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PRACTICAL BOTANY

BY

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Late Professor of Biology at University College, Southampton
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PREFACE

THIS book may be described as an elementary practical handbook of Vegetable Histology and Physiology, containing in addition a short course of practical work on selected types of Cryptogams and Gymnosperms It is divisible into three sections, namely, (1) Histology and Biochemistry—Chapters I. to III., (2) Physiology—Chapters IV. to VII , and (3) Life Histories—Chapters VIII. to XII

In the first section (Histology), I have exercised special care in giving clear and practical directions for microscopic work. Chapter I. is devoted to this purpose and to general instructions regarding the fixation and preservation of material, the cutting of sections, the application of reagents, and other practical matters.

In Chapter II. I have worked out a plan which has proved thoroughly satisfactory in practice. In my opinion no candidate should be allowed to pass in Botany at any recognised standard examination unless able to produce satisfactory proof of having worked through a practical course in Organic Chemistry. Until examining bodies insist upon this, the teacher of Botany must include in his course a few lessons on the Biochemistry of plants. The student ought to know something more about proteins, for instance, than that they contain nitrogen and are coloured brown by iodine solution!

The best makeshift plan I have been able to devise is that of working through a series of test-tube reactions for each of the important classes of vegetable organic bodies, in each case proceeding to apply the knowledge thus gained

to the identification of the substance in the tissues of the plant itself. It has been difficult to decide just how much to give and how much to withhold in this part of the course, but I regard the contents of Chapter II. as representing the minimum amount of work of this kind which should be done by the student.

In the second section (Physiology) I have outlined a thoroughly practical, but on the whole easy and elementary, course of Plant Physiology. In this course there are very few experiments that cannot be performed without the use of expensive pieces of apparatus Before beginning this part of the work, the student should refer to §§ 20 to 25 in Chapter I., noting carefully the general instructions there given regarding apparatus and methods.

I have begun Chapter IV. with the study of seeds and seedlings, because (1) the structure of seeds follows most naturally upon the floral histology and embryology given at the end of Chapter III., (2) I cannot suggest any better method of starting systematic work in Physiology than that of studying the germination of seeds, and (3) the growing of seedlings provides at once a stock of material especially well suited for many experiments

While much good work may be done with makeshift apparatus, teachers and students should realise that in many cases it is simply waste of time to fit up the makeshift apparatus. In the teaching of Plant Physiology a certain amount of special ready-made apparatus is just as essential as in the teaching of Physics. The botanical teacher should at any rate have at his disposal the chief pieces in Ganong's set of Normal Apparatus for Plant Physiology made by the Bausch and Lomb Optical Company.

In Section III. on Life Histories, I have not used pre-

viously published descriptions, but have repeatedly and thoroughly examined the various types for myself—as indeed I have done for the whole of this book. As a matter of fact, some of these type plants have hitherto been very inadequately and inaccurately described—this is especially the case with *Pellia* and *Funaria* In order to help the teacher and the student alike, I have in this section given full directions for the collection and culture of the typical plants dealt with.

Apart from figures representing various pieces of apparatus I have mainly given drawings to serve as models of the sort of sketches to be made—on a much larger scale, of course—in the student's note-book, which should be of good size and consist of drawing-paper with or without interleaved writing-paper. For the photographic illustrations, I am indebted to Messrs Flatters and Garnett; and for various blocks illustrating apparatus, to Messrs Flatters and Garnett, to the Cambridge Instrument Co , to Messrs. Leitz, to Messrs. Baird and Tatlock, and to the Bausch and Lomb Optical Company. Lecturers and students should write to these firms for catalogues of apparatus and materials.

NOTE TO THE FOURTH EDITION

In the Third Edition an Appendix on microtechnical method was included. For the present (Fourth) Edition certain alterations have been made in various parts of the Text, and a number of new diagrams have been incorporated. These additions and alterations have been carried out by Mr. L. C Fox, M.A.

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PRACTICAL BOTANY.

CHAPTER I.

APPARATUS AND METHODS.

1. Simple Lenses.—Much useful work can be done with simple lenses, and it is often advisable to examine specimens with a lens before proceeding to use the compound microscope.

(*a*) The best kind of simple lens is the **aplanatic** or "platyscopic," which gives a flat field of view, without distortion of the margins, but an ordinary double or triple folding or pocket lens will suffice.

(*b*) For various purposes it is useful to have a watchmaker's lens, with a piece of string or elastic to fasten it round the back of one's head, and thus leave both hands free in examining the specimen.

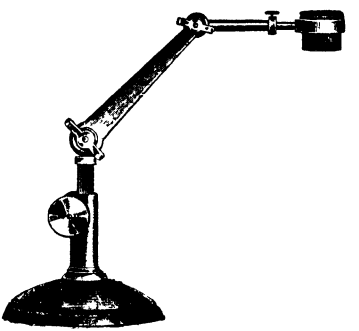


Fig 1.—A Lens Holder, with movable arms and rack and pinion focussing adjustment.

2. Lens Stand.—It is easy to make a simple stand to carry the lens and allow of both hands being used in dissection. The lens can, for instance, be fixed to a cork which slides up and down a vertical rod inserted into a firm and fairly heavy base;

a knitting-needle may be used as a rod, and it may be either fixed into a piece of wood or passed through the cork of a short wide bottle filled with shot. Two simple forms of lens holder are shown in Figs. 1, 2.

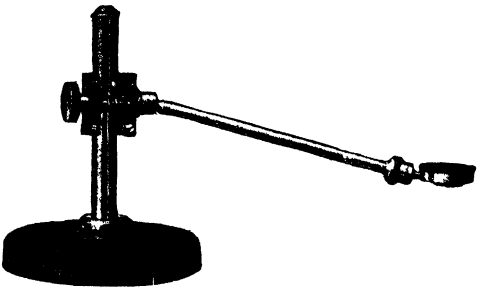


Fig 2 — A Lens Holder, on heavy base, with slow-focussing adjustment and two ball-and-socket joints.

3. Black and White Plate for Dissections, etc. (Fig. 3) —Get a piece of thick glass, and paste or glue on one side of it a piece of white paper or card, half of which is painted black. Keep the papered side down, place on the upper side the objects to be examined with the lens, and move them along so as to see their appearance against the opaque white and black surfaces. Specially prepared glazed black-and-white tiles can be purchased

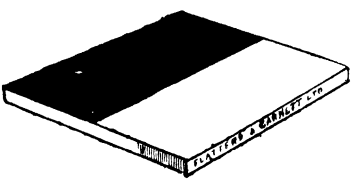


Fig. 3 —Black-and-white Tile, for dissections, teasing-out, etc. The tile may also be obtained mounted in a frame, with cork on one side, and with concentric circles for arranging parts of flowers in the form of a floral diagram.

A glass plate prepared in this way, but with the middle third left transparent, can be mounted on a wooden frame made by removing two sides of a box; in the bottom of the frame place a piece of mirror, slanted so that the transparent middle portion

of the plate receives the light. An excellent "photophore" or simple dissecting microscope can be made in this way, the rod carrying the lens being fixed to one side of the frame.

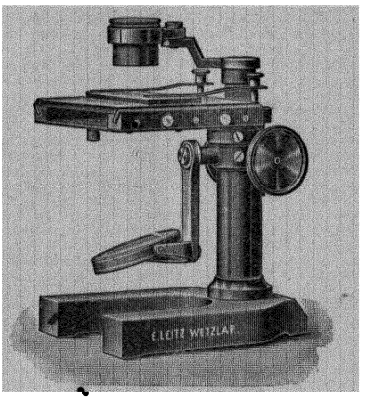
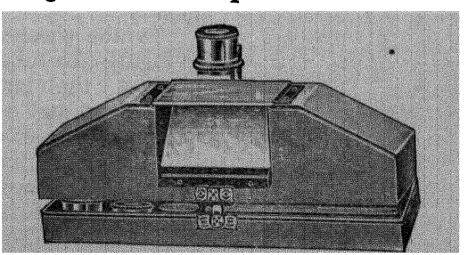


Fig 4.—Dissecting Microscope.

4. Dissecting Microscope.—This extremely useful instrument (Fig. 4) consists of a stand mounted on a heavy metal base and carrying (1) a glass plate or stage for the object to be examined, (2) a lens carrier in which the lens can be raised or lowered by a rack-and-pinion adjustment, (3) an illuminator below the stage with a mirror on one side and a white plate on the other, (4) arm-pieces which can be attached on each side of the stage to serve as rests for the hands when teasing out tissues or otherwise manipulating the specimen examined. The form shown in Fig. 5 is much cheaper.


Fig. 5.—Cheap form of Dissecting Microscope.

5. Compound Microscope.—The simple microscope can be used for all purposes where a magnification of not more than 20 diameters is required, and is an extremely

convenient instrument for this low-power work. When higher magnification is desired we must use the compound microscope, in which the image of the object is obtained by one lens (or a set of lenses) called the **objective,** and this image is magnified by a second lens, the **eye-piece.** The objective is screwed into the lower end of the brass **body-tube,** which is blackened inside (why?); the objective consists usually of several lenses screwed together. The **eye-piece,** which magnifies the inverted image of the object produced by the objective, consists of two lenses, the one next the observer's eye being called the eye-glass and the lower one the field-glass.

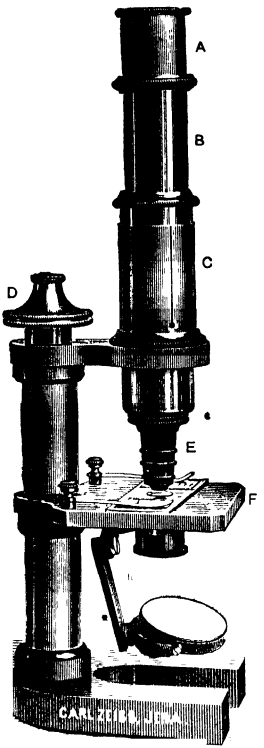


Fig 6.—Compound Microscope with sliding coarse adjustment.
A, eye-piece, B, draw-tube; C, body-tube, D, adjustment, E, objective, F, stage

In the cheaper form (Fig. 6) the tube which carries the lenses is moved up and down, to bring the objective near the object and thus bring the latter clearly into focus, inside another tube fixed to the stand; this is called the "sliding coarse adjustment."

In the more expensive microscopes (Fig. 7) there is a rack-and-pinion movement for raising or lowering the body-tube. The **coarse adjustment** brings the outlines of the

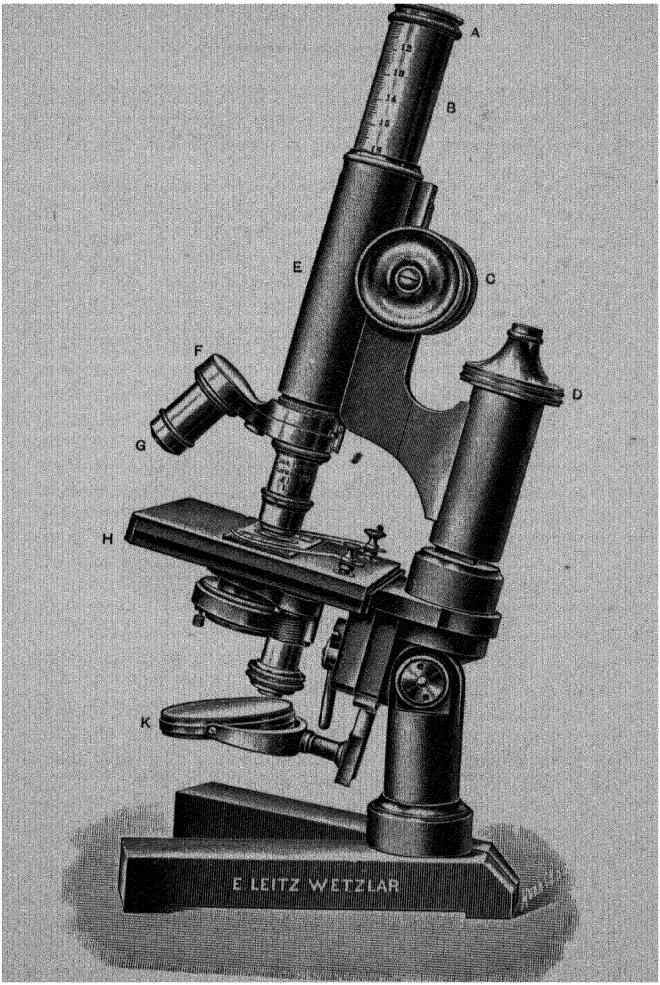


Fig 7.—Compound Microscope with rack-and-pinion adjustment, double nose-piece, and two objectives.

A, eye-piece; B, draw-tube; C, coarse adjustment, D, fine adjustment; E, body tube, F, nose-piece; G, objective; H, stage; K, mirror.

object dimly into focus, but to get more accurate focussing (especially when using a high-power objective) we use the **fine adjustment,** by turning a screw at the top of the stand, behind the body-tube.

The object to be examined is placed on the stage, which has two clips for fixing a slide in a definite position, but these need not be used except for high powers, or while sketching. There is usually a black plate (*diaphragm*), with holes of different sizes, under the stage; this can be rotated so as to bring the desired size of hole under the central opening of the stage. More expensive microscopes

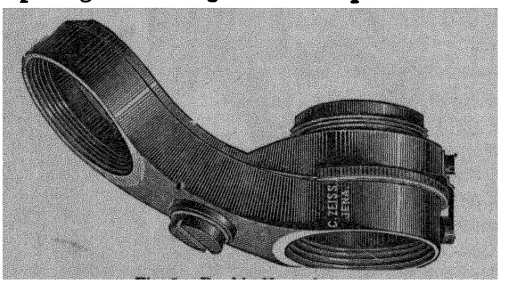


Fig 8.—Double Nose-piece.

have an *iris diaphragm.* A small hole is used with high power and a large one with low power.

For ordinary work two objectives are required, one for low power (magnifying 60 to 80 diameters), and the other for high power (300 to 400 diameters). The most useful are 1 inch or $\frac{2}{3}$ inch low-power objective and $\frac{1}{4}$ or $\frac{1}{6}$ inch high-power objective. Two eye-pieces should also be used; the one with shorter body and narrower eye-glass is the more powerful.

In most modern instruments the magnifying power can be increased by having the body-tube constructed like a telescope; the upper part (**draw-tube**), carrying the eye-piece, can be drawn out. To avoid the inconvenience of having to screw and unscrew a lens every time a change of magnifying power is required, it is worth while to get a **nose-piece** (Fig 8), which is screwed to the lower end of

the body-tube, the nose-piece carries the two objectives, and by rotating it we can quickly change from low to high power and *vice versâ*.

6. Notes on Use of Compound Microscope.—If the stand is without rack and pinion, see that the tube moves easily, but not too easily, up and down, if stiff, take out the tube, rub it with a little olive oil or vaseline.

See that the lenses are clean; dust the mirror, adjust it so as to send light through the body-tube, and insert first one eye-piece and then the other. Rotate each eye-piece; if any specks are seen to rotate with it, they must be on the eye-piece lenses, and should be removed with a chamois leather or soft cloth. If the specks are dim, the dirt must be on the objective; wipe the latter very carefully, and if necessary wash its front lens with a jet of water from a wash-bottle and wipe it dry. Do not rub lenses much, or unnecessarily; do not unscrew the separate lenses of a high objective unless it becomes absolutely necessary, and then do it with great care; in cleaning the lenses do not remove the black coating on the inside of the tube.

Always use the low-power objective first, and never use the high power unless the object is covered with a cover-glass.

With the low power use the flat mirror and a large hole of the diaphragm below the stage; with the high power use the concave mirror and a small diaphragm, otherwise (though the field may look brighter) the outlines of the cells, etc., will not be so sharply defined.

Never use the fine adjustment until the focus has been obtained with the coarse adjustment, whether by sliding or by rack and pinion. With the low-power objective (the one with the larger front lens), slide or rack down the tube to about $\frac{1}{2}$ inch from the object; then, looking through the eye-piece, slide or rack the tube upwards till the object comes into view, and focus clearly by turning the milled head of the fine adjustment screw to right or left. With the high power lower the tube to about $\frac{1}{4}$ inch from the object, then very carefully slide or rack the tube down while looking through the eye-piece, till the object just becomes visible, and focus with the fine adjustment.

Great care is necessary in using the high power, since the objective when in focus is so close to the object. Do not let the high objective touch the slide, and above all do not go on ramming or racking the tube down after passing the position of focus, or you may ruin the objective, besides breaking cover-glass and slide and damaging the specimen. Always use a cover-glass with the high objective; if you cannot see anything clearly, stop at once, move the tube upwards, wipe the objective, remount the specimen (if examined in a drop of water, which is liable to flow over the cover-glass and wet the objective), clean the cover-glass, and start again. If glycerine or other mounting fluid or reagent gets on the objective, wipe the latter with a cloth wetted with water, then dry it thoroughly.

Keep both eyes open when using the microscope; this lessens the fatigue of microscope work, and is not at all difficult if you practise for a few minutes each time you start work. Accustom yourself to using either eye indifferently.

7. Accessories for Microscope Work.—The following articles are necessary for work with the microscope:—

(1) A few dozen glass slips, 3 in. × 1 in.

(2) An ounce of $\frac{7}{8}$ in square cover-glasses, No. 2 thickness.

(3) A pair of fine-pointed forceps.

(4) A pair of fine scissors with sharp points.

(5) A few camel-hair brushes.

(6) A few mounted needles; these can be made by fixing a needle, by means of pincers, into one end of a pen-holder, or a handle adjustable for any needle can be bought for about 9d.

(7) A few flat-bottomed watch-glasses

(8) A few ointment pots with lids.

(9) A small spirit-lamp, about 4 ounce size.

(10) A coarse duster, a finer cloth (*e.g.* an old but clean handkerchief), and a small chamois leather—the last to be kept for cleaning up the microscope only.

(11) Small reagent bottles with dropping-rods, one for each of the reagents most commonly used, e g. (*a*) glycerine diluted with equal volume of water, (*b*) a 5 per cent. solution of caustic potash in water, (*c*) iodine solution, (*d*) aniline sulphate solution, (*e*) a 5 per cent. solution of common salt, (*f*) chlor-zinc-iodine.

(12) Two wash-bottles, one for 50 per cent. alcohol (methylated spirit diluted with equal volume of water), the other for water.

(13) A bundle of dried Elder-pith for section-cutting.

(14) Razors, including at least one thoroughly good hollow-ground razor—see § 10.

Various other articles and reagents are required for special purposes; these are referred to in the text—see also Appendix and Index.

8. Fixation and Preservation of Material.—In all cases fresh material should be used, both for the mounting of entire specimens and for section-cutting—at least as a preliminary to the examination of material preserved in alcohol or treated with various reagents or stained with dyes. Such specimens as starch-grains, filamentous Algae (*e.g.* Spirogyra), leaves of Mosses, etc, which do not require to be sectioned, are simply mounted in water for examination

Where thin sections must be taken, as in the investigation of solid organs (stems, roots, etc.), it is often an advantage to use material preserved in alcohol, since this reagent drives out air-bubbles besides rendering the tissue more readily cut; but it must not be forgotten that alcohol dissolves out such cell-contents as chlorophyll, oil, resin, etc., and it is therefore necessary to examine fresh material first whenever possible.

If plant tissues are placed in ordinary (methylated) alcohol, this reagent may cause plasmolysis of the cells For rough purposes this is no great disadvantage, but even for the simple freehand sectioning, with which alone we are concerned here, it is much better to be able to see more in a section than a network of cell-walls and here and there the shrunken and disorganised cell-contents. If we wish to see the cells in something like their living condition, we must use reagents which will kill the protoplasm rapidly and fix it and the other contents of living cells in as nearly as possible the natural condition—apart from their having been killed.

Various killing and fixing reagents are used for fine work, or for precise staining and double staining, but for general

purposes it is sufficient to use either strong alcohol or formalin, or (better) one of the acid fluids. (1) Put the material into 4 per cent. formalin; *i.e.* 9 vols. of distilled water to 1 vol. of 40 per cent. formalin, as sold; or, better, into formalin-alcohol, which is 100 c.c. of 70 per cent alcohol, to which 6 c.c. of 40 per cent. formalin have been added. Material will keep in this indefinitely, but should be washed in water or alcohol before use. (2) Put the material for 24 hours in 300 c.c. of water containing 2 grams chromic acid and 3 c.c. glacial acetic acid; wash in running water, or in a vessel of water changed frequently, for about two hours; then place for a day in succession in 30 per cent., 50 per cent., and 70 per cent. alcohol; and finally preserve in strong methylated spirit (= about 95 per cent. alcohol).

Objects like filamentous Algae, Mosses, Liverworts, Fern prothalli, root-tips, etc., may be placed entire in the fixing and preserving fluids, but larger specimens should be cut into pieces about $\frac{1}{2}$ cubic centimetre in size.

9. Section Cutting.—In examining the structure of a solid mass—*e.g.* a stem, root, or leaf—we can learn a good deal by crushing, teasing, or macerating the tissues, but these simple methods should be supplemented by the preparation of thin sections cut in different directions. Instructions as to the direction in which sections should be cut are given in connection with the various types. It is only necessary to remember that for the complete study of a solid cell-mass, it is necessary to cut sections in three planes at right angles to each other.

For instance, three sets of sections are required in the case of a cylindrical stem: (1) **transverse,** exactly at right angles to the long axis; (2) **radial longitudinal,** including the long axis; (3) **tangential longitudinal,** parallel to a radial plane but not including the axis. Obviously, in a cylindrical organ it will be only the central part of the tangential section that will give the desired plane—at each side of the section the radii will be cut obliquely and not at right angles.

In most cases it is necessary to keep both the razor and

the material thoroughly wetted in order to prevent the inclusion of troublesome air-bubbles and the sticking of the section to the razor. In cutting fresh material, or material preserved in formalin, moisten the razor with dilute (50 per cent) alcohol—since water is apt to collect in drops instead of spreading over the blade. In cutting alcohol material, wet the razor with alcohol of the same strength as that in which the material has been preserved. Have a saucer of alcohol at hand to dip both razor and material into while cutting the sections.

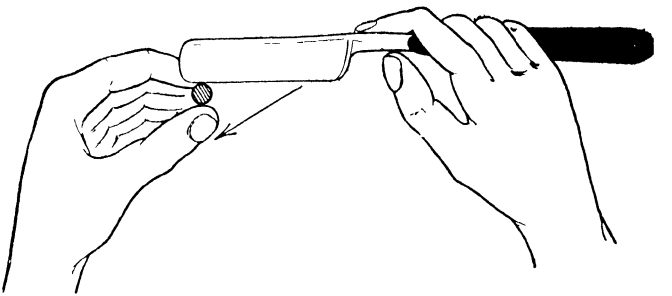


Fig 9.—Method of holding Razor and Specimen in cutting Sections.

In cutting sections (Fig 9), open the razor so that the blade is in line with the handle. Hold the specimen between thumb and forefinger of left hand, and grasp the razor tightly with the right hand so that the blade is horizontal with its edge directed towards you; place the tips of the four right fingers on the back of the razor, and the thumb in front; place the left wrist and forearm firmly on the table; rest the blade of the razor on the bent forefinger of the left hand, with the edge against the specimen and the left thumb well down and out of the way in case the razor should slip. Then draw the razor through the specimen with a sliding movement, making a long oblique stroke and cutting as thin sections as possible, dip the razor into the dilute alcohol for each stroke.

Before cutting sections, trim off the specimen with a

sharp knife or rough-work razor, also prepare the surface —whether transverse, radial, or tangential—of the stem or other solid specimen by cutting off a slice and thus exposing the right surface from which sections are to be cut with your good razor.

At first you will find that the sections are rather thick and often obliquely cut. Thick sections are sometimes useful for the general arrangement of the tissues, but *oblique* ones are generally quite useless. With practice and care, extremely thin sections can be cut, along exactly the desired plane.

After the sections have been cut, they must not be allowed to become dry. If you do not at once mount them on a slide, transfer them from the razor—by means of a wet camel-hair brush, or a jet from a wash-bottle—to a watch-glass of weak alcohol. For Mounting see § 14.

10. Razors.—In section-cutting, success depends so largely on having good razors that it is important to select these judiciously, use them carefully, and keep them clean and in good cutting condition. It is advisable to have several different razors for different purposes; in each case, it is false economy to select inferior razors on account of initial cheapness.

(1) For rough work, such as cutting off pieces of stem, trimming off the surfaces from which thin sections are to be cut, etc., have one or two strong razors ground flat on one side or on both sides. These rough-work razors should be kept sharp and free from notches; rub them on a hone moistened with water, alcohol, or olive oil.

(2) For cutting sections of somewhat hard tissues, such as those of a woody stem, and also for cutting somewhat thick and large sections so as to see the general arrangement of the tissues in a fairly stout herbaceous stem, etc., use a finer razor, preferably with both sides only slightly hollow-ground.

(3) For very thin sections of soft tissues, use a thoroughly good quality hollow-ground shaving razor, taking care not to cut a section of large area. Only small sections must be attempted with this razor, otherwise the sections will be curved, and there will be serious risk of ruining the razor by having bits broken out of its edge.

Keep the three kinds of razor separate—above all, never use the hollow-ground razors for rough work.

The hollow-ground razors must be kept as sharp as possible. The razor should be able to clip across a hair at a single touch; if it will not do this, it requires either stropping on leather or honing on a stone and then stropping. Moisten the ball of the left

thumb and, holding the razor in the right hand, draw the moistened thumb lightly over the edge of the razor from the heel (end nearest handle) to the point. If the edge gives the sensation of taking hold of the skin along its whole length, only stropping is required; if not, the razor must be honed.

In **honing**, place the razor as shown in Fig 10, with the back as well as the edge of the blade against the stone; push the blade along, edge foremost, and at the same time slide it from point to heel in the direction of the arrow. Then turn the other side of the blade to the stone, and repeat the stroke from point to heel towards the other end of the stone, and so on for several times in each direction. Keep the stone well covered with oil, or with soap and water. When the edge is ground enough, so that it passes the thumb test, strop the razor.

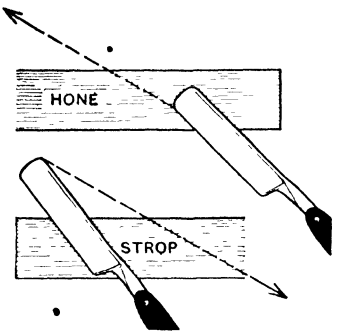


Fig. 10.—Honing (above) and Stropping (below). The arrow in each case shows the direction in which the Razor should be passed over Hone and Strop

In **stropping**, draw the razor blade over the strop back foremost from heel to point, as shown in Fig. 10; reverse the face for the backward stroke, and repeat several times, until the razor will readily clip a hair across when tested.

Never leave a razor open on the table when not in use. Always clean and dry the blade after cutting sections; rinse it in water or dilute alcohol to remove acid plant juice, etc. It is a good plan to oil the razor before putting it away; before using it again, wash off the oil with alcohol.

11. Embedding in Elder Pith.—A fairly large and firm specimen, *e.g.* a piece of Marrow stem for transverse sections, can easily be held in the hand and cut without further preparation. If the specimen is too small or too delicate or flexible to be held in the hand in this way, it becomes necessary to embed it and thus have something firm enough to handle.

Get dried Elder pith—supplied ready prepared by dealers. Cut the pith into lengths of 1·5 or 2 cm, and split each piece longitudinally; to do this without causing

the pith to crack, lay the pith on the table, hold it between thumb and fingers, and cut it in halves with a sharp knife—do not use your section-cutting razors for any rough work like this. With the knife, cut a groove in the pith so that the stem or root, etc., may be placed securely in position and held firmly, yet not so tightly as to compress the tissues much. In the case of a leaf, simply hold a strip of the leaf between the two halves of pith.

Having placed the specimen in position between the two pieces of pith, treat the whole as if it were a piece of solid tissue like a stem. Trim off the end and cut thin sections from it, judging the thickness of each section of the specimen by the opacity or transparency of the pith sections cut along with it. Cut a good number of sections, transfer them to weak alcohol in a watch-glass or saucer, and pick out the sections of the specimen for further treatment.

With the exercise of patience and ingenuity in the making of suitable grooves and other excavations for the reception of the specimens to be cut in pith, extremely good results may be obtained.

12. Mounting.—In the following directions the term "specimen" applies to all objects examined, whether entire or in thin sections.

First, see that the slide and cover-glass are dry and clean. Take a slide by its edges with thumb and forefinger of left hand, dip one half of it in water, withdraw it, and with a clean cloth (an old handkerchief is better than a duster, being freer from fluff) rub both wetted surfaces at once until they are quite clean and dry; then lay the slide on a clean suitable background, as white or black paper.

Cover-glasses, as bought from dealers, often have a cloudy film on them; to get rid of this, put the cover-glass in 50 per cent. sulphuric acid for a minute, then rinse it in water; take it between two folds of the cloth held between thumb and forefinger of right hand, and carefully rub both surfaces at once until clean and dry; do not lay

the clean cover flat, but prop it against some clean and dry object until it is required for mounting. Always clean and dry the slides and covers when done with; do not put them away wet or dirty.

In mounting, place on the centre of the slide a drop of the mounting fluid. Place the specimen in this, and carefully lower the cover-glass, in such a way that no air bubbles shall be included in the preparation. To do this, hold the clean dry cover at one side of the drop of fluid, in a tilted position, then place a mounted needle under the cover at the other side and gently lower the cover by withdrawing the needle. If this is done carefully, very little fluid will flow from below the edges of the cover, and no air bubbles should be included.

If water flows over the upper side of the cover, or if air bubbles get in, remove and dry the cover and try again. With a little practice one can accurately judge the size of the drop of fluid according to the size of the cover-glass and the thickness of the specimen. Any slight excess of fluid on the slide around the edges of the cover can be soaked up with torn bits of blotting-paper.

It is of the utmost importance that no water or other mounting fluid should reach the upper side of the cover or the surface of the objective; if either of these get wetted, or if liquid gets on the lower side of the slide or on the stage, draw up the tube of the microscope, and thoroughly clean and dry the objective, the slide, and the stage, also removing the cover-glass and making a new preparation. Absolute cleanliness and care in the use of mounting fluids and reagents must be observed, otherwise much time may be wasted. *A cover-glass must invariably be used with the high-power objective*

Some other points worth noting are the following. Never use more than one cover on a slide, and place this as nearly as possible at the middle of the slide. Never press upon the cover, unless there is some definite object in doing so; if a section is too thick, pressure will only make it worse, and if the section is thin it will simply be ruined by pressure. A section must not be allowed to get

dry in the interval between cutting and mounting it; transfer it from the razor to a watch-glass of dilute alcohol, and have the drop of water or other mounting fluid ready on the slide before transferring the section to it. Always examine the specimen in water first, before applying special reagents. If air bubbles are entangled in the tissues in a section, moisten the section with weak alcohol, or leave it for some time in a watch-glass of weak alcohol—this will at any rate remove some of the air.

13. Application of Reagents.—It is always advisable to have several specimens, whether whole objects or sections; in the case of sections this is especially necessary, so that the thinnest may be used for examination with the high power. The various reagents—iodine, aniline sulphate, chlor-zinc-iodine, etc.—may be placed directly on a slide, the specimen being then placed in the drop of reagent, and a cover lowered on the preparation, in exactly the same manner as in mounting the specimen in water or dilute glycerine

If the specimen has been mounted in water and examined and sketched, any one of the special reagents may be applied by simply raising the cover-glass with a needle, placing on the specimen a drop of the reagent, and lowering the cover again, after washing away the superfluous fluid by means of water and wiping the slide dry a little outside of the specimen all round. It is often desirable, however, to watch the action of the reagent without removing the cover-glass from the specimen, and this can be done by irrigation.

14. Irrigation.—To irrigate a specimen with any reagent, place a drop, or several successive drops, of the reagent on the slide close to one edge of the cover—taking care that it does not get on to the upper side of the cover—and place a small torn bit of blotting-paper at the opposite edge of the cover so as to draw the reagent through, watching meanwhile for any effect produced by the reagent on the specimen. Since in irrigation the reagent may fail

to penetrate the specimen or reach only its edges, before concluding that the result of irrigation is negative it is advisable to raise the cover-glass and apply a drop of the reagent to the specimen directly.

15. Clearing Reagents for Temporary Mounts.—Sometimes it is difficult to see the cell-walls in a section on account of the dense cell-contents; or it may be desired to make an entire leaf transparent. For any such purpose various clearing reagents are used; the mode of action of such reagents differs in different cases, but the result is to make the specimen more transparent

(1) **Glycerine** is frequently used instead of water for the mounting of specimens, partly because it does not evaporate and partly because it makes sections more transparent, hence it is a clearing reagent as well as a mounting medium.

(2) **Caustic Potash** causes swelling and partial disorganisation of the cell-contents, and is especially useful with such preparations as sections of growing-points, embryos *in situ* in ovule or archegonium, etc. A 5 per cent solution in water answers for most purposes, but for denser tissues a concentrated solution in alcohol may be used. If the solution does not quickly make the tissues transparent warm the slide. If the specimen becomes too much swollen, so that even the cell-walls are not seen clearly, check the action of the potash by treating the specimen with 10 per cent. acetic acid In any case, it is as well to rinse the specimen in water after treatment with potash

(3) **Eau de Javelle** (see Appendix) is often preferable to caustic potash Either mount the specimen in this reagent and put it aside for a few minutes, or warm the slide to hasten the action, then wash with water, followed by acetic acid, and mount in glycerine Eau de Javelle has much the same action as potash, but it does not cause so much swelling, and the cell-walls are left more distinctly visible.

(4) **Chloral Hydrate** (see Appendix) is a useful clearing reagent for pollen-grains, embryos, fairly thin entire leaves, etc. The specimen may be either left in the solution overnight, in a covered vessel, or may be heated in the solution to hasten the action See also Chloral Hydrate Iodine in Appendix.

(5) **Carbolic Acid (Phenol)** is sometimes used for clearing. It may be used instead of chloral hydrate for such specimens as entire leaves which have been decolorised in alcohol; the leaves are transferred from the alcohol to either pure carbolic acid or a mixture of three parts turpentine and one part carbolic acid. Pollen-grains, etc., may be cleared in this way

16. Permanent Glycerine Mounts.—Preparations mounted in water or in iodine or aniline sulphate solution are purely temporary, since these liquids quickly evaporate. Mounts made in glycerine or chlor-zinc-iodine do not evaporate. A glycerine mount may be made permanent by (1) sealing, or (2) transference to glycerine jelly.

To seal a glycerine mount, either unstained or after staining (see § 17), place the specimen in 10 per cent. glycerine in a watch-glass or on a slide (without cover-glass), and put the preparation in a covered dish, to let the water evaporate from the glycerine gradually in a place as free as possible from dust. When the glycerine has become about as thick as pure glycerine, cover the preparation, taking care to have just enough glycerine to come to the edge of the cover-glass—if any comes beyond the edge carefully wipe it away. Then seal the mount, and with a brush paint around the edge of the cover-glass a ring of Canada balsam, gold size, or other cement. Gold size answers well; apply it with a camel-hair or sable brush; a turn-table may be used with advantage; on three or four successive days, or at shorter intervals, apply the size again as the previous portion sets, so as to have a fairly thick ring—not thick enough to be in the way in using the high power objective; if the size gets too thick, thin it with turpentine—if it is too thin, leave the cork out of the bottle till it thickens.

An excellent method, devised by Prof. Lagerheim:—Take equal parts of hard paraffin wax (melting-point 55° to 60° C) and mastic; powder the mastic and heat it in a porcelain dish (on a tripod over a Bunsen or spirit-lamp) until melted; then add the paraffin in small pieces, stir the mixture till free from lumps and quite homogeneous; then pour it into a flat dish which can be covered (a Petri dish answers well), and let it cool; to apply the wax, fix into a wooden handle the long arm of a L-shaped piece of thick copper wire, the short arm of which is just under 1 inch long (*i. e.* a little longer than the length of the square cover-glass used; heat in a spirit or Bunsen flame, dip into the wax, and apply the wax-covered wire along each edge of the cover-glass in turn—the melted wax solidifies at once on contact with the glass, forming a strong join; then paint a thin coating of gold size over the wax.

If carefully sealed a glycerine mount is fairly permanent, but it is a useful plan to transfer the specimen from glycerine to glycerine jelly, especially if the object is of such thickness that the glycerine oozes out beyond the cover and thus makes it difficult to seal the preparation. Place the specimen in 10 per cent. glycerine, let this evaporate and become thick, then put the glycerine-jelly bottle into hot water until the jelly melts, put a drop of melted jelly on a warmed slide (using a glass rod, which may be passed through a hole in the cork of the jelly bottle), and transfer to it the specimen; cover, and set aside to cool. It is as well to seal jelly mounts, in the same way as glycerine mounts. A simpler method is to put a bit of the cold jelly on a slide, heat the slide till the jelly melts,

place in it the specimen, and cover—but this may damage the specimen, and it is better to apply as little heat as possible.

17. Staining with Dyes and Mounting in Balsam.—In addition to the various "microchemical" reagents which give characteristic reactions with certain cell-contents and cell-walls—*e g.* iodine, chlor-zinc-iodine, aniline sulphate, Millon's reagent, alkannin—it is often useful to stain specimens with dyes in order to see clearly certain structures which are otherwise not readily distinguished on account of transparency or lack of colour, or to bring out differences between bodies of nearly the same general appearance.

It is not proposed to give here a full account of the various stains used, the majority of which are aniline dyes, the chief non-aniline stains being the haematoxylins and carmines. Delafield's haematoxylin is perhaps the best general dye to use when single staining is required; other useful stains for this purpose are safranin, eosin, and aniline blue, all of which may be used for specimens which are to be mounted in glycerine as temporary preparations, or made permanent by sealing or by transference to glycerine jelly.

For various purposes specimens may be stained with two or even more dyes in succession. A simple form of double staining is that which has for its object the production of one colour in cellulose walls and a second colour in lignified walls. Beginning with sections of Marrow stem, for instance, we may either (1) transfer the sections from one liquid to the next in a series of watch-glasses or pots, or (2) perform all the processes on the slide, applying drops of the various liquids in turn by means of the glass rod belonging to each bottle.

First, treat the section with strong alcohol for a minute or two; then drain this off and add some safranin; after ten or fifteen minutes treat with 50 per cent. alcohol, and examine the specimen until you find that the red colour has nearly disappeared from the cellulose walls, though still present in the lignified walls. Treat the section for two or three minutes with Delafield haematoxylin—this will stain the cellulose walls, but should not displace the safranin from the lignified walls. Treat with water; if the purple colour is very deep, add a trace of hydrochloric acid (a drop to 50 c.c. of water), and as soon as the sections begin to turn reddish rinse them in plain water. Treat with ordinary alcohol for two or three minutes, then with absolute alcohol for five or ten minutes—to dehydrate the section, which is very important; then drain off the alcohol, and cover with a drop of clove oil, to clear the section; then drain off the oil, put on a drop of balsam, and cover. In this way we get a permanent double-stained balsam preparation; the lignified and suberised walls are stained red, the cellulose walls violet.

Various other combinations of stains are used for double staining, on the same general principles, sections with lignified and cellulose

walls. In each case apply first the dye which is to remain in the lignified walls. In the following list the dye named first in each pair is that which stains the lignified walls, while the second stains the cellulose walls: safranin and aniline blue; safranin and acid green; iodine green and acid fuchsin; iodine green and carmalum; cyanin and Congo red.

Various dyes are referred to in the Appendix and in other parts of this book, but it should be remembered that very good general

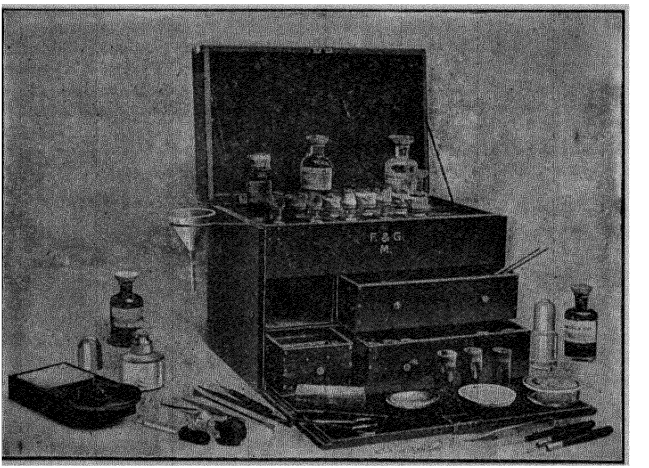


Fig. 11 – Mounting Cabinet (Flatters & Garnett), fitted with accessories for microscope work, stains, reagents, etc

work may be done without having resort to more than a very few of these stains, in addition to the reagents used in making temporary preparations. All the stains can be purchased in solution, ready for use.

Fig. 11 shows a most convenient and well-fitted mounting cabinet, supplied by Messrs. Flatters & Garnett. It contains a large selection of reagents and stains, in addition to a complete outfit of accessories for microscope work. Full particulars may be obtained from the makers (see Preface).

18. Moist Chamber Slides.—For the germination of spores and the growth of pollen-tubes, kept under observation in a hanging drop of culture solution, there are

various methods of fixing up a moist chamber slide. A simple plan is to cement a glass or rubber ring to a slide —slides with such rings can be bought ready prepared

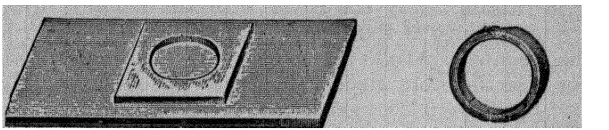


Fig 12.—A Moist Chamber Slide; on the right, a glass ring which can be cemented to an ordinary slide, to form a moist chamber.

(Fig. 12); then place in a small drop of liquid the object to be examined, invert the cover so that the liquid does not get on the upper side of the cover, and lay the inverted cover on the other ground edge of the ring—which should be smeared with vaseline to make the chamber air-tight.



Fig. 13.—A Ward's Tube (Moist Chamber) which can be cemented to a slide.

Another plan, even better for many purposes, is to cut a square or round hole 5/8ths of an inch in diameter in a piece of cardboard 1/8th inch thick, 1 inch wide, and 1½ inches long; boil the card to sterilise it—the boiling also makes it fit more closely to the slide; while still wet press the card to the slide, and invert the cover-glass, with its hanging drop, over the hole.

19. Ward's Tube (Gas Chamber) Slide.—This apparatus, which is especially suitable for experiments on

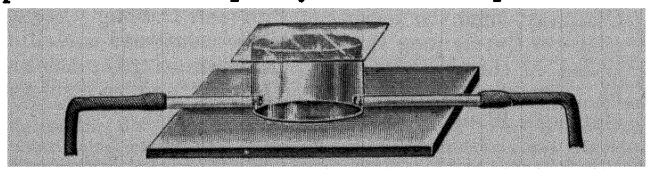


Fig 14.—Gas Chamber Slide, the side tubes are shown fitted with rubber tubing.

protoplasmic streaming (see §§ 33-35) and also for cultures of pollen-grains, spores, etc., can be bought ready fitted up (Fig. 14); or the tube itself (Fig. 13) can be

obtained, consisting of a ring of glass with two glass tubes annealed to it on opposite sides, and fitted up—simply cement one of the ground edges of the ring to a glass slide with balsam. Lay the cover with the hanging drop on the upper edge of the ring, smeared with vaseline to make the chamber air-tight; gases can be led through the chamber, and therefore made to penetrate the specimen.

20. Apparatus for Plant Physiology.—Apart from expensive "precision apparatus," made for research purposes, apparatus for Plant Physiology may be either (1) Normal or Standard Apparatus, made specially for its particular purpose, giving quantitative results of approximate accuracy, and obtainable ready made from supply firms who specialise in this kind of apparatus; or (2) Adapted Apparatus, made up carefully from various appliances and articles sold for work in Chemistry and Physics, these being altered to suit the special purpose, giving qualitatively correct results and therefore serving for elementary work and also in many cases for advanced work in Plant Physiology; or (3) Makeshift Apparatus, put together from common appliances for temporary purposes, giving only crudely qualitative results, and only justifiable in most cases in a Nature Study Course. We need only consider here the Normal and the Adapted Apparatus.

21. Normal Apparatus.—The best set of apparatus of this kind is that supplied by the Bausch and Lomb Optical Company, from the designs of Professor Ganong. Various pieces of the Ganong Apparatus are mentioned and illustrated in this book—for full descriptions reference may be made to the Company's catalogues and to Professor Ganong's *Plant Physiology*.

22. Adapted Apparatus.—Much useful information on the fitting up of apparatus in general is given in works on Practical Chemistry (see Preface). The Chemical Catalogue issued by Messrs. Baird and Tatlock should be consulted for particulars of the various articles and

appliances mentioned in the following lists Other articles are mentioned in various parts of this book in connection with special experiments.

23. General Appliances and Articles Required.—The following lists include various articles which should be available, since they will all be required, especially in the putting together of adapted apparatus.

A set of carpenter's tools; a soldering outfit; round and triangular files (three sizes of each) and a large flat file; pliers, including wire cutter. A set of cork borers; a cork presser An air-pump, or an exhausting and condensing syringe. A pair of strong scales. A good (not necessarily expensive) balance, to carry 100 grams and sensitive to 5 milligrams; a set of gram weights. A drying (hot air or water) oven; a sand bath. A meat-juice press. A spectroscope. Retort stands; filter stand; clamps; test-tube stands; test-tube brushes; Bunsen burners or spirit lamps; tripod stands; mortar and pestle.

