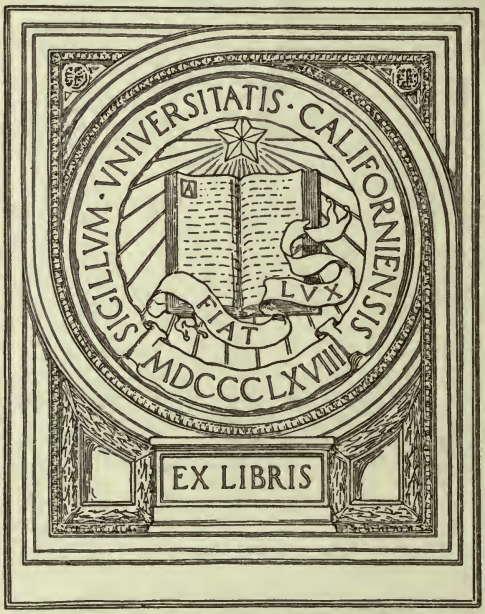
ELEMENTS OF

VEGETABLE HISTOLOGY

BY

DANIEL BASE



ELEMENTS OF

VEGETABLE HISTOLOGY

For the Use of Students of Pharmacy, Preparatory to the Study of Pharmacognosy

---

WITH 62 ILLUSTRATIONS

BY

DANIEL BASE, Ph.D.,

Professor of Chemistry and Vegetable Histology in the Maryland College of Pharmacy, Department Pharmacy, University of Maryland, Baltimore

---

SECOND EDITION, REVISED AND ENLARGED

BALTIMORE, MD.
THE AUTHOR
1905

PREFACE TO THE FIRST EDITION

This little book is intended to serve as a guide for beginners in the study of plant tissues with the microscope, with supplemental instruction from the teacher.

The greater portion deals with the tissues and their arrangement in the higher plants, but it was thought advisable to introduce a few lessons on the simplest plants, namely, some of those of the Thallophyte series, not only on account of their great importance in the life economy, but also to show the gradual increase in complexity of structure in passing from the extremely simple and minute plants of the Thallophyte series to the complicated highly organized members of the Phanerogams, thus giving the student a complete view of the structure and characteristics of the whole range of the vegetable kingdom.

The knowledge of the tissues of the higher plants gained in this book, which may be designated as the Junior Course, will find practical application in the recognition of official vegetable drugs, the detection of adulterations, the study of ground drugs and their adulterations. This branch of study is called Pharmacognosy and is daily increasing in importance.

The opening pages deal with a few physical principles and a description and explanation of the action of a compound microscope, its defects, the requirements of a good instrument, etc. The author is well aware that it is possible for one to work with a microscope without understanding its action, just as a man may know how to start and stop an engine without knowing anything of its mechanism and theory of action. But it needs no argument to convince anyone that a student who knows the theory and structure of a microscope is far better

equipped than one who merely knows how to bring an object into focus and look at it. For this reason, the subject-matter in the beginning of the book was introduced.

The matter contained in these pages is the outcome of the author's needs in his classes. No one book was found entirely satisfactory. All contained valuable material, but lacked other matters which were desirable. Some were too extensive for use in an elementary course. In preparing these pages, the author consulted a number of books, to which he desires to acknowledge his indebtedness. The books consulted were: *Encyclopædia Britannica;* Ganot's *Physics;* Behrens' *Botanische Mikroskopie;* Bessey's *Botany;* Goodale's *Physiological Botany;* Bastin's *College Botany* and *Laboratory Exercises;* Bower's *Practical Botany;* Huxley and Martin, *Practical Biology;* Gray's *Lessons in Botany.*

SEPTEMBER, 1897.

PREFACE TO THE SECOND EDITION

While the number of chapters has not been increased in this edition, considerable new matter has been added in several of the exercises, and in some instances better directions for work have been given. The chapter on stains, section cutting, mounting sections, etc., has been placed at the end as an appendix. Better illustrations and some new ones have been provided, and these have been introduced into the respective chapters instead of being grouped at the end of the book, as in the first edition. Whenever a cut has been taken from the works of other authors, I have made acknowledgment in the cut. It is hoped that in its improved form the book will meet with the approval of those into whose hands it may fall.

SEPTEMBER, 1905.

CONTENTS

THE MICROSCOPE

CHAPTER I.

VEGETABLE HISTOLOGY

CHAPTER II.

CHAPTER III.

ELEMENTS OF
VEGETABLE HISTOLOGY

THE MICROSCOPE

CHAPTER I.

Preliminary Considerations.

Before describing the mechanical and optical parts of a compound microscope, it is essential to know something about the action of the transparent bodies, as prisms, lenses, etc., on light rays—how a lens forms an image of an object, and how the image is magnified, etc.; in other words, a little of the elementary physics of light.

Light travels through homogeneous, transparent media, as air, water, glass, in straight lines, and a very narrow cylinder of light is called a *ray,* or better, a *pencil* of light. Rays are represented in geometric illustrations by straight lines. The fact that light travels in straight paths may easily be shown by admitting a small beam through a hole in a shutter into a darkened room in which dust particles are floating around. The illuminated particles will be seen to lie in a straight line. The formation of sharp shadows by obstacles in the path of light is another evidence that light travels in straight lines. When the rays come from a distant source, as the sun, moon, stars, a distant flame, they are practically parallel, and a beam of such light is spoken of as parallel light or beam.

A convergent beam of light is one in which the rays come together in a point or focus.

A divergent beam of light is one in which the rays emanate from a point or focus.

Mirrors.—When light falls upon bodies, it is in general reflected. If the surface be rough, the rays will be reflected in every conceivable direction, each point of it becoming, as it were, a new source of light. It is for this reason that rough bodies are seen, and also from any position. When the surface of a body is smooth, the light rays falling upon it are reflected in a definite direction according to fixed laws, and such surfaces are called *mirrors.* "Smoothness" is a relative term, but with reference to light rays, it means that there are no unevennesses which are at all comparable in size with the wave-length of the waves of light, which is very small. When the surface is plane,

it is called a *plane mirror,* and when spherical, it is called a *spherical mirror.* These are the commonest forms, and both are used on microscopes for illuminating objects examined.

PLANE MIRROR.—The manner in which this reflects light is illustrated in Fig. 1. AB is a vertical section of the mirror, CD is a perpendicular to the surface, OD is an incident ray, and the angle ODC is the angle of incidence, DP is the reflected ray and CDP is the angle of reflection. It has been shown experimentally, as well as deduced theoretically from the wave theory of light, that in a plane mirror the reflection follows a fixed law, namely, that the angle of reflection is always equal to the angle of incidence; that is, the angle CDP is always equal to the angle ODC.

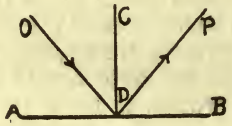


Fig. 1.

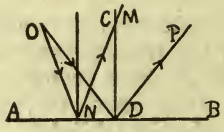


Fig. 2.

What is true for one ray, as illustrated in Fig. 1, is true for any number of rays. Hence if a beam of parallel rays of light be reflected from a plane mirror, the angles of reflection of the rays will all be equal, consequently the reflected beam will consist of parallel rays. Similarly, by geometrical construction based on the above law, it may be shown that divergent rays, after reflection, remain divergent to the same degree as before reflection. This is shown in Fig. 2. It follows, therefore, that the only function of a plane mirror is to change the path of light that falls upon it. For example, on the microscope it causes a beam of light that is received from a window to be thrown up vertically through the object and the lenses.

SPHERICAL MIRROR.—As indicated by the name, the reflecting surface of this mirror is part of a sphere, and may be either convex or concave, but only the latter is of interest in connection with the microscope.

In Fig. 3, ABD is a section through the middle point B of the spherical surface, C is the center of curvature. The line GCB through the center, C, and the middle point, B, of the mirror, is called the axis. Applying the law of reflection, it may be shown by geometric construction that, in the case of a spherical surface, any ray AO, parallel to the axis BCG, will be reflected to a point F on the axis. This point is called the principal focus and lies half-way between C and B. It will be seen that a beam of light, consisting of

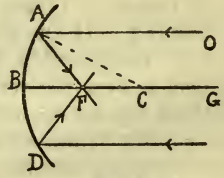


Fig. 3.

rays parallel to the axis, is concentrated or converged to the focus, and hence the illumination at the focus is greatly intensified. If a beam of parallel rays fall upon the mirror at an angle to the axis, it will also be converged to a focus, which, however, does not lie on the axis BCG.

Fig. 4 shows the direction of reflection in this case. This is the condition that obtains when the concave spherical mirror is used on a microscope. It has the additional property, as compared with a plane mirror, of concentrating light at the same time that it changes its direction, thus producing a much stronger illumination of objects to be examined.

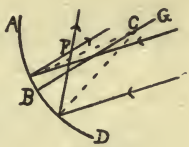


Fig. 4.

REFRACTION.—It was noted above that a light ray travels in a straight line. This is true only when the medium remains the same. Light passing from one medium into a different medium is bent out of its course, still moving, however, in a straight path in the second medium, but in a different direction from that in the first. The bending of light rays is known as *Refraction,* and the action of microscopes depends on this important property.

Fig. 5 illustrates the principle of refraction. CD is an incident ray of light passing from air into glass at the surface of separation ADB; DP is the refracted ray, NDS is a perpendicular to the surface of the glass, NDC ($=a$) is the angle of incidence, PDS ($=b$) is the angle of refraction, and HDP is the angle through which the ray has been deviated from its original path, CDH. The angle of refraction, b, for a given angle of incidence, a, in air, varies for different media, as glass, water, glycerin, etc., hence the amount of deviation of the refracted ray from the original path is variable for different media. A ray passing from air (which is usually taken as the standard of reference) to a denser medium is always bent toward the perpendicular NDS; that is, the angle of incidence is greater than the angle of refraction. The incident and refracted rays, and the perpendicular to the surface, lie in the same plane. In any medium, for example glass, the size of the angle of refraction varies with the angle of incidence, which means that the direction a ray will take after refraction depends on the slant with which the incident ray meets the refracting medium. There is, however, a constant relation between the angles of incidence and refraction which

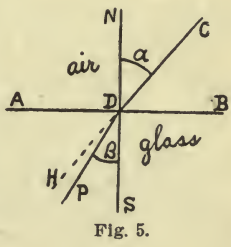


Fig. 5.

is expressed by the Law of Refraction. The law is expressed mathematically thus:

$$\frac{\text{sine of angle of incidence}}{\text{sine of angle of refraction}} = \text{n, a constant.}$$

This ratio, n, is called the *index of refraction,* and is different for the various transparent substances, but fixed for each one. It may be accurately determined by physical experiments, and is usually taken with reference to air as standard. There is only one position in which light is not bent out of its path in passing from air into any other medium, namely, when the light rays are perpedicular to the surface of separation of the two media. The index of refraction of water and glass are n = 1.33 water, n = 1.52 glass (crown). Substances for which n is greater than unity are said to be more refracting than air. The refractive index has reference to light of one color only, i. e., to monochromatic light. The yellow light obtained by holding salt in a Bunsen flame is usually employed.

When light passes from a denser medium into air the figure and discussion is just the reverse of the above. A familiar illustration of refraction is shown by dipping a stick obliquely in water, when it will appear bent. The well-known fact that an object under water is not in the place where it seems to be is also explained by refraction.

REFRACTION BY A PRISM.—In optics a prism is any transparent medium comprised between two plane faces inclined to each other. The intersection of the two plane faces is called the *edge* and their inclination is called the *refracting angle.* Triangular glass prisms are generally used. Every section perpendicular to the edge is called a *principal section,* which is a triangle in the case of a triangular prism. Fig. 6 represents such a section, A is called the summit and BC the base of the triangle. Let OI be a ray of light falling upon the prism in the plane of the section ABC. It will be refracted twice according to the law of refraction, first as it enters the prism at I, then as it leaves the prism at D, so that the direction of the ray after emerging from the prism will be DS, instead of the original direction OIP. The amount of this deviation depends on the size of the angle of the prism, its material and the angle of incidence of the ray. The angle PRS measures the deviation of the refracted ray from the original path and is called the *angle of deviation.* It is seen that a ray of light is deflected towards the base of the section ABC, hence its source O appears to be elevated or deflected towards the summit A.

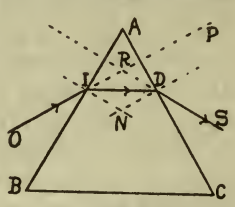


Fig. 6.

Light rays of different colors are bent by different amounts, since the refractive indices for the various colors of light are different. White light is a combination of numerous colors, and if a beam of sunlight falls upon a prism it does not come through as white light, but the constituent colors are refracted by different amounts, giving rise to a band of light containing all the colors of the rainbow, namely, red, orange, yellow, green, blue, indigo, violet, red being least refracted, violet most. Such a band of colors is known as a spectrum. An instrument has been constructed for conveniently observing the spectrum of white light which is known as the *Spectroscope.* It has proved to be of the greatest value in chemical analysis. Many new elements were discovered by its aid, for example, calcium, rubidium, thallium, indium, gallium and others.

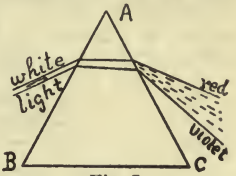


Fig. 7.

The separation of the various colors, due to the unequal refrangibility of the differently colored rays, is known as *dispersion.* We shall speak again of the unequal refrangibility of differently colored rays in connection with lenses and one of their defects, known as chromatic aberration.

LENSES.—A lens is a piece of transparent substance bounded by curved surfaces (one surface may be plane), and, according to the curvature, it may be spherical, cylindrical, elliptical or parabolic. Lenses used in optics are always *spherical* or approximately so. They are usually made either of crown glass, which is free from lead, or of flint glass, which contains lead, and is more refractive than crown glass. In virtue of the spherical surfaces, a lens has the property of causing rays of light which traverse it either to converge or diverge. There are six types of lenses according to the manner of combining concave, convex and plane surfaces. These are represented in section in Fig. 8.

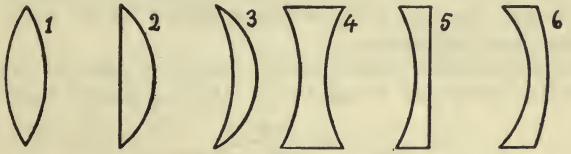


Fig. 8.

They are named, in the order of the numerals, *double convex, plano-convex, converging concavo-convex, double concave, plano-concave, diverging concavo-convex.*

Lenses 3 and 6 are also called *meniscus* lenses, from the resemblance to the crescent-shaped moon. The first three are thicker in the middle than on the edges and have the power of

converging light rays, the last three are thinner in the middle than on the edges and diverge light rays; the first are called *converging* lenses, the latter *diverging* lenses.

For our purpose it will suffice to consider the properties of the two simplest lenses of the series, namely, the biconvex and the biconcave, as the general behavior of the others of the same class is the same.

BICONVEX LENS.—Fig. 9 represents a section through the center of a biconvex lens. The faces ADB and ANB are spherical and their centers of curvature are C and C′ respectively,

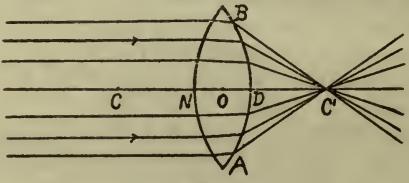


Fig. 9.

which may or may not be equally distant from the center O of the lens. Usually they are equally distant. The line joining C and C′ is called the *optical* or *principal axis.*

If a beam of parallel rays fall upon the lens parallel to the principal axis the rays will be converged to a point called the *principal focus,* which, for a lens of crown glass having surfaces of equal or nearly equal curvatures, coincides very nearly with the center of curvature C′. Of course, there are two focal points, one on each side of the lens, equally distant from the center. This distance C′O is called the *focal length* of the lens, and it varies with the index of refraction of the glass and the radius of curvature of the faces. The shorter the radius of curvature, i. e., the thicker the lens, the shorter is the focal length.

Conversely to the above case, if a divergent beam of rays, emanating from a source placed at the principal focus, fall upon the lens, the rays will emerge parallel to the axis. Fig. 9 illustrates this condition.

If divergent rays emanate from a point on the axis anywhere between the principal focus and a distant point, they will be

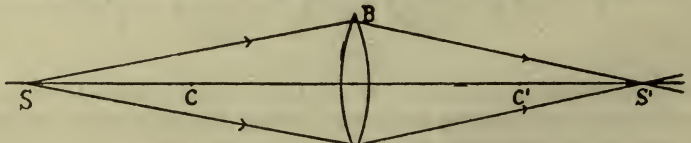


Fig. 10.

converged after passing through the lens to a point on the axis, as shown in Fig. 10.

There are any number of such related points, and they are called *conjugate foci.* If the source of light S be moved away from the lens the focus S′ will approach the lens until it reaches the principal focus C, which happens when S is at a very great distance. On the other hand, if the rays diverge from a point

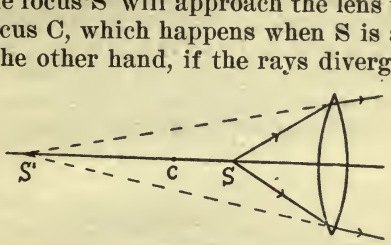


Fig. 11.

between the principal focus C and the lens, they will still diverge after emerging from the lens, but less so than before, and hence will have no real focal point, but will seem to come from a point S′ behind the lens, as shown in Fig. 11. This apparent focus is known as a *virtual* or *imaginary focus.*

BICONCAVE LENS.—This is just the reverse of the biconvex lens, the spherical surfaces being turned inward, and rays of light diverged or scattered. It has two radii of curvature and a principal axis. Rays of light parallel to the axis are diverged, but if the rays were prolonged backward they would meet in a point or focus, as shown in Fig. 12. The lens has never a real focus, but only an imaginary one. The focus C corresponding to rays parallel to the axis is called the *principal imaginary focus.*

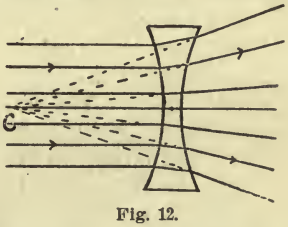


Fig. 12.

If rays diverging from any point on the axis fall upon the lens, after emerging they will be still more divergent, and will seem to emanate from a point between the principal focus and

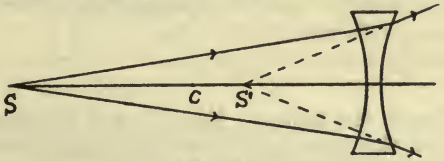


Fig. 13.

the lens. As in the case of a biconvex lens, there are any number of such reciprocally related *conjugate* foci. Fig. 13 represents two such points, S and S′.

The focus S′ approaches the lens more and more as the source of light S is brought nearer to the lens, and vice versa.

FORMATION OF AN IMAGE. (1) By double convex lens.—Without describing the geometric construction, the following is the fact: That when a small object is placed near the principal focus but a little distance in front of it, the image formed is at a great distance, is inverted and much larger, and that in proportion as the object is nearer the principal focus. This is shown in Fig. 14, where the arrow A represents a bright body and the arrow B its inverted image, much larger and at a great

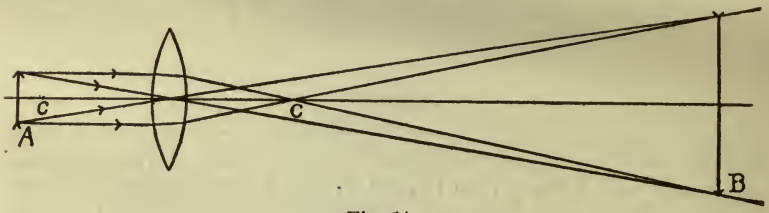


Fig. 14.

distance. C is the focus, the body being a little beyond it. The rays of light coming from every point of A are converged by the lens to a corresponding point in the image B, and the latter is real and can be caught upon a screen held at B. The figure represents what takes place in a compound microscope, as will be shown later. The object and image have the same proportion as their distances from the lens.

When the object is very near the focus, but between it and the lens, the rays from the various points of the object are not converged to a corresponding point as in the previous case, but pass through the lens still diverging, but less so than before, and in such a way that if they were prolonged backward they would meet and form an image behind the object on the same

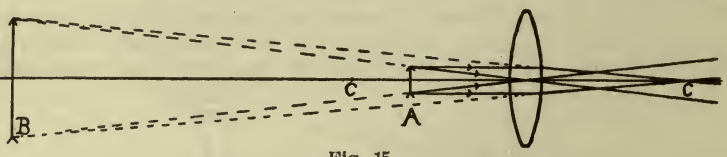


Fig. 15.

side of the lens as the latter. Fig. 15 will illustrate. An eye held in front of the lens would see the light coming from the object A as if it came from B, and B, therefore, is the image of A, erect, larger, but *unreal* or *imaginary*.

A lens used in this manner to form, with the help of the eye, an erect magnified image, constitutes a *simple microscope* or *magnifier*. In both cases considered, the magnification is greater according as the object is nearer the focus and the focal length is decreased, i. e., the lens is more convex. Magnification

may also be increased by combining two or three lenses into a "system."

There is a third position which the object may occupy, namely, the principal focus. In this case no image at all is formed, because rays diverging from any point of the object emerge from the lens parallel to one another, and, therefore, have no focus or image. The effects produced by a lens for the three positions described may readily be verified by slowly moving a lens, lying on a printed page, vertically upward, when the letters will first appear erect, then vanish and finally return in an inverted position.

(2) By double concave lens.—No real image is ever formed by a concave lens, because it never forms a real focus; it is always virtual, erect, smaller than the object and nearer the

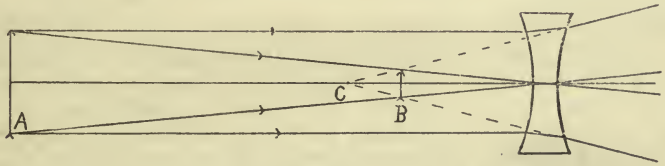


Fig. 16.

lens than the object. If A be an object in Fig. 16, the rays of light coming from any point of it will emerge from the lens more divergent than before, and will appear to the eye to come from a point nearer to the lens and to the axis as shown.

Concave lenses are not used in the microscope except in combination with convex lenses in some "objectives."

Spherical and Chromatic Aberration.—These are two serious inherent defects in all simple lenses. They are detrimental to the formation of a perfect image of an object and must be approximately overcome in compound microscopes if these are to be of any value at all.

Cause of Spherical Aberration.—It was said above that parallel rays of light falling upon a double convex lens are converged to a point, but this is not quite true. The rays falling on the edge of the lens are brought to a focus nearer to the lens than those rays falling near the center of the lens, so that the rays, instead of coming together in a point, are focused over a small circle. The diagram (Fig. 17) will illustrate.

The rays around the axis of the lens will meet in a focus C, while those near the edge will meet in C′, and a screen placed at C will not receive a mere point of light, as would be the case if the lens were perfect, but a small circle of light. The result of this is that the image of any object is not sharply defined, but is somewhat blurred in such a manner that when the center of the image is sharp the edge is indistinct, and when the edges are sharp the center is indistinct. This defect is due to the spherical nature of the lens, hence its name. As the edge rays are most effective in causing this aberration, the latter can be greatly corrected by cutting out the edge rays by means of a *diaphragm* or perforated disc placed in front of the lens. This is done in objectives and eye pieces of compound microscopes.

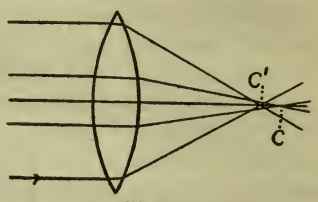


Fig. 17.

Mathematical calculation has shown that spherical aberration is greatly reduced when the radii of curvature of a lens bear a certain ratio to each other, namely, 6:1, the face with longer radius being turned towards the object. Aberration is also corrected in part by combining several lenses of suitable curvatures into a system, the lens next the object being plano-convex, with the plane face towards the object. (Absolute correction for spherical aberration is impossible.)

CAUSE OF CHROMATIC ABERRATION.—We have seen that rays of different colors have different indices of refraction, i. e., unequal refrangibilities, so that if white light is passed through a prism the constituent colors are separated by it into a spectrum. A lens acts like a prism in this respect; in fact, it may roughly be considered as two prisms with their bases together. There is a different focus for each of the seven different colors composing white light; violet being most refracted, is focused nearest the lens, while red, being least refracted, is focused farthest from the lens (Fig. 18). The red rays will meet at R, the violet ones at V, and the other colors at points intermediate, in the order, orange, yellow, green, blue, indigo. The result of this defect is that the image of an object is bordered by a color fringe instead of being perfectly colorless, as it should be. Chromatic aberration is more perceptible in proportion as the lenses are more convex, i. e., as the magnifying power increases. It is corrected by combining lenses made from crown and flint glass. The refractive indices of these are very nearly the same, being 1.751 for flint and 1.53 for crown, but the power to separate the colors of white light is nearly twice as great for flint as for crown glass. Hence a biconcave or plano-concave flint glass may be so combined with a biconvex

crown glass that the dispersion of one is corrected or compensated by that of the other, while the two still act like a double convex lens in magnifying the object (Fig. 19). Chromatic aberration cannot be corrected absolutely; there will always be a little color, but it may be so little that the image is practically colorless. For optical purposes the blue and orange are corrected or combined. If the image is bordered by a light blue fringe, the lens is said to be *overcorrected*; if by a reddish one, it is *undercorrected*.

A lens free from chromatic aberration is called *Achromatic*, and one free from both spherical and chromatic aberrations is called *Aplanatic*.

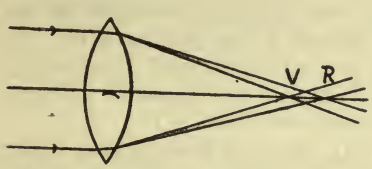


Fig. 18.

Fig. 19.
a—crown lens.
b—flint lens.

SIMPLE MICROSCOPE.—This is nothing but a convex lens used as a magnifier as described under double convex lens. There may be one lens or several combined into a system and mounted in a suitable stand. Corrected lenses and diaphragms may be used to get rid of spherical and chromatic aberrations. A good example of a simple microscope is a reading glass or a watchmaker's magnifier.

CONDITION OF DISTINCTNESS OF THE IMAGE.—There is for each person a *distance of most distinct vision*, a distance at which an object must be placed before the eye to be seen with greatest distinctness. This distance is, for the average eye, between 12 and 14 inches. It differs for different observers, and the two extremes are found in near and far-sighted persons. When an object is looked at through a lens the latter must be moved back and forth until the image is formed at the particular observer's distance of distinct vision, and this operation is called *focusing*. This explains why two persons looking through a microscope will have quite different foci, since the two eyes have different distances of distinct vision.

The human eye is, in one respect, an optical instrument, because it consists of a combination of lenses which focus upon the retina light rays coming from illuminated objects. The eye has but little spherical aberration, owing to its peculiar shape and to the action of the iris, which takes the place of a diaphragm; but it does have considerable chromatic aberration. Most eyes have power of *accommodation*, that is, of altering

their focal length at will so as to perceive objects at different distances away. There are, however, several possible optical defects in eyes, which may arise from various causes:

1. The rays may be brought to a focus in front of the retina instead of on it. Such eyes are called *near-sighted,* and may be helped by the use of diverging lenses, which cause the rays to become less divergent in the eye and thus to meet in a focus farther back on the retina.

2. The rays may be focused back of the retina. Such eyes are called far-sighted, and may be helped by the use of converging lenses, which act in a manner oposite to that stated for diverging lenses in 1.

3. The focus may be different for different sections of the eye. If the dial of a clock be looked at an eye may see the figures 2 and 8 clearly, but may not see the 5 and 11 sharply. Such eyes are called *astigmatic,* and may be helped by the use of cylindrical lenses. (Ames' Theory of Physics.)

