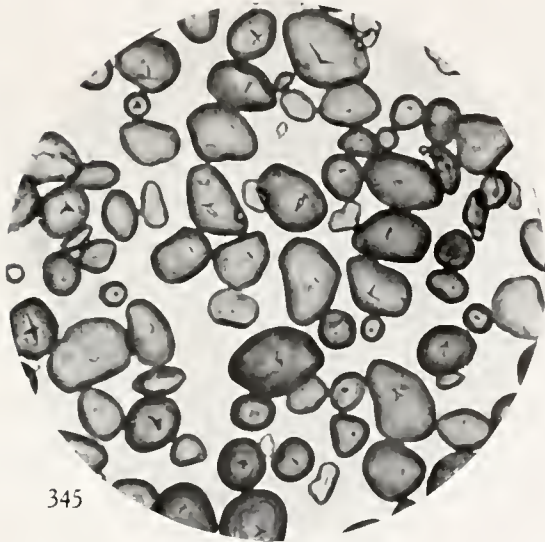




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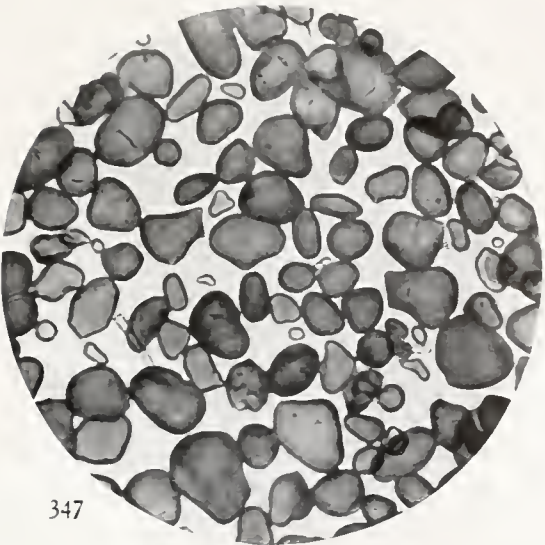
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345



346



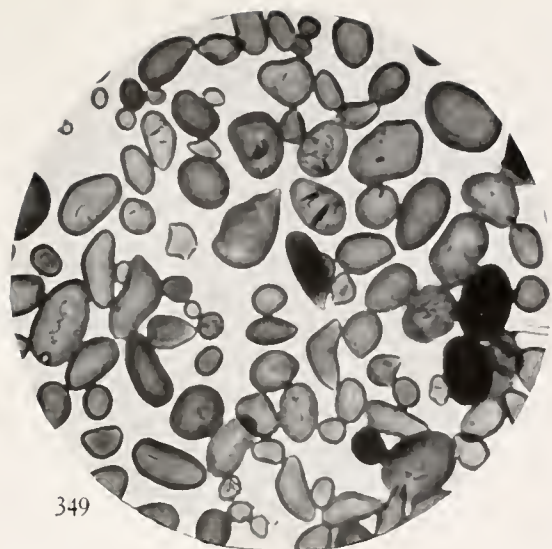
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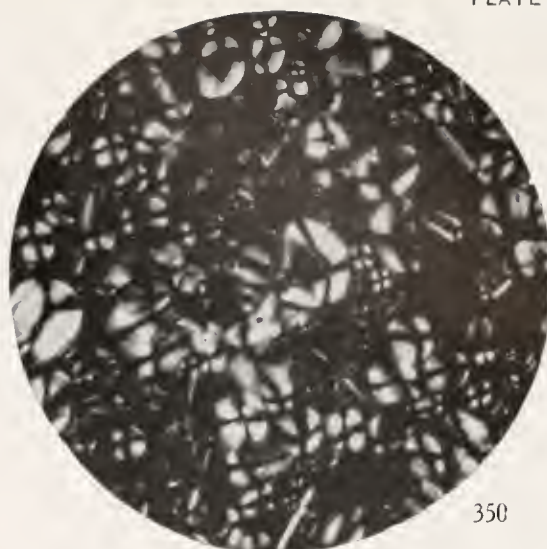
348

343 and 344. *Sternbergia lutea*.  
345 and 346. *Narcissus horsfieldii*.  
347 and 348. *Narcissus maximus*.

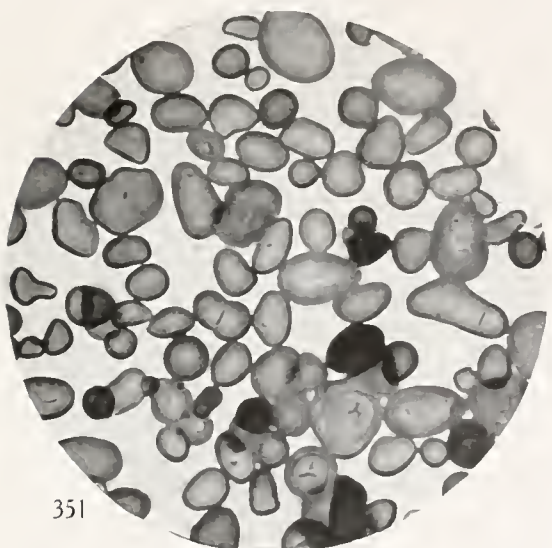




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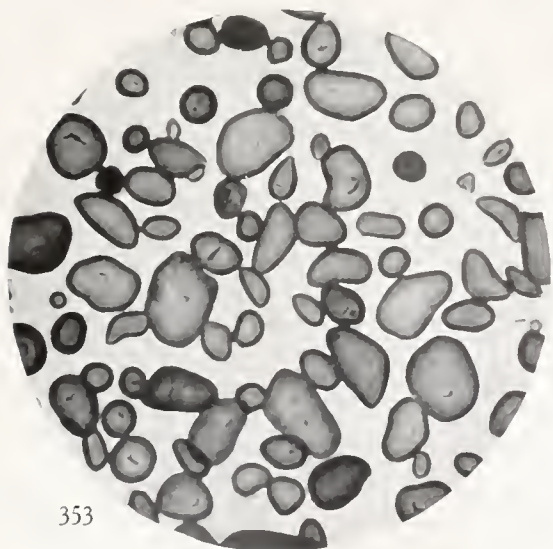
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351



352



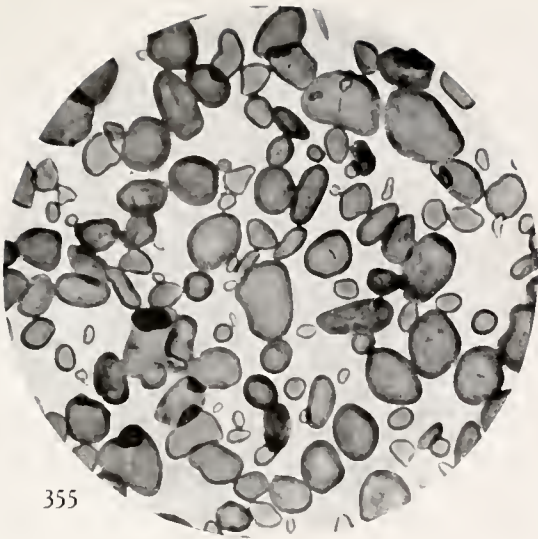
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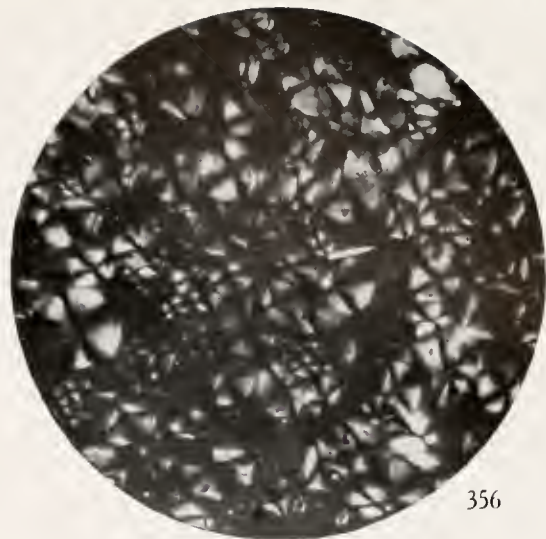
354

349 and 350. *Narcissus bulbocodium*.  
 351 and 352. *Narcissus bulbocodium* var. *conspicua*.  
 353 and 354. *Narcissus bulbocodium* var. *monophyllus*.

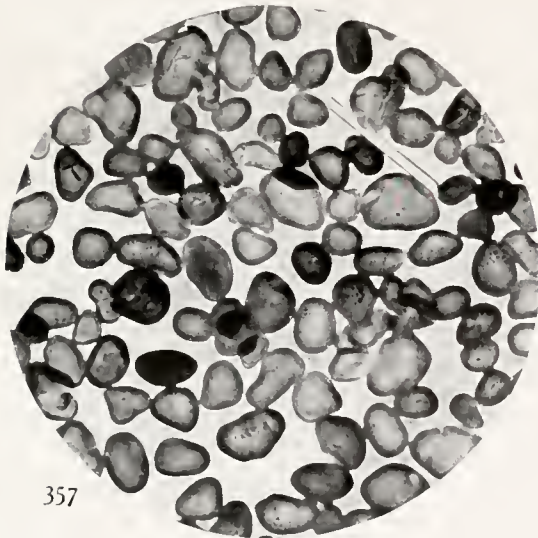




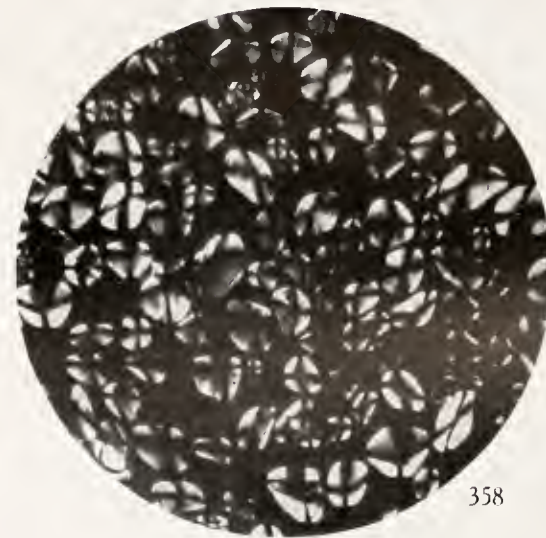
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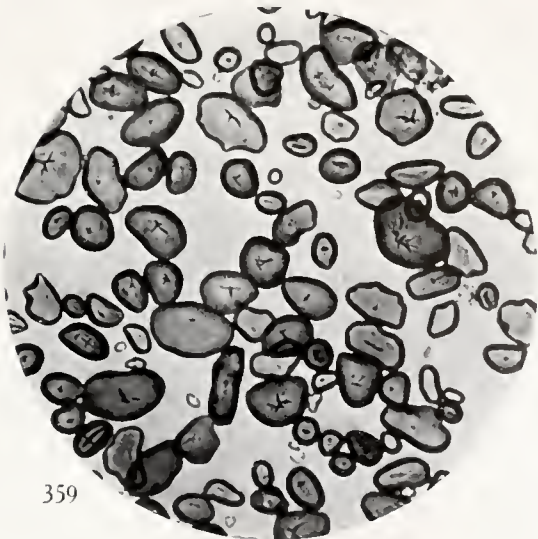
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358



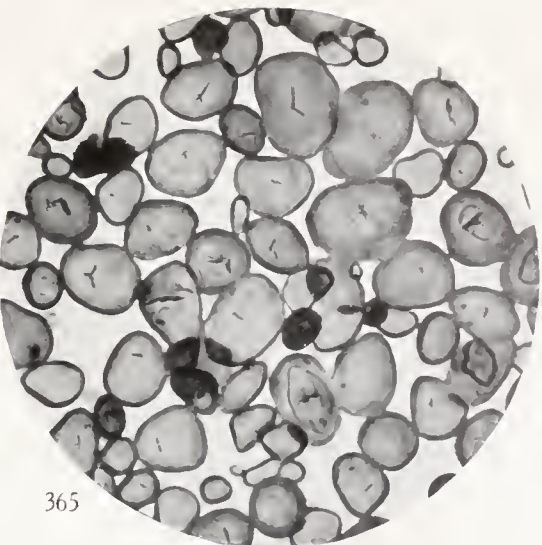
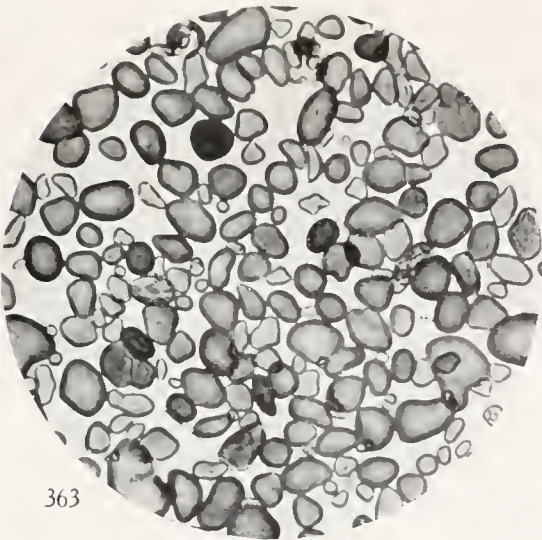
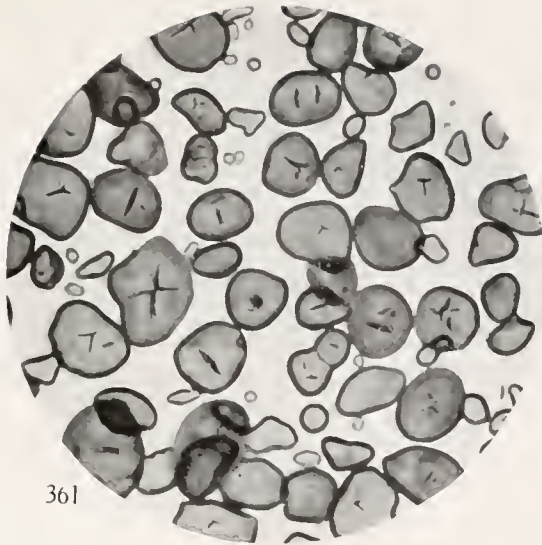
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360

355 and 356. *Narcissus incomparabilis*.  
357 and 358. *Narcissus odorus*.  
359 and 360. *Narcissus poeticus*.



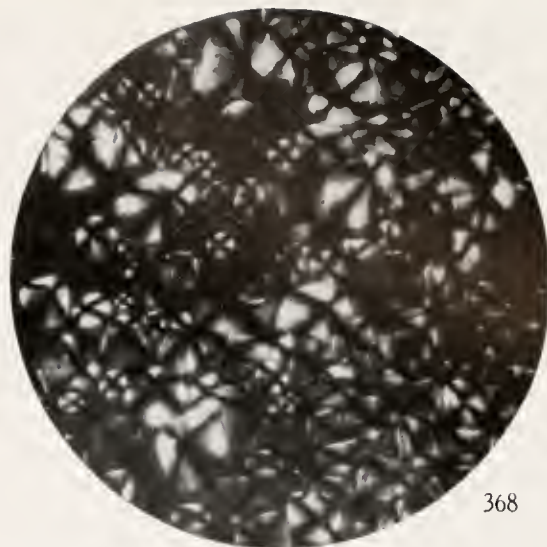


361 and 362. *Narcissus biflorus*.  
363 and 364. *Narcissus jonquilla*.  
365 and 366. *Narcissus jonquilla* var. *rugulosus*.

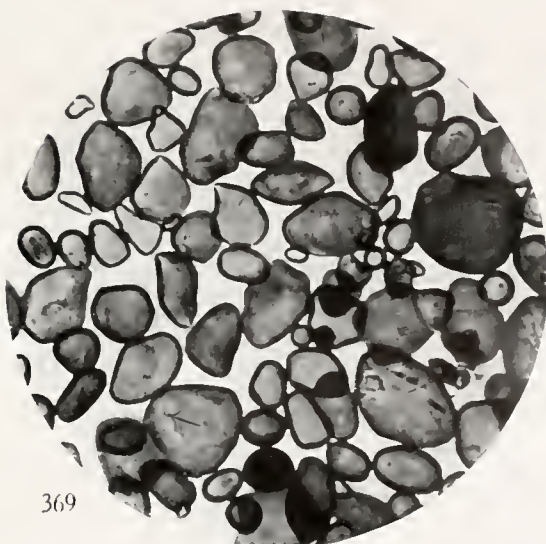




367



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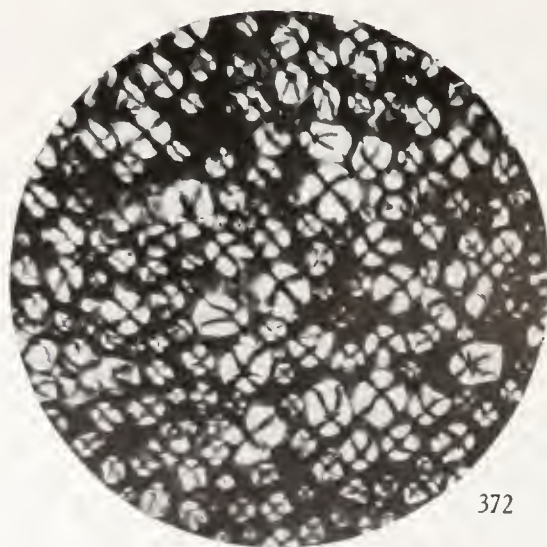
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370



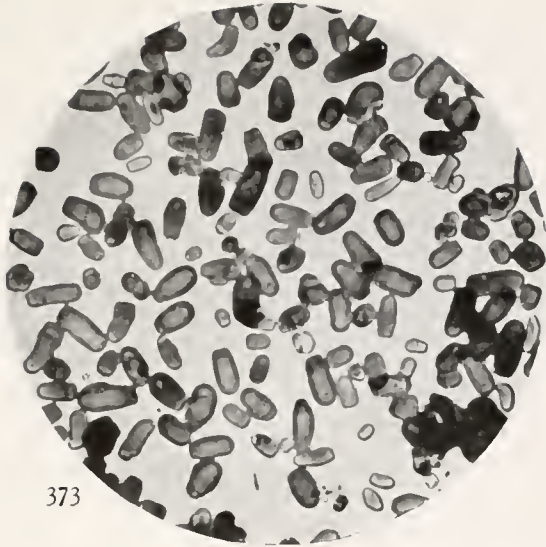
371



372

367 and 368. *Narcissus jonquilla* var. *campernellii rugulosus*.  
 369 and 370. *Narcissus tazetta* var. *orientalis*.  
 371 and 372. *Tacca pinnatifida*.

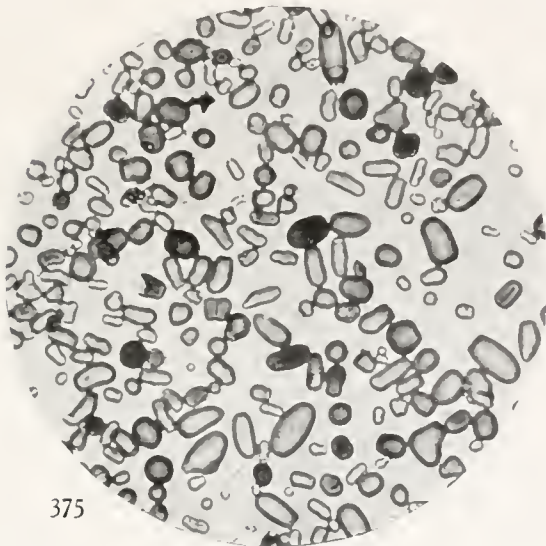




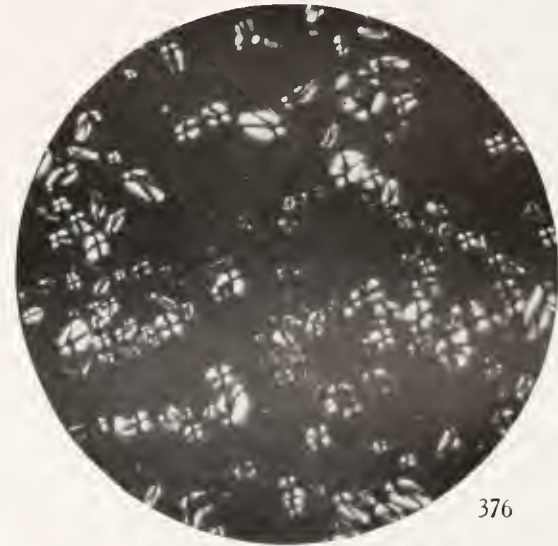
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374



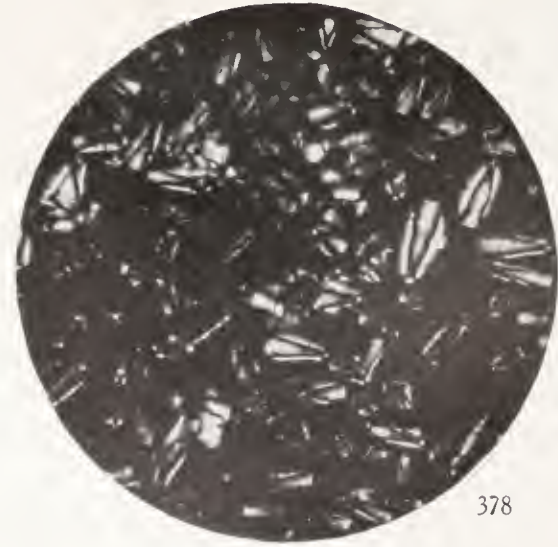
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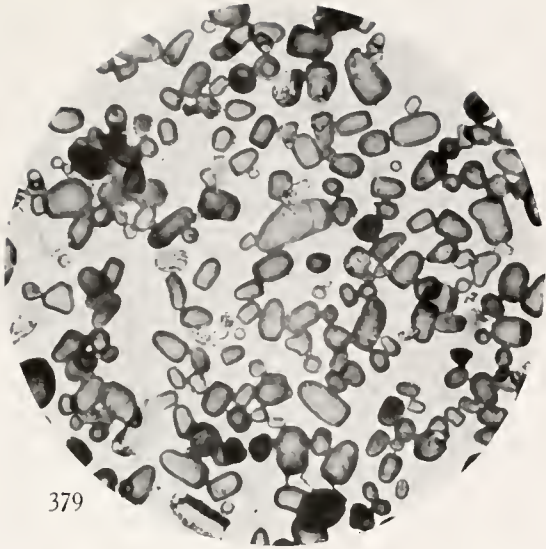
377



378

373 and 374. *Iris florentina*,  
 375 and 376. *Iris pallida speciosa*,  
 377 and 378. *Iris pumila* var. *cyanca*.