Aspirator; beakers of various sizes; bell jars (for many purposes the cheap "cloches" used by gardeners will answer); bottles of various forms and sizes; burettes; desiccator; dialyser ring; Erlenmeyer (conical) flasks; fat extraction apparatus (Soxhlet's); filtering flasks; funnels; glass rods; glass sheets; glass tubing of various diameters, including some barometer tubing and some capillary tubing; graduated vessels; separating funnels; Petri dishes; pipettes, plain and graduated; Soyka flasks; test-tubes; thermometers; thistle funnels; U-tubes; vacuum flasks and bulbs; wash-bottles; watch-glasses; white saucers.

Corks of various sizes; rubber stoppers, to fit large flasks, etc.; rubber tubing, including some stout tubing; tinfoil; sealing-wax; plasticine; vaseline; beeswax; filter-papers; litmus papers, red and blue; gummed labels; parchment membrane; diffusion shells, of test-tube form; black paper; pins; thread; copper wire and iron wire; hard paraffin. Wood blocks and wedges for supporting apparatus. Porous flower-pots and saucers of different sizes.

Various chemicals are also required, *e g.* alcohol (methylated spirit); ammonia; ammonium molybdate solution; baryta water; caustic potash; coco-butter for making joints air-tight, etc.; copper sulphate; corrosive sublimate (mercuric chloride), as an antiseptic; distilled water; eucalyptus oil, as an antiseptic; hydrochloric acid, strong and 10 per cent.; iodine solution; lead acetate; lime-water; magnesium sulphate; nitric acid, strong and in 10 per cent. solution; potassium dichromate solution; potassium nitrate; soda lime; sodium chloride; sulphuric acid, strong and in 10 per cent. solution; thymol, as an antiseptic; wax mixture. The use of each is mentioned in connection with various experiments; see also Appendix.

24. Hints on Fitting up Apparatus.—To be successful in experiments with adapted apparatus, careful attention should be paid to such details as the boring of corks, the bending of glass tubing, the accurate fitting of tubing into corks, etc Only a few general hints can be given here Since it is frequently necessary to fit flasks, etc., with corks and to bend glass tubing to various angles, we shall take as an example the making of a wash-bottle.

(*a*) **Fit a Flask of Medium Size with a Cork.**—Select a cork a little too large; wrap it in a piece of paper, and using gentle pressure with your foot, roll it to and fro upon the ground—or a cork presser may be used This softens the cork, and the risk of breaking the neck of the flask is lessened. If still too large, file down the cork equally all round.

(*b*) **Bore a Cork Lengthwise and Fit a Glass Tube tightly into the Hole made.**—Select a cork-borer (Fig. 15) slightly less in diameter than that of the tube to be fitted into the cork. The cork-borer is a brass tube about 5 in. long sharpened at one end. At

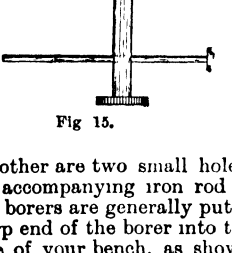


Fig 15.

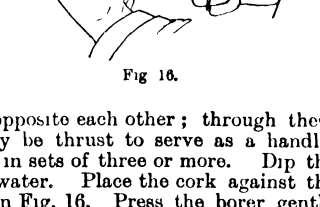


Fig 16.

the other are two small holes opposite each other; through these the accompanying iron rod may be thrust to serve as a handle The borers are generally put up in sets of three or more. Dip the sharp end of the borer into the water. Place the cork against the edge of your bench, as shown in Fig. 16. Press the borer gently into the narrower end of the cork and twist the borer round (always in the same direction) until it emerges at the other end of the cork.

Now take the cork prepared in (*a*) and bore two parallel holes in it similar in position to those in the wash-bottle (Fig. 19).

Well sharpened borers can also be used for rubber stoppers. In this case they are moistened with either alcohol or glycerine, and pressed through more slowly.

Glass tubes should always be dipped in water before being pushed through the hole in the cork or stopper.

(*c*) **Cut some Glass Tubing about $\frac{1}{4}$ in. in Diameter into Lengths 4 to 6 inches.**—Lay the tube flat on the bench and with a sharp triangular file make a scratch across it where required, the

pressure used being regulated by the thickness of the tube Now hold the tube in both hands, with the scratch away from the body and the tips of the thumbs touching each other just opposite the scratch. Break the tube by bending it, giving a pull at the same time. Round off the sharp ends by fusing them in the Bunsen flame—hold the tube vertically until the flame is coloured strongly yellow by the sodium of the glass.

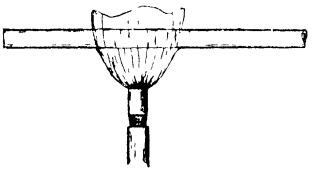


Fig 17.

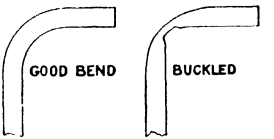


Fig 18

(*d*) **Bend some pieces of Glass Tubing to form Right Angles.**—Use an ordinary spreading gas flame lowered until it is about 2 in. across. Place the tube over the flame for a few seconds, and gradually bring it down into the hottest part, as shown in Fig. 17. Turn the tube round and round till it softens, then allow one end to fall until it makes the required angle. The bend should be round and smooth ; the Bunsen flame is apt to give buckled bends (Fig. 18). Do not remove the soot until the tube is cool

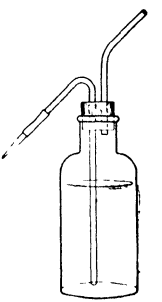


Fig 19

(*e*) **Bend some Tubing twice at Right Angles so as to form Three Sides of a Rectangle.**—When laid down all three sides must touch the bench.

(*f*) **Make two Nozzles** —Hold a piece of tubing by both ends in a flame ; soften the middle, and pull the ends slightly apart. Cut the tube through and round off the ends

(*g*) **Complete the Wash-bottle.**—Bend suitable pieces of tubing to form angles equal to those seen in the wash-bottle in Fig. 19. Push them through the cork prepared in (*b*), and attach a nozzle by means of an inch or so of rubber tube.

25. Experiments.—In making experiments, sketch the apparatus used. Make notes of the materials experimented with (name of plant or part of plant, number, condition, stage of growth, etc.); the duration of the

experiment, date, time of day, the external conditions (temperature, light-intensity, barometer-reading, etc); the precautions which seem necessary, and the sources of error which may spoil the results.

Always make "control" or "check" experiments, using the same form of apparatus, set up at the same time, but with one or other of the conditions different, *e g.* in darkness instead of light; with the plants omitted; with killed instead of living plants; with plants in different stages of growth. Also make "repeat" experiments, using different plants under similar conditions or the same plants at different times of year or day, etc.

If your experiments do not succeed, try again, if they give discordant results, try to account for these and to think out a method for a repeat experiment under different conditions, with special precautions, or for making a new experiment altogether. In drawing conclusions, try to distinguish between probability and actual proof.

CHAPTER II.

CELL-CONTENTS AND CELL-WALLS.

I. The Cell; Nuclear and Cell Division.

26. The Vegetable Cell.—The body of the higher plants consists of various forms and modifications of cells. A normal uninucleate cell consists of two series of parts:—(1) the protoplast, (2) ergastic or secondary structures.

(1) The **protoplast** or protoplasm body is again divisible into (*a*) **protoplasmatic** organs and (*b*) **alloplastic** organs. The former are distinguished by the fact that they do not arise *de novo*, but are multiplied by division. (*a*) To the protoplasmatic bodies belong (i) the **cytoplasm,** or general protoplasm; (ii) the **nucleus**, with the chromosomes; (iii) the **trophoplasts,** which are either **autoplasts** (usually **chloroplasts**) capable of photosynthesis, or **leucoplasts,** or **chromoplasts.** (*b*) The alloplastic organs, which arise by slight alteration of the ordinary protoplasm, include (i) the **surface layer** of the cytoplasm, lying immediately within the cell-wall; (ii) the **tonoplast,** or layer lining vacuoles; (iii) the **cilia** of motile cells (zoospores, etc.).

(2) The **ergastic structures** are formed by the protoplasm, cannot multiply by division, and arise *de novo* as either (*a*) inclusions of the protoplast—*e.g.* **cell-sap, oil drops, calcium oxalate crystals, starch grains, protein crystals,** or as (*b*) excretions of the protoplast—*e.g.* the **cell-wall.**

27. Protoplasm, Nucleus, Mitosis, Cell-Division.—In order to make out the minute details of protoplasmic and especially of nuclear structure, the materials must be

carefully selected, fixed, and stained. However, there are a few cases in which it is possible to trace some of the stages of nuclear division (mitosis, or karyokinesis) and cell-division without the use of fixative or stains

(*a*) The processes of division can be, in part at any rate, observed in *living* cells. Carefully open a flower-bud of Tradescantia which is almost ready to open (choose a warm day or use a plant that has been kept for some time in a warm place, so that growth has been vigorous), remove the stamens, mount them in 2 per cent. sugar solution, cut off the anthers, cover the filaments, and examine the hairs on the latter with high power. Each hair consists of a row of cells, having relatively large nuclei. In most of the cells the nucleus will appear rounded and definite in form (resting nucleus), but in the longer cells at or near the end of the hair the nucleus has an elongated form and ill-defined appearance (dividing nucleus).

In a **resting nucleus** note (1) the fine chromatin threads forming a network and giving the nucleus a granular appearance, (2) the highly refractive nucleoli—usually one or two in number, sometimes more.

In the **dividing nuclei** the following stages can be made out:—(1) the nucleus grows larger; (2) the threads become thicker; (3) the network breaks up into a number of rod-like chromosomes, at first curved; (4) the dividing nucleus becomes spindle-shaped, with the chromosomes straightened and arranged in two groups, one group on either side of the equator of the spindle (each original chromosome has split longitudinally into two, one half passing to one side of the equator and the other half to the other side, but this is not easily observed), the cell meanwhile having grown in length; (5) the chromosomes of each group become curved again, and join up to form the chromatin network of the new nucleus; (6) the cell-plate is formed at the equator of the spindle, by the fusion of granules which have appeared here; (7) the spindle widens out, so that the cell-plate reaches the outer wall of the cell, which is thus divided into two cells by the new cell-wall.

(*b*) Some hairs of Tradescantia should be **stained,** in order to bring out the details clearly. For this purpose we may either use a single stain, or two stains of which one will show up the chromatin and the other the fine spindle-threads. Of single stains, methyl green or haematoxylin should be used. For double staining use first safranin and then gentian violet; the former stains the chromosomes and nucleoli, the latter the threads of the spindle connecting the two new nuclei. In the case of living cells it is better to use a single stain, and methyl green answers well with the hairs of Tradescantia.

(*c*) In order to study both nuclear division and the changes undergone by young cells, root-tips afford good material. The

roots of Hyacinth or of Onion, obtained by growing the bulbs in hyacinth-glasses containing culture solution, may be used. The tip (about an inch) of a growing root is cut off, and the tips are at once transferred either to absolute alcohol (or strong methylated spirit), or (if the mitotic figures are to be obtained with certainty) to a fixing solution containing 10 parts by volume of 2 per cent osmic acid, 4 parts of 10 per cent. chromic acid, 3 parts of glacial acetic acid, and 20 parts of water. If the latter fixing solution is used, the tips must be left in it for about 12 hours, then transferred to water and thoroughly washed for several hours, then hardened by being placed in increasing strengths of alcohol—70, 80, 90 per cent., and finally absolute alcohol, for a few hours in each case. After this, they are transferred to methylated spirit, and sections cut in split pith. After staining, the sections should be treated with absolute alcohol, cleared with clove oil, and mounted in balsam

Preparations of root-tips, cut with the microtome and doubly stained, may be purchased The details of mitosis are given in text-books, and most of the stages may be traced in successful preparations made from root-tips

(*d*) **Direct division (fragmentation)** of the nucleus may be observed in the large internodal cells of *Nitella*, or in longitudinal sections of the stem of *Tradescantia* and various other plants It takes place chiefly in old cells, which have ceased to undergo cell-division. The nucleus becomes elongated and dumbbell-shaped, and finally constricted into two, in much the same way as a dividing chloroplast.

II Streaming Movements of Protoplasm.

28. Streaming Movements of living protoplasm, rapid enough to be watched under the microscope, are well shown in the long cells of the Stoneworts Nitella and Chara, and in the leaf of Elodea. This streaming, or **cyclosis,** may also be studied in the plasmodium of Myxomycetes; in the Desmid genus Closterium; in the mycelium of Mucor; in the epidermis torn from the inner scales of an Onion bulb; and in hairs found on the roots, stems, leaves, and flowers of various plants It will usually be found that the movements can be started or, if already in evidence, hastened by warming the preparations or by using warm water to mount the objects in.

29. Cyclosis in Elodea.—Mount in water a few leaves of Elodea, which grows abundantly in many rivers and canals, having long submerged stems and leaves arranged in whorls of three. Look for the streaming of the protoplasm in the leaf-cells. The long narrow cells of the midrib show a continual **rotation,** which by careful focussing is seen to be confined to the inner portion of the "primordial utricle"—this portion of the protoplasm flows round the lateral and end walls of the cell, carrying with it the chloroplasts. The outer portion, in immediate contact with the cell-wall, is at rest, as is also the whole protoplasm layer lying along a line ("indifferent" or "neutral" line) in the middle of the upper and lower walls—these points are more easily seen in Nitella or Chara.

In the shorter and broader cells on each side of the midrib there are strands of protoplasm running across the vacuole, some being attached to the central nucleus; in these cells the strands, as well as the primordial utricle, show streaming movements in all directions—these movements of **circulation** may also be seen in the staminal hairs of Tradescantia (§ 31).

30. Cyclosis in the Stoneworts.—Examine specimens of Chara and Nitella, which grow in stagnant or sluggish water, rooting in the mud and sending up shoots often a foot long which bear whorls of appendages ("leaves"). Each "internode" contains a single very long cell, but in Chara this is covered by a layer of cortex filaments (except in the terminal cells of the "leaves")—the rotation can be observed in these naked "leaf" cells of Chara or (better) in the long naked internodal cells of Nitella, which has no cortex.

Note that here the chloroplasts, which lie in the outer layer of protoplasm just within the cell-wall, remain stationary; the movement, which is confined to the colourless inner layer, is shown by the sweeping along of the granules embedded in this inner portion of the protoplasm. Note the very conspicuous "indifferent line" which runs spirally along the cell and is sharply defined

by the absence of chloroplasts. Carefully watch the rotation movement; on the two sides of the colourless "indifferent line" the protoplasm moves in opposite directions.

31. Cyclosis in Staminal Hairs of Tradescantia.—Take a newly opened flower, preferably on a warm day; cut off the stamens, mount in water, and examine the hairs on the filaments—each hair consisting of a row of cells with violet sap. Note that the protoplasm in these cells is in active movement in various directions; that in the thickest of the strands extending across the vacuole two currents may be seen flowing simultaneously in opposite directions; and that in any part of the protoplasm the movements may stop for a time and then start again—sometimes in the reverse direction.

32. Influence of Temperature on Protoplasmic Streaming.—While watching movement in Elodea or Nitella, place a piece of ice at the edge of the cover-glass, and a strip of filter-paper at the opposite edge, so as to draw cold water through; the movement slows down and stops, but starts again as the water gets warmed. Heat the slide over a flame; with gentle warming the rate of streaming is hastened, but if the slide is heated further movement stops, and the protoplasm is of course killed if the water is heated still further.

A better method is to use a Ward's tube (§ 19) and draw through (*a*) air heated in a U-tube held over a flame, (*b*) air chilled in a U-tube placed in chopped ice.

33. Effect of Chloroform.—To observe the effect of anaesthetics, etc, use a Ward's tube cemented to a slide, placing the specimen in a drop of water on a cover-glass, inverting the cover, and sealing it air-tight over the chamber. For experiments in which it is not desired to lead gases through the apparatus, use either the Ward's tube with the ends open or an ordinary moist-chamber slide (§ 18).

Half fill a wash-bottle with water, add a few drops of chloroform—about 1 per cent.—cork tightly, and shake the bottle. Fix a rubber tube to the short tube of the bottle and to one end of the gas-chamber; join the other end of the chamber by rubber tubing to an aspirator, and let the air charged with the chloroform pass through the chamber, on which is inverted a cover-glass with a drop of water containing an Elodea leaf or other object showing active protoplasmic streaming. The chloroform vapour causes the movement to slow down and finally stop. Disconnect the wash-bottle, so as to let fresh air pass through, the movement will be resumed—if the chloroform vapour has not been allowed to kill the protoplasm

34. Effect of Carbon Dioxide.—Lead carbon dioxide through the gas-chamber—*e.g.* by placing plain water in the wash-bottle and joining its long tube to a bottle in which carbon dioxide is generated by pouring dilute hydrochloric acid on marble chips or chalk. The movement is quickly arrested, but is renewed on disconnecting the apparatus to let fresh air pass through.

35. Effect of Exclusion of Oxygen.—Repeat the preceding experiment, using hydrogen generated by pouring hydrochloric acid on zinc filings. Or oxygen may be excluded by simply placing with a pipette some freshly made potassium pyrogallate in the gas-chamber, after sealing the tubes up, and quickly laying the inverted cover-glass preparation on the upper edge of the chamber. In absence of oxygen the movement continues longer than in the preceding experiments and is only gradually slowed down; if hydrogen is used, it acts by simply excluding oxygen from the protoplasm, not as a poison or narcotic.

III. Effects of Heat, Cold, etc., on Protoplasm.

36. Effects of Heat, Cold, Poisons, etc., on Protoplasm.—We have already noted the effect of these agencies on protoplasmic streaming. That protoplasm alters when killed can be shown in various ways.

(*a*) The leaves of most plants change but little in colour when plunged into water at 60° C. or over, but they become limp, owing to the cells losing their turgidity on being killed, and cannot be restored to the normal condition of turgescence However, some leaves, *e.g.* Oxalis, Vine, Begonia (especially B. manicata), quickly become discoloured by hot water, owing to the chlorophyll being decomposed by the acid sap which, on the death of the protoplasm, is allowed to come into direct contact with the chloroplasts.

(*b*) Tie an Oxalis leaf to the bulb of a thermometer and hold it in water in a beaker above a Bunsen, or in a large test-tube, and gradually heat the water. Try several times, and carefully note the average temperature at which the colour change occurs—usually about 50° C. or a little over.

(*c*) Place Oxalis leaves, some entire and others cut into pieces, in a bottle of 1 per cent. chloroform water; note the time taken for the colour change in each case. Try other poisons instead of chloroform in the water, *e g* carbolic acid, formalin.

(*d*) Cut out two pieces of living Begonia leaf-stalk, rinse them in water, then place one in a beaker of cold water (either distilled water, or water that has been boiled and allowed to cool) labelled *A*. Kill the second piece (*B*) by immersion in very hot water; when it is discoloured, put it in a second beaker of water. After half an hour remove the two pieces, and pour into the water in each beaker an equal quantity of strong calcium chloride solution. In *A* the water remains clear; that in B becomes turbid, owing to the formation of calcium oxalate produced by the oxalic acid which has escaped from the killed cells.

(*e*) The effect of **mechanical injury** on the protoplasm can readily be shown by firmly squeezing between the fingers or a pair of forceps a Begonia leaf; the crushed parts at once become brownish. Cut and mount in water a tangential section of the injured part, and note that the chloroplasts have lost their green colour and become

brown. The pressure has destroyed the protoplasm and made it permeable to the acid sap, which then decomposes the chlorophyll.

(*f*) Another indication of the death temperature of protoplasm is afforded by cells with coloured sap, *e.g.* those of Beetroot. We have already noted that when Beetroot sections are heated the red sap escapes from the cells. Cut a thin slice (3 or 4 mm.), rinse it in cold water (to remove any sap on cut surfaces), and suspend it, with a thermometer, in a beaker of cold water; then gradually heat the water. The red sap does not, as a rule, escape until the temperature exceeds 55° C.

(*g*) Here is another proof of the difference between living and dead protoplasm as regards permeability. Cut two fairly thick slices of Beetroot and rinse them thoroughly in water. Place one slice in some cold water in a beaker (*A*). Plunge the other slice in boiling water to kill it, and then place it in water in a second beaker (*B*). After an hour take some water from each beaker; add in each case a few drops of sulphuric acid and boil, then pour in some Fehling's solution and boil again. Sugar is present in the water in *B*, but not in *A*.

(*h*) Cut out two pieces of fresh Turnip, rinse them in water; kill one piece by immersion in very hot water. Mince a piece of Beetroot, boil with water, and pour the red juice into a shallow dish, and lay in the juice the two pieces of turnip. Note that, after a day's immersion, the killed piece of turnip is stained right through, while the living piece is unstained or only slightly stained on the surface.

(*i*) Cut a fairly thick (about 1 cm.) slice of Beetroot, rinse it in water, wipe it dry, and place it in a glass jar with a cork, through which passes a thermometer. Put the jar in a larger vessel containing a freezing mixture of snow (or broken ice) and salt, giving a temperature of −6° C. or lower. After a time quickly remove and examine the frozen slice; its surface is covered with a layer of ice, consisting of parallel rods, most abundant on the lower side where the slice was in contact with the

glass of the jar. Note that the ice is colourless, showing that only water, not the coloured sap, has been frozen out of the cells

(*j*) Freeze another Beetroot slice, and suspend it in a beaker of water at the ordinary temperature; arrange a slice of unfrozen root in a similar beaker for comparison; the frozen slice yields its coloured sap to the water, the other does not.

(*k*) Mount filaments of Spirogyra in water on a slide, place the preparation in the freezing apparatus, and note that the cells are strongly plasmolysed and shrunken, but on thawing the cell-wall is seen to be intact. Freezing does not cause rupture of the cell-wall.

(*l*) To illustrate the fact that freezing causes a molecular change in the protoplasm—a rearrangement of the molecules—make some starch paste in a beaker or test-tube. Freeze the paste; when it thaws it is no longer a homogeneous liquid, but has become spongy, the "pores" being filled with fluid.

(*m*) Our experiments with frozen Beetroot slices and Spirogyra threads suggest that on freezing the formation of ice takes place not inside the cells themselves, but on the outside—in the intercellular spaces in the case of a mass of tissue. To demonstrate that this is usually the case (though under some conditions ice is formed within the cell), cut off the upper part of a Beetroot, scoop out a cavity in the lower part, and fix the upper part on again, like a lid, with thread. Freeze to about $-8°$ C., and on removing the lid-like upper part note that ice has accumulated in the cavity.

(*n*) That ice usually forms, at any rate at first, in the intercellular spaces may be directly observed. Freeze a Potato or a Carrot, and with a very cold razor (chilled by being put in the freezing apparatus or in ice-water) cut sections and mount them on a chilled slide. Observe quickly, and note that the ice crystals have been formed between the cells. As thawing proceeds (check its rate by placing a bit of ice at the side of the cover-glass), note that the intercellular spaces have expanded as the ice

accumulated, so that the cells have been disturbed and thrust asunder. As the tissue freezes water is drawn from the cells, and this on freezing collects as films on the walls abutting on the intercellular spaces As the water is withdrawn from the cell-sap these films accumulate and cause disruption of the tissue, the cells at the same time shrinking

(*o*) From experiments like the preceding, it has been suggested that death from freezing is really due to the resulting withdrawal of water from the protoplasm, and that unless and until ice-formation occurs the cold is not fatal—that for sudden death on cooling ice-formation is essential, whether it acts on the protoplasm directly or indirectly The reverse, however, is not true, for many plants readily recover after being frozen solid It has also been suggested that it is only on the thawing of the cell that the fatal disorganisation occurs, and that if thawing proceeds slowly recovery may take place even in cases where quick thawing would lead to death. But this view has been disproved by experiments, of which the two following should be made

(1) The cells of the red sea-weed Nitophyllum, on being frozen to $-5°$ C, show orange-red fluorescence; in the living cells the pigment shows no fluorescence, and its appearance is a sign of death. (2) Treat in the same way the leaves of the commonly cultivated Ageratum mexicanum; on freezing the characteristic smell of coumarin is perceived This aromatic substance, which occurs in Sweet Woodruff and some other plants, is not present in living Ageratum leaves, but is produced on the death of the cells.

IV. Proteins and their Digestion.

37. Vegetable Proteins.—Various proteins occur in plants. The proteins contain Carbon (50 to 55 per cent.), Hydrogen (about 7 per cent), Nitrogen (15 to 20 per cent.), Oxygen (about 20 per cent.), and Sulphur (0 1 to 2 per cent.). The nucleoproteins and phosphoproteins contain Phosphorus in addition to these five elements. The proteins show various reactions in common. All, except the

prolamins, are insoluble in alcohol; some are soluble in water, others insoluble, others, again, are soluble in saline solutions All are soluble in strong acids and alkalies, but undergo decomposition in the process.

The constitution of the proteins is very complex. When decomposed in various ways, *e g.* by acids, alkalis, or proteolytic enzymes, they yield a great variety of substances When acted upon by Bacteria they undergo putrefaction, offensive gases (ammonia, sulphuretted hydrogen, phosphoretted hydrogen, etc) being given off.

Proteins are composed largely of amino acids (§ 60), which form the chief units of the protein molecule These include leucin, tyrosin, aspartic acid, glutamic acid, arginin, tryptophane, etc. By synthesis compounds have been prepared which contain from two to about twenty amino acid units The most complex of these compounds, or polypeptides, would be regarded as true proteins if they were found in nature Proofs of the polypeptide constitution of the proteins are (1) the isolation of polypeptides from the natural proteins; (2) the hydrolysis of polypeptides by trypsin into their constituent units or amino acids, in the same way as the natural proteins are hydrolysed. The various amino acids can be combined together in many different ways, hence an enormous number of isomers is possible among the polypeptides, while the proteins found in nature show still greater variety.

The known proteins are classified mainly according to their origin, solubility in different reagents, coagulability on heating, and other physical characters, without strict reference to their chemical composition, though the classification is borne out by their actual composition so far as this is known. For our purposes we may divide the vegetable proteins into (1) primary proteins, (2) conjugated proteins, (3) derived proteins.

38. Primary proteins.—These, including the chief proteins found in seeds, are divided into **albumins** (soluble in water and coagulated on boiling), **globulins** (insoluble in water, but soluble in saline solutions), **prolamins** (insoluble in water or saline solutions, but soluble in alcohol), and **glutelins** (insoluble in water or saline solutions or alcohol, but soluble in alkalis).

Of these the **globulins** include the majority of seed proteins, *e.g.* the **legumin** of Broad Bean and Pea, the **phaselin** of Phaseolus, the **conglutin** of Lupin, and the **crystalline globulins** found in many oily seeds.

Of **albumins**, **leucosin** occurs in Wheat, etc., **legumelin** in Broad Bean and Pea, **ricin** in Castor Oil.

The chief **glutelin** known is the **glutenin** of Wheat.

The chief **prolamins** are **gliadin** in Wheat, **hordein** in Barley, **zein** in Maize

39. Conjugated Proteins.—The **nucleo-proteins**, belonging to this class, are important constituents of the cells of both animals and plants, occurring especially in the nucleus They contain phosphorus, and consist of protein combined with nucleic acid. They probably do not occur as reserve proteins in seeds; in the Wheat grain, for instance, they are found in the embryo but not in the endosperm

40. Derivatives of Proteins.—Proteins when acted upon by acids and alkalis, and by enzymes, are converted into (1) the **metaproteins—acid albumin** and **alkali-albumin**; (2) **proteoses**, formed from proteins by further action of acids and alkalis, and by enzymes; (3) **peptones**, formed from proteins by prolonged action of acids, alkalis, and enzymes; (4) **amino acids**, the ultimate products in tryptic digestion of proteins. Several **polypeptides** (most of which are, as already stated, synthetic substances) have been obtained from proteins by hydrolysis.

The metaproteins, proteoses, and peptones still show the properties of proteins, but the amino acids do not. See §§ 43, 56, 60.

41. Experiments with Egg Albumin.—For a study of the general reactions of proteins use white of egg, which contains about 10 per cent. of protein, the greater part being soluble albumin.

Break three or four fresh eggs into a basin, keeping back the yolks; then beat up the white with an egg-beater, or snip it in all directions with scissors so as to cut the membranes in it and make it more readily soluble. Add about 100 c.c. of water for each egg used, transfer to a flask, and shake vigorously; the solution formed is somewhat opalescent, but becomes clear on addition of some common salt. Test a portion of the solution with red litmus paper: the reaction is faintly alkaline. Divide the solution into the required number of portions, in test-tubes, for the following colour and precipitate reactions (*a* to *r*), most of which apply to proteins in general. For *s* and *t* have ready a hard-boiled egg.

Dried powdered albumin may be bought ready for solution in water from chemical supply firms.

(*a*) **Biuret Reaction of Proteins.**—Add excess of caustic soda (or potash), then drop by drop some 1 per cent solution of copper sulphate—a violet colour, which deepens on heating. Compare with the rose pink colour given with this test in the case of peptones (§ 43). In making the Biuret test, take care to use very little of the copper solution, adding it drop by drop, otherwise its blue colour masks the reaction.

(*b*) **Iodine Reaction of Proteins.**—Add a little iodine solution (see Appendix), and note the yellowish brown colour given. Pour an equal volume of the iodine solution into an equal volume of water in another tube, as a control, and compare the colour with that given in the case of the albumin.

(*c*) **Xanthoproteic Reaction.**—Add some strong nitric acid—a white precipitate which on boiling turns yellow. Cool, and add strong ammonia—the yellow precipitate becomes orange. Instead of a coloured precipitate there may be merely a yellow colour, but in any case this is a good test for proteins.

(*d*) **Millon's Reaction.**—Add some Millon's reagent (see Appendix)—a white precipitate, which on boiling turns red. If very little protein is present in a tested liquid, no precipitate but only a red colour may be given. Millon's reaction is due to the presence of tyrosin in the protein molecule.

(*e*) **Sulphur (Cystin) Reaction.**—Add a drop of lead acetate solution, then caustic soda or potash sufficient to redissolve the precipitate first formed, and boil. A brown or black colour appears, due to the separation of sulphuretted hydrogen from the amino acid cystin in the protein molecule (unlike most amino acids, cystin contains sulphur), this giving lead sulphide with the lead acetate

(*f*) **Tryptophane (Adamkiewicz) Reaction.**—Add excess of glacial acetic acid to the solution, then (using a thistle tube) run in strong sulphuric acid to the bottom of the test-tube. Gently shake the tube, or simply let it stand for several minutes; at the junction of the liquids there appears a violet colour, which gradually spreads through the solution. This reaction is due to the amino acid tryptophane present in the protein molecule.

Several other reactions are due to the presence of tryptophane, *e.g.* (1) proteins give a reddish violet colour, afterwards turning brown, when heated with strong hydrochloric acid; (2) proteins give a blue colour when precipitated by alcohol, then washed with ether, and treated with strong hydrochloric acid; (3) proteins give a green or blue colour when heated with benzaldehyde, a drop of ferric chloride, and strong hydrochloric acid.

(*g*) **Molisch Reaction.**—Add a few drops of α-naphthol solution to the albumin solution, shake up, then run some strong sulphuric acid to the bottom of the tube. A violet ring is formed at the junction of the two liquids. This reaction is of special interest, since it depends upon the carbohydrate radicle present in protein (see § 63, *g*). In the reaction, furfural is formed from the carbohydrate radicle.

In addition to the preceding **colour reactions** of proteins (*a* to *g*), some of the chief **precipitation reactions** may now be studied (*h* to *r*).

(*h*) **Coagulation by Heat.**—Heat some albumin solution. Since its reaction is alkaline, no clot is formed, but only an opalescence or perhaps a slight precipitate on the inside of the tube. (*i*) Now slightly acidify another portion of the albumin solution by adding a few drops of dilute acetic acid, and heat. The solution turns cloudy, and then a precipitate of coagulated albumin is formed; note that this precipitate is not soluble in cold acids and alkalis, but gradually dissolves on heating with caustic soda. (*j*) Make some of the solution faintly acid; immerse the test-tube in a beaker of water, with a thermometer, and heat gradually—the tube may be fixed in the clamp of a retort stand, so that it dips into a beaker of cold water placed on a sand-covered plate over a Bunsen or spirit lamp. Note the temperature at which a cloudiness appears in the solution, and when (usually about 70° C.) coagulation becomes complete.

Note that a precipitate of coagulated albumin is given on adding each of the following reagents to portions of the solution:—(*k*) alcohol; (*l*) nitric or hydrochloric acid; (*m*) mercuric chloride solution; (*n*) lead acetate solution; (*o*) tannic acid, or strong tea that has been stewed for about half an hour.

(*p*) A white precipitate is formed—not given with peptones—on adding a little glacial acetic acid, then potassium ferrocyanide solution drop by drop.

(*q*) Add excess of acetic acid, then an equal volume of saturated sodium sulphate solution, and heat; the precipitate formed removes all proteins (except peptones) from a solution.

(*r*) Saturate the solution with ammonium sulphate, by adding crystals or the powdered salt until no more will dissolve on shaking—a white precipitate, not given with peptones. This throws down all proteins (except peptones) from solution; filter, and note that the filtrate now contains no proteins

(*s*) Dry the clotted albumin of a hard-boiled egg, mix it with about twice as much powdered soda-lime, and add a little water to form a paste of the mixture. Roll this paste between the fingers into small pellets, and place these in a dry warmed tube of hard glass. Heat over a Bunsen, and into the mouth of the tube place first (A) a moist red litmus paper, then (B) a lead acetate paper The escaping vapours turn A blue and B black ; the former change is due to ammonia (which can also be smelt), the latter to the formation of lead sulphide—proving the presence of **nitrogen** and of **sulphur** in the albumin

(*t*) Put a bit of hard-boiled egg on a needle, and hold in a Bunsen flame ; it becomes charred, showing that **carbon** is present.

42. Proteoses and Peptones.—These derivatives of proteins (§ 40) are formed in nature by the action of proteolytic enzymes (pepsin, trypsin) on the primary proteins. It is doubtful whether they occur as reserve food in resting seeds, but they appear when germination begins

The proteoses (soluble in water, not coagulated on boiling, but precipitated by acids) are intermediate digestion products between primary proteins and the peptones (soluble in water and neither coagulated by boiling nor precipitated by acids). The peptones are readily soluble in water, and are not precipitated by acids, alkalis, neutral salts, and many of the other reagents that precipitate the primary proteins. The proteoses are less diffusible than the peptones; some proteoses are not readily soluble in water, and they are distinguished from peptones by being precipitated when their solutions are saturated with ammonium sulphate. Proteoses yield precipitates with many of the reagents that precipitate other proteins; the precipitates they give with nitric acid, and with potassium ferrocyanide in presence of acetic acid, disappear on warming and reappear on cooling.

43. Experiments with Commercial Peptone.—Get some Witte Peptone, which in reality contains more proteose than true peptone. Dissolve in warm water, and make the following tests for **proteose** and for **peptone,** after dividing the solution into portions in test tubes.

(1) Heat the solution and acidify it with dilute acetic acid—no coagulation. (2) Saturate with ammonium sulphate—a white precipitate, which partly disappears on heating and reappears on

cooling. (3) Add nitric acid—a white precipitate, which dissolves on heating, the liquid turning yellow, and reappears on cooling. (4) The Biuret test—a rose pink colour. (5) Add acetic acid and potassium ferrocyanide, (6) saturate with common salt—in each case a precipitate, which disappears on heating and reappears on cooling.

Now add ammonium sulphate to saturation to the remainder of the solution, filter, and to the filtrate (which contains peptone but not proteose) apply the general protein tests—xanthoproteic, Millon's, biuret (rose pink colour given); note also that the filtrate gives no precipitate with acids, or with acetic acid and potassium ferrocyanide.

44. Dialysis Experiments with Albumin and Peptone.—Fit up two dialysers (Fig. 20), each floating in a dish of distilled water. Into *A* place some of the albumin solution, into *B* some peptone solution; to each add a little thymol or other antiseptic Let the two dialysers stand for three days; then test the water in each for proteins, using (with different samples) the xanthoproteic, Millon's, biuret, and other tests.

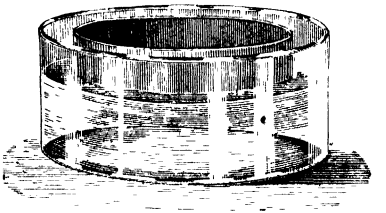


Fig 20 —A Dialyser, made by binding parchment paper over a hoop of vulcanite

Note that albumin is indiffusible, while peptone is diffusible, though somewhat slowly, through a membrane.

45. Proteins in Pea Flour.—Pea flour contains starch, dextrin, and several proteins. The chief protein is a globulin (legumin), but there is also another globulin (vicilin) and an albumin (legumelin).

(*a*) Place 10 grams of Pea flour with 50 c c. of water in a flask, shake vigorously, let it stand for several hours, and filter. Test the residue for starch with iodine Divide the clear filtrate, containing the albumin, into several parts and apply to these the chief protein tests: (1) Xanthoproteic test; (2) Millon's test; (3) Biuret test; (4) acetic acid and potassium ferrocyanide; (5) heat and note the coagulation of the albumin, especially if a few drops of acetic acid be added.

(*b*) Treat some Pea flour with 10 per cent salt solution for several hours, and filter; test the residue for starch. To portions of the clear filtrate apply the chief protein tests; then drop some of it into a beaker of water—note the precipitate of globulins.

46. Proteins in Potato Tuber.—Scrape the surface of a potato into a beaker; to the scrapings add some salt solution, stir well, and strain through calico into another beaker. On standing, a deposit of starch is formed; examine this with the microscope, and test a portion of it with iodine. Pour off the liquid, and apply to it the chief protein tests.

47. Proteins in Wheat Flour.—Make extracts of ordinary wheaten flour with (1) water, (2) salt solution, (3) alcohol. In each case filter, and test the filtrate for proteins.

Separate the gluten (a mixture of proteins) from the starch, as follows. Enclose a tablespoonful of flour in a piece of fine muslin, and knead it in a basin of water. Note the deposit of starch grains; examine these with the microscope and compare with those of Potato and Pea. Remove the starch entirely by kneading under a running tap until the water—at first whitened by the starch—passes off clear; open the muslin and note the yellowish sticky mass of gluten left behind.

Extract the gliadin from the gluten by boiling with alcohol, filter, evaporate the alcohol from the filtrate, and apply the protein tests to the residue (gliadin) The insoluble remainder left on the filter contains glutelin; note that this is insoluble in water and in salt solutions, soluble in dilute acetic acid and in dilute caustic soda.

Prove the presence of carbon, nitrogen, and sulphur in (1) Pea flour, (2) the "gluten" just isolated from Wheaten flour—in the same way as with egg-albumin (§ 41, *s*, *t*).

48. Proteins in Brazil Nut.—Remove the shells from some seeds, grind up the seeds, and extract with ether to remove the oil; this may be done best with a Soxhlet fat extraction apparatus (§ 97). Allow the ether to evaporate, and note the residue of oil.

Extract about 10 grams of the oil-free nut meal with 50 c.c. of 10 per cent salt solution. Pour some of the extract into about 20 times its volume of water in a beaker; a cloudiness is produced which on standing separates into flakes and falls to the bottom. Then pour off the greater part of the water, and filter the remainder; to the precipitate apply the chief protein tests.

The crystalline globulin (excelsin) of Brazil nut can be obtained in fine hexagonal plates by dialysing the saline extract; by this method the globulin separates out more slowly than by simply pouring the extract into water.

49. Microchemical Tests for Proteins.—All the tests that give a colour reaction may be readily used as microchemical methods of detecting proteins in plant tissues.

(*a*) Cut thin sections from a Pea, Bean, or Lupin, cotyledon. (1) Treat a section with iodine; the starch-grains turn blue, the small protein grains turn brown or yellow. (2) Lay a section in strong copper sulphate solution for a minute, rinse in water, and transfer to a little potash in a test-tube, heat to boiling; mount, cover, and note the violet colour of the protein cell-contents (3) Apply the xanthoproteic test, by placing a section first in strong nitric acid in a watch-glass, then in strong ammonia; note the intense yellow colour of the protein contents. (4) Place a section in a little Millon's reagent, if the protein contents do not turn red quickly, warm the slide. See also § 74.

(*b*) Cut transverse and longitudinal sections of the grain ("seed") of Wheat and of Maize and apply the above tests. Note that the greater portion of the endosperm consists of cells packed with starch-grains, but the outermost layer ("aleurone" layer) consists of cubical cells containing protein grains. See also § 75.

50. Protein Grains ("Aleurone" Grains) are found in various parts where food is stored, but are especially abundant and large in seeds. In some cases the grains are small and of simple structure (§ 51). In other cases, especially in oily seeds, they are large (§ 52) and contain one or more angular "crystalloids" (protein crystals) and also rounded "globoids" consisting of mineral substance (double phosphate of calcium and magnesium). Protein crystals may also occur in cells quite apart from definite protein grains.

51. Simple Protein Grains.—Get dry seeds of Almond, Apple, Bean, Pea, Lupin, Sunflower. Moisten the razor with glycerine, cut sections of the cotyledons, and mount in glycerine.

Note the numerous small refractive protein grains, which at first sight may resemble starch grains, but are not stratified and turn brown, not blue, with iodine Of these simple grains, some are soluble in water (Almond, Apple); others, insoluble in water, are soluble in saturated salt solution either at once (Beans, Peas, Lupin), or after treatment with alcohol (Sunflower).

In each case treat different sections with (1) water—even when this does not dissolve the grains, it usually makes them swell and lose their bright appearance; (2) potash—this makes the grains swell and dissolve; (3) iodine—this turns the grains brown; (4) Biuret test; (5) Xanthoproteic test; (6) Millon's reagent.

52. Protein Grains with Crystalloids and Globoids.—Brazil nut and Castor Oil seeds form good material for the study of the larger and more complex protein or "aleurone" grains, which are embedded in the oil-containing protoplasmic matrix of the cells These grains are not soluble in water, but are dissolved by strong salt solution, either at once (Brazil nut) or after treatment with alcohol (Castor Oil, Walnut). In each case remove the shell, and make the following preparations In each case cut the sections with the razor dry, except where otherwise directed

(*a*) Mount dry sections in thick glycerine—the oily matrix of the cells will be seen, with the oil drops; note the protein grains, in which the crystalloids and globoids may be seen

(*b*) Mount sections in olive oil (which may in this case be used for wetting the razor). Note that the oil makes the oily matrix of the cells transparent and almost invisible

(*c*) Wet the razor with alcohol, cut sections, soak them in alcohol to dissolve out the oil (ether will do this more quickly—wash out the ether with alcohol), and mount in thick glycerine

(*d*) Cut dry sections, and mount them in water—this makes the grains swell, but the crystalloids should become more conspicuous Irrigate sections, mounted in water,

with (1) iodine solution—the grains turn yellow; (2) dilute potash—the crystalloids swell and dissolve, leaving the globoids behind; (3) dilute sulphuric acid (note that this destroys the grains), then iodine solution (this stains the matrix left behind in the cells); (4) a drop or two of 1 per cent. osmic acid—the crystalloids slowly swell, while the rest of the cell-contents, especially the oily matrix, rapidly becomes blackened.

(*e*) Cut dry sections, and transfer them to a watch-glass containing two parts of alcohol and one part of castor oil, with enough eosin to make the mixture light red After a few hours, mount in castor oil and alcohol (without the eosin). This treatment brings the grains out clearly; they are seen embedded in vacuoles in the cytoplasm of the cells.

(*f*) Place some dry sections in alkannin (§ 86) for several hours, and mount in dilute glycerine; the oil is stained red.

(*g*) The structure of the grains is well brought out by fixation in alcoholic picric acid, and staining with eosin. Place the sections in concentrated alcoholic solution of picric acid in a watch-glass for several hours; then wash them in alcohol, and stain for a few minutes in eosin dissolved in alcohol. It is best to wash the sections next in absolute alcohol, transfer them to oil of cloves, and mount in Canada balsam. The matrix of the grains is stained dark red, the crystalloid yellow, and the globoid remains colourless

(*h*) Note that the globoids are (1) insoluble in alcohol and in dilute potash, but (2) soluble in dilute mineral acids (hydrochloric, nitric, or sulphuric) and in acetic acid; (3) in an ammoniacal solution of ammonium phosphate the globoids are replaced by crystals of ammonium magnesium phosphate; (4) on being treated with ammonium oxalate, they are replaced by crystals of calcium oxalate; (5) after extracting the oil from sections by treatment with alcohol, or alcohol and ether, the globoids can be made to stand out clearly on adding some dilute (1 per cent.) potash solution which will dissolve the ground substance of the protein grains.

55. Experiments with Pepsin.—Boil an egg hard, and chop the clotted white into small pieces. Label six test tubes *A*, *B*, *C*, *D*, *E*, *F*. Half fill each tube with water, and drop in some of the chopped albumin. To *A* add some pepsin extract or some pepsin powder, with a pinch of bicarbonate of soda to make the liquid distinctly alkaline; to *B* and *C* add some pepsin and a few drops of dilute hydrochloric acid; to *D* add a few drops of acid, but no pepsin; to *E*, some acid together with pepsin extract (or dissolved pepsin powder) which has been boiled; and leave *F* with nothing added to the albumin.

Set all the tubes, except *C*, in a beaker of warm water, and keep at 40° C. on a bath for an hour. Put *C* in a freezing mixture, or ice and water, for the same period. Note that in *A*, *C*, *E*, and *F* the albumin is unchanged; in *B* it has disappeared, having become swollen up and clear.