MEASURE OF MAGNIFICATION IN A SIMPLE MICROSCOPE.—The apparent magnitude of an object is the angle it subtends at the eye of the observer. In the case of two objects seen at the same distance, the ratio of the apparent diameters is the same as that of their absolute magnitude. Hence, in a simple microscope (also in a compound one), the magnification is equal to the ratio of the apparent diameter of the image to that of the object,

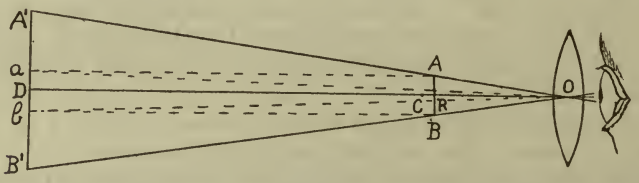


Fig. 20.

both being at the distance of most distinct vision. But as the apparent diameters are not easy to measure, a simpler method is used which gives an approximate measurement (Fig. 20). AB is an object and A'B' its image, formed at the distance of distinct vision for the eye E. Since the eye is always very close to the lens, the angles subtended by the object and image may be taken as A'OB' and aOb, and the magnification $= \frac{A'OB'}{aOb}$. This is approximately equal to $\frac{A'B'}{ab} = \frac{A'B'}{AB}$, and by similar triangles, $\frac{A'B'}{AB} = \frac{DO\ (\text{dist. of distinct vision})}{RO\ (\text{dist. of object from lens})} = \frac{12 \text{ to } 14 \text{ inches}}{CO\ (\text{focal length})}$, since the object is very nearly at the focus. Hence, magnification by convex lens = ratio of distance of distinct vision (say

13 inches as average) to the focal length of the lens. It will be seen that magnification is greater as the focal length is smaller and as the observer's distance of distinct vision is greater.

COMPOUND MICROSCOPE

The simplest form would consist of two simple microscopes or magnifiers, one with short focus, placed near the object, called *the objective,* the other with longer focus, placed next the eye, and called *the eye-piece* or power. The objective forms an inverted real image of the object, and, by means of the eye-piece, we see a virtual, erect, magnified image of the real image.

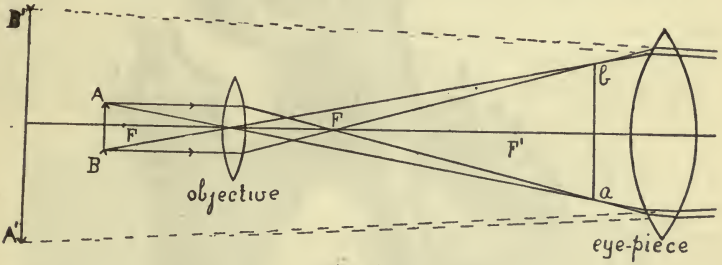


Fig. 21.

Mode of Action.—In Fig. 21, AB is an object; an image is formed at ab, real, inverted and magnified. The eye-piece forms an imaginary, erect, magnified image of ab at A′B′. This is the principle of all compound microscopes. This form would be very defective on account of spherical and chromatic aberrations, and we will now study the more perfect microscope.

Description of Compound Microscope.—Fig. 22, A is the base; B, pillar; C, pillar and arm; D, body; E, nose-piece; F, objective; G, ocular; H, draw-tube; I, collar; J, rack and pinion; K, coarse adjustment; L, fine adjustment; N, spring clips; O, mirror; P, mirror bar; Q, diaphragm and substage; R, substage screw; S, stage; T, pillar hinge-joint.

Only a few words need be said about the mechanical parts of the instrument, as the figure will explain sufficiently.

By the rack and pinion movement K, the body D is given a large up-and-down motion and a body is quickly brought into rough focus. Then by the micrometer screw L the fine adjustment is made, a very small motion of the body D being produced by one turn of the screw.

The draw tube H carries a scale so that any tube length can be obtained by pulling out or pushing in. The ocular G slips

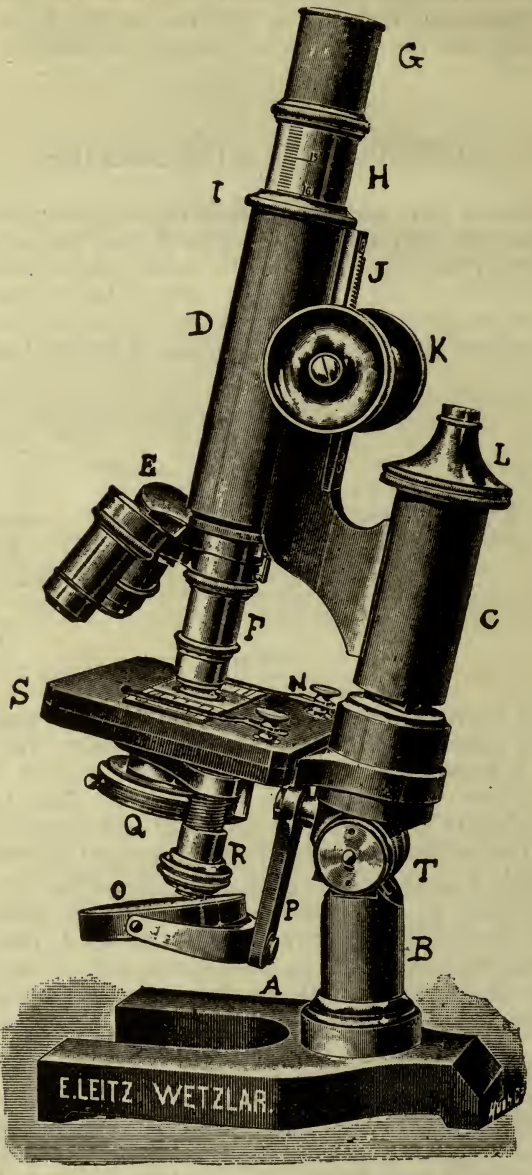
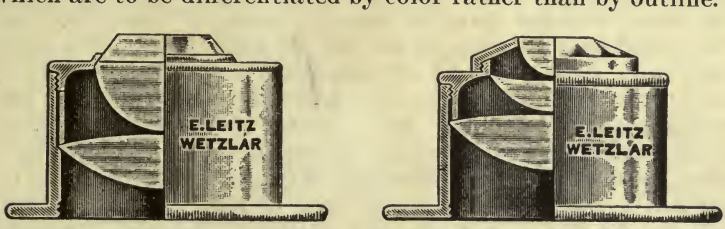


Fig. 22.

into the end of the tube H. The triple nose-piece E is a convenience for sliding one or the other objective into place as desired. The stage is perforated in the center for transmitting light, reflected up by the mirror O. In the opening there may be fitted little cylinders with smaller openings, known as diaphragms, the object of which is to regulate the amount of light. There is a series of three or four of these. On the stage are two clips for holding a glass slide, on which the object is examined.

The iris diaphragm Q is much more convenient than the cylinder diaphragms, as the opening can be made gradually larger or smaller by simply turning a small lever back or forth.

If a greater concentration of light is desired than is produced by the concave mirror O, a condenser is used, which is placed in position beneath the stage. The best form is the *Abbe type* (Fig. 23), consisting of one lens or a system of lenses for converging a large beam of light. The condenser is used for great magnification and is invaluable in studying stained specimens, which are to be differentiated by color rather than by outline.



Condenser of 1.20 num. apert. Condenser of 1.40 num. apert.

Fig. 23.

ILLUMINATION.—No fixed rule can be laid down in regard to the size of opening in the diaphragm to be used for any given magnification, as the amount of light to be passed through a specimen depends somewhat on its nature and thickness. As a general rule, large diaphragms are used for low powers, with weaker illumination and small ones for high powers with strong illumination. Weak illumination is brought about by the plane mirror, stronger by the concave mirror and the use of a condenser if desirable. Actual laboratory practice is better than many words in teaching the student what is the best illumination of an object.

OBJECTIVES.—The objectives are the most important parts of the whole microscope. Instead of one lens they consist of a system of two, three or four lenses, some of which are simple, others compounded of a convex crown lens and a concave flint lens, as described under chromatic aberration. The front lens of the system always has a plane face which is turned towards the object, and is usually a simple lens (plano-convex). Such

a system of lenses is almost free from aberration defects. According to the method adopted by the maker, objectives are designated by letters, as A, B, C, etc., or by numbers, as 1, 2, 3, etc., or by figures which represent focal lengths. In the latter method, which is the most rational, if an objective is marked, say 1 inch or $^2/_3$ inch, this means that its magnifying power is the same as that of a simple lens whose focal length is 1 inch or $^2/_3$ inch. In order to know which is high or low power, the student should remember this rule: *The smaller the number or fraction representing the focal length of an objective, the greater is its magnifying power.* The same rule applies to oculars or eye-pieces. The objectives mostly used in vegetable histology are those of 1 inch, $^2/_3$ inch and $^1/_6$ inch focal length. The distance between the front lens of the objective and the object when in focus is about equal to the focal length of the objective, and is known as the *working distance.*

Objectives are either *dry* lenses or *immersion* lenses. If, as is usually the case, there is an air space between the objective and the object, the lens is called a *dry* one; if a liquid is between the object and the lens, the lens is called an *immersion lens.* The liquid may be water or an oil. If water, we have a *water immersion* lens; if oil, an *oil immersion* lens. If the index of refraction of the oil is about the same as that of glass, we have a *homogeneous immersion* lens. Cedar oil thickened by evaporation is an example of such. Lenses intended for immersion must be constructed accordingly. The great advantage of immersion is that the angle of the cone of light that can be utilized by the lens is considerably increased, thereby increasing the illumination and the efficiency of the microscope.

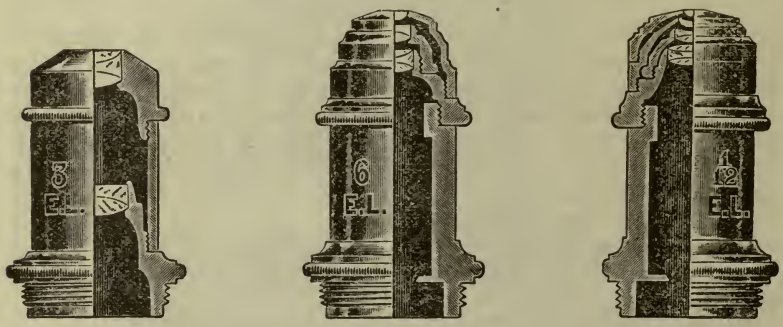


Fig. 24.

Fig. 24 represents the construction of two dry objectives (3 and 6) and an oil immersion objective ($^1/_{12}$ inch focal length). Piece 3 has ¾ inch focal length and consists of two compound lenses or doublets, in which concave and convex lenses are combined. Piece 6 has one single lens and two doublets and a focal

length of $^1/_6$ inch. The $^1/_{12}$ inch objective has two single lenses and two doublets. The compound lenses in the pieces serve the purpose of correcting aberration defects. The objectives are achromatic.

ANGULAR APERTURE OF A LENS.—The efficiency of an objective is in great part dependent upon the size of the cone of light it can take in from a point of the object to form its image. The cone of light utilized is approximately measured by the so-called angular aperture. For a single lens this is the angle formed by lines joining the focal point with the edges of the lens. In an objective it is the angle formed by the lines from the focal point to the edges of the uppermost lens of the system. More accurately, the cone of light utilized is measured by the product of the index of refraction of the medium between the objective and the cover-glass lying over the object, and the sine of half the angular aperture, which is expressed thus, n. sine u, where n is the index of refraction and u is half the angular aperture. This expression is known as the *numerical aperture.* In the case of dry objectives n is one (index of refraction of air); for water-immersion objectives n is 1.33; for cedar-oil immersion objectives n is 1.52. It will be seen that the cone of light that can be utilized by an objective of a given angular aperture is considerably greater for an immersion lens than for a dry lens. As the angular aperture varies inversely as the focal length, it follows that the shorter the focal length is the greater is the cone of light that the lens can utilize; in other words, lenses of high magnification can utilize a greater cone of light than those of low magnification.

OCULARS OR EYE-PIECES.—The Huyghens' eye-piece is universally used. It is known as a *negative* eye-piece. Its construction is shown in Fig. 25, which consists of two plano-convex crown lenses, the lower one being the larger, less magnifying (its focal length being three times that of the upper lens). It is known as the *field lens.* It increases the *field of vision,* i. e., the number of points of the object that are made visible through the instrument. The upper lens is known as the *eye lens.* It magnifies the image formed by the objective. Both lenses have their convex surfaces turned towards the object. Midway between them is a perforated diaphragm, the object of which is to cut out edge rays from the image and thus decrease spherical aberration. The virtues of the Huyghens' eye-piece are that it corrects chromatic aberration, enlarges the field of vision and forms a flat image, i. e.,

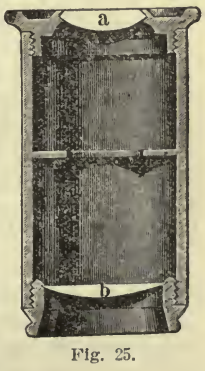


Fig. 25.

all points of the image are in focus at the same time. This latter quality is essential in all good microscopes. In all negative eye-pieces the image of the object is formed between the two lenses, and is then further magnified by the *eye lens.* The lenses taking part in the formation of the first image are, therefore, the objective and the field lens of the eye-piece. In *positive eye-pieces* the first image is formed below the field lens, i. e., the ocular takes no part in its formation. An example of such is Ramsden's eye-piece.

Eye-pieces are designated by methods like those stated for objectives. Those most commonly used have focal lengths of 2 inches, 1½ inches and 1 inch.

There is a particular order that should be observed in changing the lenses in passing from a low power to a high one. For example, there are two objectives, $^2/_3$ and $^1/_8$ inch, and two eye-pieces, 2 and 1 inch. The following is the best order for changing these:

Objective.	Eye-piece.
$^2/_3$ inch	2 inch—Low power.
$^1/_8$ inch	2 inch—Medium power.
$^1/_8$ inch	1 inch—High power.

In other words, it is better to increase magnification at the objective end than at the eye-piece end of the microscope. The reason for this is that the eye-piece magnifies any defects of the objective. In good instruments a high objective is not likely to have more defects than a low one, hence in increasing magnification by changing to a high objective the image will have no more defects than before, although much more magnified.

As the magnifying power is increased the field of view becomes smaller, illumination of the image decreases and the image is increased in size.

TUBE LENGTH.—Magnification may also be increased by drawing out the inner tube, which increases the distance between the objective and the plane of the real image formed in the barrel of the instrument, and, consequently, the size of the image. The tube length, however, should be kept constant, because the objectives are prepared to suit a definite tube length, which is sometimes fixed at 160 mm. (6.3 inches), sometimes at 216 mm. (8½ inches). The scale on the inner tube regulates the length.

CAMERA LUCIDA.—This is a drawing apparatus which is attached to the eye-piece, and is used whenever it is desired to make accurate delineations of the object. By means of it, a white surface of paper, on the table alongside of the instrument, is reflected into the eye while it receives the image, and thus a pencil point can be traced on the paper along the lines of the image, giving an accurate drawing. The Abbe camera

is the best form in the market at the present time. In drawing, the microscope must be erect and the paper horizontal and at the distance of distinct vision, about 12 inches.

Determination of Magnification in a Microscope.—The magnifying power for certain combinations of objectives and eye-pieces and tube length is usually stated by the makers, so that it is hardly necessary now to determine the magnifying power. But sometimes the rating of the makers is not correct and we might want to use a different tube length, and, again, the distance of most distinct vision for our eyes might not be the average distance, namely, 12-14 inches, in which case a new determination must be made, which may be done in the following manner:

A Stage Micrometer—a piece of glass, accurately ruled to hundredths of a millimeter—is placed on the stage and brought into focus. By means of a camera lucida, the magnified scale and an accurate mm. scale, placed at the distance of distinct vision alongside the microscope and parallel with the micrometer scale, are brought into superposition. The number of mm. divisions covered by a definite number of the micrometer scale divisions is then noted. Suppose each magnified scale division covers 5 mm. of the rule, what is the magnifying power? One mm. is equal to 100 micrometer divisions, 5 mm. covered by one micrometer space are equal to 500 micrometer divisions. Hence one micrometer division has been magnified so as to cover a space 500 times as wide, i. e., it has been magnified 500 times. This, then, is the power of the instrument for the particular combination.

Source of Light.—The best source of light is a white cloud or the diffused light reflected from a white wall or other white object. *Never* use direct sunlight. Light from the blue sky is not so good as that from a white surface. There is a tendency among beginners to use the strongest light possible. This is injurious to the eyes and often obscures details of the object by its dazzling glare. A window facing north is best.

Requisites of a Good Microscope.—It goes without saying that the best workmanship must be found in the mechanical parts. The foot, pillar, arm, stage, etc., must be of sufficient weight and strength and size. For the optical parts five points must be considered:

1. Working Distance.—This is the distance between the front lens of the objective and the object. The lower the magnifying power the larger the working distance in general. Working distance has no fixed relation to the focal length, but varies with the mode of construction and the aperture of the objective. Of two objectives having the same focal length, that one with the larger working distance is to be chosen. As the

power is increased the working distance is decreased. It is often advantageous to gain working distance at the expense of magnification, as the manipulation of objects on the section slide is made easier.

2. PENETRATING POWER OR FOCAL DEPTH.—This is the vertical range through which the parts of an object not precisely in the focal plane may be seen with sufficient distinctness to enable their relations with what lies exactly in that plane to be clearly traced out. It is larger the smaller the magnifying power and numerical aperture are, and vice versa. Of two objectives having the same power, but different working distances, that one will have the more focal depth whose working distance is the greater. It is often desirable to see for a considerable distance into an object. In such cases low power must be used.

3. FLATNESS OF FIELD.—All parts of the image must be in focus at the same time.

4. DEFINING POWER.—The power to form an image in the highest degree sharply defined, and free from color. This quality is governed by the objectives only and depends on accurate centering of the lenses and completenss of correction for spherical and chromatic aberrations. Want of defining power is indicated by blurring of clearly-marked lines or edges and by general fog.

5. RESOLVING POWER.—By which very minute and closely approximated markings, whether lines, striæ, dots or apertures can be separately discerned. This power varies directly as the aperture of the objective. High powers have the greatest resolving power.

CARE OF THE MICROSCOPE.—The stand should *never* be wetted with such substances as alcohol, soap, etc., which dissolve lacquer. If it be necessary to clean the stand, moisten with water and dry with an old linen rag, rubbing with the grain of the brass. Never examine objects lying in acids or alkalies or other chemicals without putting on a "cover" glass. If liquid happens to get on the objective, rinse off at once with water and dry with an old linen rag or Japanese filter paper.

Be careful not to force the lens down on the cover glass. Exercise great care in putting objectives and eye-pieces on or off, lest they be dropped and injured.

DIRECTIONS FOR USING THE MICROSCOPE.

1. The instrument should be placed directly in front of the observer, with the pillar facing backward. Wipe the mirror with a soft rag and turn it so that a beam of light is thrown up through the diaphragm. *All work should be begun with the*

low-power objective. The body of the microscope should be about vertical, so as not to interfere with mounting in fluid media.

2. The object mounted on a glass "slide" in a suitable liquid and covered with a "cover glass" is brought to the center of the diaphragm and focused by means of the coarse adjustment in the following manner: Using the left thumb and forefinger to adjust the slide, with the right hand the objective is brought down so that it all but touches the cover glass, then, while looking through the eye-piece, slowly raise the tube by the coarse adjustment until the object is in view; from this point the exact focus can be made by turning the fine adjustment screw.

3. Never lift the slide from the stage, but, having raised the objective, especially in case of high powers, slide it off the stage without upward movement.

4. Accustom yourself to use both eyes indifferently and always keep *both* eyes open. It is preferable to observe with the left eye, as it is more convenient in making drawings.

5. To mount an object, place it in the center of a slide in a drop of liquid, say water,; rest a cover glass on its edge near the object in a slanting position and gradually lower it by means of a teasing needle or forceps, in order to avoid entrapping air bubbles. The cover glass should be previously cleaned with a soft rag or lens paper and then handled by the forceps only. Any superfluous water on the slide is taken up by a camel's hair brush or blotting paper.

6. Cleanliness should characterize all the work of the microscopical laboratory. All apparatus, slides, cover glasses, etc., should be kept scrupulously free from dirt. The glasses of the objectives and eye-pieces should never be touched with the fingers. Whenever they need cleaning, breathe upon them and wipe with a soft, clean linen rag or a piece of Japanese filter paper.

7. All objects observed should be drawn. Drawings are useful, not only in explaining to others the structures observed, but they are themselves great aids also to accurate observation, and are equally helpful in giving vividness and permanency to knowledge.

Each student should provide himself with a drawing book and a medium pencil. It is excellent practice to keep a record in writing of work done in the laboratory, besides making drawings.

SOME ACCESSORY APPARATUS NECESSARY IN HISTOLOGICAL WORK.

1. Micrometer, preferably metric scale. Convenient scale is hundredths of a millimeter.

2. Section razor, flat on one side, slightly hollow on the

other, for making thin "sections" or slices of bodies; also a hone and a strop.

3. A graduated ruler, having both English and metric scale.
4. Dissecting needles.
5. Sharp-pointed scissors preferably bent.
6. Delicate forceps or pincettes.
7. Watch glasses for holding sections.
8. Small porcelain evaporating dish.
9. Camel's hair brushes, assorted sizes.
10. Glass section slides, 3″x1″, not too thick, ground edges.
11. Cover glasses, ¾″ circles No. 2.
12. Camera lucida for drawing.
13. Polariscope.
14. Draughtsman's dividers, for drawing.
15. Microtome, for section cutting.
16. Turn-table for ringing sections.
17. Pipettes, glass rods, blotting paper.

Microscopical apparatus may be obtained from any large dealer, as Bausch & Lomb Optical Co., Rochester, N. Y.; Queen & Co., Philadelphia. Cuts of apparatus may be seen in catalogues or in larger works on Microscopy, as Behrens' Botanical Microscopy.

VEGETABLE HISTOLOGY

CHAPTER II.

In order to acquire some familiarity with the manipulation of the microscope before studying vegetable objects, it is well to study some simple things like cotton, silk, wool and linen fibres, and these are chosen because they sometimes occur accidentally on the slide when we are studying other things, and also because of their great practical importance.

Linen, Cotton, Silk, Wool.

Linen.—Scrape a linen thread on a glass slide with a knife blade to a woolly mass, mount a *little* of this on a slide in a drop of water, taking care that the fibres are wetted and no air adheres to them, then cover with a cover glass as described above. *The student should guard against an error that beginners are apt to fall into, namely, putting too much material on the slide.* A very small quantity will suffice. Examine with low power (²/₃″ objective and 2″ eye-piece). Very little will be made out. Some clear, smooth, tangled threads will be seen.

Put on high power. The linen will be seen to consist of long, cylindrical fibres, thickened at intervals into nodes, with a small canal looking like a line running lengthwise of the fibre. At intervals there are faint cross-lines (Fig. 26, B). The walls are faintly striated and rather thick, and the canal, which is uniform in width, may contain granular remains of protoplasm. The faint cross-lines become more prominent when the fibres are mounted in a concentrated aqueous solution of chloral hydrate.

Linen fibres are of vegetable origin, their material is *cellulose*, a substance which is one of the chief materials found in plants. Remove the cover glass, add a drop of a solution of iodine in potassium iodide (see reagents), after a few minutes render the fibres nearly dry by removing the liquid with filter paper, then add a few drops of sulphuric acid (see reagents), replace the cover glass and examine again. The fibres are stained a deep blue color and swollen. This is a characteristic test for cellulose material. Iodine alone does not color it, but the acid acts on it, converting it into a starch-like body called *amyloid*, which stains just like starch itself with iodine. Cellulose and starch belong to the same group of chemical compounds, known as *carbohydrates*.

Mount some of the fibres in ammonio-copper hydroxide solution (see reagents) and note that they swell and dissolve quickly, except a slender thread from the center (contents of the canal). The reagent is one of the few solvents for cellulose.

Linen fibres are the so-called *bast fibres* found in the inner bark of the stem of the Flax plant, Linum usitatissimum.

COTTON.—Mount a little raw cotton or non-absorbent cotton wool in a drop of alcohol; let most of the latter evaporate, then add sufficient water and cover with a glass. With low power, slender, clear fibres, not very different in appearance from linen fibres, will be seen. Some of them are marked by what appears to be constriction. With high power, long, flat bands, which have caved in, often twisted like a corkscrew, at times striated diagonally, will be seen (Fig. 26, C and D). The fibres do not possess cross-lines. They are the long hairs on the seeds of the cotton plant, Gossypium, the hairs being plant cells, consisting at maturity only of cellulose walls which fall together, giving the fibres the appearance of a flat band. The filaments are about 2 cm. ($^4/_5$ inch) long in short staple to 4 cm. ($1\ ^3/_5$ inch) long in long staple cotton, and about 0.02 mm. (0.0008 inch) broad. There is a central canal running through each fibre, much larger than in linen fibres. The fibres respond to tests for cellulose as in case of linen. With ammonio-copper hydroxide solution, when the swelling action is moderated, there often appear constrictions alternating with large swellings. This is due to the cuticle which covers the surface of the fibres.

Linen has no cuticle and does not give the appearance mentioned. The cuticle is insoluble in the reagent, not being cellulose in nature.

WOOL.—Mount some fibres from white woolen yarn in water. With low power the fibres are clear, slightly roughened on the surfaces with faint cross-lines. Under high power the fibres are cylindrical, containing a central axial substance, called the medulla (not present in all hairs or wool). The surface is covered by imbricated scales, like tiles on a roof, giving to the edges of the fibres a barbed appearance (Fig. 26, E). Compare a human hair with wool. If raw wool be examined, globules of fatty matter (wool fat) will be seen adhering to the fibres.

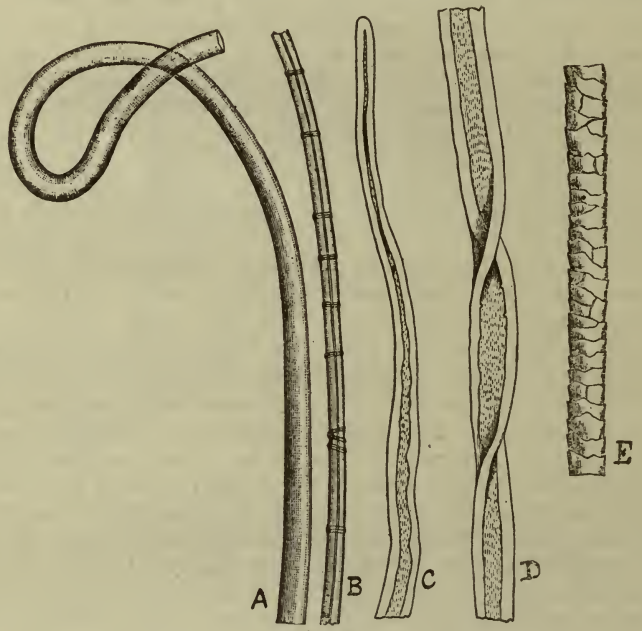


Fig. 26.

Wool being of animal origin does not give the cellulose reactions, as do linen and cotton fibres, but shows proteid reactions. Treated with iodine solution and sulphuric acid, as described under linen, the fibres are stained a deep yellow and do not dissolve even when warmed. When warmed on the slide with a saturated aqueous solution of picric acid the fibres are stained yellow; cellulose does not stain with this reagent. Warmed with ammonio-copper hydroxide solution, wool turns bluish-violet and its structure becomes more distinct, but it does not dissolve or swell.

Wool varies in its details of structure, and the identification

of its source is not always an easy task, being sometimes well-nigh impossible.