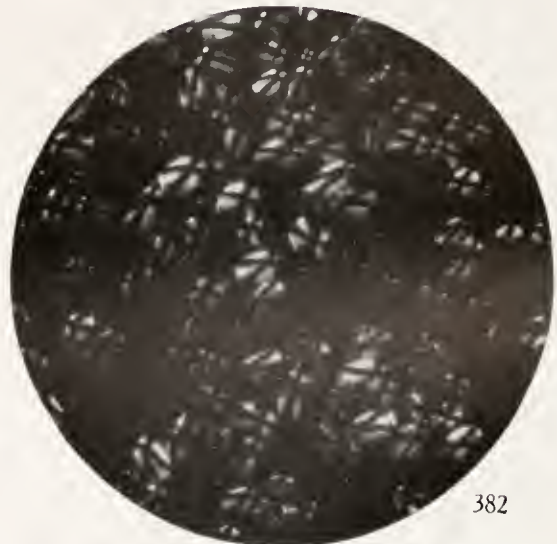
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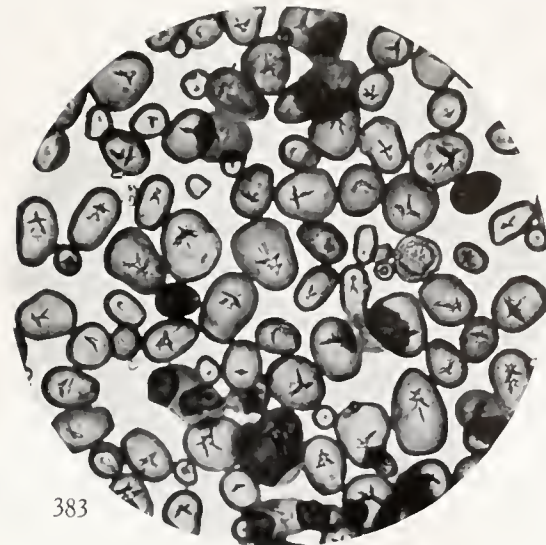
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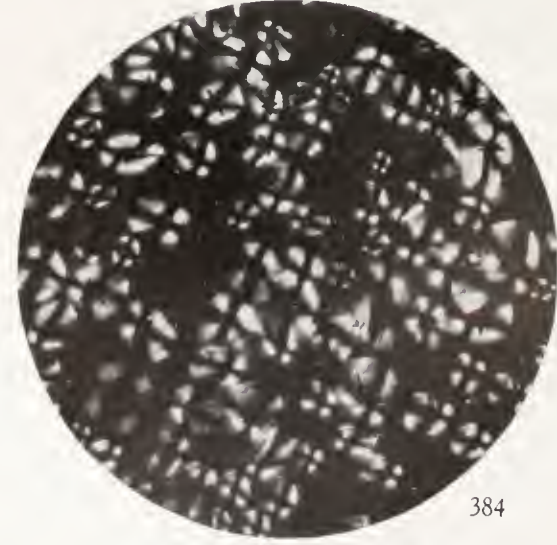
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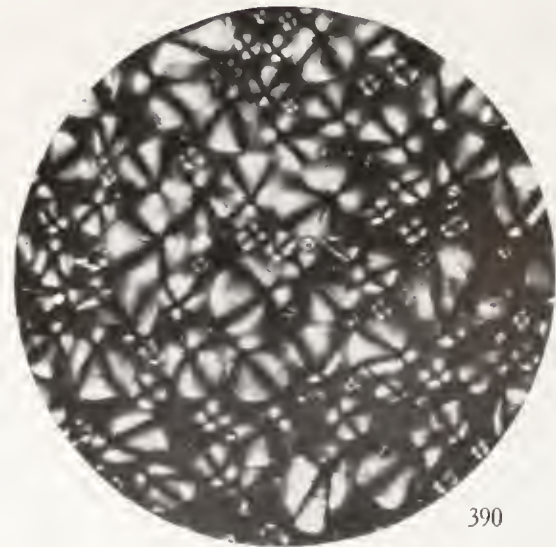
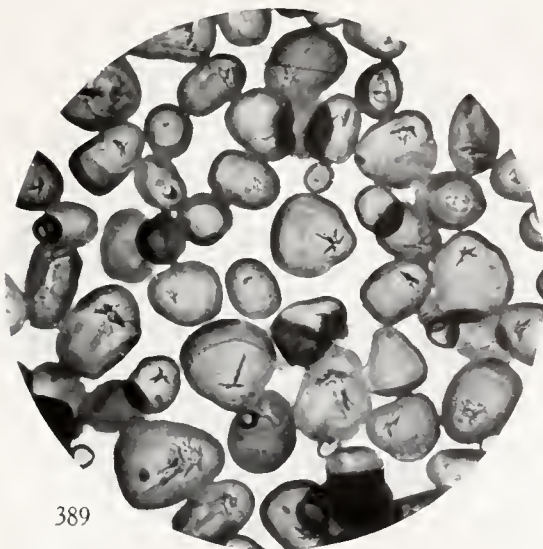
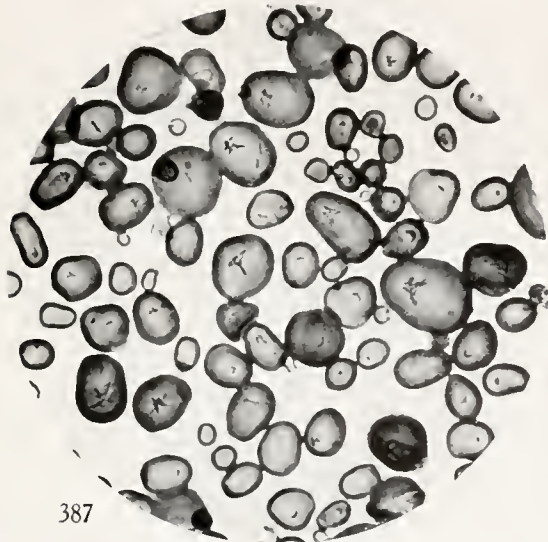
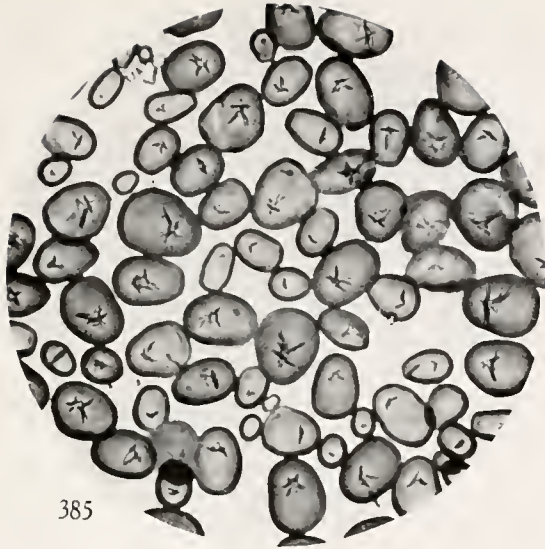
383



384

379 and 380. *Iris bismarckiana*.  
381 and 382. *Iris iberica*.  
383 and 384. *Iris zippium* var. *grand tresorier*.





385 and 386. *Iris xiphium* var. *wilhelminc.*  
387 and 388. *Iris xiphium* var. *lusitanica.*  
389 and 390. *Iris tingitana.*





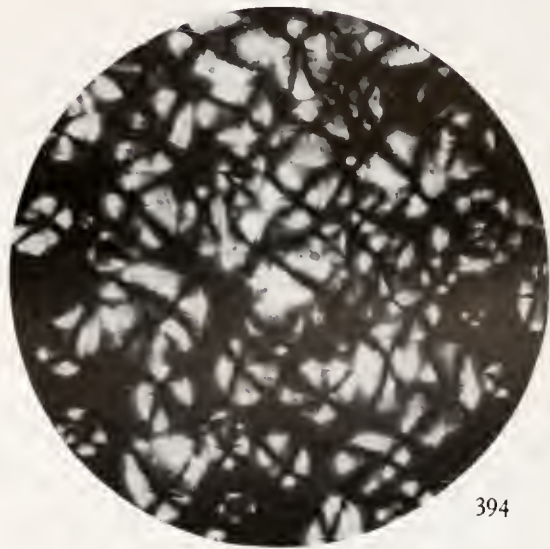
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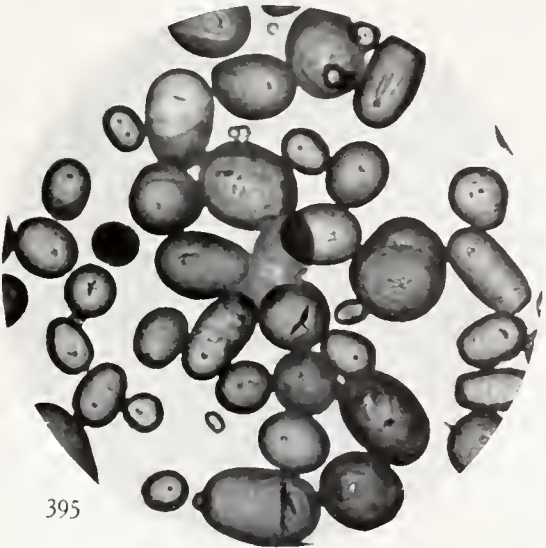
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393



394



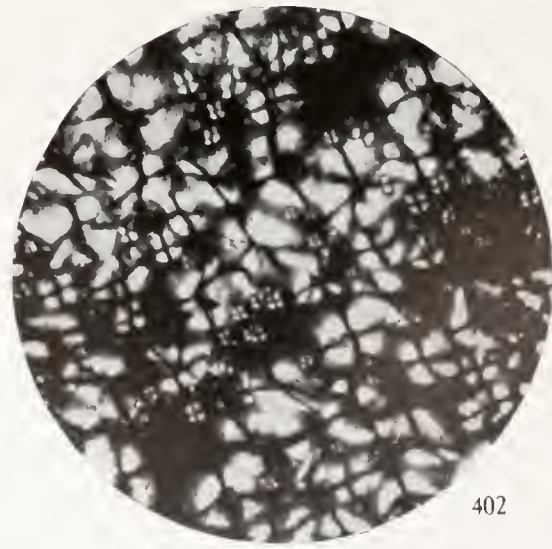
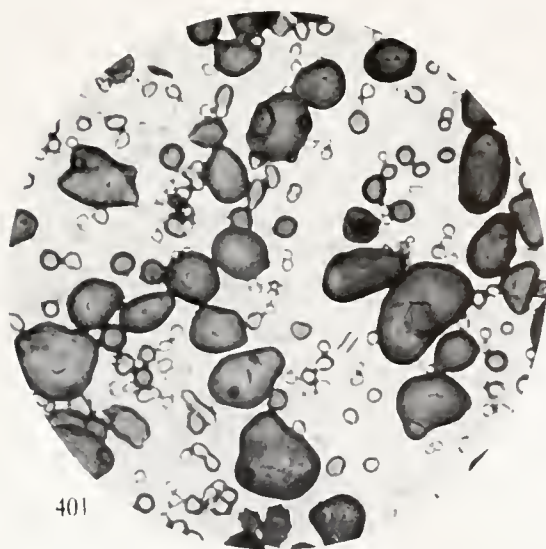
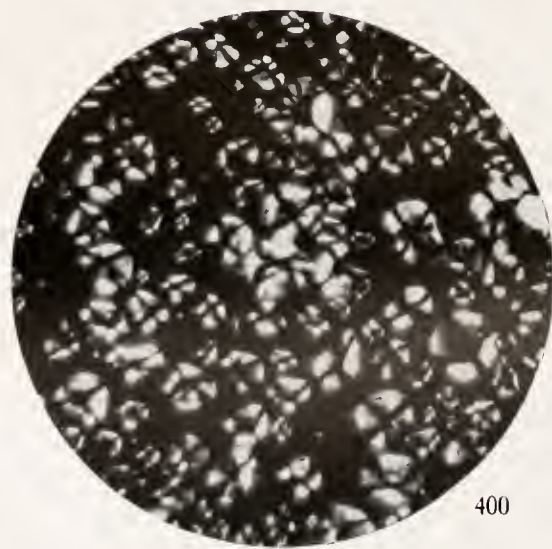
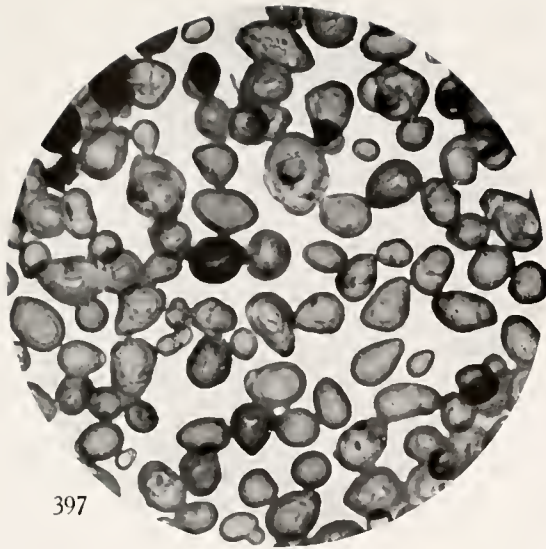
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396

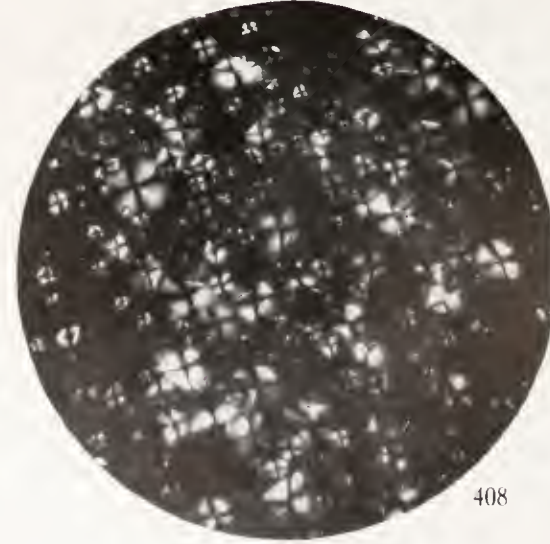
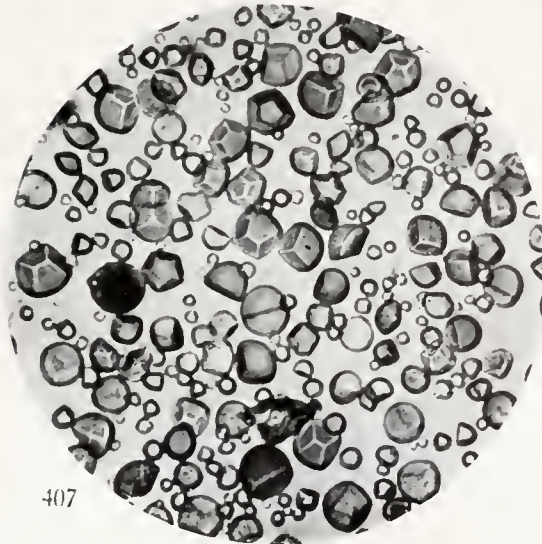
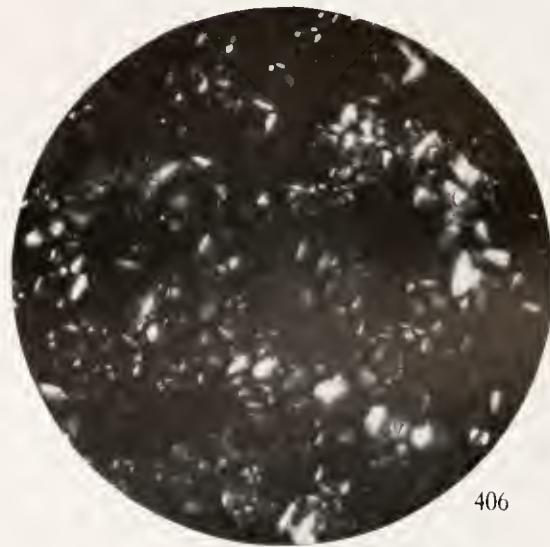
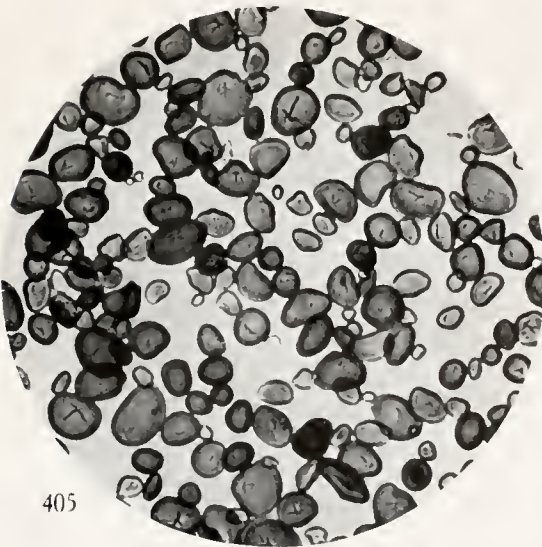
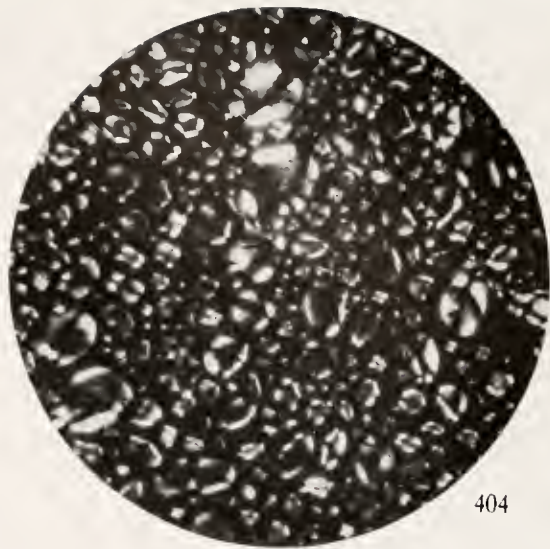
391 and 392, *Iris reticulata*.  
393 and 394, *Iris histrio*.  
395 and 396, *Iris alata*.





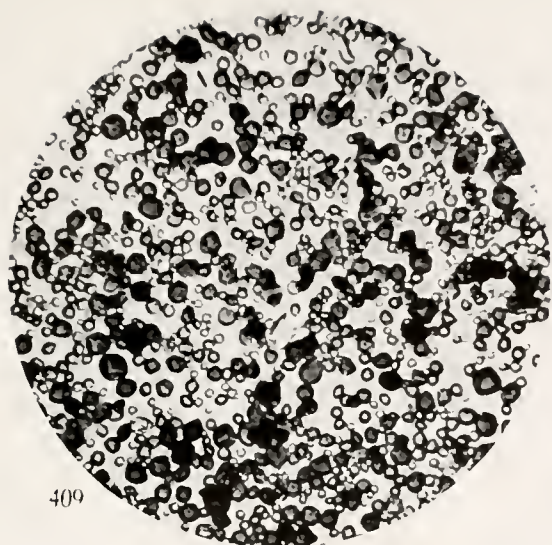
397 and 398. *Iris caucasica*.  
399 and 400. *Morva tristis*.  
401 and 402. *Homeria collina*.



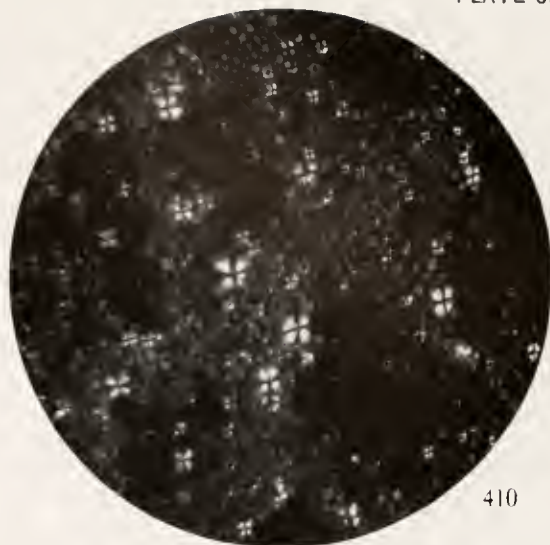


403 and 404. *Tigridia pavonia* var. *grandiflora* alba.  
405 and 406. *Tigridia pavonia* var. *conchiflora*.  
407 and 408. *Gladiolus byzantinus*.