Now apply to a few drops of liquid from each tube the xantho proteic and the biuret tests Peptone is present in *B*, but not in any of the others. In *E* the pepsin has been destroyed by the boil ing. In *A* the action of the pepsin has been prevented by the alkaline medium; on adding acid to the liquid and keeping the tube at 40° C again digestion takes place In *C* the action has been prevented by the cold; on transferring the tube to the bath at 40° C. digestion takes place. In *D*, the weak acid used, without pepsin, has only changed the albumin into acid-albumin, but not into peptone.

56. Products of Peptic Digestion.—Repeat the preceding experiment on a larger scale, so as to get more material to test for the products of pepsin action. This time place in a flask some pieces of albumin, dilute hydrochloric acid (add 4 c c of strong acid to 300 c.c of water), and some pepsin extract or powder. Keep at 40° C for an hour; if the liquid is cloudy, filter it

(A) To the liquid, or filtrate, add dilute caustic soda solution until it becomes neutral—a precipitate is given, consisting of acid albumin; filter off this precipitate, dissolve it in dilute acid, and note that the acid solution gives protein reactions and does not coagulate on boiling

(B) Test part of the filtrate from A for proteose. It gives the protein reactions. On adding nitric acid and common salt, a pre cipitate is formed, which is re-dissolved on heating but reappears on cooling. It is precipitated by (*a*) acetic acid and potassium ferrocyanide, and by (*b*) acetic acid and saturated sodium sulphate solution, neither of which precipitates peptones. It gives the same biuret reaction (rosy pink) as peptones and, like them, is soluble in water.

(C) Saturate another portion of the filtrate from B with ammonium sulphate crystals, or the powdered salt; this precipitates the pro teoses, while the peptones remain in solution—test with biuret, using a large amount of soda.

(*i*) Place some sections in a watch-glass containing either pepsin or trypsin, *e.g.* liquor pepticus (§ 54) or liquor pancreaticus (§ 57); for comparison, place others in a watch-glass of water. Set both in a warm place, and note that the ground substance of the protein grains is first dissolved, then the crystalloid more slowly, while the limiting membrane of the vacuole occupied by the grain persists.

53. Digestion of Proteins.—In mammals the primary proteins are acted upon by the gastric juice of the stomach and by the pancreatic juice and the intestinal juice (succus entericus) of the small intestine. The hydrolysis of the proteins is effected by the three enzymes, **pepsin, trypsin,** and **erepsin,** present in these three juices respectively. Pepsin hydrolyses the primary proteins into peptones; trypsin also acts upon the primary proteins, but it carries the hydrolysing process further and changes the peptones into amino-acids; erepsin is peculiar in that it does not attack the primary proteins, but is only capable of acting upon proteoses and peptones, changing them into amino-acids.

For our purposes we may regard the proteolytic enzymes of plants as corresponding to trypsin in their mode of action. The vegetable trypsin called papain is obtainable commercially, being used in medicine, but for the following experiments we may use either pepsin prepared from gastric juice, or preparations of pancreas containing the enzymes diastase and lipase in addition to trypsin.

54. Preparation of Pepsin.—(*a*) Pepsin may be purchased in the form of Benger's "liquor pepticus," or the dried pepsin (Burroughs and Wellcome). (*b*) Artificial gastric juice may be prepared as follows—Get a fresh pig's stomach from the butcher, cut it open, rinse with water, cut out the cardiac (broader) end, spread it out, scrape the mucous (inner) surface, rub up the scrapings in a mortar with sand, add water, rub up again, and filter; the filtrate is to be used. Another method is to scrape the mucous membrane off, dry the scrapings between folds of blotting-paper, put them in a bottle, and cover with glycerine which will dissolve out the pepsin; after a day, filter, and use the filtrate (glycerine extract).

57. Preparation of Trypsin.—There are various commercial preparations which contain trypsin, *e g.* Benger's "liquor pancreaticus" (which often contains a sediment of tyrosin), the "Holadin" of Fairchild Bros. (a very active preparation containing also lipase and diastase). The vegetable trypsin, papain, can also be obtained; it contains only trypsin.

To make a glycerine extract of pancreas, which will serve also for experiments on the hydrolysis of starch (§ 74) and that of oils (§ 86), mince up a fresh ox or pig pancreas ("sweetbread") in the same way as directed for the gastric extract (§ 54).

58. Experiments with Trypsin.—Repeat the experiments directed for pepsin (§ 55), but instead of acid use 1 per cent. sodium bicarbonate solution To prevent putrefaction, add some antiseptic such as thymol, or toluene, or chloroform water (5 c c. of chloroform shaken with a litre of water).

Label three test-tubes *A*, *B*, *C*. Half fill each with 1 per cent sodium carbonate solution, and add some hard-boiled egg white, with a few drops of the antiseptic Boil *B*; make *C* acid with dilute hydrochloric acid. Plug the three tubes with cotton-wool, and place them in a bath at 40° for an hour. In *A* the liquid becomes more or less clear, the albumin being digested; in *B* and *C* there is no change.

Filter the liquid in *A*, neutralise the filtrate with dilute acid; alkali albumin is precipitated—filter this precipitate off and test the filtrate for peptones

Filter *B* and *C*, and neutralise *B* with acid and *C* with sodium carbonate; no precipitate is formed. Test for peptones—none are present In *B* the trypsin has been destroyed by the boiling, in *C* its action is prevented by the presence of the acid.

59. Products of Tryptic Digestion.—Make a tryptic digestion on a larger scale, so as to study the products more fully. Two-thirds fill a large flask (1 or 2 litres capacity) with 1 per cent. sodium carbonate solution; add the chopped white of a hard boiled egg; then some trypsin solution or pancreas extract; and finally some antiseptic—this is essential since tryptic digestion is otherwise accompanied by active putrefaction or bacterial decomposition, by which evil-smelling products (indol, skatol, sulphuretted hydrogen, etc.) are formed. After two or three days, filter the liquid.

(*a*) The sediment or precipitate in the liquid contains tyrosin. After filtering, dissolve a portion of the precipitate in dilute hydrochloric acid, and test with Millon's reagent—the solution becomes red.

(*b*) Acidify about 5 c.c. of the filtrate with acetic acid, then add bromine water drop by drop—a reddish colour appears, which gradually deepens, then disappears as more bromine water is added. When the colour is no longer deepened on adding bromine water

add a few c.c. of amyl alcohol, and shake, then allow to stand—the amyl alcohol separates, coloured red or violet. This reaction is due to the presence of the amino acid tryptophane.

(*c*) Concentrate some of the liquid to small bulk by heating on a water bath; after a day, examine the residue with the microscope for crystals of **leucin** and **tyrosin.** The leucin is chiefly in brownish spheres showing radiate and concentric markings, the tyrosin in bundles or rosettes of long white needles.

(*d*) The leucin is also obtained as a sticky residue if the filtered liquid is treated with alcohol until no more precipitate comes down; filter and concentrate the filtrate on a bath

(*e*) Treat a portion of the filtered liquid with Millon's reagent, which precipitates any proteins present; filter, and boil the filtrate —a red colour indicates tyrosin.

60. Amino Acids and their Derivatives.—The amino compounds (amines, amino acids, amides), containing carbon, hydrogen, oxygen, nitrogen, and in some cases (cystin) also sulphur, may be formed either in constructive or in destructive metabolism That is, they are intermediate bodies formed either on the up-grade towards protein, or on the down-grade from protein to simpler bodies. In either case they are important for translocation, being soluble and diffusible. Many of these substances are present in plants—*e.g.* asparagin, which is abundant in seeds of Leguminosae. Asparagin (and other amino compounds) combines with non-nitrogenous substances to form proteins; it often accumulates in those parts of plants where there is not sufficient non-nitrogenous material at hand for the formation of proteins. Asparagin may accumulate in plants which are grown in darkness, so that photosynthesis cannot take place. Lupin seedlings germinated in darkness contain a large amount of asparagin, which disappears when the seedlings are placed in the light. If, however, the seedlings are exposed to light in an atmosphere deprived of carbon dioxide, the asparagin persists in the seedlings. Both asparagin and tyrosin occur in Dahlia tubers. Leucin is associated with asparagin in seedlings of Lupin and other Leguminosae. In Cruciferae, Cucurbitaceae, etc., asparagin is replaced by an allied substance, glutamin.

(*a*) Make a strong aqueous solution of commercial asparagin, and divide it into three portions. (1) Dissolve some copper sulphate in water, and add dilute potash; collect the precipitate on a filter, and wash it with water. Add this precipitated copper hydroxide to the asparagin solution—asparagin (and other amides) gives a deep blue colour; evaporate the liquid down—crystals of a copper compound of the amide are formed. (2) Boil with dilute sulphuric acid —ammonia is formed; add excess of magnesia and heat—fumes of ammonia are given off. (3) Add alcohol—the asparagin is precipitated

(*b*) With a dry razor cut rather thick sections of Dahlia tuber, mount in alcohol, and cover. On the evaporation of the alcohol, rhombic plate-like crystals of **asparagin** are deposited on the cover-glass and slide. Raise the cover, place on the section a completely saturated solution of asparagin, and place the cover-glass on again; if the crystals are really asparagin, instead of being dissolved they will increase in size—substances other than asparagin would dissolve in the saturated asparagin solution just as they would in water. In this way we can distinguish the asparagin crystals from the deposits of inulin (§ 77), which also occurs in the Dahlia and is precipitated by alcohol.

(*c*) The **tyrosin** in Dahlia tubers may be thus demonstrated:— (1) Keep sections mounted in glycerine for several days; needle-like crystals of tyrosin are deposited in radiating groups (2) Place a fairly thick slice of tuber in a dish of about the same size as itself, and nearly cover it with alcohol; the tyrosin crystals will appear on the cut surface of the tuber (3) Note that the tyrosin crystals are coloured deep red by Millon's reagent. (4) Tyrosin gives a yellow colour when warmed with nitric acid, becoming orange on addition of ammonia. (5) Place some of the crystals in a dry test-tube, add a few drops of strong sulphuric acid, and place on a bath at 100° for half an hour; then add about 5 c.c. of water, neutralise with barium carbonate, filter, and to the filtrate add two or three drops of ferric chloride—a blue or violet colour is given

(*d*) Cut sections of (1) dry seeds, (2) seedlings, of Lupin; mount in water, and test some for asparagin, others for proteins. If the seedlings are preserved in alcohol for some weeks, sections mounted in glycerine will often show large asparagin crystals; on irrigation with water the crystals are dissolved.

Note that the dry seed contains no asparagin, but abundant proteins. When the radicle is 1 to 3 cm. long, and the hypocotyl 2 to 5 mm. long, these organs contain some asparagin, but none is present in the cotyledons. When the radicle is 5 or 6 cm long, but the cotyledons not yet expanded, the radicle and hypocotyl contain larger quantities of asparagin, which is still absent from the cotyledons. In older seedlings, with expanded cotyledons, the latter, as well as the other organs, still contain asparagin As germination advances and the plumule elongates, this organ is found to contain asparagin, which gradually disappears from the other parts.

Cut sections of the various parts of the seedling, at different stages, and note that the protein diminishes in amount during germination as the formation of asparagin increases. Eventually, asparagin practically disappears from all the organs

(*e*) Germinate some Lupin seeds in darkness, and compare them with those grown in light as regards their asparagin and protein contents. Note that after a few weeks the seedlings grown in darkness contain asparagin, while those grown in the light contain little or none.

(*f*) Get two similar Lupin seedlings, germinated in darkness in a water culture jar, with a well developed root-system. Place one (*A*) on a wooden board or glass plate, along with a bottle containing baryta-water, or a dish of soda-lime; cover with a tubulated bell-jar, sealing the edges of the jar to the plate with wax mixture, putty, or plasticine Fit the mouth of the jar with a cork, bored with three holes. Through one hole pass a tube through which water can be poured to replenish that lost by the culture solution owing to evaporation; for this purpose join the upper end of the tube by rubber tubing to a funnel, placing a clip on the rubber tubing Through the other two holes pass pieces of glass tubing, one joined up to a bottle containing baryta-water, the other to an aspirator The general arrangement of the apparatus is somewhat similar to that shown in Fig 44. The object is to grow the seedling in air deprived of carbon dioxide, but to give it daily aeration by drawing through the bell-jar a current of fresh air (deprived of carbon dioxide by passing through the vessel of baryta water). For comparison, place the second seedling (*B*) on a similar plate and cover with a bell-jar, but leave the neck of the jar open, so that the seedling is supplied with the ordinary air. After a few weeks, note that *A* still contains large amounts of asparagin, though this will have practically disappeared from *B*

V. Starch, Sugars, Dextrin, Inulin.

61. Carbohydrates contain Carbon, Hydrogen, and Oxygen, with the hydrogen and oxygen in the same proportion as in water (two atoms of hydrogen to each atom of oxygen) Formaldehyde (CH_2O) has the empirical formula of a carbohydrate, and is in fact the first member of the series, which includes members with 2, 3, 4, 5, 6, etc, carbon atoms The chief carbohydrates—those of physiological importance—are the members with six carbon atoms, or some multiple of six. The simple six-carbon-atom compounds, or hexoses, *e.g* **glucose,** belong to the

monosaccharides. The hexoses are combined together as units in the disaccharides, trisaccharides, and polysaccharides, named according as they contain two, three, or more of the monosaccharide units Cane sugar (**sucrose**) and malt sugar (**maltose**) are disaccharides; **starch, dextrin, inulin,** and **cellulose** are polysaccharides. These complex compounds are converted into the simple monosaccharides by hydrolysis with acids, or by the action of enzymes (*e g* diastase, invertase, inulase, cytase).

Of the following general characters and reactions of the carbohydrates, one or other may fail or only be shown after the substance has been treated in some way, *e g* by hydrolysing agents or by enzymes. (1) They reduce alkaline solutions of copper; (2) they are coloured yellow by alkalis; (3) they rotate the plane of polarised light either to right or left; (4) in contact with Yeast they are split into alcohol and carbon dioxide; (5) when strongly heated they are decomposed, charred, and yield various products; (6) on being heated with mineral acids they are decomposed, with formation of formic acid and other substances; (7) they give a deposit of needle-like crystals with phenyl-hydrazine; (8) some are insoluble in water, while others are readily soluble, and those which are insoluble can be converted into soluble carbohydrates by hydrolysis; (9) in absolute alcohol most of them are either insoluble or only slightly soluble.

62. Glucose, Maltose, and Sucrose. — Examine specimens of these three sugars.

(1) **Glucose** (grape sugar) occurs in commerce in warty uncrystallised yellowish masses, but is readily crystallised —*e.g* on dissolving it in hot alcohol and cooling the solution; on being treated with caustic soda it turns yellow; it reduces various metallic oxides in alkaline solutions; it forms a characteristic osazone with phenylhydrazine.

(2) **Maltose** (malt sugar) occurs as a white warty mass of needle-like crystals; it is the chief sugar formed by the action of diastase upon starch (§ 74, *i*); it reduces metallic oxides in alkaline solutions, but it does not give Barfoed's test and is therefore easily distinguished from glucose.

(3) **Sucrose** (cane sugar) occurs in crystals, it is only slightly soluble in alcohol; on being heated with caustic soda it does not become yellow, though it slowly darkens; it does not reduce alkaline solutions of metallic oxides; it gives no osazone. After hydrolysis by acids or by the enzyme invertase, sucrose is converted into "invert sugar" which gives the same reactions as glucose with caustic soda, alkaline metallic solutions, and phenylhydrazine.

63. Reactions of Glucose.—Dissolve some glucose in water, and to portions of the solution in test-tubes apply the following tests.

(*a*) Add caustic potash or caustic soda, and boil. The solution turns yellow, then dark brown, and smells of caramel; the smell becomes more distinct on acidifying with dilute sulphuric acid

(*b*) **Trommer's Test.**—Add some caustic potash or soda; then add copper sulphate solution, drop by drop, shaking after each addition until the solution becomes deep blue. (Excess of copper sulphate causes the precipitation of copper hydrate, *i e.* it is no longer dissolved by the sugar solution; a few drops of Rochelle salt cause this to redissolve—see Fehling's test). Heat nearly to boiling—a yellowish red precipitate of cuprous oxide is formed.

(*c*) **Fehling's Test.**—Add equal quantities of Fehling A and Fehling B (see Appendix). No precipitate is formed with the excess of copper sulphate present (compare Trommer's test). Heat to boiling—cuprous oxide is precipitated.

(*d*) **Barfoed's Test.**—Add Barfoed's solution (see Appendix), and boil. Red cuprous oxide is precipitated. This test is not given with maltose or sucrose.

(*e*) **Reduction of Silver.**—Prepare some ammoniacal silver nitrate in a test-tube, by adding dilute ammonia to silver nitrate until the precipitate first formed is just redissolved. Add some of this solution to the sugar solution, and warm in the water bath. A mirror of metallic silver is formed on the inside of the tube.

(*f*) **Phenylhydrazine Test.**—To some sugar solution add equal quantities of phenylhydrazine and glacial acetic acid (about 10 drops of each). Place in a water bath at 100° for half an hour,

when a yellow crystalline mass of phenyl glucosazone should be deposited. Cool; filter off the crystals, and examine them with the microscope. They are needle-like, and arranged in feather-like tufts or in rosettes.

(*g*) **Molisch Test.**—To some sugar solution add a drop or two of α-naphthol solution, then run into the bottom of the tube a little (about 2 c c) strong sulphuric acid. A violet ring appears at the junction of the two liquids, either at once or in a short time. This reaction is chiefly of importance in proving the presence of a carbohydrate radicle in the molecule of proteins (§ 41, *g*).

64. Reactions of Sucrose.—Dissolve pure cane sugar in water, and note that (1) it does not give a brown colour with potash; (2) it does not reduce alkaline solutions of metallic oxides, hence no precipitate is given with the Fehling, Barfoed, and Trommer tests, nor is silver thrown down; (3) it gives no osazone with phenylhydrazine and acetic acid.

If, however, cane sugar is boiled for a long time in water, or for a shorter time in dilute mineral (*e g.* sulphuric) acid, it is converted into a mixture of the monosaccharides glucose and fructose, and the solution on being neutralised gives reduction of copper oxide from Fehling, etc.

65. Reactions of Maltose.—Note that maltose agrees with glucose in (1) being coloured brown when heated with soda or potash; (2) reducing metallic oxides in alkaline solution; (3) forming an osazone with phenylhydrazine. It does not, however, reduce Barfoed's solution. With the phenylhydrazine test, maltosazone is not deposited while the solution is hot, but only when after being heated for half an hour the solution is allowed to cool; the crystals are shorter and thicker than those of glucosazone.

66. Microchemical Tests for Sugar. — Fehling's test is readily applied to tissues in which the presence of sugar is to be detected. Rather thick sections should be cut, so that a good many of the cells will remain intact Soak the sections in Fehling in a watch-glass for a few minutes, rinse them quickly in water, and heat the slide

so that the water boils gently for about a minute, then cover (adding a drop of water if necessary) and examine; if grape sugar is present, the granular red precipitate of cuprous oxide will be seen in the cells. To test sections for cane sugar, boil them in 10 per cent sulphuric acid in a test-tube, then test as before with Fehling; or place the Fehling at once on the sections, add a few drops of the acid, and boil gently on the slide.

(*a*) Squeeze the juice of some grapes into a test-tube, add Fehling, boil, and note the precipitate formed.

(*b*) Cut sections of a ripe Grape; mount in water, cover, and note the transparent colourless thin-walled cells with large vacuole and scanty protoplasm (1) Place some sections in strong alcohol in a watch-glass for a few minutes; mount in alcohol, cover, and note the numerous sugar crystals in the cells. Irrigate with water; the crystals are dissolved. (2) Test sections for grape sugar with Fehling's solution.

Make similar experiments with pear, apple, and other fruits

(*c*) Mince up some Beet-root, boil in water, pour the red juice into two test-tubes. To one add Fehling and boil—no precipitate; to the other add acid, boil, add Fehling, boil again—precipitate formed.

(*d*) Cut sections of Beet-root; the cells are transparent, with scanty protoplasm, the sugar-containing sap is coloured (1) Place some of the sections in alcohol for a few minutes, mount in alcohol, and note the sugar crystals —smaller than those seen in the cells of the Grape. (2) Test other sections for grape-sugar with Fehling (no result), and for cane sugar by boiling with acid and then adding Fehling (precipitate formed).

67. Experiments with Solid Starch.—For experiments with starch use ordinary laundry starch or (better) the starch powder sold by chemists.

Heat some dry starch in a test-tube Note the condensation of water in the upper part of the tube This proves the presence of *hydrogen* and *oxygen* in starch (since water is composed of these

elements). Note also that the starch soon begins to *blacken*, proving that it contains *carbon*, and at the same time dirty white fumes are evolved, having a pungent odour somewhat resembling that of burnt sugar.

Apply a light to the mouth of the test-tube—the fumes are *inflammable*. Introduce a piece of moist blue litmus paper into it—the litmus becomes red, showing that the fumes are *acid*. Introduce a glass rod, on the end of which is a drop of lime-water, into the test-tube. The lime-water becomes milky, showing that *carbon dioxide* is one of the products of decomposition of starch. This confirms the presence of carbon in starch (since carbon dioxide is a compound of carbon and oxygen).

When all the volatile matter has been driven off, a black residue of *charcoal* remains

68. Experiments with Starch Solution.—Shake up some powdered starch with cold water, it is not dissolved. Filter, test the filtrate with iodine—no blue colour is given. Stir up some dry starch with a little cold water, then add boiling water, and boil until an imperfect opalescent "solution" is obtained; this, on cooling, will, if strong enough, "set" or gelatinise to form a paste or mucilage.

(*a*) Add iodine solution to the starch solution; the blue colour produced will disappear on heating (the "iodide of starch" is destroyed by heat) and reappears on cooling (run a tap of cold water over the test-tube to cool it).

(*b*) To another tube of starch solution add Fehling's solution, and heat. There is no reduction.

(*c*) Hydrolyse a portion of the starch solution by boiling with a little dilute sulphuric acid for a few minutes; neutralise with soda or potash, and test with Fehling's solution. Reduction occurs, owing to the conversion of starch into glucose.

(*d*) Note that starch is precipitated from solution by alcohol, also by basic lead acetate solution.

69. Starch Grains in Potato Tuber (Fig. 21) —Cut across a Potato tuber, apply some dilute iodine solution to the surface, and note the deep blue or almost black colour due to the abundant starch. In testing for starch, it is

better to use a weaker iodine solution than that used in testing for proteins.

(*a*) Put a drop of water on a slide, dip into it a cut piece of tuber, and note the small white starch-grains that escape from the opened cells. With the microscope, note that the grains show delicate lines, corresponding to the

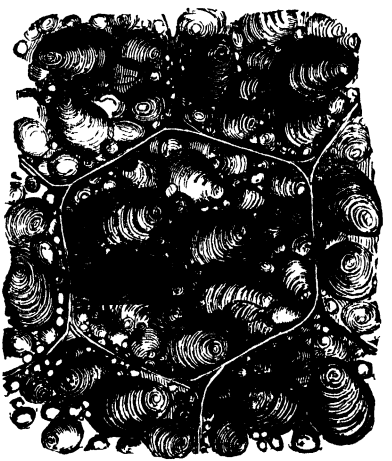


Fig 21 —Part of a Section of Potato Tuber, with Starch Grains × 200.

thin layers built up around the first-formed portion of the grain, which appears as a clear spot (hilum) placed excentrically.

(*b*) Cut thin sections from the tuber, and note that the cells of the parenchyma have thin walls and are almost filled up by the numerous grains, there being very little protoplasm. Add iodine, and examine again; to see the stratification more clearly, use very weak iodine solution.

(*c*) Mount some grains in water, and treat with chlorzinc-iodine; the grains turn blue, as with iodine, but also swell and become less bright and refractive.

(*d*) Treat another preparation with potash; the grains swell and become dull, losing their highly refractive properties owing to the additional water which the potash causes them to absorb. Irrigate with water, and treat with iodine; the swollen grains turn blue, though not so intensely coloured as usual.

(*e*) Heat another preparation, holding the slide over a flame till boiling occurs; the grains swell and become dull in appearance. Add iodine; the grains turn blue, as in the preceding case.

(*f*) To bring out more clearly the striations in the grains, scrape some of the contents of a Potato tuber into a watch-glass containing some 5 per cent. silver nitrate solution. Let them remain in this solution for about 15 minutes, then transfer them to a watch-glass containing some 1 per cent. solution of common salt, and expose to direct sunlight, in order to reduce the chloride of silver which has been formed within the grains. The less dense layers of the starch will take a grey colour, due to the reduced silver.

70. Half-compound and compound grains are sometimes found in Potato tuber, in addition to the simple grains with a single hilum. A half-grain compound grain consists of two or more small grains fixed together (usually by their broader ends) and covered by a common outer layered envelope of starch A compound grain consists of an aggregate of several grains without any common envelope; in the endosperm of Oats and Rice all the grains are compound, with as many as 100 to 300 small grains.

The starch-grains of other plants should be examined. Those of Bean and Pea cotyledons are rounded or ovoid but centric. Those of Wheat endosperm are rounded discs, and those of Maize polygonal and densely packed in the endosperm cells.

71. Leucoplasts are colourless plastids. They occur in various tissues in which starch is being stored. In the "pseudo-bulb" of Phajus, of which prepared sections can be bought, the leucoplasts are long disc-like bodies. They can be found, however, in the rhizome of Canna or Iris, or in Potato tubers.

Get some young Potato tubers. To harden the tissues thoroughly cut the tubers in pieces, not larger than a Pea, and place these in strong picric acid. A rapid method is to cut sections from the surface of the young tuber (the leucoplasts are most abundant in the

cells just within the cork-layer) and mount them in a drop of the acid; but it is perhaps better to soak the pieces of tuber in the acid for an hour, then wash them with weak alcohol, and keep them in strong alcohol for a few days. Then cut sections near the surface, treat with iodine, and mount in glycerine. Note the small rounded starch-grains (blue), each with a small leucoplast (yellow) attached, the leucoplasts are usually found near the nucleus of the cell, and in the outer cells of the tuber there may be seen leucoplasts which have not yet formed a starch-grain.

72. Dextrin.—This name is given to a series of soluble carbohydrates, formed in the processes by which starch is converted into reducing sugar, and therefore found in plant tissues where starch has been stored. In the hydrolysis of starch the intermediate products formed differ considerably, some giving various colours with iodine, while others are not coloured by iodine.

(*a*) Examine some commercial dextrin It is a yellow-brown powder, soluble in either cold or hot water; the solution is clear Pour into one test-tube some of the dextrin solution, and into another tube an equal volume of water, then add to each an equal volume of iodine solution. The water is coloured yellow only, but the dextrin solution becomes reddish-brown—the colour disappears on heating and reappears on cooling.

(*b*) To some dextrin solution in a test-tube add alcohol; the dextrin is precipitated.

(*c*) To some dextrin solution add basic lead acetate solution; dextrin is not precipitated (cf. starch).

(*d*) Dextrin can be obtained, as a sticky mass, by moistening a little starch with hydrochloric acid and heating gently in a dish.

(*e*) Make 10 grams of starch into paste with 20 c.c. of water, add 30 c.c. of 20 per cent. sulphuric acid, and boil for several minutes Cool, add alcohol, collect the white precipitate of dextrin, wash it with alcohol, dry it in a watch-glass, and test with iodine.

(*f*) Boil some starch in water (about 1 gram starch to 100 c.c water); cool, add a few drops of 20 per cent. sulphuric acid, and again heat—the fluid becomes clear, and on adding iodine to a cooled sample of it the blue colour is still given. Continue to boil the solution, and remove from it every five minutes a small sample to which when cool iodine is added. The first samples, containing dextrin, turn violet with the iodine, the later ones reddish-brown, then yellowish, as the conversion of the successive dextrins into sugar proceeds.

(*g*) Make a watery extract of Pea flour, or of pulverised Peas, let the turbid liquid stand for an hour, and filter it. Pour some of the filtrate into a watch-glass and place in it a crystal of iodine; the liquid gradually turns brown. For comparison pour into two other watch-glasses, each containing an iodine crystal, (1) a little water—the iodine only turns the water yellow; (2) some of the dextrin you have prepared from starch—note the brown colour produced in the dextrin. A few drops of iodine solution may be used in each case instead of iodine crystals

(*h*) Pour some of the filtered Pea extract into a test-tube, add some Fehling's solution, and boil; no reduction occurs, since no reducing sugar is present in dry Peas.

(*i*) To another portion of the Pea extract in a test-tube add a little sulphuric acid and boil for a few minutes, then add some Fehling and boil again; the red copper precipitate appears, because the dextrin has been converted by the action of the acid into a reducing sugar.

73. Digestion of Starch.—Starch is converted into sugar by hydrolysis, which may be brought about either by (*a*) simply boiling starch in water or in mineral acids, or (*b*) by the action of diastase enzymes. Of the latter several varieties occur in both plants and animals; in mammals, for instance, the digestion of sugar is effected by the ptyalin of saliva and the amylopsin of pancreatic juice.

(*a*) Heat some starch in water, and put a little of the cooled paste on the tongue. After a time the sweet taste shows that part of the starch has been converted into sugar by the diastase (ptyalin) of the saliva.

(*b*) For experiments on the digestion of starch use either saliva, or (better) malt extract or commercial diastase. To obtain saliva for the experiments induce secretion by rinsing the mouth with water and then chewing a bit of rubber. Collect the saliva in a test-tube, and dilute with about five times its volume of water; if it is very turbid or frothy filter it. Make starch paste by rubbing up 10 grams of starch with 30 c c. of cold water, adding 200 c c of boiling water, and cooling the thin mucilage formed in this way.

(*c*) Make experiments with a dialyser (§ 44) to ascertain whether or not (*a*) starch mucilage and (*b*) sugar solution can pass through a membrane. From time to time take out some of the water and test it with iodine in the case of the starch; with Fehling's solution in the case of sugar.

(*d*) Repeat the dialysis experiment with starch solution to which some saliva or diastase has been added; after an hour test the water with Fehling (sugar present). As a control, set up a second dialyser containing saliva which has been boiled before being added to the starch

(*e*) Label three test-tubes *A*, *B*, *C*. In *A* put some starch solution; in *B* saliva only, in *C* one part of saliva and three parts of starch solution. Place the three tubes in a beaker of water at 40° C. for about ten minutes; to maintain the temperature, set the beaker on a sand-bath with a thermometer suspended in the water. Then test portions of the three liquids for reducing sugar with Fehling's solution; *C* reduces Fehling, *A* and *B* do not. Also test a portion of each with a few drops of iodine; only *A* gives a blue colour; the starch in *C* has been changed into maltose.

(*f*) Label two test-tubes *A* and *B*, and place in each some thick opalescent starch paste; to *B* add some saliva, and keep both *A* and *B* at 40° C. *A* remains unchanged, but in a minute or so *B* begins to become liquid and clear —a process preparatory to the conversion of the starch into sugar.

(*g*) Label three test-tubes *A*, *B*, *C*. Into *A* put some saliva and boil it, then add thin starch paste, into *B* put starch paste, saliva, and a little hydrochloric acid; into *C* starch paste, saliva, and a little potash. Keep all three at 40° C., and after ten minutes test each with Fehling; no sugar is present in either—diastase is destroyed by boiling, and its action is arrested by acids and alkalis. If *B* and *C* are very carefully neutralised (*B* with potash, *C* with acid), the diastase may be enabled to act as usual.

(*h*) Into a test-tube place some thick starch paste, add saliva, and place the tube in a freezing mixture. After

an hour test some of the liquid with Fehling; no sugar is present, the action of the diastase having been arrested by the low temperature. Now keep the tube at 40° C. for ten minutes; the paste becomes clear and will soon reduce Fehling, showing that the enzyme has not been destroyed by the cold to which it has been exposed.

(*i*) That *maltose* is the form of reducing sugar produced by the action of saliva or of malt extract can be proved. To starch solution add some saliva or malt, keep at 40° C., and at intervals of two minutes take out a drop or two of the liquid with a glass rod, place it in a white saucer, add iodine, and note the colours given—indicating the stages between starch and maltose. At first, blue (soluble starch); then violet (a mixture of red due to dextrin and blue to starch); then reddish-brown (dextrin alone); then yellowish brown, and finally no reaction at all (dextrin mixed with maltose). Now test a portion with Fehling (this might be done with the successive stages if the experiment is made in a large tube with plenty of material), which will prove the presence of a reducing sugar. After the liquid has ceased to give any iodine reaction, add to it alcohol, which precipitates the dextrin; filter, and test the filtrate for maltose (§ 66), which is not precipitated by the alcohol.

74. Translocation of Starch in Peas and Beans.—(*a*) Cut transverse sections of a cotyledon of Bean or Pea; treat some with iodine. Note that the cotyledon is made up of (1) a layer of small-celled **epidermis;** (2) the general **parenchyma,** consisting of larger cells separated by intercellular air-spaces; (3) the **veins,** appearing as patches and streaks of small-celled tissue. The cells of the parenchyma contain large starch grains and much smaller protein grains, but these are absent from the epidermis and veins. Test sections for sugar with Fehling's solution.

(*b*) Cut sections of the radicle and plumule, and of the young foliage-leaves (detach some of these and mount them entire), and note that the cells contain little or no starch in the resting seed. Test sections with Fehling; no sugar (or only a trace) is present.

(*c*) Now examine seedlings, treating sections with iodine, and note that when the root is about 5 cm. long numerous starch grains appear in the cortex and pith of the root and

hypocotyl. Test sections of these with Fehling: sugar is now present. As germination proceeds, starch disappears (being replaced by sugar) from the older and fully elongated tissues—remaining, however, in the starch-sheath around the ring of vascular bundles—and appears in the younger tissues. When the two primary foliage-leaves of Phaseolus emerge they contain starch, but as they develop it disappears from them, and by this time the amount of starch in the cotyledons has become greatly reduced—as can be seen by testing sections (note that the starch grains show extensive corrosion, with cracks and cavities).

(*d*) Remove the coats from seedlings of Peas or Beans in which the radicle has grown about 5 cm., grind or pound up the seedlings with water, and filter. Put starch paste into three saucers; into *A* pour some of the filtered extract from the seedlings, into *B* some extract that has been boiled, and leave *C* as a control. After the three have been in a fairly warm place for an hour or two, note that a sample of the liquid from *A* gives only a reddish colour with iodine, and ultimately remains uncoloured; while *B* and *C* become blue on adding iodine—the diastase in *B* has been destroyed by boiling. Transfer some of *A* to a test-tube, and apply Fehling's test; note the abundant sugar.

75. Translocation of Starch in Wheat.—(*a*) Cut and examine **transverse and longitudinal sections of a Wheat grain** softened in water for an hour. Note (1) the **coat,** consisting of a distinct epidermis, about two layers of thick-walled cells, a layer of large flattened cells, and then several layers of cells with more or less completely obliterated cavities—these tissues, starting from the outside, are derived from the ovary-wall (*pericarp*), the integuments (*testa*), and the nucellus (*perisperm*) of the ovule; (2) the **aleurone layer,** or outermost layer of the endosperm, consisting of cubical cells containing abundant protein grains but no starch; (3) the **starchy endosperm** tissue, consisting of polygonal cells with crowded starch grains; (4) the **embryo.** To see the successive layers of the grain coat more distinctly, mount sections in potash.

(*b*) In the **embryo** (compare transverse and longitudinal sections of the grain) note (1) the **scutellum,** abutting on the endosperm and consisting chiefly of small cells but showing on the surface a very distinct **epithelium** layer of narrow vertically elongated cells; (2) at the upper end of the embryo, the growing point of the **shoot,** covered by the young foliage-leaves and enveloped by the **plumule-sheath;** (3) the **radicle,** showing very regular longitudinal rows of cells, with the distinct **root-cap** covering the growing-point, and the **radicle-sheath** surrounding the whole root; (4) the **vascular bundles** seen at the junction of scutellum, plumule, and radicle, with veins diverging into these three organs, (5) a small appendage—**epiblast**—opposite the scutellum at the junction of the plumule and radicle sheaths, (6) the spiral and annular **vessels** of the bundle which enters the base of the grain—look for these in both longitudinal and transverse sections; (7) the fine tapering hairs at the apex of the grain.

(*c*) Test sections for starch, sugar, and proteids; there is no sugar in the dry resting Wheat grain; the embryo contains proteins, but no starch; the endosperm contains both starch and proteins.

(*d*) Examine **Wheat seedlings** from time to time, and test sections for starch, sugar, and proteins. At an early stage sugar appears in the endosperm; soon afterwards transitory starch grains appear in the scutellum (except the epithelium), and starch is also detected in the cells of the elongating plumule-sheath and of the young growing leaves within it.

(*e*) Squeeze out the milky contents of germinating Wheat grains on a slide, and note the corroded starch grains. Also squeeze some germinating grains into a test-tube, shake with water, and filter; test the filtrate for sugar with Fehling's solution.

76. Inulin.—This carbohydrate is found as a reserve substance in many plants. It is soluble, but not readily, in cold water, though it occurs in the cell-sap in solution,

and it is precipitated, often in spherical crystalline masses, on extraction of the water by alcohol or glycerine.

(*a*) Examine commercial inulin. Place some of it in a test-tube, add cold water, shake up, filter, and apply to the filtrate the tests given below—it is only slightly soluble. On being treated with hot water, however, it dissolves readily.

(*b*) To the cold-water solution add Fehling, and boil: no reduction occurs To the solution made with boiling water add hot Fehling, and boil for a few minutes: a little cuprous oxide is thrown down, because the hot water converts some of the inulin into glucose. To another portion of the hot-water solution add a little sulphuric acid, boil, and test with Fehling: a copious precipitate is given

(*c*) Allow some of the hot-water solution to cool, and set the test-tube aside: the inulin is precipitated, but very slowly. To a little of the cooled solution add excess of alcohol: the inulin is quickly thrown down.

77. Tests for Inulin.—(*a*) Test the inulin solution with iodine: only a faint brownish colour is given. (*b*) Add caustic soda or potash to dry inulin in a test-tube: it dissolves without being coloured. (*c*) Warm some inulin solution, then add a few drops of alcoholic solution of orcin: an orange-red colour is given (*d*) To some inulin solution add a few drops of strong hydrochloric acid, and boil; cool, and add a few drops of alcoholic solution of phloroglucin: a yellow-brown colour is given. Inulin is readily distinguished from sugars by reactions (*b*), (*c*), and (*d*).

(*e*) Cut sections from the pith of a fresh Dahlia tuber, and examine in alcohol; note the scanty cell-contents, with transparent sap. Lay the sections in strong alcohol for about an hour, and mount in glycerine; note that the inulin has separated out in the form of spherical crystal-like masses.

(*f*) Cut a Dahlia tuber into pieces, and steep them in alcohol for at least a week. (1) On examining sections in glycerine, note the large sphere-crystals seated on the cell-wall and often extending from cell to cell; the longer the material has been in alcohol the larger will these masses be. (2) To sections showing these inulin-masses add iodine: the inulin is scarcely coloured. (3) Treat other sections with water: the inulin is slowly dissolved. On heating, the process of solution is hastened; and during solution the masses show a radiating structure. (4) Treat other preparations with potash: they are dissolved more quickly than with water. (5) Treat a section with 20 per cent. α-naphthol solution, then add two or three drops of strong sulphuric acid: the crystals dissolve with a violet colour.

(*g*) In addition to, or instead of, Dahlia tubers, the following may be used for the demonstration of inulin: tuber of Jerusalem Artichoke, root of Dandelion. Cut sections of fresh material, examine in water, then add alcohol, and note the granular precipitate formed in the cells; on irrigating with water the precipitate will be again dissolved. Pieces of the tissue should also be placed in alcohol for a week or more, as directed for Dahlia, in order to obtain the sphere-crystals

VI. Cell-walls; Mucilages

78. Tests for Cellulose.—Soak some cotton-wool in alcohol, to remove air-bubbles, and then in water.

(*a*) Mount some of the soaked cotton in water, and with high power note that the long hairs are unicellular, with thick colourless walls and scanty remains of the protoplasm

(*b*) Place some in strong iodine solution in a watch-glass for a few minutes, mount in iodine, and note that the walls are stained faint yellow.

(*c*) Transfer a little of the iodine-treated material to a drop of 50 per cent. sulphuric acid, and note that the walls swell up and turn blue.

(*d*) Treat some of the material with chlor-zinc-iodine (see Appendix): the walls become blue or violet.

(*e*) Mount some in aniline sulphate solution: the walls are *not* stained—this solution is used as a test for lignin (§ 79).

(*f*) Place some dry cotton-wool in ammonio-cupric hydrate ("cuprammonia," see Appendix), and note that the hairs fuse into a gum-like mass and eventually dissolve.

(*g*) Mount some of the cotton in "cuprammonia" on a slide, and note the swelling of the walls that precedes their solution.

The blue colour given with iodine and sulphuric acid, or with chlor-zinc-iodine, is the best positive microchemical test for cellulose, and both of these reagents should

always be tried; the negative reactions with iodine and with aniline sulphate should also be noted when examining tissues.

N.B.—In some cases no blue colour is produced either with chlor-zinc-iodine or with iodine and sulphuric acid, hence the failure of these two reactions must not always be taken as a proof that cellulose is absent, though they are positive proofs of its presence when they are obtained. Sometimes these reactions are not given until the tissues have been treated for some time with potash. Occasionally walls turn blue with iodine alone.

79. Lignified Walls show certain well-defined colour reactions. The chief reagents used are the following:—

(*a*) **Aniline Sulphate (or Chloride) Solution.**—Dissolve aniline sulphate, or chloride, in water, and add a little acid—sulphuric or hydrochloric. Dip a wooden match into the solution—the wood turns bright yellow, more rapidly on warming it; if the reaction is not given readily, add more acid.

(*b*) **Phloroglucin Solution.**—Dissolve phloroglucin powder in alcohol, making a 5 or 10 per cent. solution. Add strong hydrochloric acid until a precipitate just begins to appear; the solution is then ready for use. The solution may be made up without acid, and the acid applied to the tissue simultaneously with the alcoholic (or aqueous) phloroglucin solution. Dip a wooden match into the solution—it turns bright red; if the colour is not given at once, add acid or apply heat.

(*c*) **Carbolic-Hydrochloric Acid Mixture.**—Dissolve some carbolic acid in warm hydrochloric acid; if a precipitate is formed, add enough hydrochloric acid to redissolve it, and the mixture is ready for use. Into some of this mixture in a watch-glass lay broken pieces of a wooden match; on exposure to the light for a short time the wood becomes bright green.

(*d*) In addition to these three very characteristic reactions (which are very readily applied to sections as

microchemical tests) try the following tests Dip separate wooden matches into (1) iodine—it turns yellow only; (2) chlor-zinc-iodine—it turns yellow; (3) first iodine and then sulphuric acid—it turns brownish.

(*e*) Cut transverse sections from a wooden match, soak them in alcohol to remove air-bubbles. (1) Mount a section in water, and note the network of walls, which are practically colourless or sometimes very faintly yellow; (2) add iodine—the walls turn yellow; (3) next add sulphuric acid—the walls swell up and turn brownish, (4) mount another section in chlor-zinc-iodine—the walls turn yellow, (5) mount another in aniline sulphate—the walls turn bright yellow; (6) mount another in phloroglucin—the walls turn bright red; (7) mount another in carbolic-hydrochloric acid, and expose to the light—the walls turn green; (8) treat another section with potassium permanganate solution, followed by ammonia—the walls turn red.

(*f*) Note also that lignified membranes are insoluble in cuprammonia, but are swollen and finally dissolved by strong sulphuric acid. After treatment with Schultze maceration fluid (§ 80 *b*), they react like cellulose.

80. Corky Walls do not give well-defined reactions, except for the relatively great resistance which they offer to the action of strong acids.

(*a*) Cut thin sections of an ordinary bottle cork, and soak them in alcohol to remove air-bubbles. (1) Mount a section in water, and note the cork cells, regularly arranged in rows, with thin yellowish walls; (2) treat with iodine—the walls turn more distinctly yellow; (3) next add sulphuric acid—the walls turn deep brown, but they retain their sharp outlines and do not swell; (4) treat a section with chlor-zinc-iodine—the walls either remain unchanged or turn deeper yellow; (5) treat a section with potash for a few minutes, then add chlor-zinc-iodine—the walls turn violet.

(*b*) Dissolve crystals of potassium nitrate in strong nitric acid in a test-tube—this gives **Schultze maceration**

fluid. Put into the solution some rather thick sections of cork, and boil for a short time—the sections lose shape and fuse into a mass, on cooling, pour off the solution and replace it by alcohol—the mass is dissolved.

(*c*) Make this experiment with thin sections, taking great care not to let the acid fumes injure the microscope Warm the sections gently with a little maceration fluid on a slide, and note that the corky walls turn bright yellow, then boil the liquid on the slide, allow to cool, and note that the walls have fused into drops (consisting of ceric acid).

(*d*) Corky walls also turn red with alkannin, but not so deeply as in the case of oils—for which alkannin is also used as a test.