SILK.—Scrape some threads, as in case of linen, and mount in water. With both low and high powers the fibres appear about the same—shining, dense, cylindrical, structureless, without central canal, easily distinguished from all other spun fibres (Fig. 26, A). Silk is animal in origin and gives the same reactions as wool. Several other bast fibres are very valuable in textile industries and present appearances similar to that of linen.

Hemp is derived from Cannabis sativa, jute from Corchorus olitorius and Corchorus capsularis, Manila hemp from Musa textilis. More detailed study of various fibres may be found in some large work, for example, *Die Mikroskopie der technisch-verwendeten Faserstoffe,* by von Höhnel.

CHAPTER III.

YEAST (TORULA OR SACCHAROMYCES CEREVISIÆ).

This is a plant and is that which causes alcoholic fermentation in sugar solutions. It is a plant of the simplest kind, consisting of a single cell. Plants are divided into four series according to their complexity of structure and functions.

Thallophyta—(Thallus plants)
Bryophyta—(Moss plants)
Pteridophyta—(Fern plants)
} Cryptogamia or Flowerless plants.

Spermaphyta or Phanerogamia or Flowering plants.

The Thallophyta are a large group of plants in which there is no clear differentiation of the plant body into root, stem and leaf. A vast number of forms are included, which differ greatly among themselves in complexity, but even the highest forms never have true roots, and in the great majority of cases there is no differentiation into stem and leaves. There is never a clear differentiation into epidermal, fundamental and fibro-vascular systems of tissues as in the ferns and flowering plants.

The Thallophyta are divided into a number of classes, one of which is called *Fungi,* or Moulds. It is thought by some that yeast belongs to the fungi, being a degenerate form. Yeast occurs both *wild* as well as *cultivated,* the former living upon fruits or in fruit juices and occurring in the air, the latter being employed in brewing and for making bread, etc. There are a number of species of wild and cultivated yeasts, and it is probable that cultivated yeasts are descended from similar forms of wild yeasts. Saccharomyces cerevisiæ, or brewers' yeast, is

one species, but ordinary commercial yeast seldom consists of this species alone.

Sow some fresh baker's yeast in Pasteur's fluid and keep in a warm place. As soon as the solution begins to froth and the yeast is manifestly increasing in quantity it is ready for study. Fermentation is most active between 28° and 34° C. At 38° C. growth ceases.

Mount a drop of the liquid and examine with low power, minute specks will be seen. With high power numerous rounded or ellipsoidal bodies will be seen, either single or loosely united into short chains. The diameter of the cells varies from $^1/_{2500}$ to $^1/_{7000}$ inch (average, $^1/_{3000}$ inch). Each torula consists of a well-defined homogeneous transparent sac or cell-wall of cellulose material, enclosing a semi-fluid granular substance called *protoplasm,* within which there is often a space full of a more watery fluid than the rest, termed a *vacuole.* The whole structure is known as a *cell.* The cell-wall is comparatively tough, but may easily be burst and the contents thrown out; it is thicker in old cells than in young actively growing ones. Minute shining dots, thought to be fat globules, may be seen in the protoplasm, but there is neither chlorophyll nor starch present. By the use of special reagents, a nucleus has been demonstrated to be present in the cells, but it is never seen in the living cells.

Torulæ break down sugar mainly into alcohol and carbon dioxide gas, and at the same time increase in number. Multiplication takes place in this way. A small protuberance begins to form on the parent torula, which grows larger, forming a bud. The bud increases until it attains the size of the parent torula and eventually becomes detached, though generally not until it has developed other buds on itself and these still others. The torulæ produced thus by *gemmation* or budding are apt to adhere to each other for a long time and thus produce heaps and strings (Fig. 27).

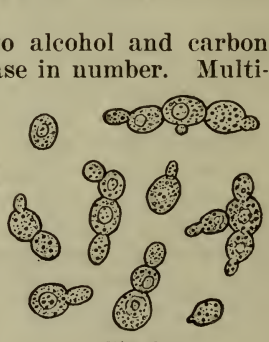


Fig. 27.

Mount a drop of the yeast culture in a small drop of fuchsin stain. Note which cells stain most rapidly and deeply. Actively-growing protoplasm is not stained readily by many dyes, while dead or passive protoplasm is colored quickly by the same dyes. The cell-wall is unaffected and the vacuole also, although the latter may appear purplish, because it is seen through a colored layer of protoplasm.

Make another mount and apply iodine solution at the edge of the cover glass. As the iodine diffuses under the glass the

protoplasm of the cells is stained yellowish-brown. This is one of the best tests for proteid matter. The absence of blue-stained particles is proof that the cells contain no starch. The cell-wall is not stained.

Yeast occurs in the market in a dry or pasty condition as "yeast cakes." Make an emulsion of a bit of one of these cakes in water and examine a drop under high power. Cells similar or identical to those seen in the yeast culture are in abundance. In the pasty cake there is also present rounded bodies many times larger than the yeast cells. These are starch grains, usually of potato starch, which are added to absorb the water of the mass of yeast cells in order to render it semi-solid and capable of being moulded into cakes.

In brewing industries two well-defined varieties of yeast are used, known respectively as *top* and *bottom* yeast. Top yeast is employed in making English ale, stout and porter, fermentation taking place at ordinary summer temperature and producing carbon dioxide rapidly enough to cause the yeast to collect at the surface of the liquid, hence the name top yeast. Bottom yeast is used in making "lager" beer, and grows quietly at the bottom of the vat at a temperature of about 4° C., which is kept constant by artificial means. Besides this difference in conditions of growth, the two yeasts also differ in form, size and structure when seen under the microscope.

Torula is classed among the plants, because it has a cellulose cell-wall and the power of constructing protoplasm (living matter) out of comparatively simple substances, such as ammonium tartrate, which is distinctively a vegetable peculiarity. But though a plant, it contains neither starch nor chlorophyll, and cannot obtain the whole of its food from inorganic compounds, thus differing widely from green plants.

PASTEUR'S SOLUTION.—Potassium phosphate, 2 parts; calcium phosphate, 0.2 parts; magnesium sulphate, 0.2 parts; ammonium tartrate, 10 parts; cane sugar, 150 parts; water, 838 parts.

CHAPTER IV.

BACTERIA (SCHIZOMYCETES, OR FISSION MOULDS).

One of the classes of the Thallophyte series of plants is the Schizophyta. This class is composed chiefly of the Schizomycetes or Bacteria. These are extremely low forms of plant life, being exceedingly simple in structure and always minute, some of them being the smallest of known organisms. They are mostly unicellular, or, if consisting of cell-aggregates, as is sometimes the case, the cells are united in a simple way and have very little dependence upon each other. They are the most abundant of organisms, the largest being not more than $^1/_{10000}$ inch in diameter and the smallest not more than $^1/_{10}$ of that. All are chlorophylless, i. e., without coloring matter. The cells agree in having mostly rigid transparent walls and colorless cell-contents, but different species differ considerably in form, size, etc. Their usual mode of increase is by *fission* or splitting, but they also produce very minute so-called *spores* by a method known as *internal cell-formation.* (See later.)

In some species the cells, after fission, immediately become independent; in others they remain united for a time, to form filaments or chains of various lengths. Many of the species in some stage of their development have the habit of secreting a jelly and increasing rapidly by fission, forming large gelatinous colonies. These are called *zoöglœa-masses.* "Mother-of-vinegar" and the so-called "blood-rain," consisting of red gelatinous spots often found on putrefying bread, are examples of zoöglœa-masses.

In all putrefying fluids or solutions that contain decaying organic matter bacteria swarm in myriads. They are, in fact, the inciting cause of putrefaction. By their agency also milk sours, wine is converted into vinegar, etc. So far as animal life is concerned, some of the species are harmless or perhaps even beneficial, while others are the source of some of the most dreaded and most fatal of diseases. Chicken cholera, splenic fever, typhoid fever, diphtheria and leprosy are examples. A peculiar interest therefore attaches to the study of these organisms.

Bacteria are killed at about 70° C. or above, but the spores can, in many cases, survive a temperature above 100° C. (Spores are little specialized cells which have the power of developing into cells or plants in all respects like the ones from which they were derived. In the case of bacteria they are formed for protective and not for reproductive purposes; they can withstand conditions of temperature, etc., under which the ordinary cells die and thus ensure the survival of the bacteria.)

Bacteria are conveniently grouped according to their shapes,

as illustrated in Fig. 28, in which a is the *Micrococcus* or spherical form; b the *Bacterium* or rod-like form; c the *Bacillus* or filiform form, and d the *Spirillum* or coiled form. In growing, bacteria are often grouped in different arrangements, to which special names are applied, thus *Streptococcus,* a moniliform or necklace-like grouping of cocci; *Diplococcus,* cocci in pairs; *Staphylococcus,* single cocci; *Leptothrix,* a filament of bacilli; *Sarcina,* a plate of cocci.

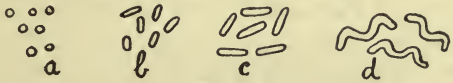


Fig. 28.

Make an infusion of fresh hay by steeping it in water warmed to between 40° and 50° C. for one-half hour or more, filter and set aside for 36 hours or more. The liquid becomes cloudy, due to swarms of bacteria. Examine a drop of the liquid under the highest power at command. Focusing must be done very carefully because of the extreme minuteness and transparency of the cells. The illumination must be somewhat dimmed, else the bacteria will be almost invisible in the glare. A convenient way to find the focal plane is to move the slide about until a coarser object, like a speck of dust, comes into view, and with this as a guide to make the fine adjustment of the focus.

Note the moving bacteria, elliptical or rod-like, sometimes forming short, jointed rows. The cells have an outer, more transparent wall, enveloping a more opaque substance.

Apply a drop of iodine solution at the edge of the cover glass. The bacteria are killed, all forward motion ceases, the contents of the cells are stained and become more conspicuous. The cell-wall does not stain. Other forms that may be found are micrococcus, bacillus and spirillum.

Resting Bacteria or Zoöglœa Stage.—The hay-infusion after a time develops a scum or zoöglœa. Mount a little of this and examine with high power. Myriads of bacteria resting in a gelatinous mass will be seen. Although they do not move away from their places, the bacteria will be seen to have a wiggling or oscillatory motion, known as the *Brownian movement.* This motion is not a vital one, but is characteristic of very small bodies, whether dead or alive. Fine clay, pumice, lamp-black, gamboge, show the same motion. The cause is not definitely known.

Raise the cover glass and add a drop of iodine solution to the scum. The bacteria stain, but not the gelatinous material in which they are imbedded.

Mount a little of the scum composing "mother-of-vinegar." Nearly the same appearance will be seen as in the case of the scum of the hay-infusion. The scum is known as *mycoderma*

aceti, and the bacteria cause the oxidation of dilute alcohol to acetic acid (vinegar).

In order to show the position of the plants already studied, as well as those to follow, in the system of classification of plants, a table of the Thallophyta, with the subdivisions, is here appended.

THALLOPHYTE SERIES.

Class.	*Sub-class.*	*Order.*	*Genus.*
1. Myxomycetes.			
2. Schizophyta.	1. Schizomycetes. (Bacteria.)		
	2. Cyanophyceæ.		
3. Algæ.	1. Diatomaceæ.		
	2. Chlorophyceæ.	1.	
		2.	
		3.	
		4.	
		5. Conjugatæ.	1.
			2. Spirogyra.
			3. Zygnema.
			4.
		6.	
	3.		
	4.		
4. Fungi.	1.		
	2.		
	3. Phycomycetes.	1. Zygomycetes (Mucor mould).	
		2.	
		3.	
	4. Ascomycetes.	1.	
		2. Erysipheæ (Penicillium mould).	
		3.	
		4. Pyrenomycetes (Ergot of rye).	
		5.	
		6. Saccharomycetes (Yeast).	
	5.		
	6. Basidiomycetes.	1.	
		2.	
		3. Hymenomycetes.	1.
			2.
			3.
			4.
			5. Agaricineæ (Mushrooms).
5. Lichenes.			

The table is incomplete, only those sub-divisions being given with which the plants studied in these lessons are concerned.

CHAPTER V.

SPIROGYRA.

This plant belongs to the third class of the Thallophyte series, which is known as *Algæ*. The class includes nearly all the Thallophyte plants which contain chlorophyll.

The algæ are an assemblage of quite simple plants, none of the members attaining any great degree of complexity. For the most part, the plant body consists of an elongated filament composed of united cells; sometimes, however, they form surfaces, and in other cases the plants are unicellular or aggregated into communities. In these plants we find the first examples of undoubted sexuality, and throughout the group the organs and methods of fertilization are nearly enough uniform to enable us to use them as distinguishing characters.

The algæ are for the most part aquatic plants and inhabit either fresh or salt water. They abound in ponds and slow-running streams.

Spirogyra will illustrate the characteristics of the class (Fig. 29). Its position in the system of plants is given in the table (Chapter IV). It belongs to the order Conjugatæ. This order differs from all other algæ in the peculiarly complex structure of the chlorophyll bodies and the mode of sexual reproduction (except some of the Diatomaceæ) which consists in the direct conjugation or union of two ordinary vegetative cells, hence the name *Conjugatæ*. Spirogyra is a filamentous plant, very common in ponds and ditches as a green scum composed of silky, green threads, which sometimes attain a length of six or eight inches. The filaments are unbranched and composed of a row of cylindrical cells all alike and independent of each other and loosely joined together. The name is given in allusion to the fact that the chlorophyll bodies, i. e., the bodies bearing the green coloring matter, form spiral bands winding around the cell on the interior of the cell-wall. Sometimes the bands are single, at other times double or treble (Zygnemas have stellate chlorophyll bodies, two in each cell, arranged axially). At intervals along each band are to be seen highly refractive lenticular bodies called *pyrenoids*. When exposed to the light for some time the pyrenoids would be found, on appropriate treatment, to be surrounded by starch grains.

The cells are bounded by well-marked, refractive cellulose

walls. Next to the wall is a thin layer of protoplasm, better seen by staining with iodine solution. The chlorophyll bands are in contact with this layer of protoplasm. The greater part of the interior of the cell is occupied by a large vacuole containing *cell sap,* i. e., water with substances in solution. Each cell has a usually centrally-placed, distinct protoplasmic body known as a *nucleus,* with radiating extensions of protoplasm passing from it to the outer layer of protoplasm next the cell-wall. The growth of spirogyra in length is brought about by cell division. Each cell is repeatedly divided into two equal parts by the appearance in it of a cross-partition. This process takes place during the night, and special precaution must be taken in order to study it. This method of cell formation is the general mode throughout the vegetable kindom.

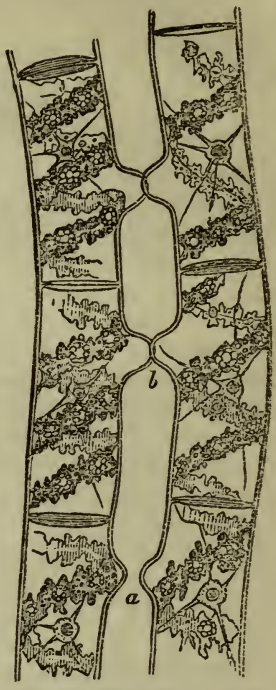


Fig. 29.—Spirogyra longata (Bessey).

The method of reproduction in spirogyra is a sexual one and known as conjugation. This process occurs from early spring to June and July, but can be induced when the plant is under cultivation by allowing the water in which it is growing to slowly evaporate. Two filaments arrange themselves side by side, and the cells lying opposite each other send out each a process or tube; these unite and the protoplasm from one cell passes over and coalesces with that in the cell opposite. In Fig. 29 two such tubes about to unite are shown at b, while the beginning of formation of two other tubes is shown at a.

The result of the process is a new cell called a *zygospore.* This is set free by decay of the walls of the old cell and falls to the bottom of the water and rests until proper time for growth.

It is not an easy matter to find conjugating forms of spirogyra, and their study is not well suited for class work.

There are a number of species of spirogyra, and the student should keep a lookout for different kinds of filaments in the specimen studied. Mount some filaments in water and, under low power, note the great length of the filaments, as well as of the individual cells, the uniform diameter, the well-defined cell-walls and the conspicuous green chlorophyll bands, the shape and relative length and breadth of the cells.

With high power, note more especially the crenulated and wrinkled margin of the bands and the refractive nodules at intervals along them (pyrenoids). Look for a nucleus. This is sometimes hidden by the chlorophyll bands and then not easily seen, but there will always be found some cells in which the nucleus stands out clearly.

Apply iodine solution. The bands stain deeply, especially the dense pyrenoids, which appear almost black. The nucleus also appears much more conspicuous when stained.

It may not be possible to obtain spirogyra in a growing state at the time it is wanted for study. In such a case it should be collected at some other time, when a supply is found, and preserved for future use in dilute formaldehdye solution (one volume of 40 per cent. "formalin" to 10 or 15 volumes of water). In this solution the color is bleached only slowly, and if the specimen has not been kept too long it will still show a green color.

Another large-sized alga often found in slow streams is the *Hydrodictyon* or *Water-net.* The cells are united into network, which has the shape of an elongated bag, often 8 or 10 inches long. The cells are quite large, cylindrical and filled with dense granular chlorophyll matter. The ends of three or four cells meet at a common point. Several nets may be entangled in one another, representing different generations, the cells of the younger nets being much smaller than those of the old and matured nets.

The structure is best studied under low objective in a watch glass containing water, as thus crushing of the net is avoided, which would happen under a cover glass.

CHAPTER VI.

Reproduction.

This is the power that plants possess of giving rise to new individuals, and the process takes place by one of three ways, namely, Division, Rejuvenescence and Union. The first two modes are *asexual,* the last *sexual.*

There are three varieties of reproduction by division:

Division. { Fission. Gemmation. Internal cell formation.

Fission.—The most common mode of division. This is the separation of a cell into equal portions.

a. A constriction takes place in the middle of the cell and along the plane of this constriction; the cell-walls may grow

inward until the cell contents become separated into two equal portions. This mode has been observed in some of the lower algæ (Spirogyra).

b. A delicate partition of cellulose may at once be formed through the middle of the cell. This is the usual mode by which "tissues" are formed and growth takes place in all the higher plants.

GEMMATION.—This method is found in the yeast plant and its relations. (See Torula for description.)

INTERNAL CELL FORMATION.—The protoplasm of a cell breaks up into two or more rounded masses, each of which eventually acquires a cell-wall of its own and escapes from the parent cell by the rupture or decay of the old cell-wall. Example, ascospores in lichens and some fungi and pollen grains in the anthers of flowering plants.

REJUVENESCENCE.—The protoplasm aggregates into a rounded mass, escapes through the cell-wall and subsequently forms a new cell-wall. Commonly, before the new cell-wall forms, the protoplasm forms cilia and moves about. Rejuvenescence is found only among lower forms of plant life; for example, Œdogonium, one of the algæ.

As was said above, Division and Rejuvenescence constitute asexual reproduction. There are two modes of asexual reproduction:

1. Vegetative reproduction.
2. Spore reproduction.

In the former the parent plant throws off from itself ordinary vegetative cells; in the latter, specialized cells called spores are formed. Examples of the first are bacteria, cell multiplication in higher plants, multiplication in case of many plants by bulbs, tubers, stolons, offsets, etc.

Spore reproduction by the asexual process is exemplified in many flowerless plants. Examples, spores on the gills of the common mushroom, motile spores so commonly produced by the mosses and ferns. Spores are commonly borne in a special organ called a *sporangium.*

UNION OF CELLS.—SEXUAL REPRODUCTION.

This consists in the coming together and blending of the protoplasm of two distinct cells to form a new one.

a. The uniting cells may be alike and the process is then known as *conjugation,* and is found only in certain low forms of plant life, as Mucor (a mould), Diatoms, Spirogyra, Desmids, all of which, except the first, belong to algæ.

b. The uniting cells may be unlike, the process being then

known as *fertilization.* One cell (the male or sperm cell) is commonly not only smaller, but more active than the other (called the female or germ cell). Example, all higher plants.

CHAPTER VII.

MOULDS (FUNGI).

Moulds belong to the class of plants known as Fungi, which latter, as we have already seen, form one of the divisions of the Thallophyta. The fungi are, in their habits, chlorophylless *saprophytes* or *parasites.* (A saprophyte is a plant which derives its sustenance from decaying organic matter. A parasite lives on other organisms.) In all but a few instances (see Torula), their vegetative parts consist of slender segmented or unsegmented, usually colorless filaments, each one being known as a *hypha.* These ramify among decaying organic debris or invade the tissues of living organisms, plant or animal, and derive their sustenance from them. In the simpler hyphal forms the hyphæ occur singly or more or less interwoven into a tangled felt-work, but they are not gathered into definite forms and have little or no dependence on each other. In the higher groups, however, there is more or less division of labor among the hyphæ, and they become consolidated into false tissues, which acquire definite shapes according to the species. Of this character are the fructifying organs or *carpophores,* which constitute the above-ground parts of the agarics, puff-balls, cup-fungi, etc., and the *sclerotium,* a compact hard mass of thick-walled hyphæ, which serves as a resting stage in the development of some species, for example, *Ergot of rye.*

Fungi reproduce asexually by means of spores, known as *gonidia* or *conidia.* These are, as a rule, thick-walled cells, which become separated from the parent hyphæ in ways which are more or less characteristic in the different groups. In all hyphal fungi the hyphæ consist of two portions—the *vegetative,* which ramifies in the substratum, often forming tangled, felt-like masses of threads called the *mycelium;* and the *reproductive,* which comes to the surface. The latter produces the conidia, which may be borne on isolated filaments, as in the bread-mould (Penicillium) or on a carpophore, which produces a spore-bearing *hymenium.* The common mushroom (Agaricus campestris) is an example of the latter, the plate-like bodies or gills on the under surface of the cap constituting the hymenium.

In a large number of fungi, including some of the most highly organized forms, sexual reproduction is unknown. In other

species sexual reproduction takes place, and this may be by several methods, namely, conjugation, and formation of so-called oöspores.

PENICILLIUM GLAUCUM (BREAD MOULD).

This mould is familiar to everyone from its forming sage-green crusts upon bread, jam, old boots, etc. It may be obtained at any time by placing a moist piece of bread under a bell-jar in a moderately warm place. When spores appear, sow some in Pasteur's fluid (see under Torula). Moulds growing in this fluid are easier to examine than when growing on bread. On examining a patch of mould on the surface of the fluid, it is found to consist of a horizontal felt work of delicate tubular filaments, the hyphæ, forming a crust like so much blotting paper, which is known as the *mycelium.* Hyphæ project from this into the air, b, and bear a green powder, the spores, c, Fig. 30. These hyphæ are called *aërial.* From the mycelium other hyphæ grow down into the liquid and are called *submerged hyphæ,* corresponding somewhat to the roots of higher plants.

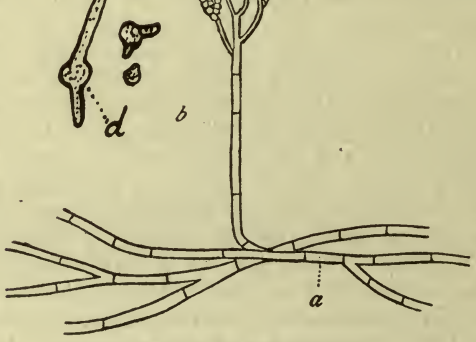


Fig. 30.—Penicillium glaucum.

Carefully make a thin section of the mycelium by cutting between two pieces of pith. Moisten a section on a slide with alcohol, allow the latter to nearly evaporate, then add water and cover. Examine first under low power and observe the appearances described above.

With high power, observe that each hypha has a transparent wall and protoplasmic contents and is divided by transverse partitions into a number of cells. Each cell has several large clear spaces, the vacuoles and a number of nuclei which, however, are only visible by staining properly.

The hyphæ frequently branch and are inextricably entagled with one another, but every hypha with its branches is quite distinct from every other one. If the section be a little too thick and obscure, gentle tapping on the cover glass over the section will spread the parts, so that they may be more easily seen.

Note the aërial hyphæ, with brushes or branches, which become constricted on their ends into a series of rounded spores like a row of beads. These hyphæ which bear the spores or conidia are called *conidiaphores.* The conidia form the loose green powder characteristic of the mould. The spore is a round, transparent sac enclosing a mass of protoplasm and is in all essential respects similar to a torula. When sown in an appropriate medium (Pasteur's solution) it germinates and forms hyphæ from several points, forming a new plant like the original one (Fig. 30, d).

Stain different sections with fuchsin, hæmatoxylin and iodine and note results.

Besides Penicillium, usually other moulds will be found on mouldy bread or other matters. The most prominent among them will probably be *Eurotium Aspergillus glaucus,* which may be distinguished from Penicillium by its higher growth, less velvet-like appearance and the olive-green color of the spores. The conidiaphores of Eurotium are about $^1/_{16}$ inch long, visible to the eye, and bear roundish white (unripe) or pale-green heads closely packed.

A pure growth of either of the fungi described above may be obtained as follows: Place a few thoroughly boiled (and thus sterilized) French plums on a sterile glass plate and infect them with spores taken from as pure a patch of the mould as can be found by a previously heated and cooled needle. Cover the plate with a sterile bell glass and keep in a moderately warm place. (The plate and bell glass may be sterilized by placing them in an air-bath heated to 105° or 110° C. for one-half hour or more).

MUCOR STOLONIFER.

This mould may be grown by keeping some bread very moist and warm under a bell jar or by placing some moist Poke-root in a bottle and closing. Mucor is similar to Penicillium in its growth, consisting of a mycelium from which grow erect or aërial hyphæ, each one bearing a rounded, dark head or spore case, looking like a pin head and called a *sporangium.* The wall of the spore case is beset with minute asperities of oxalate of lime, and inside the case are a great number of minute oval bodies, the spores, held together by a transparent intermediate substance. When ripe, the thin and brittle coat of the case bursts at the slightest pressure, setting free the spores. A lit-

tle portion of the wall of the spore case remains adhering to the stalk as a collar. The cavity of the stalk does not communicate with the sporangium, but is cut off by a bulging partition, forming a central projection known as the *columella*. This may be mistaken for the spore case itself.

The spores are oval and larger than those of Penicillium, consisting of a sac enclosing protoplasm and a nucleus. When sown in a proper medium they send out hyphæ and produce a new plant. The spores are at first colorless, but when ripe are colored and give the black appearance to the sporangia.

The hyphæ are cylindrical threads, longer and larger in diameter than those of Penicillium, and when young have no dividing partitions, so that each hypha, however long, with all its branches, forms a single cell. In old growths, partitions may be found after the production of sporangia. The hyphæ contain granular protoplasm with vacuoles and nuclei.

Carefully remove some hyphæ, white (unripe) and dark spore cases with a teasing needle or forceps to a slide and keep them spread apart. Add a drop or two of 70 or 80 per cent. alcohol and cover. Water causes the spore cases to swell and burst, hence should not be used. Examine with low and high power. Note the hyphæ, spore cases (both whole and broken), columella, spores, protoplasm, etc. Also apply fuchsin and iodine stains and note the effects. Compare with Penicillium as to its dimensions of parts.

When moist bread is allowed to become mouldy Mucor is apt to be the first growth. Later on this mould will die away

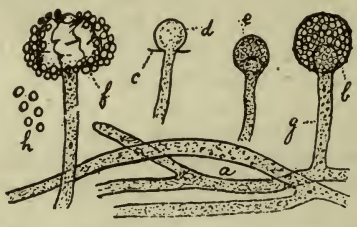


Fig. 31.—Mucor stolonifer. a, hypha; b, ripe spore case; c, collar or remains of broken wall of spore case; d, columella or dome-like partition separating spore case from the cavity of its stalk, g; f, broken spore case with spores; e, young spore case, spores not yet formed; h, spores.

and Penicillium or Eurotium will get the upper hand and flourish. The source of the mould is the spores which float about in the air or are present in water.

CLAVICEPS PURPUREA (ERGOT OF RYE).