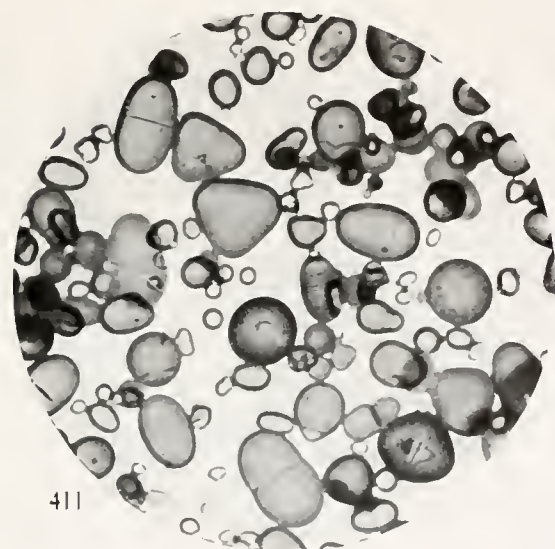




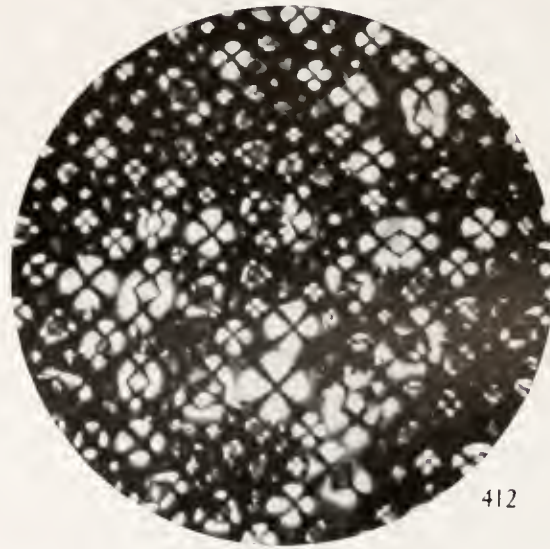
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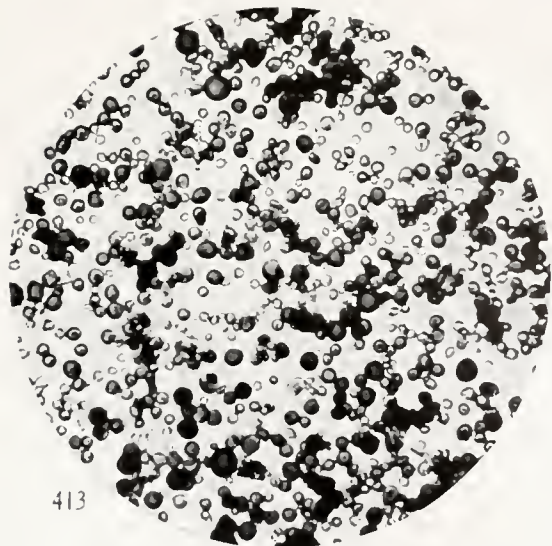
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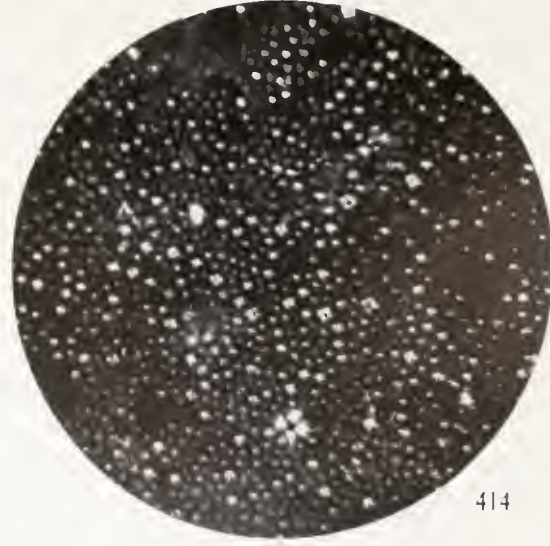
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412



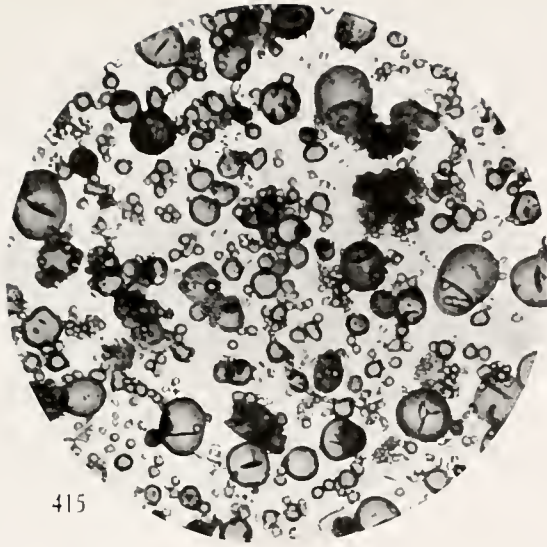
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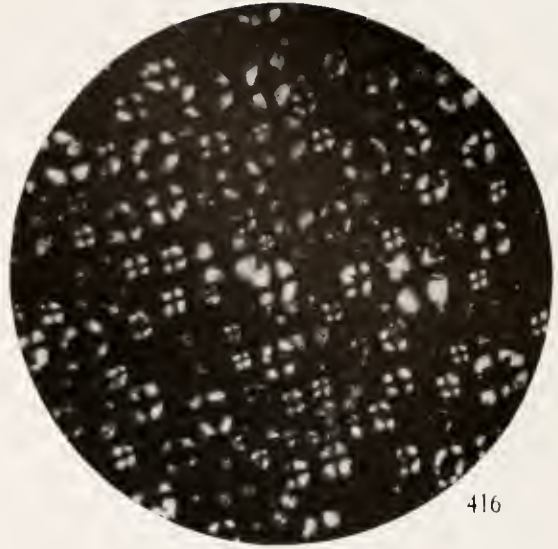
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409 and 410. *Gladiolus primulinus*.  
411 and 412. *Gladiolus cardinalis* (Blushing Bride).  
413 and 414. *Gladiolus floribundus*.





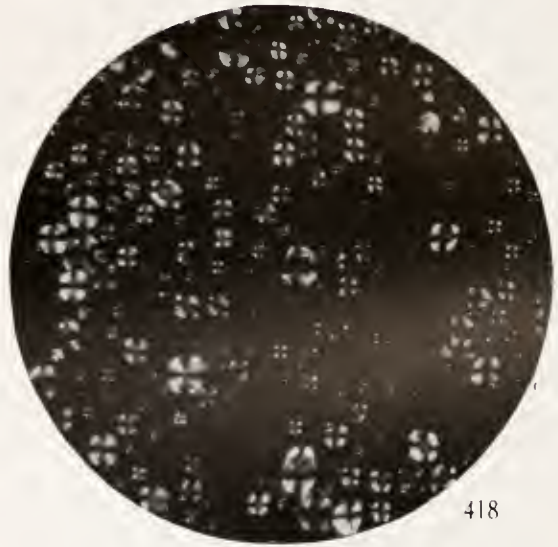
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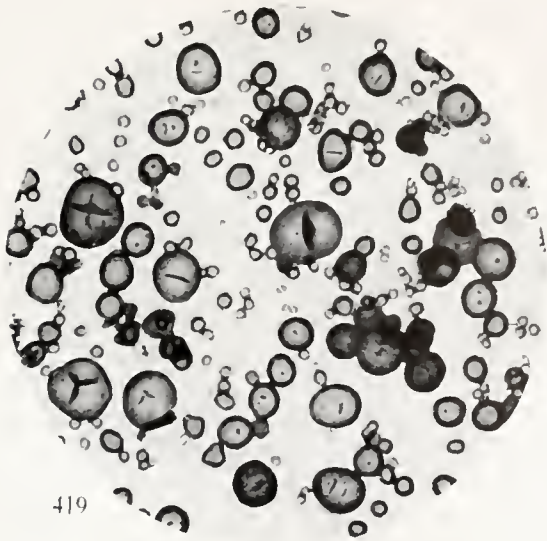
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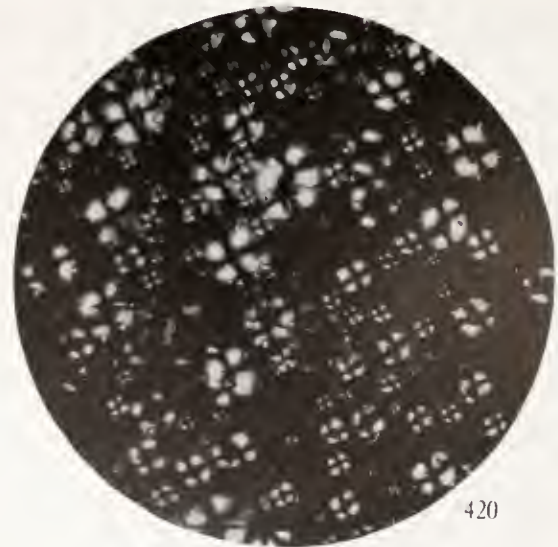
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418



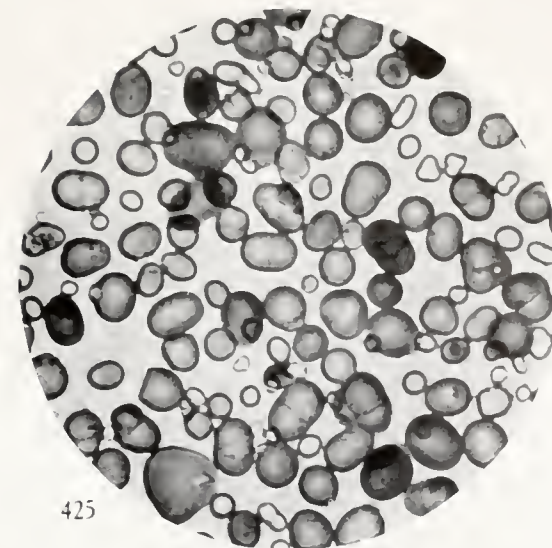
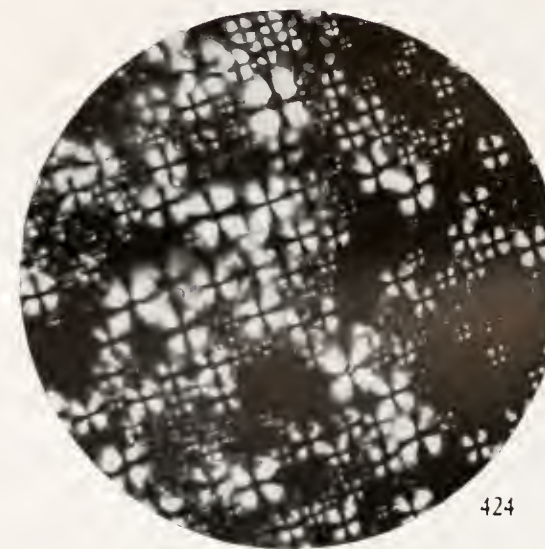
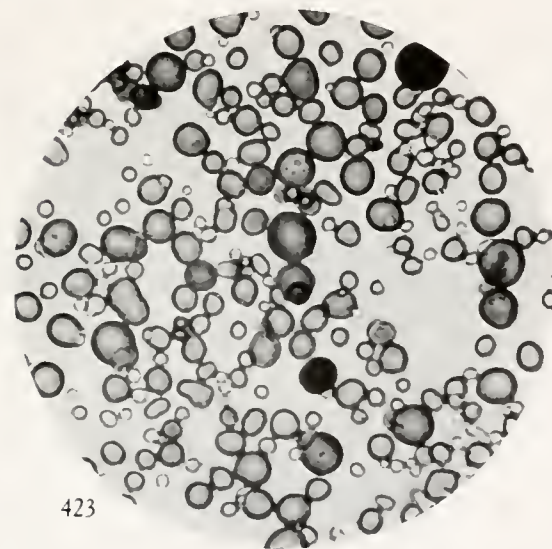
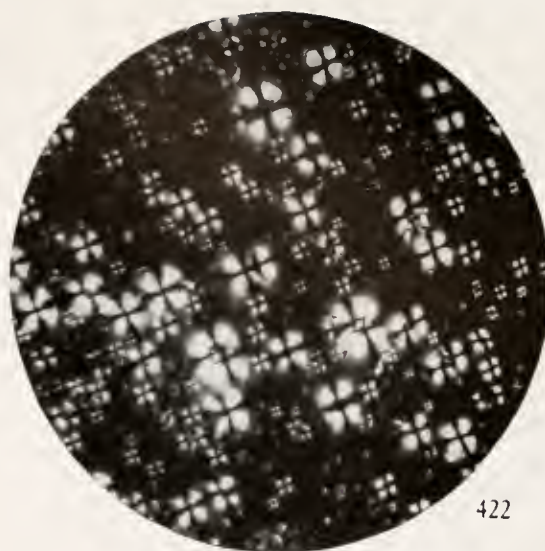
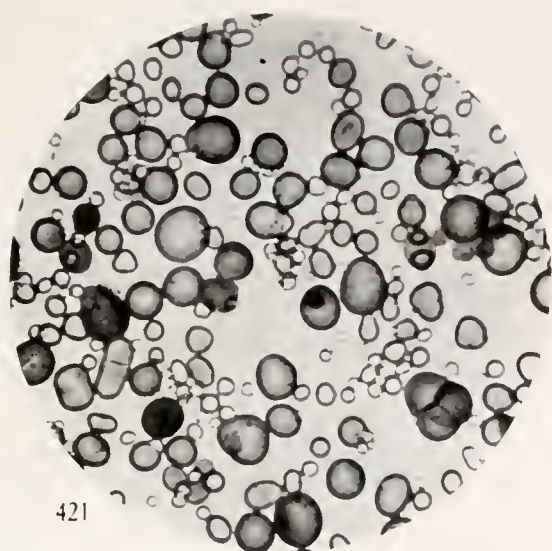
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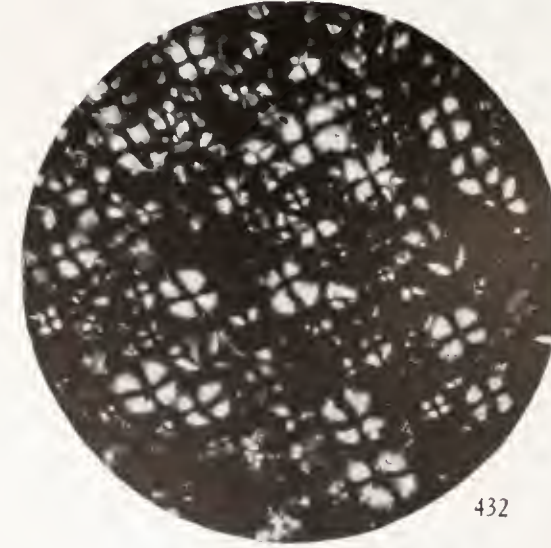
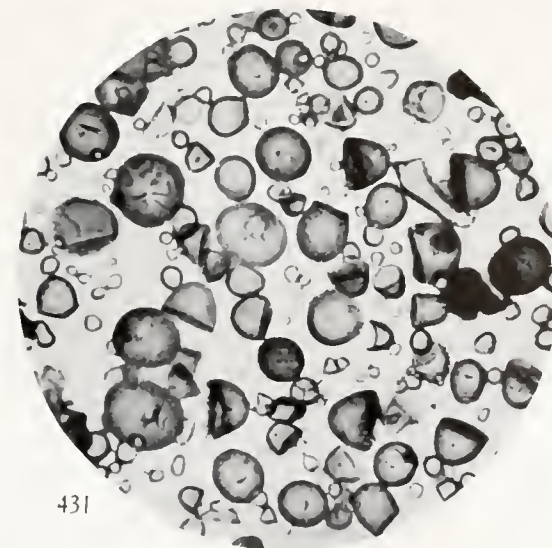
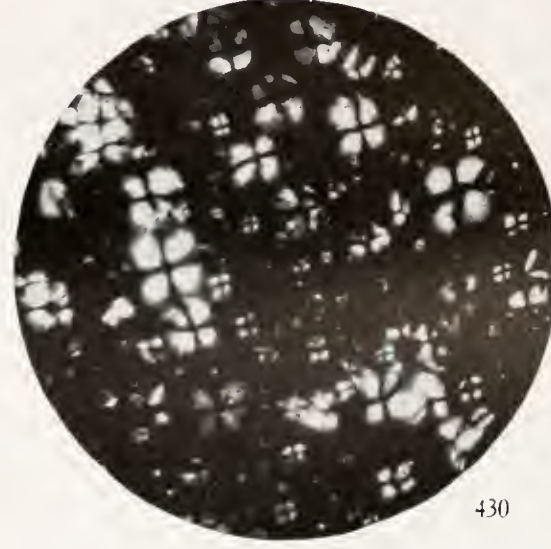
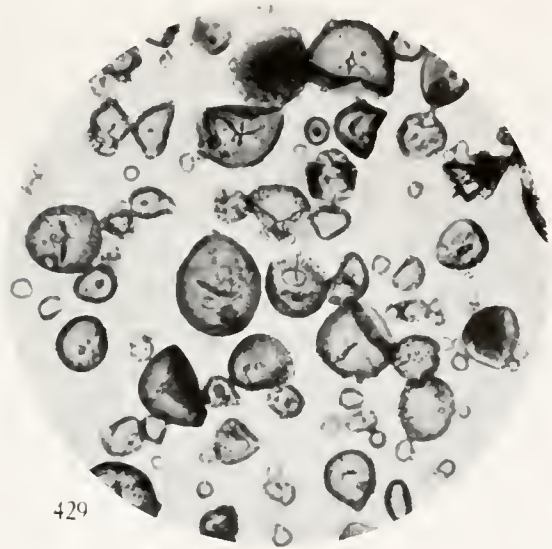
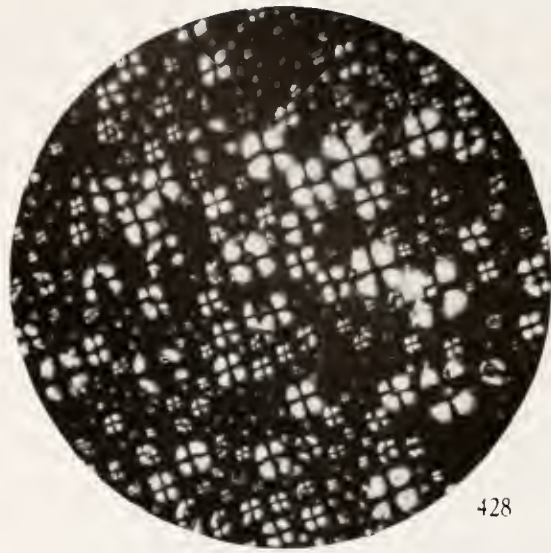
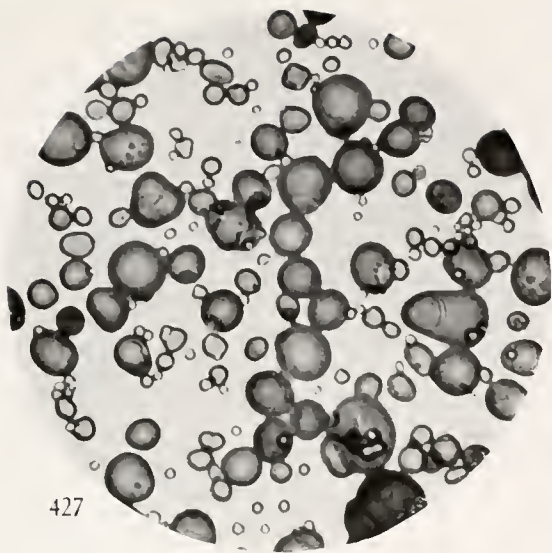
415 and 416. *Watsonia humilis*.  
417 and 418. *Watsonia iridifolia* var. *o'brici*.  
419 and 420. *Watsonia meriana*.





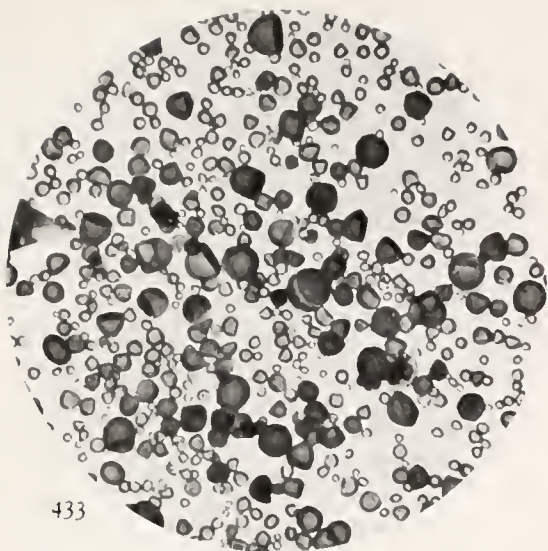
421 and 422. *Tritonia crocata*.  
423 and 424. *Tritonia crocata* var. *lilacina*.  
425 and 426. *Tritonia crocata* var. *rosca*.