81. Cutinised Walls resemble corky walls in their general reactions. If sections of stems, etc., are placed for an hour or so in strong freshly-made chlorophyll solution, the cutinised and the suberised walls are stained deeply green, while lignified and cellulose walls remain unstained. Cutin is typically developed in the outer walls of epidermal cells, which often show a stratified clear or yellowish **cuticle.**

82. Gums and Mucilages may be treated here, since they are often, though not always, derived from cell-walls. They are greatly swollen by potash, dissolve in water, and are insoluble in alcohol Vegetable gums may or may not give the same reactions as ordinary cellulose; they are stained deeply blue with methylene blue and in some cases Hoffman's blue, pink with corallin-soda Most of these bodies are allied to carbohydrates; they are converted into dextrin by treatment with sulphuric acid; on treatment with nitric acid they yield oxalic and mucic acids; and they are quite amorphous, not being crystallisable like the sugars.

(*a*) Examine commercial gum-arabic (obtained from an Acacia). (1) Treat with warm water—it dissolves; (2) add alcohol to the solution—it is precipitated; (3) treat with iodine—brown colour; (4) treat with sulphuric acid and then with iodine—brown colour.

(*b*) Soak seeds of Linseed in water for an hour or so, and note that the surface of the seed is covered by a thick transparent gum. (1) Cut transverse sections of a dry seed (wet the razor with alcohol

or glycerine), mount in strong glycerine, and note that the epidermis of the seed-coat consists of cells which have thick walls and are covered externally by a distinct cuticle. (2) Irrigate with water (or lift off the cover-glass and place a water drop on the section), and note that the walls of these cells become swollen, the stratified structure of the outer walls becoming more marked; the cuticle is ruptured as the swollen mass bulges out; the middle lamella of the walls between adjacent cells does not swell up, but remains distinct. The swelling of the walls may be hastened by warming the slide. (3) Treat the section, which has been soaked in water, with iodine solution—the gummy walls are not stained or only slightly (4) Treat a section with iodine and sulphuric acid—a bluish colouration is produced. (5) Treat a section with Hoffman's blue—the gummy walls are not stained, or very slightly. (6) Treat a section with corallin-soda—the gummy walls turn pink (7) Treat a section with potash—the swelling of the walls occurs much more rapidly than with water.

(*c*) Examine commercial salep, or make it by drying and crushing the tubers of Orchis mascula (or O. maculata, or O latifolia); treat the salep, or the pounded tubers, with cold water, and filter (1) To the clear filtrate add alcohol—the white flocculent precipitate consists of Orchid mucilage, insoluble in alcohol. (2) Evaporate the liquid, and treat the residue with iodine and sulphuric acid—the blue or violet colour produced is distinctive of the so-called "true vegetable mucilages."

(*d*) Cut transverse sections of the tuber of an Orchis. (1) Mount in alcohol, and note that the ground tissue (parenchyma), in which the vascular bundles are embedded, consists of small starch-containing cells, together with larger cells each of which contains a bundle of needle-like crystals (raphides) of calcium oxalate embedded in mucilage. (2) Treat a section with corallin-soda, mount in glycerine, and note that the large cells have their mucilaginous contents stained pink. (3) Treat a section with iodine and sulphuric acid, and (4) another with Hoffman's blue, and note the results.

VII. Glucosides and Tannins.

83. Glucosides are combinations of glucose, or more rarely of other sugars, with various classes of organic compounds, especially those of the aromatic series. In general chemical properties they resemble cane sugar and the polysaccharides, and various glucosides have been prepared synthetically from glucose. The glucosides yield glucose on being hydrolysed by means of acids or of special enzymes (glucosidases or glucoside-splitting enzymes).

For instance, the glucoside **amygdalin** occurs in Almonds, and is obtained by extraction with alcohol and precipitation with ether. The enzyme **emulsin** (found in germinating Bitter Almond seeds, also in the leaves of Cherry Laurel, Bird Cherry, etc) decomposes amygdalin into prussic acid, benzaldehyde, and glucose. **Salicin,** found in the twigs of Willows and Poplars, and obtainable in the same way, is converted by emulsin into glucose and saligenol (salicylic alcohol).

(*a*) Examine commercial salicin; note its bitter taste. Dissolve some salicin in warm water, and note that (1) it does not reduce Fehling's solution; (2) it gives a red colour on addition of strong sulphuric acid—if water be then added, a red precipitate is given, (3) it gives no colour on addition of dilute ferric chloride solution.

(*b*) Using a Soxhlet fat-extraction apparatus (§ 97), extract salicin from some chopped-up twigs of Willow or Poplar with water. Test the watery extract as in the preceding experiment

(*c*) Add dilute sulphuric acid to some salicin solution, and boil. Neutralise with caustic soda, and apply Fehling's test—a reducing sugar is now present

(*d*) Grind up some Bitter Almonds in a mortar with sand and water. Filter the liquid, which will contain emulsin. To salicin solution in test-tubes add (A) some of the emulsin solution; (B) some emulsin solution that has been boiled; (C) some diastase solution; leave (D) with nothing added to the salicin solution. Place the tubes on a bath at 40° C. for half an hour or an hour. Test with Fehling's solution: glucose is present in A, though absent in the other cases. Note that in A the addition of some dilute ferric chloride gives a deep purple colour (destroyed by acids or by alkalis); this is due to the presence of saligenol

(*e*) Grind up in the same way some Sweet Almond seeds, and note that the watery extract in this case contains emulsin, but there is no amygdalin. To some of the extract of Bitter Almond seeds, which has been boiled so as to destroy the emulsin present in it, add some of the Sweet Almond extract, and note that hydrolysis occurs—glucose, prussic acid, and benzaldehyde (oil of Bitter Almond) being formed.

(*f*) In transverse sections of the leaf of Cherry Laurel note that there is a layer of cells surrounding the vascular bundles, marked by the finely granular character of their protoplasm and their freedom from chloroplasts and starch. (1) Apply the tests for tannin (§ 84) to some sections—these cells contain tannin. (2) Treat sections with Millon's reagent—on warming the slide, note that these cells turn deep orange red, while the ordinary parenchyma

cells are faint pink. (3) Treat other sections with copper sulphate and caustic potash—these cells become violet The contents of these cells therefore give somewhat similar reactions to those of proteins; whereas cells containing only tannin do not react to Millon's and the biuret test more than do ordinary parenchyma cells with their protoplasm lining, which stains a pale pink.

(*g*) For comparison with Cherry Laurel, make similar sections of the leaf of Portugal Laurel Note that in this species there is a corresponding layer of cells around the bundles, and prove by tests that in this case the leaf contains tannin but no emulsin.

(*h*) Grind up leaves of Cherry Laurel and of Portugal Laurel and make a watery extract, which will contain emulsin only in the former case. Prove this by adding each extract to some salicin or to some amygdalin containing extract of Bitter Almonds.

(*i*) The glucoside phloroglucin (which is used with acid as a test for lignin, see § 79) is obtained from the wood of various plants. Make a phloroglucin solution, and note that it gives (1) a violet colour with ferric chloride, (2) a violet colour to a freshly-cut piece of Pine wood dipped into the solution after adding hydrochloric acid.

(*j*) Pound up some Horse Chestnut bark with glacial acetic acid to extract the glucoside aesculin, and note that a fine blue fluorescent colour is given on making the solution alkaline with potash.

84. Tannins.—Under this name are included various substances found chiefly in bark and in pathological gall formations. The best known is tannic acid, which occurs along with the allied gallic acid in "gall nuts" (oak galls). The tannins are probably related to the glucosides, and in some cases are of similar importance in metabolism, yielding glucose on being hydrolysed. Like most glucosides, they have an astringent taste, and their most characteristic reaction is the dark blue or green colour which they give with salts of iron.

(*a*) For the general reactions of tannin use commercial tannin (tannic acid) dissolved in water. (1) Add a few drops of ferric chloride—a deep blue or blue-black colour. (2) Add a few drops of potassium ferricyanide and some ammonia—a red or brown colour. (3) Add potassium dichromate—a reddish-brown colour (4) Add some ammonium chloride solution, followed by some ammonium molybdate solution—a yellow precipitate.

(*b*) The tannins are widely distributed in plants, and their presence is easily recognised on testing cut surfaces with ferric chloride, ferrous sulphate, or potassium dichromate As material

use young oak galls; acorns (cut across the cotyledons, which also contain abundant starch); twigs of Hazel, cut in winter; young Rose stems. Rose leaves contain abundant tannin; fold up several leaves and crush with the fingers between folded white paper to press out the sap, then touch the moistened portions of the paper with ferric chloride solution, and note the dark blue colour.

(*c*) Cut thin sections of any of the above (1) Irrigate with ferric chloride, or with ferrous sulphate—note that at first a deep blue precipitate is formed, which soon dissolves and imparts its colour to the surrounding liquid. (2) Place sections in 10 per cent. potassium dichromate solution—a reddish brown precipitate is formed in the tannin-containing cells. (3) Place sections in strong solution of ammonium molybdate in strong ammonium chloride—a brown or yellow precipitate. (4) Place sections in lead acetate solution—a white precipitate. (5) Place sections in strong copper acetate solution for a week; then place them on a slide in a drop of 1 per cent solution of ferrous sulphate; after a few minutes, wash with water, transfer to a watch-glass of alcohol (to remove air-bubbles and to extract chlorophyll if present), and mount in glycerine. An insoluble brown precipitate is found in the cells containing tannin If the sections are taken from the alcohol and placed in iron acetate solution, a blue or green colour is produced, according to the kind of tannin present.

(*d*) It can be shown that in various plants tannins are produced in green leaves exposed to light and supplied with carbon dioxide, but not in darkness or in absence of carbon dioxide. They are probably formed as bye-products in the process of proteid-formation, rather than as primary products of photosynthesis. They probably migrate from the leaf during the night and are ultimately deposited in the stem tissues, but it is doubtful whether the primary tannins thus formed enter largely again into metabolism So-called "secondary" tannins are, however, formed in many plants when kept in darkness. Seeds of Broad Bean and Scarlet Runner contain no tannin; but seedlings grown in darkness are rich in tannin. Apply the tannin tests to sections of (1) dry seeds, (2) the stems of darkened seedlings of these plants, and note the results.

VIII. Oils, Resin, Latex.

85. Oils.—Under this name are included two series of substances, which give certain reactions in common but differ considerably in chemical properties, and in their functions.

The **fatty oils,** or **fats,** occur in many seeds, and less frequently in other parts of plants (*e.g.* stems of Lime,

Birch, and some other trees in winter) as a store of reserve food Chemically, the fats are compounds—esters—of higher fatty acids (oleic, stearic, etc.) with glycerine (glycerol). They are quite insoluble in water, cold or hot; hardly soluble (except castor oil) in alcohol; readily soluble in ether, benzine, chloroform, etc. They can be extracted from seeds by simple pressure, or by distillation with their solvents, but not by distillation with water (cf ethereal oils).

The **ethereal oils** differ from the fatty oils in that they may be distilled (from the leaves, etc, in which they occur) along with water vapour; also in being soluble in glacial acetic acid and in chloral hydrate. At 130° C all ethereal oils are driven from sections, while the fatty oils remain behind. Ethereal oils are only slightly soluble in water, but they impart their smell strongly to it. They are easily soluble in ether, chloroform, etc.; the spot produced on paper by ethereal oils soon disappears, these oils being volatile; they agree with fatty oils in being browned or blackened by osmic acid, and in being stained red by alkannin and blue by cyanin.

(*a*) Allow drops of (1) turpentine, (2) olive oil or castor oil, to fall on different parts of a sheet of white paper. The turpentine (a volatile or ethereal oil) soon disappears; the olive or castor oil remains. Other ethereal oils are oil of eucalyptus, clove oil, lavender oil.

(*b*) Test the solubilities of (1) fatty oils, (2) ethereal oils, by placing a drop of oil on a slide in each case and adding the following solvents. Olive oil, and most other fatty oils, are only slightly soluble in ordinary alcohol, but are soluble in methyl alcohol as well as ether, chloroform, and carbon bisulphide. Castor oil, however, is readily soluble in ordinary alcohol Ethereal oils are soluble in both ordinary alcohol and in ether. In each case pour a few drops of the solution on filter paper, and note the grease stain left.

(*c*) Place a drop of fatty oil on a slide, add a mixture of ether and absolute alcohol (equal parts), which dissolves the oil. When the ether and alcohol evaporate,

drops of oil are left on the slide. Examine with the microscope; on focussing down, note that the dark-looking ring around each drop becomes bright—compare with air bubbles, the dark ring around which simply becomes broader on focussing down.

(*d*) Pound up dry oily seeds (*e.g.* Sunflower, Linseed, Castor Oil, Brazil Nut—remove the coats from the larger seeds) between folds of blotting-paper, and note the greasy stain produced; this dissolves in ether. Castor Oil and Brazil Nut seeds are so rich in oil that the oil drops are readily seen on cutting across the seed with a heated knife

(*e*) Place drops of various oils on a series of slides. In each case add a drop of 1 per cent. osmic acid; the oil is coloured brown or black

(*f*) Examine commercial ground Almonds, a rather greasy powder, and apply to it the tests for oils and for proteins.

(*g*) Cut sections of oily seeds, *e.g.* Castor Oil, Almond, Brazil Nut, Sunflower, Walnut. (1) Mount in water, and note the bright-looking oil drops, both in the cells and in the water. (2) Mount dry-cut sections in a mixture of equal parts ether and absolute alcohol; the oil drops are dissolved, but separate out again on letting the solution evaporate. (3) Treat a section with 1 per cent. osmic acid; the oil drops become blackened. (4) Treat a section with alkannin solution (see Appendix); this stains the oil drops red, but an hour or more may be required. It is often better to cut a section of dry alkanna root and lay it on the section. In the case of Castor Oil, since the oil is soluble in alcohol, it is advisable (unless the dry alkanna root is used) to mix the alkanna tincture with an equal volume of glycerine, and to examine the section in glycerine.

(*h*) Ethereal oils may be examined in sections cut with a dry razor from fresh material, such as Orange rind, fruits of Umbellifers, etc. Note that these oils are stained by osmic acid and by alkannin, are soluble in ordinary alcohol, and being volatile disappear on being warmed.

(*i*) The vapour of hydrochloric acid may be used to distinguish between ethereal and fatty oils. Cement to a slide a large glass ring, such as are used for hanging-drop cultures (§ 18), and a small glass ring shallower than the large one. Place hydrochloric acid in the space between the two concentric rings; place the sections to be tested on a cover-glass in a drop of glycerine containing strong sugar solution, then invert the cover and place it on the larger ring. Note that in a short time any ethereal oil in the sections takes the form of bright yellow drops which finally disappear. Fatty oils do not form yellow drops on treatment in this way with hydrochloric acid vapour.

(*j*) Compare the reactions of oils and those of resins (§ 88). The alkannin test for oil is not decisive, since resins take the same red colour; suberised and cutinised walls also give a red colour with alkannin.

86. Digestion of Fatty Oils.—The fats are hydrolysed into their constituent fatty acids and glycerine by boiling with water or treatment with steam, and by boiling with acids and alkalis. The alkali method of decomposing fats is a special kind of hydrolysis, called saponification, since it was first used in the making of soap.

The fatty oils undergo hydrolysis during digestion. They are decomposed by the enzyme lipase, present in the pancreatic juice (§ 57) and also in germinating oily seeds, and hydrolysed into their constituents (fatty acids and glycerine).

(*a*) Boil a small quantity of lard with about 20 c.c. of alcoholic soda solution (1 gram sodium solution in 50 c c alcohol), or with caustic soda solution, for about five minutes The fat is converted into soap (sodium stearate, etc.). Then pour the solution into an evaporating basin to evaporate the alcohol, if alcoholic soda is used. Add some water; if oil drops are seen, saponification (hydrolysis by alkali) is incomplete and should be completed by boiling with more soda. Acidify with dilute sulphuric acid A precipitate of fatty acid is formed from the soap. Filter this precipitate off through a wet filter paper, and wash it with water till free from acid. Keep the filtrate to test for glycerine (see *c* and *d* below).

(*b*) Prove that the precipitate from the preceding experiment consists of fatty acid, as follows. (1) Dissolve some of it in ether, and add some alcohol containing a drop of dilute soda and a drop of phenolphthalein; the red colour of the indicator disappears. (2) Dissolve some precipitate in caustic soda and divide the solution into three parts:—(1) shake up with warm water—a soap

lather is produced; (ii) add some sodium chloride—the soap is separated and rises to the surface as a curd; (iii) add calcium chloride—a precipitate of calcium soap (calcium stearate, etc.) is produced.

(*c*) Neutralise filtrate from *a* with dilute soda; evaporate it to a syrup on a water bath. Add alcohol, which precipitates the sodium sulphate, and pour off the liquid (alcoholic solution of glycerine). Evaporate this, and test for glycerine as below.

(*d*) **Tests for Glycerine.**—(1) Heat a little glycerine with powdered potassium hydrogen sulphate, and note the pungent smell of acrolein—this indicates the presence of glycerine. (2) Add a few drops of copper sulphate solution, then some potash—a deep blue colour is produced but no precipitate, since glycerine prevents the precipitation of cupric oxide by alkalis. (3) Add drop by drop a 20 per cent. aqueous solution of glycerine to a 5 per cent. solution of borax, to which enough phenolphthalein has been added to produce a distinct red colour; the red colour disappears, but on boiling it returns if excess of glycerine has not been used. This reaction is also given by other polyhydric alcohols.

(*e*) Grind up a few Castor Oil seeds with about 30 c.c. of water to which a drop of chloroform has been added. Divide the liquid into two exactly equal portions, place them in two test-tubes, and at once boil one to destroy the enzyme (lipase). Then add to each 1 c.c. of dilute acetic acid, and place both tubes in a bath at 40° C. for half an hour or an hour. Then add to each tube a few drops of phenolphthalein and titrate with decinormal caustic soda solution. Note that the number of c.c. of soda solution required to neutralise the tube with unboiled enzyme will be greater than in the tube with boiled enzyme.

(*f*) In the hydrolysis of oils in the intestine, **emulsification** occurs. (1) To some Linseed, Olive, or Castor oil in a test-tube, add a little water; close the tube with the thumb or a cork, and shake vigorously. On letting the tube stand the milky appearance is lost, the oil and water separating again into two layers—this is only temporary emulsification. (2) Repeat the experiment, but this time add a little carbonate of soda to the water before shaking up—the emulsion produced this time is of a more permanent character.

(*g*) The emulsification produced by alkalis is due to the presence of free fatty acids in most oils. If a perfectly neutral oil is shaken up with alkali, no emulsion is formed.

To detect free fatty acid in a fatty oil, add a drop of phenolphthalein to a little alcohol in a test-tube, then a drop or two of very dilute soda—just enough to produce a red colour. Then add a little olive oil dissolved in ether, or castor oil dissolved in alcohol. The presence of fatty acid is shown by the disappearance of the red colour.

To prepare neutral olive oil, dissolve the oil in ether, shake it up with dilute sodium carbonate, wash free from alkali, and evaporate off the ether. In each of five test-tubes place 10 c c. of water, then add (1) 2 c.c neutral olive oil; (2) 2 c c. neutral oil and 1 drop 10 per cent. caustic soda; (3) 2 c.c. neutral oil and 2 drops oleic acid; (4) 2 c.c. neutral oil, 2 drops oleic acid, 1 drop 8 per cent soda; (5) 2 c c ordinary olive oil and 1 drop 8 per cent soda. Shake the tubes, place them in a stand, and note that only in (4) and (5) is a permanent emulsion formed; in the others, separation occurs after a short time.

87. Digestion and Translocation of Oils in Germination.—Test both the cotyledons and the endosperm of dry **Castor Oil** seeds with iodine: starch is absent. When the root and hypocotyl have grown considerably, but the latter is still curved and the cotyledons are embedded in the endosperm, remove the cotyledons from the endosperm.

Test for oil (osmic acid or alkanna), for starch (iodine), and for sugar (Fehling), and note that the endosperm still contains only oil and proteids; abundant starch and sugar are present in the cortex and pith of the upper portion of the hypocotyl, but the amount diminishes further down and is there confined to the starch-sheath (endodermis) around the bundle-ring; in the root there is no starch, but sugar is present, especially in the secondary roots; in the cotyledons (which before germination contain no starch), starch is now present in the parenchyma cells around the veins, but oil also occurs in the rest of the parenchyma tissue.

As germination advances, the amount of starch increases in the upper portion of the hypocotyl and in the cotyledons, and diminishes as the hypocotyl elongates, until the development of these organs is completed; then both starch and sugar disappear from their cells, having been used up in the process of respiration as well as the formation of new cell-contents and cell-walls.

88. Resins.—Many ethereal or volatile oils consist of solid oxygenated compounds dissolved in liquid hydrocarbons called terpenes. Turpentine oil or spirit (ordinary "turps"), which consists chiefly of a terpene (pinene), is obtained from the volatile oil of various Conifers by distillation with steam, common resin ("rosin") being left behind. Oil of camphor consists of solid camphor dissolved in a terpene. Most of the volatile oils are converted by oxidation into more or less solid compounds called resins or (if they still contain unaltered ethereal oils) balsams (*e g* Canada balsam).

(*a*) Examine common resin, and note that it is a yellow translucent amorphous substance, insoluble in water, soluble in turpentine, benzine, alcohol, and ether. It is coloured red by alkannin.

(*b*) Cut transverse sections (wetting the razor with water) from a young Ivy stem or Pine stem. (1) Mount in water, and note the highly refractive resin drops, found chiefly in and around the resin ducts (2) Irrigate with alcohol; the drops are dissolved. (3) Test a section with alkannin, using either the alcoholic solution or (better) laying a section of dry alkanna root on the stem section; cover and leave for about an hour—the resin drops are stained red. (4) Test other sections with osmic acid Compare with the reactions of oils.

(*c*) Place a piece of Ivy or Pine stem in strong copper acetate solution for about a week; then wash the pieces in water or dilute alcohol, cut sections, and note that the resin is stained green

89. Latex.—This is a liquid found in many plants. Occasionally it is watery and colourless (Banana, etc), but it is generally a milky emulsion owing to the presence of suspended particles, and sometimes it is coloured (Chelidonium) In the fluid of latex there occur dissolved salts, sugars, etc.; the suspended particles consist chiefly of rubber or caoutchouc, but sometimes there are starch grains (Spurges); various nitrogenous organic substances also occur in latex, *e.g.* proteins, enzymes, and alkaloids (opium, etc).

Cut across the stem of a Spurge freshly pulled up the latex escapes as a white juice, which was evidently under pressure (exerted by the turgid parenchyma around the latex tubes), since it escapes in considerable quantity. (1) Quickly cover and examine fresh latex on a slide: at first it is like milk, containing numerous suspended particles, but after a time a clot is formed and the materials originally distributed uniformly in the liquid collect into masses. (2) To another fresh portion of latex add alcohol; clotting occurs much more rapidly and completely. Hence it is necessary, in the study of laticiferous tissue, to place an entire plant, or pieces cut from it, at once in alcohol, in order to coagulate the contents and prevent their escape. (3) To another portion add iodine; the granular masses stain brown (proteins), while here and there are rod-like or dumbbell-like starch grains. (4) Treat another portion with alkanna: the rubber particles are stained red.

For the structure of laticiferous tissue see § 139.

IX. Non-nitrogenous Organic Acids; Mineral Deposits.

90. Non-nitrogenous Organic Acids are frequently formed from carbohydrates, usually by processes of oxidation, and are present in the cell-sap, either in the free

state or, more commonly, combined with bases to form acid or neutral salts.

The chief non-nitrogenous organic acids in plants are oxalic, malic, citric, and tartaric. Soluble potassium oxalate occurs in Rumex (Docks, Sorrel Docks) and Oxalis (Wood Sorrel), sodium oxalate in Salsola and Salicornia, while crystals of insoluble calcium oxalate are the most frequent mineral deposits found in plant tissues. Malic acid and malates occur in the juice of many fruits (*e g* Apple, Gooseberry, Rowan, where they are abundant), in the tissues of various succulent plants (especially in Crassulaceae), in Fern prothalli, etc., citric acid occurs in the juice of Lemons, Oranges, etc, and in Lycopodium prothalli; tartaric acid (generally as acid potassium tartrate) in Grapes, Pine-apples, etc

91. Oxalic Acid.—Any soluble calcium salt, added to a solution of oxalic acid or a soluble oxalate, gives a white precipitate of calcium oxalate, soluble in hydrochloric or nitric acid, but almost insoluble in potash or ammonia This is a delicate test, and is hastened by warming, if the oxalic solution is very dilute

Repeat this test with oxalic acid or potassium oxalate, using calcium chloride for the reagent. Apply this test to juice pressed from leaves and petioles of Rumex, Oxalis, Salicornia, Salsola

92. Tartaric Acid.—Potassium chloride produces in a solution of free tartaric acid a white precipitate of hydrogen potassium tartarate, readily soluble in mineral acids and alkalis; calcium chloride, added to tartaric acid or an alkaline tartarate, gives a white precipitate of calcium tartarate, distinguished from calcium oxalate by being soluble in potash; silver nitrate gives a white precipitate of silver tartarate—filter, dissolve the precipitate off the filter with a little dilute ammonia, heat the solution in a test tube for a few minutes, when the glass becomes coated with a silver mirror (characteristic reaction for tartaric acid).

Apply these tests (especially that with silver nitrate) to a solution of tartaric acid or a tartarate. Repeat the tests with some Grape juice pressed into a test-tube, after addition of a little caustic potash to the juice.

93. Citric Acid is readily distinguished from tartaric, since no precipitates are given with potassium salts, nor with cold lime-water (on heating with lime-water, white calcium citrate is thrown down); the silver citrate precipitate (given on adding silver nitrate solution) does not form a mirror when dissolved

with ammonia and heated, but gives a black deposit after boiling for some time.

Try these tests with citric acid or a soluble citrate. Repeat the tests with juice of Orange or Lemon

94. Malic Acid, usually combined with lime, is abundant in various Crassulaceae, etc. The calcium malate, which may form nearly half the dry weight of the sap in Sempervivum, Echeveria, and other plants of this family, can be extracted by bruising or pounding up some fresh leaves, filtering the pulp, and adding to the filtered sap four or five times its volume of strong alcohol—the malate is precipitated as a white powder.

See Text-books on Organic Chemistry for detailed reactions of these acids, methods of detecting each in mixtures, etc.

95. Mineral Deposits may occur either in the cell-contents or in the cell-walls. The commonest of these deposits consist of calcium oxalate, calcium carbonate, and silica, which are easily distinguished from each other

(*a*) **Calcium oxalate** is chiefly found in the cell-sap as crystals of various forms, of which the chief are (1) single prismatic, flat, or diamond-shaped crystals; (2) more or less spherical aggregates (sphaero-raphides) with numerous small pyramidal crystals on the free surface; (3) needle-like crystals (raphides) arranged in bundles and generally embedded in mucilage. Calcium carbonate often occurs on cell-walls as an incrustation, the most striking of which are those called cystoliths. Silica occurs chiefly as incrustations on the cell-wall

(*b*) **Calcium carbonate** is soluble in acetic acid, and in weak nitric acid, with evolution of gas-bubbles (carbon dioxide). Calcium oxalate is insoluble in acetic acid; soluble in dilute nitric acid, but without evolution of bubbles; soluble in sulphuric acid, with formation of a crystalline precipitate of calcium sulphate. **Silica** is insoluble in acetic or nitric acid, and remains as a flinty residue after strongly igniting the tissue on a cover-glass or on platinum-foil and treating the ash with nitric acid.

(*c*) Cut transverse sections of the leaf of India-rubber Plant (Ficus elastica), mount in water, and note the large pear-shaped cystoliths, each occupying one of the large cells below the upper epidermis. Add a drop of acetic acid: the cystoliths become transparent and dissolve, bubbles of gas being given off. When the carbonate is dissolved, a mass of cellulose (on which the carbonate is deposited) is left, showing concentric stratification and radial striation.

(*d*) Since calcium oxalate crystals are so abundant in plants, they will be frequently found in sections of stems, leaves, etc., often occupying special cells. On testing the sections, note that the

sections are not stained by iodine, chlor-zinc-iodine, etc.; not dissolved by potash or by acetic acid; dissolved by nitric acid without evolution of bubbles; dissolved by sulphuric acid, and then replaced in the cell by small crystals of calcium sulphate. On adding barium chloride, crystals of calcium sulphate become covered by a granular layer of barium sulphate, while crystals of calcium oxalate are not affected by barium chloride.

(*e*) Cut tangential sections of the stem of Horsetail, so as to remove the epidermis, mount in water; note the numerous small projections covering the epidermal cells, and the radiating bands covering the cells around the stomata. Soak a section in nitric acid in a watch-glass for an hour, then ignite it over a flame on platinum-foil or on a cover-glass; treat the residue with a little acetic acid, mount in water, and note that a siliceous skeleton remains, showing the markings on the epidermis.

X. Methods for Extraction of Organic Substances.

96. Extraction of Non-nitrogenous Organic Substances.—The chief non-nitrogenous plastic substances can be extracted from plant tissues by the following methods.

(A) Dry the fresh tissue—after cutting stems, roots, etc., into short lengths—at a high temperature (100° C. if possible) in an oven until it ceases to lose weight. Reduce the dried material as nearly as possible to powder, using if necessary a hand-mill or domestic chopping-machine, and grinding and pounding the material in a mortar. Then place it in a Soxhlet fat-extracting apparatus (§ 97), using ether as the extracting solvent, and boil for some time. Filter, and place the filtrate (ether extract) in a corked bottle labelled A.

(B) Dry the residue, and place it again in the extraction apparatus, this time using 60 per cent. alcohol, and boil for some time. Filter, and place the filtrate (alcoholic extract) in a bottle labelled B.

(C) Dry the residue, place it in a bottle, add water, cork, and shake vigorously and repeatedly for some time, and allow to stand for several hours or until next day. Then filter, and place the filtrate (watery extract) into a third bottle labelled C.

(D) Boil the residue with dilute (1 per cent.) sulphuric acid for a few minutes. Filter the acid extract into a fourth bottle labelled D.

Extract A contains the oils and resins, in addition to chlorophyll and other pigments Extract B contains glucosides, tannins, and some sugar. Extract C contains dextrins and other soluble carbohydrates not dissolved by the alcohol. Extract D contains reducing sugars formed by action of acid on starch.

Each extract should now be tested, using the tests for the substances mentioned above.

(A) Distil off most of the ether, then evaporate the rest down on a bath Warm the residue with strong potash for an hour, add water, and filter if there is a residue (of resins, etc.). Then add hydrochloric acid until the solution gives an acid reaction with litmus, and cool. The fatty acids become solid in most cases; filter, and examine the filtrate for glycerine, after evaporating it down to small bulk.

(B) Evaporate off the alcohol, treat the residue with water, filter. If the solution is acid, neutralise with dilute soda, and test portions of it for tannins and glucosides (1) If woody tissue has been extracted, phloroglucin will probably be present; remove it by shaking up the solution after adding ether (in which tannins and ordinary glucosides are insoluble); pour off the ether layer, evaporate down the ether, dissolve the residue in water, and test the watery solution thus obtained for phloroglucin (2) There are no reliable general tests for glucosides; special tests must be used for the glucoside likely to be present. For instance, extract of Willow or Poplar stem will contain salicin, extract of Horse Chestnut bark will contain aesculin; the tests for these have been given. (3) Test parts of the watery solution for tannins, with ferric chloride, etc.

(C) (1) Concentrate the watery extract to small bulk by heating on a bath, add strong alcohol until no more precipitate is formed, and filter Examine the precipitate for dextrin and inulin, after dissolving it in water. (2) Evaporate the filtrate from (1) to remove the alcohol, dissolve

the residue in water, and test for (*a*) reducing sugar, (*b*) cane sugar

(D) Examine for reducing sugar, with Fehling's solution. Since some of the sugar may be present in the alcoholic extract, test for sugars some dried material which has been extracted directly with water.

97. Soxhlet Apparatus.—This apparatus (Fig 22) consists of a dry flask, which should be weighed accurately, a special extracting tube in which is placed a paper thimble containing the tissue to be extracted, and a short condenser. The extracting tube consists of a wide upper piece of glass tubing shaped like a test-tube and fused at its closed end to a narrower tube which is cut off at an angle at its lower end. Below the join of these two pieces of tubing, a side tube is fused into the lower piece; the other end of this side tube is fused into the wider piece. At the base of the wide tube is fused one end of a narrow siphon tube (on the right in Fig 22), the other end of which is fused to (and passes through) the narrow tube.

Place the material in the paper thimble; place this at the bottom of the wide upper part of the extracting tube, as in Fig. 22; fix the narrow lower end of the extracting tube through a cork into the flask; attach the condenser, connecting its two tubes to tap and sink Place ether in the flask, which is to be gently heated over a Bunsen. The volatilised ether passes through the side tube and reaches the condenser; the condensed ether falls in drops on the thimble; when this is covered, the ether passes back into the flask through the siphon tube, and the process is repeated The apparatus can be left for two or three hours if necessary, without constant attention

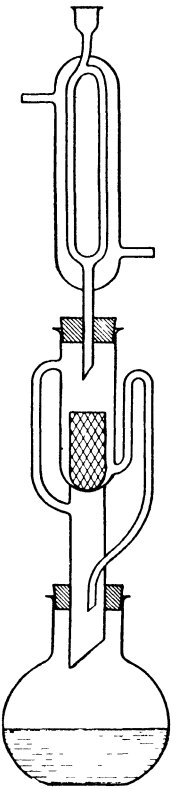


Fig 22.—Soxhlet Fat Extraction Apparatus.

98. Extraction of Proteins and Enzymes.—These substances should be extracted from material dried at a low temperature. Enzymes are destroyed by drying at 100° C.

(A) Pulverise the material, and dry it in an oven at a

temperature not above 30° C, or simply let it dry in the air without applying heat Extract the dried material with cold water, and after repeated shaking filter the extract into a bottle labelled A.

(B) To the residue from A add some 2 per cent. caustic soda solution; shake repeatedly, and filter into a bottle labelled B.

Extract A contains soluble proteins, proteoses, peptones, amido compounds. Apply the tests for these.

Extract B contains proteins insoluble in water but soluble in dilute alkali. Apply the tests for proteins.

To test for the presence of enzymes in the watery extract (A) add portions of the extract to (1) starch solution—test for diastase; (2) albumin solution—test for pepsins and trypsins; (3) peptone solution—test for erepsin; (4) neutral olive oil—test for lipase; (5) salicin solution—test for emulsin. In each case set up a control, adding extract which has been boiled to destroy any enzyme present; place the test-tubes in a bath, or in a beaker of water kept at 40° C., for an hour, and test (1) for reducing sugar, (2) for peptone, (3) for tyrosin, (4) for free fatty acid and glycerine, (5) for glucose and saligenol.

CHAPTER III.

HISTOLOGY OF ANGIOSPERM STEM, ROOT, LEAF, AND FLOWER.

99. Vegetable Marrow Stem (General Anatomy).—Material for study may be obtained by raising Marrow (or Cucumber) plants from seed in large pots or boxes of soil; when the plant is six to eight weeks old, turn it out of the pot and place it entire in a large pan of boiling water for three or four minutes. Then cut the stem—especially the lower part, starting from about 18 inches below the apex—into short lengths and place these in alcohol.

(*a*) Note that the stem is hollow, with (usually) five ridges and furrows; the bundles (usually ten) are in two rings—a smaller outer bundle to each ridge and a larger inner bundle to each furrow. (Fig. 23)

(*b*) Scrape the outer surface of the stem, so as to remove part of the epidermis with its hairs; note the soft tissue which lies between the bundles—the cells of this ground tissue parenchyma can be seen with a lens.

(*c*) After removing the epidermis, scrape away the soft tissue below it, and note the shiny hard tissue (sclerenchyma) which forms a wavy tube around the stem outside of the bundles.

(*d*) Slit a piece of stem by a longitudinal cut, and isolate a strip of the sclerenchyma; note that the strip is very flexible, is easily split longitudinally, but is difficult to break by pulling at the ends.

(*e*) Examine with a lens the bundles; the hard middle portion (wood) of each bundle shows the large open vessels.

(*f*) Place a living piece of Marrow shoot with its cut end in red ink and when the red colour has appeared in the leaves cut the stem and note that the ink has passed through and stained these wood vessels.

(*g*) Cut across a piece of fresh living stem with a dry knife or razor, and note the juice which oozes out of the

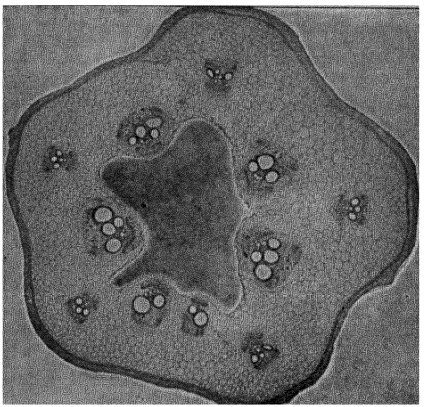


Fig 23 —Transverse Section of Stem of Vegetable Marrow.

soft outer and inner portions (phloem) of each bundle; collect some of this juice on a slide and test it for (i) sugar—with Fehling's solution; (ii) starch—with iodine; (iii) proteins—with Millon's reagent, etc.

100. T. S. Marrow Stem (Figs 23, 24, 25).—Cut transverse sections of the stem; mount some at once in glycerine, others after treatment with one of the following reagents: iodine, chlor-zinc-iodine, aniline sulphate, Millon's reagent. Also test a fairly thick section with Fehling's solution—or boil in Fehling a short piece of

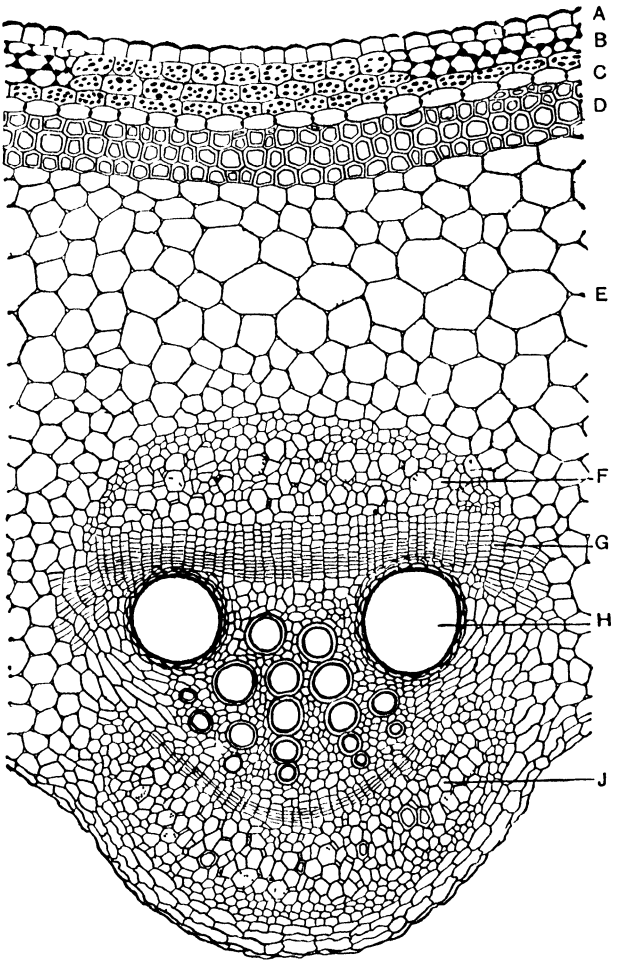


Fig 24 —Part of a Transverse Section of Marrow Stem, including one of the Vascular Bundles. A, epidermis, B, collenchyma (at sides) and parenchyma (in middle) of the cortex; C, endodermis, D, sclerenchyma; E, parenchyma (intra-stelar), F, outer phloem, G, cambium, H, xylem; J, inner phloem (note the cambium between this and the protoxylem)

stem and then cut sections from it—and note which tissues, if any, contain sugar.

(*a*) With the **low power,** starting from the outside of the stem, note (1) the **epidermis,** consisting of one layer of cells and here and there passing out into (2) large **hairs,** with thickened bases, the tissue of which is continuous with the epidermis and the underlying stem-tissue while the upper part of the hair is a row of cells—besides these there are thinner hairs with smaller bases; (3) below the epidermis a zone of **collenchyma**—thick-walled but not lignified tissue; (4) a narrow zone of thin-walled **parenchyma,** abutting internally on (5) a zone of **sclerenchyma** with thick lignified walls—the tissues lying between this zone and the epidermis form collectively the **cortex;** (6) the internal **parenchyma** in which are embedded (7) the **vascular bundles;** (8) the central pith **cavity.** (See § 102.)

(*b*) Examine one of the larger bundles in detail, and note:—

(1) The very conspicuous wide **vessels** of the xylem—stained yellow with aniline sulphate—embedded in thin-walled tissue (**xylem parenchyma**); the outer vessels are very wide, while the inner ones are narrower and are in fairly regular radial rows.

(2) On the outer side of the wood, the **cambium,** consisting of thin-walled cells elongated tangentially and narrow radially, showing very regular arrangement in radial rows; the tangential walls are especially thin, thus indicating recent and repeated divisions in this direction.

(3) The **outer phloem,** into which the cambium merges on its outer side, with very conspicuous **sieve-tubes** embedded in small-celled tissue.

(4) The **inner phloem,** resembling the outer in structure and forming in cross section a crescent-shaped patch on the inner side of the xylem—the Marrow is rather exceptional in having *bicollateral* bundles, with inner phloem as well as the normal outer phloem found in collateral bundles.

(*c*) Examine the various tissues with the **high power.** Note that in the **collenchyma,** below the epidermis, the walls of the cells are strongly thickened at the angles between adjacent cells, though thin at the middle, so that the cell-cavity appears rounded or oval in section; at places the collenchyma is interrupted by the underlying

thin-walled parenchyma, and at these places **stomata** occur in the epidermis; chloroplasts occur in the cortical parenchyma, less abundantly in the collenchyma; air-spaces occur between the parenchyma cells, but not in the collenchyma; the innermost layer **(endodermis)** of the cortex—lying immediately outside the sclerenchyma zone—consists of cells with the radial walls wavy, and the cells contain starch grains.

In the **phloem** note the protein contents of the sieve-tubes in sections treated with iodine or with Millon's reagent, and look for places where the section has passed just above or just below a transverse wall **(sieve-plate)** in a sieve-tube; the plate has a dotted appearance, the dots being pores in the plate—these are often seen better on treating a section with eau de Javelle, which removes the contents of the sieve-tube. Note also that each sieve-tube is associated with a narrow cell **(companion-cell)** which has been cut off from the sieve-tube by a longitudinal wall, and that the remaining tissue of the phloem consists of parenchyma cells differing from the sieve-tubes in their smaller size and the absence of sieve-plates. Also note carefully the appearance of the cells in the cambium.

101. L. S. of Marrow Stem (Fig. 26).—Cut radial longitudinal sections of the stem, passing through one of the larger bundles; mount some sections unstained in glycerine, and treat others with iodine, chlor-zinc-iodine, aniline sulphate, and Millon's reagent respectively—leaving the rest in alcohol for further treatment.

(*a*) Starting from the outside, note the various tissues (compare carefully with what has been seen in the transverse sections):—epidermis; collenchyma and parenchyma of cortex; endodermis; sclerenchyma of very long fibres with thick lignified walls and tapering pointed ends; the inner ground tissue parenchyma within the sclerenchyma; the bundles.

(*b*) In a single vascular bundle, with high power, note:—

(1) The **outer phloem**, in which the **sieve-tubes** are easily recognised by their conspicuous transverse walls **(sieve-plates)**.

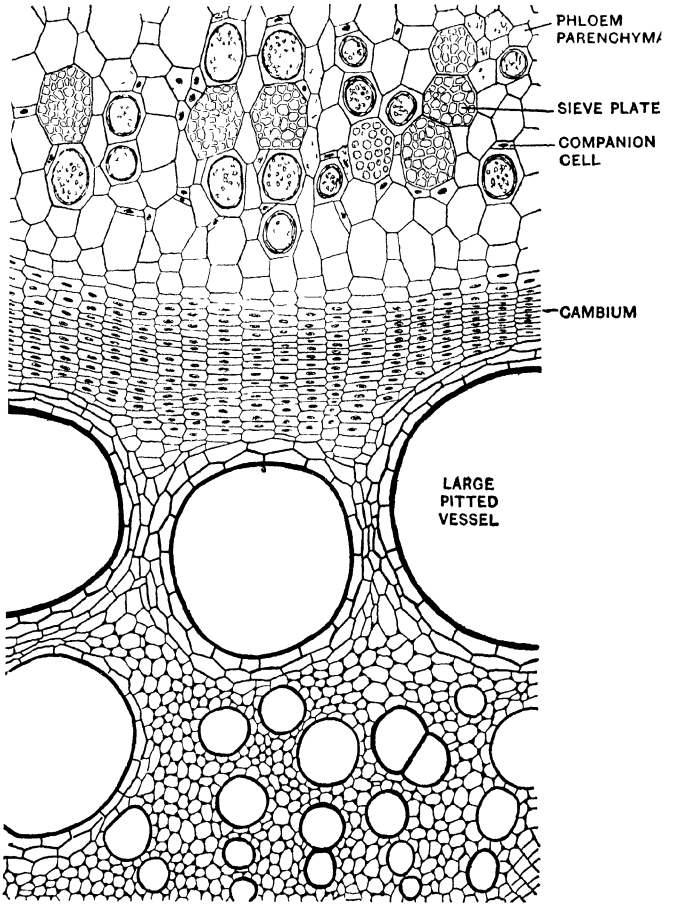


Fig 25.—Transverse Section of Marrow Stem, showing Part of a Vascular Bundle highly magnified. In the Phloem several Sieve-plates are shown in surface view; in some Sieve tubes the contents are shown contracted by the reagents used for fixation and preservation, in others they are omitted. In the Cambium only the nuclei of the cells are shown.