Claviceps is a genus of the fungi whose different species produce Ergot grains on various kinds of grasses. The hyphæ of the species Claviceps purpurea begin their development on the

surface and interior of the ovary of the flowers of Rye as delicate filaments. After a certain time the fungus begins to form a dense mass of thick, hard, dark purple hyphæ, which gradually destroy and take the place of the cells of the ovary until finally there is scarcely anything left of the latter. This hard mass, known as the *sclerotium* stage, constitutes the official Ergot of Rye. This is a resting stage, the grain lying dormant until spring, when, if placed in warm, damp soil, there arises a number of stalked bodies with globular heads in which spores are produced. If these spores be carried by the wind to the flowers of Rye they develop and produce new grains in the manner just described.

Wrap a few large grains in moistened filter paper and keep in a corked bottle for several hours. By this time they will

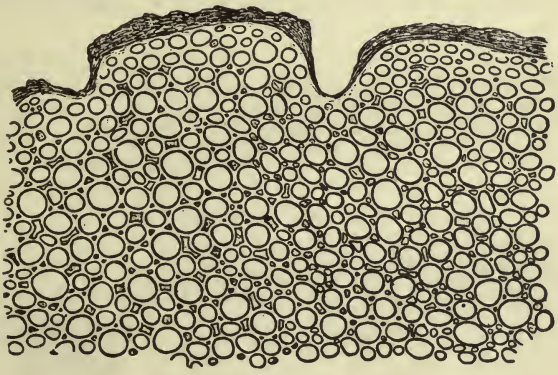


Fig. 32.—Ergot of Rye, Transverse Section (Vogl).

have changed from a brittle to a flexible state. Place half of a grain between the two halves of a piece of elder pith, clamp in a microtome and cut very thin transverse sections through the pith and place them in water. Mount in water on a slide and examine with low and high power. The margin of the section consists of smaller cells with brown contents. Within this border the cells are larger and lighter in color, rounded or oval, having thick cell-walls and oily contents. In chloral hydrate solution the cells become clearer and the oil collects in larger globules. In longitudinal section the appearance is nearly the same as in the transverse.

CHAPTER VIII.

THE TISSUES OF THE HIGHER PLANTS.

The lessons thus far have been given to the study of some of the simple plants, for the purpose of giving an idea of the nature of the lowest forms of plant life as well as familiarizing the student with the use of the microscope and the manipulation of objects on the slide. Some of the plants studied play an important role in the life economy, for example, yeast, bacteria, moulds, and thus deserve close study. While studying these plants we have learned what is meant by a plant cell, and the subsequent lessons will be devoted to a study of the various kinds of cells and webs of cells known as "tissues," found in the most highly developed and complex plants, the Phanerogamia or flowering plants.

The peculiarity of these is that there is a great division of labor, with corresponding tissues and organs, which have been differentiated from a fundamental tissue. Thus we have a leaf, an organ for manufacturing protoplasm and starch; the flower, which is the reproductive organ; the stem, the channel for conveying sap; the roots for imbibing water and nourishment. The cells are differentiated into distinct tissues. On passing down to the lower series of plants these tissues become simpler until, finally, in the Thallophyta and most of the Bryophyta we have no distinction of tissues at all. Those plants consist of a homogeneous mass of cells, as we have seen in the case of algæ and moulds.

The various tissues or cell-webs of the flowering plants, namely, epidermal, ground, fibro-vascular, stony, etc., are all derived from cells that were at one time all alike. By various physical and chemical modifications the cells come to differ from one another and thus to give rise to the different tissues. The cells of stony tissue, as found in shells of nuts, were once like the soft cells of a leaf, but they became subsequently hardened and modified.

A cell has been defined as a *nucleated mass of protoplasm.* It may or may not possess a cell-wall of different composition. With rare exceptions in vegetable cells such a wall is present, while most animal cells are destitute of it; but in all essential respects animal and vegetable cells resemble each other. Cells are the structural units of the organism. All plant bodies are composed of cells or of these together with the products of cell activity. Within the compass of the cell occur all those essential phenomena which are called *vital*; the life of a plant resides in its cells; the sum of the activities it exhibits is the sum of the activities of its component cells.

Vegetable cells are, on the average, not more than one five-hundredth or one six-hundredth of an inch in diameter, though in some cases they are large enough to be distinctly seen by the unaided eye, as in the flesh of the Watermelon and the pith of Elder; in rare instances, as the internodal cells of Chara, they may even be more than an inch long. Some cells, on the other hand, are so small as to be barely visible under the highest powers of the microscope, for example, some bacteria.

The primary form of cells appears to be that of a sphere or spheroid, but commonly, especially in the tissues of the higher plants, they acquire forms quite different from this, and even within the limits of the same organism the shapes may be exceedingly various. This may be due to mutual pressure, to unequal growth caused by the unequal operation of various physical forces, as gravitation, light, etc., or to other influence. Cells, like the organs of which they are components, undergo many modifications of form and structure, adapting them to different uses. The cells which make up the body of a plant are comparable to the human units which make up society. A plant is a community or republic of cells, and, to understand it, one must understand the individuals that compose it.

TYPICAL VEGETABLE CELL.

As all the different kinds of cells that go to make up the various tissues of a plant are derived from cells that are at one time all alike, we will begin by a consideration of these primitive or *typical* cells, and afterwards study the various modifications.

Peel off the skin or *epidermis* from the convex surface of an onion scale by making a cross incision and catching the skin between the thumb and the knife or razor edge. Be careful not to draw along with the epidermis any of the thick underlying flesh of the scale. Mount a piece of the skin about a quarter-inch square in water on a slide, cover carefully with a glass so as not to include any air-bubbles.

Examine with low power. Very little will be made out. There is a fine and somewhat irregular network. This is due to an aggregation in a single layer of a number of cells, the network of lines being the bounding cell-walls, which are so nearly transparent as to be almost invisible. If the light be properly dimmed there may be seen in each cell a small denser-looking body which is called the nucleus, and perhaps faintly granular matter. The cells are filled with a semi-liquid matter, which, however, is too transparent to be seen.

Examine the various parts of a cell with high power. The details are somewhat difficult to make out because of the transparency of the cell contents. This is very often the case with

living cells, but the difficulty can be overcome by killing the protoplasm or staining it.

Raise the cover glass, add a drop of iodine stain and allow a few moments for it to penetrate the cells, then cover and examine again.

The cell-walls, scarcely stained, are distinctly visible. In mature cells aggregated to form tissues, the common cell-wall between two cells is made up of two like portions separated by a layer of a slightly different chemical substance, which is more soluble in reagents than the rest of the wall and shows different reactions with test reagents and is known as the *middle lamella.* Next to the cell-wall is a layer of protoplasm, granular and deeply stained yellowish brown, called the primordial utricle. Somewhere within the cell will be seen a dense body, the nucleus, surrounded by protoplasm and connected by strings of protoplasm with the utricle. Between the strings

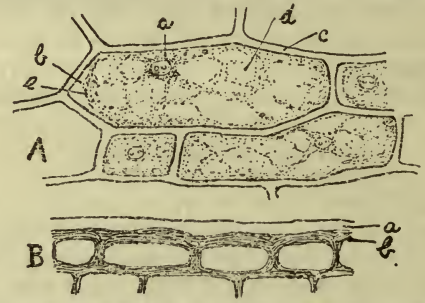


Fig. 33.—Cells of Onion epidermis. A, surface view. a, nucleus; c, cell-wall; d, vacuole; b, space where protoplasm has shrunken from cell-wall; e, primordial utricle. B, cross-section. a, cuticle; b, cellulose portion of cell-wall.

are *vacuoles,* clear spaces filled with cell-sap. The nucleus contains several smaller bodies, which are little nuclei or *nucleoli* (plural of nucleolus). In some cells the protoplasm may have shrunken away from the cell-wall at one end, leaving a clear, apparently empty space. The cells dovetail into one another, leaving *no intercellular spaces,* but forming a close impervious layer.

Like epidermal cells in general, the cells just described are rather flat, but that is not apparent in surface view. This fact is brought out in sections cut vertical to the surface of the epidermis, which is best done by cutting through several scales of the onion. The appearance is given in Fig. 33. The cells are oblong in shape and the outer wall is somewhat thickened.

Raise the cover glass from the section that was stained with iodine, remove the excess of fluid and add a drop of sulphuric acid (2 vols. conc. acid to 1 vol. water), after a moment replace the cover glass and examine. The cell-walls are stained a deep

blue, proving that they are cellulose in nature. A light line, *not* blue, but of a yellow color, is in the middle of the cell-walls and marks off the boundary of each cell. This line is the middle lamella. It is composed chiefly of calcium pectate. The cellulose portions, as well as the middle lamella, gradually swell, finally dissolve and disappear.

If a cross-section of the epidermis be treated as above described the thickened outer wall will be seen to consist of two layers, an inner one, which is cellulose and stains blue, and an outer one, which stains yellowish or brownish. This outer layer is called the cuticle and is composed of a substance known as *cutin* (cork substance), which does not dissolve in the acid. Cutin is very resistant to reagents and thus forms an excellent protecting layer. The cuticle is represented in Fig. 33.

The typical cell just described is somewhat advanced from the earliest stage of a cell, known as the *primary meristem* cell. In such very young cells the wall is exceedingly thin and apparently homogeneous, the vacuoles are absent and the entire area enclosed by the wall appears to be filled with protoplasm (Fig. 34). As the cell grows older its wall becomes thicker and differentiated, as described above. By the expansion of the wall the cavity of the cell increases faster than the contained protoplasm, the latter imbibes more water than it is capable of holding in solution, and thus sap cavities or vacuoles are formed, which, at the maturity of the cell, often occupy more space than the protoplasm itself. Finally, when the cell is quite old its living contents disappear altogether and the cell is dead matter.

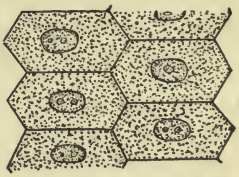


Fig. 34.—Very young cells.

As the cells develop to form the various tissues, a number of substances make their appearance in the contents. Some of these are chlorophyll bodies, aleurone grains, starch, fatty oils and fats, calcium salts, glucosides, alkaloids, sugar, bitter principles, tannin, resins, gums, inulin, etc. Some of these will be studied in later lessons.

CHAPTER IX.

TISSUES OF VARIOUSLY MODIFIED CELLS AS FOUND IN HIGHER PLANTS.

While it is true that all the essential phenomena which we call vital are manifested within the compass of a single cell, it is also true that the manifestation is feeble in comparison with that exhibited by cell aggregates, where there is division of labor among cells. All the higher plants are such aggregates of cells. They are made up of millions of them, and their life is not the mere aggregate life of cells precisely alike, but rather that of sets of cells that have come to differ from each other in form and function, but all subserving the life of the whole organism.

These cell groups, which differ from each other in ways more or less important, but each of which is composed of similar cells, are called tissues. There is a great variety of tissues, the individual cells of which differ more or less markedly from the typical cell already described.

It must be remembered, however, that all these tissues originate from a single cell, and that each cell of the mature plant, however great its deviation from the typical form, approximates the latter very closely in its early stages of development.

The various kinds of tissues are classified into four groups or series.

I. Parenchymatous Series.
1. Parenchyma; ordinary soft ground tissue.
2. Collenchyma; thick-angled tissue.
3. Sclerotic parenchyma; stony tissue.
4. Epidermal tissue.
5. Endodermal tissue.
6. Suberous or corky tissue.

II. Prosenchymatous Series.
1. Wood or libriform tissue.
2. Tracheids or vasiform cells.
3. Ducts or vascular tissue.
4. Hard bast or bast-fibres.

III. Sieve Series, including only sieve or cribriform tissue.

IV. Laticiferous Series, including laticiferous or milk-tissue, which may be simple or complex.

Not all of the tissues are alive at maturity. Some are dead and merely serve as supporting tissues. All of the prosenchymatous series are of this nature, being devoid of protoplasm and mechanical in function. Likewise sclerotic and suberous tissues. Any one of the higher plants will contain most of the above tissues. If we examine stems of plants we find that the tissues are not arranged indiscriminately, but always follow a definite order. This order in Phanerogams is different from that in Cryptogams, in exogenous flowering plants it is different from that in endogenous ones. But in any particular

case the order is always the same. *Exogens* are dicotyledonous plants and are characterized by having their stems sharply divided into bark and central wood cylinder, while *endogens,* or monocotyledonous plants, have no sharply-defined bark and wood core.

To get an idea of the various kinds of tissues and the particular order of arrangement always met with in exogens, the stem of the common Geranium serves very well. The stem of most any other exogen would answer, as the Bittersweet, Elder, Willow, Sycamore, Maple, Yellow-Parilla, etc.

Make several thin cross-sections of a common Geranium stem, free-hand or by the microtome; place one on a slide, add a few drops of chlor-zinc-iodine solution and cover. Examine with low power.

The first thing that strikes one is that the cells differ in size and shape, in compactness of arrangement, in thickness of cell-walls, in contents, besides that some stain blue while others stain brown.

Going from the exterior towards the center the very first layer of cells is the

1. *Epidermis* or external bounding tissue, a single tier of closely laid and similar cells, interspersed with hairs and having their outer walls thickened into a cuticle. If the stem be too old, the epidermis may not be present.

2. Beneath the epidermis are several tiers of tabular, brick-shaped cells in radial rows, the cells of the outer layers are empty and their walls stain brown, while the inner cells may contain protoplasm and the walls show some blue color. This is the *cork layer.*

3. *Collenchyma,* next to the cork, consisting of cells quite different in shape and arrangement from the cork cells, with cellulose walls, as shown by the blue color with chlor-zinc-iodine, rounded or polygonal and thickened at the angles where the cells join. The cells are rich in protoplasm. They are not as well developed nor as conspicuous in the Geranium as in some other plants.

4. The next layer of cells is *ordinary parenchyma* or *ground tissue,* a broad zone of large cells, with walls very thin and uniform and stained blue (cellulose), rich in protoplasm and starch contents, globular in shape, with small angular interspaces at the angles where the cells meet.

5. *Bast fibres,* next to the parenchyma, a zone of much smaller, angular, very thick-walled cells, stained a deep brown (lignified walls), compactly arranged and free from protoplasmic and starchy contents. The bast fibres are dead and act only as mechanical tissue. The cell-walls are stained brown like those of the cork cells, which might indicate that they are composed of the same material. That this is not so may be shown by adding to a fresh section a drop of phloroglucin

reagent, followed after a time by strong hydrochloric acid. The cork cells remain unchanged, while the bast cells assume a fine reddish-purple color. The chemical substance composing the bast cells is known as lignin.

The fibrous nature of the cells is seen only in longitudinal section.

6. The next layer is a not very broad zone of small-celled tissue with blue stained walls. Under high power this is composed of two sub-layers; the outer one consists of larger cells, more rounded, of *unequal* size, irregularly arranged and made up of two kinds of thin-walled cells, sieve tissue and a variety of parenchyma. These constitute the so-called *soft bast.*

7. The inner layer of the two sub-layers is composed of very small cells, rich in protoplasm, very closely packed, in radial rows. These are primary meristem cells and form the so-called *cambium zone* of the stem. It is in this zone where growth takes place most actively, by which the stem continues to increase in diameter. When the bark is stripped from a stem the rupture takes place in this succulent fragile zone.

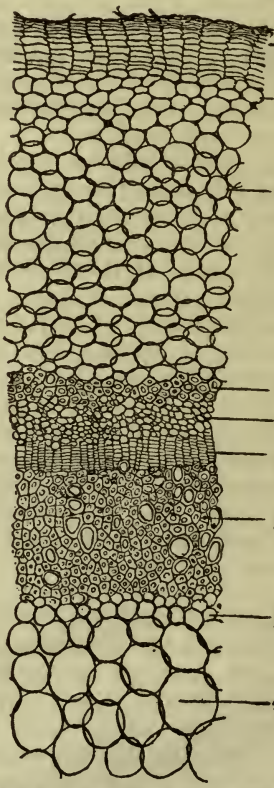


Fig. 35.—Geranium stem, cross-section. a, cork cells; b, collenchyma; c, parenchyma of middle bark; d, bast fibres; e, soft bast; f, cambium zone; g, ring of mixed wood fibres and vessels; h, i, small and large parenchyma cells of pith (Bastin)

8. Next the cambium is a layer more or less broad, according to the age of the stem, composed of cells for the most part like the bast fibres (see 5) and stained brown. These are *wood* or *libriform fibres* (lignified). Scattered among them are other cells of larger diameter whose walls are also thickened and stained brown. These constitute the *vasiform* or *vascular tissue,* composed of *ducts* and *tracheids* of various kinds. Their function is to strengthen and also to convey nutriment. All these cell-walls are lignified and behave like the bast fibres towards phloroglucin stain.

9. Interior to the wood circle is the *pith,* composed of large-celled parenchyma, with large inter-cellular spaces and starchy contents. The cells on the exterior are smaller than those towards the center and more compactly arranged. Many of the cells of the pith and the outer parenchyma layer

contain dark masses, which, under the high power, are seen to be stellate crystals.

Make a longitudinal radial section and examine as before. The cork cells look about the same as in cross-section; the collenchyma cells are elongated; the bast fibres, wood cells and ducts are *very much* elongated and oblique-ended or tapering.

Examine cross-sections of other exogenous plants.

Make permanent mounts of sections stained in hæmatoxylin and fuchsin, also in methyl-green and eosin.

CHAPTER X.

PARENCHYMA TISSUE.

In the parenchymatous series of tissues the cells are less modified, at least in shape, from very young cells than those of other tissues. They mostly retain to maturity the characteristics of the living cell, namely, protoplasm and nucleus and the power to form new cells by division. In some cases they become elongated and somewhat fibrous, but more commonly they are not much longer than broad, and have their ends square or rounded rather than oblique or tapering. In most of the tissues of the parenchymatous series the cells have thin walls, but in some instances the cell-walls are thickened by cellulose, cutinous or ligneous deposits.

Ordinary parenchyma or *soft ground tissue* is the least modified and most abundant of all the plant tissues. The walls are thin and frequently, though not always, composed of unmodified cellulose, commonly spheroidal or polyhedral in form and the longitudinal rarely exceeds the transverse diameter. It includes most of the soft tissues of plants, such as the green cells of the leaf, the cells of pith, a considerable portion of the cells of bark, etc. Sometimes the cell-walls are unequally thickened, so as to present the appearance of markings of various kinds; indeed, they are seldom of uniform thickness, but commonly their membranous character and transparency make them appear so.

For the study of ordinary parenchyma cells the young Geranium stem, studied in Chapter IX, would serve very well, but for variety sections from the soft green parts of other plants may be taken, such as the petiole of the Begonia, the Pumpkin stem, etc.

Let cross-sections be examined first in water, then stained with iodine and chlor-zinc-iodine.

The description of the parts of a typical cell given in Chapter VIII answers very well for parenchyma cells. A structure not noted there is present in all green parenchyma cells, namely, small rounded granules stained deep brown, the *chloroplasts* or

chlorophyll bodies. In the fresh unstained cell these are green. The chlorophyll granules give the green color to leaves, etc.

Another difference is the presence of small intercellular spaces between the cells, not found in the onion epidermis or any epidermis in fact. Starch grains also are often present, which assume a deep blue color with iodine, appearing almost black. Under high power the walls that have been stained blue by chlor-zinc-iodine appear finely punctate with nearly colorless dots. These are thin places or pits in the walls. Unlike the epidermal cells of the onion, parenchyma cells are nearly always globular, as may be seen from the cross and longitudinal sections of the Geranium stem (Fig. 35.)

Parenchyma cells are not always globular and closely packed, but occur at times in modifications, both as to shape as well as arrangement. These modified forms are—

Stellate, or star-shaped cells.

Folded—the cell-walls have internal folds.

Spongy—the cells are very loosely arranged.

Palisade—the cells are elongated and arranged like posts, seen in leaves.

Pitted—the cell-walls marked by thin places of various dimensions and shapes.

Folded cells will be seen later in pine needles, spongy and palisade cells in leaves. For pitted cells, several plants might be chosen, but a convenient one is the plant known as the Sago Palm (Cycas revoluta).

Examine a cross-section of the petiole unstained in water. Under high power thick-walled rounded parenchyma cells will be found with a number of transparent, rounded areas looking

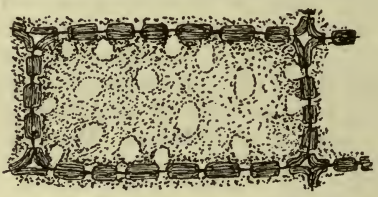


Fig. 36.—Pitted parenchyma cells.

like holes in the wall. These are thin portions of the otherwise thickened walls and not holes. On the edges the pits cause a beaded appearance (Fig. 36). Phloroglucin shows that the walls are somewhat lignified, giving a red color.

Beautiful permanent mounts may be made by staining in aqueous fuchsin solution, passing the section through 70 per cent., 90 per cent. and absolute alcohol, then into eosin oil of cloves for ten or fifteen minutes and finally mounting in balsam. The thin areas of the wall will be stained pink by eosin and the thicker portions red by fuchsin.

CHAPTER XI.

COLLENCHYMA CELLS.

Collenchyma, or thick-angled tissue, is closely related to ordinary parenchyma, but the cells are more elongated, often five or six times longer than broad, prismatic in shape and thickened at the angles. The thickenings are usually not lignified and the cells contain protoplasm, a nucleus and more or less chlorophyll. They are never found elsewhere than in close proximity to the epidermis or rarely in a similar relation to endodermal tissue, and one of the uses of the cells is evidently that of giving strength to the epidermis. Sometimes collenchyma forms a continuous circle, as in the petiole of Begonia and Grape; at other times it forms longitudinal bands, as in stem of Yellow Dock and Cow-parsnip.

The thickenings of the cell-walls in some plants are excessive and strongly diminish the cell lumen; in others they are slight. Sometimes they are confined to the angles, while at others they extend to a less degree to the rest of the cell-wall.

The petioles of Burdock, Begonia or any species of Grape afford good examples for the study of collenchyma.

The petiole of Burdock is bluntly angular, and seven ribs, darker in appearance than the rest of the surface, may be seen running lengthwise and forming the angles. These are the ribs of collenchyma cells. Make cross-sections and examine first in water with low and high power. The cells occur in patches just beneath the epidermis at the angles of the section. The cell-walls are much thickened, especially at the corners, and are strongly glistening (refractive), in this respect showing a marked contrast to the neighboring larger parenchyma cells. The cavities of the cells look dark by contrast with the shining cell-walls. At the border between collenchyma and parenchyma tissue the first line of cells of the latter tissue have one-half of their walls thickened, while the other half is thin.

The walls of the collenchyma cells are marked with delicate stratification lines. Such lines are common to thick walls generally and are due to the different layers containing different amounts of water, as may be proved by immersing in strong alcohol, which removes all the water, when the lines will disappear. Collenchyma is absent in most monocotyledonous plants. In longitudinal section the cells present quite a different appearance. The thickenings appear as long narrow bands, the cells are elongated, some being twice, others five to six times as long as broad, and blunt-ended. In some plants collenchyma tends strongly to fibrous tissue, being very long and greatly thickened and taper-pointed.

A good way to get a longitudinal view of the cells is to strip off the epidermis from a Lily petiole; the collenchyma cells will come off with it and may readily be studied in water or iodine solution.

In iodine solution the collenchyma cells are seen to contain protoplasm and a nucleus. If the excess of iodine solution be removed and sulphuric acid reagent be added, the cell-walls will swell and assume a blue color, which shows that they are cellulose in nature. Chlor-zinc-iodine solution gives the same result.

While studying collenchyma in the petiole of Burdock attention should be given to the whole section, as another illustration of the dicotyledonous type of stem structure. A number of distinct moccasin-like areas may be observed, arranged in a single circle and separated from each other by plates of ordinary parenchyma cells. These are the *fibro-vascular bundles,* consisting of bast and wood fibres and tracheary vessels, just as in

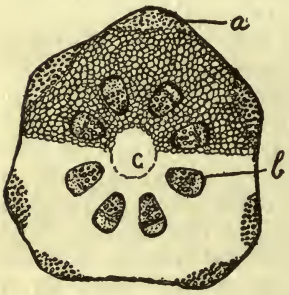


Fig. 37.—Sketch of cross-section of petiole of Burdock. a, patch of collenchyma; b, ring of bundles; c, pith with hole in center.

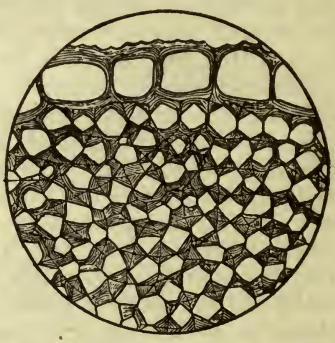


Fig. 38. — Cross-section of collenchyma of Burdock magnified, showing thickened angles of cell-walls (Bastin).

the Geranium stem. But as in the case of succulent plants in general, so here the vascular bundles have not coalesced, so that there is no continuous ring of bast fibres, cambium zone and wood fibres and vessels, as is the case in the Geranium. Next to the circle of bundles comes the pith, the center of which is occupied by a cavity. Cork cells are absent.

Let a cross-section of Burdock soak a few minutes in phloroglucin solution, then add hydrochloric acid and note that the only lignified material occurs in the vascular bundles in the fibres and vessels.

CHAPTER XII.

Sclerotic Cells.

The cells of this tissue are commonly called *stone* or *grit* cells. The cells differ from ordinary parenchyma ones in having the walls excessively thickened, so much so frequently that the cavity of the cell is nearly obliterated. Every gradation, however, may be observed between these and ordinary parenchyma. The walls of sclerotic cells are usually lignified and the thickening is deposited in layers, giving the appearance of concentric rings. These are the cells which give the great hardness to the outer coats of seeds and the shells of nuts. They constitute the gritty particles that occur in the flesh of some fruits, as the pear and apple, and are present in many barks, for example, Cinnamon, Oak, Viburnum, Cascara, etc. Stone cells are classed by some with parenchymatous tissues because of their origin and shape, but otherwise they have very little in common with parenchyma.

Sclerotic Tissue of Walnut Shell.

Use a piece of shell that has been softened by long soaking in 10 per cent. alkali and washed in acidified water. Cut thin sections in various directions, taking care not to let the razor run too deep. Mount in water and examine with low power, picking out the thinnest edge.

There will be seen a mass of rounded, somewhat polyhedral cells, pressed so closely together that no intercellular spaces are visible. The walls are extremely thickened and contain minute dots, as well as radial lines connecting the small cavity or lumen of the cell with the middle lamella. In this section the cells are almost colorless.

Put on high power. The radial lines can now be seen to be tubes, and the dots are tubes cut cross-wise, the ends appearing as dots. The tubes are known as pore-canals and are analogous to the pits in ordinary parenchyma cells. They probably serve to help the circulation of nutritive fluids from one cell to another, as is evidenced by the fact that the tubes of neighboring cells end opposite each other.

With careful examination the walls are seen to be made up of concentric layers. These are made more distinct by adding a drop of chloral-hydrate solution (5 chloral-hydrate to 2 water) and watching closely its swelling action. The lines come out plainly at first, but after a time disappear, owing to continued swelling.

Examining sections cut in various directions from the shell,

it will be found that the cells have the same general appearance, showing that they are, in form, essentially like parenchyma cells, the difference being that the walls are immensely thickened in stone cells, and they no longer take part in the vital processes of the plant, but act as mechanical tissue; in fact, in some cases they are elongated and fusiform, so that it is difficult to distinguish them from prosenchyma fibres. All gradations are found between parenchyma and stone cells and between stone cells and bast fibres.

Add phloroglucin and hydrochloric acid to a section. The walls are stained red, proving lignified material.