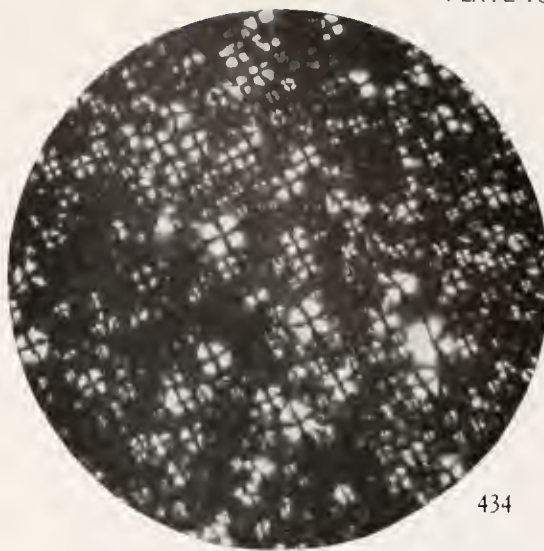


427 and 428. *Tritonia securigera*.  
429 and 430. *Tritonia pottsii*.  
431 and 432. *Tritonia crocosmaflora*.

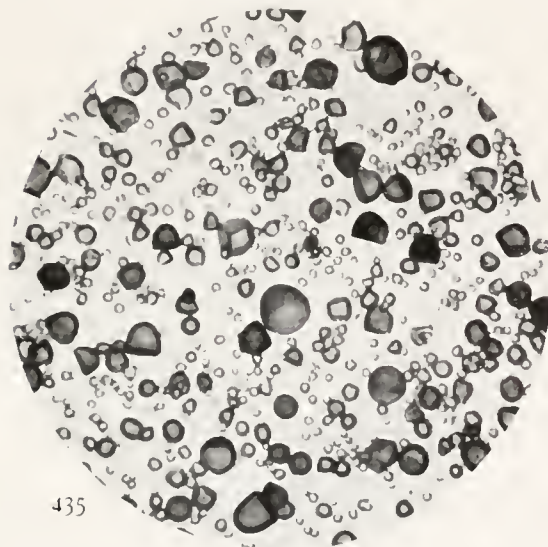




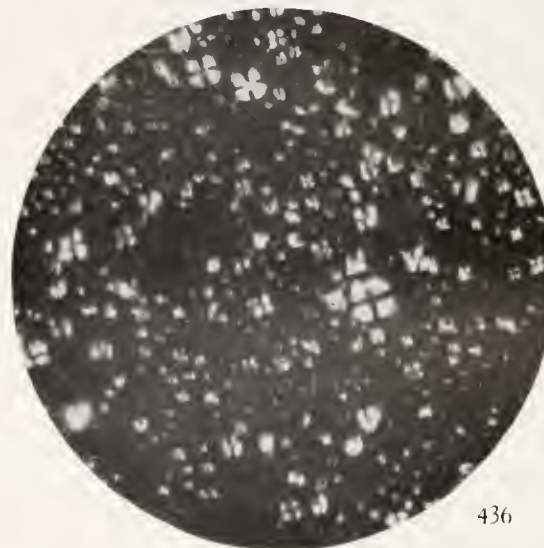
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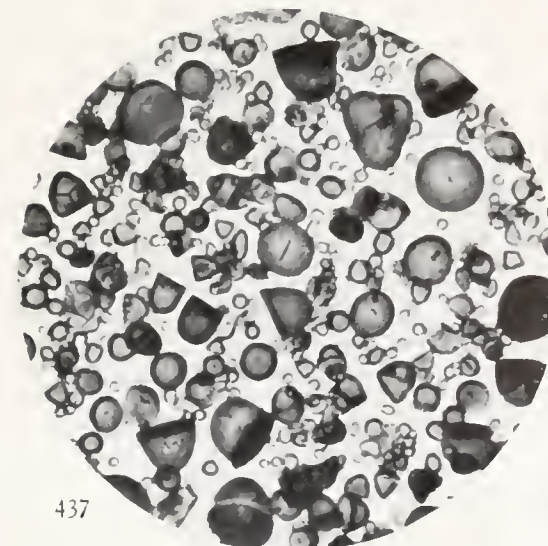
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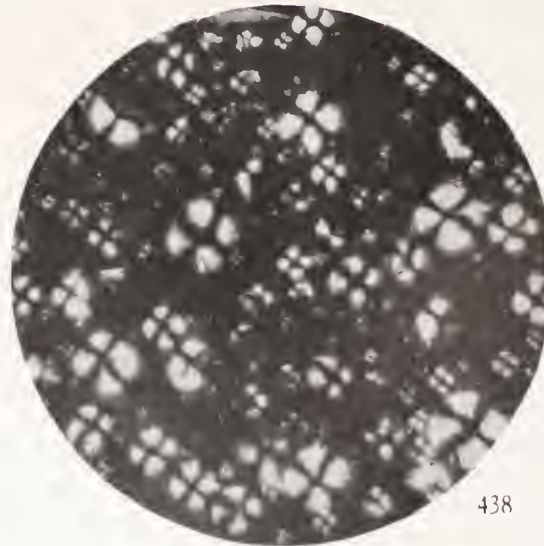
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436



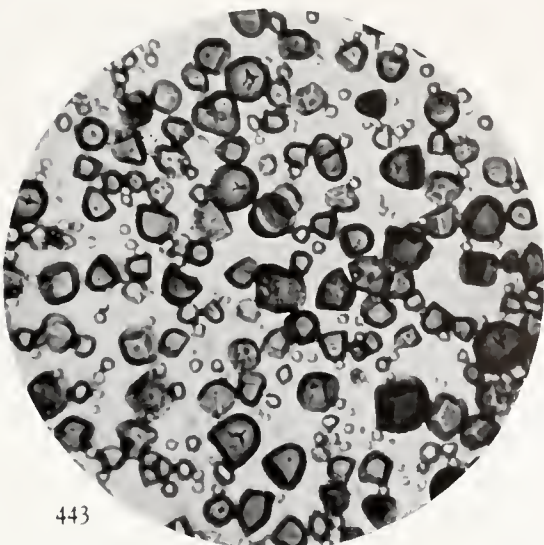
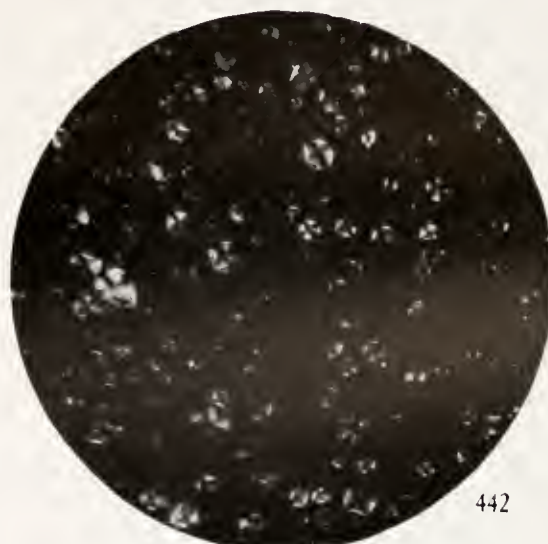
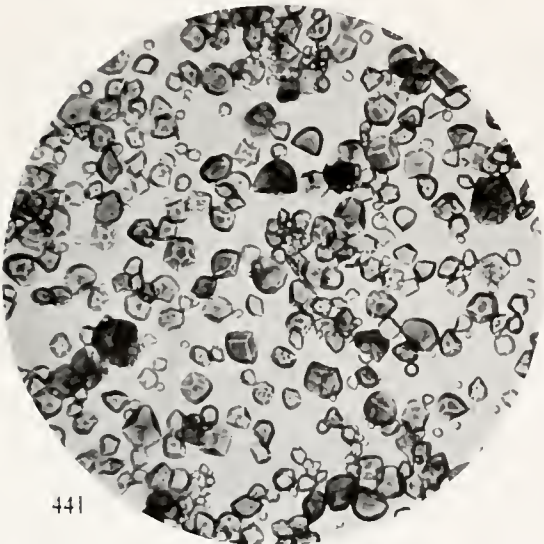
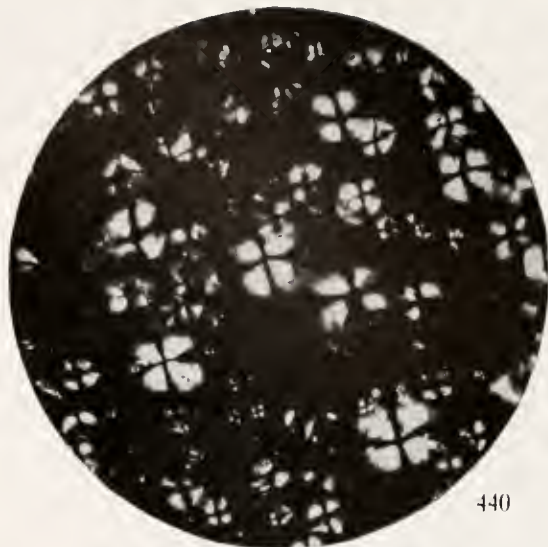
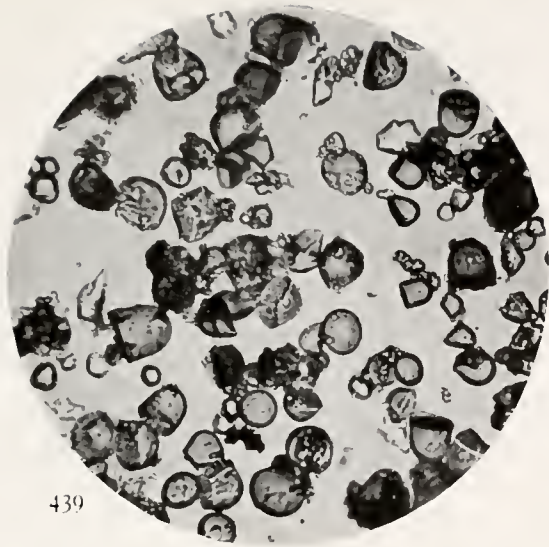
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438

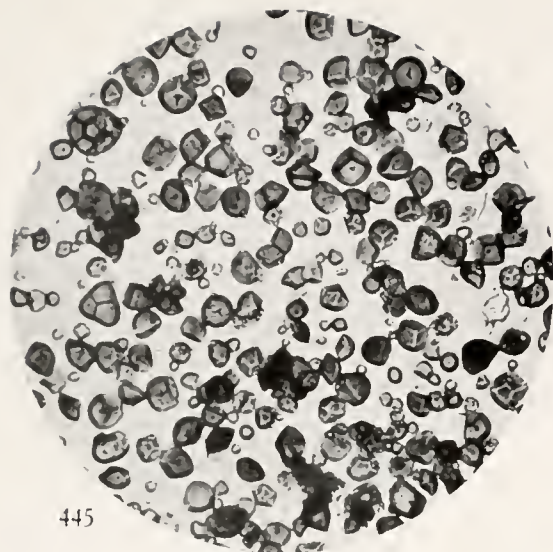
433 and 434. *Freesia refracta* var. *alba*.  
 435 and 436. *Freesia refracta* var. *leichtlinii*.  
 437 and 438. *Antholyza crocosmoides*.



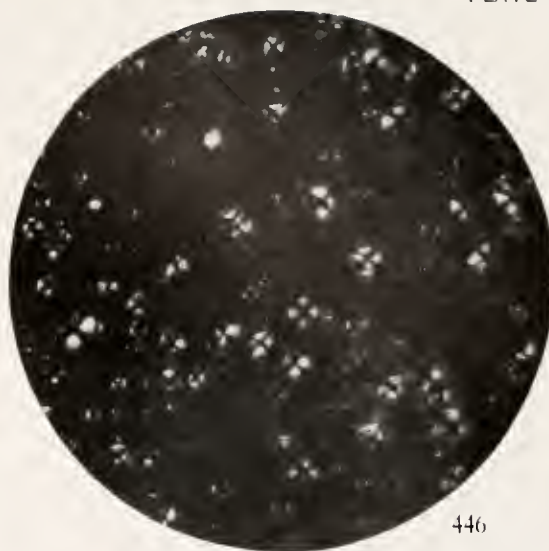


439 and 440. *Antholyza paniculata*.  
441 and 442. *Crocus susianus*.  
443 and 444. *Crocus versicolor*.

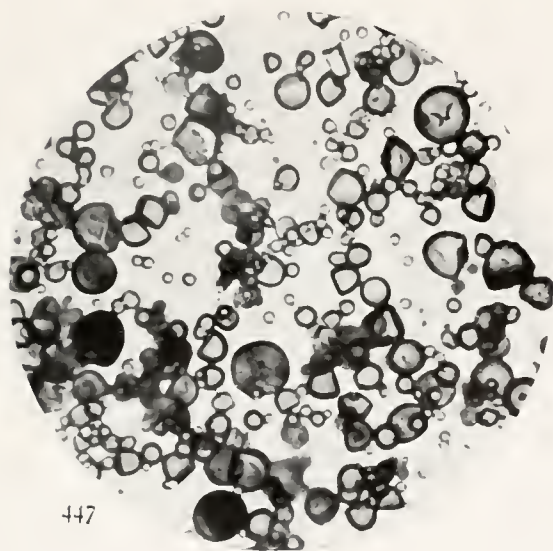




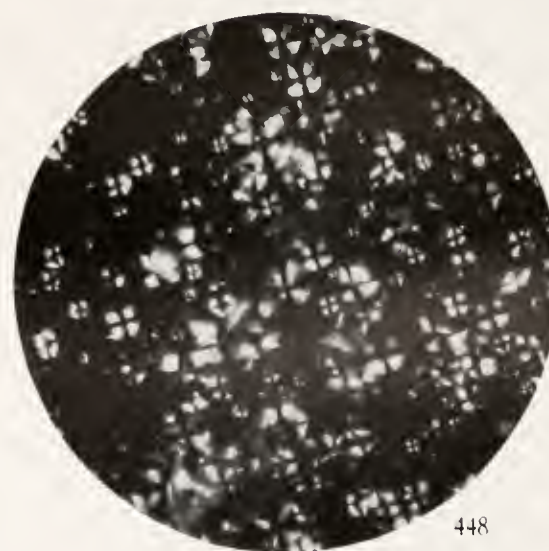
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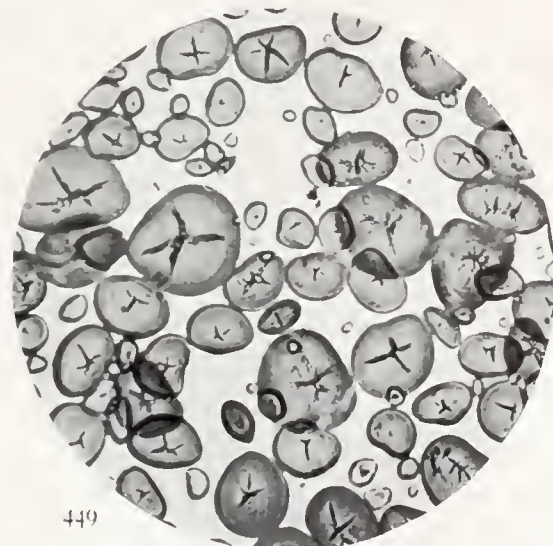
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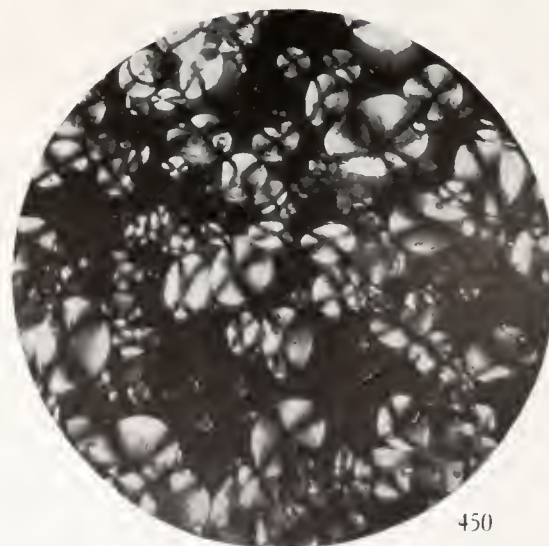
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448



449



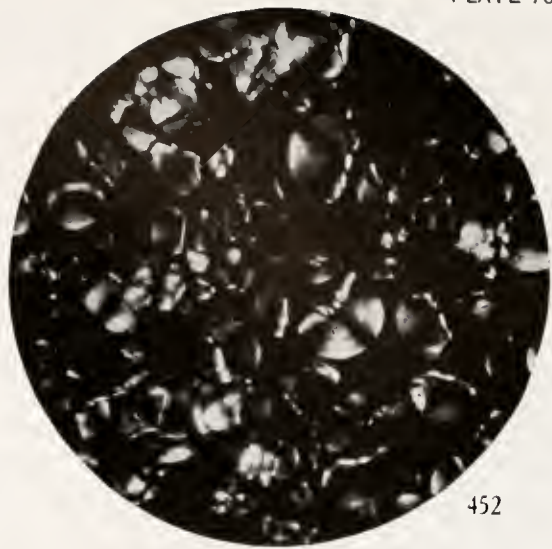
450

445 and 446. *Crocus* var. (Baron von Brunow).  
447 and 448. *Romulea rosea* var. *speciosa*.  
449 and 450. *Cypella herberti*.

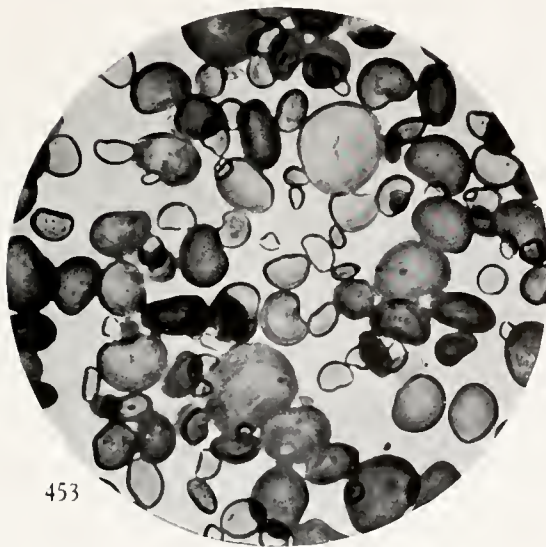




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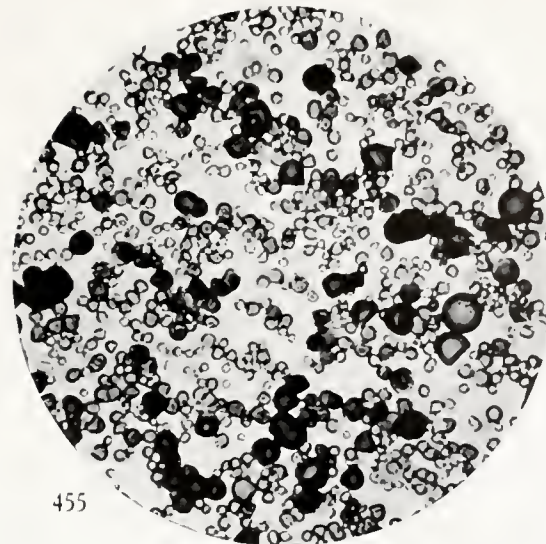
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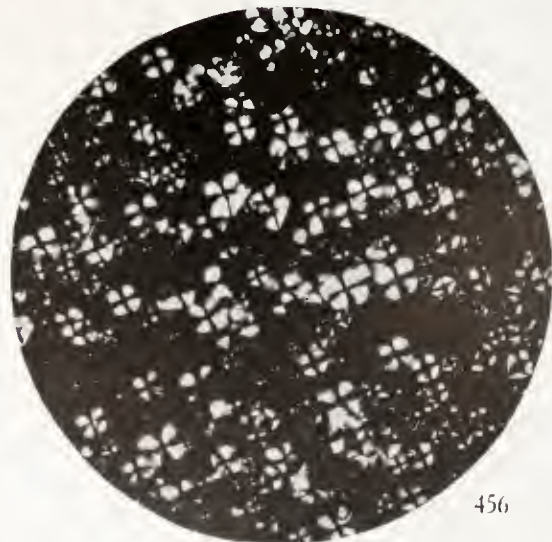
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454



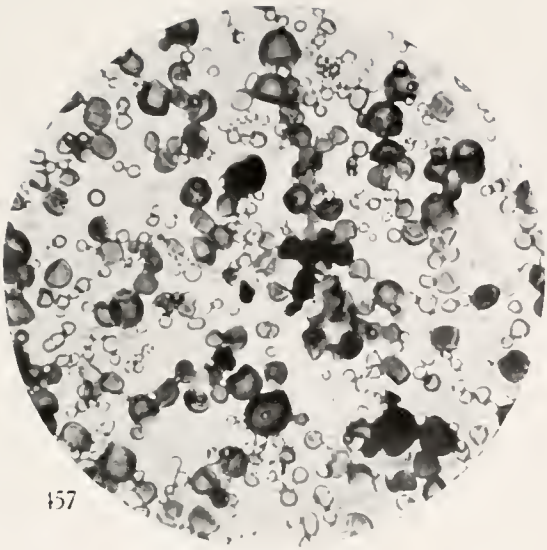
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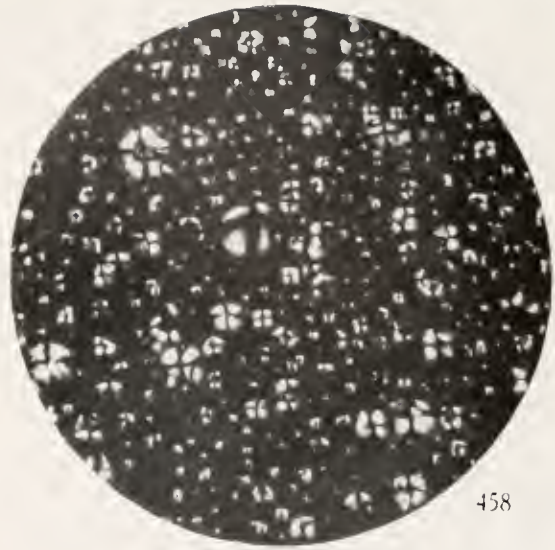
456

451 and 452. *Marica gracilis*.  
453 and 454. *Gelasine azurea*.  
455 and 456. *Sparaxis grandiflora alba*.