(2) The **cambium,** of long narrow cells arranged in regular rows and having abundant contents and very thin walls.

(3) The **xylem vessels,** embedded in parenchyma. The large outer **pitted vessels** appear to consist of a row of empty oblong cells bearing on their walls a network of thickening with thin meshes (pits); but closer examination and focussing shows that the apparent cross-walls are merely ring-like projections representing the remains of the originally complete transverse walls that have been almost entirely absorbed in the formation of the vessel from a row of cells end to end; of the inner (**protoxylem**) vessels some have spiral and others annular thickenings on the inner surface of the walls.

(4) The **inner phloem,** like the outer in structure.

(*c*) Now examine the **sieve-tubes** (as seen in longitudinal section of stem) in greater detail. In the older tubes, especially in material cut in late summer or autumn, each sieve-plate is covered with a mass of **callus,** which stains yellow-brown with iodine but is readily distinguished from the protein contents of the tube. Irrigate with potash, the callus swells and becomes transparent, so that the cellulose portion of the plate becomes conspicuous and shows the pores, the plate appearing in optical section (on focussing into it) like a string of beads—the constrictions corresponding to the pores. Other sections showing callus on the sieve-plates should be treated with callus reagent (see Appendix) which stains it brown, and with corallin (see Appendix) which stains it pink.

(*d*) That the protein contents (which often collect in a clump in contact with the plate, especially on the upper side) are continuous from segment to segment of the tube through the pores may be shown by either of the following methods—both should be tried. (1) Treat a section with iodine, wipe it with blotting-paper, mount it in a small drop of strong sulphuric acid, and very carefully cover and examine it. The acid causes the cellulose and the callus to swell up and the protoplasm to contract, so that the proteid contents appear as strands with here and there a thickening corresponding to the position of a sieve-tube—each such thickening will show the fine protein strings which pass through the pores of the plate. (2) Add some dry

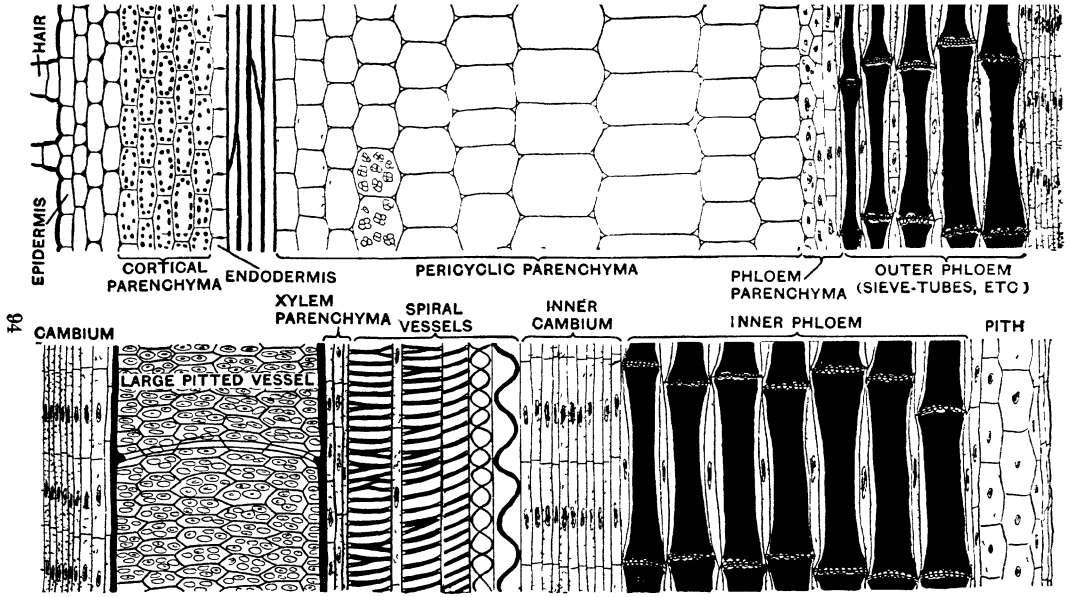


Fig 26.—Longitudinal Section of Marrow Stem. The upper drawing extends from Epidermis to Cambium, the lower from Cambium to Pith. In two of the intra-stelar (pericyclic) parenchyma cells are shown the compound starch-grains which most of these cells contain.

Hoffman's blue to a few drops of strong sulphuric acid in a watch-glass, stir with a glass rod; place sections in the liquid for a few minutes, rinse them in water, and mount in glycerine; the continuity of the sieve-tube contents is made clear by this treatment

(*e*) The companion-cells are made conspicuous by the deep staining of their contents by aniline blue. This also gives a good double stain in conjunction with safranin, place sections in safranin for 15 or 20 minutes, rinse in alcohol and transfer to aniline blue for about a minute, rinse again, dehydrate with absolute alcohol, clear with clove oil, mount in balsam. The lignified tissues (xylem and sclerenchyma) are stained red, the remaining tissues (with cellulose walls) blue.

102. Development of Vessels, etc.—In transverse and longitudinal sections through the youngest parts of the Marrow stem, notice that the ground tissue is complete right across the stem; the cavity found in the older parts is formed by the central region of ground tissue (pith) becoming torn as the stem grows thicker. Also notice that the wood contains only the narrow spiral and ringed vessels, and that the collenchyma and sclerenchyma are not yet distinguished sharply from the ordinary ground tissue.

103. Macerated Tissues (Fig 28).—Cut out about 1 cm. of Marrow stem, chop it by radial cuts into pieces including each a bundle, and heat the pieces for a few minutes in Schultze macerating fluid (§ 121). Wash in water, mount in glycerine, tease with needles, or press on the cover-glass, and note the isolated tissue constituents—cells, fibres, vessels of various kinds. Compare carefully with the appearance of the tissues as seen in transverse and longitudinal sections of the stem.

In herbaceous structures like Marrow stem, the tissue constituents can be isolated by using (instead of Schultze fluid) a mixture of one part hydrochloric acid and three parts alcohol; place sections in this mixture for a day, rinse in water, treat with potash, when the cells, etc., are readily dissociated by pressure under the cover-glass.

Herbaceous stems are readily macerated by chromic acid.

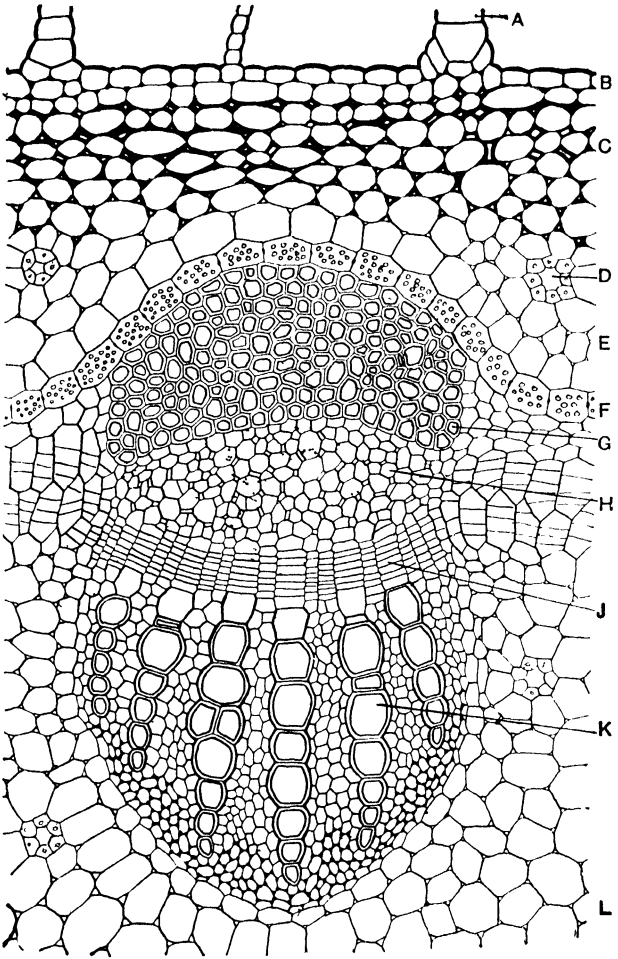


Fig. 27.—Part of a Transverse Section of Stem of Sunflower, showing one of the Vascular Bundles. A, hair, B, epidermis, C, collenchyma of cortex, D, resin duct in cortex, E, parenchyma of cortex, F, endodermis, G, sclerenchyma, H, phloem; J, cambium, K, xylem, L, intra-stelar parenchyma (note the resin-ducts).

104. Sunflower Stem (Fig. 27).—Cut transverse and radial longitudinal sections of a well-grown stem (about

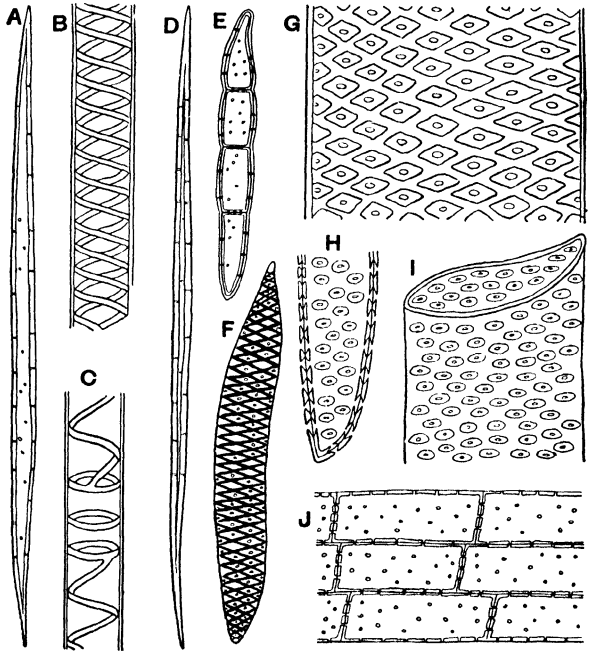


Fig 28.—Tissue Constituents isolated by Maceration. A, Fibre from Pericyclic Sclerenchyma of Marrow Stem, B, Part of a Spiral Vessel, from Marrow Stem, C, Part of a Vessel showing transition from annular to spiral thickening, from Marrow Stem; D, Fibre from Phloem of Lime Stem (a relatively short fibre drawn in order to fit size of page most of the fibres are much longer in proportion to their thickness), E, a row of Xylem Parenchyma Cells from Lime Stem, F, Spiral and Pitted Tracheid from Lime Stem, G, Part of a large Pitted Vessel from Lime Stem; H, Part of a Pitted Tracheid from Oak Stem, I, Part of a large Pitted Vessel from Oak Stem, J, Part of a Medullary Ray from Oak Stem

1 cm. diameter). Note that (1) the central ground tissue (**pith**) does not become torn to form a cavity, but remains solid; (2) the **vascular bundles** are arranged in a single uniform ring; (3) below the epidermis there is a zone of

collenchyma, then thin-walled **cortex parenchyma** in which there are **resin-ducts,** each surrounded by a small-celled **epithelium** (resin-secreting) layer; (4) the **sclerenchyma** forms separate strands, one immediately outside the phloem of the larger primary vascular bundles; (5) just outside the sclerenchyma, and traceable as a wavy band round the whole stem, is the **endodermis,** a layer of cells with wavy cutinised walls and containing starch grains; (6) **chloroplasts** occur in the cortical cells and also in the epidermis; (7) each bundle consists of **phloem** externally, **xylem** internally, and **cambium** in the middle—there is no inner phloem.

105. Interfascicular Cambium in Sunflower.—Cut, examine, and compare transverse sections taken through different parts of the stem of a Sunflower plant, or from plants of different ages Note that in all except quite young stems, or the youngest parts of a fully-grown stem, the individual bundles are more or less joined up by interfascicular cambium, which has been formed by the growth and division of the parenchyma between the primary bundles—*i.e.* the parenchyma of the primary medullary rays—and is continuous with the primary or fascicular cambium lying between the xylem and phloem of the bundles themselves. In older stems, therefore, there is a continuous cylinder of cambium round the stem, and this gives rise to secondary xylem internally and secondary phloem externally.

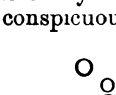
106. Study of Stem of Aristolochia.—One of the very best Dicotyledonous stems for detailed study is that of Aristolochia (Dutchman's Pipe). A well-grown plant should be obtained from a nursery and cultivated either in a greenhouse or in a sunny position in the garden, provided with trellis or other supports; or material may be obtained from dealers in botanical supplies.

The following preparations should be made.—(1) A series of transverse sections to illustrate the progressive development of the tissues from the primordial meristem at the apex downwards. Near the apex cut the sections

at short intervals in order to trace the appearance of the first xylem and phloem elements to be differentiated from the tissue of the procambial strands. Farther back take sections from successive internodes. (2) A series of longitudinal sections through the same regions

Treat the sections with aniline sulphate, etc. Note where the first elements of the vascular bundle appear in the procambium; how the earliest vessels become stretched longitudinally; how far back from the apex the original cellulose walls become lignified in the xylem and the sclerenchyma; how the cambium becomes a continuous zone by the development of interfascicular cambium; how the interfascicular cambium produces secondary medullary rays, which do not extend inwards to the pith; how the pith becomes crushed as secondary growth proceeds; how the originally continuous sclerenchyma band becomes broken up into strips; how the cork cambium arises in the cortex and produces cork; and so on.

107. T. S. Stem of Maize (Figs 29, 30).—Cut thin transverse sections of one of the lower internodes. Note that the bundles, though "scattered," are most crowded towards the periphery, and that in each bundle the phloem is external (nearest the periphery of the stem) and the xylem internal Note (1) the **epidermis** of small thick-walled cells; (2) the narrow zone of **sclerenchyma** below the epidermis; (3) the ground tissue **parenchyma** of thin-walled cells, with small intercellular spaces at the corners; (4) the **bundles,** each with a more or less complete **sheath** of sclerenchyma. In a single bundle note the (usually four) conspicuous **xylem vessels** arranged like a Y, thus:



with narrower vessels lying between; the patch of thin-walled **phloem** lying partly between the two larger xylem vessels.

With high power note that the epidermis is covered by a distinct **cuticle**; the hypodermal sclerenchyma is inter-

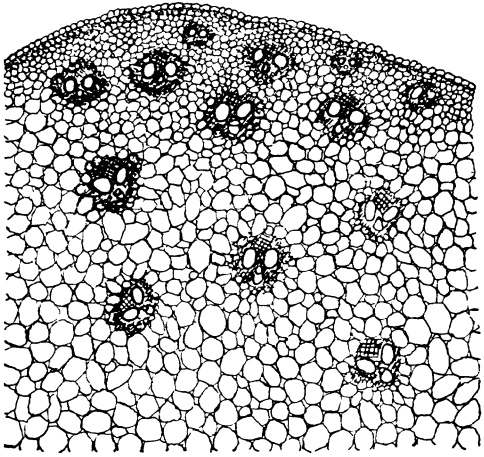


Fig 29 —Part of a Transverse Section of Stem of Maize.

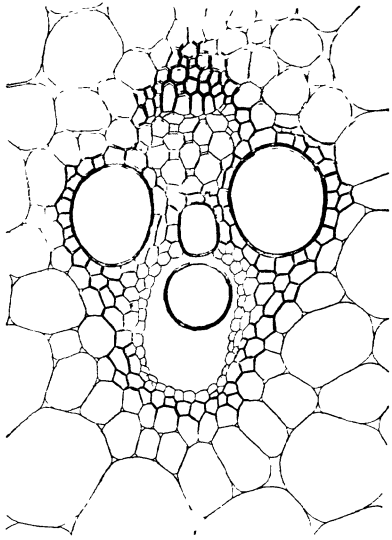


Fig 30 —Part of Transverse Section of Maize Stem showing a Vascular Bundle and parts of the surrounding Ground Tissue The Sclerenchyma and Xylem are indicated by the thickness of the walls The cell-contents are shown in the case of the Companion-cells in the Phloem and of the Xylem Parenchyma lining the Lysigenous Cavity (Protoxylem).

rupted here and there by the underlying thin-walled parenchyma, the epidermis showing at some of these places a **stoma**; the outermost portion of the phloem (**protophloem**) is frequently crushed and disorganised; the sieve-tubes and companion-cells are arranged with great regularity; the innermost portion of the xylem (**protoxylem**) is usually represented by a water-containing **cavity** (formed by expansion and tearing apart of the protoxylem during growth of the stem), to the inside of which isolated ring-fibres may be seen adhering.

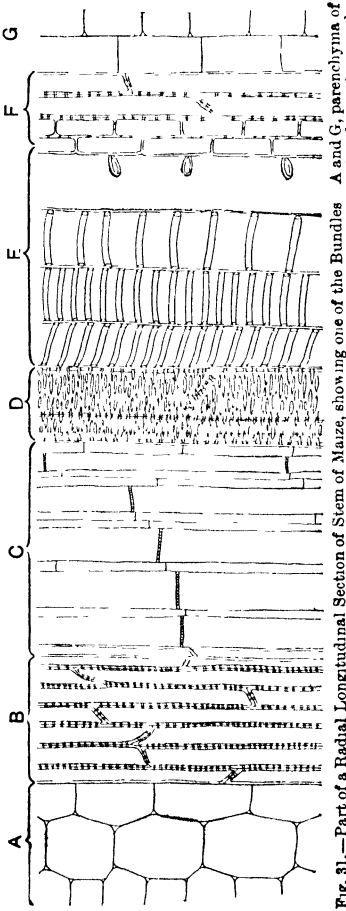


Fig. 31.—Part of a Radial Longitudinal Section of Stem of Maize, showing one of the Bundles. A and G, parenchyma of the ground tissue, B and F, sclerenchyma, C, phloem, D, pitted vessels of the xylem, E, spiral and annular vessels

108. L. S. Maize Stem (Figs. 31, 32).—In longitudinal sections note the (1) **epidermis** (oblong cells), with **cuticle**; (2) **sclerenchyma** (long tapering lignified fibres); (3) **parenchyma** (polygonal thin-walled cells); and (4) the **vascular bundles**, each surrounded by its fibrous sheath. Examine several bundles, if necessary, to make out (5) the **large pitted vessels**, (6) the small

spiral and **annular vessels,** (7) the small **pitted tracheids** (differing from vessels in not having their end-walls absorbed), and (8) the **xylem parenchyma** in

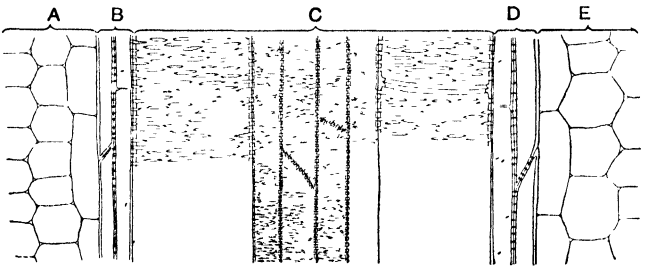


Fig. 32.—Part of a Tangential Longitudinal Section of Stem of Maize, showing one of the Bundles. A and E, parenchyma (ground tissue), B and D, sclerenchyma; C, xylem—note the large pitted vessel on either side, and the small pitted vessels in the middle.

the xylem; (9) the narrow **sieve-tubes** and (10) narrower **companion-cells** in the phloem.

109. Further Work on Herbaceous Stems.—In examining sections of various other herbaceous stems, or the youngest parts of woody stems, note any special points of structure presented.

In some herbaceous Dicotyledons, there is little or no interfascicular cambium; in Buttercup, etc., each bundle is surrounded by a more or less complete sheath of sclerenchyma, and even the fascicular cambium is scanty and soon ceases to be active.

The **distribution of the stereome** (strengthening or supporting tissue) of herbaceous stems is of great interest. This term is often restricted to the **sclerenchyma,** consisting of fibrous cells which have thick lignified walls and have lost their living contents, so that they serve a purely mechanical function. **Collenchyma,** however, is an important form of supporting tissue, found below the epidermis in herbaceous stems, young woody stems, petioles, and flower-stalks; its cells are living, usually with chloroplasts as well as protoplasm, and the walls are thickened but not lignified, hence this tissue can also perform vital functions, and it has the power of growing, especially when subjected to tension. Note that collenchyma is often developed chiefly in the projections of angular, ribbed, or winged stems.

The **hypodermal sclerenchyma** usually forms a number of isolated strands—*e.g.* various Umbellifers, Leguminosae, Sedges, Rushes. **Pericyclic sclerenchyma** may either form (1) a con-

tinuous zone separated from the vascular bundles by several layers of parenchyma—*e.g.* various Cucurbitaceae, Caryophyllaceae, Aristolochia, Honeysuckle, and many Grasses, Sedges, and Rushes; or (2) a continuous zone in direct contact with the vascular bundles —*e g.* Plantain, many Liliaceae and Iridaceae, Orchids, etc ; or (3) separate strands, one opposite and in contact with the phloem of each vascular bundle—*e g.* various Compositae, Labiatae, Leguminosae, Ranunculaceae, etc ; or (4) strands scattered without any obvious relation to the bundles—*e.g.* various Solanaceae, Privet, Mallow, etc. Sclerenchyma strands may also be developed in the cortex, between the hypodermis and the pericycle; or the interfascicular ground tissue may become sclerenchymatous where it abuts on the bundles (*e.g.* Maize and various other Monocotyledons), and these strands may either remain isolated or, more often, are joined up to the pericyclic sclerenchyma. The stereome of the stem may be built up of sclerenchyma bands and strands in any or all of these four positions—hypodermal, cortical, pericyclic, interfascicular—and these may be joined up in various ways

110. Structure of Aquatic Stems.—In sections of the stems of aquatic plants, note especially the scanty development of xylem and sclerenchyma, the tendency of the vascular tissue to be massed at the centre of the stem, and the large development of air-chambers. Compare the structure of the submerged stem with that of the aerial flowering stem in plants which send up their flowers above water—*e.g.* Water Crowfoot. Typical aquatic stem structure is also seen in Dicotyledons like Marestail (Hippuris), Water Milfoil, Water Violet (Hottonia), etc.; and in Monocotyledons like the Pond-weeds (various species of Potamogeton), Elodea, etc.

The petiole of Water Lily (Nymphaea or Nuphar) may be used for the study of aquatic stem structure as regards the characteristic development of large air-spaces. Note the feeble development of the xylem of the bundles, which is practically represented only by a cavity in each bundle; also the curious branched internal hairs, covered with small crystals of calcium oxalate.

111. Apical Meristem of Stem.—At the growing apex of the stem there is a single tissue, since the cells are essentially all alike. This **apical** or **primordial tissue** can be seen in any vegetative bud. On comparing transverse and longitudinal sections taken at successively lower planes, beginning just below the apex, we find that the primordial meristem soon becomes differentiated into three parts, the **primary meristems,** known as **dermatogen, procambial strands,** and **ground meristem.**

These three tissues undergo further differentiation. The

dermatogen gives rise to the **epidermis.** The procambial strands give rise to the **vascular bundles,** the inner tissue in each strand being the **xylem,** the outer tissue the **phloem,** while (in Dicotyledons) there remains between xylem and phloem a layer of meristem—the **fascicular cambium.** The ground meristem in Dicotyledons becomes differentiated into **primary cortex, pericycle, primary medullary rays** and **pith.** The inner limit of the cortex is the **endodermis,** or starch-sheath layer; the pericycle extends from this to the outer border of the vascular bundles; the primary rays lie between the bundles, the pith is the tissue surrounded by the ring of bundles. The whole of the tissue within the endodermis is called the **stele.**

112. Apical Meristem of Stem in Elodea, Hippuris, etc.—The growing point of various aquatic plants is especially easy to study. If possible examine Elodea, Myriophyllum, and Hippuris.

(*a*) Pick off the terminal leaves, then cut off the stem tip, place it in water, and carefully dissect away the small inner leaves, which can be readily seen and handled. With the low power note the rather long apical cone, the tip of which is quite smooth, while lower down the young leaves are seen as outgrowths on the sides of the stem, becoming successively larger as we pass farther backwards from the apex. Treat the preparation with eau de Javelle to make it more transparent.

(*b*) Cut median longitudinal sections of the apical bud of Hippuris. Treat some with potash or eau de Javelle, and mount in glycerine; stain others with haematoxylin, and mount in balsam. The procambial tissue is unusually distinct in Hippuris. Farther down, note the development in the cortex of air-passages, interrupted by solid partitions at the nodes and the origin of the young leaves. The older leaves have buds (some may be flower-buds) in their axils; the leaves bear peltate hairs.

(*c*) In sections stained with haematoxylin, or with iodine, note that the meristematic tissue at the apex consists of small cells with thin walls and dense protoplasm—these cells have obviously been undergoing rapid growth and division. Farther back, as the cells grow in length, the growth in volume of the protoplasm fails to keep pace with the extension of the cell-wall, and thus the protoplasm becomes vacuolated, the cell-sap collecting in drops (vacuoles) which later run together to form a large central vacuole, while the protoplasm becomes restricted to a peripheral layer ("primordial utricle") immediately within the cell-wall.

113. Apical Meristem of Bud of Lilac.—Remove the outer scales and leaves from a resting (winter) bud, then cut longitudinal sections, cutting in a plane joining two opposite rows of leaves. Clear the sections with potash or eau de Javelle.

Note the broad rounded apex, with the young leaves in various stages of development, the dermatogen, the procambial strands, and the ground meristem. In some of the sections may be seen the first spiral vessels of a vascular bundle

Cut a series of transverse sections and compare them with the longitudinal sections.

114. General Structure of Woody Stem.—From twigs of Sycamore, Horse Chestnut, Elder, Lime, Willow, Apple, and other woody plants, peel off (1) the **cork,** and note (2) the green **cortex,** (3) the **phloem,** a zone of colourless tissue separated by (4) the thin sticky **cambium** layer from (5) the hard **wood,** (6) the central **pith.** Note that the surface-markings of the twig include (1) **leaf-scars,** where the leaves of former seasons fell off; (2) **girdle-scars**—zones of closely-set scars, where the scales fell from the opening buds of previous years; (3) **lenticels**—usually raised patches differing from the rest of the cork in colour and texture.

Note that the lenticels are not merely surface markings or projections, but that each lenticel goes right through the cork—this is easily seen on stripping off, layer after layer, the white papery bark of a Birch, in which the dark transversely-elongated lenticels are very conspicuous A lenticel is a local modification of the cork—a place where, instead of compact impervious cork, there has been produced loose powdery tissue through which gases can pass into and out of the living tissues within the cork (§§ 115, 123).

115. Experiments with Lenticels.—(1) Dip a twig of Elder, or other plant with conspicuous lenticels, into boiling water; air-bubbles escape from the lenticels. (2) Fix one end of a cut twig (about 10 cm. long) of Elder, etc., on to the nozzle of a bicycle pump by means of stout rubber tubing; seal the free end of the twig by tying on a piece of rubber tubing, folding it, and again tying it to the twig. Put the whole into a jar of cold water, so

that the twig is below the surface, and force air through with the pump; air-bubbles escape from the lenticels.

116. Structure of Lime Stem.—The Lime-tree (Tilia) is taken here as a type in which the minute structure of the woody stem may be studied, chiefly because its wood is easily cut and its phloem is arranged in conspicuous wedge-like strands; other types—*e.g.* Oak, Elm—may with advantage be used, however. The winter-bud of Lime has few scales, but the girdle-scars can be found on careful inspection of the twig. Starting from the tip of a twig, cut out parts of each year's growth, and make thin **transverse sections** of each. It is enough to go back as far as the four-year-old portion in this way; then cut out about 1 cm. from a still older region, slice from this a wedge-shaped piece including about one-eighth of the circumference and extending right into the pith, and cut sections from this wedge.

117. T. S. Young Lime Stem.—In the **young current-year twig,** cut in early summer shortly after opening of the bud, note (1) the **epidermis,** with **cuticle;** (2) cortex **collenchyma;** (3) cortex **parenchyma;** (4) an interrupted zone of **sclerenchyma;** (5) **phloem,** more or less broken up into masses by the expanded outer ends of (6) the **medullary rays,** which extend through (7) the **cambium** and (8) the **xylem** into (9) the **pith.** In the cortex and pith note the conspicuous large mucilage-containing cells and the small crystal-containing cells.

In **older portions of current-year twig,** cut in late summer or autumn, note that (1) the xylem and phloem zones have increased in width, especially the xylem; (2) in passing outwards, the xylem elements after a time diminish in width so that the part just inside the cambium is compact and close in texture; (3) bands of sclerenchyma have been developed in the secondary phloem, alternating with bands of soft tissue—the sieve-tubes and parenchyma; (4) the phloem is more distinctly broken up into roughly triangular masses with the apex outwards, alternating with the fan-like outer portions of

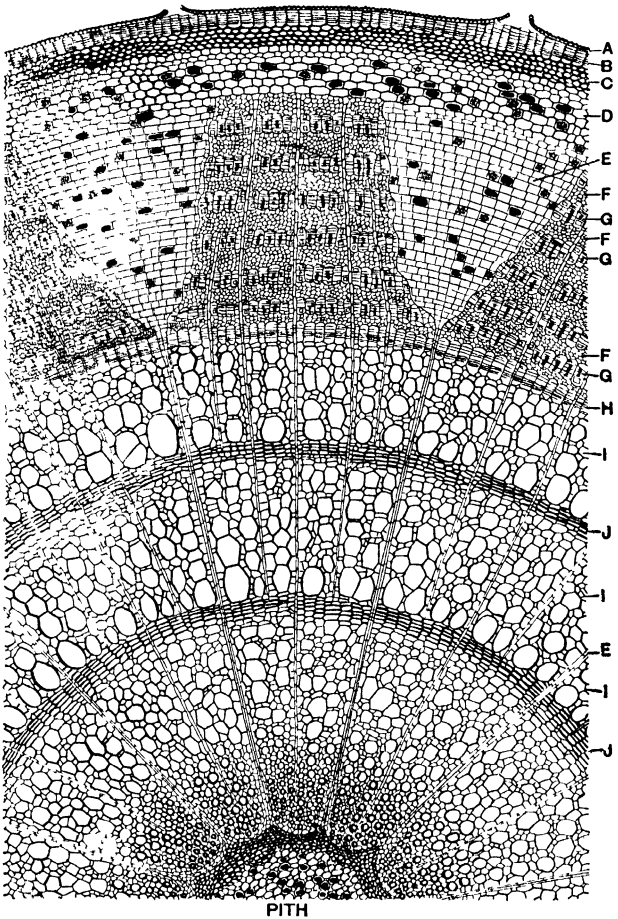


Fig 33.—Part of a Transverse Section of Three year-old Lime Stem. A, Epidermis, B, Periderm; C, Collenchyma; D, Parenchyma (C and D together make up the Cortex); E, Expanded portion of a Medullary Ray several Rays are shown traversing the Xylem and Phloem, F, G, Alternating Bands of Sclerenchyma (F) and "Soft Bast" (Sieve-tubes, Companion-cells, Parenchyma), together making up the Phloem; H, Cambium; I, Summer Wood, J, Autumn Wood.

the primary medullary rays; (5) the hypodermal cortex-layer has produced a zone of **periderm,** lying within the epidermis and consisting of flattened cells arranged in regular radial rows.

Some of these points may be seen better in sections from the older regions, to which we shall now pass.

118. T. S. Three or Four Year Old Lime Stem.—Note (1) the disorganised and torn **epidermis;** (2) the **periderm;** (3) the **cortex;** (4) the triangular **phloem** masses, consisting of the alternating tangential bands of thick-walled (fibrous) and thin-walled tissue; (5) the **cambium,** a narrow zone of flat thin-walled cells—in radial rows as usual; (6) the arrangement of the **xylem** in three or four layers (**annual rings**) which may vary a good deal in thickness; (7) the **pith,** and (8) the **medullary rays.** (Figs. 33, 34, 35)

Starting from the centre, note the following details:—The pith shows large empty (air-containing) cells tending to be arranged in rosettes around small cells, which may contain tannin or crystals; in the outer part there are large mucilage sacs; and the outermost pith tissue, into which project the primary masses of wood containing the protoxylem vessels, consists of small cells with tannin or starch.

In each annual wood-ring large vessels are produced at first, but later in the year the cambium produces only narrow xylem elements—the abrupt change from the close-textured **autumn wood** to the open **spring wood** of next year produces the ringed appearance of the secondary wood. The wider xylem elements are **pitted vessels;** the narrower ones are either **tracheids** (resembling vessels in having, where in contact with other tracheids or vessels, bordered pits on their walls) or **fibres** (with a few fine pits), or **parenchyma cells** with protoplasm and sometimes starch.

The **primary medullary** rays are two or more cells broad tangentially, and in their widened fan-like outer portions (between the phloem wedges) there are obvious signs of tangential elongation and radial division of the cells, to keep pace with the expansion of the stem as secondary thickening proceeds. The **secondary rays** are usually only one cell wide; some of them run through from pith to cortex (interrupting the sclerenchyma bands in the phloem), while others can only be traced from the cambium through part of the xylem and part of the phloem—all the secondary rays of course pass through the cambium in both directions, since they are formed by the cambium.

During the first year several alternating bands of hard and soft tissue may be formed in the phloem, but later on the cambium

usually produces each year two bands of **phloem fibres**, so that the number of fibrous bands in the phloem is roughly double that of the annual rings in the wood. In each band of soft phloem the wide **sieve-tubes** occupy the middle of the bands, while abutting on the fibre-bands are the narrower **parenchyma-cells**, some of which contain starch and others crystals

119. L. S. Old Lime Stem (Fig. 36).—In a radial longitudinal section of four-year-old (or older) stem, note the various tissues already seen in the transverse section.

In the **xylem**, note (1) the narrow **protoxylem vessels**, nearest the pith, with spiral thickenings; (2) the wide **pitted vessels**, with small bordered pits and also spiral or reticulate thickening; (3) the **tracheids**, differing from the vessels only in being narrower and in having tapering intact (not absorbed) end-walls; (4) the **fibres**, resembling tracheids in shape, but having only small pits scattered sparsely on their walls—these fibres are the most abundant of the secondary xylem constituents in the Lime; (5) the **xylem parenchyma** cells, arranged in vertical rows and usually containing starch; (6) the **rays**, seen as bands running across (in reality *between*) the xylem elements and consisting of cells with pitted walls and protein or starchy contents—the ray cells in contact with vessels have most pits and scantiest contents.

The **cambium, pith, cortex,** and **periderm** have much the same appearance as in transverse section.

In the **phloem**, note the very long narrow thick-walled lignified **fibres**; the **sieve-tubes**, the oblique end-walls (**compound sieve-plates**) of which mostly face the radial plane and are therefore seen in surface view here, each plate showing three or more **sieve-areas**—this "compound" type of sieve-plate is common in the secondary phloem of woody plants.

120. In a **Tangential Longitudinal Section** of the wood of the Lime, note especially the medullary rays, easily distinguished by their spindle-like form and their narrow and fairly thick-walled cells; some of the rays are but one cell wide throughout, others two or even more cells wide at the middle, while their height varies greatly (Fig. 36).

121. Maceration of Woody Stems (Schultze Method) (Fig. 28).—Place some longitudinal sections of stem in a test-tube, add a few crystals of potassium chlorate, and then enough nitric acid to cover them. Heat gently, at a distance from the microscope, so that the fumes may not injure it. After the fumes have ceased add water

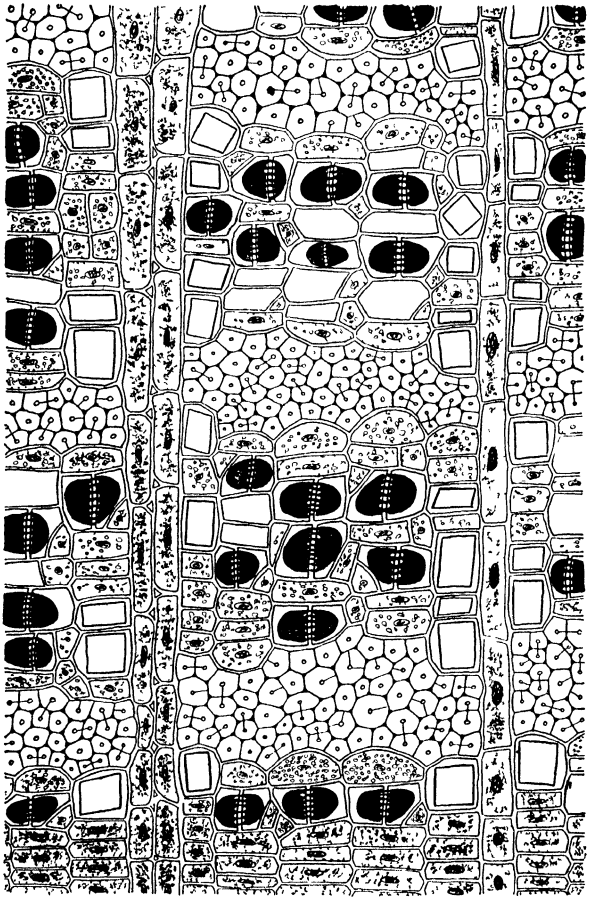


Fig 34.—Part of the Secondary Phloem of Lime Stem shown in detail The Cambium (at foot of drawing) and Phloem are traversed by two Medullary Rays (two cells broad on left, one cell broad on right). Many of the Phloem Parenchyma cells immediately bordering on the Rays are filled with a large Calcium Oxalate Crystal. In the Sclerenchyma there are indicated the "middle lamella" of the Fibres, the very thick cell-walls traversed by Pits, and the reduced cavity of the Fibres. The contents of the Sieve-tubes abutting on the radially-facing Sieve-plates are shown black.

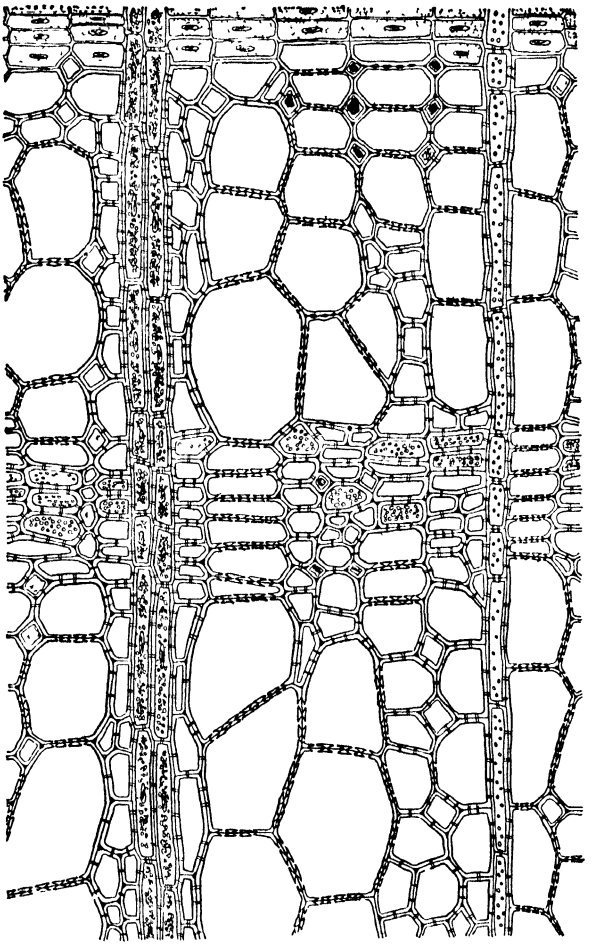


Fig 35 — Part of the Secondary Xylem of Lime Stem shown in detail (this drawing fits on the foot of Fig 34) The Xylem is largely made up of Fibres (most of the narrower elements), the elements with contents shown are Xylem Parenchyma Cells.

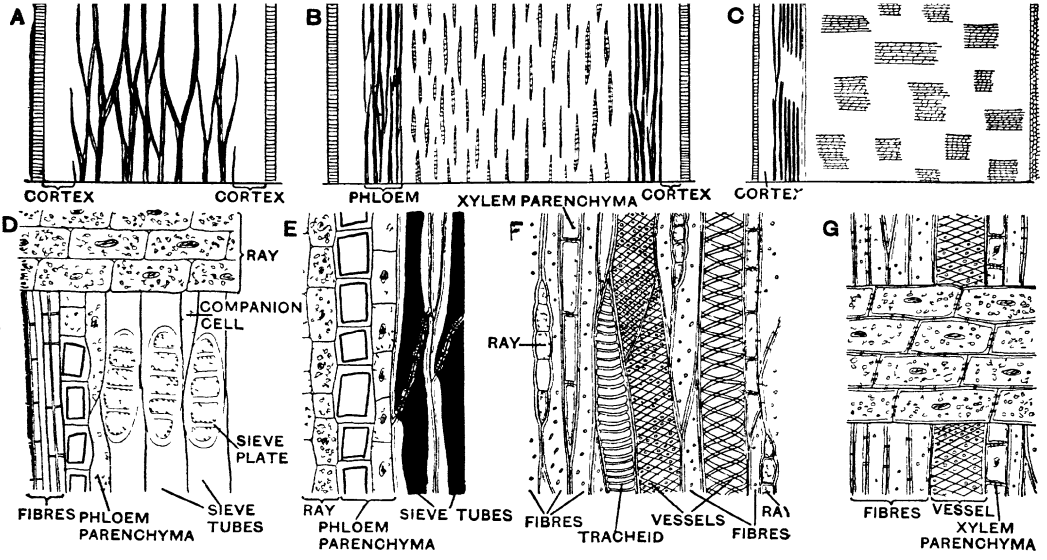


Fig 36.—A Series of Drawings illustrating the Structure of the Lime Stem as seen in Longitudinal Sections. A, Tangential Section through the Phloem only the anastomosing Sclerenchyma bands are shown, B Tangential Section through the Xylem only the Phloem Sclerenchyma and the Medullary Rays are shown, C, Radial Section through Phloem (only the Sclerenchyma shown) and Xylem (only the Rays shown) A, B and C are only slightly magnified, in each case the Periderm (Per) is shown diagrammatically at the edge of the drawing D, Part of the Phloem in Radial Section: in this section the Sieve-plates are seen in surface view, E Part of the Phloem in Tangential Sec-

and pour the contents of the tube on a filter, then wash (with the wash-bottle of water) the macerated tissue on the filter paper; or pour the contents of the tube into a large dish of water. (See § 103)

Transfer the macerated tissue to a drop of glycerine on a slide, and tease it out and cover the isolated tissue constituents. In the xylem the most abundant elements are the fibres in the case of Lime-tree; note also the vessels, tracheids, and parenchyma-cells (often still in vertical rows). The most conspicuous phloem elements are the fibres—much longer than those of the xylem and with thicker walls.

Other woody stems (*e g* Oak, Elm) should be studied by the maceration method, together with thin transverse and longitudinal (radial and tangential) sections.

122. The **Development of Phellogen** (**Cork-cambium**) is easily followed in the Elder (Figs. 37, 38). Cut

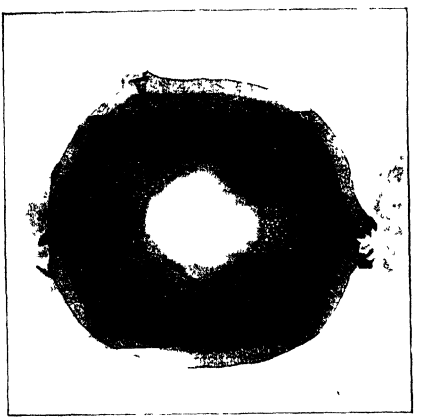


Fig. 37.—Transverse Section of Stem of Elder, showing three Lenticels.

transverse sections of an Elder twig where the surface is beginning to change from green to grey or brown, and note that tangential divisions have appeared in the outer-

most layer of the cortex, just below the epidermis. Each of these hypodermal collenchyma-cells first elongates in the radial direction, and divides by a tangential wall into an outer and an inner cell; the latter divides again in the same way, then successive tangential divisions, accompanied by radial growth, occur in the middle (**phellogen**) cell, giving rise externally to a row of **cork-cells.** Thus we get a radial row of cells—the outermost represents the outer half and the innermost the inner half of the original collenchyma-cell, the lowest cell but one being the phellogen-cell. At a later stage the phellogen cuts off cells on its inner side—these retain their protoplasm, contain chloroplasts, and add to the cortex, forming the "secondary cortex" or **phelloderm.**

123 The **Development of Lenticels** (Fig. 38) can also be studied in Elder. On the young stem the lenticels

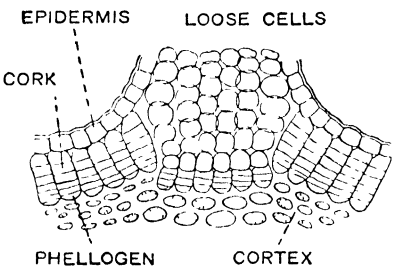


Fig 38 —Section through a Lenticel. Phellogen = Cork-cambium.

appear as projections, each forming a groove with raised lips, and on examining the younger parts of the twig the incipient lenticels appear as light-brown spots on the otherwise green surface. Transverse sections taken at these spots may show that just below a stoma divisions occur in the hypodermis, giving rise to a meniscus-like layer of phellogen, which produces on its inner side a little phelloderm and on its outer side rows of loose brown "packing tissue." Then the epidermis becomes torn to form the fissure-like lenticel. The development of the

ordinary periderm, all over the stem, begins after that of the lenticels. Comparison of sections taken at different times of the year shows that in most trees the lenticel-tissue produced in autumn is relatively compact, so that the lenticel is practically closed up in winter.