Stone cells may be studied to better advantage by isolating them by Schulze's maceration solution as directed under this reagent. Stain a section treated thus with methyl-green and mount in water, and tap the cover glass with a teasing needle, when the cells will separate in virtue of the middle lamellas, which unite the cells, being dissolved away by the maceration fluid.

Examine sections cut in various directions from cocoanut shell. These cells have a natural brownish-yellow color, and their boundaries are more easily seen than in the case of the walnut cells. While many cells are almost circular in outline, others are somewhat elongated. This fact indicates that the cells tend towards the fibrous condition and run in different directions, so that in the same section some are seen in cross-section and appear round, while others are seen lengthwise and appear elongated. The cavities are filled with a dark material. In general structure the cells resemble those of the walnut shell. Ground cocoanut shells are used frequently as an adulterant of ground spices and should, therefore, command close study by the student.

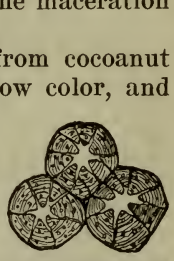


Fig. 39.—Stone cells of walnut shell.

To study the stone cells as they occur in barks, wrap pieces of the bark of cassia cinnamon and cascara in wet filter paper and keep in a closed bottle until they are soft enough for sectioning. Also immerse a piece of viburnum (stem) bark in dilute (1 or 2 per cent.) alkali until soft, and finally wash thoroughly in water. Cut cross-sections between pith or cork and mount in water or chloral solution. Cinnamon has an interrupted chain of colorless stone cells running tangentially about midway of the section. Cascara presents large masses of yellow stone cells at about one-third the width of the section from the outer edge. Viburnum has similar masses of stone

cells distributed over the whole section. The stone cells can easily be recognized by their structure as well as by the red stain they assume with phloroglucin and hydrochloric acid.

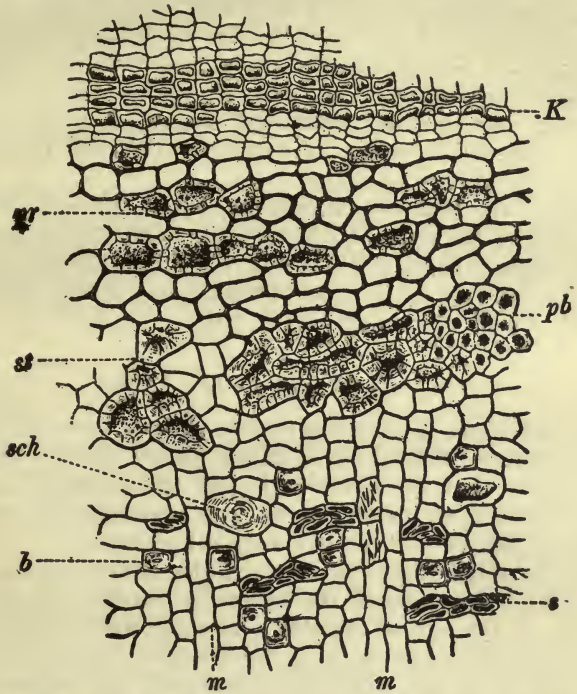


Fig. 40.—Cassia bark, cross-section. K, stony cork cells; pr, cortical parenchyma, with stony parenchyma cells; st, stone cells forming an interrupted ring; b, bast fibre; pb, a group of primary bast fibres; sch, secretion cell; m, medullary rays; s, sieve tubes (Moeller).

CHAPTER XIII.

EPIDERMAL TISSUE.

This tissue has already been met with in the exercise on the onion epidermis, but the latter is not quite a typical example, as there are no *stomata* or *breathing pores* present, which are always found when an epidermis is exposed to the air. Since the onion scale epidermis is not exposed to the air there is no need for the pores and they are consequently absent. In other respects the epidermis of other plants resembles very much that of the onion.

The tissue constitutes the primary covering of the plant. It

usually consists of one, but sometimes of two or three layers of cells. The cells are closely packed together, leaving no intercellular space except the breathing pores, and commonly they have that portion of the cell-wall which faces exteriorly considerably thickened and cutinized and are usually flattened. When seen in surface view they often appear sinuous or irregular in outline, but sometimes they are straight-sided and regular. In many plants they are somewhat elongated in the direction of the length of the organ, especially in the cells on the veins on the under surface of leaves.

The cells are rich in protoplasm. The different parts that were noted in the onion epidermis are noticed just as plainly in other cases. In most plants there are no chlorophyll bodies present in the epidermal cells, to which fact is due their transparency. Ferns are exceptions to this.

STOMATA OR BREATHING PORES.

These pores are minute apertures, usually surrounded by a pair of crescent-shaped cells called *guard cells*. These are much smaller than the epidermal cells and are much richer in proteid matters, containing a nucleus, protoplasm, numerous chlorophyll bodies and occasionally oil globules.

By means of the pores the plant exhales the superfluous water taken in by the roots and the excess of oxygen not used by it and takes in the carbon dioxide necessary for the plant's life. They always open into a large intercellular space. Thus the outside air is in free communication with the whole interior of the plant stem and leaves, since the air circulates freely through the intercellular spaces which are in communication. Communication with the interior of the plant takes place only through the pores, since the cutinized exterior of the surface of the epidermal cells is highly impregnable to water and air. Hence epidermis is an excellent protection against evaporation of moisture from the interior of the plant.

The size of the breathing pore is regulated by the guard cells, which expand or contract according as they absorb or give off moisture. The thin radial walls and the thickened outer and inner walls are so devised by nature that when, in hot, dry weather, moisture is given off by the guard cells to the air, the concave sides enclosing the opening straighten out and thus close it, thereby stopping the further evaporation. Similarly, when the air is moist, the guard cells absorb moisture, and the result is the widening of the pore and any excess of moisture in the interior may escape.

It is found by suitable tests that the outer and inner faces of the guard cells are thickened and *cutinized,* while the radial walls are *not cutinized* and very thin.

In some cases epidermis is smooth, but in the majority of cases it is roughened by hairs or glands and the walls are wavy in outline. The number and distribution of stomata varies greatly in different epidermal tissues. They are found principally on the under surface of leaves. In some leaves many are found on the upper surface, in others none at all are found.

Hold a leaf of the common trailing garden plant, known popularly as the Wandering Jew, against the side of a large cork, and with a sharp razor cut off tangentially little patches of the epidermis, both from the upper and lower surfaces. Some of the underlying green cells may be taken with the epidermis, but the edges of the sections will generally consist of epidermis alone. A few of the green cells, in fact, are desirable, as they will afford an opportunity to study incidentally chlorophyll granules.

Mount a section from the upper surface in water and examine with low and high powers. The epidermal cells are hexagonal in shape, transparent, free from chlorophyll granules and have no intercellular spaces between them. With iodine solution the protoplasm stains yellow and is more readily seen. In all essentials the cells are like those of the onion epidermis. Note that stomata *are absent.*

In the same manner examine a section from the lower surface of the leaf. The epidermal cells are pretty much the same in appearance, but scattered among them are numerous stomata, easily distinguished by the pairs of crescentic cells with dense contents and chlorophyll granules. These cells guard an opening or pore which is readily seen. With iodine solution the granules in the guard cells are very deeply stained.

Note the relative sizes of epidermal cells and guard cells and whether the latter all point in one direction or not.

In some of the sections in which the underlying green parenchyma was cut with the epidermis there may be seen long strands of spirally-marked tubular cells. These are the spiral wood vessels of a small vein of the leaf. This kind of vascular tissue will be studied in a later exercise.

Examine the epidermis from both sides of a leaf of the Cultivated Lily and compare with that of the Wandering Jew. Note the absence of plant hairs from both leaves.

Mount fragments of a Senna leaf in a few drops of chloral hydrate solution, one with the upper surface turned up, one

with the lower surface turned up and cover with a glass. Heat gently till the liquid begins to boil and keep it at that temperature for a few moments, then cool, add another drop of liquid if necessary and examine.

The leaf has become sufficiently transparent that the epidermal cells, stomata and hairs can be distinctly observed. The epidermal cells are polygonal in shape, stomata are present on both surfaces and the hairs are one-celled with thick, warty walls, often curved.

Fig. 41.—Epidermis of Wandering Jew. a, epidermal cells; b, guard cells of a stoma, with chlorophyll granules.

Often the epidermis can be separated by warming a fragment of a leaf in a solution of potassium hydroxide (about 2 per cent.) until it boils, cooling and pressing the cover glass firmly, while at the same time giving it a sliding motion. This method may be tried when chloral hydrate solution fails to clarify sufficiently.

Cross-sections of stomata will be examined in the exercises on stems and leaves.

CHAPTER XIV.

EPIDERMAL APPENDAGES.

All the outgrowths or appendages of the surface of a plant are known as *trichomes,* which means literally *hairs.* They consist of one or more cells usually arranged in a row or column, sometimes in a mass. The most common forms of trichomes are—

1. Hairs—these are the principal form.
2. Bristles—a single-pointed cell or row of cells, much thickened and hardened.
3. Prickles, like bristles, but stouter.
4. Scales.
5. Glands—generally short, bearing one or more secreting cells.
6. Root-hairs, long, thin, single-celled and subterranean.
7. Sporangia of ferns.
8. Ovules of flowering plants.

Trichomes originate mostly from the growth of single epidermal cells, and on their first appearance consist of slightly enlarged and protruding cells. These may elongate and form

single-celled hairs, which may be simple or variously branched. The most important of these hairs are those which clothe so abundantly the young roots of most of the higher plants and to which the name of *Root-hairs* has been applied. These are single cells which have very thin and delicate walls, and are the active agents in the absorption of nutritive matters for the plant. On the above-ground parts the hairs frequently have the terminal cell developed into a secreting cell, carrying gummy, resinous or other products. Such trichomes are known as *glandular hairs.*

A good example of simple hairs, which is familiar to every one, is the cotton of commerce. Cotton consists of the hairs on the seeds of the cotton plant. They have been studied in Chapter II.

HAIRS AND GLANDS AS FOUND ON A GERANIUM.

Make cross-sections of the young stem of the common Geranium, which need not necessarily be very thin. Mount several in water and examine the circumference of the sections under low power. Two kinds of hairs will be seen, simple and glandular.

Under high power the simple hairs are very long, consisting of a row of tapering cells which contain transparent protoplasm and nucleus, easily seen by applying iodine solution. The end cell is long and pointed. The hair fits in among the epidermal cells. The cell-walls are somewhat thick and the cross partitions are well marked.

There are three kinds of gland hairs.

One kind is rather long, consisting of a stalk of about six cells (count the actual number), terminated by a larger gland cell, which is round and full of contents. The two or three cells just below the gland cell are suddenly narrowed. On the top of the gland cell there is a crescent-shaped glistening mass which is found to lie in the cell-wall itself between the outer and inner layers. This mass dissolves on applying alcohol or ether, which indicates that it is probably resinous. Additional evidence of this is given by the reaction with alcannin solution, which stains the mass red. The resinous matter is secreted by the gland cell and forced out into the cell-wall where it accumulates. Iodine solution shows the presence of protoplasm and nuclei in the cells.

Soak some sections 20 to 30 minutes in strong alcohol, also

in alcannin solution and note the effect upon the resinous matter.

The other two kinds of gland-hairs are very short. One has an oblong gland while the gland cell of the other is round. Note the number of cells in the stalk of each. The cells are very short.

Note which of the three kinds is most abundant.

Submit a section to the action of chlor-zinc-iodine solution for about half an hour and then examine. The outer layer of the walls of the hair-cells is stained brown, showing cutinization; the inner portion of the walls may be blue, showing cellulose substance.

Fig. 42.—Hairs on Geranium stem. 1, simple hair; 2, 3, 4, three forms of gland hairs (reduced, from Bastin).

Try the clearing effect of chloral-hydrate solution on the gland-hairs. The contents will gradually disappear, leaving the cell-wall distinct, and the gland-cell structure may now be studied.

It is thought that the purpose of hairs and glands is to afford protection to the plant against insects, etc. The odor of the Geranium is due to the secretions of the gland-hairs.

HAIRS ON OTHER PLANTS.

For variety, let the hairs on the following plants be studied:

Clamp a piece of Mullein leaf between pith and cut several sections. Mount them in water and examine. A dense growth of branched hairs covers the leaf and beneath these, close to the surface, will be found a few short glandular hairs.

Clamp a moderately large vein (with a portion of the leaf on each side) of Stramonium, Digitalis, Belladonna and Hyoscyamus leaf respectively in pith and cut cross-sections. Examine these in water or chloral hydrate solution. Compare the hairs and make drawings. These are leaves of four important official drugs and the comparison of the hairs should therefore be of special interest.

CHAPTER XV.

STARCHES.

The green color of the leaves of plants is due to a colored substance called chlorophyll, which is diffused through certain proteid granules in the cells. The function of this substance is to utilize the energy of the sun's rays in converting carbon dioxide, the main food material of plants, into some form of carbohydrate. Starch is a carbohydrate, but that formed by the green coloring-matter from carbon dioxide is not starch. The carbohydrate in question is combined with nitrogen and sulphur (taken up by the plant in the form of salts) into a proteid by the vital processes of the cells. It is not known what the composition of the carbohydrate is nor the processes involved in building up the proteid substance.

The starch granules found in chlorophyll bodies were at one time supposed to be formed directly from carbon dioxide, but Strasburger has clearly shown that this is not true. They result from a breaking down, by the chlorophyll bodies, of protoplasm previously formed by those bodies. Starch, hence, is a result of a destructive process. It is probable also that the starch formed by amyloplasts in cells devoid of chlorophyll is also formed from proteids.

There are various reserve food materials found in the plant, and starch is one of the most important. It is found in various parts of the plant, for example, the stems of certain palms, which are gorged with it; it is the principal substance in taproots, root-stocks, corms, bulbs, tubers; many fruits and seeds, as grains, pulses, bananas.

The power of building up protoplasm from starch is possessed by all living cells, whether possessing chlorophyll or not, and independent of sunlight, but no *new* carbohydrate is ever formed without light. A tuber will sprout and grow in the dark until all the starch is used up, when growth ceases, and to renew growth it must be brought into light.

DESCRIPTION OF STARCH GRAINS.

They are hard and of various sizes and often possess shapes and markings sufficiently characteristic to identify the plant from which they come. They vary from 1 to 100 or even 200 microns. A micron or micromillimeter is one-thousandth of a millimeter and is represented by the sign μ, the Greek letter m. Starch gains are *simple* or *compound.* Compound grains consist of two or more simple grains united to form larger grains. Potato, wheat, arrow-root, corn and ginger starch are examples

of simple grains, while oat, rice and colchicum starch are compound.

Nearly all starch grains possess a *nucleus* or *hilum,* around which the granule is built up in layers, which differ from each other in transparency, owing to different amounts of water in the different layers. The layers are concentric or eccentric according as the nucleus is central or placed to one side. Examples of these are bean and potato starch respectively.

Starch grains differ from one another in a number of ways, some of which are the following:

Size and shape of grains.
Position of hilum (central or eccentric).
Number and distinctness of stratification lines.
Degree to which the hilum is fissured.
Character of fissure.

POTATO STARCH.

To see the manner in which starch is packed in the cells of the various plant organs, examine cross-sections of the potato tuber and podophyllum rhizome.

Cut an oblong block about a half-inch square on the end from a potato, having the outer coat on one side. Cut thin sections perpendicular to the coat and mount one in water. Examine first under low power and then under high power.

There will be seen on the exterior a layer of cork cells like rows of bricks. Next these are some small, closely packed parenchyma cells, rich in protoplasm, but containing few starch grains and these are small. There may also be seen cubical crystals which look like mineral crystals, but are proteid, as they stain with iodine and are dissolved by caustic potash. Farther interior the cells are very large and filled with large-sized starch grains, and the cubical crystals are wanting.

Apply dilute iodine solution. The grains assume a dark blue color, the protoplasm stains brown and the cubes the same. It will be observed that the younger starch grains in the outer small parenchyma cells are mostly found in groups. By a special method of preparation, it would be seen that the grains are grouped around a mass of proteid granules, each one of which is attached to a starch grain. These proteid granules are the starch-builders and are called *amyloplasts.* They are similar to chlorophyll bodies, and except in a few instances, all starch grains are formed by one or the other kind of these bodies.

Scrape a slice of potato with a knife and mount in water. The grains are ovate, with one end smaller than the other and a hilum or nucleus at the smaller end. The hilum is surrounded first by concentric lines which, farther away, become eccentric. This shows that at first the growth of the grain was

equal on all sides, but afterwards became much greater on one side than on the other. This view is borne out by the fact that in young grains the hilum is central. Some of the lines or striations are more strongly developed than others, and some of the grains show scarcely any lines. Nearly all the grains are single, but some double grains may be found containing two nuclei, each with concentric and eccentric markings about it and a distinct dividing line. Some grains are not double, but contain two nuclei, i. e., are bi-nucleated.

The nucleus is usually a circular spot in the potato grain, but sometimes it is fissured. The small end of the grain, where the nucleus is found, is thicker than the broad end, the grain being shaped somewhat like a clam shell. To observe the exact shape of the grains they must be seen from all sides. This is readily done by pressing gently on the cover glass with a needle, when the grains will roll over and may be seen in various positions. Another way to cause the grains to roll is to place a drop of alcohol at the edge of the cover glass, which, by mixing with the water, causes currents. When thus observed, the grains are seen to be somewhat flattened (Fig. 43, II).

Let a drop of 5 per cent. caustic potash solution run under the cover glass and watch the starch grains as the alkali comes in contact with them. They swell, and at first the layers become more distinct, but after a while they grow less distinct and finally disappear. The more watery layers at first absorb water under the action of the potash more rapidly, and thus stand out more distinctly. Finally the whole grain dissolves and disappears. Other reagents which cause starch to swell and dissolve are concentrated solutions of chloral hydrate, zinc chloride, calcium chloride, strong hydrochloric and sulphuric acids, oil of cloves.

Mount some potato starch in water and apply a gentle heat near the edge of the cover glass until the grains there become translucent, then quickly remove the flame and cool the slide. Compare the grains that have been affected by heat with those that have just begun to change and those that are still intact. Heat causes the grains to swell and ultimately to assume a gelatinous form. Intermediate granular and translucent stages occur in those grains that have not been heated too strongly. The temperature of gelatinization varies appreciably with the kind of starch.

Examine potato starch mounted in glycerin, also in oil of cloves. The grains appear brighter and the lines are almost invisible. If an air globule be imprisoned in the hilum it will appear as a dark spot. Glycerin and oil of cloves and any similar liquids are unsuited as a medium for mounting starch because of their high refractive power, which causes details of structure to be almost invisible.

OTHER STARCHES.

MARANTA.—A large proportion of the edible starches obtained from the rhizomes or root-stocks of various plants are known in commerce under the name of arrow-root. Properly the name should be restricted to the starch yielded by two or three species of Maranta, the chief of which is Maranta arundinacea. According to the country from which it is derived, maranta starch is known as Bermuda, St. Vincent, West Indian or Natal arrow-root. The grains are simple and mostly ovoid, the largest ones being marked by fine striations, but less distinct than those of potato starch. The nucleus is rounded, linear or star-shaped and usually placed eccentrically. The grains are rather large, but smaller than potato starch.

TOUS-LES-MOIS, *Tulema* or *Queensland* arrow-root is obtained from several species of canna, a genus closely allied to maranta. The grains resemble those of potato starch, but are much larger.

CURCUMA or *East Indian* arrow-root is obtained from the root-stocks of several species of the genus Curcuma (Zingiberaceæ) chiefly Curcuma angustifolia. The grains are ovate and taper to a nipple-like projection at one end, in which the hilum is located. The grains are simple, rather large and so flat that on edge they seem rod-shaped (Fig. 43, VIII). Ginger starch is shaped somewhat like that of curcuma.

BRAZILIAN arrow-root, cassava, or tapioca of commerce, is manufactured from the starch obtained from the tubers of Manihot utilissima. Most of the grains are blurred, which is due to the heating in the process of manufacture, but many grains may be found uninjured. Soak some of the starch a few hours in water, mash a little out on a slide in water, cover and examine. The unchanged grains appear as in Fig. 43, IX; the others are more or less gelatinized.

BRITISH arrow-root is potato starch, which is sometimes sold under this name. The French excel in the preparation of imitations of the more costly starches from potato starch. Its chief use, however, as an edible starch, is for adulterating other more costly preparations. It can easily be distinguished under the microscope.

SAGO, or pearl sago, of commerce is obtained by heating and stirring the moist starch of the sago palm, Metroxylon Sagu. As in the case of tapioca, many of the grains are gelatinized, but some are still intact, which appear as in Fig. 43, X.

MAIZE or corn starch is obtained from the fruits of Zea Mays. The grains are simple, rounded or polygonal and tolerably uniform. The hilum appears as a point or more often as a stellate cleft, less frequently as a large central cavity. This starch is one of the most frequently used adulterants of powdered articles, and hence should be closely studied.

WHEAT starch is obtained from several species of Triticum. It consists of large rounded grains and numerous small ones. Hilum and striations are seldom visible. The grains are oval or concavo-convex in shape. Wheat starch is also used as adulterant.

RICE starch comes from Oryza sativa, and may be examined after soaking for three hours or more in water. The grains are

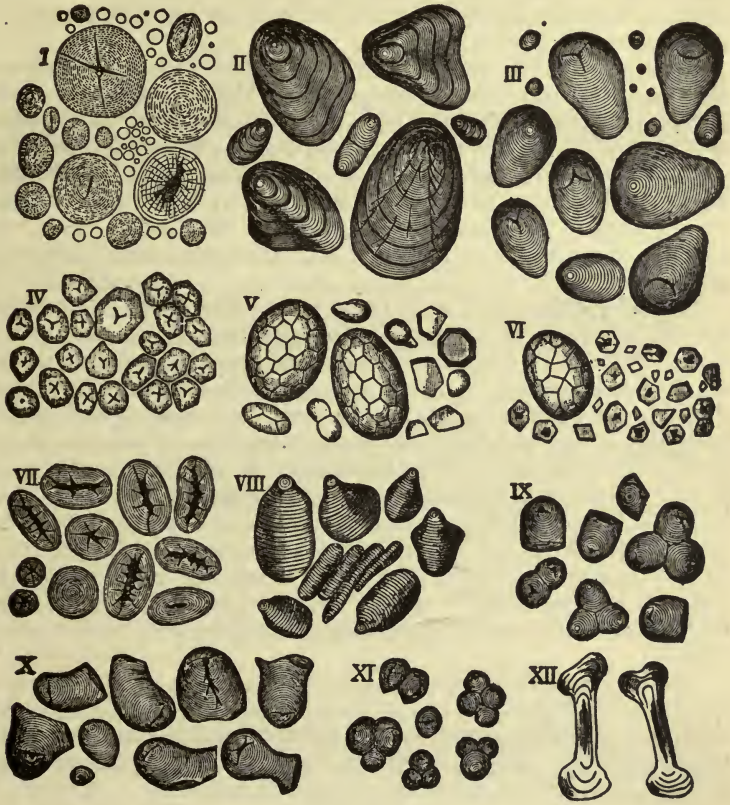


Fig. 43.—Starches.

I, Wheat; II, Potato; III, Arrow-root; IV, Corn; V, Oat; VI, Rice; VII, Bean; VIII, Curcuma; IX, Tapioca; X, Sago; XI, Sarsaparilla; XII, Euphorbia.

very small and angular, with no hilum. The small grains result from the breaking apart of larger compound oval grains, some of which may be present in the mount.

OAT starch from Avena sativa resembles rice starch very much. Some of the granules are rounded, semi-circular or lemon-shaped, which helps to distinguish it from rice starch (Fig. 43, V and VI).

CHAPTER XVI.

ALEURONE GRAINS.

Protoplasm exists both as active and inactive. In the active state, as found in actively-growing cells, it exhibits vital phenomena in a marked degree, but, as found in the cells of seeds, tubers and thickened roots, it exhibits few signs of vitality, contains comparatively little water and its condition approximates that of a solid.

Ordinary protoplasm is formless; even under the highest powers it exhibits no structure except the presence of numerous very minute granular bodies called *microsomes,* the nature and uses of which are not yet understood. It passes, however, into several modifications which exhibit a more or less characteristic structure. The most important of these is the chlorophyll body. The amyloplasts, spoken of under starch, are another form with structure. These are active forms of protoplasm.

Alerone grains are a structural form of inactive protoplasm, the use of which is to act as a reserve food material. Plants lay up a store of food in various forms, one of which we have studied, i. e., starch. Other non-proteid food materials are oil, inulin, sugar. Some albuminous forms are also stored up, the most important of which are aleurone grains. These are found chiefly in seeds and most abundantly in oily ones. They are usually rounded granules, often very small, but sometimes quite large, as in Croton and Castor seeds and in the Brazil-nut. In some cases the granules appear homogeneous in structure, as in peony, almond, cherry and apple seeds; in other cases they contain various substances, as oily matters, mineral crystals and crystalloids, as in Brazil-nut, pumpkin and castor seeds, walnut.

The ground substance of aleurone grains is dissolved or attacked by water, hence the latter is an unsuitable mounting medium. Alcohol, glycerin or fixed oils have no solvent action on the grains. Dilute alkalies readily dissolve the aleurone grains except the mineral matter in them.

CASTOR SEED.

Remove the hard seed-coat and make thin sections of the endosperm. Mount one in strong glycerin and examine with low and high powers. Not much will be made out with low power. The cells will be seen to be crowded with rounded granules, looking much like starch.

Under high power, rounded or ellipsoid bodies, imbedded in a finely granular matter fill the cells. These are the aleurone

grains. After a little time the clearing effect of the glycerin shows that the grains are not homogeneous, but contain a denser, polygonal body looking like a crystal and known as a *crystalloid* which varies in size, being often nearly as large as the grain. Lying alongside of this is seen a globular body, strongly refractive and composed of magnesium and calcium phosphates and known as a *globoid* (Fig. 44).

Add strong iodine solution to the section in glycerin. The grains stain brown, especially deep in the crystalloid, indicating the proteid nature of the latter. The globoids remain unstained.

There is no blue coloration, showing the absence of starch.

Mount a fresh section in water and watch the aleurone grains. The ground substance soon swells and dissolves, leaving the crystalloid standing out more distinct. After a time this also swells and loses its angles and finally disappears. Oil globules may also be seen to collect and run out from the ruptured cells. These have a refractive appearance, being bounded by a dark band.

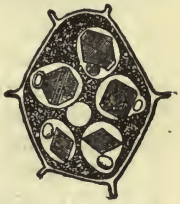


Fig. 44.—Aleurone grains in cell of castor seed, showing crystalloids and globoids (Sachs).

If fresh sections be mounted in alcannin or cyanin solution the oil globules will stain after a time deep red or blue. Oil is a reserve food for the plant, like starch and aleurone, and is found in a great many seeds and spores, often in large quantity.

Mount a section in dilute alkali (about 1 per cent.) and watch a grain closely. All parts dissolve except the globoid.

CHAPTER XVII.

CHLOROPLASTS OR CHLOROPHYLL CORPUSCLES.

When speaking of starch, it was noted what importance these bodies are to the life of plants, and that without them plants could not exist. They are the most important structural form of protoplasm. They are the bodies which give the green color to plants and are commonly rounded, oblong or flattened in shape. An odd form is the spiral bands in Spirogyra (which see).

Chloroplasts are proteid in nature, having the power of growth and division, and always closely associated with ordinary protoplasm, and are hence to be regarded as part of the living protoplasm.

In many plants chloroplasts are small and difficult to study, but in others they are easily studied. Most any moss, the

prothallia of ferns, Eel-grass, Water-weed, are excellent objects of study.

Mount a few fresh leaves of a moss in water and examine the cells lying near the edge of the leaf. The cells contain numerous rounded greenish bodies—the chlorophyll bodies. Note the closely-packed, rectangular-shaped, somewhat thick-walled cells. Try to observe chlorophyll bodies that are constricted in the middle. Such are in the act of division.