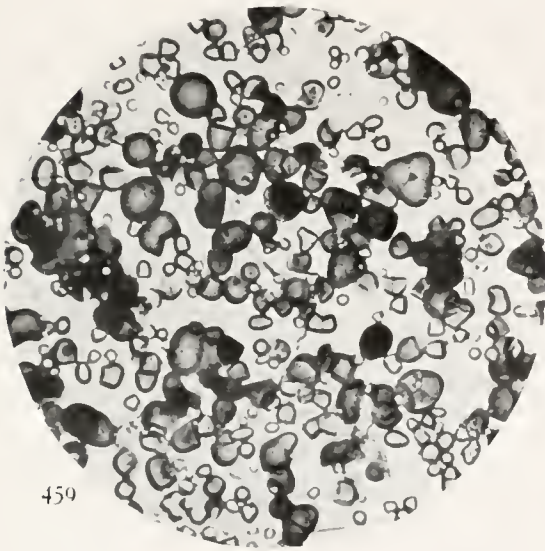




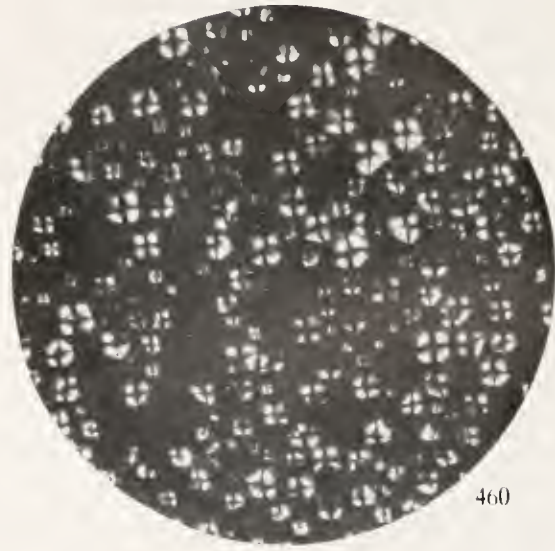
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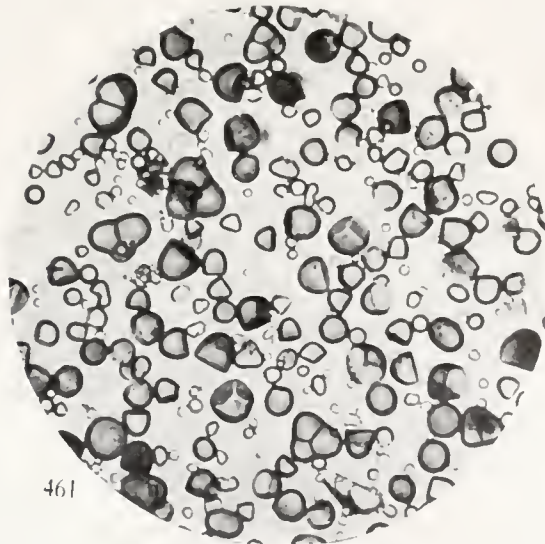
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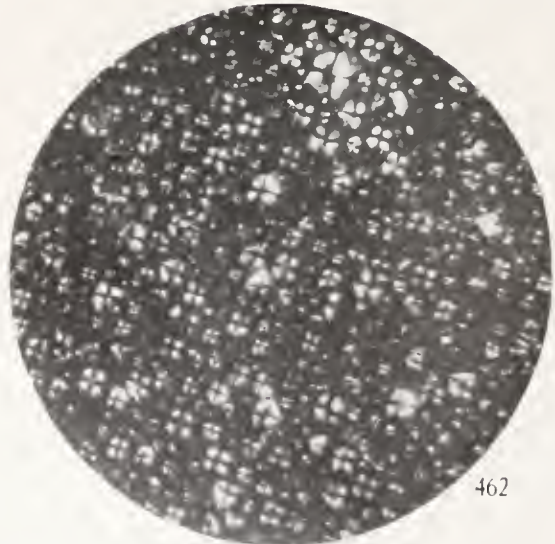
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461



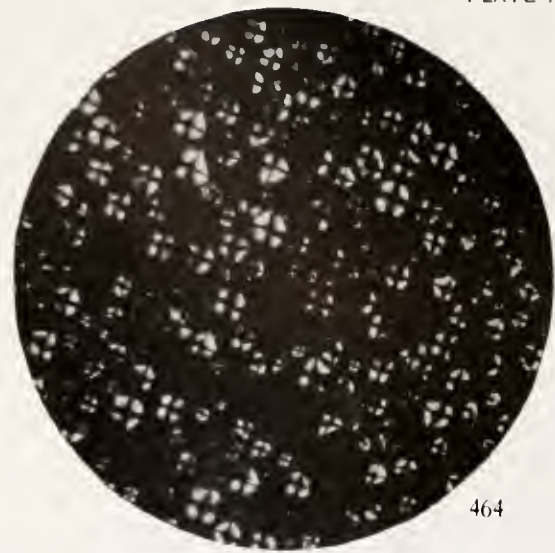
462

457 and 458. *Sparaxis* var. (*albertine*).  
459 and 460. *Ixia speciosa*.  
461 and 462. *Ixia viridiflora*.

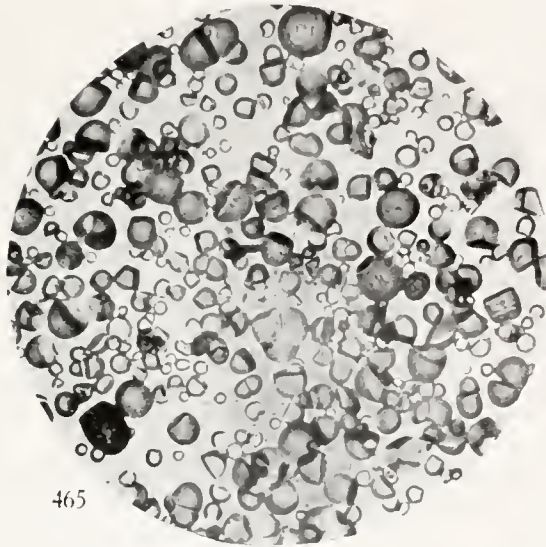




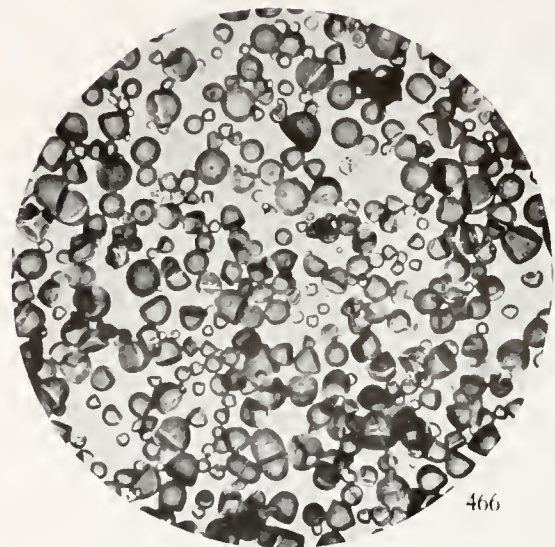
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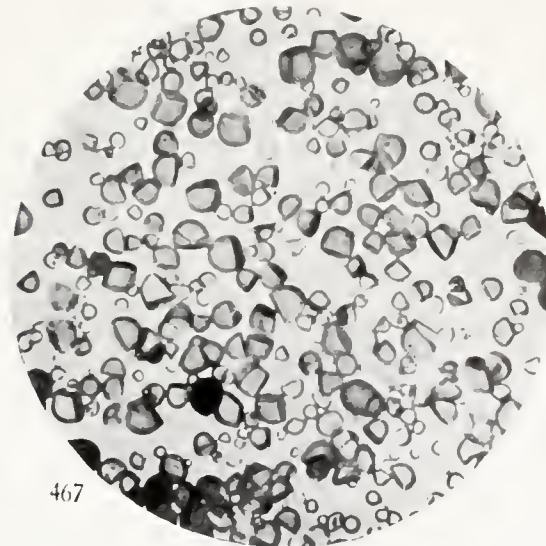
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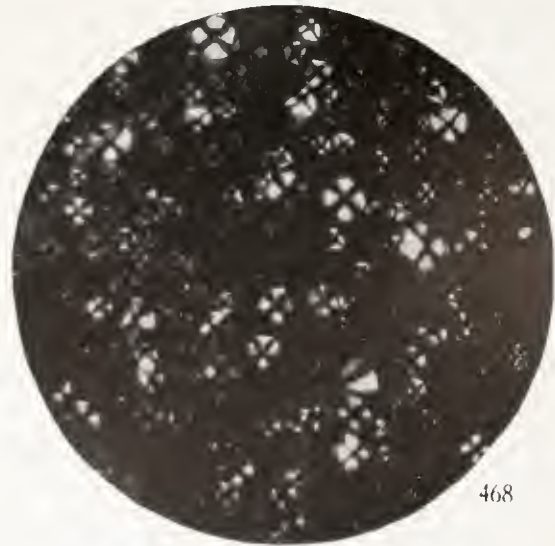
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466



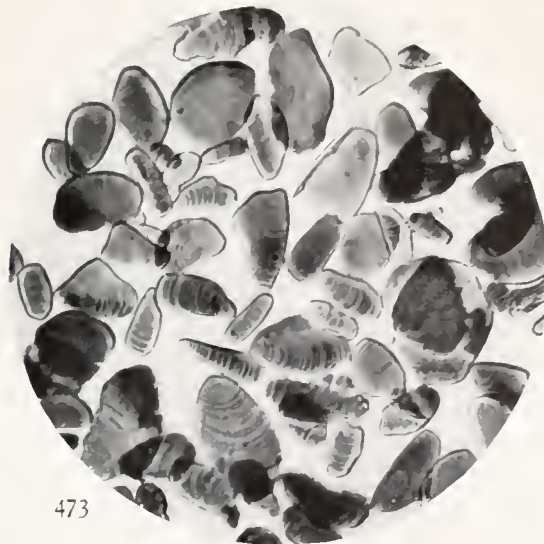
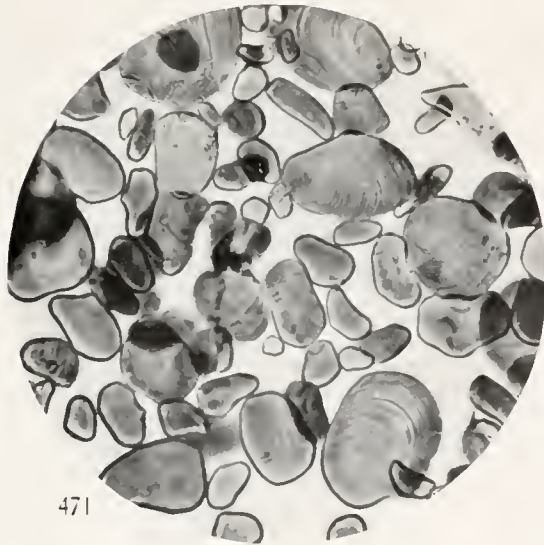
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468

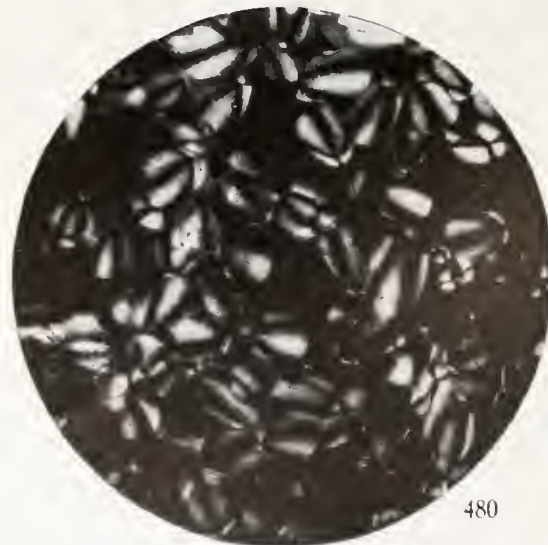
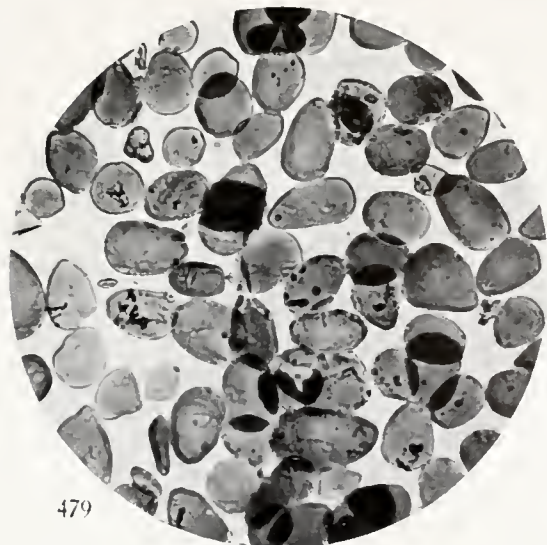
463 and 464. *Ixia* var. (Emma).  
465 and 466. *Babiana* var. (violacea).  
467 and 468. *Babiana* var. (attraction).





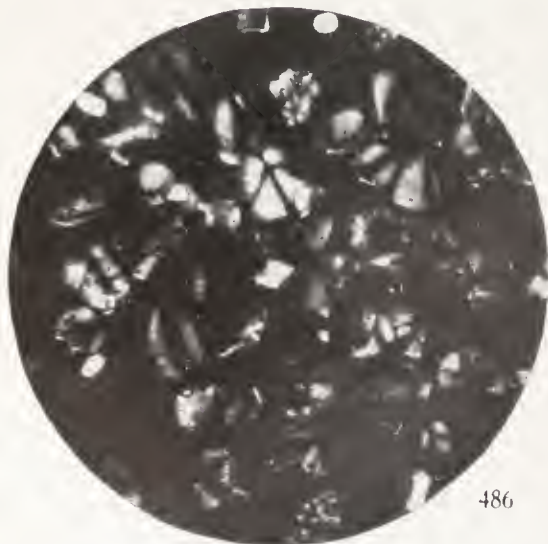
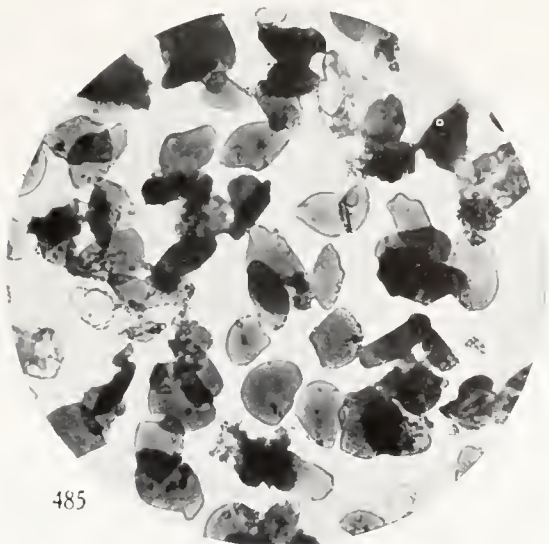
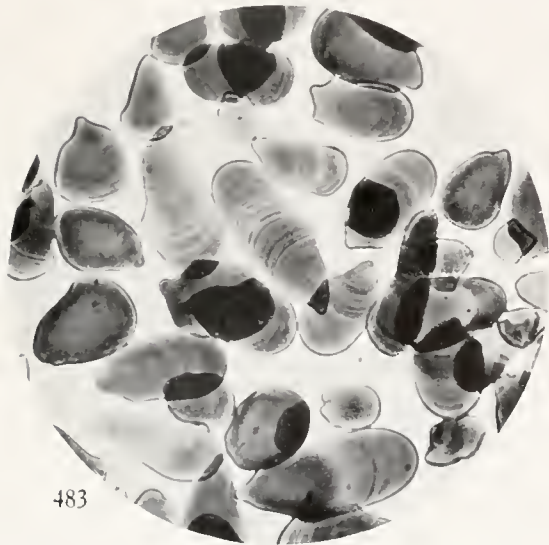
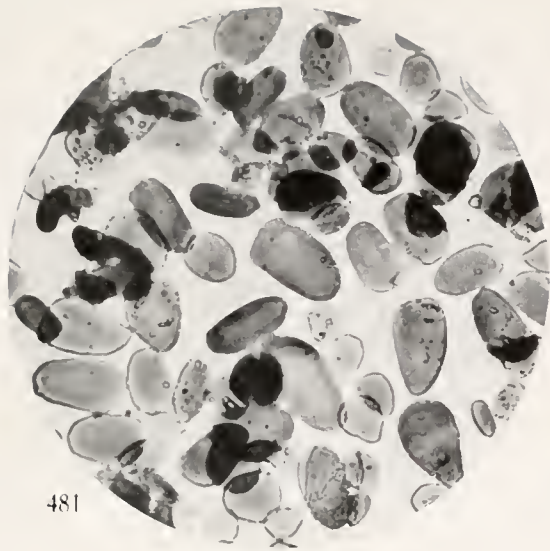
469 and 470. *Musa cavendishii* (root-stalk).  
471 and 472. *Musa cavendishii* (green fruit).  
473 and 474. *Musa sapientum*.





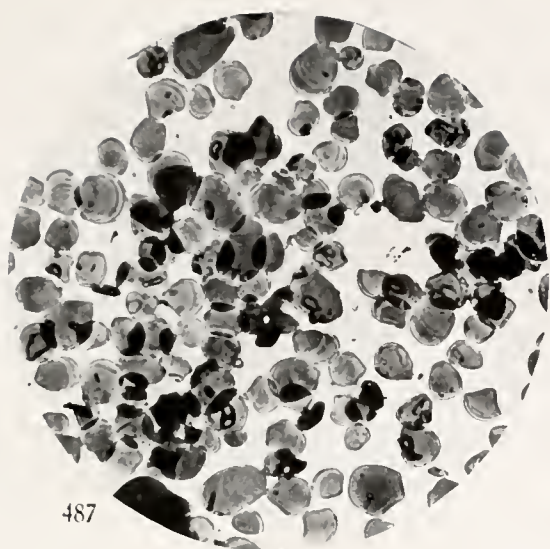
475 and 476. *Musa esculenta*.  
477 and 478. *Zingiber officinale*.  
479 and 480. *Zingiber officinale* var. *jamaica*.





481 and 482. *Zingiber officinale* var. *cockin*.  
483 and 484. *Hedychium coronarium*.  
485 and 486. *Hedychium gardnerianum*.





487



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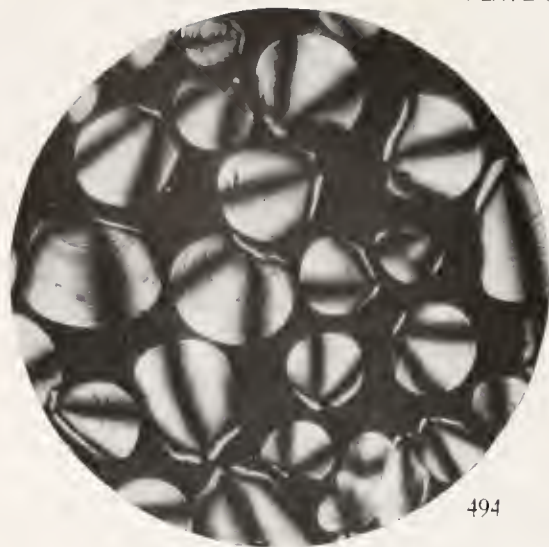
491



492

487 and 488. *Curcuma longa*.  
 489 and 490. *Curcuma petiolata*.  
 491 and 492. *Canna warszewiczii*.





493 and 494. *Canna roscoana*.  
495 and 496. *Canna musafolia*.  
497 and 498. *Canna edulis*.