124. Further Work on Cork-formation.—Examine various other trees and shrubs, and note that the phellogen arises in the hypodermis in the majority of cases, but sometimes in the epidermis itself (Willow, Apple, Pear, Jasmine, *Aucuba*, *Euonymus*, *Solanum*); or in about the third layer of cortex, reckoning inwards from the epidermis (*Laburnum*, *Robinia*); or in the pericycle (*Ribes*, *Vitis*, *Rosa*, Ericaceae, etc). As a rule, the more deep-seated the phellogen the greater the amount of phelloderm produced, hence phelloderm is well seen in sections of twigs of *Ribes* (Gooseberry, Black or Red Currant).

125. Examination of Entire Roots.—A good deal of the structure of the root can be made out without cutting sections. Good material is afforded by the slender roots of such seedlings as Cress, Mustard, Radish, Wheat, Oats, etc. Some of these should be grown in moist air—sow the seeds in moist porous seed-pans, or in loose sphagnum in a flower-pot or lamp chimney in the case of Beans and Peas; others on muslin tied over tumblers of water or culture solution; others in garden soil. For Germination Boxes and Jars see §§ 169, 170.

126. General Structure of Root.—Mount in water the entire roots of Cress or Mustard seedlings that have grown through muslin into water.

Note (1) the **tip** of the root, covered by the conical and usually distinctly stratified **root-cap,** the superficial cells of which may be seen lying loose, isolated or joined in rows, and evidently in the act of becoming shed or peeled off the cap; (2) the **root-hairs,** beginning at some distance behind the root-tip as unseptate and unbranched outgrowths of the epidermis cells, becoming longer on being traced backwards from the tip, and disappearing still farther back; (3) the dense inner tissue of the **vascular cylinder,** running through the root; (4) the more transparent outer tissue or **cortex;** (5) the **rootlets,** clearly

arising as outgrowths of the central cylinder and passing through the cortex to the surface, from which they protrude—examine several roots, to see various stages in the development of the rootlets.

127. Root-hairs.—These should be examined in roots of seedlings grown in damp air, in water, and in soil. In Bean and Pea the hairs are readily seen in seedlings grown in loose moist sphagnum (see § 170) instead of soil.

(*a*) Mount in water the slender roots of such seedlings as Cress, Mustard, or Wheat, grown through muslin into water, and examine the root-hairs with the high power Run in salt solution and note the plasmolysis of the cell-contents Treat with iodine solution, which will stain the protoplasmic lining of the hair.

(*b*) Germinate Wheat grains in two pots of fine garden soil, 4 or 5 grains to each pot. When each seedling has produced 4 or 5 roots, turn out the soil of one pot and carefully remove the plants, noting the mass of soil adhering to the roots; shake the plant and note that most of this soil falls away, but some of it remains clinging closely to the roots, though the root-tip is free from soil. Rinse the roots in water, examine with the microscope, and note that the tip of each root is free from root-hairs, which are abundantly present over the rest of the root, and that the finer soil-particles cling so closely to the hairs as not to be removed by the rinsing in water. Let the other plants grow for 5 or 6 weeks; then remove a plant and note that no soil-particles cling to the older parts of the roots, from which the root-hairs have now disappeared.

(*c*) Soak seeds of different plants—*e.g* Mustard, Wheat, Pea—in water until the radicle is 1 cm. long. In each case get three tumblers or jars with muslin tied over the mouth; fill *A* with distilled water, *B* with culture solution, and into *C* pour a little water to keep the air moist Transfer a seedling of each kind to each tumbler, and also sow some (*D*) in a pot of good soil Cover the tumblers with a bell-jar, add water daily to make up for that lost by evaporation and transpiration. After a week or two

examine the roots, and note the presence, absence, and relative abundance of root-hairs in the four cases. In the roots grown in soil, note that the root-hairs are irregular in form and are often branched at the tips, to which soil-particles may cling even after the roots have been well rinsed in water.

128. Xylem Vessels in the Root.—Cut the slender root of a seedling—*e g.* Mustard or Cress—into pieces about 1 cm. long. Mount in water all the pieces, or at any rate some taken from different regions starting at the youngest (that nearest the root-cap), and crush them under the cover-glass, and examine. Treat with aniline sulphate and note especially the xylem vessels: some are narrow spiral (protoxylem) vessels, others wider and pitted.

Prove, by crushing the root in this way, or by clearing it with potash, that only the spiral vessels are present in the younger parts of the root, also that these first-formed spiral vessels lie near the outside of the vascular cylinder, while the later-formed pitted vessels are developed internally to them—towards the centre of the cylinder.

129. General Anatomy of Bean Root.—Examine well-grown roots (about 15 cm. long) of seedling Beans; or dig up Broad or Runner Bean plants growing in garden soil, and rinse the roots in water.

(*a*) Note that each rootlet emerges from a slit in the surface of the main root. Cut across the main root so as to cut one or more of the rootlets longitudinally, clear with potash if necessary, and note that each rootlet arises from the central cylinder.

(*b*) Scrape the soft outer tissue (cortex) from an old part of the root, and note the hardness of the cylinder; mount a piece of the latter in aniline sulphate, tease it out or crush it on the slide, and look for the spiral and pitted vessels By scraping the cortex from the place where a rootlet is given off, and treating with aniline sulphate, prove that there is continuity between the vascular cylinder of the rootlet and that of the main root.

(*c*) Cut a **series of transverse sections** from various points on the main root, at intervals of about 1 cm. starting from the tip; arrange the sections in order on the slide, and treat them with aniline sulphate. Note the different appearances presented by the vascular cylinder in the different regions.

From this series of sections you will learn

(1) That for some distance behind the tip the central cylinder shows as many xylem strands as there are longitudinal rows of rootlets (usually four in *Phaseolus*, five or six in Broad Bean)

(2) That in passing backwards from the apex these strands of primary xylem increase in size by the formation of additional vessels on the inner side of those first formed, the later-formed (inner) vessels of each strand being wider than the first-formed vessels.

(3) That the young rootlet begins as a projecting mass of tissue immediately outside one of these primary xylems.

(4) That the young rootlet pushes its way through the cortex as it grows, eventually bursting through the surface.

(5) That the xylem of the rootlet is joined on to that of the xylem strand opposite which it arose.

(6) That in the older part of the root additional xylem vessels appear in tangential bands alternating with the primary xylem strands, and that the cells on the outer side of each of the bands of secondary xylem show the appearance of a cambium (cells arranged in radial rows, with closely-set tangential walls).

(7) That, still farther from the root-tip, the secondary vascular tissue increases in amount, though the primary xylem strands can still be seen towards the centre of the root.

130. T. S. of Young Bean Root (Fig. 39).—Cut thin transverse sections of a seedling Broad Bean root, at about 5 or 6 cm. from the tip; treat different sections with iodine, chlor-zinc-iodine, and aniline sulphate. Note

(1) The **epidermis,** or **piliferous layer,** some cells of which give out a root hair.

(2) The parenchymatous **cortex,** of thin-walled and rounded cells, which may contain starch grains, and which are separated at the corners by intercellular spaces.

(3) The **endodermis,** a single layer of cells showing the characteristic radial walls.

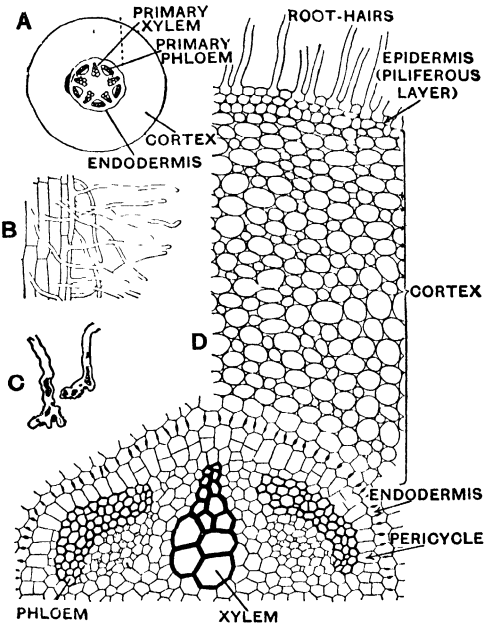


Fig 30.—Young Root of Broad Bean. A, Transverse Section, showing the general arrangement of the Tissues; the portion indicated by the dotted line is shown on a larger scale in D. B, Part of the Epidermis (Piliferous Layer with Root-hairs; C, Ends of two Root-hairs, showing adhering Soil-particles; D, Part of Transverse Section, more highly magnified, showing one Primary Xylem Bundle and two Primary Phloem Bundles.

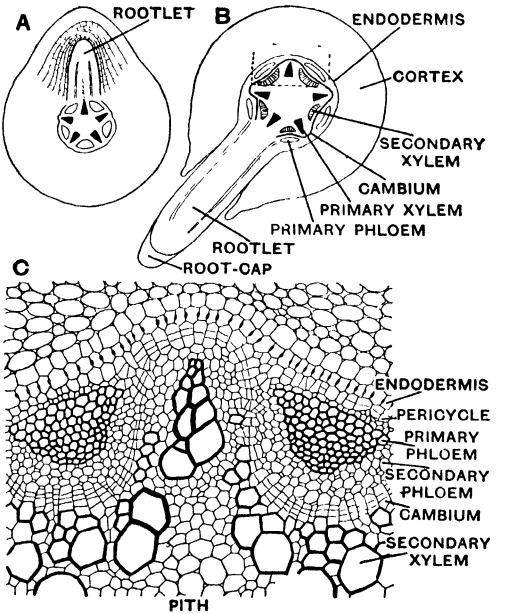


Fig 40.—Older Root of Broad Bean (compare with Fig 39) A, Transverse Section showing a young Rootlet growing outwards through the Cortex; B, Transverse Section of an older Root than that shown in A, with a Rootlet which has emerged from the surface; C, Part of B more highly magnified, showing the Cambium and Secondary Xylem and Phloem: the Cambium ring is becoming completed by the Pericycle cells outside the Primary Xylem becoming meristematic.

(4) The **pericycle,** a layer of cells some of which (especially those outside the xylem strands) show division by a tangential wall (so that there is a double layer at these points).

(5) The radiating **primary xylem bundles,** usually five (sometimes 4 or 6) in number, each roughly triangular with the narrowest (**protoxylem**) vessels at the apex of the triangle, which points outwards and lies immediately within the pericycle—the development of the wood is centripetal, and new vessels may be seen in course of formation on the inner side of the bundle.

(6) The **primary phloem bundles,** alternating with the primary xylems—the outer part of each phloem bundle consists of thick-walled tissue, while the inner thin-walled portion is not readily distinguished from

(7) The parenchyma of the **conjunctive tissue** or ground tissue of the vascular cylinder.

131. Structure of old Bean Root (Fig. 40).—Cut a series of sections across the older parts of the root—avoid the oldest part near the seed itself, where the hypocotyl transition region between root and stem begins. Note that the ground tissue lying within the phloem bundles shows repeated division by tangential walls and is therefore arranged in radial rows, forming cambium bands which produce secondary xylem internally and secondary phloem externally. Then the portions of pericycle lying outside the primary xylem bundles also become meristematic, so that the cambium now forms a continuous zone. The piliferous layer dies off and the hypodermal cortex layer becomes cutinised, forming the exodermis.

Cut transverse sections from the oldest part of the root of a large seedling or an adult plant, in which considerable secondary thickening has taken place. Note (1) the primary xylem bundles, still in their original position at the outside of the central ground tissue; (2) the broad rays of parenchyma, on the same radii as the primary xylems; (3) the secondary xylem, in masses alternating with the rays; (4) the cambium, forming a continuous zone; (5) the secondary phloem, lying outside the cambium; (6) the dis-

organised primary phloem, seen as patches of thick-walled or crushed cells on radii alternating with the rays and primary xylems; (7) the cork-cambium, which has arisen from the pericycle and produced a cork layer—the endodermis, cortex, and piliferous layer have, of course, been cast off.

132. In **Woody Dicotyledons** secondary thickening in the root begins as in herbaceous forms, but after the first year annual rings are formed in the secondary xylem; the primary xylem can, however, be recognised at the centre of the root owing to its radiate or star-like appearance. The root of Horse Chestnut shows the tissues clearly, but others should be tried.

133. Roots of Monocotyledons.—Suitable material is afforded by the roots of Wheat, Maize, Oats, and other seedlings, some of which should be grown in water or culture solution, others in soil; the roots may be mounted entire and examined as directed for Cress, Mustard, etc. For sections, use the roots of Onion or Hyacinth grown in water and also in soil; Iris and other roots should also be tried.

The roots of Monocotyledons resemble those of Dicotyledons in primary structure, but there is no secondary thickening In Dicotyledons the number of xylem bundles varies from two to six, rarely more; in Monocotyledons a limited number—five to eight—is sometimes found (*e.g.* Onion, Hyacinth), but there are typically more than often a very large number. The endodermis and pericycle are usually very sharply defined, each consisting of a single layer. The endodermis is often strongly thickened, especially on the lateral and inner walls, but here and there we find a thin-walled "passage-cell" in the endodermis, opposite a xylem-bundle—this is usually well shown in Iris. The exodermis, or hypodermal layer, the cells of which become cutinised and persist after the piliferous layer has disappeared, is usually well marked in Monocotyledons.

134. Apical Meristem of Root.—For the structure of the growing point of the root cut median longitudinal sections of the radicle of the embryo in the seed of (1) Broad Bean, (2) Sunflower, (3) Maize.

In each case treat the sections with potash or eau de Javelle, rinse in water, mount in glycerine, and note (*a*) the root-cap, (*b*) the "piliferous layer," (*c*) the "periblem," (*d*) the "plerome."

In Broad Bean all these tissues appear to arise from a general mass of meristem, *a* and *b* being formed by cells

cut off on the outer side of the meristem, *c* and *d* by cells cut from the inner side.

In Sunflower, which is typical of Dicotyledons in general, *c* and *d* are distinct, the "periblem" being traceable to a single layer of cells covering the apex of the "plerome" and being itself covered by a layer which gives rise to the root-cap and the piliferous layer.

In Maize, however, the piliferous layer when traced towards the apex is seen to be continuous with the "periblem," so that the root-cap tissue alone is developed towards the outer side of the apical meristem, the layer from which it arises being termed the "calyptrogen."

135. Aerial Root of Tropical Epiphytic Orchid.—In transverse sections of the aerial root of a tropical epiphytic Orchid (e g. Oncidium, Vanda, Dendrobium), note (*a*) the **vascular cylinder** with its alternating xylem and phloem bundles, pericycle, endodermis—strongly thickened but with passage-cells opposite the xylems; (*b*) the **cortex,** consisting of rounded cells containing **chloroplasts;** (*c*) the **exodermis,** a layer of cells mostly with thickened walls, but some thin-walled and forming passage-cells; (*d*) the **velamen,** consisting of several layers of transparent empty cells which serve to absorb and store water.

If fresh material is available, note that the aerial root appears white when dry (the velamen then containing only air) and green when moist (the velamen being then transparent and making the green colour of the cortex visible)

In tangential sections, note the fibrous thickenings on the walls of the velamen-cells.

136. Haustorium of Dodder.—Get material of plants (Gorse, Heather, etc.) infested with Dodder, and with scissors cut it into pieces which can be held in pith so that sections may be cut passing through both plants at the places where the Dodder stem is attached to its host; arrange that some sections shall cut the Dodder stem transversely, others longitudinally.

Note that the Dodder stem is fixed to the host stem by a disk, the superficial cells of which are often greatly enlarged, and that from this disk a haustorium has grown into the tissue of the host stem. Some of the haustoria will be seen applied to the vascular bundles of the host. Note that each haustorium has a central xylem strand with spiral vessels, continuous with the bundles of the Dodder stem. In favourable sections this strand may be traced right into the xylem of the host plant, while the haustorium has also elements which join on to the phloem of the host.

Similar, but smaller, attachment organs and haustoria can be

seen on digging up a large clump of soil from which is growing a patch of Yellow Rattle, Eyebright, or Cow-wheat; set the mass in a large basin of water, wash it gently, and clip out the roots of the parasite and the host-plant (a Grass) at points where they are in contact. Sections will show that the parasitic root forms a swollen mass of tissue, from which there proceeds a haustorium containing a strand of xylem vessels, much as in the Dodder.

137. Endotrophic Mycorhiza of Bird's-Nest Orchid.—Dig up a plant of Neottia, or at any rate remove portions of the thick fleshy roots. In transverse sections of a root, note (*a*) the **central cylinder** with its alternating xylem and phloem strands; (*b*) the thick **cortex**; with the **fungus-zone** near the periphery, within (*c*) the **epidermis.**

Examine the fungus-zone more closely, and note that it is usually in three layers of cells. In the outer layer (that immediately within the epidermis) and in the inner layer of the fungus zone, the fungus hyphae are slender and usually clustered round a central mass of proteid in the cell; while in the middle layer the fungus hyphae are stouter and practically fill up the entire cell cavity. As a rule the fungus, after infecting the three outermost layers of the cortex, thrives only in the middle one of the three layers, while in the outer and inner layers its growth is checked by the living protoplasm of the cortex-cells, which absorb the food-materials provided by the fungus.

138. Exotrophic Mycorhiza of Beech, etc.—Dig up a Beech seedling, rinse in water, and note that the rootlets bear (*a*) fine white fungus-threads—clearly not root-hairs, since they branch freely and arise from (*b*) a **fungus mantle** of interwoven threads (hyphae) covering the surface of the root; (*c*) masses of humus attached to the fungus threads. Cut and examine transverse and (more instructive) longitudinal sections of the root. A similar fungus mantle may be found on various other humus-loving plants, *e.g.* Heather.

139. Laticiferous Tissue.—The latex (§ 89) found in the various plants is contained in special tissues, of which the chief forms are (1) **latex vessels** or **syncytes,** produced by the fusion of original separate cells; (2) **latex cells** or **coenocytes,** which branch but do not fuse or anastomose. Latex vessels occur in Dandelion and some other Composites, Campanula, Chelidonium, Poppy, etc.; latex cells in various Euphorbiaceae, Apocynaceae, Asclepiadaceae, etc.

(1) **Latex Vessels.**—Cut (*a*) transverse sections of the root of Dandelion, (*b*) tangential longitudinal sections passing through the phloem; treat some with potash, others with alkanna, others with potassium dichromate, and mount in glycerine. In (*a*) note the

latex vessels, circular in cross-section, arranged in rings outside of the cambium and distinguished by their dense contents. In (*b*) the latex vessels appear as a network, the main parallel longitudinal tubes being connected by horizontal branches. The origin of the vessels can be traced in sections traversing the cambium ; the latex-containing cells are at first separate, but their cavities become continuous owing to fusion of the terminal and lateral walls

(2) **Latex Cells (Coenocytes).**—Cut (*a*) transverse sections of the stem of a Spurge (Euphorbia) and (*b*) tangential longitudinal sections passing through the cortex. In (*a*) note the thick-walled **latex tubes,** lying in the cortex outside the ring of vascular bundles. In (*b*) note the long tubes running chiefly in the longitudinal direction through the cortex, here and there branching but never showing fusion. These coenocytic tubes are formed by the continued growth and branching of single cells which are present in the embryo itself. Note the dumbbell-shaped starch grains embedded in the granular contents of the tubes. Carefully cut away the entire cortex from a piece of Spurge stem, boil in a test-tube in potash for a few minutes, and tease out with needles the latex tubes, noting their branching. Cut longitudinal sections of the apex of a Spurge stem, and look for the tips of the tubes, which are rarely seen in the older parts of the stem ; stain with safranin or haematoxylin, and look for the numerous small nuclei at these growing tips.

140. The Bifacial Leaf.—The detailed structure of a bifacial foliage-leaf can be made out by (1) the maceration of entire leaves—small entire leaves are most suitable for this purpose; (2) the removal of the upper and lower epidermis by tearing-off; (3) examination of tangential sections, cut parallel to the upper and lower surfaces of the leaf; (4) examination of vertical transverse sections, cut at right angles to the surface of the leaf.

141. Maceration of Leaf.—Boil some Box or Privet leaves for about five minutes in 10 per cent. potash. Hold a leaf under water in a saucer or dissecting-dish, and with scissors cut off a strip of tissue round the margin—where the upper and lower epidermis layers are joined. If the leaf has been boiled sufficiently, it will separate readily into three parts :—(1) **upper epidermis,** (2) **mesophyll** with the **veins,** (3) **lower epidermis.** Mount these in water —the upper side of the leaf is usually convex and the lower concave, hence the two sides can be distinguished.

The epidermis of both sides is thin and transparent and consists of a single layer of cells. In the **upper epidermis** note the closely fitting polygonal cells; in the **lower epidermis** the numerous **stomata** scattered about, each stoma with two curved **guard-cells.**

Tease out the middle portion, or crush it under the cover-glass, to separate the tissues Note the two forms of mesophyll-cells—some cylindrical (**palisade mesophyll**), others branched in a star-like manner (**spongy mesophyll**); some of the cylindrical cells may be found attached to the inside of the upper epidermis, and some of the branched cells to that of the lower epidermis. Note also the **veins,** which run out on either side from the median vein in the **midrib,** and the fine branching veins forming a network; in each vein note the **sheath** of narrow cells, and the **vessels** of the xylem (spiral, annular, pitted).

142. Isolated Epidermis.—Remove some leaflets from a Broad Bean seedling. Hold a leaflet, with the lighter green lower side towards you, between forefinger and thumb of each hand and, starting from a point of the edge, tear the leaflet across obliquely. The thin colourless lower epidermis can be torn off in this way, exposing the green inner tissue or mesophyll; mount the piece of epidermis in water on a slide. Now turn the leaflet over and try to tear off in a similar way a piece of the upper epidermis—it does not come off so readily, and more of the green mesophyll is torn off with it, since the mesophyll is relatively compact above and loose below. In this case the epidermis bears stomata on both upper and lower sides of the leaf.

The epidermis can readily be torn from various other leaves, *e.g.* Lily, Tulip, Narcissus, Hyacinth, Tropaeolum, Ivy-leaved Toadflax.

143. Intercellular Air-spaces in the Mesophyll.—Dip the leaves of various plants into very hot water; the water should be boiled and immediately poured into warmed tumblers. Note whether the air-bubbles, driven out of the

mesophyll air-spaces by the heat, escape from both sides or only from the lower side. Cut or tear across a leaf before dipping it into the hot water; note the streams of bubbles issuing from the cut edge. This simple experiment shows that the leaf contains air, and that the air-spaces in the mesophyll communicate with the atmosphere by means of the stomata.

144. Microscopic Examination of Air-spaces in Leaf.—Fold a large leaf (*e g* Laurel or Rhododendron) several times, or cut it into strips, and cut transverse sections, keeping the razor dry. Mount in water, and with the microscope note the numerous irregular air-bubbles between the cells of the spongy mesophyll; then run in some alcohol, and note the expulsion of the air in the form of spherical bubbles.

145. Tangential (Horizontal) Sections of Leaf (Fig 41).—Fold a leaf (*e g.* Beech, Privet, Laurel, Rhododendron, Ivy) over one finger and, wetting the razor with dilute alcohol, cut thin sections parallel with the upper surface of the leaf; then turn the leaf over and cut sections parallel with the lower surface.

In each case mount some of the sections with the epidermis side upwards, and others with the mesophyll side upwards, so as to have preparations (each mounted in water or dilute glycerine on a separate slide) of (1) **upper epidermis,** surface view; (2) upper or **palisade mesophyll,** cells cut transversely and therefore appearing circular with narrow air-spaces at the corners between adjacent cells, (3) lower or **spongy mesophyll,** the cells of which appear like starfish, being joined up by their diverging arms so as to form a network, the meshes of which are occupied by air; (4) the **lower epidermis** in surface view. Note that the mesophyll-cells contain chloroplasts, and that the vertical walls of the epidermis are usually wavy.

146. Structure of Petiole.—Cut transverse sections of the petiole of various stalked leaves. A stout petiole, if examined by itself, might sometimes be mistaken for a stem, especially when

the bundles are arranged in a ring (*e g* Ivy, Horse Chestnut), but as a rule the petiole is more or less flattened, or grooved, on its upper surface, and if several bundles are present they are usually arranged in a curved band, the xylems being on the concave side which faces upwards. Collenchyma is generally present below the epidermis, and in Dicotyledonous petioles there is a rudimentary or for some time functional cambium between the xylem and phloem of the bundles.

147. Vertical Transverse Section of Leaf Blade.—Remove a strip of Laurel or of Ivy leaf by making a cut down each side of the midrib, including a portion of the thin wing. Hold the strip in pith, and cut thin sections at right angles to the midrib. The midrib projects on the lower side of the leaf, hence it is easy to distinguish the upper and lower sides of the section when mounted. In the midrib note the large **bundle,** or curved band of bundles, with the xylem facing upwards; in Laurel there are numerous brown cells around the bundles; within the epidermis on both sides there is a zone of collenchyma. (Fig. 42.)

In the thin lateral parts, on each side of the midrib, note—

(1) the **upper epidermis,** the upper and lower cell-walls usually convex, and the upper wall covered with cuticle;

(2) the **palisade mesophyll,** consisting of cells elongated vertically and containing abundant chloroplasts—on being traced downwards the cells of the palisade layers (often two or three in number) become shorter and less closely packed and pass into

(3) the **spongy mesophyll,** in which the cells are of irregular shape, contain chloroplasts, and are loosely arranged, large intercellular air-spaces being present;

(4) the small **bundles** or veins, lying between the palisade and spongy zones of the mesophyll;

(5) small cells here and there in the mesophyll, containing either single crystals or spherical crystal clusters consisting of calcium oxalate;

(6) the **lower epidermis,** showing at places the two small guard-cells of a **stoma.**

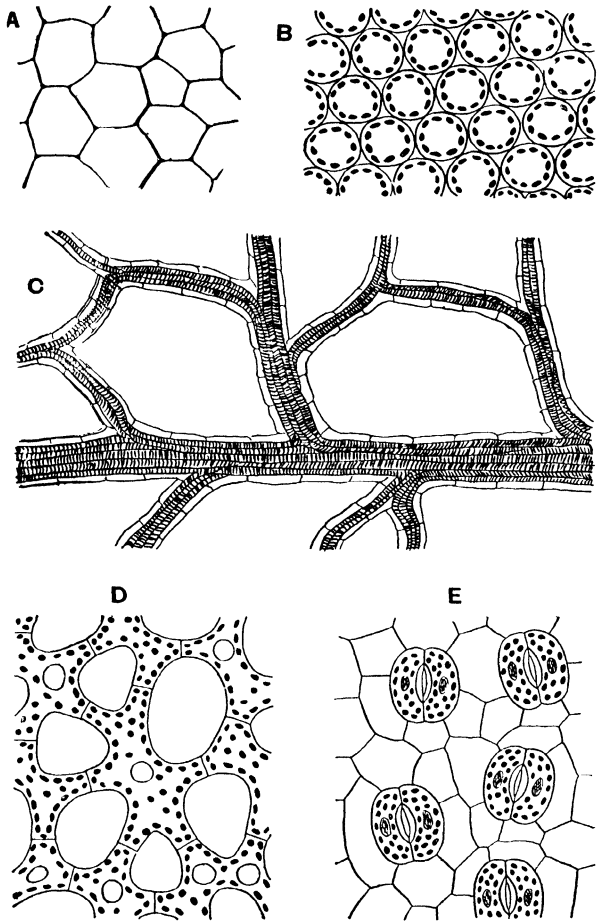


Fig 41.—Tissues of Box Leaf as seen in Horizontal Sections A, Upper Epidermis (in many Dicotyledonous leaves the vertical walls of the Epidermis Cells are wavy, not straight as in Box), B, Palisade Mesophyll Cells, cut transversely to their long axis, C, Part of the anastomosing Vascular System, showing Vessels and Bundle Sheath the Mesophyll Cells between the Bundles are omitted, D, Spongy Mesophyll Cells : the circles shown in some of the cells are arms of the star-shaped cells cut across, E, Lower Epidermis with Stomata.

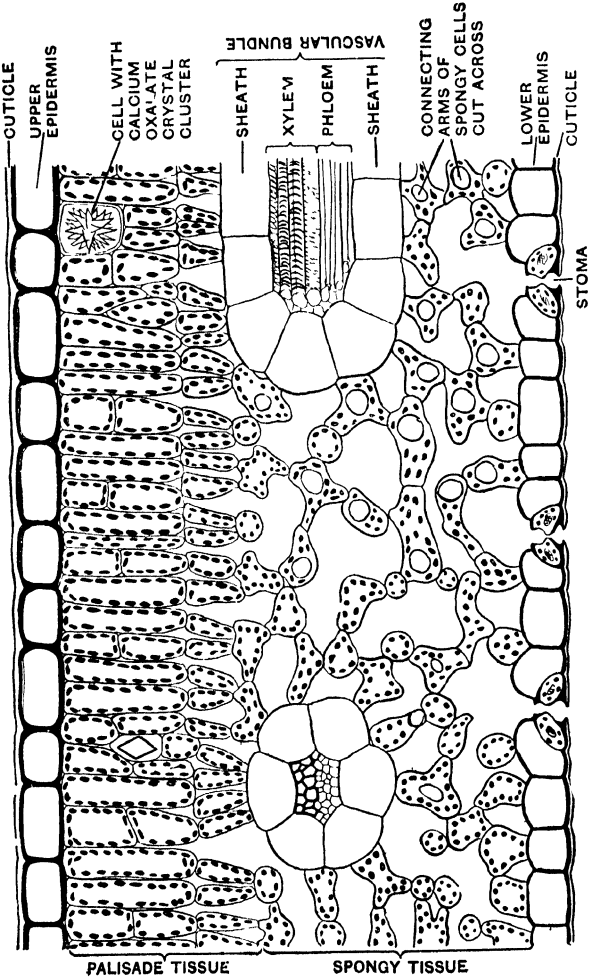


Fig 42.—Part of Vertical Transverse Section of Cherry Laurel Leaf. The cells of the Bundle (Vein) Sheath are very large in this leaf and are mostly filled with deeply staining contents (not shown), the circles seen in some of the Spongy Mesophyll cells are the arms of the star-shaped cells cut across; three Stomata are seen in the Lower Epidermis.

148. Structure of Stomata and Guard-cells.—Since the stomata of various Monocotyledons are very large, transverse sections and strips of epidermis should be taken from the leaves of Lily, Tulip, Narcissus, or Hyacinth.

(1) In surface view, note that each stoma in these plants has a definite position, being intercalated between the ends of two of the elongated ordinary epidermis cells. Focus down on a stoma with high power, and note that the opening, which is flush with the surface of the leaf, narrows downwards like a funnel and then opens out again below.

(2) In section, note that the wall of each guard-cell is thin where it adjoins the other epidermal cells, while on the side adjoining the pore it is thickened (except at the middle where the pore is narrowed) and is produced into a ridge above and below—these ridges, with the projection that narrows the pore at the middle, divide the pore into an outer and an inner chamber.

149. The **development of stomata** is readily followed in the young leaves of Hyacinth or Narcissus. Dissect a resting-bulb or one just beginning to sprout, and from the young leaves tear off strips of epidermis, or cut tangential sections, at different points of the leaf. Starting from the base and working up to the apex of the young leaf, note that the cells are in longitudinal rows and differ in size, some being elongated and others short and square, further up, each short cell divides by a longitudinal wall into two cells (guard-cells); this median wall then becomes thickened and finally splits to form the pore, while the guard-cells curve outwards on either side.

150. Fall of the Leaf.—The formation of the **abscisslayer,** by which the fall of the leaf is effected in autumn, and the cork which closes over the stem and forms the **leaf-scar,** may be studied in various deciduous trees, *e.g.* Horse Chestnut or Sycamore. In autumn cut across the base of the petiole of a leaf which has changed colour; after cutting across the stem above and below, split the whole in halves by a longitudinal cut passing through petiole base and stem, and prepare longitudinal sections as shown in Fig. 43.

Note that the cork of the stem does not run on to the petiole (which has collenchyma below its epidermis), and that where it stops short a cork-layer runs across the base of the petiole. On the outer side of this cork-layer is the absciss-layer, a zone of loose rounded yellowish cells. The cork-layer is at first interrupted by the bundles that pass into the petiole, but on the disorganisation of the absciss-

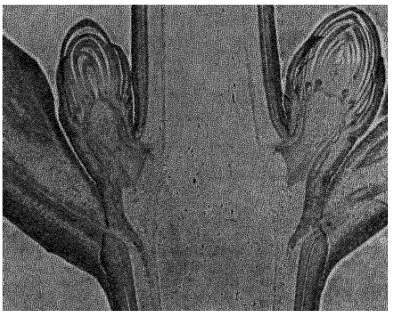


Fig 43.—Longitudinal Section of Node of Sycamore Stem, showing the Abscise Layer across the base of the Leaf on each side. Above each leaf-base an axillary bud is seen.

layer (which is continued through the parenchyma of the bundle) the cork-layer is completed by the formation of cork over the projecting stumps of the bundles, and then the leaf is separated, the vessels of the exposed bundles being compressed and closed while the cork-layer is left covering the leaf-scar. The cells in the petiole contain, as a rule, abundant calcium oxalate crystals, while those in the stem cortex usually contain starch.

151. Isobilateral Leaves, Phyllodes, Centric Leaves.—In some leaves which grow nearly erect, *e.g.* Hyacinth, the tissues have much the same arrangement on the two sides, but the bundles have the xylem facing the upper surface; in Hyacinth itself, for instance, both sides have stomata, and there is no distinct palisade tissue. In other cases, however, **isobilateral** structure is shown, *e g* in Iris, where palisade tissue occurs within the stoma-bearing

epidermis of both sides and merges into spongy tissue in which are embedded vascular bundles arranged somewhat irregularly but having the phloem turned towrads the leaf surface to which the bundle is nearest. This structure is also shown by **phyllodes** (laterally flattened petioles) in various species of Acacia, etc, sections of which should be examined. For the more or less markedly **centric** type of leaf, which is cylindrical or prismatic and shows little or no distinction of upper and lower surfaces, examine sections of the leaves of Onion, some species of Juncus, Stonecrop, Sea Blite (Suaeda), Prickly Saltwort (Salsola), etc.

152. Water-stomata.—Mount in water pieces cut from the margin of a Tropaeolum leaf, and on the upper side at the margin note the numerous water-pores in groups at the ends of the chief veins. The guard-cells of these pores having usually lost their living contents at an early stage in the development of the leaf, the water-stoma remains wide open—it has lost the power of movement. These pores can be seen more clearly in tangential sections cut from the margin of the leaf.

153. Water-Glands (Hydathodes)—For the structure of the water-glands which are often associated with water-pores examine a Fuchsia leaf, in which these glands appear as swellings on the edge, each gland being on a tooth at the termination of a vein. Cut off, mount, and examine the tip of a tooth, to see the large water-stoma.

Cut vertical sections, so as to traverse tooth, vein, and gland longitudinally, and note (1) the **epidermis** on either side, interrupted at the tip of the tooth by (2) the **water-stoma**; (3) the widening out of the **bundle** and its termination in the **glandular** (epithem) **tissue**—a mass of colourless parenchyma; (4) the **water-cavity** at the end of the gland, below the stoma.

154. Chalk-glands.—These are modified water-glands, found in various Saxifrages, etc. Note the white masses on the leaf-margin in one of these plants; treated with acetic acid, the masses dissolve with effervescence, since they consist of calcium carbonate deposited on the evaporation of the water excreted by the gland The structure is much the same as in Fuchsia; note the short **hairs** on which the lime is deposited.

155. Structure of Grass Leaf.—Cut transverse sections of the plumule of a Wheat seedling, and note (1) the tubular sheath, consisting of colourless parenchyma and containing two opposite vascular bundles; (2) the young foliage-leaves enclosed in the sheath.

In a foliage-leaf note the ridges on the inner (upper) side of the leaf; each ridge contains a bundle, and the green parenchyma, with stomata, occupies the lower part of each ridge, lining the furrows. A similar structure may be seen in the leaves of various other Gramineae.

The leaves of Marram Grass (Psamma) and of some moorland Grasses (Festuca, Aira, Nardus, etc.) are of special interest from being rolled up; in Psamma the leaf can become partially unrolled in moist air, becoming closely rolled up again in dry air.

156. Xerophilous Leaf Structures.—In various xerophilous plants the leaves show characteristic structural adaptations for the reduction of transpiration or the storage of water.

Among adaptations for checking transpiration note the following.—thick cuticle, often stratified; sunken stomata, lying below the general level of the leaf and often overarched by the surrounding epidermal cells; development of colourless "aqueous tissue" for water-storage, and of hypodermis, above or below the mesophyll or in both positions; dense hairy covering; waxy coating on the cuticle; rolling-up of the leaf with the stoma-bearing surface (upper surface in Grasses, lower surface in most other plants) placed internally. In many cases, of course, a xerophilous leaf shows *several* of these features.

For (1) **wax layers,** in the form of grains or rods on the surface, examine the leaves of Iris, Echeveria, Eucalyptus, etc.; for (2) **thick cuticle,** Holly, Agave, Aloe, India-rubber Plant; for (3) **sunken stomata,** India-rubber Plant, Oleander (stomata sunk in groups in chamber-like infoldings of the lower surface); for (4) **aqueous tissue,** India-rubber Plant, Begonia, Peperomia; for (5) **hairy covering,** Woolly Mullein, Hippophaé; for (6) **rolled-up leaves,** Erica, Calluna, Empetrum, Nardus, Psamma, etc.

157. Aquatic Leaf-structures.—Examine and compare the structure of (1) floating leaves, like those of Pondweed (Potamogeton natans); (2) finely-divided submerged leaves, like those of Water Crowfoot (often also with floating leaves), Water Milfoil, etc.

Note that floating leaves have the stomata on the upper side, and very large air-spaces in both the palisade and spongy mesophyll—often forming wide air-chambers below the stomata; while submerged leaves have chloroplasts in the ordinary epidermal cells, no stomata, feebly developed vascular bundles, and small air-spaces.

158. Hairs, Glands, etc.—The leaves (and also the stems) of various plants should be examined for different types of hairs and glands. In each case strip off the epidermis (or make tangential sections) and also cut transverse sections of the leaf or stem.

The following show interesting hairs —Wallflower (hairs compass-shaped); Shepherd's Purse (hairs star-shaped); Stinging Nettle (large unicellular stinging hairs, sunk in a multicellular base and provided at tip with a detachable swelling); Goosefoot or Orache (large spherical or ovoid shortly stalked hairs, giving the leaves a mealy appearance); Hop (compass-like hairs with stalk sunk in a raised multicellular basal outgrowth—these hairs help the plant in climbing); Goosegrass (curved and pointed hairs, which help the plant to climb); Mouse-ear Hawkweed (shaggy hairs, consisting of several longitudinal rows of cells cohering laterally).

Various forms of **glandular hairs** should also be examined, *e.g.* those on petiole of Chinese Primrose, leaves of various Labiates, etc, which have a multicellular stalk and a rounded glandular terminal cell; the short thick rounded multicellular glands on the bud-scales of Horse Chestnut. In these and various other cases, the copious secretion of the gland may be seen in sections mounted in water. This secretion may be resinous or oily. Of special interest are the gland hairs found on the leaves of Sundew and Butterwort, which produce enzymes for the digestion of insects caught by the sticky secretion.

159. Structure of Perianth-leaves of Flower.—Examine the perianth-leaves of various Monocotyledons, and the sepals and petals of various Dicotyledons. In some cases these leaves are so transparent that they may be mounted entire, or made transparent by treatment with chloral hydrate, potash, etc In other cases, make tangential and transverse sections as in the case of foliage-leaves.

Note that the perianth-leaves of Tulip, etc, have stomata in the epidermis; this is often the case also with the sepals and even the petals of various Dicotyledons, but the

internal structure of floral leaves is, on the whole, simpler than that of foliage-leaves.

Note that the epidermis-cells often have the outer wall dome-like or conical, and marked by striations, in some cases (*e.g.* Pansy) the epidermis-cells of the petals are produced into long finger-like processes, given the velvety or satin-like appearance of the petal surface. The lateral walls of the epidermis-cells are often very wavy, or have ingrowths resembling those seen in the mesophyll-cells of a Pine leaf.

160. Chromatophores and Coloured Sap.—Strip the epidermis from the perianth-leaves and petals of various flowers. Note that in green floral leaves the colour is due to chloroplasts; in white leaves the cells contain colourless plastids (leucoplasts) and colourless cell-sap In most yellow flowers the colour is due to yellow **chromoplasts,** chiefly in the epidermis, but sometimes (*e.g.* Narcissus perianth-lobes) in the mesophyll-cells In a few cases, however, the yellow pigment is dissolved in the cell-sap, *e.g.* Mullein. Blue, violet, and some red colours are due to **coloured sap,** but some reds are due to chromoplasts.

Examine the flowers of Narcissus, Pansy, Tropaeolum, Buttercup, red Rose, Poppy, Wallflower, Myosotis, Crocus, etc. Investigate cases of mixed colouring, *e.g.* red and yellow Tulips and Zinnias (some epidermis-cells with red sap, others with yellow chromoplasts). In each case mount in water or glycerine strips of tissue torn or shaved from the floral leaves.

161. Structure of Mature Anther (Fig. 44).—The flower of a Lily or a Narcissus may be used with advantage for the structure of the anther and the ovule, but various other flowers that are available should be tried.

For **transverse sections** of the mature but intact anther it is necessary to use young flower-buds. In the case of Narcissus cut across (*a*) flower-buds still enclosed in the resting bulb, (*b*) young flowers that have been carried up in spring but have not yet opened. Note the

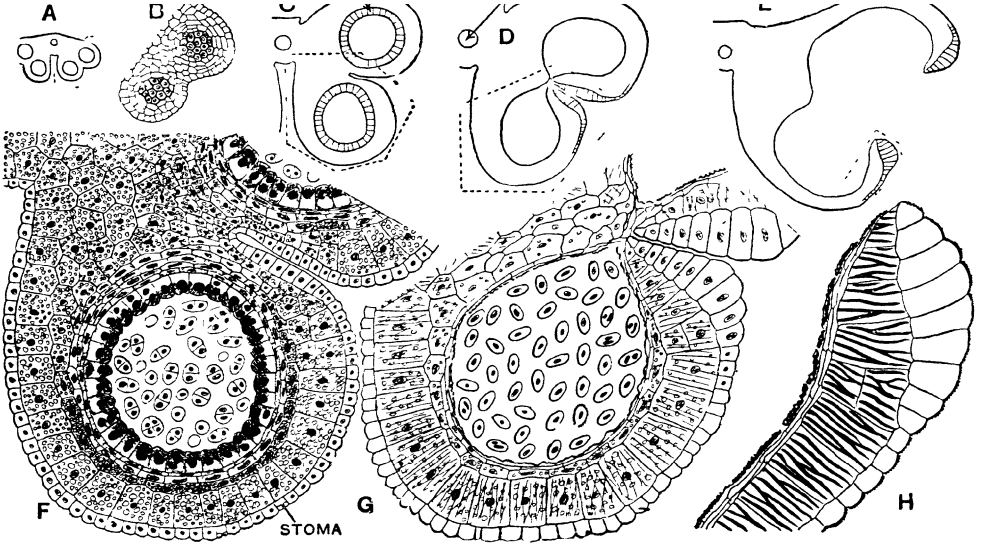


Fig 44 —Structure of Anther of Lily (Transverse Sections). A, Young Anther , B, details of same, showing the Sporogenous Tissue of two of the Pollen-sacs , C, Older Anther , F, details of same, showing Epidermis, Fibrous Layer (at this stage filled with starch grains), and Tapetum-layer (large cells) lining the Pollen-sac which contains dividing Pollen-mother-cells , D, still older (Mature) Anther, just before Dehiscence note the large size of the Epidermis Cells forming the "Stomium" at the junction of the two Pollen-sacs , G details of same · the cells of the Fibrous Layer have lost much of their contents and now show the thickening fibres, and the Tapetum is disorganised, while the Pollen-grains are

general outline of the section and compare with an entire anther; there are three longitudinal grooves—one along the middle of the inner face and one along either flank, while along the middle of the outer face there is a whitish band, the connective.

With high power, note (1) the tissue of the **connective,** with a **vascular bundle** in a nearly central position; (2) the four **pollen-sacs,** two on either side, containing the **pollen-grains;** (3) the **epidermis** covering the entire anther and consisting of small cells—along the outer side of the connective **stomata** may be seen cut through, and at the points where the two pollen-sacs on either side of the anther meet there is a band of large epidermal cells; (4) the **fibrous tissue,** consisting of cells with the walls thickened by spiral or annular bands arranged transversely to the long axis of the anther. Below the epidermis of the pollen-sacs this tissue consists of a well-defined layer of large cells; along the inner side of the anther there are several layers of smaller cells; while at the point where the partition between the two pollen-sacs meets the anther-wall the fibrous tissue is absent (**dehiscence line**).

Also examine **longitudinal sections** of an anther, cut parallel to the plane of flattening. Starting with a surface section of the connective, note the numerous **stomata** in the epidermis. A section a little deeper will show the vascular bundle of the connective, with its xylem vessels, also the thickening bands in the cells of the fibrous tissue.

162. Structure of Pollen-grains.—Note the oval or bean-shaped form of the ripe Narcissus pollen-grain; the outer surface shows granular thickenings. With iodine, or (better) with acetic methyl green, note the **two nuclei**—one spindle-shaped and the other spherical. Pollen-grains may be rapidly cleared and made transparent by treatment with either chloral hydrate or carbolic acid.