Run strong alcohol under the cover glass. The green bodies are slowly bleached, the chlorophyll is dissolved out and forms a greenish solution in the cells. From this experiment it is concluded that the bodies consist of two parts, a proteid ground work, through which is diffused the chlorophyll or green color substance.

Add iodine solution to a leaf that has been bleached with alcohol. The transparent protoplasm of the cells is now stained brown and made visible. The ground substance of the chloroplasts also stains brown, indicating a proteid substance.

Starch grains in chloroplasts. Put some leaves of a vigorously-growing moss that has been exposed to sunlight for two or three hours in alcohol until bleached. Then mount a leaf in water, focus it and apply a drop of chloral-hydrate iodine solution at the edge of the cover glass and watch closely the chlorophyll body as the reagent comes in contact with it. The ground substance is seen to swell rapidly and becomes transparent, leaving the starch grains stained blue, visible. The latter are small and elongated. By the continued action of the chloral the whole structure gradually disappears. The starch that is formed in the chlorophyll bodies in the daytime is dissolved during the night and transferred to other parts of the plant.

CHAPTER XVIII.

SECRETION SACS, INTERCELLULAR AIR SPACES AND SECRETION RESERVOIRS.

Some cells at maturity lose their protoplasm and their proper cell character and become filled with secreted matters. These form the *secretion sacs*. They are of various forms, but more commonly resemble parenchyma cells in appearance and character of their walls. Sometimes they are much elongated and resemble latex tissue. The sacs are given names according to the secretion they contain, thus, resin sacs, mucilage sacs, etc.

Intercellular air spaces are more or less abundant in nearly all multicellular plants, their probable function being to supply air to the interior tissues for respiratory purposes. In aquatics the spaces are usually large and often regular in shape, while

in most terrestrial plants they are small and angular. Air spaces are of two kinds, *schizogenous* and *lysigenous*. The former are formed by the splitting of the cell-wall common to two or three cells. The latter result from the breaking down of some cells, leaving a space. Secretion reservoirs or canals and intercellular air spaces differ from each other only in their contents, the former containing resins, gums, oleo-resins, etc., the latter only air.

INTERCELLULAR AIR SPACES AND RESIN SACS.

CALAMUS (SWEET FLAG) RHIZOME.—Make thin cross-sections of the fresh rhizome or of the dried one after having soaked it in water to soften it. Mount a section in water and examine the outer third of the section. The parenchyma cells are loosely arranged in chains with large and tolerably regular intercellular air spaces. Most of the cells are filled with starch grains, together with protoplasm and nucleus, but scattered here and there among these are larger spherical cells filled with a

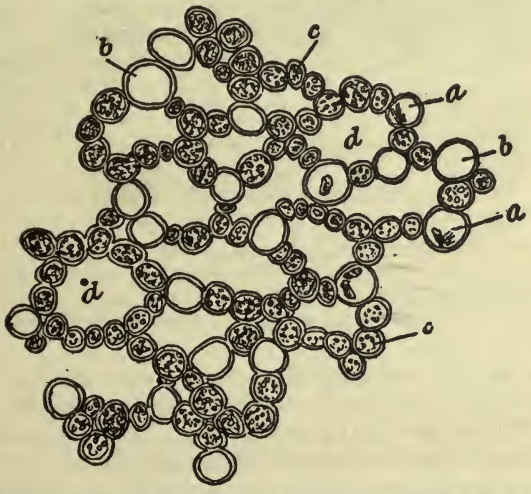


Fig. 45.—Part of cross-section of Calamus rhizome. a, volatile oil and resin sacs; b, sacs with transparent secretion; c, starchy parenchyma cells; d, air space (Bastin).

refractive or glistening material, sometimes intermixed with a brownish solid. These are the secretion sacs (Fig. 45). The refractive contents of the sacs are not saponified by caustic potash, which fact indicates a volatile oil.

Further evidence in favor of this conclusion is given by applying cyanin solution to a section, when after some time the contents are stained blue. The solid brown matter found in some of the cells is resin and also stains blue with cyanin.

GINGER RHIZOME.—Cut cross-sections from the dry rhizome, mount a thin one in water and examine with low power first. The section is made up for the most part of large starch grains in ordinary parenchyma cells, but here and there may be seen groups of thick-walled light yellow cells, the fibres and vessels of the wood bundles. In addition to these there will be seen more or less distinctly lighter or darker yellowish homogeneous masses. They are the oleo-resinous contents of secretion sacs which are scattered over the whole section. If dilute iodine solution be added the section turns dark, but the groups of woody cells now stand out more conspicuously in contrast by their deep yellow walls and the resin sacs also.

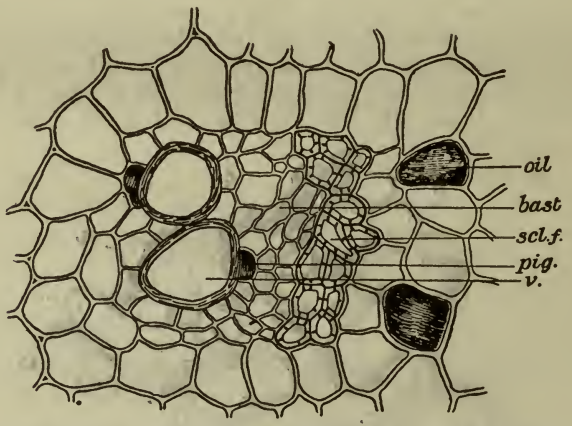


Fig. 46.—Cross-section of Ginger rhizome showing a vascular bundle and parenchyma; oil, oleoresin sacs; scl. f., bast fibres; v, wood vessel (Vogl).

To remove the starch grains which interfere by their great numbers mount a section in chloral hydrate solution and warm gently. The shape of the parenchyma, wood bundles and the secretion sacs can now be seen more easily.

Mount a section in caustic alkali (5 per cent.). The starch dissolves and the secretion sacs turn a deep reddish-brown, because of the union of alkali with the resin. In the liquid of the sacs unchanged oil drops are visible. Use high power also in the above experiments and also study the starch grains carefully.

MUCILAGE SACS IN MARSH-MALLOW ROOT.

Wrap a piece of the root in wet filter paper until it is soft enough to cut. Make cross-sections and place them in dilute

alcohol (not water, because it acts on the mucilage). Mount a section in dilute alcohol or glycerin. Smaller cells densely filled with starch constitutes the greatest proportion of the section. Scattered irregularly among these are numerous larger rounded sacs containing a transparent substance—the mucilage sacs.

Add a drop of iodine solution to a fresh section. Most of the cells turn dark (starch), but the mucilage sacs remain as before. In the large central area groups of yellow-stained wood bundles are visible, while in the cortical layer groups of similarly stained bast fibres are seen. A distinct circle of small cells, free from starch, divides the cortical layer from the larger central area. It is the cambium zone.

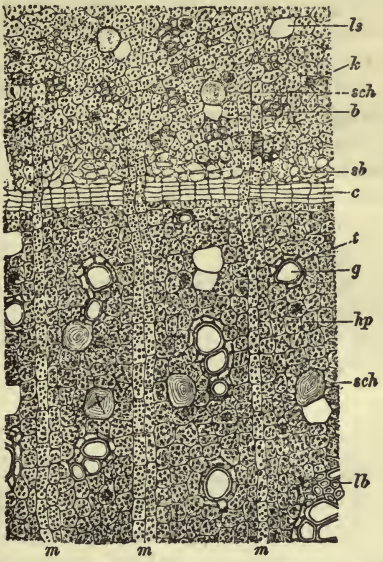


Fig. 47.—Cross-section of Althæa root. sch, mucilage sacs; c, cambium; b, bast fibres; lb, a bundle of wood vessels and fibres; m, medullary rays (Tschirch).

Remove nearly all of the liquid from the iodine section and add a drop or two of sulphuric acid (diluted with one-fourth water). The mucilage is stained a deep brown and the walls blue (cellulose). Under favorable conditions delicate concentric lines may be seen in the mucilage.

Soften a piece of Elm bark and examine cross-sections in the same manner as in the case of marsh-mallow root. It has large and numerous sacs.

MUCILAGE AND RAPHIDE SACS IN SQUILL BULB.

Crystals are of such general occurrence in widely different orders of the higher plants that there are perhaps none in which they may not be detected. They have been found in nearly all parts of the vegetable structure, more commonly in the interior of parenchyma cells, sometimes in specialized crystal cells.

They occur either singly or in groups. The most common forms are the octahedron and the prism. Sometimes the crystals are much elongated and pointed, like a needle, and are then known as raphides (from raphis, a needle). These are gener-

ally massed in a compact bundle, like a wheat-sheaf, occupying a large part of the interior of the cell. Raphides are by no means of such general occurrence as ordinary crystals, being restricted to certain orders.

Soak a slice of the scale of a squill bulb in water just long enough to soften it and then place in a mixture of alcohol and glycerin 6-8 hours. Make thin sections and mount in glycerin. The tissue is composed of large, clear, typical, parenchyma cells, with here and there a cell filled with long needle crystals, together with mucilage.

Apply a drop of acetic acid. The needles do not dissolve. To another section add hydrochloric acid; the needles dissolve without effervescence, showing that they are calcium oxalate. Had they been carbonate they would have dissolved in acetic acid with effervescence.

SECRETION RESERVOIRS.

These differ from intercellular air space only in being filled with secretions instead of air. They differ from secretion sacs in that they are spaces surrounded by a number of cells, while the sacs are single cells. The reservoirs are often merely irregular spaces left by the breaking down of one or more cells, but they sometimes have a remarkable regularity of form and clearness of outline.

It has been observed that these spaces are not, as a rule, met with in plants having the simple secretion sacs. The cells which surround the more complete cavities are quite different from the other parenchyma cells, and they are collectively called the *epithelium* of the spaces. These are the secreting cells and the secreted matters are discharged in some manner into the reservoirs, where they accumulate.

Cut cross-sections of a pine needle between pith and mount in water or chloral hydrate solution. Arranged around the section near the periphery are five or six cavities, each having a lining epithelium of flattened, thin-walled cells, and next to these is a circle of glistening, very thick-walled cells (Fig. 62). These structures are channels which carry the turpentine secretion. Also make cross-sections of the stem of a softened clove bud and mount in water. Near the circumference will be found a double row of large cavities, each lined with a membrance of secreting cells. The cavities carry the "oil of cloves."

CHAPTER XIX.

Wood (Libriform) Fibres and Bast (Liber) Fibres.

These fibres belong to the prosenchymatous series of tissues. This series embraces those cells which at maturity lose their nuclei and protoplasm, and, therefore, their distinctively cellular character, and have their walls thickened by secondary deposits. They sometimes contain starch and traces of proteid matter, but take no active part in the nutritive processes of the plant. They serve it mainly for strengthening or supporting, and hence have been called mechanical tissues. They are serviceable also in conducting the sap. The elements of these tissues are, for the most part, elongated and oblique-ended or taper-pointed. Among the shorter cells transitions occur. Between them and sclerotic parenchyma, and between the fibrous forms and collenchyma, every gradation may be found.

Bast and wood fibres are found in the so-called fibro-vascular bundles of the plant. These bundles constitute the fibrous framework of the plant, corresponding somewhat to the bony skeleton of the human body. In the leaf they are the system of veins, and in the stem and root the tough resistant portions. Their function is partly to give strength and partly to conduct the fluids of the plant. The cells composing the bundles, therefore, for the most part, have their walls thickened and are elongated in the direction of the length of the organ bearing them. They belong chiefly to the prosenchymatous series, although other tissues are commonly included in the bundles. In some plants, as the stem of the Indian Corn and the petiole of the Plantain, the bundles may be readily separated in the form of tough, stringy masses from the softer surrounding tissue. (Break the petiole of a Plantain leaf and note the tough threads protruding from the broken end. These are the bundles).

Although a number of kinds of tissues are usually found in the fibro-vascular bundles, only two kinds are really essential, namely, *ducts* (and tracheids, which may be regarded as imperfectly-formed ducts) and *sieve* cells. These and their associated tissues always constitute separate longitudinal portions of the bundle. The nature of ducts and sieve cells will be explained later. The portion of the bundle to which the ducts belong is called the *xylem,* which means the *wood,* and that to which the sieve cells belong is called the *phloem* or *bast* (phloem means *bark*). The reason for calling this the bast or bark is the fact that the inner bark of gymnosperms and dicotyledons, also called the bast layer, is composed of the phloem portions of the bundles, which are arranged in a circle. Within these

are the xylem portions of the bundles, forming the cylinder of wood.

The term bast was originally given to the inner bark of the Linden tree, which was called Bass-wood tree. It is now applied to the inner layer of any bark. The term liber, which is also given to the inner or bast layer, was applied in a more general way to any smooth, inner bark upon which one could write (liber is the Latin for book).

The peculiar, long, tough, thick-walled cells which impart toughness to the inner bark, making it valuable in the arts, are hence known as bast or liber fibres. It is in the bark of dicotyledons that liber fibres occur most abundantly in the phloem portions of the bundles.

Some plants, for example, the monocotyls, have no true bark, consequently the bast or phloem portions of the bundles do not lie in the inner or bast layer of the bark, and the bast fibres of such bundles are apparently misnamed. However, the term liber or bast fibres has been extended to embrace all those fibres that occur in the phloem portions of the bundles, whether they occur in the inner bark or elsewhere in the plant and whether they occur in gymnosperms, dicotyls or monocotyls.

The term libriform has reference to the general resemblance of the wood fibres to bast or liber fibres. Wood fibres usually differ from liber fibres in being relatively less elongated, less tough and flexible and less strongly lignified at maturity, but there are many exceptions, especially in monocotyls, where the two tissues are often indistinguishable by structure alone. The fibres vary often in length.

Wood and bast fibres may be conveniently studied in a moderately stout Geranium stem, in which they have already been noted superficially in Chapter IX. Make thin cross-sections of a Geranium stem and study without staining and also by staining with phloroglucin and hydrochloric acid (red).

The wood circle lies within the cambium zone and surrounds a central area of parenchyma cells, the pith. It is composed of the xylem portions of numerous bundles which have grown together into a solid ring of woody tissue. The wood fibres, in cross section, are seen to be very thick-walled, compactly arranged, more or less compressed laterally by mutual pressure, so as to appear angular. They lie next to the cambium zone. The cells are separated by a distinct line, the middle lamella. The walls are lignified, as shown by the red stain, the middle lamella being deeper red than the rest of the walls. There are no intercellular spaces. The cells are unequal in size and irregularly arranged. There are delicate stratification lines and occasionally pore-canals in the walls, though these are seen with difficulty.

In the wood ring there are other cells besides those just described, with much larger openings, the ducts or tracheids, which will be described a little later. Wood fibres do not occur in all fibro-vascular bundles, but are nearly always present in woody and herbaceous dicotyls.

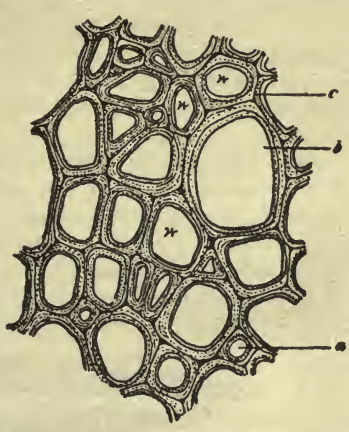


Fig. 48.—Portion of cross-section of wood ring of Geranium stem. w, wood fibres; b, duct; c, middle lamella (Bastin).

BAST FIBRES.—Outside the cambium zone is another circle of thick-walled cells, looking in all respects very much like the wood fibres just described. These are the bast or liber fibres. The description of the wood fibres answers also for the bast fibres. With phloroglucin and hydrochloric acid they stain red and the middle lamella deepest. Stratification lines and pore canals can also be seen with care. In many cases bast fibres are not as strongly lignified as wood fibres and take the stains less deeply. In such cases the lignification is chiefly confined to the outer layers of the cell-walls, while the inner layers are more or less cellulose in nature; with double stains, as methyl-green and eosin the outer layers will stain green and the inner ones reddish.

Bast fibres are confined not alone to the phloem or bast portion of fibro-vascular bundles, but are often found in other portions of the plant, for example, the strengthening fibres beneath the epidermis of some leaves; in the ground tissue of many vascular cryptogams. As a rule, bast fibres are thicker-walled than wood fibres, but both kinds may vary considerably in the thickness of the walls, in their lengths as compared with their diameters, in the number of pore canals.

WOOD AND BAST FIBRES IN LONGITUDINAL SECTION.

Treat several longitudinal radial sections of the Geranium stem with Schulze's macerating fluid, as directed under this reagent. Wash them in water and place them in very dilute methyl-green solution for a long time, say 24 hours or more, which will stain only the lignified walls and the cork cells. Mount in water and examine first with low power. On the outer edge is a green layer—the cork cells—scarcely at all affected by the macerating fluid. Next to this is a transparent layer of unstained parenchyma cells more or less injured by the

corrosive fluid—the middle bark. Then comes a narrow strand of deeply-stained cells, which on the ends of the section may be seen to be fibrous, and also farther in if the section be not too thick, in which case there may be observed how the fibres are spliced over one another. Next to the bast is another colorless, narrow layer composed of the soft bast and cambium. Adjacent to this is the broader strand of green wood fibres and vessels.

Now tap gently with a teasing needle on the cover glass directly over the sections. The macerating fluid has dissolved the middle lamellas, so that by tapping the cells spread apart and are easily studied individually. The fibres are very long, the wood fibres being often as much as 20 or 30 times as long as wide and the bast fibres even longer; the ends taper to a point, sometimes, however, abruptly, and at times are forked.

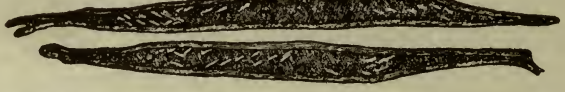


Fig. 49.—Longitudinal view of fibres from stem of Geranium (Bastin).

The walls are smooth, and with careful staining and good light delicate slits are seen running obliquely across the walls. Fig. 49 illustrates both bast and wood fibres in longitudinal section.

Sometimes bast and wood fibres are found which are made up of a row of two or three cells and then they have cross-partitions in the lumen or cavity of the fibre.

BAST FIBRES OF CINCHONA BARK.

Bast fibres vary greatly in length and lignification. There are some extremely long, flexible, slightly lignified fibres, for example, Flax, Hemp, Mezereum, on one extreme, and very short, brittle, much lignified fibres on the other extreme, as found in Cinchona bark.

Make cross and longitudinal radial sections of Cinchona bark (calisaya or succirubra) that has been softened in water or alkali.

Examine first a cross-section with low power. In the inner bark there are numerous yellowish, solid-looking quadrangular cells, the cavities of which have been reduced nearly to a point by the great thickening of the cell-walls. These are the bast fibres. They occur singly or in small radial groups of three or more. Incidentally, the student should note carefully the structure of the whole section, as an example of a typical well-developed bark. On the outer edge is a thick layer of small cork cells, deeply colored with natural plant pigment. These have a tendency to flake off in places and are divided here and

there by transverse clefts. Next to the cork is the middle bark, consisting of lighter colored, tangentially elongated parenchyma cells. In it is a tangential row of secretion channels. Next to the middle bark is the inner or bast bark, which is divided into compartments by distinct radial lines of cells, two or three rows wide, which are the medullary rays. Between these rays occur the bast fibres and a tangled network of smaller thin-walled cells, consisting of ordinary parenchyma and sieve tubes, the latter not being easily made out.

Under high power the excessively thickened walls of the bast fibres are made up of layers which are finely stratified. The walls are also crossed by radiating fine canals (Fig. 50). In a section stained with methyl-green solution the walls are seen to be strongly lignified, but more so in the exterior layers than in those next to the cavity, as is shown by the different depths of color.

Fig. 50.—Bast fibres of Cinchona calisaya in cross and longitudinal section, showing narrow cavity, pore canals and stratification of walls (reduced, from Bastin).

LONGITUDINAL SECTION.—In this the cork and parenchyma look pretty much as in cross-section, but the secretion space, if the section contain one, appears as a long, empty channel, and the medullary rays appear as isolated plates of cells lying across the section. The bast fibres, which are seen best at the ends of the section, are relatively very short, being about six times as long as wide, fusiform or wedge-shaped on the ends. The cavity appears as a line and the stratification is distinct. The fibers are also marked by cross-lines radiating from the cavity (Fig. 50).

Macerate a section in Schulze's solution, as in the case of the Geranium section, wash, mount and separate by tapping on the cover glass. The bast fibres are separated and may be studied more easily. Stain a macerated section in methyl-green, tap out on a slide and note the effect of the stain.

CHAPTER XX.

TRACHEARY TISSUE.

This includes tracheids and ducts. When speaking of the fibro-vascular bundles, it was said that the xylem portion of the bundle was that which contains the wood cells and tracheids or ducts. The peculiarity of tracheary cells is that the walls are thickened unevenly, the thick parts being on the inside of the walls and arranged in various forms, giving rise to *spiral, reticulate, scalariform, annular, dotted* ducts, etc.

The cells of tracheary tissue are usually less thick-walled than wood fibres and of larger diameter, and mostly oblique-ended or blunt. They are more widely distributed than wood fibres, as they are found in all vascular plants, i. e., plants containing bundles. (The name fibro-vascular means that there are both fibres and vessels or ducts in the bundles.)

Ducts and tracheids are alike in regard to the markings of the walls. The difference is that a duct is composed of a row of tracheids in which the separating partitions have been absorbed, leaving a duct or vessel. A tracheid is a single cell complete on all sides. The name tracheary signifies the resemblance between some of the ducts and the trachea or wind-pipe of man.

TRACHEARY TISSUE IN GERANIUM STEM.

Make longitudinal radial sections and macerate in Schulze's solution, wash and stain in methyl-green, mount in water. The following ducts may be found in the xylem of the bundles, especially if the cells be separated by tapping upon the cover glass.

RETICULATE DUCTS.—So called because the thickenings in the cell-wall are in the form of a network, giving a pitted appearance to the wall. The walls are somewhat prismatic and the pits occur on the flat sides. By means of them a lateral osmotic circulation is kept up with other ducts. Notice in some of the ducts, on the oblique ends, large openings where one cell communicates with another. Reticulate ducts are the most numerous in the Geranium, as well as most plants (Fig. 51).

SPIRAL DUCTS.—Widely distributed in plants. The thickening in the wall takes place in a perfect spiral, the part between the turns being very thin and almost invisible and cellulose in nature. There may be two spirals in a duct, one within the other. In other plants ducts with more than two spirals are found. Sometimes the turns of the spirals are connected by cross-thickenings, and then the duct merges into a reticulate one. Again, the thickenings may be spiral on one end of the

duct, but pass into separate circles on the other, forming an *annular duct*. This kind of duct is found associated with the spirals in the Geranium. The two are closely related. The spirals and rings may be close together or wide apart and the rings may be at various inclinations to the length of the duct. The spiral ducts are smaller in diameter than the reticulate (Fig. 51).

SCALARIFORM DUCTS IN FERNS.

These ducts have their thickenings arranged like the rounds of a ladder, hence the name *scalariform*. They occur in many plants, but they are seen to best advantage in the ferns, where they are beautifully developed and almost to the exclusion of all other woody tissue in the bundles.

Make thin longitudinal radial sections of the rhizome of the official Male Fern or of the Eagle Fern (Pteris aquilina), stain in methyl-green or phloroglucin and hydrochloric acid and mount in water. The ducts will be stained green or red; they are mostly prismatic or flat-sided where they press upon one another, and at the edges where the sides meet there is a thickened ridge. Crossing the flat faces are numerous parallel thick ridges separated by very thin places looking like slits. The faces have the appearance of a ladder. The ducts are oblique or taper-pointed, large in diameter and the ends splice over one another (Fig. 51).

Isolate the ducts by maceration in Schulze's solution as in the previous cases.

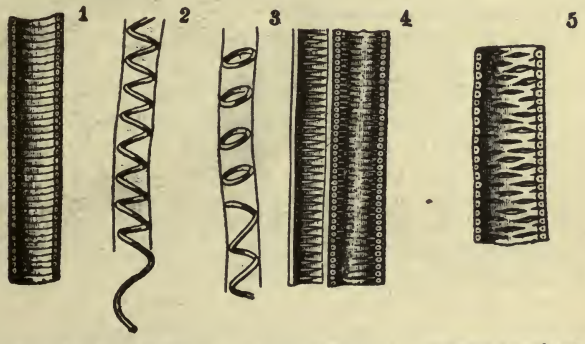


Fig. 51.—Various tracheary ducts. 1 and 2, spiral; 3, combined annular and spiral; 4, scalariform; 5, reticulated.

TRACHEARY TISSUE OF GYMNOSPERMS.

TRACHEIDS WITH BORDERED PITS. — These peculiar cells, although occasionally found in other plants, are characteristic of gymnosperms. In these plants ducts and wood cells are rare, being replaced by tracheids, which constitute nearly the whole

of the wood. The tracheids are so peculiar in structure that one may distinguish a gymnosperm from other plants. A few genera of gymnosperms can be recognized by the number and regularity of the markings on the tracheids.

Soften a piece of pine wood by soaking a long time in dilute alkali. After washing thoroughly in water, make longitudinal radial sections by cutting at right angles to the rings of growth. Mount a thin section in water or glycerin. The tracheids are long, tapering fibres, similar to wood fibres, but larger. The surfaces of the cells are marked by a row of pits, each pit being surrounded by a smaller circle inside of a larger one. At times the pits are close together; at other times they are wide apart. Lying across the fibres at intervals are rows or plates of cells of the medullary rays. Try the action of phloroglucin and hydrochloric acid on a section.

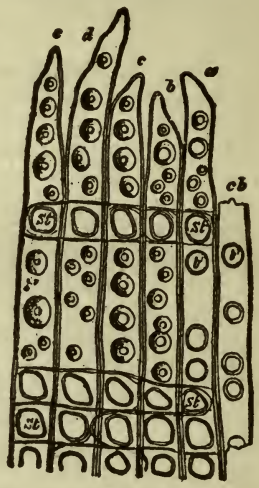


Fig. 52.—Tracheids of pine showing bordered pits, with medullary rays crossing the tracheids (Sachs).

Now make longitudinal tangential sections by cutting parallel with the rings of growth. In such a section no pits are found on the faces of the cells, but they may now be seen in section on the edge in the cell-wall. In other words, the peculiar pits occur only on the radial faces of the cell-walls.

Find a pit cut exactly through the middle. It forms a lens-shaped cavity in the wall between two cells, opening into the two cell cavities by circular orifices which in flat view appear as the inner small circle of the pit (see above). Running lengthwise through the pit and closing off the cavities of the two neighboring cells is the middle lamella. Here and there between the tracheids occur rows of two or three rounded cells, which are not to be mistaken for the pits. They are much larger, being the lignified parenchyma cells which form the so-called medullary rays.

CHAPTER XXI.

Latex or Milk Tubes.

Many plants, when wounded, emit a milky fluid varying in color, copiousness, consistency and chemical composition in different plants. This is called the *latex,* hence the name laticiferous tissue. This tissue differs considerably in different plants and is not confined to any particular region or tissue system, but it is most common and abundant in ordinary parenchyma.

The cells of milk tissue are of two kinds—simple and complex or branching.

The simple latex tubes consist of long cells of indefinite length running lenthwise of the plant with only a few branches. Each tube with its few branches is believed to be a single cell. In cross-section they are distinguished from parenchyma cells by their smaller diameter and by being filled with opaque and densely granular substances. The cell-walls are cellulose. The complex tissue consists of greatly branching tubes, the branches uniting cross-wise and forming a complex network in the plant.