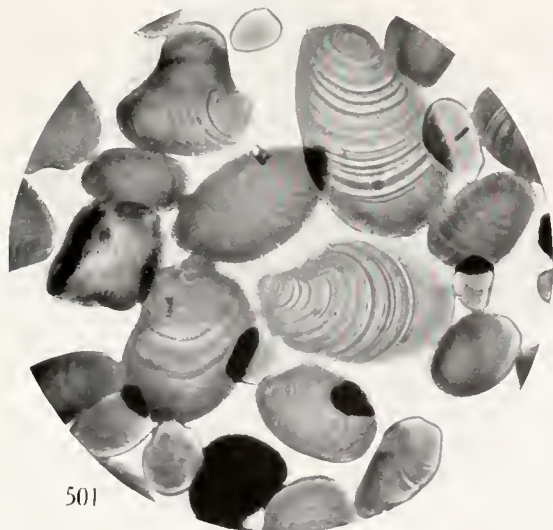




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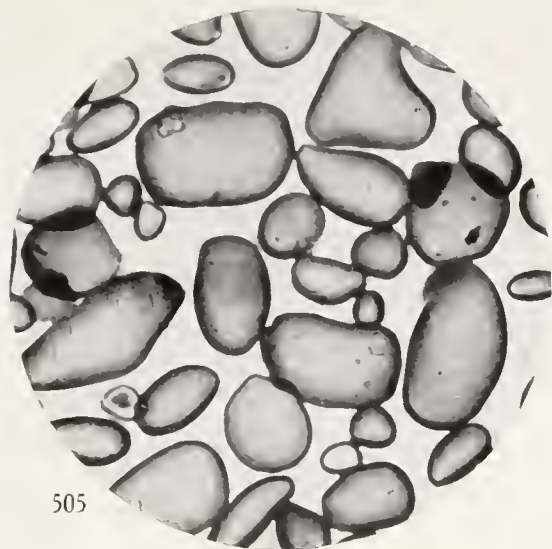
503



504

499 and 500. *Canna* var. (Königen Charlotte).  
501 and 502. *Canna* var. (President Carnot).  
503 and 504. *Canna* var. (L. E. Bally)

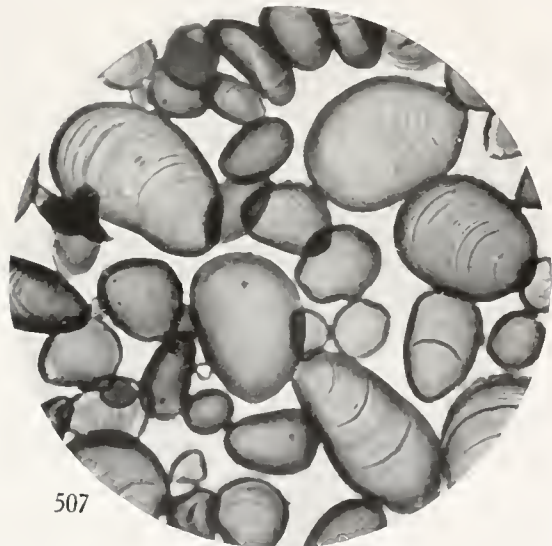




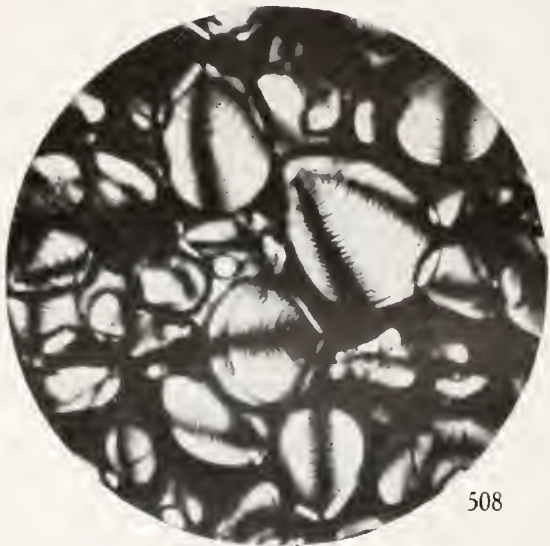
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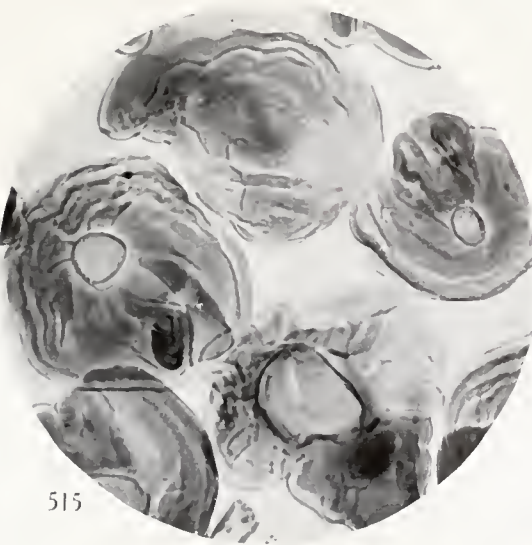
509



510

505 and 506. *Canna* var. (Mrs. Kate Grey).  
507 and 508. *Canna* var. (Jean Tissot).  
509 and 510. *Canna* var. (J. D. Eisele).





511 to 516. Effects of chloral hydrate-iodine on *Canna warszewiczii*.





517



518



519



520



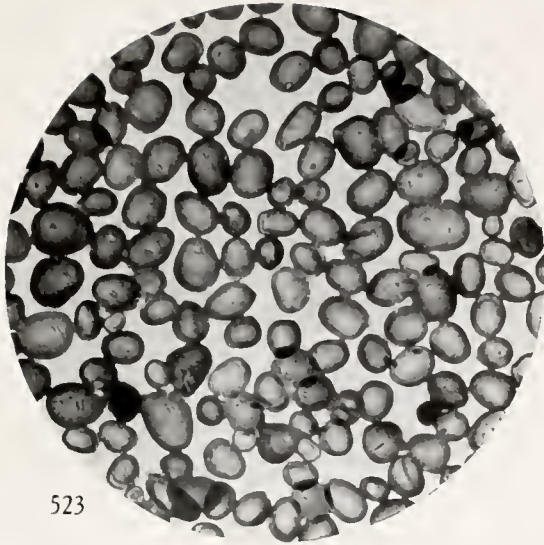
521



522

517 and 518. Effects of chloral hydrate-iodine on the starch of *Solanum tuberosum*.  
519 to 522. Effects of chromic acid on *Canna warszewiczii*.

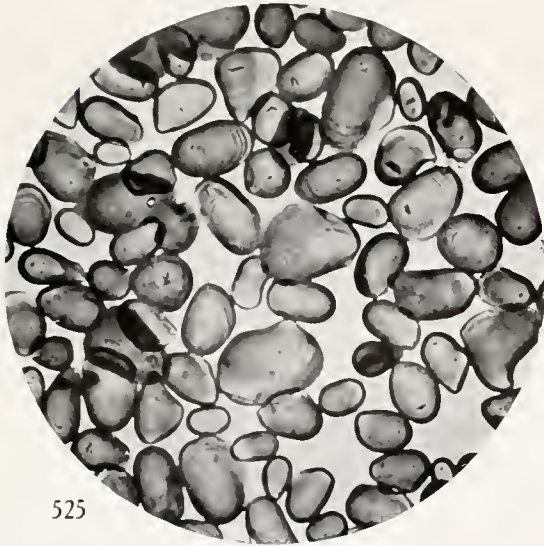




523



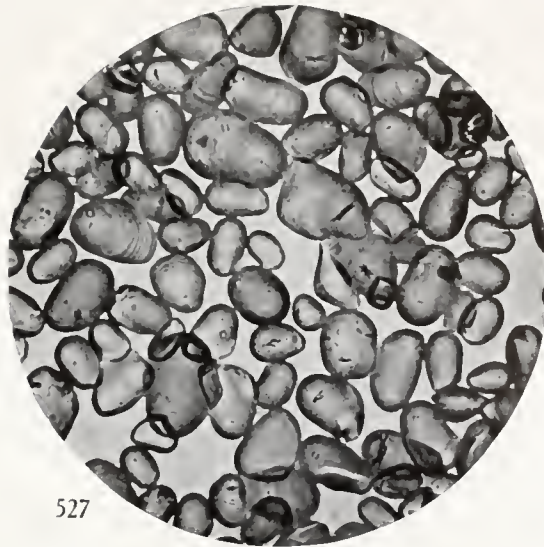
524



525



526



527



528

523 and 524. *Maranta arundinacea*.  
 525 and 526. *Maranta arundinacea* var. No. 1.  
 527 and 528. *Maranta arundinacea* var. No. 2.

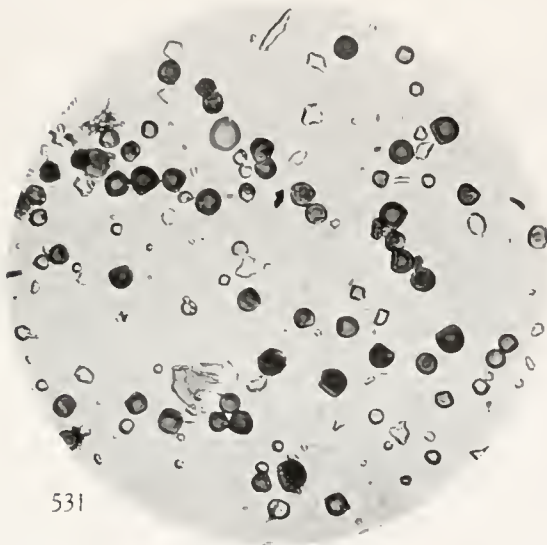




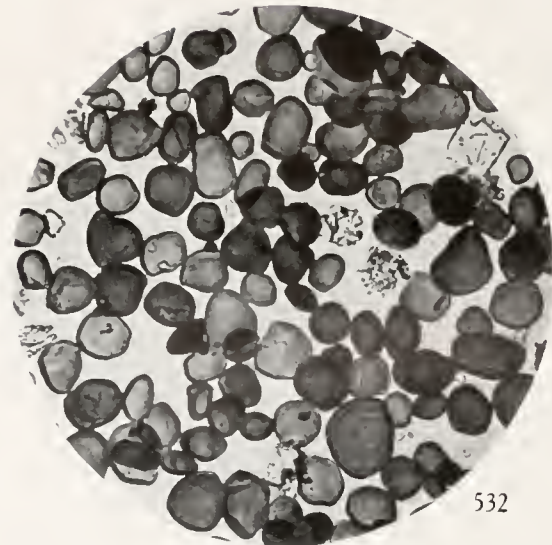
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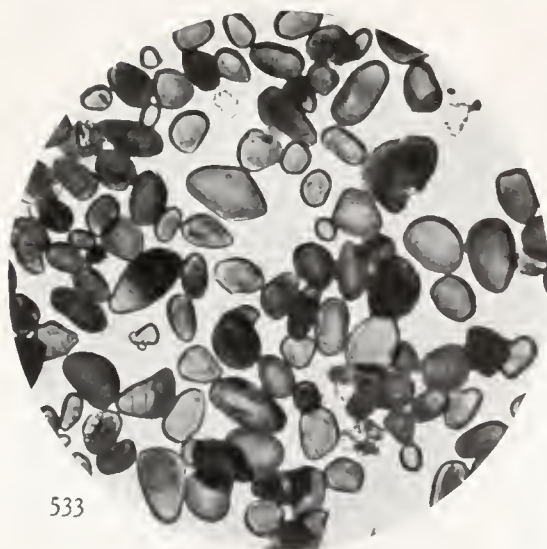
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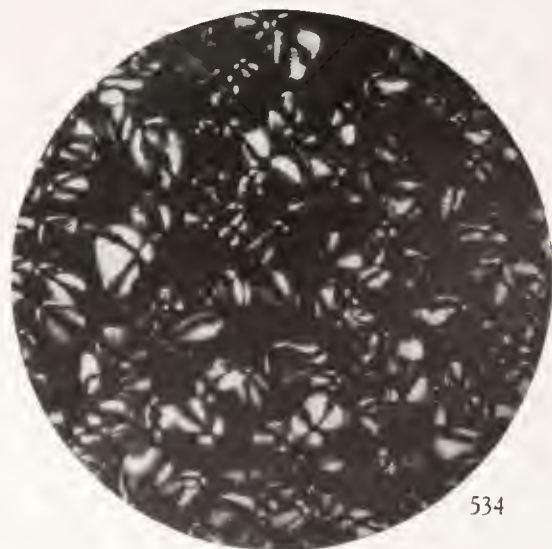
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532



533



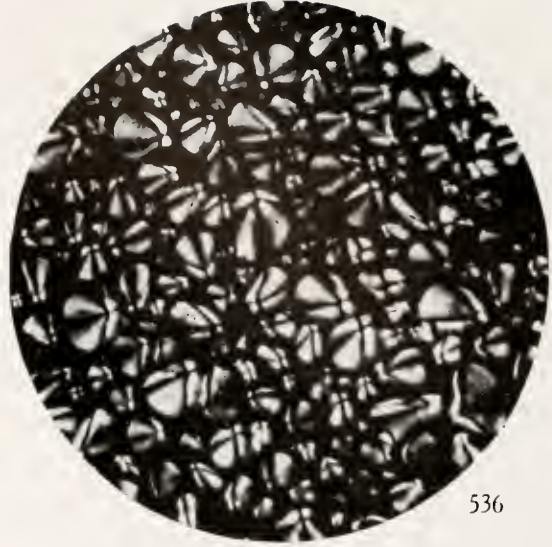
534

529 and 530. *Maranta massangana*.  
 531. *Maranta leuconura*.  
 532. *Maranta goreniana*.  
 533 and 534. *Maranta musaica*.





535



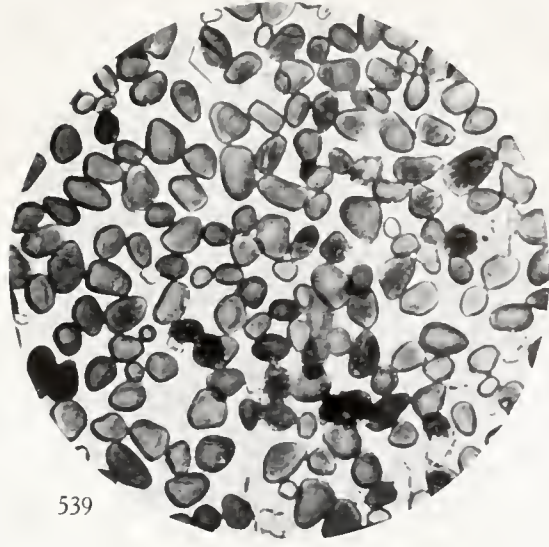
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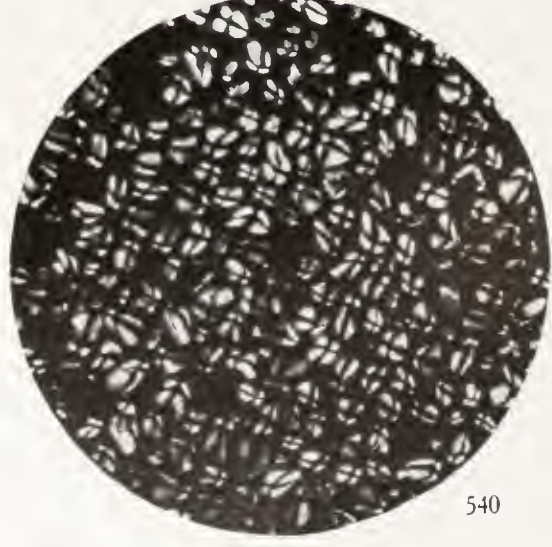
537



538



539



540

535 and 536. *Calathea litzet*.  
537 and 538. *Calathea vittata*.  
539 and 540. *Calathea viottiana*.

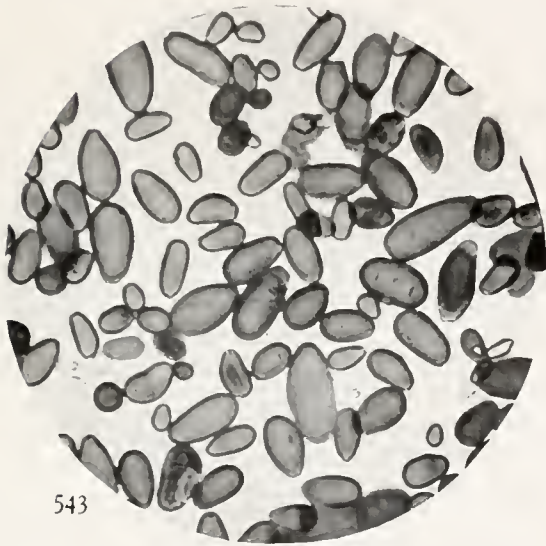




541



542



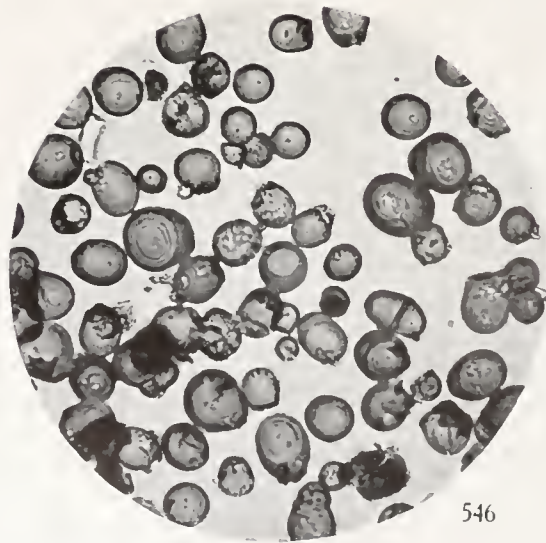
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544



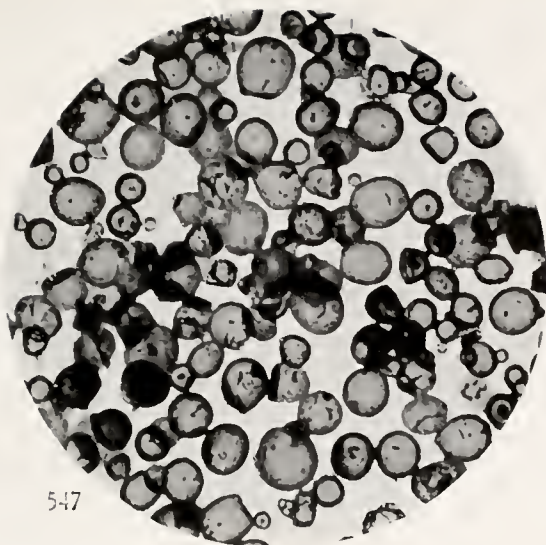
545



546

541 and 542. *Calathea rondonbeckii*.  
543 and 544. *Stromanthe sanguinea*.  
545. *Nymphaea alba*.  
546. *Nymphaea mexicana*.





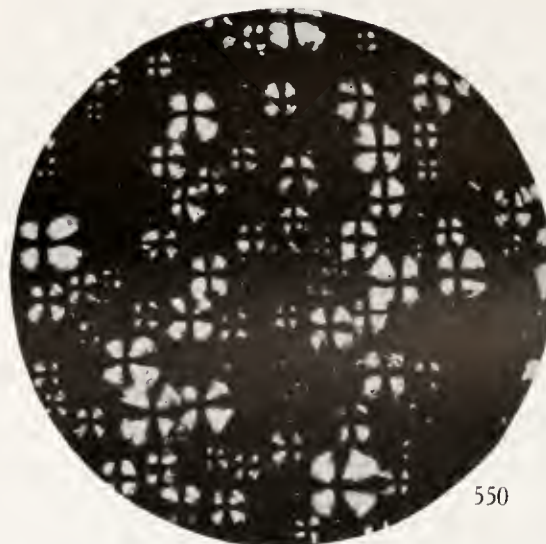
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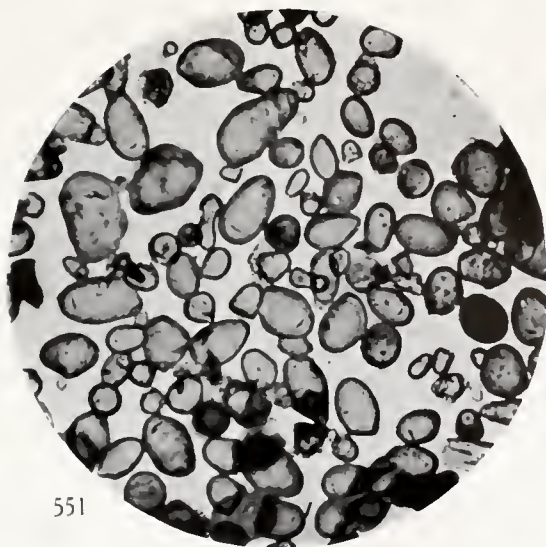
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549



550



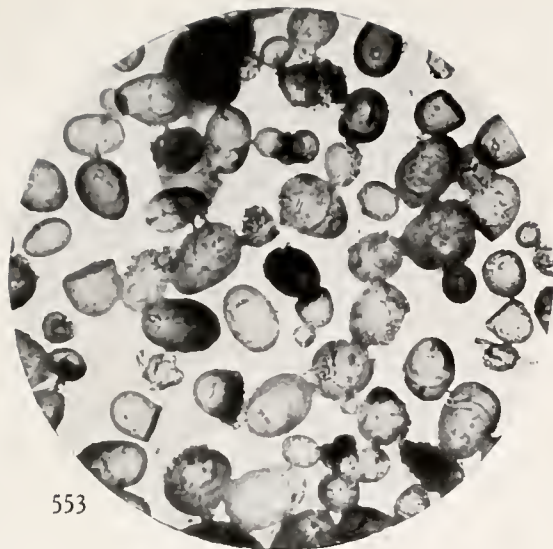
551



552

547 and 548. *Nymphaea marliacca* var. *albida*.  
 549 and 550. *Nymphaea marliacca* var. *carnea*.  
 551 and 552. *Nymphaea gladstoniana*.