For comparison with Narcissus, examine the pollen-grains of various other plants, and note the great differences in size, form, surface sculpturing and outgrowths, etc. A good selection would be the following:—Mallow or Hollyhock, Marrow or Cucumber, Broad Bean, Crocus, Chicory. Wallflower, Rhododendron, also the pollinia of Orchis.

163. Growth of the Pollen Tube.—To follow the germination of pollen-grains, sow the grains in a drop of 5 per cent. sugar solution on a cover-glass and invert over a moist-chamber (§ 18), or place the grains in sugar solution in a watch-glass and examine from time to time. It is often necessary to use a 10, 15, or even 20 per cent. sugar solution; try some pollen in each strength of solution.

The tubes appear within a few hours, especially if the culture is kept in a warm place in darkness Note that the protoplasm in the tube may show marked streaming movements. In Narcissus, after two days, the tube is seen (on being stained with iodine or acetic methyl green) to contain **three nuclei;** of these, the one nearest the tip of the tube is the rounded vegetative nucleus, while the two others (which stain more deeply) have arisen by division of the spindle-shaped generative nucleus of the pollen-grain As the tube grows in length, the protoplasm passes into the apical region, and sometimes walls appear in the tube shutting off the hinder protoplasm-free portion.

164. Structure of Style and Stigma.—In a Narcissus flower, remove the small three-lobed stigma by cutting across the style just below it; note the short finger-like outgrowths (**stigmatic papillae**) of the epidermal cells of the three lobes, and the central opening In transverse sections of the style at different points note the three angles answering to the three stigma-lobes, and the central **canal;** this canal, opening at the apex, may be seen in longitudinal sections through the upper portion of the style and the stigma.

Also examine, by mounting entire or by means of sections, the styles and stigmas of various other flowers. The style is not always hollow, as in Narcissus, but the central tissue is often sharply distinguished from the outer or cortical tissue—this central **conducting tissue,** transparent and often mucilaginous, is traversed by the pollen-tubes on their way from stigma to ovary.

165. Structure of Ovary and Ovule (Fig 45).—Cut numerous transverse sections of the ovary of Narcissus. In this plant the contents of the embryo-sac are

usually seen quite readily in unstained sections from fresh material; but it is perhaps better to cut across the ovaries of a number of flowers and place them in alcohol or an acid fixative before cutting sections, and to stain sections with acetic methyl green, or iodine, or other stains

In T S. of ovary note (1) the division of the ovary into three **chambers;** (2) the presence of **vascular bundles** in the outer wall of the ovary and also in the partitions—note the six larger bundles in the outer wall, three corresponding to the midribs of the carpels and three to the outer ends of the partitions, (3) in each chamber, where the partitions meet, two anatropous **ovules.**

In a single **ovule** note (1) the short **stalk** or funicle, traversed by a slender **bundle** which comes from one of the bundles in the "axile placenta" and ends in the base or **chalaza** of the inverted ovule; (2) the **two integuments,** which start from the chalaza and end at the apex of the ovule in the fine canal or **micropyle;** (3) along one side the outer integument is united to the stalk of the ovule, this portion of the stalk being termed the **raphe;** (4) the **nucellus,** an ovoid mass of tissue lying within the integuments and bounded above by the micropyle and below by the chalaza; (5) the **embryo-sac,** appearing as a large cavity in the micropylar half of the nucellus.

In the **embryo-sac** (examine a number of ovules in order to see all these points) note (1) the vacuolated **protoplasm** of the sac; (2) the large **central nucleus,** connected by protoplasmic threads with the peripheral layer of protoplasm of the sac; (3) at the micropyle end of the sac, three cells forming the "egg apparatus"—the **egg** (oosphere) and the two **synergids;** (4) at the opposite end of the sac, the three **antipodal cells.**

Good preparations showing the structure of the embryo-sac may also be made from Marsh Marigold, White Lily, etc.

166. Pollination and Fertilisation.—It is fairly easy to trace the passage of the pollen-tube from stigma to micropyle. Various plants should be tried, observations being made just after the flower has faded. In each case

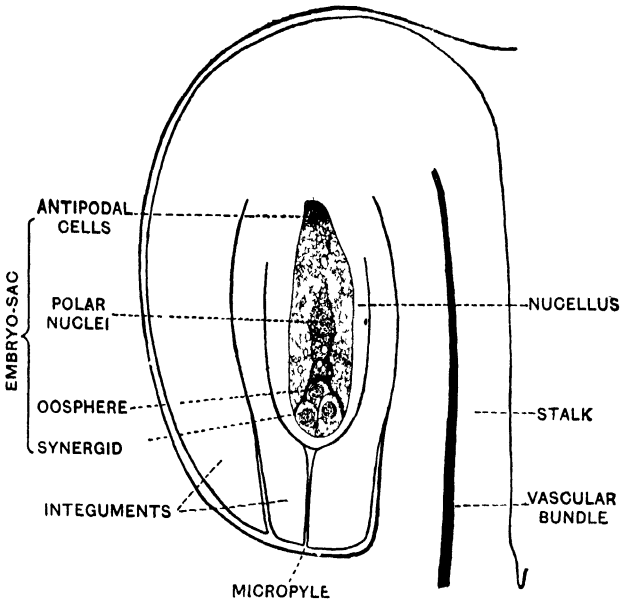


Fig 45.—Longitudinal Section of nearly Mature Ovule (from Transverse Section of Ovary) of Lily. The Embryo-sac is shown in detail, the other parts in outline only. It will be noted that here (as in various other anatropous ovules) the two Integuments are not distinguishable as distinct layers on the side nearest the Stalk (Funicle). In the Embryo-sac the following features should be noted (see also description in Art. 165). At the micropylar end of the Sac are the three cells making up the Egg-apparatus, the Synergids, and the somewhat larger central Oosphere (Egg-cell). The Oosphere is connected with the Polar Nuclei (centre of Sac) by a strand of denser protoplasm (in which the second Male Gamete travels to fuse with the Polar Nuclei). The Polar Nuclei have not yet fused to form the so-called "Definitive Nucleus" or "Secondary Nucleus" of the Sac, which after fusion with the second Male Gamete gives rise to the Endosperm. The three Antipodal Cells have not yet become cut off by cell-walls. The Protoplasm of the Embryo-sac contains numerous Vacuoles.

moisten the preparation with alcohol, to remove air, and mount in glycerine; to make the tissues more transparent, heat the sections in the glycerine, since clearing reagents like potash obscure the dense granular contents by which the pollen-tubes are recognisable.

Pick the pistil from a faded Chickweed flower, mount, and note (1) the ovary wall with three vascular bundles; (2) the three curved styles, with stigmatic hairs on their convex sides; (3) the yellow pollen-grains held between the stigmatic hairs; (4) the pollen-tubes passing from the grains into the tissue of the style Examine faded flowers of different ages, and look for ovules with a pollen-tube applied to the micropyle.

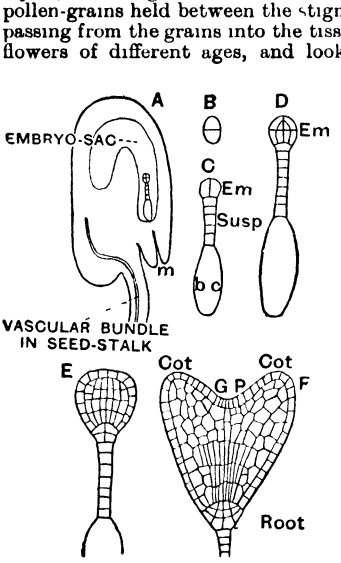


Fig 46.—Development of Embryo in Shepherd's Purse.

A, young seed (treated with caustic potash) showing embryo at micropyle end of curved embryo-sac, m., micropyle, B-F, stages in growth of embryo, G P., growing-point of stem, Susp, suspensor, b c, basal cell, Cot, cotyledon; Em., embryo.

Similar observations may be made on many other plants For the passage of the pollen-tubes down the central canal or the central conducting tissue of the style, examine longitudinal sections of stigma and style of Foxglove, Rhododendron, etc.

The entrance of the pollen-tube into the micropyle of the ovule is easily seen in faded flowers of Speedwell, Chickweed, Shepherd's Purse, etc. Treat the teased-out ovules with chloral hydrate or carbolic acid to make them transparent.

167. Development of Embryo (Fig 46).—This is very easily studied in the Shepherd's Purse Remove the fertilised ovules from fresh ovaries 3 to 6 mm. long, and study (*a*) the embryo *in situ*, (*b*) isolated embryos, in ovaries of different ages.

In ovules mounted entire in potash note the stalk, integuments, micropyle (a pollen-tube may be seen entering this), and curved embryo-sac of the campylotropous ovule;

in the embryo-sac note the **embryo,** which is attached to the end of the sac nearest the micropyle

Mount a number of ovules in potash, then press on the cover-glass with a needle so as to burst the ovules without damaging the embryos which are thus isolated. If the embryos are too transparent run in some acetic acid.

With patience one can get a series showing various stages in the **embryogeny** of this typical Dicotyledon, *e g* (1) a short row of cells, at one end the elongated **basal cell** of the **suspensor,** at the other the rounded **embryo cell;** (2) the embryo cell divided into **octants,** the suspensor elongated and with greatly enlarged basal cell; (3) the octants divided by tangential walls cutting off the **dermatogen,** the **hypophysis cell** of the suspensor pushing in between the lower octants of the embryo, the basal cell still further enlarged, (4) the inner tissue of embryonic mass differentiated into **periblem** and **plerome;** (5) the formation of periblem and dermatogen of **root** at expense of the hypophysis cell; (6) formation of the two **cotyledons** by outgrowth from the upper octants, and of **hypocotyl** from the lower ones; (7) formation of stem **apex** or **plumule** between the cotyledons.

CHAPTER IV.

GERMINATION, GROWTH, TISSUE TENSION

I. Typical Seeds and Seedlings.

168. Types of Seeds and Seedlings.—The chief types selected for study may be classified as follows.—

Dicotyledons
- Non-endospermic. Hypogeal:—Broad Bean (§§ 171-173).
  Epigeal:—Sunflower (§ 175).
- Endospermic:—Castor Oil (§ 177).

Monocotyledons.
- Endospermic. Hypogeal:—Maize (§§ 178-180).
  Epigeal:—Onion (§ 182)

Other seeds and seedlings should also be studied for comparison with these and for special points in the structure and biology of seeds and seedlings. Soak the various seeds in water, and make successive sowings both indoors and in a garden border so as to have plenty of material for observation and experiment.

169. Germination Jars.—(*a*) Take a large wide-mouthed glass jar wiped dry inside, and a piece of thick blotting-paper cut rectangular with one side equal in length to the height of the jar and the other a few inches longer than the circumference of the jar. Roll the paper and insert it in the jar, then fill up the jar with sawdust, keeping the paper pressed against the inner side of the glass. Place seeds in different positions between paper

and glass, and pour in enough water to wet thoroughly the sawdust and the paper.

(*b*) Sphagnum moss is better than sawdust; lamp-glasses, supported in the vertical position by being stuck in a pot of soil or sand, are better than glass jars or tumblers. Root-hairs are well seen in seedlings germinated in moist air; a simple method is to soak a flower-pot, throw on to its inner surface some seeds whose coats become sticky when wet (Cress, Mustard), then invert the pot (with the seeds sticking to it) in a dish of water.

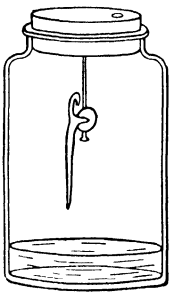


Fig 47.

Into a wide-mouthed glass jar pour enough water to form a layer about 3 cm deep. Stick a long pin through a soaked Bean or Pea, and fix it into a cork (or a piece of wood to cover the mouth of the jar), inverting the cork so as to suspend the seed in the moist air of the jar, in which it will germinate; the inside of the jar should be kept moist—*e.g* with strips of wet blotting-paper. Keep this simple piece of apparatus (Fig 47) for later experiments

170. Glass-sided Box.—Besides flower-pots and boxes of the ordinary kind, get a few boxes of different sizes—one at least a foot deep for the long roots of Bean seedlings—and make them into glass-sided germination boxes as follows: Remove one of the longer sides and replace it by a sheet of glass sloping downwards and backwards, so that the roots in growing vertically downwards will press against the glass and thus be more readily observed. The glass side may be simply held in position by a series of tacks or nails at either side; it will be quite easy in this way to make the glass side movable so that it may be inserted vertically or at different angles.

Fill the boxes with moist sawdust, good garden soil, or sphagnum, and plant the seeds close to the glass. The sawdust or soil should be renewed now and then, since they are apt to become foul; the sphagnum should at

intervals be taken out, sterilised by being boiled in water, then rinsed in water and replaced in the box. These germination boxes will also be useful for various other purposes—*e g.* experiments on geotropism.

171. Broad Bean Seedling.—Examine an entire well-grown seedling, at least a foot in total length. Note the **root** which has grown downwards from the seed, and the **shoot** which has grown upwards from the seed.

(*a*) In the **root** note (1) the main root **axis**, gradually tapering to the free end or root tip; (2) the **rootlets**, arising from the main root in regular longitudinal rows—usually five in Broad Bean—and differing from it only in their smaller diameter and different direction of growth; (3) **root-hairs**—well seen in seedlings grown in moist air in germination jars or lamp-glasses; (4) **root-tubercles**, often seen in seedlings grown in soil.

(*b*) In the **shoot** note (1) the axis or **stem**, four-sided and hollow; (2) the **leaves**, in two rows corresponding to two opposite ridges of the stem; (3) the **buds**, which in a well-grown plant may have grown out as lateral **branches**, each bud or branch arising in the **axil** of a leaf.

(*c*) In a **leaf** from the upper part of the shoot note (1) the **petiole** or leaf-stalk, grooved above; (2) the **stipules**, a pair of outgrowths at the base of the petiole, each like half of a spear-head in form and having near the centre a dark spot—this consists of minute gland-hairs in a patch on underside of stipule; (3) the **leaflets**, thin flat oval appendages with a pointed tip; (4) the prolongation of the petiole above the leaflets—this outgrowth, sometimes developed as a small terminal leaflet, is evidently a **rudimentary tendril**, as may be inferred by comparison with the tendril-bearing Vetches and Peas related to Broad Bean.

(*d*) Trace the root upwards and the shoot downwards to their junction with the two large **cotyledons** or "seed-leaves," which lie within the ruptured **seed-coat.** The lower foliage-leaves are simpler in form than the upper ones; the two lowest (first formed) leaves above the cotyledons are rudimentary and consist of three lobes joined at the base.

Also examine younger seedlings, working back to the earliest stages in germination. Note that in the axil of each cotyledon there is a **bud**; hence the cotyledons are morphologically *leaves*, though in this plant differing markedly from ordinary (foliage) leaves.

172. Broad Bean Seed.—Examine (1) dry seeds; (2) seeds that have been soaked in water for two days; (3) pods of different ages, containing fresh seeds in different stages of development.

(*a*) Note the shape of the ripe seed At the thicker end there is a black or brown mark (**hilum**)—obviously the **scar** formed when the seed became detached from the stalk which fixed it to the inside of the pod.

(*b*) Examine from time to time dry seeds that have been placed in water. At first the surface is thrown into folds —evidently the coat at first absorbs water and swells more rapidly than the seed-contents, hence it becomes loosened and is easier to remove in a well soaked seed. The wrinkling of the coat is very marked in Phaseolus (Scarlet Runner and French or Haricot Bean)

(*c*) Drop some dry seeds into very hot water, or fix some seeds into a spirally coiled piece of copper wire and put this in a beaker of water boiling over a Bunsen, and note the air-bubbles that escape from near the hilum Wipe dry the hilum end of a soaked seed, and squeeze the seed—water oozes out of a small slit-like pore (**micropyle**) at one end of the scar. The micropyle is very conspicuous in Phaseolus, having a raised margin.

(*d*) Remove the coat from a soaked seed, starting at the end opposite the scar. Note the two large whitish **cotyledons,** whose slightly concave inner sides are pressed against each other. After stripping off the upper half of the coat, pull off the rest of it (the part covering the scar end) entire like a cup. Note the smooth tapering **radicle,** projecting from between the cotyledons and pointing towards the micropyle end of the hilum; also note the little **pocket** on the inner side of the seed-coat, into which the radicle fits.

(*e*) Pull apart the cotyledons, and remove one by breaking across the short stalk by which it is joined to the thickest part of the radicle. Note the curved **plumule,** lying between the cotyledons, fitting into a groove on the inner surface of each cotyledon, and forming a continuous curved line with the radicle. Examine the plumule care-

fully with a lens, and with a pin turn back the minute **foliage-leaves** which it bears

(*f*) Make **sketches,** at least twice the natural size, of (1) the entire soaked Broad Bean seed, from the scar end; (2) same from the front—*i.e.* thicker edge—showing the micropyle and the bulge caused by the radicle; (3) same in side view; (4) side, and (5) front views of embryo after removing seed-coat; (6) scar end portion of empty seed-coat, showing the pocket into which the radicle fits; (7) side, and (8) front views of embryo with one cotyledon broken off; (9) section of whole seed, cut between the cotyledons, to show pocket with radicle fitting into it.

173. Stages in Germination.—Study and sketch various stages in the germination of Broad Bean. Note (1) that the radicle emerges from the seed in advance of the plumule; (2) that there is a V-shaped split in the coat along the edge of the radicle-pocket—this is caused by the root swelling and raising the outer wall of the pocket as a triangular flap, the apex of the triangle *not* reaching the micropyle; (3) that in whatever position the seed has been planted, the radicle grows downwards and the shoot upwards—curving, if necessary, in order to take the vertical direction.

Note also (4) that the stalk of each cotyledon lengthens, pushing the cotyledons apart and helping the plumule to emerge from between them; (5) that the plumule remains for a time strongly hooked at the top, but gradually straightens out as it grows upwards; (6) that the cotyledons remain in their original position, covered by the torn seed-coat, and gradually shrivel as germination proceeds; (7) that the bud in the axil of each cotyledon may grow out to form a leafy branch, especially if the plumule itself has been injured; (8) that roots may grow out from the base of the plumule, especially if the radicle has been injured.

174. Seeds and Seedlings of Phaseolus and Pisum.—Examine seeds and seedlings of **French Bean** (Phaseolus vulgaris) and **Scarlet Runner** (Phaseolus multiflorus). In both note the position of the hilum, the conspicuous micropyle, the wrinkling of

the seed-coat during soaking, and the two large primary foliage-leaves carried on the first internode (epicotyl) of the plumule. In Runner the cotyledons are **hypogeal**, remaining below ground; while in French Bean they are **epigeal**, being carried above ground by the elongation of the hypocotyl—the region of the young plant's axis which lies between the root proper and the insertion of the cotyledons

Most seedlings are epigeal, and it is easy to prove—*e.g.* by making Indian ink marks on the axis of the very young seedling and noting the position of these marks at a later stage—that the hypocotyl grows rapidly in length, carrying up the cotyledons and the plumule. Epigeal cotyledons sooner or later turn green on reaching the light; they are larger, thinner, and more like foliage-leaves than in the case of hypogeal cotyledons, which do not turn green (unless they happen to be exposed to light) and which soon shrivel up instead of persisting and growing. Note that in Phaseolus the first two foliage-leaves are simple and heart-shaped and stand opposite each other; while the later foliage-leaves are compound with three leaflets, and arise singly from the stem.

In the **Garden Pea** (Pisum sativum) the transparency of the coat enables one to see clearly in the soaked seed the hilum, micropyle, and radicle, all lying in the same line, with the tip of the radicle pointing to the micropyle; the cotyledons are hypogeal, and the earlier foliage-leaves resemble those in Broad Bean seedling, but the uppermost leaflets of the later leaves are developed as tendrils.

175. Sunflower Seed and Seedling.—Get "seeds" of this plant, also flower-heads of different ages, and note that the "seeds" are in reality one-seeded fruits, or *achenes*, each being formed from the ovary of one of the flowers in the flower-head. The hard shell is not seed-coat, but pericarp or fruit-wall. The upper parts of the flower fall off after fertilisation has occurred, leaving a ring-like scar at the broad upper end of the achene—the hole often seen at the narrow end is (obviously) not the micropyle, but is simply due to the breaking of the achene from the disc of the flower-head.

Soak some achenes in water for a few days, and open one or two to examine the seed that lies inside; the shell (pericarp) is readily split open along the edge. Note that the seed is attached by a fine short stalk to the inside of the shell at the pointed end. Remove the thin seed-coat, and note the radicle, the flat oval cotyledons, and the small plumule.

In germination the radicle grows out, splitting the pericarp, and the hypocotyl grows vigorously, carrying up the cotyledons—often with the split pericarp over their edges like a clip. The hypocotyl is at first bent downward, or coiled in a loop, at the top. This appearance is seen in many seedlings, whether the cotyledons are hypogeal or epigeal—in the former case the epicotyl (plumule-axis) is hooked, in the latter case the hypocotyl The cotyledons turn green, diverge (throwing off the empty pericarp if it has not fallen already), and spread out to the light, also growing larger. At first the plumule grows very slowly, as is usual in seedlings with epigeal cotyledons which function as foliage-leaves; note the hairiness of the epicotyl as compared with the smooth hypocotyl.

176. Other Non-endospermic Seeds.—Examine seeds and seedlings of Linseed, Radish, Cress, Mustard, Turnip, "Nasturtium" (Tropaeolum), Lupin, Marrow or Cucumber, Horse Chestnut; also the achenes and seedlings of Oak and Sycamore, and the seedlings of Beech and Gorse Test cut surfaces of the seeds for starch, proteids, oil; examine thin sections with the microscope; dissect the seeds; sketch stages in germination

Sow the seeds in moist sawdust or soil; note the temperature required (or most favourable) for germination in each case; examine and sketch the seedlings from time to time. In moistened seeds of Linseed, Cress, Mustard, and Turnip, notice the jelly formed by the swelling of the gummy seed-coat when it absorbs water

Small seeds—*e.g.* Cress, Mustard, Wheat—should be grown on muslin stretched across a tumbler filled with water—examine the roots for rootlets and root-hairs.

In nearly all cases the cotyledons are carried up into the air by the lengthening of the hypocotyl. In Horse Chestnut the large cotyledons are partly fused together; on germination the young stem and root are pushed out of the seed by the lengthening of the cotyledon stalks. In Vegetable Marrow and Cucumber note that an outgrowth ("peg" or "heel") is formed to hold down the lower half of the seed-coat against the soil, while the growing hypocotyl raises the upper half of the seed-coat and thus gets free.

In Mustard the cotyledons are two-lobed, in Cress they are three-lobed. In the "Nasturtium" (*Tropaeolum majus*) the later leaves have a nearly circular blade with even margin, and the stalk is inserted at the centre of the lower side of the blade, but in the earliest leaves of the seedling the leaf-blade is lobed and the stalk inserted at the lower margin, as in the adult leaves of the closely-allied leaves of the Canary Creeper (*T. canariense*). In Gorse the

youngest foliage-leaves are trifoliate or three lobed; those formed later are simple, narrow, and spine-tipped

In Brazil "nut" (really a seed) the hard shell is the seed-coat; the minute cotyledons occupy one end of the embryo, the root being at the other end. The greater part of the embryo consists of the swollen axis (hypocotyl). The two cotyledons and the plumule can be seen in a section examined with the microscope—if the section has been cut in exactly the right place.

177. Castor Oil Seed and Seedling.—In the seed note the hard and usually mottled black or brown seed-coat, bearing at one end an appendage (*aril*) which absorbs water readily and becomes soft when the seed is soaked. Place a seed in hot water, and note that air-bubbles arise from beside the aril, which lies just outside of the micropyle. Remove the coat, dissect the seed contents, and make transverse and longitudinal sections; note the **embryo** which lies in a cavity in the middle of the white oily **endosperm** and consists of two very thin flat **cotyledons** (pressed against the endosperm but easily separated from it by means of a knife point), the small **plumule** between the bases of the cotyledons, and the **radicle** below the cotyledons and reaching the surface of the seed at the micropyle-and-aril end; with care the embryo can be dissected from the endosperm; the cotyledons show a distinct midrib with veins arising from it on either side.

On germination the hard seed-coat splits into three valves, the hypocotyl emerges at the other end of the seed and, after the radicle has grown into the soil, elongates and pulls up the seed into the air; the elongating hypocotyl is hooked at the top, rootlets grow out—usually in four regular longitudinal rows—from the top of the radicle, the endosperm becomes swollen and gradually thins out to a papery film covering the outer (lower) surfaces of the two cotyledons, which meanwhile grow larger; then the shrivelled film of endosperm is ruptured by the cotyledons, which spread out in the air (the hypocotyl becoming straightened) as heart-shaped leaves with short stalk and prominent veins—between the cotyledons the plumule is plainly seen.

178. Maize Grain.—Get some Maize "seeds," also a "cob" (female inflorescence); the seeds of the White Horsetooth variety are much better (being larger and more regular in shape) than the ordinary Indian Corn.

(*a*) In a young cob note that the thicker end of the young grain (ovary) bears a long feathery stigma; the ripe grain is a one-seeded fruit, differing from an ordinary achene in having pericarp and seed-coat fused together to form the "husk."

(*b*) In a soaked grain note the oval patch on one side, indicating the position of the **embryo**; with knife or forceps catch at the pointed end of the grain and tear off the thin tough skin (husk) and note the two appendages fixed to the middle of the oval patch—the free tip of the **plumule** is towards the broad end and that of the **radicle** towards the narrow end of the grain

(*c*) Lay the grain on the table with the embryo uppermost, and make a clean slice down the middle of the plumule and radicle; note that these organs are attached to a shield-shaped structure—the **scutellum**—which projects into the grain and runs obliquely across its interior Make sure of the general structure and relationships of these three parts of the embryo; dissect the plumule and radicle with needle or knife, noting that the former contains rolled-up young leaves within a sheath, while the latter is a solid body also within a sheath.

(*d*) To see the form of the scutellum better, (1) cut transverse sections of the grain at different levels; (2) remove the whole embryo from a well-soaked grain; (3) cut a grain longitudinally and smear the cut surface with iodine, this brings out in sharp contrast the brown-stained embryo (radicle, plumule, scutellum) and the blue or almost black-stained starch-bearing region **(endosperm)**. Also treat with iodine the series of transverse sections of the grain.

179. Wheat Grain.—The general structure is the same as in Maize. In a soaked grain note the deep furrow down one side, the small embryo at one end of the op-

posite side, and the patch of hairs at the other end; remove the embryo from the endosperm, to see the small rounded convex scutellum; cut transverse and longitudinal sections, and treat with iodine.

180. Maize and Wheat Seedlings.—Wheat germinates more readily than Maize, but seedlings of both should be examined

(*a*) Note that the husk breaks open at the embryo end of the grain, the radicle growing out first but not giving rise to the whole root-system of the plant (as normally occurs in the Bean, for instance), and later roots arising from the hypocotyl region of the embryo, *i.e* from the base of the plumule.

(*b*) Note that all the primary roots agree with the radicle itself in bursting from a sheath which remains as a collar at the base of the root; this is especially well seen in Wheat, where a first and a second pair of roots, right and left, succeed the radicle, then a fifth root—these five roots can all be recognised in the resting grain (examine series of transverse as well as longitudinal sections of grain)

(*c*) Note the tubular sheath through the burst apex of which the first foliage-leaf makes its appearance. Compare this with earlier stages of germination, noting that the sheath is at first closed at the top but is burst by the rapidly elongating foliage-leaf after the tip of the cone is carried well up into the air.

(*d*) Make a longitudinal section of the grain and of the young shoot, and note that the endosperm, especially near the young plant itself, is reduced to a pulp; the cotyledon remains in its original position and acts as a digesting and absorbing organ. In Wheat the grain soon becomes shrunken and the endosperm reduced to a milky fluid; in both seedlings examine some of the endosperm and note that the starch grains are being corroded and broken up under the action of diastase. Remove the pulpy endosperm from a seedling, and note the shape of the convex shield-like cotyledon—oval in outline in Maize, circular in Wheat.

181. Date.—Examine a Date seed (*i. e.* the "stone"). Notice the deep groove along one side. Scrape the surface on the other side, to see the small embryo embedded in the stone (endosperm). Cut the stone across at this point; then dip the stone in dilute sulphuric acid and apply iodine (test for cellulose). Plant some Date stones in damp sawdust or soil, set in a warm place (a heated greenhouse, if possible), and sketch stages in their germination. Open the stone in some of the seedlings, and then notice the softening of the stone and the extent to which the cotyledon has grown inside it. Notice in sections of the stone that the cell-walls become thinner, and that starch appears in the young root and shoot, in darkness as well as in light. The digestion (conversion into sugar) of the reserve food (cellulose) is due to the secretion of a ferment (cytase) by the cotyledon.

182. Onion.—Examine a seedling of Onion before the embryo has finally withdrawn its cotyledon from the seed.

Observe (*a*) the long slender *root*, (*b*) the slight swelling at the base of the root marking the position of the relatively short *stem* from which arises (*c*) the long, hollow *cotyledon* whose tip is still within the seed-coat.

Remove the testa and observe the colourless end of cotyledon coiled like a watch-spring as it lies within the seed. During germination the cotyledon absorbed the food from the endosperm and passed it on to the growing parts.

In older specimens observe how the air-exposed tip of the cotyledon withers; also note the formation of secondary roots from the base of the short stem. Slit open the hollow leaf-sheath at its base and discover the delicate pale-green plumule within. In still older specimens the plumule itself has split the sheath as a result of its growth and development.

II. Some Experiments on Germination.

183. Water present in "air-dry" Seeds.—(*a*) Are the "dry" seeds sold by the seedsman quite dry, or do they contain any water at all? Into a dry test-tube (warm the tube all over to make sure it is quite dry) put a few "dry" Peas or Beans and heat over a Bunsen or spirit lamp, applying the flame to the bottom of the test-tube.

Notice the drops of water which condense in the colder upper part of the tube.

(*b*) Weigh about 30 Peas or Beans, and then dry them thoroughly without scorching or charring them at all. This is best done by placing the seeds for a few hours in an oven, or by means of a sand-bath or a water-bath. Then compare the weight of the thoroughly dried seeds, and the percentage weight of water which the "dry" seeds originally contained (usually about 10 per cent). This amount of water, though not sufficient to allow of germination taking place, is evidently necessary for the seed to remain alive and capable of germinating.

A simple water-bath consists of two tin cups and an iron tripod to rest them on; half fill one cup with water, and into it put the other cup containing the seeds to be dried. A simple sand-bath consists of a shallow tin or pan filled with sand, supported on a tripod and heated below as usual, the seeds being placed in a smaller tin or a saucer resting on the sand.

184. Absorption of Water by Seeds —(*a*) Keep some "dry" seeds in a drying-oven or drying-bath until they show no further loss in weight, and then find out whether they swell up in water and whether they germinate The results will show that killed seeds still have the property of absorbing water

(*b*) When a dry seed is placed in water, how much does it absorb, and what proportion do the volume and weight of the absorbed water bear to the volume of the dry seed? Weigh twenty dry Beans; pour water into a graduated vessel until it reaches the 150 c c. mark, then drop in the beans, and shake the vessel to get rid of any air present; the rise in level gives the volume of the Beans. Take them out and place them in moist sawdust for two days, then wipe them dry, weigh them, and find their volume as before. If you have no graduated vessels, use a glass jar with a strip of paper, marked into inches or centimetres, gummed on the outside of the jar. Beans absorb about 130 per cent. of their own weight of water.

(*c*) The swelling of seeds by imbibition of water can be easily demonstrated to a class. Put about 30 grams of dry Peas and an equal amount of water into a narrow cylindrical glass jar. Cover the Peas with a cork; smear the edges of the cork so that it can slide inside the jar, and pass a thermometer through a hole bored in its centre. Weigh the cork down with lumps of lead or a number of weights and mark its position by gumming a strip of

paper on the outside of the jar Fit up a "control" experiment in which a cork with a thermometer hangs into a jar containing some water but no seeds. Note the rise of the cork as the Peas swell and push it up, and compare the temperatures, at the beginning and end of the experiment, in the jar containing the Peas and that containing water (or that of the surrounding air).

(*d*) Does imbibition cause rise of temperature in dead substances as well as in seeds? Put some powdered starch into a tumbler, to form a layer about an inch deep, put an equal amount of water into another tumbler, and set a thermometer into each When the two temperatures are equal, pour the water over the starch, stir with the thermometer, and note the rise in temperature (how many degrees?).

(*e*) If a small wooden box (*e. g.* a cigar-box with the lid fastened down by tacks) is filled with dried Peas and then immersed in water, it will burst as the Peas absorb water and swell. Try this experiment. A large mass of swelling Peas may lift a weight of more than 100 lb

(*f*) The force exerted by swelling seeds can also be shown by filling an ordinary narrow-necked bottle with Peas, and placing it under water in a basin; the bottle should be left uncorked, and some rubber bands should be put round it to prevent the shattered glass from being thrown out. Another method is to fill with dry Peas an empty rabbit-skull and let it lie in water; the bones will be torn apart along the seams (sutures) where they join each other.

(*g*) How is the absorption of water by seeds affected by temperature? Weigh about 30 grams of dry Beans or Peas, place them in a beaker of water at 35° C., set the beaker on a sand-bath with a thermometer in the water, and keep the temperature steady at 35° C for two hours. At the same time place an equal weight of seeds in cool water, with a thermometer; first let the water stand for a time till it acquires the temperature of the room. At the end of two hours, wipe dry both lots of seeds and compare the increase in weight in each case. The seeds that have been kept in water at 35° C. will have absorbed from two to three times as much as those kept in the cool water.

(*h*) Weigh about 30 grams of dry Peas and place them in a 10 per cent. solution of salt in a beaker or tumbler At the same time put a similar weight of Peas in distilled water (or tap water). Compare the weights of the two lots of seeds after two hours, wiping them dry before weighing. Which lot has increased most in weight?

185. Effects of Heat on Seeds.—When a seed is exposed to a fairly high temperature for a few hours all the water it contains is driven off, and the young plant is killed,—we can only tell whether a seed is alive or not by ascertaining whether it will germinate when

exposed to suitable conditions, placing along with it other seeds of the same species for comparison.

Place some dry Beans or Peas in a dry, large test-tube, and an equal number of soaked seeds in a test-tube half filled with water Cork both tubes and immerse them in a beaker of water kept at 60° C. for two hours on a bath—other temperatures and periods of exposure should be tried. Then soak the dry seeds in water, and sow both lots, labelled, in your germination jars and boxes, and expose the two batches to the same conditions. Find out in this way how dry and soaked seeds differ in their ability to withstand the effects of high temperatures

186. Effects of Cold on Seeds.—We find that dry seeds can withstand high temperatures which are fatal to soaked seeds. On placing seeds among ice or a freezing mixture, we find that dry seeds can also resist low temperatures that kill soaked seeds. Dry seeds can germinate after being exposed for a long time to the most intense cold that can be obtained, while soaked seeds are often killed by exposure to the freezing temperature of water or a few degrees below this. Repeat § 185, but immerse the two tubes in a freezing mixture, or place the two lots of seeds on ice instead of using hot water.

187. Is Air necessary for Germination?—This is easily tested either by depriving the seeds of air, or by confining them in a series of closed vessels containing different volumes of air and comparing the results.

(*a*) Drop some seeds into a glass jar or wide-necked bottle, fill up with water and cork tightly. As a control, put some soaked seeds into a similar jar, leaving it open and adding a little water each day to prevent the seeds from becoming dry, but not enough to cover them. Ordinary tap-water contains dissolved air, but as a rule seeds immersed in it, in a corked bottle, do not germinate; to make quite sure that no air reaches the seeds, the water should be previously boiled to expel the dissolved air, and the cork sealed air-tight with vaseline or plasticine. To hold the seeds down, fix them into a spiral coil of wire, easily made by winding iron or brass wire round a tube or a stick.

(*b*) Take four glass jars, all of the same size, and provided with well-fitting corks. Fill these jars to different heights with moist sand, marking each jar into five equal parts, and putting into the first jar enough sand to reach the lowest mark; into the second, sand up to the next mark; and so on The fourth jar will thus contain four times as much sand, and therefore only a quarter as much air, as the first. Into each jar now place a dozen soaked seeds (*e.g.* Cress, Wheat), cork tightly, and seal with plasticine and vaseline. In which jar do the seeds germinate best? Do the results

suggest that germinating seeds cause some change in the air, that they use the air up?

After three or four days carefully remove the cork from one of the jars and lower a lighted taper or match into it: note what happens. Open another of the jars, and dip into it a glass rod which has been dipped into clear lime-water (or baryta-water); note the white precipitate indicating the presence of carbon dioxide.

These experiments show that germinating seeds *respire*—they absorb oxygen and release carbon dioxide, thus changing the composition of the air around them in the same way that animals do by their breathing or respiration.

188. Growth of Seedlings in Light and in Darkness.—Experiments on the respiration of germinating seeds show that the seedling loses carbon, which is released in the form of carbon dioxide To estimate this loss we must dry the seeds and the seedlings before weighing them, since the water present must not be taken into account. Does this loss in dry weight occur both in light and in darkness?

(*a*) Take about forty Beans as nearly alike in size and weight as possible; select four of them as samples, and find their weight after thoroughly drying them on a water or sand bath or in a slow oven. Take the dry weight of a seed, found in this way, as the average. Sow half of the seeds in sifted garden soil in a box which is kept in darkness, the other half in a box kept in full light; water both lots about equally.

At the end of each week measure and record the average height of the shoot in each lot of seedlings; remove three seedlings from each box, wash the roots in running water (do not leave any in the soil or lose them in any way), and dry them thoroughly without charring any part. When quite dry and brittle, weigh each lot and obtain the average weight of the solid matter in each plant. Get a piece of squared paper, as in Fig. 48 (spaces *representing* inches need not, of course, *be* inches). As the weekly observations proceed, trace two lines across the sheet, one (a continuous line) to show the weight, the other (a dotted line) the height of the seedlings grown in light; draw two other lines in red ink to show the dry weight, and the height, of the seedlings grown in darkness.

(*b*) Another method is to use Wheat grains, and grow them with the roots in water. From some Wheat count out thirty-six good sound grains, and divide them into batches of a dozen each; see that the weight of each batch is as nearly as possible the same Dry one batch (*A*) and record the dry weight. Tie a piece of muslin over a tumbler or bowl filled with water, and put a batch (*B*) of seeds on the surface of the muslin, which should be kept wet. Another plan is to use a piece of flannel, stab twelve holes in it, and in each hole place a seed. Keep the tumbler in a warm, dark place, and renew the water every second or third day. Plant the third batch (*C*) as in (*B*) and keep both at about the same temperature, but when the young shoots appear expose (*C*) to the light. When the shoots have grown several inches, carefully remove the seedlings from (*B*) and (*C*), noting the difference in colour between the two sets. Dry them thoroughly, without charring even the finest rootlet, and then weigh each lot and compare the weights of (*A*), (*B*), and (*C*).

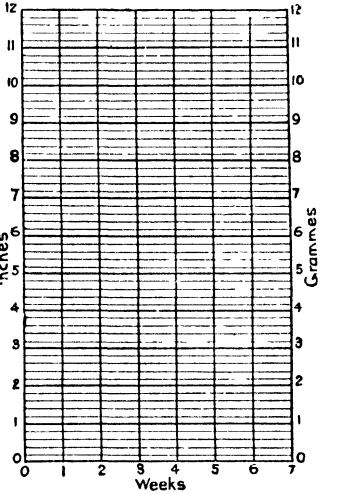


Fig 48.—Chart on which to plot the Curves of Height and Weight

189. Growth in Distilled Water.—We shall see later that green plants get their food from the air and the soil. The young plant in a seed has a store of food for its early growth, a store which is sometimes very scanty and sometimes (as in Pea and Bean) very abundant or even extravagant. Tap-water and rain-water are not pure, but contain dissolved substances, while soil-water and river-water are

much richer in dissolved salts. In order to find out how long the stored food lasts, we should therefore use distilled water, so that we know exactly what the roots are supplied with.

Grow various seeds in jars containing distilled water, fixing them either into holes in muslin or flannel, or into split or bored corks, fill up the water as required, but always use *distilled* water. Keep some of them in darkness, expose others to the light, and compare their growth and their increase or decrease in dry weight Another method is to let the roots grow into sand that has been washed thoroughly with tap-water and then with distilled water, using the latter for watering afterwards.

Seedlings grown with their roots in pure water do not live very long as a rule, especially if they are kept in darkness, when their dry weight diminishes, and they die after using up the stored food. In the light, however, the seedlings live longer, and for a time increase in dry weight. Bean and Pea seedlings exposed to light, with their roots in distilled water, grow for several months and may even produce flowers, though they are small and weakly as compared with seedlings grown in soil. Small seedlings, with scanty food-stores—*e g.* Mustard—may live only a few weeks when exposed to light, with the roots in distilled water, and die still earlier if kept in darkness

190. Energy Expended in Growth of Root and Shoot.—We know that the radicle and plumule of a Bean seedling, for instance, must exert considerable force in growing through the soil—the root protected by its cap, the shoot by its recurved tip (or by its pointed form in seedlings like Maize and Wheat). We can roughly measure the force exerted, and by calculation we can roughly determine the amount of energy that is set free by the oxidation of the carbon contained in the seed's store of reserve food.

The combustion or oxidation of 1 gram of carbon—the Broad Bean seed contains roughly 1 gram of carbon—sets free enough energy to raise 8 kilograms of water from 0° to 1°C., and about 2 litres of carbon dioxide are given off; if all this energy were used in mechanical work, it would suffice to raise 3,400 kilograms through 1 metre, but the energy is used up by the plant in the form of heat and of chemical work, in addition to mechanical work.

(*a*) Plant some Beans about 3 in. deep in moist soil or sawdust in a flower-pot, and pack stiff clayey soil (or plasticine) firmly above them. Watch them to see whether they emerge at the sides or whether they push the whole mass of clay upwards.

(*b*) Invert a short test-tube over a Bean seedling with a plumule about 3 in. long, then place over this a vertical glass tube open at both ends, inside which the test-tube can slide freely, and clamp this tube to a support. Into the upper end of the open tube place a second test-tube containing mercury or shot Mark with a paper strip the level of the top of the shoot, and see what weight of mercury or shot is required to prevent the shoot from continuing to grow upwards. Another method is to use a spring inside a closed tube in place of the mercury or shot; measure how much the force of the shoot, pushing up its tube, compresses the spring, then find what weight is needed to compress it to the same extent.

(*c*) You have probably used mercury in various experiments, and know that it is a very heavy liquid (13½ times heavier than water). Fix a seedling (Bean, Pea, etc, should be tried) to the side of a small dish containing mercury with a layer of water above it, and see whether the root will grow down into the mercury. The seeds may be pinned to a cork which is securely fixed to the rim of the dish (*e.g.* a saucer) by making a slit in it and jamming it tightly on the rim; each seed should of course be fixed by *two* pins.

(*d*) Fix a young Bean seedling so that its root grows in a small tube filled with moist soil or sawdust, and place this tube within a larger one containing a spring. The root grows downwards with a force equal to over 300 grams (about 11 oz); measure the diameter of the root and calculate the force it exerts per square centimetre or square inch.

191. Effect of Removal of Cotyledons.—Deprive Beans, Peas, and other germinating seeds of both of their cotyledons—in some cases just after the seed has been soaked, in others after the radicle has grown 5 cm. long, in others after the plumule has grown 5 cm. long. In each case place some of these seeds, along with untouched seeds for comparison, in the light; and place others, also with untouched control seeds, in darkness.

192. Effect of Removal of Foliage-leaves.—Remove the foliage-leaves from (*A*) a young Bean plant which has not yet used up the food in its cotyledons, (*B*) an older seedling whose cotyledons have fallen off (if they have

shrunk considerably, pull them off). Does the removal of the foliage-leaves check the growth of the plant, as compared with that of similar plants left untouched? In which case (*A* or *B*) is the effect greater?

III Some Experiments on Growth.

193. Measurement of Rate of Growth.—Seedlings of Broad Bean, Pea, and Phaseolus (French Bean or Scarlet Runner) afford excellent material for experiments of the rate of growth of roots and stems. The Broad Bean and Pea seeds should in most cases be placed with the hilum downwards; the Phaseolus seeds should be laid horizontally so that the root will grow out at right angles to the long axis of the seed. To avoid heliotropic curvature, grow the seeds in darkness; the temperature should be kept as uniform as possible, at about 20° C.

194. Daily Growth of Root.—Place six soaked Peas in a shallow dish of wet sphagnum, or simply with water half covering them; label each seed with a number or letter on a small piece of paper fixed by a pin through the cotyledons. Keep in darkness, and at the same hour each day measure off and record the length of each root. Note that (1) there are individual differences between the seedlings in the daily increments in length; (2) in each case the daily growth of the root is at first slight, then gradually increases until it reaches a maximum (usually by about the eighth day in Peas at 20° C.), and then gradually falls off again. Plot the measurements on squared paper and construct the curve showing the rise and fall in the rate of growth in length.

195. Grand Period of Growth.—A similar result is obtained with all growing organs. The rate of growth of a growing organ (root, stem, leaf, etc.) is not uniform, and the same applies to each of its constituent cells. A growing structure, even under constant external conditions, does not undergo equal amounts of growth in equal successive time intervals. When growth begins, its rate is at first slow; then it gradually becomes accelerated until a maximum

rapidity is reached, after which it gradually diminishes until growth ceases altogether. This rise and fall in the growth rate, extending over the whole of a growth period, is called the "grand period of growth."