Latex is of the nature of a waste or excretory product, although it contains albumin and carbohydrates. It contains resins and gums in solution and oily matters, often alkaloids and organic acids. It coagulates and forms a sticky mass upon exposure to the air. India rubber is an example of such dried latex. Latex varies in color in different plants; it may be white, yellow, orange, etc. In Bloodroot it is reddish.

Stems of plants in which latex tissue is to be studied should be cut into pieces and immediately put into strong alcohol, which coagulates the latex and prevents it from running out of the tubes.

Simple Latex Tubes.

These may be studied conveniently in Euphorbia and Milkweed plants. Make cross-sections of the stem of one of the Milkweeds, stain in methyl-green and mount in water. The milk tubes occur in the pith and bark and are distinguished from the neighboring parenchyma cells by their smaller size and densely granular, more deeply-stained contents.

Examine a longitudinal section stained in methyl-green. Lying among the parenchyma cells will be found long tubes filled with dense, granular matter, wavy and with only an occasional branch. The branches, when present, do not unite with those of a neighboring tube. Each tube, however long, may be looked upon as a single cell.

Apply chlor-zinc-iodine. The cell-walls stain blue and the contents a brown, showing presence of albuminous matter. Alcannin or cyanin solutions would show the presence of oily or resinous matters. These are held in suspension by emulsion and give the latex the milky appearance.

COMPLEX OR BRANCHING LATEX TUBES.

These occur in a number of plants, for example, Dandelion, Chicory, Celandine, Poppy, etc.

Make cross-sections of Dandelion and Chicory roots, stain with hæmatoxylin solution and wash thoroughly in water.

The milk cells are arranged in small groups, and these form, in the Dandelion, concentric circles throughout the whole bark, which, to the naked eye and under low power, seem complete. Under high power the circles are interrupted here and there. In Chicory the milk tubes are arranged in radiating lines through the bark, and by means of the milk tubes alone Dandelion and Chicory can readily be distinguished under the microscope. The milk cells stain more deeply than the surrounding parenchyma cells and thus stand out conspicuously.

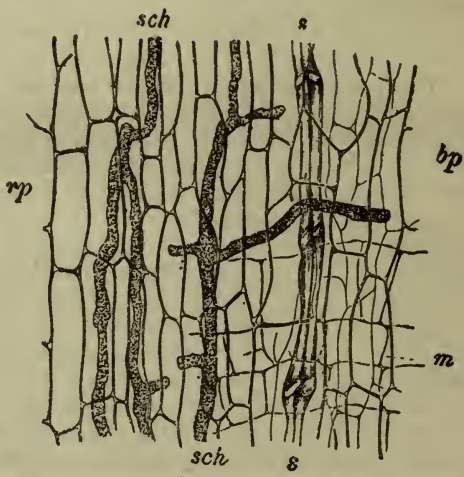


Fig. 53.—Longitudinal radial section of Chicory root, showing branching latex tubes, sch (Moeller).

Study longitudinal radial section of Dandelion and longitudinal tangential section of Chicory.

The milk ducts form a tangled network in the elongated parenchyma cells of the bark of the root. There are numerous cross-branches connecting the tubes.

CHAPTER XXII.

Fibro-Vascular Bundles and Types of Stems.

The nature of a fibro-vascular bundle was considered in the lessons on wood and bast fibres and tracheary tissue, but the various types of bundles were not discussed. These will be considered now.

According to the relative arrangement of the xylem and phloem masses three kinds of fibro-vascular bundles are distinguished, namely, *collateral, concentric* and *radial.*

The collateral type is characterized by having the xylem and phloem masses lying side by side with the xylem facing towards the pith or center of the stem and the phloem towards the exterior. In the veins of leaves the xylem faces the upper or ventral surface, the phloem the lower or dorsal surface. Collateral bundles are characteristic of the stems and leaves of nearly all flowering plants. They seldom occur in roots. There are two varieites of the collateral type:

The *ordinary* bundle, containing one phloem and one xylem mass, and the *bicollateral* bundle, in which there is one xylem mass between two phloem masses or vice versa. The second variety is found only in the stems of gourd plants (Cucurbitaceæ) and a few others. Some collateral bundles continue to increase in thickness during the life of the plant, and the growing layer is located at the junction of the xylem and phloem, forming a cambium or meristem zone of the bundle. Such bundles are called *open bundles,* while those which have no cambium zone, and thus soon cease to grow, are called *closed bundles.*

The open bundles are characteristic of the *stems* of woody dicotyls. An illustration of this kind of bundle has been seen in the Geranium stem. The stems of most monocotyls contain the closed collateral bundles.

Concentric Bundles.—These have a central xylem mass surrounded by a phloem mass or vice versa. There is no cambium zone in this type. The bundle with xylem central is characteristic of nearly all ferns and club mosses. The one with phloem central occurs only in stems and leaves of some monocotyls.

Radial Bundles.—In these the xylem tissues are arranged in radial masses and are separated from one another by the phloem masses, together with some parenchyma cells. Such bundles are characteristic of the roots of all phanerogams and pteridophytes and stems of Lycopodiaceæ.

The following scheme shows the types of bundles and their distribution:

COLLATERAL BUNDLES. — Stems and leaves of nearly all flowering plants, a few ferns, as the genera Ophioglossum and Osmunda and stems of Equisetaceæ.	I. *Ordinary Bundles.* — Having one phloem and one xylem mass.	*a. Open Bundles.* —Stems and leaves of woody dicotyls. (Cambium zone present.)
		b. Closed Bundles. — Most monocotyls, the ferns mentioned and stems of Equisetaceæ. (No cambium zone present.)
	II. *Bicollateral Bundles.* — Chiefly stems of Cucurbitaceæ (gourd family).	
CONCENTRIC BUNDLES.	I. Bundles with xylem central. Stems and leaves of nearly all ferns and club mosses.	
	II. Bundles with phloem central. Stems and leaves of some monocotyls.	

RADIAL BUNDLES.—Roots of all phanerogams and pteridophytes and stems of Lycopodiaceæ.

COLLATERAL BUNDLES.

CLOSED BUNDLES.—Harden a stout piece of the stem of Spiderwort (Tradescantia Virginica) in alcohol and make thin cross-sections. (This is a monocotyl). Stain with phloroglucin.

The greater portion of the section is made up of large ordinary parenchyma cells containing starch. Scattered among these cells over the entire section are numerous rounded areas of smaller cells containing no starch. These are the closed collateral bundles. In the xylem, which faces towards the center of the section, will be found from three to five thick-walled ducts, which in many of the bundles are arranged in the form of a V. In some of the bundles the xylem completely surrounds the phloem. On the side of the xylem towards the center of the section there is usually found a large intercellular space. This is often found in closed collateral bundles.

The phloem is composed of small cells, which are mostly sieve cells, accompanied by some parenchyma cells. There is no growing or cambium zone between the phloem and xylem.

The bundles are enclosed by a single row of cells smaller in diameter than the other parenchyma cells of the section and containing little or no starch. This is the *endodermis* or *bundle sheath.* It is poorly developed in this type of bundle and is often not present at all. It will be met with later in perfectly developed form in the concentric and radial bundles.

Note the arrangement of the whole section of this stem. It represents the type of structure of all monocotyl stems. The bundles are scattered without any definite order over the whole cross-section, though they are more numerous in the outer part than towards the center of the section. There is no true bark as exists in stems of dicotyls.

The student should be careful to differentiate between *type* of structure and *details* of structure. Other monocotyl plants have the same plan of arrangement as in the one just studied, but perhaps none looks exactly the same. The case is very much like that of human beings, all of whom are constructed on the same plan, but very rarely do two tally so closely that they cannot be distinguished.

To emphasize the above point, study a cross-section of the young stem of Greenbrier stained with phloroglucin. If the stem be too hard to cut easily, soften it by soaking in dilute alkali (1 or 2 per cent.) sufficiently long. Greenbrier is a monocotyl plant of the harder or more woody kind, while Spiderwort represents the soft, herbaceous plants. In the section will be seen numerous bundles of the closed collateral variety containing, besides vessels, both wood and bast fibres. The bundles are scattered almost over the whole area of the section and are imbedded in a groundwork of parenchyma cells, which, however, have their wall considerably thickened, thus differing from the parenchyma of Spiderwort. The area containing the bundles is sharply divided from a narrow, outer rim of the section, which resembles in a way the bark of a dicotyl plant. Most monocotyl stems have such a more or less distinct dividing line. In some it is a single chain of cells, in others it is strongly developed; it is known as the *cylinder sheath.* In the Greenbrier it consists of numerous hard fibrous cells, which result from the crowding of incomplete bundles in this region.

Note the two very large ducts and several smaller ones in the xylem or wood portion of the bundles. The xylem portion is directed towards the center of the section. In the outer portion next to the two large ducts is an area of softer smaller cells, the phloem, made up of sieve tubes and some parenchyma cells. The whole bundle is enclosed by fibres which, on the inner edge, are called wood fibres, while on the outer edge they are called bast fibres.

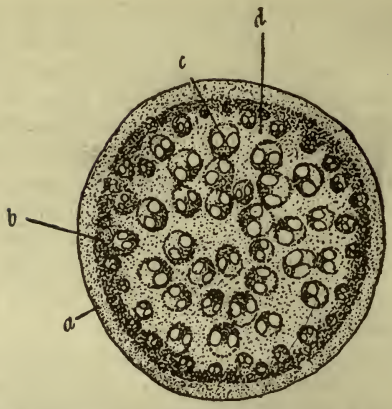


Fig. 54.—Cross-section of stem of Greenbrier. a, cortex; b, cylinder sheath, made up of incomplete bundles; c, a bundle; d, parenchyma cells of ground tissue (Bastin).

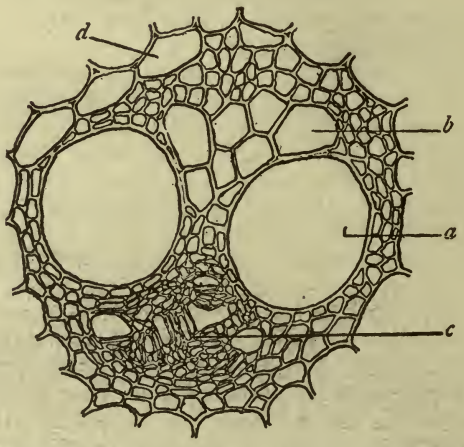


Fig. 55.—Section of a bundle of Greenbrier, magnified. a, large duct in xylem; b, smaller duct; c, phloem mass; d, parenchyma cell (Bastin).

OPEN BUNDLES.—These are found in stems of dicotyledonous plants. In herbs they are more or less isolated, and the xylem portions do not form a solid continuous woody cylinder, as in shrubs and trees. The cross-section of the Geranium stem furnished an example of open collateral bundles.

To study the bundles in a woody type of stem, make cross-sections of the stem of Bittersweet and stain with phloroglucin. Surrounding a central area of pith cells will be found a thick ring of cells that stain red, composed mainly of thick-walled wood fibres, which are interspersed with cells of much larger diameter, the ducts. This solid ring of cells is composed of the

xylem portions of numerous bundles which have grown together, and the only evidence left of their having once been separated is a number of radial rows of elongated cells running through the ring from the pith to the outer edge. These rows of cells are the *medullary rays*. The cells were once soft parenchyma cells between the bundles, but have subsequently become lignified.

At the outer edge of the ring of wood cells is a narrow zone of small cells, thin-walled, unstained, rectangular in shape and more or less in radial rows. These form the cambium zone or growing layer.

Outside the cambium is a zone of unstained cells composed of sieve tissue and parenchyma cells. This zone is bordered

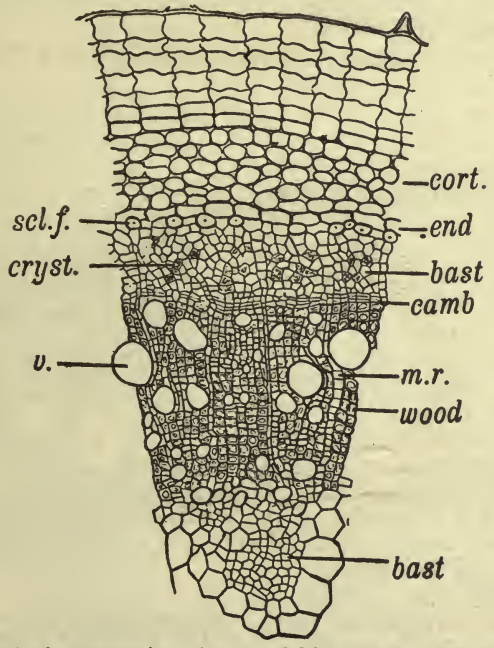


Fig. 56.—Segment of cross-section of stem of Bittersweet. cort, parenchyma of middle bark; scl.f., bast fibres; m.r., medullary ray (Greenish).

exteriorly by a broken circle of small thick-walled bast fibres. The area between the cambium and bast fibres, including the latter, is composed of the phloem portions of the bundles. The medullary rays are continued through the phloem areas. This region forms the so-called inner or liber bark, spoken of in the lesson on bast and wood fibres. Next to the inner bark is the middle bark, composed of large ordinary parenchyma cells. Beyond this is the outer bark, composed of a layer of cork cells. Only dicotyls have a true bark, and the structure of such a bark is seen in this section. When the bark is peeled off the rupture takes place at the cambium zone, which is soft and easily torn.

In perennial dicotyl stems the cambium zone forms yearly a new layer of woody tissue on its inner edge, thus increasing the diameter of the plant. Moreover, the beginning and end of the year's growth usually differ in appearance, and thus the "rings of growth" are distinguished, and by their number the age of the plant also. Monocotyl stems have no cambium in the bundles after they are mature, hence such stems soon cease to increase in diameter. They are, as a rule, slender stems.

Study the whole cross-section as a type of the more woody dicotyledonous stems and contrast it with the stem of the Spiderwort studied above. It is the definite arrangement of the bundles in a single circle, with xylem ends centrally and phloem ends exteriorly, that gives rise to a true bark and a central woody cylinder in dicotyls.

Make cross-sections of stem of Lizard's Tail as a type of herbaceous dicotyledonous plants.

Stain with phloroglucin. The bundles are arranged in a circle, but are not grown together. They are separated by soft parenchyma tissue, which forms the greater portion of the section. There is a cambium zone between phloem and xylem. In the xylem, next the cambium, are some large ducts with a few parenchyma cells mixed in. Next to these are some ducts of smaller diameter and then a semicircular zone of thick-walled wood fibres marking the limits of the xylem of the bundle. In the phloem, next to the cambium, is a mass of soft tissue composed of sieve cells, parenchyma cells and a few secretion cells. The phloem is bounded by a layer of thick-walled bast fibres with pore-canals. This layer passes around to meet the layer of wood fibres of the xylem, forming thus a sort of sheath to the bundle.

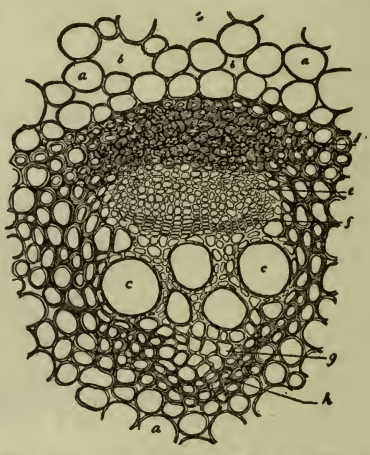


Fig. 57.—Cross-section of a bundle of Lizard's Tail. a, parenchyma; b, air space; c, large duct; d, bast fibres; h, wood fibres; e, soft bast; f, cambium; g, smaller duct (Bastin).

Note that the arrangement of the tissues in this stem is the same as that in the Bittersweet, the difference being one of degree rather than kind. There is far less wood in the section. The type of bundles and the arrangement in a single circle is the same.

Sketch the whole cross-section of Bittersweet and Lizard's Tail and make a more detailed drawing of a segment of each section.

BI-COLLATERAL BUNDLES.

Make cross-sections of the hardened stem of Pumpkin, Squash or Watermelon.

The bundles consist of a xylem mass betwen an outer and an inner phloem mass. In the xylem are some very large ducts, looking like large holes, with some smaller ones in the inner portion. There is a large quantity of small-celled parenchyma tissue. The phloem masses consist of large sieve cells and accompanying parenchyma cells. There are no bast fibres present nor any wood fibres in the xylem. There are two layers of cambium cells, one between the xylem and outer phloem mass, the other between the xylem and inner phloem mass. The rest of the section is made up of very large-celled parenchyma tissue.

CONCENTRIC BUNDLES.

I. Variety with phloem surrounding xylem.

Make cross-sections of the rhizome of the Eagle Fern (Pteris aquilina) and stain with phloroglucin.

On the exterior is the epidermis, brown in color, then several layers of thick-walled fibrous cells, also brown, known as the *hypoderma*. Interior to this is a zone of large ordinary parenchyma cells with starch grains. Then comes a circle of bundles, separated from one another by parenchyma. The bundles may be round or elongated, but never radially. Within this circle of bundles are two elongated masses of thick-walled, fibrous cells, dark colored. Lying between these are two bundles, elongated and larger than those of the circle. The spaces between the objects just described are filled up with parenchyma cells.

In the center of the bundles is the xylem, composed of large scalariform ducts stained red and a few small parenchyma cells. Surrounding the xylem is the phloem, unstained, consisting of a layer of small parenchyma cells with fine starch grains immediately next to the xylem; then a layer of sieve cells and their companion cells, finally another layer of small starch-bearing parenchyma cells. The bundle is sharply divided off from the surrounding parenchyma by a well-developed ring of elongated prismatic cells, known as the *endodermis* or *bundle sheath*.

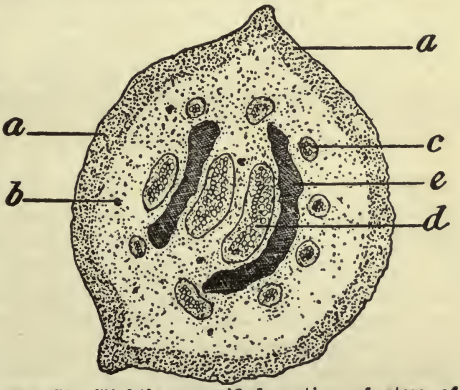


Fig. 58.—Slightly magnified section of stem of Eagle Fern. a, hypoderma; b, small group of dark fibres in cortex; c, one of bundles in a circle; d, one of the two bundles in the center; e, brown mass of fibrous cells (Bastin).

There is no cambium in the bundles, hence fern stems, just as those of monocotyl plants, do not increase in diameter from year to year. They remain rather slender.

Note the plan of this stem. It is the type on which all ferns are built. Note also that it differs from the monocotyl and dicotyl types of stem. The two large masses of dark fibrous cells in the central portion of the section are not present in all ferns.

II. Variety with xylem surrounding phloem.

Make cross-sections of stems of False Solomon's Seal or Sweet Flag, both monocotyl plants, and stain with phloroglucin.

The structure of the bundles is just the reverse of that of the bundles of the fern, in that the xylem cells are on the outside and the phloem is central, but there is no endodermis present. In a few bundles the xylem ring is incomplete and the phloem is continuous with the parenchyma outside the bundle. The concentric bundles, with phloem central, may be regarded as closed collateral bundles in which the xylem has completely grown around the phloem mass.

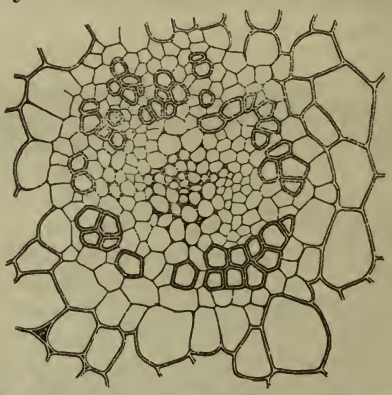


Fig. 59.—Cross-section of a concentric bundle of the rhizome of Sweet Flag, with central phloem surrounded by the xylem vessels (Tschirch).

Both these plants have the arrangement of the parts characteristic of monocotyl stems. In both there is a cylinder sheath dividing an outer border or cortex from the broad central area in which the bundles are scattered. The parenchyma tissue of Sweet Flag (Calamus) has been studied earlier (Chapter XVIII). The parenchyma of False Solomon's Seal is large, thin-walled, closely packed. Neither section contains cork cells.

RADIAL BUNDLES.

These vary considerably among themselves in regard to the number of xylem rays and their length, the amount of lignification of the cells, the structure of the pericambium layer and of the endodermis. The number of rays varies from two to forty or fifty in different roots. The number of rays is indicated by the word *arch* with a numeral prefixed. Thus a bundle with two xylem rays is called a *diarch* bundle, one with three a *triarch* bundle, etc. As a rule, dicotyl and gymnosperm roots have fewer rays and a thinner-walled endodermis than roots of monocotyls.

Root of a Dicotyl Plant.—Make cross-sections of the root of the May Apple (Podophyllum peltatum) and stain in phloroglucin. In the center of the section will be found the bundle, which in this plant is usually pentarch, i. e., has five xylem rays. The rays are wedge-shaped, with the broad ends towards the center. At the outer narrow ends of the rays the ducts are smaller in diameter, but are much larger at the broad ends and mostly scalariform. The central portion of the bundle is made up of parenchyma cells, among which may be a few scattered ducts.

The phloem masses lie between the xylem rays, towards their outer ends, and are separated from them by several layers of parenchyma cells. The cells of the phloem have glistening walls and may be told by these. The bundle is enclosed by an endodermis of elongated cells, which are thin-walled, as is usual in dicotyl plants. Immediately next to the endodermis are two layers of cells, larger in diameter than the cells of the endodermis or of the phloem and containing some fine-grained starch. These cells are known as the *pericambium* or phloem-sheath. The cells have the power of multiplication and root branches have their origin from them, opposite the xylem rays.

The area outside of the bundle is filled with parenchyma cells densely filled with starch grains.

Root of a Monocotyl Plant.—Make sections of the root of Yellow Lady's Slipper (Cypripedium pubescens), a monocotyl plant, and stain with phloroglucin. The xylem rays are about eight in number, longer and better developed than in the previous case. They meet at the center of the section, where there are numerous large ducts and smaller thick-walled cells. The ends of the rays are surrounded by thick-walled narrow cells which reach out to the endodermis, interrupting the pericambium layer in places. The phloem masses lie between the rays; the walls of the cells are thin and glistening. The endodermis is peculiar in that the cells opposite the phloem masses have their radial and inner walls much thickened, giving to these parts the appearance of a crescent, while the outer walls remain thin. The other cells of the endodermis opposite the xylem rays are thin-walled. This is a peculiarity of the endodermis of monocotyl roots. Make a drawing of the bundle.

Compare with the section of Cypripedium, one from the root of the corn plant, which is also a monocotyl. There are about fifteen xylem rays, somewhat like those of podophyllum in appearance. They do not reach to the center of the bundle. This is filled up with parenchyma cells, in which there is a circle of five very large vessels.

The roots of monocotyls undergo very little change as they grow older, but while the *young* roots of dicotyls present the appearance described under the root of Podophyllum, the older

ones undergo radical changes and assume the structure of dicotyl stems. In fact, the section of old dicotyl roots looks so much like that of a stem that it is often difficult to distinguish it from a stem section. These changes can easily be followed by making sections of a root at various distances behind the growing point.

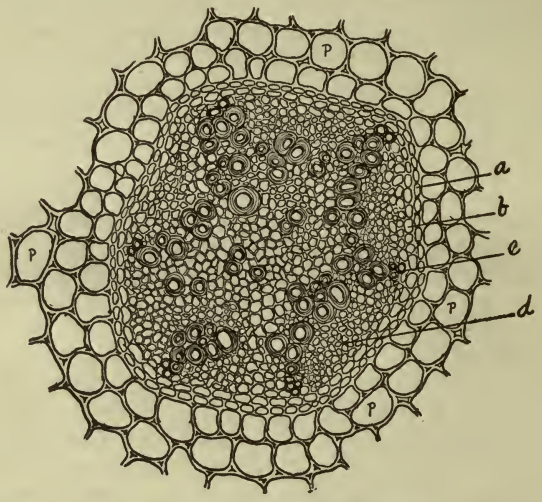


Fig. 60.—Cross-section of root of May Apple, showing radial bundle. a, endodermis; b, pericambium; c, xylem ray; d, phloem; p, parenchyma of cortex surrounding the bundle (reduced, from Bastin).

CHAPTER XXIII.

LEAVES.

The leaf consists of: (1) the fibro-vascular system or framework of veins; (2) the parenchyma or filling; (3) the epidermis, which covers the whole leaf. The parenchyma or mesophyll of the leaf is arranged differently in different leaves, giving rise to two types of leaves, namely, *bifacial* and *centric.*

BIFACIAL LEAF.—These are always flat leaves, and in section present a distinct upper and lower surface, which are quite different in structure. The parenchyma cells next the upper surface are compactly arranged and elongated perpendicular to the surface. Such cells are known as *palisade* parenchyma. They contain numerous chlorophyll bodies, which give to the upper surface of such leaves the deeper green color, as compared with the lower surface.

The parenchyma next to the lower surface is loosely arranged

and scarcely elongated at all, and is known as *spongy* parenchyma.

Most any flattened leaf will serve for the study of the bifacial type. An excellent leaf is that of the Rubber Tree (Ficus elastica), because of its toughness and thickness. Leaves bleached in alcohol will be better, as the sections will be more transparent.

Make sections perpendicular to the lateral veins of the leaf and mount in water or glycerin. Sections of the fresh leaf may be cleared in carbolic acid or chloral-hydrate.

The epidermis on both surfaces is composed of three layers of cells. This is not common to all leaves, but is usually found in tough evergreen leaves. The triple layer affords greater protection. Here and there along the upper surface, and sometimes on the lower also, occur very large cells with a mass hanging from a stalk attached to the cell-wall, like a bunch of grapes, in the cavity of the cell. The hanging masses are called *cystoliths*. They are not of common occurrence.

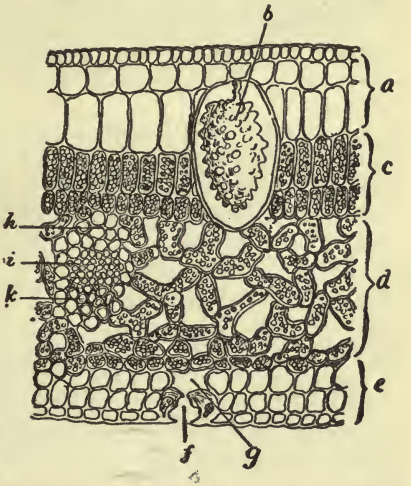


Fig. 61.—Cross-section of portion of leaf of Rubber Tree. a, e, three-layered upper and lower epidermis; c, palisade cells; d, spongy cells; h, xylem; i, soft bast; k, bast fibres, of a small vein; f, stoma; g, air space (reduced, from Bastin).

Next to the upper epidermis are two layers of elongated cells, the cells of the outer layer being much longer than those of the inner layer, and all are filled with chlorophyll granules. These are the palisade cells.

The rest of the space below the palisade cells is filled in with spongy parenchyma. The cells are not elongated and contain much less chlorophyll than the palisade cells. The cells next the lower epidermis are somewhat compactly arranged.

In the lower epidermis will be found stomata or breathing pores. Some of the pores will be found cut through the middle, giving a clear view of the guard cells. Each pore is seen to lead into a large air space in the leaf. (See Fig. 61.)

The cystoliths consist of a groundwork of cellulose infiltrated with calcium carbonate. On adding a drop of acetic acid to a section the carbonate will dissolve with effervescence, leaving the cellulose mass, which stains blue with chlor-zinc-iodine.

At intervals along the section will be found the collateral

bundles of the veins with the xylem always towards the upper side and the phloem towards the lower side of the leaf. The upper and lower faces of a leaf can always be told by noting the position of the xylem and phloem of the bundles of the leaf.