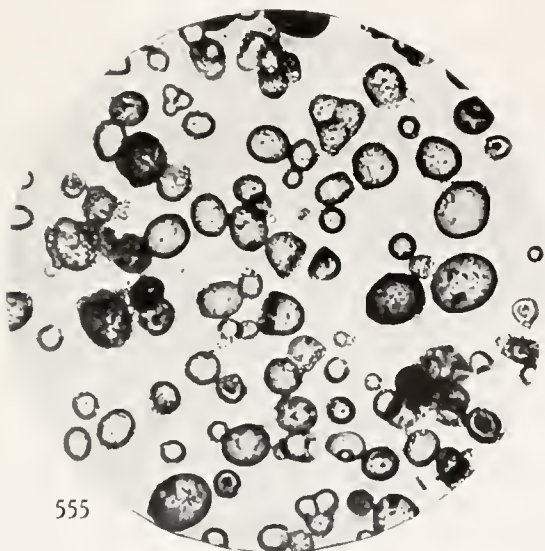




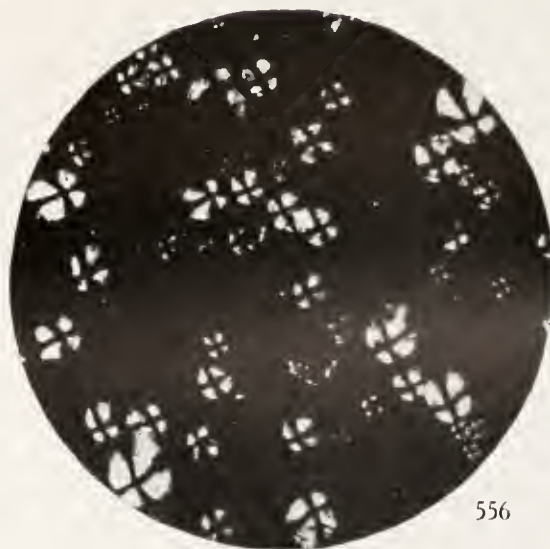
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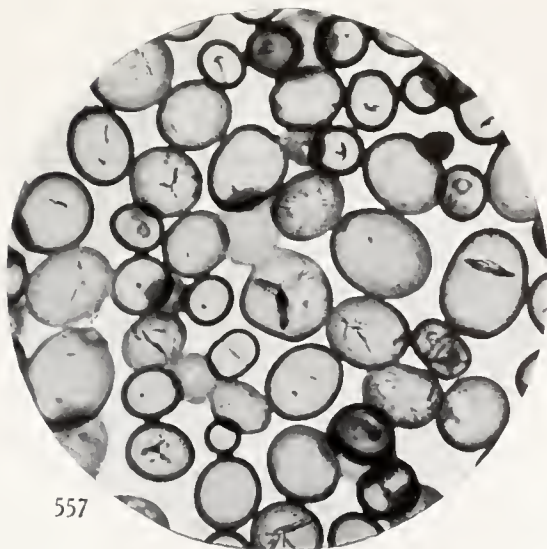
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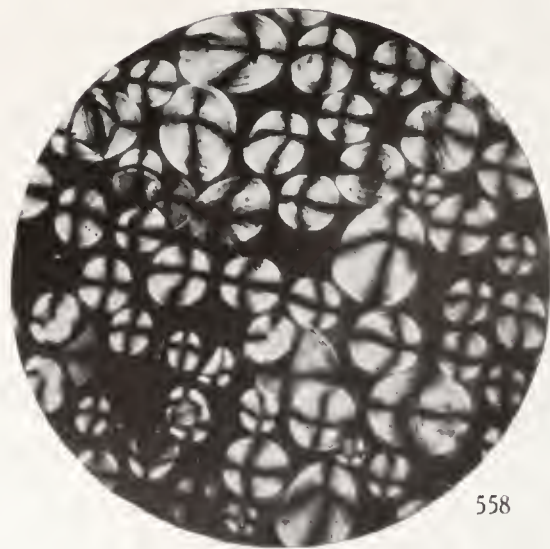
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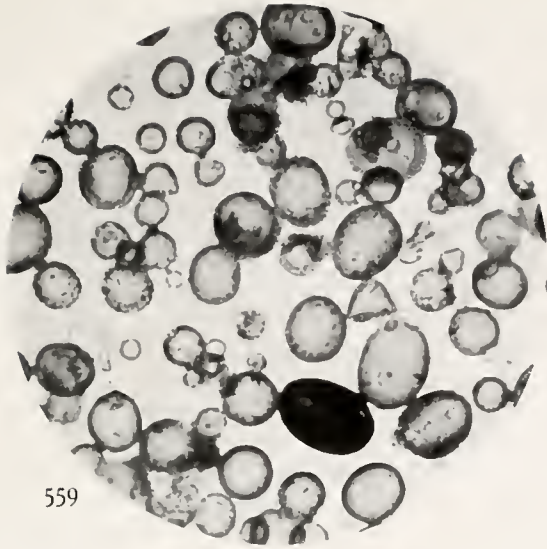
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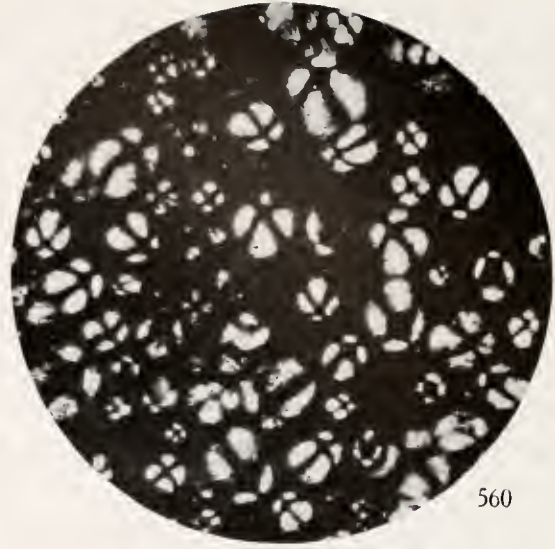
558

553 and 554. *Nymphaea odorata*.  
555 and 556. *Nymphaea odorata* var. *rosca*.  
557 and 558. *Nelumbo nucifera*.





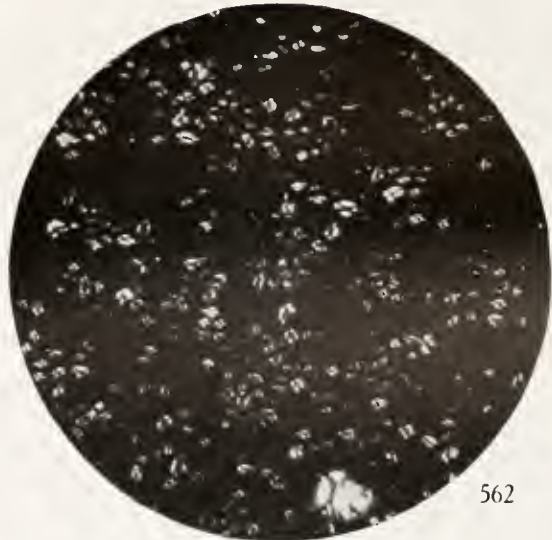
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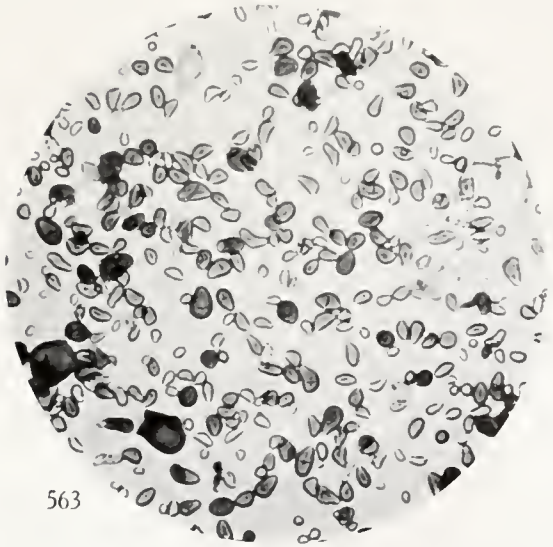
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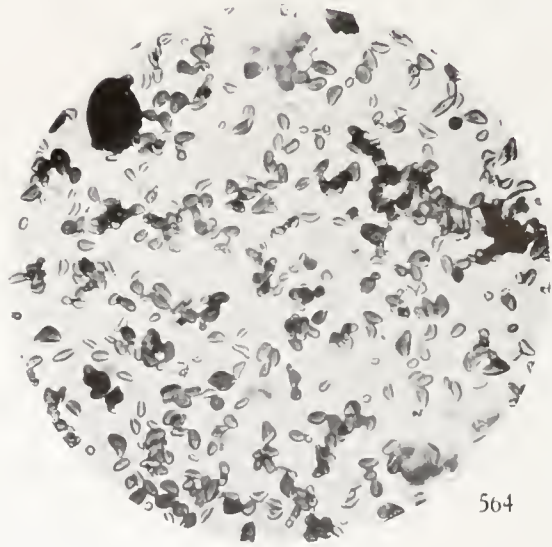
561



562



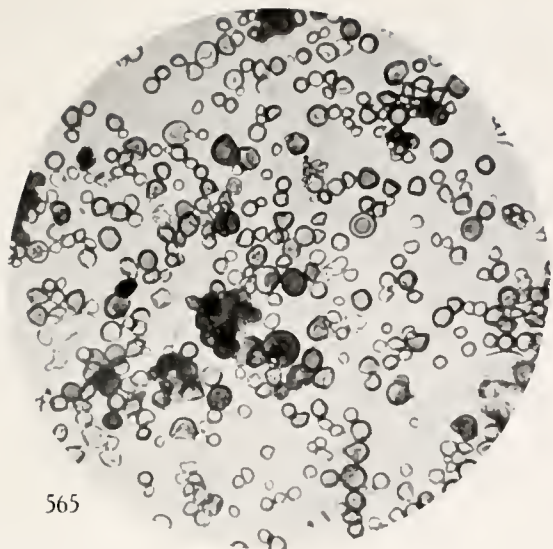
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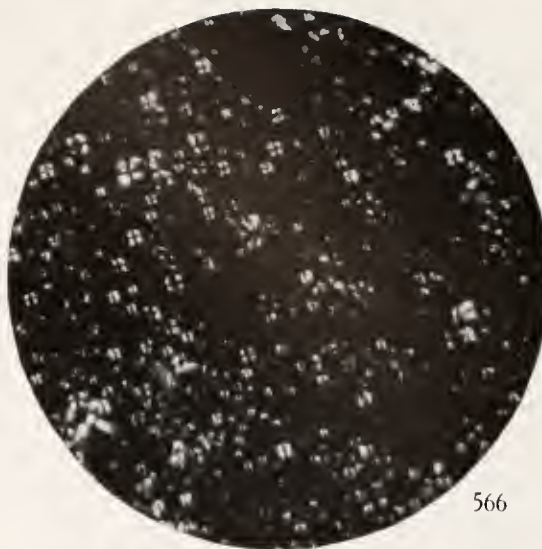
564

559 and 560. *Nymphaea lutea*.  
561 and 562. *Anemone apennina*.  
563. *Anemone fulgens*.  
564. *Anemone blanda*.

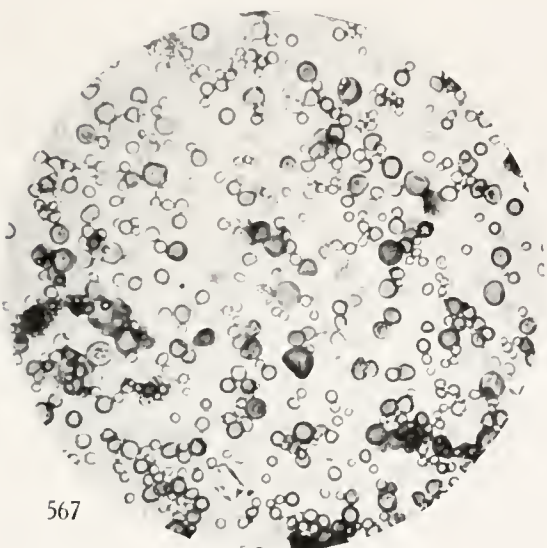




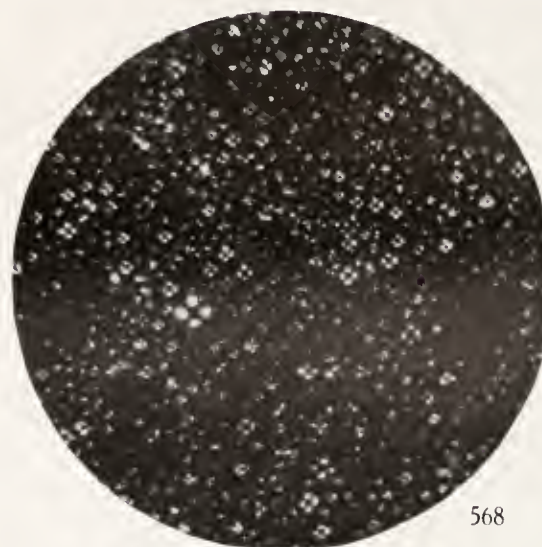
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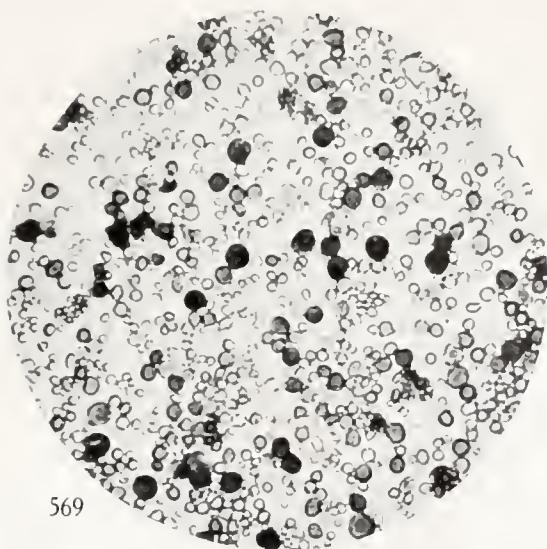
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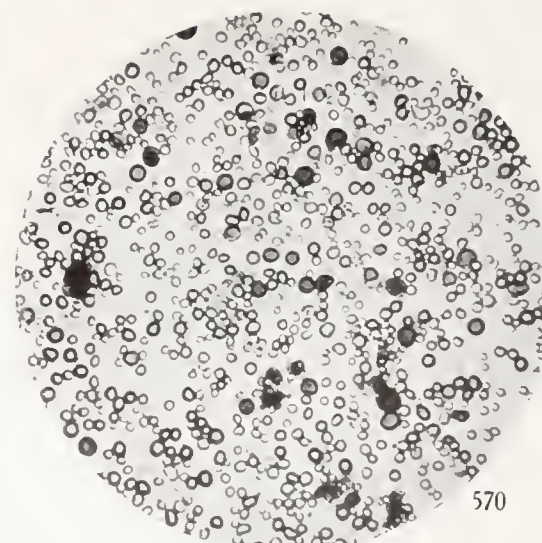
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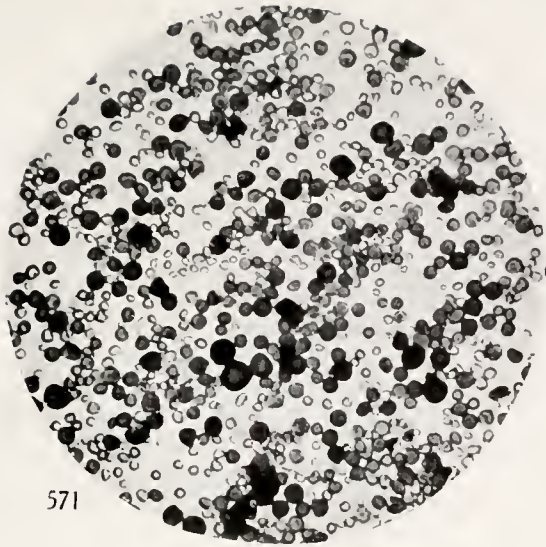
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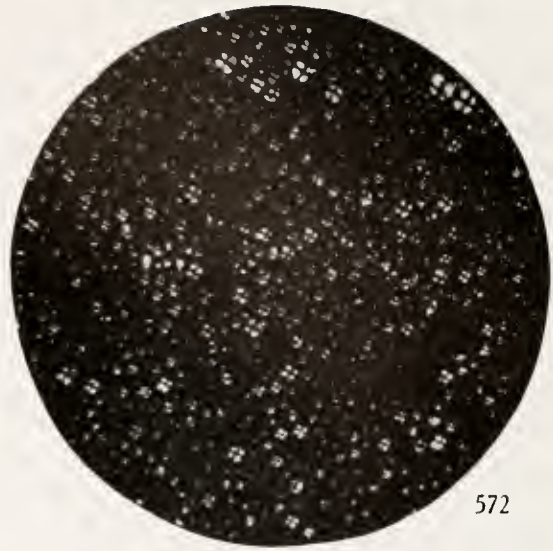
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565 and 566. *Anemone japonica*.  
 567 and 568. *Aconitum napellus*.  
 569. *Actaea alba*.  
 570. *Actaea spicata* var. *rubra*.