CENTRIC LEAF.—This type of leaf is symmetrical, i. e., the structure on one side is the same as on any other side. Palisade cells are not present. Centric leaves are terete, acicular or succulent, and, occasionally, flattened leaves belong to the type.

Most any pine needle will illustrate the type; also leaves of Lady's Slipper, Sweet Flag, Hyacinth, Daffodil.

Make cross-sections of a pine needle by holding it between pith and cutting through the latter. If necessary, clear them in carbolic acid, chloral hydrate or Labarraque's solution.

The leaf is flat on one side, which is the upper or ventral, and nearly semi-circular on the other. The epidermis is a single layer of thick-walled cells which possesses stomata on all sides of the leaf.

Next to the epidermis are two or three layers of thickened fibrous cells, and next to these comes the parenchyma of the leaf, consisting of thin-walled cells, whose walls have been infolded, forming a variety of parenchyma known as *folded*. The cells contain chlorophyll bodies. Arranged at nearly equal intervals in the parenchyma are about five secretion reservoirs, in which the circle of secreting cells is enclosed by one of thick-walled cells.

A bundle sheath separates the central portion from the rest of the section, next to which are parenchyma cells surrounding two collateral bundles in the center of the section.

Apply phloroglucin and hydrochloric acid and note result; also iodine solution.

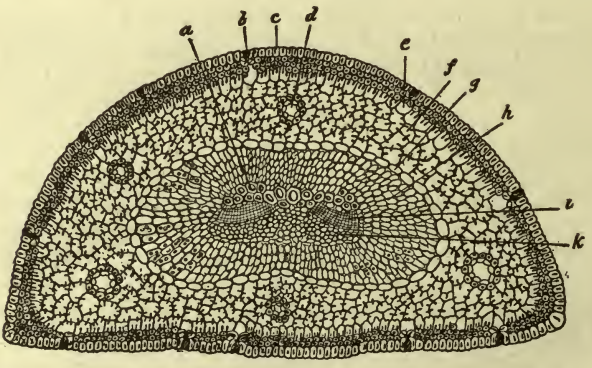


Fig. 62.—Cross-section of a pine needle (centric leaf). a, bast fibres of bundle; b, stoma; c, epidermis; d, secretion channel; e, air space; f, folded parenchyma; g, bundle sheath; h, parenchyma; i, phloem, or soft bast of bundle; k, xylem of bundle (reduced, from Bastin).

APPENDIX.

A.—Various Reagents Used in the Study of Plant Tissues.

PERMANENT STAINS.

Kleinenberg's Hæmatoxylin.—Saturate some 70 per cent. alcohol with calcium chloride, let the mixture stand 12 to 24 hours over powdered alum, shaking occasionally; add 8 parts of 70 per cent. alcohol, filter and then add a saturated solution of hæmatoxylin in absolute alcohol until a purple-blue color is produced. Let stand in a corked bottle in sunlight for a month; it is then ready for use. The liquid is to be diluted as required with dilute alum solution. Over-stained sections are brought back to proper degree of staining by washing in acidified 70 per cent. alcohol (4 to 6 drops hydrochloric acid to 100 cc. alcohol). Since acids are incompatible with the stain, it is best to wash the section next in alcohol or water containing a trace of ammonia before making the final mount.

Hæmatoxylin is an excellent *nuclear* and *cellulose* stain. It scarcely stains lignified material. Alcoholic sections should first be washed well in water and also thoroughly washed after staining.

Beale's Carmine.—Dissolve 0.6 gram of carmine in 2 cc. of hot ammonia water; let the solution stand 1 to 2 hours to cool and to allow the excess of ammonia to escape. Then add 60 cc. of distilled water, 60 grams of glycerin and 15 grams of absolute alcohol. Allow the mixture to stand for some time and then filter it. Over-stained sections are brought back to proper color by washing in acidified 70 per cent. alcohol, then in alcohol free from acid. Carmine is a protoplasmic and nuclear stain.

Fuchsin.—Dissolve 0.1 gram of fuchsin in 160 cc. of water, add 1 cc. of absolute alcohol. Keep in a well-closed bottle. Fuchsin stains lignified and corky tissues, but is easily washed out of cellulose walls.

Methyl-green.—Dissolve the dye in water to deep green color. This stains lignified and cutinized tissues more rapidly than cellulose tissue. It also stains protoplasm and the nucleus.

Safranin.—Equal parts by volume of aniline water (water saturated with aniline oil) and concentrated alcoholic solution of safranin. Sections stained and then washed in acidified 70 per cent. alcohol have only lignified and cutinized walls colored.

Gentian-violet.—Three parts by weight of aniline, 1 part of gentian-violet, 15 parts by weight of alcohol and 100 cc. of water. It stains lignified and cutinized walls.

IODINE-GREEN.—A deep green aqueous solution is used. It acts like methyl-green. It is much employed along with carmine, fuchsin or eosin for double staining of tissues. The stains are better used successively than mixed together.

EOSIN.—Oil of cloves solution of eosin is used for clearing and at the same time staining sections that have previously been treated with gentian-violet, iodine-green or methyl-green. The violet or green goes to the lignified and cutinized tissues, while the cellulose walls are stained red by the eosin.

PICRO-NIGROSIN SOLUTION.—Add enough of a strong aqueous solution of nigrosin to a saturated solution of picric acid in water to produce a deep olive-green color. This is a good nuclear stain and a good double stain, the nigrosin going to the cellulose and the picric acid to the lignified tissues. A comparatively long time is required for staining.

TEMPORARY STAINS.

POTASSIUM IODIDE-IODINE.—Dissolve 1 part of iodine and 4 parts of potassium iodide in 10 cc. of water, then dilute with 185 parts of water. It is one of the most useful stains. It colors starch blue, protoplasm and proteids yellowish-brown, lignified cell-walls deep brown; together with sulphuric acid it stains cellulose blue.

CHLOR-ZINC-IODINE.—Dissolve 10 grams of potassium iodide and 0.15 gram of iodine in 10 cc. of water. Add this solution to 100 grams of a solution of zinc chloride of specific gravity 1.8 and mix thoroughly. This reagent may be used either on fresh or alcoholic material, and the specimen on the slide should be nearly dried before applying it. Cellulose is colored (often slowly) blue or violet, lignified walls yellow, cork yellow to brown, protoplasm brown, and starch swells and is colored blue.

PHLOROGLUCIN.—A solution of 1 gram in 100 cc. of 90 per cent. alcohol. The solution in time turns dark and should not be kept more than three or four months. The section is first immersed in the reagent, say for five minutes, after which it is nearly dried and a drop of strong hydrochloric acid added to it. Only lignified substance is colored. The color varies from pale to dark red, according to the amount of lignification.

ANILINE HYDROCHLORIDE.—A 5 per cent. alcoholic solution is employed in the same way as phloroglucin, with hydrochloric acid, as a test for lignified tissues, which it stains a deep yellow. It is not as good a stain as phloroglucin.

CYANIN.—A solution of cyanin in equal parts of alcohol and water is used to test for fats, which are colored a beautiful blue after one-half hour's immersion. Glycerin may be used to wash out the superfluous stain.

ALCANNIN.—Macerate 20 grams of alcanet root in 100 cc. of 90 per cent. alcohol for a week and filter. Dilute the tincture with an equal volume of water just before using and immerse the sections for several hours. The reagent is a test for fats, resins and volatile oils, which assume a red color. Cutinized and suberized cells are also stained red, though not so deeply.

AMMONIUM FERRIC ALUM.—A saturated aqueous solution is used as a test for tannins, which form a bluish-black or greenish-black precipitate. It should be remembered, however, that occasionally other substances, usually related to the tannins, may be present, which are capable of forming dark-colored precipitates with ferric salts.

FIXING AND HARDENING REAGENTS.

ALCOHOL.—This is universally used for hardening plant tissues. It hardens by abstracting water. Strong alcohol possesses also in a high degree the power of fixing the protoplasmic contents of cells. Some plant organs may be placed at once into absolute alcohol, while other more tender parts *must be placed at first into weak* alcohol (60 per cent.), then into grades of increasing strength, as 70 per cent., 90 per cent., absolute alcohol. Tissues may be kept in alcohol for any length of time. They acquire the best condition for cutting if they are placed in a mixture of equal volumes of water, absolute alcohol and glycerin 24 hours before sectioning. Other less frequently used solutions are—

CHROMACETIC ACID.—A mixture of 1 part of 0.1 per cent. acetic acid and 1 part of 0.2 per cent. chromic acid solution. It is a fixing reagent and objects must remain in it from several to 24 hours. They are then thoroughly washed in water and hardened in alcohol.

CHROMIC ACID.—A 1 per cent. solution is used for the same purpose as the previous solution. All chromic acid mixtures should be kept in the dark, as sunlight decomposes them.

OSMIC ACID.—A 1 per cent. solution fixes protoplasm immediately. Objects may remain in it from a few seconds to several hours according to their nature, and are then washed thoroughly in water and hardened in alcohol.

PICRIC ACID.—In concentrated aqueous or 50 per cent. alcoholic solution for algæ and higher plants.

SOFTENING REAGENTS.

In some exceptional cases objects are too hard for sectioning and, therefore, must be rendered soft before they can be studied. Such objects are wood, hard seeds, barks, dried drugs. In some

cases mere soaking for a shorter or longer time in cold or hot water will suffice to soften the specimen. In other cases weak alkalies are necessary. A very good solution is 2 per cent. ammonia water (24 to 48 hours' immersion). Very hard objects are placed in 5 per cent. caustic potash solution.

CLEARING REAGENTS.

To make sections more transparent so that the parts may be better seen various reagents are used. Those most generally used are *oil of cloves, creosote, carbolic acid* (liquid). These are used before mounting the section permanently in balsam or dammar. Other reagents that are sometimes used are caustic potash (dilute), chloral-hydrate, a mixture of creosote and turpentine (1:3), or creosote and alcohol (1:1). Delicate objects are gradually made clear even in glycerin.

Starch is dissolved in dilute mineral acids, protoplasm in dilute alkalies, oils and resins in alcohol, ether and alkalies.

Alkalies, acids, alcohol or chloral hydrate solution are used when it is desired to clear out the contents of cells so that the cell-walls alone may be studied without the interference of the contents.

LABARRAQUE'S SOLUTION (sodium hypochlorite) is also used as a clearing and bleaching agent, especially for cells rich in protoplasm, for example, meristem cells. Time of action 5 to 15 minutes. It is excellent for bleaching sections, etc., in which the natural plant pigment is too dark to allow a clear view of details. The reagent should not be allowed to act longer than necessary.

CHLORAL HYDRATE SOLUTION.—Chloral hydrate crystals, 5 grams, dissolved in 2 cc. of water. It is a very valuable reagent. It causes shrunken cells to expand, and dissolves starch, proteids, resin, volatile oils, chlorophyll, etc. When saturated with iodine, by keeping a few crystals of the latter in it, it is employed to detect small starch grains.

PERMANENT MOUNTING OR ENCLOSING MEDIA.

CANADA BALSAM.—This is a thick solution of the resin in benzene, turpentine, chloroform or xylene. If the solution becomes too thick it is diluted with benzene, etc., respectively. The resin is incompletely soluble in absolute alcohol, hence addition of alcohol to the clear solution in benzene, etc., causes a cloudiness. The balsam should be kept in glass-capped, wide-mouthed bottles. Before mounting in balsam sections must be soaked in solutions which are miscible with it. Such solutions are clove oil, turpentine, benzene, chloroform, xylene, creosote, carbolic acid. It would not do, for example, to take a section from

alcohol into balsam. Balsam hardens gradually and hence the slide is finished when the cover glass is placed on.

DAMMAR.—This is a resin from which solutions are made similar to those of Canada balsam, the same kind of solvents being used.

GLYCERIN-GELATIN.—This is a very convenient medium and is often used for mounting vegetable sections. Preparation: 42 cc. water, 38 cc. glycerin, 7 grams gelatin, 1 gram carbolic acid. Soften the gelatin (best French or German) in the water two hours, add the glycerin and warm; add the acid, warm and stir one-quarter hour. Filter hot through glass wool and let cool. It is solid when cold, but melts at 35° to 40° C., and will keep for years. Before mounting in this medium, tender objects must be gradually brought from weaker to strong glycerin. The gelatin is then melted, a drop placed on a warm slide, the section, freed from most of the adhering glycerin, placed in it and covered with a warm cover glass. When cold the gelatin solidifies. The slide should then be "ringed" with a circle of cement at the edge of the cover glass. This is done by means of a centering turn-table, a camel's hair brush dipped in the cement being held at the edge of the cover glass while the slide rotates with the turn-table.

Carmine-stained sections cannot be mounted in gelatin as the carmine is soluble in it.

FARRANT'S MEDIUM.—Equal parts by weight of gum acacia, saturated solution of arsenous acid and glycerin. Soak acacia in solution of arsenous acid for several days, then add the glycerin. *Avoid* shaking, which causes air-bubbles. The same method of mounting is employed in this as in the case of glycerin-gelatin. Slides should be finished with a ring of cement.

GLYCERIN.—This is used sometimes as a mounting medium, but is troublesome on account of the difficulty of enclosing it with cement.

FLUIDS FOR TEMPORARY MOUNTING OF OBJECTS.

Water is oftenest used. Glycerin, either concentrated or diluted to various degrees, is an excellent medium and very often used. A good fluid is a mixture of equal parts by volume of glycerin, alcohol and water.

OTHER MICRO-REAGENTS.

SULPHURIC ACID.—Strong acid diluted with one-fourth its bulk of water.

PHENOL (CARBOLIC ACID).—Used as a clearing agent, also for dehydrating specimens when it is not desired to use alcohol.

Sections may be mounted directly from this into balsam. Aniline oil may be used in the same way. Aniline is kept free from water by placing in it a stick of solid caustic potash.

SCHULZE'S MACERATION MIXTURE.—One gram of potassium chlorate dissolved in 50 cc. of nitric acid, generally of specific gravity 1.3, but the strength of the acid may be varied to suit the specimen. It is used for the isolation of cells. Sections are placed in the solution and gently heated until the reddish color which first appears in the tissue has disappeared. The whole is then poured into a large quantity of water to stop action and washed well with water. The cells will now be found easy to separate. Sections should not be carried from alcohol to the mixture, but *always from water,* to avoid violent action. Care is needed to stop the action at the right point. The work should be done under a fume-hood.

AMMONIO-COPPER HYDROXIDE (Schweitzer's reagent).—This should be freshly prepared when needed by dissolving some precipitated copper carbonate in concentrated ammonia water. The copper carbonate is obtained by adding sodium carbonate solution to a solution of copper sulphate and thoroughly washing and drying the precipitate by exposure to the air. Schweitzer's reagent is a good solvent for cellulose.

PRESERVING FLUIDS.

1. Alcohol. Pass objects from weaker to stronger solutions —50 per cent., 70 per cent., 90 per cent.
2. Glycerin. Pass from weaker to stronger glycerin.
3. One per cent. solution carbolic acid in water.
4. Aqueous solution corrosive sublimate.
5. Aqueous solution of formaldehyde, 2 to 3 per cent.

B.—SECTION CUTTING AND PROCEDURE IN MAKING A PERMANENT MOUNT.

SECTION CUTTING.

Only very thin objects are suited for examination under the microscope, and the higher the power the thinner must be the object. It is evident that in order to study large bodies, as the organs of plants, thin slices or "sections" must be made. Such sections should be of as nearly equal thickness in all parts as possible. A *transverse* section is one at right angles to the long axis of the object. A *longitudinal* section is one parallel to the long axis of the object. In the case of cylindrical objects, as a stem, there are two kinds—

1. *Longitudinal radial section,* which lies in the plane of the radius.

2. *Longitudinal tangential section,* which is parallel to a plane tangent to the cylinder, and cuts the latter near the surface.

The razor must always be keen-edged and should be stropped frequently to keep it thus, and, when necessary, honed. It is impossible to cut a thin section with a dull razor. A razor flat on one side is the best. It should always be cleaned after cutting sections. It should be pushed, rather than drawn, through the object, with an oblique or sliding motion, even and steady and never with a to-and-fro or sawing motion.

Sections may be cut free-hand or by the use of a so-called microtome or section-cutter, a machine in which the object is clamped in a jaw, which is raised by an accurate screw, while the razor, either held by the hand or clamped in a carriage, slides through it, giving very even and thin sections.

Free-hand Sections.—If the object is large it is held in the left hand between the thumb and forefinger, the latter being extended slightly, so as to form a rest for the razor-blade, which is held in the right hand. Small objects are held in elder or sunflower pith, which is split longitudinally in halves. The object is left protruding slightly, or both object and pith are cut together.

In most cases it is best, in cutting, to keep the knife-blade wet with alcohol or a mixture of equal parts of alcohol and glycerin. Sections of fresh tissues or of those that have been kept in any of the preserving fluids should, immediately after cutting, be transferred—best by means of a camel's hair brush—to water or alcohol, otherwise air will get into the cells and seriously impair the value of the section for study. In regard to cutting sections with the microtome, practice under the eye of an instructor is the best teacher.

While in the majority of instances, certainly in those mentioned in this book, the method of cutting sections above described gives satisfactory results, in the case of very delicate objects, such as anthers, very young ovaries, longitudinal sections of root tips, etc., more careful manipulation is required. The objects are imbedded either in paraffin or celloidin.* The paraffin method requires less time and is preferred by many to the celloidin method, especially in animal histology. Celloidin is especially applicable to delicate plant tissues. The two methods are described in Sedgwick and Wilson's *General Biology,* as follows:

Paraffin Method.—After hardening and staining, the object is soaked in alcohol (95 per cent. or more) until the water is thoroughly extracted (2 to 12 hours, changing the alcohol at least once), then in chloroform until the alcohol is extracted

*Celloidin is the best quality of gun-cotton, and occurs in the market in pieces looking somewhat like cartilage.

(2 to 12 hours), and then in melted paraffin (not warmer than 55° C.) on a water-bath for 15 to 30 minutes (too high a temperature or two long a bath causes excessive shrinkage). Some of the paraffin is then poured into a small paper box or into adjustable metal frames. The object is transferred to it, and after the mass has begun to set it is placed in cold water until quite hard. It is then cemented (by paraffin) to a square piece of cork and placed in the microtome. In cutting the knife is kept dry. The sections should be fixed on the slide by the collodion method. (Collodion mixture consists of 1 part of ether-collodion and 3 parts of oil of cloves. In mounting sections a slide is smeared with the mixture by means of a camel's hair brush, the sections laid on and the slide placed on a water-bath for a few minutes to evaporate the oil of cloves. The slide is then placed in turpentine (to dissolve the paraffin), then drained, after which a drop of balsam is placed on the section and a cover glass put on.)

CELLOIDIN METHOD.—After dehydrating the object thoroughly in alcohol, soak it 24 hours in a mixture of equal parts of alcohol and ether. Make a thick solution of celloidin in the same mixture and soak the object for some hours in it. It may then be imbedded as follows: Dip the smaller end of a tapering cork in the celloidin solution, allow it to dry for a moment (blowing on it if necessary), and then build upon it a mass of celloidin, allowing it to dry a moment after each addition. Transfer the object to the cork and cover it thoroughly with the celloidin. Then float the cork in 82 to 85 per cent. (0.842 specific gravity) alcohol until the mass has a firm consistency (24 hours). It may then be cut in the microtome, the knife being kept very wet with alcohol of above strength. Keep the sections in 85 per cent. alcohol until ready to mount them, then soak them for a minute in strong alcohol, transfer to a slide, pour on chloroform until the alcohol is removed, drain off the liquid, quickly add a drop of balsam and cover.

OPERATIONS INVOLVED IN MAKING A PERMANENT MOUNT OF A SECTION.

FIXING AND HARDENING.—In many cases plant tissues are sectioned and examined in the fresh state. But often the contents of cells in the living state are too transparent to be seen distinctly and must be killed in order to make them more opaque and easily seen under the microscope. Again, portions of plants may be too soft to be cut without first being put through a hardening process. In all cases where it is desired to make permanent mounts or slides of tissues the protoplasm is first killed and then hardened, unless the tissue consists of cells already dead, for example, wood cells, stone cells of nuts, etc.

The object of killing the protoplasm is, as just stated, to make it more opaque, and, at the same time, to preserve its structure for a long time. The process is termed *fixing*. The protoplasm is fixed or coagulated.

After fixing the object must often be hardened for cutting. There are various reagents for fixing and hardening, some of which do both at the same time, while others only fix or kill. (See under the respective reagents for fixing and hardening tissues, page 103.)

Although there are a number of such reagents, those actually used, especially in botany, are few. Alcohol is used in nearly all cases. *Tender objects must never be placed at once in strong alcohol.*

Staining.—It often happens that some objects, even after fixing, are so transparent that their structure cannot easily be made out. The more a transparent body approaches in its refraction of light the medium in which it lies the more difficult it is to be seen. Finally, when the refracting power is the same as that of the medium, the object is invisible. Shells of Diatoms in glycerin are invisible, the refractive indices of both being 1.43. By staining or coloring the transparent parts of an object these become visible and their structure is easily made out. In a heterogeneous section, like that of a plant stem, for example, the chemically different materials in it select different stains, so that by a contrast of colors a great deal more is learned than by study of the unstained section. The different stains require different lengths of time for action, which is best learned by actual work with them. Some of them are permanent, others only temporary. The stains fall into groups, as aniline, carmine, hæmatoxylin stains, etc.

Clearing.—In most cases, even after making very thin sections, these are too opaque and obscure for observation with the higher powers of the microscope and, consequently, must be made more transparent, that is, must be *cleared*. Some objects are naturally very opaque, as pollen grains, spores; such objects must always be cleared before studying them. It often happens that it is desired to study the cell-walls only of a section, but this is impossible because of the dense mass of cell contents, as starch, protoplasm, oil, resin, milk-sap, etc., which must first be removed by special clearing reagents. (See the various clearing reagents, page 104.)

Mounting.—If objects are not intended to be kept for a long time they are mounted in water, alcohol, dilute glycerin or concentrated glycerin, or some other suitable material. For permanent mounting they are usually placed in a medium which solidifies after a time, such as balsam, glycerin-gelatin, which are commonly used. The handiest is the second, because of the little preliminary treatment necessary. The object, in what-

ever medium it is to be mounted, must be saturated with a fluid not very different from the mounting medium. For example, to mount in gelatin, the object must be transferred from glycerin; in balsam, from cloves, turpentine, etc.; in Farrant's medium, from glycerin. Media which do not harden or which absorb moisture, as glycerin-gelatin, must be closed in with a ring of cement. (See mounting media, page 104.)

SCHEME FOR MAKING PERMANENT MOUNTS.

The specimens are supposed to be alcoholic; if not, they are to be placed for a time in dilute alcohol. Alcoholic objects take the stains better than fresh tissues. In the following scheme the sections are supposed to be free from any imbedding material. The procedure for mounting paraffin or celloidin sections has already been stated under Section Cutting.

BALSAM MOUNTS.—If the section be lying in alcohol—

1. Wash in water if it is to be stained in aqueous stains.
2. Transfer to staining liquid. Choose stains according to the nature of the cells that it is desired to stain. Aniline stains color chiefly woody or lignified material. If the stains are in alcoholic solutions take objects from alcohol of about same strength. The time required will vary according to the nature and strength of the stains and is best learned by actual practice with them. The sections should be examined at intervals for depth of color by transferring them to water or alcohol and then back again to the staining liquid. After one has stained a few sections he obtains an idea of the exact time required to attain proper color effects.
3. Wash off the superfluous stain by moving the section about in water.
4. Dehydrate the section by passing it into 70 per cent. alcohol 3 minutes, 90 per cent. alcohol 5 minutes, absolute alcohol (distilled from lime) 5 or more minutes.
5. Place the section next in a clearing agent, as oil of cloves or turpentine, 5 minutes or more. If section be not completely dehydrated it will now appear opaque. It must then be passed back to the absolute alcohol and back again to the clearing reagent.
6. Transfer the section from the clearing agent to a slide and remove the excess of the former. Place upon it a drop of balsam and then a cover glass, avoiding air-bubbles.

GLYCERIN-GELATIN MOUNTS.—Steps 1, 2, 3 are the same as above.

4. Pass the section into weak glycerin 3 to 5 minutes.
5. Then into stronger glycerin 3 to 5 minutes.
6. From 5 into concentrated glycerin 5 minutes.

7. Remove excess of glycerin and mount in glycerin-gelatin, as directed under the latter medium.
8. Enclose the cover glass with a ring of cement (asphaltum, Brunswick black, etc.).

C.—MORE IMPORTANT TEST REACTIONS OF THE PARTS OF VEGETABLE CELLS. (BOWER'S *Practical Botany.*)

CELLULOSE CELL-WALLS—
1. Colored faintly yellow by iodine.
2. Swollen and ultimately dissolved by sulphuric acid.
3. Colored blue with iodine and sulphuric acid.
4. Colored blue or violet with chlor-zinc-iodine.
5. Stained by solutions of carmine or hæmatoxylin, by methylene-blue and in various degrees by other aniline colors.

LIGNIFIED CELL-WALLS—
1. Colored distinctly yellow by iodine and by chlor-zinc-iodine, but in case of bast fibres the tint may vary to sherry-brown or even pink.
2. Colored brown and swollen by iodine and sulphuric acid.
3. Colored bright yellow by acidulated solution of aniline sulphate.
4. Colored red with acid solution of phloroglucin.
5. Stained slightly or not at all by solutions of carmine and hæmatoxylin, but readily by aniline colors.

CUTICULARIZED OR CORKY CELL-WALLS—
1. Yellow by iodine.
2. Yellow or brown by chlor-zinc-iodine.
3. Yellow by strong potash; on gradually warming (without boiling) bright yellow.
4. Resist action of sulphuric acid, retaining their clearly-marked outlines.
5. Are not stained by solutions of carmine or hæmatoxylin, but are colored by aniline stains.

MUCILAGINOUS WALLS—Resemble cellulose in many reactions.
1. Swell with water and to a greater extent with potash.
2. Do not stain with iodine.
3. Stain red with Hanstein's aniline-violet, blue with methylene-blue.

CALCIUM OXALATE—Occurs in cells in form of crystals.
1. Insoluble in acetic acid.
2. Soluble without evolution of gas in nitric or hydrochloric acids.
3. Soluble in sulphuric acid, with formation of fresh crystals of calcium sulphate if only small bulk of fluid be present.
4. Are not stained with iodine.

CALCIUM CARBONATE—Occurs as incrustations or crystals; it is soluble in acetic acid with evolution of gas (CO_2).

PROTOPLASM OR PROTEIDS GENERALLY—

1. Yellow or brown by iodine solutions.
2. Yellow by nitric acid; on addition of potash or ammonia a bright yellow color is produced (xantho-proteic reaction).
3. Swell and lose details of structure on treatment with potash, ammonia or Labarraque's solution.
4. Stain readily with carmine, hæmatoxylin, bright red with Hanstein's aniline-violet.
5. Best stains for nucleus are hæmatoxylin, safranin and methyl-green.

STARCH GRAINS—

1. Blue with solutions of iodine in presence of water.
2. Swell in potash and in water above 65° C.
3. Swell in dilute sulphuric acid.
4. Swell and are colored blue with iodine in chloral hydrate.

INULIN—

1. Soluble, but not readily, in cold water.
2. Precipitated as sphere-crystals by alcohol or glycerin.
3. Not colored by iodine, and soluble in potash.

FIXED OILS—

1. Black with osmic acid.
2. Saponified with potash; soluble in ether.
3. Pink with alcannin solution.

RESIN—

1. Soluble in alcohol or ether.
2. Red by alcannin solution and blue by Hanstein's aniline violet.

TANNIN—

1. Deep brown by potassium dichromate or chromic acid.
2. Greenish blue by ferric salts.