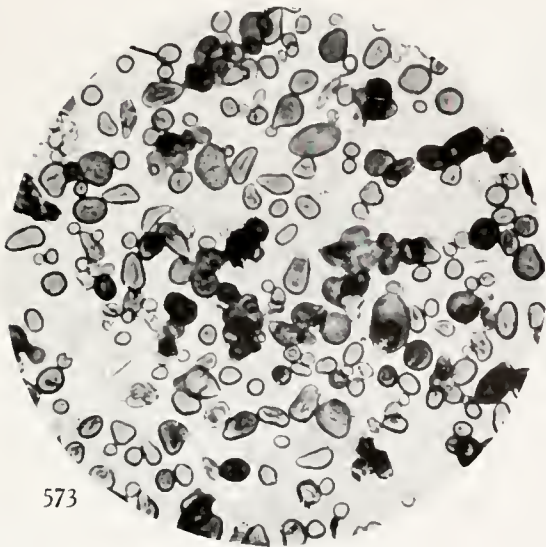




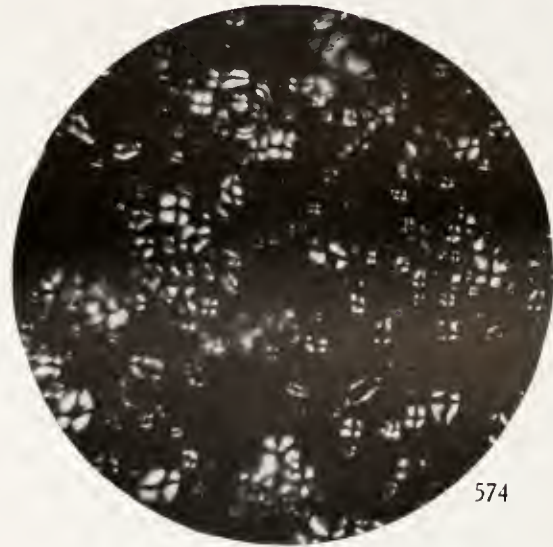
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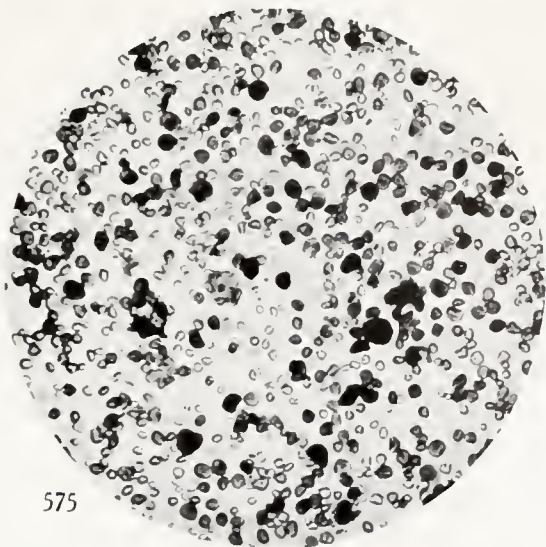
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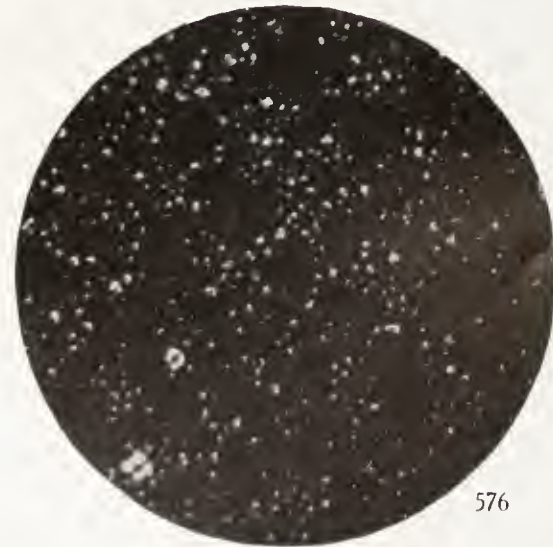
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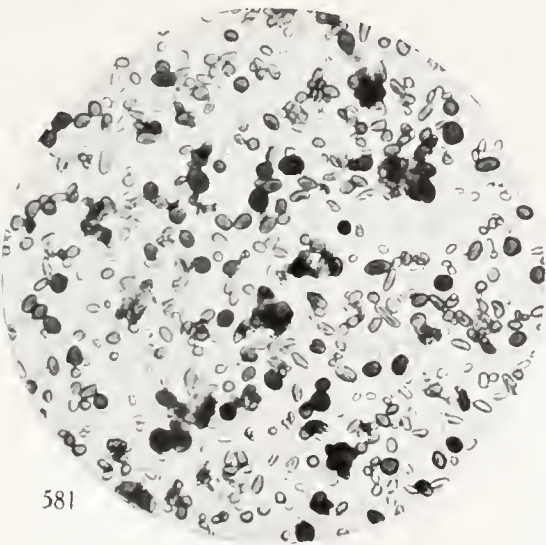
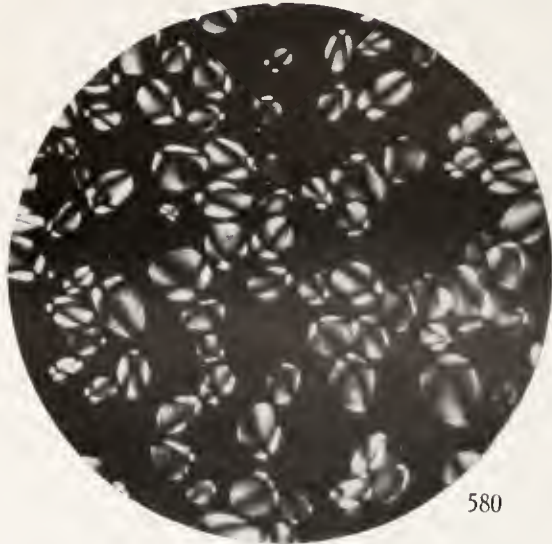
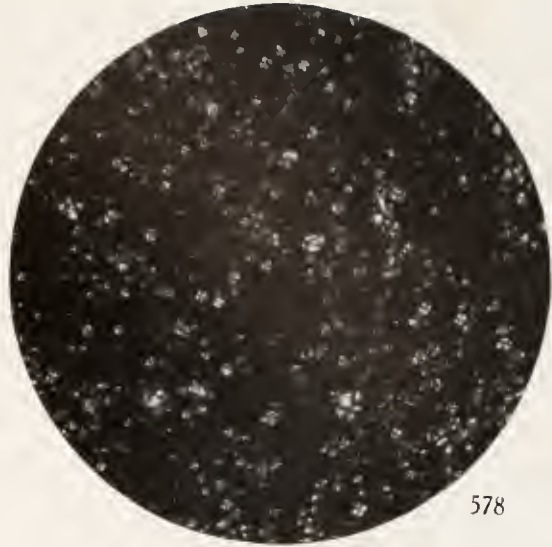
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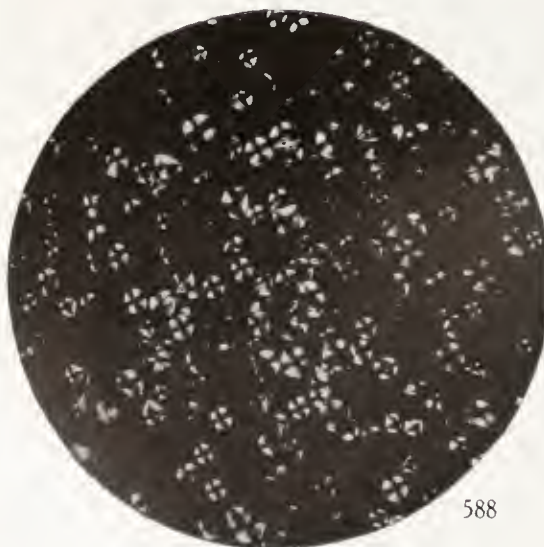
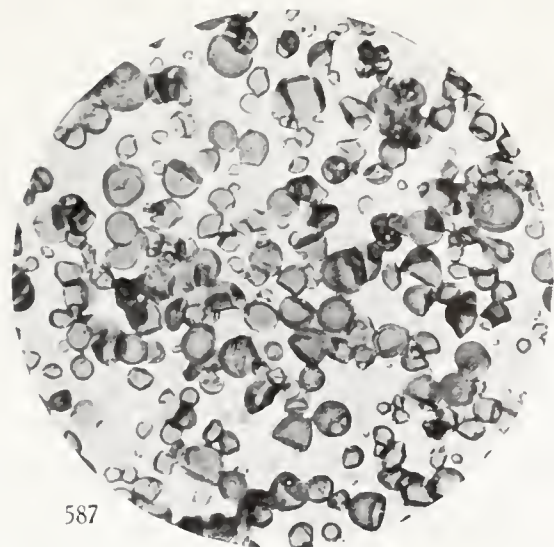
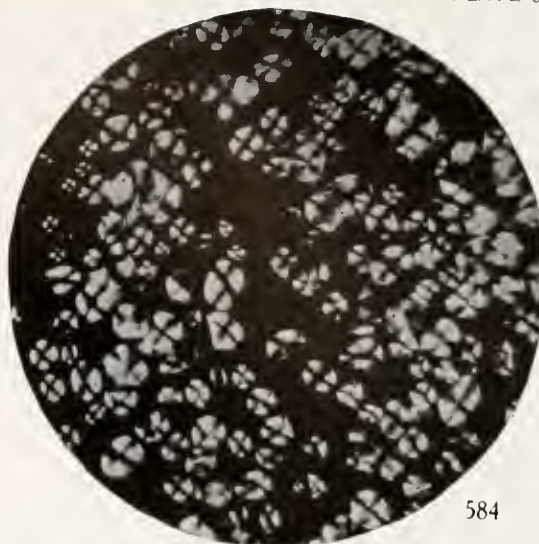
571 and 572. *Cimicifuga racemosa*.  
573 and 574. *Eranthis hiemalis*.  
575 and 576. *Adonis amurensis*.





577 and 578. *Ranunculus bulbosus*.  
579 and 580. *Ranunculus ficaria*.  
581 and 582. *Cochlearia armoracia*.



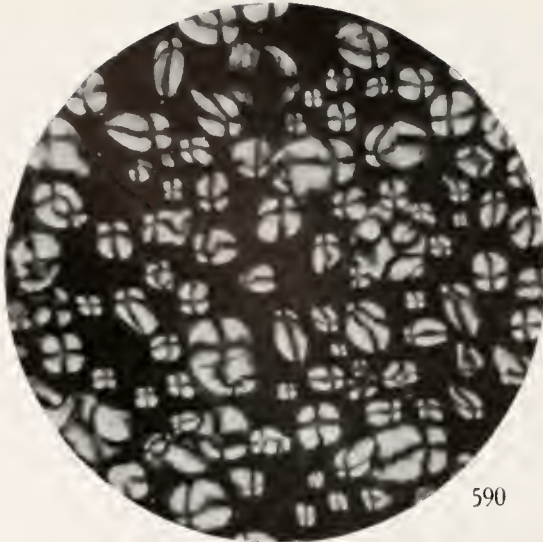


583 and 584. *Atropa curcas*.  
585 and 586. *Manihot utilissima*.  
587 and 588. *Trianosprma ficifolia*.

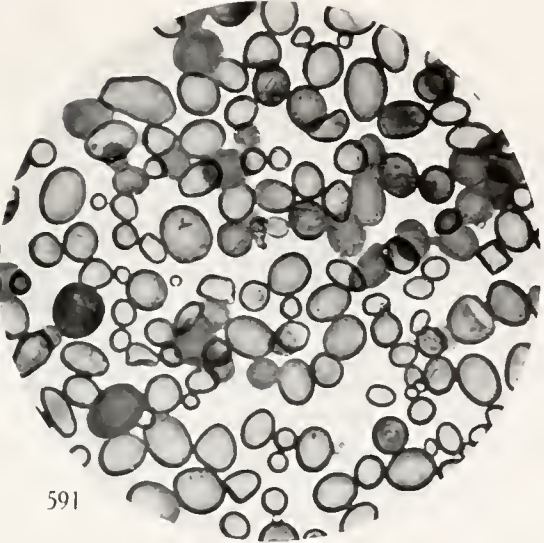




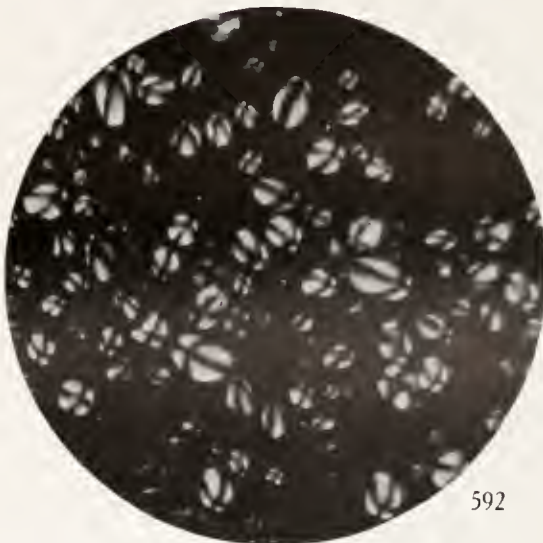
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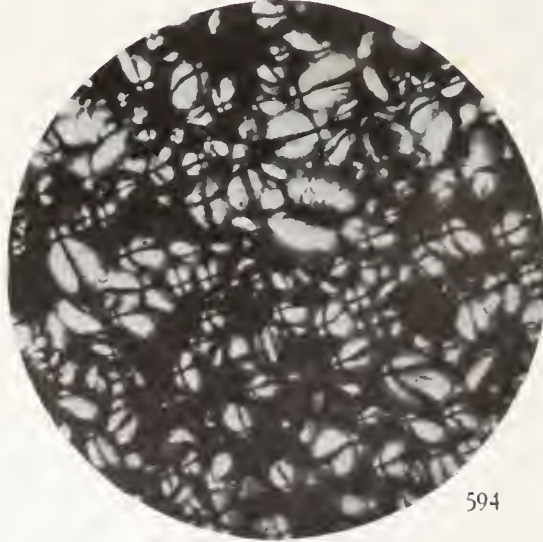
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589 and 590. *Cyclamen repandum*.  
 591 and 592. *Cyclamen coum*.  
 593 and 594. *Cyclamen cilicium*.

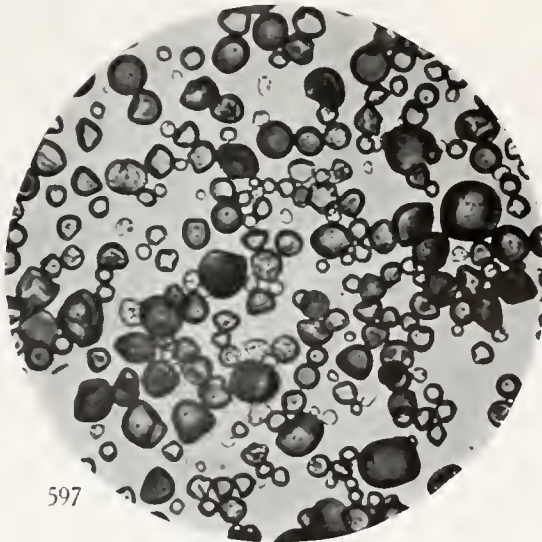




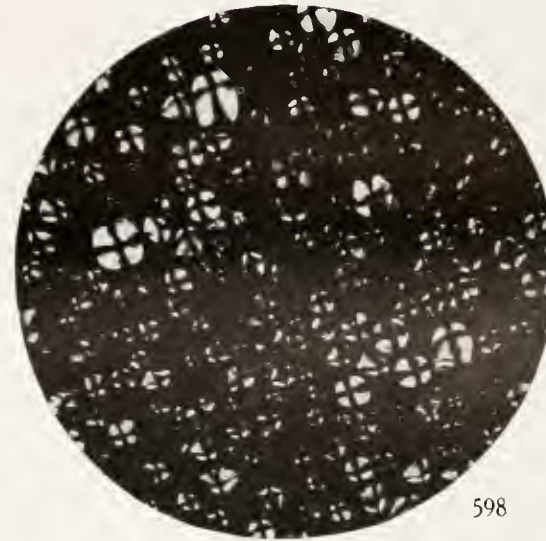
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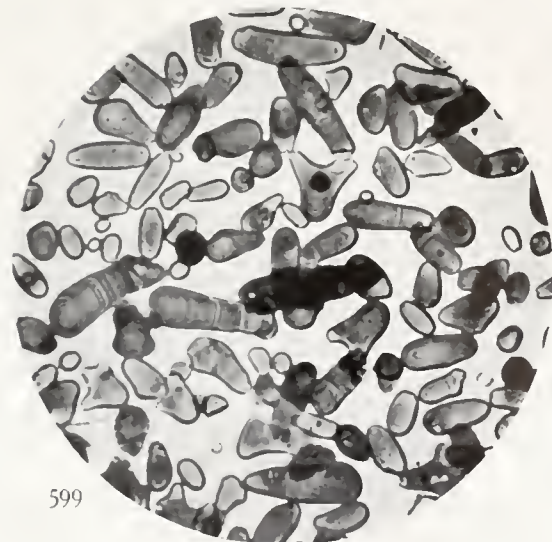
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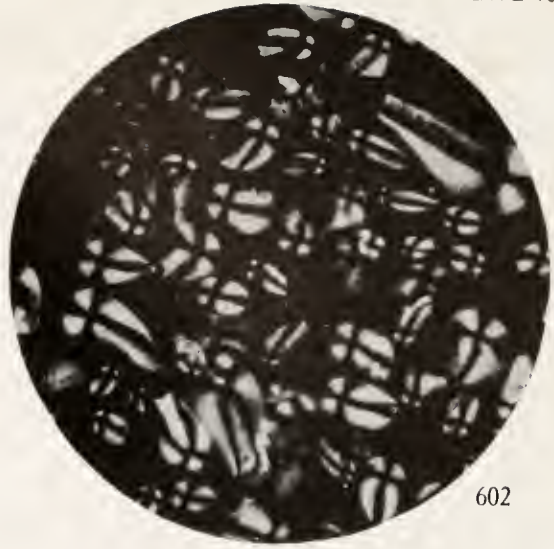
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595 and 596. *Solanum tuberosum*.  
 597 and 598. *Batatas edulis*.  
 599 and 600. *Gesneria tubiflora*.

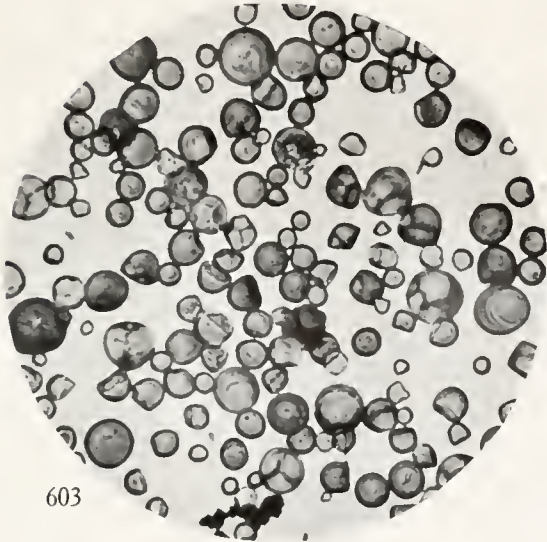




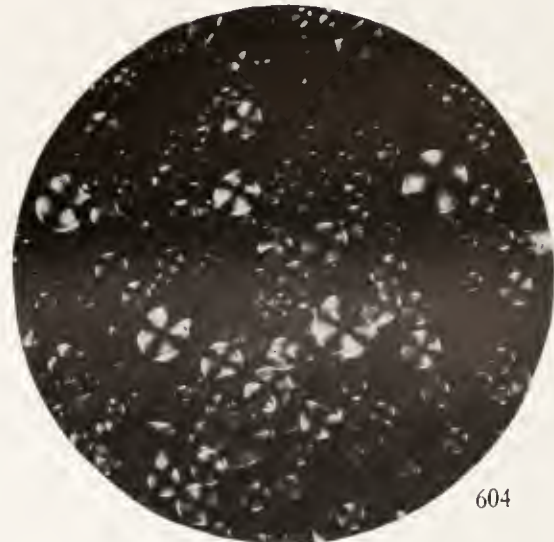
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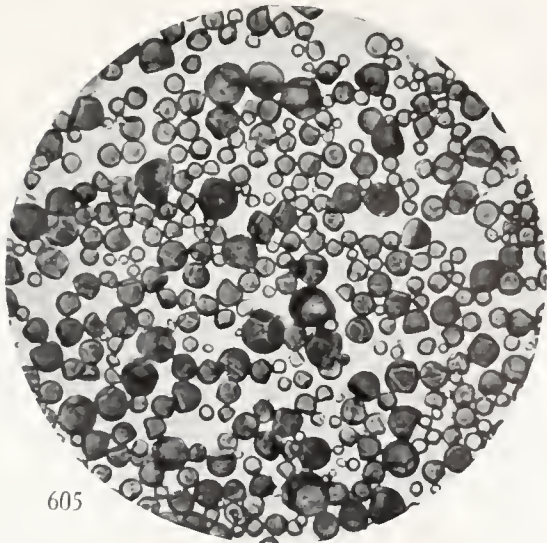
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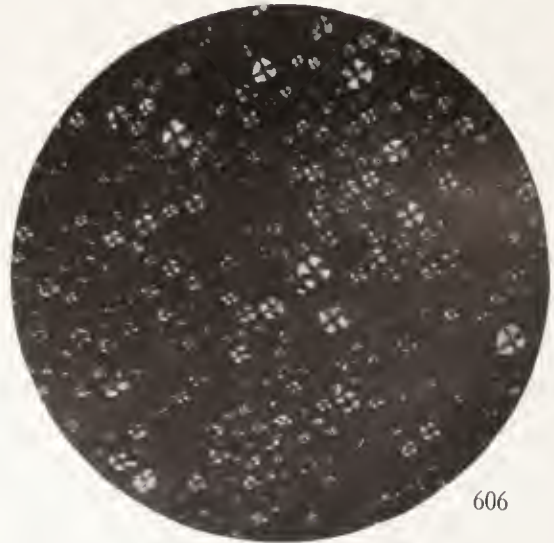
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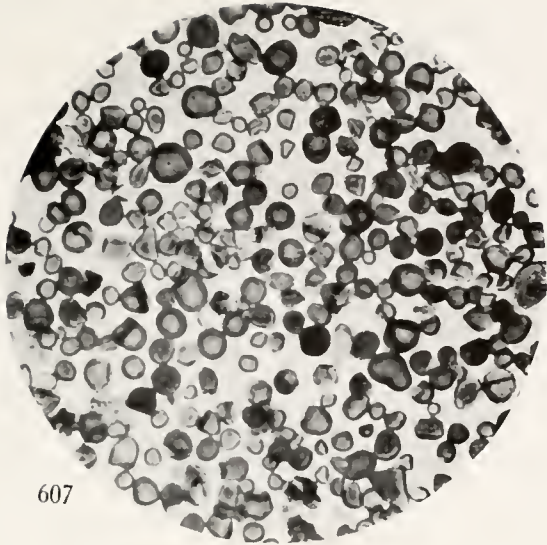
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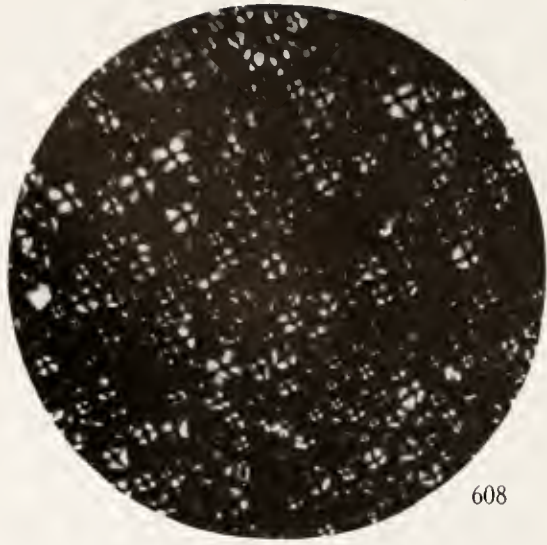
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601 and 602. *Gloxinia* var.  
603 and 604. *Cycas revoluta*.  
605 and 606. *Cycas circinalis*.

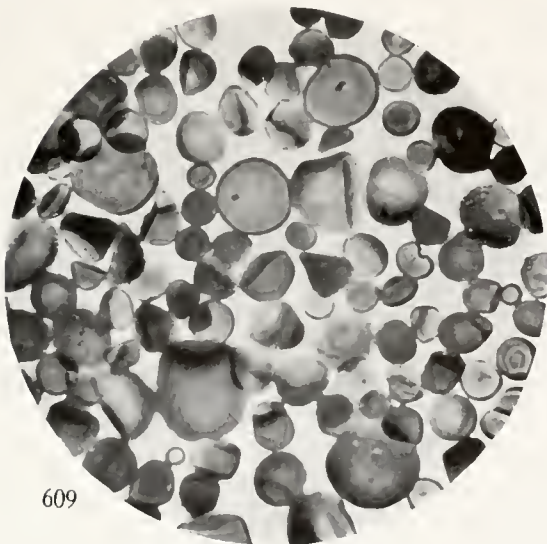




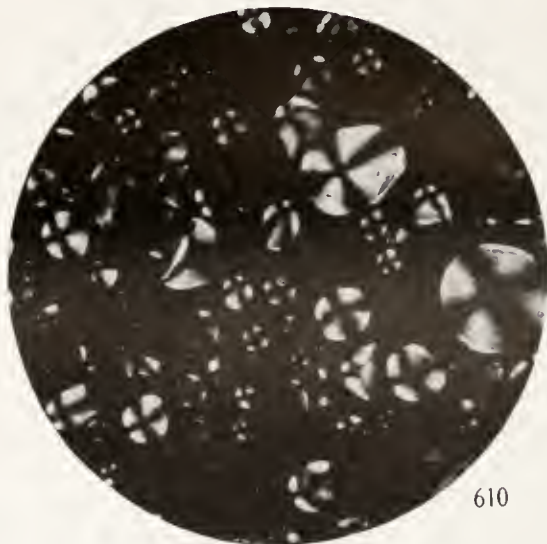
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607 and 608. *Dioon edule*.  
609 and 610. *Zamia integrifolia*.  
611 and 612. *Phoinix waltlichii*.











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