196. Grand Period in Roots.—Some additional simple experiments on the grand period of growth should be made. Place a germinating Bean or Pea in the bulb of a long thistle-tube, so that the root can grow down the tube Set the tube in a bottle containing water, put wet sphagnum or cotton-wool in the bulb with the seed. Read off the length of the root daily with a scale, or gum a strip of paper along the tube, each day at the same hour mark the position reached by the root-tip and measure the intervals (the daily amounts of growth).

197. Grand Period in Shoots.—Grow Phaseolus seedlings in pots of soil, and make daily measurements of the epicotyl (the stem region between the cotyledons and the paired primary foliage-leaves); as long as the tip of the epicotyl remains curved, measure with a strip of paper.

Also measure separately the daily growth in length of the successive internodes of a Bean or Pea seedling, and note that (1) each internode shows a grand period; (2) when the internodes have fully elongated the oldest are usually relatively short, then come longer ones (the fifth, counting upwards, is generally the longest in the Pea), while the youngest internodes are again shorter—this is another example of the grand period.

Since these results are obtained with plants kept in darkness and at constant temperature, we may infer that the growth energy of the different internodes varies owing to *internal* causes.

198. Distribution of Growth in Growing Organs.—The preceding experiment suggests a simple method for finding out whether or not any portion of a growing organ elongates uniformly, *i. e.* for investigating the distribution of rate of growth in length of roots, stems, etc All we have to do is to mark the organ with parallel transverse lines at regular short intervals,

limited is much longer (3 or 4 cm. in Phaseolus epicotyl) than in roots (4 to 8 mm as a rule). Hence in dealing with stems and flower-stalks it is sufficient to make the marks 5 mm. apart.

201. Grand Periods of the Growing Zones.—The two preceding experiments show that in both root and stem the youngest part grows very little, then comes a region of vigorous growth, and farther back there is again little growth. On continuing the observations, we note that growth soon ceases in the older zones, while the maximum is shifted forward to the younger zones, and still later the rate of growth in these zones in turn diminishes.

That each individual zone passes through a grand period in this way—the zones nearest the apex being at the beginning of their grand period and those farthest away from it at the end of theirs—is strikingly shown as follows. Mark a single transverse line on a Bean root at a point between 2 and 3 mm. from the tip, then carefully mark a second line 1 mm. behind the first, so as to have a zone 1 mm. long in the most rapidly elongating region. At the same hour each day measure this zone and record its daily increase in length. At first the rate of growth is slow, but soon it becomes rapid, reaches a maximum about the third or fourth day and maintains this for about three days, then falls off and by about the tenth day ceases altogether.

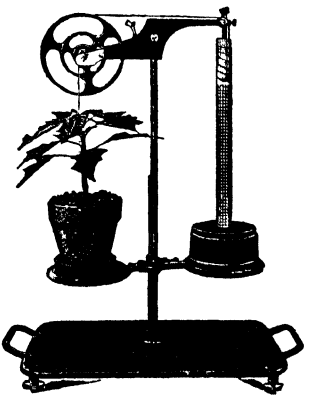


Fig. 50.—Ganong's Auxograph (Self-recording Auxanometer).

202. Growth Measurement Instruments.—Various special instruments have been devised by means of which the growth of organs may be magnified and simply demonstrated (**auxoscopes**), or measured accurately with or without magnification (**auxano-**

meters, or measured and recorded automatically at hourly or other short intervals (**auxographs**, or self-recording auxanometers).

In most of these instruments (Fig. 50) there is a wheel or pulley over which passes a cord attached by one end to the stem or flower-stalk (special devices are necessary in the case of roots and leaves), the other end carrying a small weight to keep the cord taut. The wheel is fixed above the plant, and the growth in length is observed in various ways · (1) The descent of the weight—equalling the elongation of the plant—is read off daily on a graduated scale fixed vertically alongside it ; (2) a simple apparatus for magnifying the movement is afforded by attaching to the wheel a light pointer which moves over a graduated arc or a disc of cardboard.

Either of these simple auxanometers can easily be made, the arc-pointer form being useful for demonstration during a lesson : rapidly growing stems should be used, *e.g.* the young flowering stem of a sprouting Narcissus bulb. See Appendix III., Fig. 115.

203. Influence of External Factors on Growth.—Observations on growth—*e.g.* auxograph records—show that there are great variations in the rate of growth. These are largely due to changes in the varying external conditions, of which the most important are (1) temperature, (2) light; others are (3) humidity of the air, (4) water content of the soil.

The influence of food supply on growth is readily seen on comparing the growth of Bean seedlings from (*a*) seeds with both cotyledons removed, (*b*) seeds with one cotyledon removed, (*c*) intact seeds (see §§ 191, 192); or of Wheat seedlings from (*a*) grains with endosperm removed, (*b*) intact grains. This leads to the consideration of energy supply, and this again is connected with respiration. To study the dependence of growth upon respiration, we may simply compare the growth of similar organs (*a*) when supplied with oxygen, (*b*) when deprived of oxygen (§ 205).

The consideration of the pressure exerted by growing parts (§ 190) as the result of turgescence set up by osmosis leads naturally to an important aspect of the relation of osmotic pressure to growth—namely, the relative tensions of the tissues in growing stems and roots (§§ 207-210).

204. Influence of Temperature on Growth.—For exact work it is necessary to use instruments by which a constant temperature may be maintained. This is done by means of thermostats —constant-temperature chambers or ovens. (1) We may expose a

single plant to various degrees of temperature for equal periods of time, keeping all other conditions constant; this is done either by using a single thermostat and altering the temperature at intervals, or by transferring the plant from one thermostat to another at a different temperature (2) We may expose a series of similar plants to different degrees of temperature; this is done by using a differential thermostat, consisting of a series of chambers cooled (by ice or by circulating water) at one end and heated at the other, one plant being placed in each chamber and the temperatures of the chambers ranging from, say, 5° to 60° C

As a rough experiment, sow a number of seeds of the same kind in a series of three or four pots, giving equal light, air, and water to each. Place the pots in different positions known to vary in temperature, in one of the following ways :—

(*a*) Place some soaked seeds in a glass jar and cover them with moist sawdust; plunge the jar into a box containing pieces of ice, which must be renewed as they melt. The ice will last longer if the box containing it is set into a larger box, and the space between the two boxes is packed with dry sawdust (why ?)

(*b*) Another method is to use two boxes as in the preceding, but to place in the smaller box a single bit of ice, with dry sawdust below and around it; place the seeds directly on the ice and cover them with dry sawdust, which will be kept moist by the melting ice.

(*c*) In winter and spring the minimum temperature for germination should be determined for as many seeds as possible. Into a large flower-pot or seed-pan put some bits of broken earthenware at bottom, and fill up the rest of the pot with sifted soil. Plant in the pot a few seeds of different kinds, and bury the bulb of a thermometer at the depth of the seeds, tying the thermometer stem to a stick thrust into the soil. Sink the pot up to its rim in the soil of a garden bed and record the temperature each day, looking for any signs of germination. After two or three weeks bring the plants indoors; keep the soil moist; make notes of your observations Other pots should be kept in different parts of the house or school, in addition to those kept outside. Such experiments will show that warmth hastens germination, while cold retards it.

205. Growth dependent on Oxygen.—(1) Soak six Peas in water, and let them germinate until the root is about 1 cm. long. Measure the length of the root of each seedling from an ink mark on one cotyledon, then pass three of the Peas (*A*) up into an inverted test-tube of mercury, as in the experiment on intra-molecular respiration. Place the other three Peas (*B*) in wet sawdust or sphagnum. After a day or two, measure the roots again and note that in *A* very little growth has occurred.

(2) Take six germinating Beans with roots from 2 to 3 cm. long; mark each root with a transverse ink line at 1 cm. from the tip. Fix the seeds by long pins to the corks of two tall wide-mouthed jars, placing three seeds in each jar. Fill *A* with water, so that the seeds are submerged; in *B* place only a little water, so that the seedlings will be growing in damp air Measure the roots again after a day or two Then fill up *B* with water, and note that the rate of growth of the roots is diminished during the succeeding days.

IV. Turgor, Tissue Tensions.

206. Wilting due to Plasmolysis.—(*a*) Pull up whole seedlings or cut off their shoots, and let them lie on the table; they become limp (wilted, flaccid), and it is easy to prove (*e.g* by weighing before and after) that they have lost water in wilting. Put the limp shoot into water; it becomes firm again.

(*b*) Cut off the shoot of a seedling and put it into 5 per cent. salt solution. When the shoot has become limp, wash it under a tap, set it in water, and note that it turns firm (turgescent) again.

(*c*) The shoots used in these two experiments are not necessarily killed unless they have been allowed to become dry, or unless the salt solution is too strong or they have been kept in it too long. Prove this by pulling up whole seedlings, making them flaccid by means of salt solution, and re-planting them in wet sawdust or soil.

207. Longitudinal Tissue Tension.—In addition to the three supporting or "skeletal" tissues—wood-vessels, sclerenchyma, collenchyma—the ordinary thin-walled tissue (parenchyma) plays an important part in maintaining the rigidity of herbaceous stems, as well as of petioles, leaf-blades, and flower-stalks, by the turgidity of its cells. In a herbaceous stem the pith has a strong tendency to elongate, but this is hindered by the outer tissue, and the state

of strain thus set up tends to keep the stem rigid and erect. The outer tissue is on the stretch, tending to shorten, while the inner tissue is under compression.

(*a*) Cut short longitudinal slits in the cut end of a seedling stem, or the flower-stalk of Dandelion, Tulip, etc., and set it in water The slit parts curl outwards, evidently because the inner cells absorb water more rapidly than the outer ones.

(*b*) Cut off about 50 cm. from the youngest part of a vigorously growing Elder shoot. Slice off the tissue from two opposite sides, so as to obtain a flat strip the whole length of the original piece of stem. Bisect this strip, and note that each half bends outwards.

(*c*) Cut off about 6 cm. of internode from a stout young Elder stem, and measure it accurately. Isolate the pith, by slitting the outer tissue and then removing the hard woody cylinder ; measure the pith, and note that it has become longer on being isolated. Now place the pith in water, and after a few minutes measure it again : it has increased further in length. Next, place the pith in 10 per cent. salt solution for some time ; measure and note the decrease in length Then rinse the pith in water and place it in a large vessel of water for some time : it becomes longer again.

(*d*) Cut from vigorously growing shoots (*e g.* Elder, Tobacco-plant, Sunflower) some straight young internodes 4 or 5 cm. long. Draw four straight parallel lines on a card, lay an internode on each line in turn, and mark off on the line its two ends. Then remove from the whole length of the internode (1) the epidermis, (2) the cortex, (3) the wood, (4) the pith. Mark off the length of each of the four strips of tissue on one of the four lines, and note that the lengths of the isolated strips of tissue increase from without inwards ; as compared with the intact internode, the pith is longer, the epidermis shorter, and the intermediate tissues are of about the same length.

Hence the pith is in a state of compression, and the epidermis in one of tension. It is sufficient in experiments of this kind to compare the lengths of the intact stem, the isolated epidermis, and the isolated pith. The amount of the tension in the intact internode may be expressed as a percentage ; if the length of the intact internode is 50 mm., that of the isolated epidermis 49 mm., and that of the isolated pith 54 mm., the tension percentage is 10.

(*e*) In the same way determine the lengths of (1) the intact internode, (2) the isolated epidermis, (3) the isolated pith, in several internodes of a growing shoot, and calculate the percentage tension in each internode. Note that the tension in the youngest internodes is small, rises in those rather older, and again falls off in the still older internodes. This shows that the longitudinal tension is due chiefly to the turgescence of the pith cells, which absorb much water, so that the pith tends to elongate and therefore

to stretch the extensible outer tissues, but the latter are elastic and therefore tend to compress the pith. As the stem grows older, the pith loses its water and stops growing. Hence the longitudinal tension disappears, but in its place there appears transverse tension (§ 208).

(*f*) By finding what strength of salt or sugar solution is needed to bring about plasmolysis, we get a rough idea of the osmotic force of the cell-sap. Saltpetre solutions are generally used; a 1 per cent. solution of this salt (nitrate of potash, KNO_3) exerts a pressure of $3\frac{1}{2}$ atmospheres.

(*g*) Split a Dandelion stalk longitudinally into four strips and notice that each strip at once becomes curved, with the epidermis on the concave side: why? Place some strips in water, others in strong (about 10 per cent.) salt solution, and observe the differences in the curvature caused by the changes in the turgidity of the inner tissue—*i.e.* that nearest the centre of the stalk.

(*h*) Cut a long narrow strip of Dandelion stalk and fasten the ends securely, by threads or pins, close together to a piece of wood. Dip the strip into water and carefully watch how it coils; part of it twists in one direction, part in the opposite direction, and between these there is a part where the spiral reverses. This gives an excellent illustration of the coiling of a tendril, which shows a similar reversed spiral when the free end has become fixed to a support.

(*i*) Split a Dandelion stalk and cut the curled-up strips into rings. If the ring is placed in water it will become more tightly coiled; if in a very strong solution of salt or sugar, it will open out. In this way we can find out what strength of solution produces neither increase nor decrease of curvature and therefore equals the osmotic force of the soft tissue, *i.e.* the osmotic strength of the cell-sap.

(*j*) Prepare a 5 % solution of common salt, by stirring 25 grams of salt into 500 c.c. of water. Get ten saucers ready, and into one pour 100 c.c. of the solution. Then, using a graduated beaker, dilute the 5 % solution with water, so as to make 4 %, 3 %, 1 %, 0·5 %, 0·4 %, 0·3 %, 0·2 %, and 0·1 % solutions, pouring 100 c.c. of each into one of the saucers. In each saucer place two or three rings, and find out in which saucer the rings become neither more nor less curved. For comparison place some rings into a saucer containing plain water.

(*k*) Measure an "internode" of young Sunflower stem, then extract the pith by using a cork-borer, and measure (1) the isolated pith, (2) the outer tissue: the former has elongated, the latter contracted.

(*l*) Another and simpler method is to use the long leaf-stalks of Rhubarb or of "Arum Lily." Lay the stalk down, cut the ends

squarely, and measure the length carefully. Then remove a strip of the outer tissue and measure: it will be shorter than the whole stalk. Next strip off the whole of the outer tissue and measure the pith, which will be longer than the whole stalk.

208. Transverse Tension in Stems.—Cut transverse slices from a fairly old portion of a woody twig, *e.g.* Willow, and measure its circumference with a strip of paper. Make a vertical slit in the stem and carefully remove the outer tissue. Now try to replace the ring of cortex on the wood; the ends of the ring will not meet now, showing that the cortex was in a state of tension in the intact stem.

Measure the distance between the two ends of the split cortex ring after replacing it, and from this calculate (1) the length of the isolated ring; (2) the tension to which the cortex was subject, as a percentage of the circumference of the intact stem. For instance, from a Willow twig, a slice was cut with circumference 200 mm.; the distance between the cut ends of the isolated cortex ring was 9 mm.; therefore the percentage tension of the cortex was 4·5.

209. Distribution of Transverse Tensions in Stem.—It is interesting to determine simultaneously the transverse tension in different portions of the same stem. Cut out slices from the top, middle, and base of a Sunflower stem, for instance; in each case measure the circumference, then remove and measure the isolated cortex ring. Note that the tension in the youngest parts is small, and that it increases progressively in the older parts.

210. Tension dependent on Water in Tissues.—That the tension of the outer tissues in a woody stem depends upon the amount of water present is easily shown. Cut six slices from a Willow branch, and determine the tension of the cortex in three of them at once; determine it in the other three slices after leaving them in water for a day. Note that the tension increases considerably, owing to the absorption of water.

211. Extensibility and Elasticity of Tissues.—Cut fresh pieces of stem of Elder, Honeysuckle, Vine, or Aristolochia. Make a transverse mark with Indian ink at the upper and the lower ends of (A) a young internode near the apex of the shoot, (B) an older internode. Now lay the stem against a scale, and stretch it as much as possible without breaking it. Note that the younger internodes are much more extensible than the older; that the stem shortens again when left to itself after having been stretched—hence the tissues are more or less elastic; and that they do not regain their original length, but remain permanently longer—hence the tissues are incompletely elastic.

212. Flexibility of Tissues.—That growing tissues are flexible, but incompletely elastic, is easily shown. From one of the plants just named choose a straight flexible internode. Mark a card with concentric circles, and bend the stem over the card until its axis coincides with one of the circles: note the radius of curvature Leave the stem to itself for some time; it does not become straight, but remains permanently bent—determine its radius of curvature.

213. Relation between Turgidity, Growth, and Extensibility.—Determine the distribution of growth in the shoot and root of a seedling (§§ 199, 200). Then lay the seedling in a 10 per cent. solution of salt; after an hour or two the tissues will be completely plasmolysed. Measure the zones again, and note that they have become shorter through loss of turgescence, and that the plasmolysis is greatest in the zones which have been growing most rapidly.

The preceding experiment suggests that the rate of growth of the cells depends on the amount of their turgor tension. This tension is determined by (1) the amount of the osmotic pressure and (2) the amount of the resistance offered by the stretched cell-walls owing to their extensibility.

Take a Phaseolus seedling with epicotyl about 4 cm. long; mark it into zones 5 mm long; after two days, measure the zones again,

and record the lengths. Then cut off the epicotyl, and plasmolyse it with 10 per cent. salt solution. On a piece of cork or soft wood make two marks corresponding to the length of the marked portion of the epicotyl. Lay the plasmolysed epicotyl on the cork, so that the highest ink mark on it corresponds with one of the marks on the cork. Hold this end of the epicotyl down, and pull at the other end so as to bring the lowest ink-mark on the epicotyl to the other mark on the cork. Pin the stretched epicotyl to the cork, lay alongside it a scale, and measure off the lengths of the zones. Note that the tissue is more extensible in the younger than in the older zones

The result of this experiment shows that there is a direct relation between the rate of growth, the amount of turgor tension, and the extensibility of the tissue in the different zones.

CHAPTER V.

WATER CULTURE, PHOTOSYNTHESIS, RESPIRATION.

I. Ash Analysis and Water Culture.

214. Proportion of Water in Fresh Tissues.—In order to analyse a plant, it is necessary to determine the percentage weights of (1) the water it contains in the fresh state, (2) the carbon present in the dried material, (3) the incombustible ash left after strongly heating the dried material. In order to determine the proportion of water, and at the same time the "dry weight" of the plant, it is only necessary to dry the fresh plant thoroughly without charring it. If the plant is now burnt, the carbon, hydrogen, and nitrogen which it contains are given off in the form of gases (carbon dioxide, water, oxides of nitrogen); hence these three elements may not be present in the ash that remains after complete combustion.

215. Carbon and Ash.—Dry and weigh a porcelain crucible or evaporating dish, place in it half of the oven-dried leaves, and weigh the crucible again, to obtain the weight of the dried leaves placed in it. Heat the crucible strongly: the dry material chars, and in ten minutes or so it is reduced to fine ash, which should not be allowed to glow. Find the weight of the ash, weighing the crucible and its contents twice or thrice until no further loss occurs on heating it. To ascertain roughly the amount of carbon,

weigh the rest of the dried leaves, place them in a weighed crucible, cover them with a weighed quantity of dried sand, and after about ten minutes' heating turn out the contents and find the weight of the charcoal (carbon + ash).

216. Ash Analysis —The chief elements to be tested for in analysing the ash of plants are Calcium, Potassium, Magnesium, Phosphorus, Sulphur (the two latter being present as acids) The ash should *not* be heated so strongly as to make it burst into flame.

(*a*) Is the ash soluble in (1) water, (2) dilute hydrochloric acid, (3) strong hydrochloric acid? Find out in each case by boiling some of the ash in a test-tube with water or acid, allowing the undissolved part to subside and evaporating some of the liquid, or heating it to dryness, on a watch-glass or evaporating-dish. The insoluble residue, after treatment with strong acid, contains chiefly silica and carbon.

(*b*) Place about 10 grams of ash in a 500 c.c. flask, moisten it with a small quantity of strong nitric acid, then add about 20 c.c. strong hydrochloric acid and heat on a tripod (or "digest" it for half an hour on a water or sand bath at boiling-point). Rinse the contents of the flask into an evaporating basin and heat to dryness. Moisten the residue with strong hydrochloric acid, add about 200 c.c. of water, and filter. Make the filtrate up to 600 c c. with water and divide it into four parts :—

(i) To one part add, in a large test-tube, some barium chloride solution. The finely divided white precipitate (barium sulphate) indicates the presence of **sulphur** (as sulphuric acid). Verify this by mixing some dry ash with carbonate of soda, heat on charcoal with the reducing blowpipe-flame, and (1) put a few drops of dilute hydrochloric acid on the fused mass (the sulphuretted hydrogen given off is easily recognised by its odour), (2) put a little of the mass on a silver coin and add a drop of dilute acid (a black stain of silver sulphide is formed). These "dry" tests may fail, however, if but little sulphuric acid is present.

(ii) To some ash solution in a test-tube add an equal bulk of strong nitric acid, then three or four times its bulk of ammonium molybdate. A yellow precipitate indicates presence of **phosphorus** (as phosphoric acid).

(iii) To test for **iron**, add potassium ferrocyanide : a dark blue precipitate (Prussian blue) is produced.

(iv) It is necessary to remove the phosphates from the ash solution, as follows. Neutralise with ammonia, then add acetic acid

till the solution is distinctly acid again, and then ammonium acetate in excess. Now add ferric chloride till no further buff-coloured precipitate (ferric phosphate) is produced and the solution becomes red (owing to ferric acetate). Boil the solution till it is colourless, filter, and reject the precipitate.

To the solution thus obtained add ammonium chloride, ammonia, and ammonium carbonate; a white precipitate indicates the presence of **lime**. Filter, and to the filtrate add sodium phosphate: a white precipitate (often formed only after shaking the liquid and letting it stand for some minutes) shows that **magnesia** is present.

Filter, evaporate the filtrate to dryness, and test the residue for soda and potash. Add a few drops of platinic chloride to the residue, evaporate again, then add some alcohol: a yellow crystalline precipitate shows that **potash** is present. Or dip a clean platinum wire into hydrochloric acid and hold it in a Bunsen or spirit-lamp flame until it no longer colours the flame yellow (owing to presence of soda). Then dip the wire, moistened with hydrochloric acid (strong), into the residue and put it in the flame. **Potash** turns the flame *violet*, but if the *yellow* (**soda**) colour is too strong look at the flame through a *thick* piece of blue glass: the soda colour is cut off and the reddish-violet potash flame is seen.

217. Water Culture.—Analysis shows that in all plants at least 13 elements are present—Potassium (K), Sodium (Na), Calcium (Ca), Magnesium (Mg), Iron (Fe), Carbon (C), Hydrogen (H), Oxygen (O), Nitrogen (N), Sulphur (S), Phosphorus (P), Silicon (Si), and Chlorine (Cl).

Since various other elements occur in different plants, analysis alone leaves it doubtful whether all these elements are essential for life and healthy growth—although this is obviously the case with the elements C, H, and O which are present in all the organic compounds found in plants, with N and S which are present (along with C, H, and O) in proteins, and also with P which is an essential element (along with C, H, O, N, and S) in nucleo-proteins.

However, in order to ascertain definitely which elements are indispensable for the nutrition of a green plant, we must offer its roots a solution of salts containing (1) all the essential elements, (2) solutions in which one or other of these elements is omitted. This method of research is called "water culture," and the solutions used are called "culture solutions."

218. Water Culture Jars.—Get about 20 large glass jars of equal size—if possible to hold 4 or 5 litres, certainly not less than 2 litres. Fit each jar with a wooden cover or a cork, having in the centre a hole for the plant, a slit running from this hole to the edge of the cover (so that the plant can be inserted or removed easily when necessary), and a second and smaller hole for a stick to which the plant can be lashed for support. Take care to keep the cover, as well as the part of the plant in contact with it, quite dry—failure in water cultures is often due to damping-off caused by Fungi. If a stick is used to support the plant, it is hardly necessary to put in any packing material round the base of the shoot—if any packing is used, soft asbestos is much better than wet cotton-wool.

It is also advisable to cleanse the jars thoroughly before starting the cultures—wash with water, rinse with strong nitric acid, wash again with water, rinse again with water, then with strong solution of mercuric chloride, and lastly with boiled water.

219. Water Culture Solution.—Various recipes have been given for the making up of culture solutions for green plants. The following **(Knop's Solution)** has the advantage that it provides just the essential elements, neither more nor less. Weigh out—

(*a*) Potassium nitrate, KNO_3 . . .. 1 gram.
(*b*) Potassium phosphate, KH_2PO_4 1 gram
(*c*) Magnesium sulphate, $MgSO_4$ . . 1 gram.
(*d*) Calcium nitrate, $Ca(NO_3)_2$ . . . . . 3 grams

Dissolve *a*, *b*, and *c* in 1 litre of water, then add the calcium nitrate—a precipitate of calcium phosphate is formed, but this is gradually dissolved and utilised by the roots This is a solution of 0 6 per cent. strength, and may be diluted to the extent required; when a dilute solution is made from this stock solution, shake the bottle well so as to include a right proportion of the precipitate in the dilute solution.

To make a 0·1 per cent. solution, which is quite strong enough for water culture experiments, add 5 litres of water to 1 litre of the stock solution; then add a few drops of 5 per cent. solution of an **iron** salt, *e.g.* chloride, so that the complete solution contains a trace of iron.

220. Water Culture Experiments.—In order to get good average results, we require to set up nine sets of cultures with two or three similar plants in each set—in case of anything going wrong as well as to eliminate individual differences. The nine sets are wanted for growth in (1) full culture solution; (2) solution minus Iron; (3) solution minus Calcium; (4) solution minus Magnesium; (5) solution minus Potassium; (6) solution minus Nitrogen; (7) solution minus Sulphur; (8) solution minus Phosphorus; (9) distilled water.

One kind of plant must, of course, be used throughout the experiments. Good results may be obtained with seedlings of Bean, Pea, Wallflower, Maize, Oats; or cuttings of Willow, Fuchsia, Horse Chestnut, Tradescantia, etc. Try different plants each time you start a series of cultures. If seedlings are to be used, select seeds as nearly alike in weight as possible, weigh a batch of seeds, determine the average dry weight; then soak the seeds and sow them in a germination jar or in moist sphagnum or sawdust. When the roots are several centimetres long, select seedlings as nearly alike in development as possible; fill the jars with distilled water to within about 5 cm. of the top—so that the cover and the seed itself shall not be wetted—and fit a seedling into each jar so that its root dips into the water.

For four or five days allow the plants to grow in water. Then take the jars in pairs and treat each pair as follows:—

(1) **Full solution**—replace the water with the complete culture solution.

(2) **Full solution minus Iron**—omit the iron compound.

(3) **Full solution minus Calcium**—substitute (in equal amount) potassium nitrate for the calcium nitrate.

(4) **Full solution minus Magnesium**—substitute potassium sulphate for the magnesium sulphate.

(5) **Full solution minus Potassium**—omit potassium nitrate and substitute calcium phosphate for the potassium phosphate.

(6) **Full solution minus Nitrogen**—omit potassium nitrate and substitute calcium phosphate for the calcium nitrate.

(7) **Full solution minus Sulphur**—substitute magnesium nitrate for the magnesium sulphate.

(8) **Full solution minus Phosphorus**—substitute potassium sulphate for the potassium phosphate

(9) As a control to all the other cultures, leave one pair with **distilled water** only.

221. Progress of the Cultures.—Various steps must be taken in order to ensure success with water cultures. Tie a roll of black paper or cloth round each jar, to darken the roots. Each day add distilled water, to replace that lost by evaporation and absorption —take care not to let the cork get wet—and stir up the liquid with a rod; also force air into it with a bicycle pump—otherwise the roots may suffer from lack of oxygen.

The solution must not be allowed to become alkaline in reaction; if it turns blue with litmus paper, add a little 5 per cent. phosphoric acid until it is slightly acid in reaction. Once a month pour off the solution in each jar, rinse out the jar with distilled water, fill up the jar with distilled water and let the plants grow in this for a few days before filling the jar again with fresh culture solution.

Label each jar—"Full Solution," "Minus Iron," "Minus Nitrogen," and so on. Keep a record of the progress of the plants, noting in each case their general characters—height, number of leaves, size of leaves, etc.

In the case of seedlings with large food-containing cotyledons, *e.g.* Broad Bean, the plant will often show healthy though somewhat meagre growth, and eventually produce flowers and fruits when the roots are supplied only with distilled water. In this plant the cotyledons contain quite sufficient of some at least of the necessary elements, without depending upon external sources. The cotyledons should therefore be removed from all the plants, when Beans or Peas are used, and this should be done simultaneously when the seedlings are placed in the jars.

In the absence of iron, the plant produces normal leaves at first, but after a time the new leaves formed are white—this condition of **chlorosis** is readily remedied by either adding an iron salt to the culture fluid or by applying it in very weak solution to the chlorotic leaves, which then turn green owing to the formation of chlorophyll. Chlorotic culture plants are easily obtained in the Maize or Sunflower; in the case of Beans, the cotyledons contain enough iron for the whole plant.

Besides noting that in the absence of the essential elements the plant grows badly, a rough comparison of the amount of increase in each case should be made by drying the seed-raised plants in the incomplete solutions, as soon as they show obvious signs of arrested growth and approaching death, and noting their dry weight as compared with that of the seeds from which the plants started.

II Photosynthesis.

222. Iodine Test for Photosynthetic Starch in Foliage-leaves, etc.—Pick leaves from various plants which have been exposed to light in the usual way. To test the leaves for **starch,** which is in most green plants the first *visible* product of photosynthesis, the chlorophyll should be removed by means of alcohol, and the blanched leaves placed in dilute iodine solution. The extraction of the chlorophyll is hastened by boiling the leaves in water for a few minutes before steeping them in the alcohol, also by using warm alcohol or placing the alcohol, containing the leaves, in a large test-tube set in a beaker of water and warming over a sand-bath (to prevent the ignition of the inflammable alcohol or its vapour). It will be found that the leaves of Tropaeolum, Primrose, and Fuchsia are readily decolorised and otherwise well suited for photosynthesis experiments, but other plants should be tried.

Since the alcohol makes the decolorised leaves brittle, soften them by steeping in hot water for a minute or two; place them in iodine (dissolved in potassium iodide) for a few minutes, rinse them in water, then place them in clean cold water in a saucer. A yellow or brownish colour indicates absence of starch; if the leaves contain starch they will turn blue or almost black. The colour obtained depends upon the amount of starch present in the tissue and the strength of iodine solution used The leaves may be preserved in alcohol, which destroys the blue colour, and may afterwards be again treated with iodine after being rinsed in hot water—but it is always better in class work to start each experiment from the beginning.

Since the colour given with the iodine test is often by no means blue but a purplish brown, it is a good plan to place the leaf for a few minutes in benzole after treating it with iodine. The benzole removes the iodine from the protoplasm and the cell-walls, but does not affect the blue "starch iodide," hence this method causes the blue colour to show up clearly, being no longer masked by the brownish colour of the iodine-stained protoplasm and cellulose.

223. Microscopical Detection of Photosynthetic Starch.—To detect the presence of the small starch grains formed by photosynthesis in the chloroplasts of assimilating cells—*e g.* the mesophyll-cells and the guard-cells of leaves—cut vertical or tangential sections of the leaves if thick, or mount entire thin leaves, and treat by one of the following methods.

(1) If the tissue is very transparent, simply treat with iodine solution, and note that the starch grains are associated with the chloroplasts.

(2) Place the leaf in hot alcohol, until decolorised; then place it in potash solution; rinse in water, treat with dilute acetic acid to neutralise the potash; rinse again in water; then treat with iodine and mount in water.

(3) Another method is to make up Schimper's **chloral-hydrate-iodine,** by dissolving chloral hydrate crystals in as much water as will cover them, and then adding to the solution a little iodine tincture; on placing this solution on a thin leaf, the chlorophyll is dissolved, the tissue becomes transparent, and the starch grains swell up and are stained blue

224. The Quantity of the Photosynthate, or carbohydrate product of photosynthesis, may be roughly determined as follows. Choose a pot plant with a large number of leaves or leaflets of about equal size, *e.g.* Fuchsia, Broad Bean, or Clover; or use a Tropaeolum plant, in which the area of the roughly circular leaf can be estimated by measuring the radius. Place the plant in darkness till the leaves are starch-free.

Remove a number of the leaves, before exposing the plant to light; kill them (to prevent loss of photosynthate by respiration) by holding them, impaled on a mounted needle, in the steam issuing from a kettle or in the upper part of a test-tube containing water boiled over a Bunsen flame; dry them in an oven until on weighing them no further loss in weight occurs, and determine their dry weight. Expose the plant to light for a few hours; then remove an equal number of leaves, treat them in the same way, and find their dry weight. The difference will give a rough idea of

the increase in weight due to the accumulated products of photosynthesis formed during exposure to light.

A better method is to compare pieces of the same leaves, using plants with large leaves, *e g.* Sunflower, Tobacco Plant. Select a number of symmetrical leaves—the halves on either side of the midrib approximately equal—and divide each in two longitudinally by cutting with scissors close to the midrib. Find the area of the removed half-leaves by cutting out a paper model of each half-leaf and weighing these paper models against measured rectangular pieces of similar paper, until by balancing you get the total area of the half-leaves. Kill the leaves by steam, dry them, and record their dry weight Expose the plant to light for a few hours; then remove the remaining halves of the same leaves (cutting along by the midrib) and kill, dry, and weigh them. Reduce the resulting weight-increase to grams per square metre per hour.

225. Ganong's "Leaf-area Cutter."—Fig 51 shows an extremely useful instrument invented by Prof. Ganong and supplied by the Bausch and Lomb Optical Company, for rapid and accurate cutting-out of discs 1 sq. cm. in area from leaves An iron frame, which can be held in one hand, carries steel dies operated by pressure of the thumb, the dies cutting discs from a leaf held between them and the discs then falling into the perforated aluminium cup attached below the lower die.

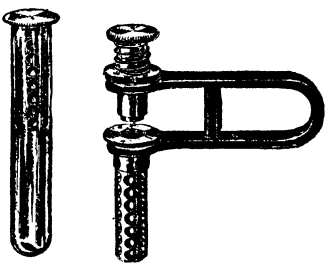


Fig 51.—Ganong Leaf Area Cutter. (Bausch and Lomb Optical Company.)

The arms of the frame are slipped above and below the leaf, which is guided by the other hand; any desired number of discs may be cut from the leaf, care being taken to avoid the larger veins and the discs being cut alternately from the two sides of the midrib; the cup containing the discs is then unscrewed and covered by its own cap, so that the cup will hang into a test-tube which is partly filled with water and heated over a flame; the steam enters the perforations of the cup and kills the leaf discs; the cup with its contents is then placed in the drying-oven.

A second cup is provided, so that after an interval of exposure of the plant to light, or to darkness, according to the object of the experiment, an equal number of discs is cut from the same leaves; these discs are treated like the first set, and the second cup placed with the first in the drying-oven.

When thoroughly dried, both cups are weighed; then the weights of the cups (stamped on them, with the letters M and N to distinguish morning and night, or light and darkness, experiments) are subtracted, giving the dry weights of the two sets of leaves, which are of equal known total area.

226. The Time required for the Appearance of Starch may be determined by bringing into bright light a plant with starch-freed leaves, and then taking discs from the leaves at intervals of, say, ten minutes; mark each disc, test with iodine, and note the increase in amount of starch. Also experiment with threads of Spirogyra, kept in darkness until starch-free, then exposed to light in water in a watch-glass; mount pieces in choral-hydrate-iodine at intervals, and note that about five minutes' exposure to bright light may be sufficient for the appearance of some starch, while in about half an hour abundant starch will usually be found.

227. That **only a portion of the photosynthate accumulates in the leaf,** when in light, is readily proved. Determine, as directed above, the dry weight of a certain area, say 200 sq. cm., of (1) leaves freed from starch by keeping the plant in darkness; (2) an equal area of leaves after exposure of the plant to bright light for four hours, say from 10 a.m. to 2 p.m.; (3) an equal area of leaves from same plant after transferring it to darkness from 2 p m. to 6 p.m.

On adding the increase in dry weight during the four hours of light and the decrease in dry weight during the four hours of darkness, we get a rough estimate of the total product of photosynthesis. A large proportion of photosynthate migrates from the leaf and is used up in respiration, even in leaves which accumulate a relatively large amount of starch. Even in decidedly starchy leaves, like those of Sunflower, not more than one-sixth of the photosynthate consists of starch.

228. Disappearance of Photosynthetic Starch in Darkness.—On a bright day expose to light a healthy pot-plant, *e.g* Tropaeolum or Fuchsia, or a young Sunflower or Phaseolus raised in a pot, and in the afternoon remove from two or three of the leaves a piece about 1 cm square; if the plant has plenty of leaves, remove larger pieces or even half of the leaf. Place these pieces in boiling water for a minute or two, then into a tube of alcohol labelled *A*. Now set the plant in darkness, after watering it. Next day remove similar pieces from the leaves, and place them in a second tube of alcohol labelled *B*. Repeat this on the third day (*C*). Then test with iodine the pieces in *A*, *B*, and *C*, placing each lot separately in a saucer. Note that as the result of keeping the plant in darkness the starch present in the leaves diminishes until in two or three days it has disappeared altogether.

Now expose the plant to the light again, and after a few hours remove pieces of leaf; note that they contain starch.

This experiment is made more striking if each time we cut out differently shaped pieces from each leaf, and then place the different pieces together in the saucer of iodine. Note that the leaves do not change in colour, or suffer any other change (excepting the disappearance of the starch) when kept in darkness for two or three days.

In investigating the conditions required for photosynthesis, we must begin with *starch-free* leaves, and in order to do this all we simply set in darkness for a few days a plant whose leaves have been found to contain starch under normal conditions.

229. Isolated Leaves (Appearance and Disappearance of Starch).—Repeat the preceding experiment with isolated leaves instead of the entire plant: Tropaeolum is especially suitable. Cut off the long-stalked leaves and set them with the stalk dipping into a bottle of water. For comparison, set a pot-plant along with the isolated leaves. The results will show that (1) the starch which appears in the leaves of plants exposed to light is actually made in the leaves, and is not derived from sugar or other substances carried to the leaves from other parts of the plant; (2) starch disappears more slowly from isolated leaves than from those remaining on the plant; (3) evidently starch accumulates in the leaves because it is formed more rapidly than it can be removed by translocation.

230. Translocation of Photosynthetic Starch by Diastase.—Is the disappearance of starch from a foliage-leaf due to its conversion into sugar by means of the ferment diastase—as in the translocation of starch from the cotyledons of a Bean seedling or from the endosperm of a Wheat seedling ?

In the evening of a bright day remove a number of leaves from a Tropaeolum plant that has been exposed to good diffused light; dry them in an oven, and rub them up with some water in a mortar—or cut the fresh leaves up, add some water, and squeeze out the leaf-juice with a meat-juice press. Filter the extract thus obtained, treat it with absolute alcohol to remove the sugar present in it and divide it into two portions; boil one portion in a test-tube. Make some thin starch-paste, place it in three saucers marked *A*, *B*, *C*. To *A* add some unboiled extract; to *B*, some boiled extract; leave *C* as a control. After a time test portions of all three with iodine: in *A* the starch is converted into sugar by the diastase in the extract; in *B* the diastase has been destroyed by boiling.

231. The Giving-off of Oxygen during Photosynthesis may be demonstrated in various ways, but is, perhaps, most readily observed in submerged water-plants (e.g. Elodea, Marestail, Water Milfoil). Collect some of these plants, place them in a large vessel—if necessary tie them to a stone to keep them sunk at the bottom—and set the vessel in good but not too strong light Note the bubbles of gas which are given off, especially if the stems are cut across and the shoots inverted when sunk in the water. See Appendix III , Fig 116.

Graduate a large test-tube roughly by gumming along one side of it a strip of paper marked into inches or centimetres. Invert the tube so that it dips below the surface of the water in a jar, and fix it in this position by means of a retort stand

Loosely tie a number of Elodea shoots, with thread, to form a bundle, and place this in the jar, with the cut ends of the shoots projecting into the test-tube. When a good deal of gas has collected, slip your hand or a glass plate

under the mouth of the test-tube and transfer the latter, still inverted, to a vessel containing potassium pyrogallate, freshly made (§ 233). Note what proportion of the collected gas is absorbed by the pyrogallate, and therefore consists of oxygen; the gas not absorbed is nitrogen, but that the collected gas is richer in oxygen than ordinary air is shown by the fact that more than one-fifth of it is absorbed by the pyrogallate.

232. That **Atmospheric Carbon Dioxide is essential for Photosynthesis** is readily proved. The most satisfactory method is to analyse the air in which plants have been confined under conditions favourable for photosynthesis. This direct analysis method is difficult because of the small amount of carbon dioxide concerned, but convincing results are given by the indirect method of placing similar green leaves in light under conditions exactly alike except that atmospheric carbon dioxide is allowed access in one case and is excluded in the other, and noting whether starch is produced.

Another method is to deprive water-plants of carbon dioxide by boiling the water (and thus driving off all dissolved gases) before placing the plants in it and exposing them to the light, and noting whether oxygen is given off.

(*a*) A rough method of excluding air, and therefore atmospheric carbon dioxide, from the leaf is to treat starch-free leaves of Fuchsia, Tropaeolum, or other hypostomatal (with stomata confined to lower surface) leaves as follows. Smear different leaves with vaseline (*a*) on the lower side only; (*b*) on the upper side only; (*c*) on both sides; (*d*) on a band-like area of both sides. These experiments are not, however, conclusive, since the smearing necessarily interferes with respiration and transpiration.

(*b*) Tie the stalks of starch-free Tropaeolum leaves to a stone, and sink them under water in a large jar; expose to light for several hours, then test with iodine. A little starch may be formed, since the leaves of land-plants are

covered by a film of air, but the experiment shows that a land-plant cannot make much starch when deprived of free air by being submerged in water. For comparison, set a few of the starch-free leaves with only their stalks dipping into water in a bottle; expose to light alongside the jar containing the submerged leaves for the same length of time; then test with iodine.

(*c*) Test with iodine the leaves of a submerged Elodea plant that has been exposed to light: starch is present. Place the vessel in darkness until the leaves are starch-free, then place one shoot (*A*) in a jar of water that has been boiled in order to expel the dissolved gases, and a second shoot (*B*) in a jar of ordinary water. Expose the two to bright light, and after some hours test sample leaves of *A* and *B* for starch.

(*d*) Tie together two large flat medicine-bottles of exactly the same size. Into one (*A*) pour some water, into the other (*B*) place some carbon dioxide absorbent—either caustic potash, or freshly-made soda-lime, or baryta-water. On the rim of the neck of each bottle place some plasticine and vaseline, then press down on the two necks the two halves of a large leaf of Tropaeolum or Tobacco Plant (previously kept in darkness so as to be starch-free), and put over each a glass slip with a weight to keep the leaf down. If Tropaeolum is used, let the stalk of the leaf dip into a bottle of water. The circular portion of leaf over bottle *A* receives carbon dioxide from the air in the bottle; the portion over bottle *B* receives air deprived of carbon dioxide. Expose to light for several hours, then test the leaf with iodine: on one side of the leaf there will be a circular patch (*A*) containing starch, on the other side (*B*) a patch without starch.

(*e*) Put a Tropaeolum leaf in a small bottle of water, so that its stalk dips into the water while the blade rests on the neck of the bottle. Pour some caustic potash into a large jar, place the bottle in the jar, cork the jar tightly and make it air-tight with plasticine or vaseline and wax. The leaf is now exposed to air whose carbon dioxide has been absorbed by the potash. Set up a control experi-

ment in which the arrangements are the same, but leave the jar open to admit air, and omit the potash.

(*f*) Fit a wide-mouthed glass jar with a cork cut in two across the middle. Smear with vaseline and wax the edges of the two half-corks; pour some caustic potash or clear baryta-water into the bottle Lay the bottle on its side and place between the halves of the cork a starch-free Primrose leaf, so that part of the leaf is inside the bottle and the rest outside (Fig 52). Cover the apparatus with a large bell-glass, and set it in a good light After some hours remove the leaf, decolorise it and test with iodine If care has been taken in fitting up the apparatus, the part of the leaf that was inside the bottle (in air free from carbon dioxide, which the baryta-water absorbs readily) contains no starch, while the part outside does. This is often termed "Moll's experiment"

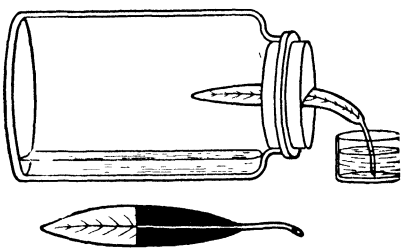


Fig 52.—Moll's Experiment.

Instead of the jar and split cork, we may use two similar glass dishes with ground edges, placing some baryta-water or potash in the lower dish, smearing the edges of both dishes with vaseline or wax, and inverting the empty dish over the other one, so that the leaf is held between the edges of the two dishes.

233. Gas Analysis.—In experiments on photosynthesis **carbon dioxide** may be tested for, or absorbed from the air or the gases to be analysed, by means of (1) caustic potash, (2) soda-lime, (3) baryta-water, (4) lime-water. **Oxygen** is readily absorbed by (1) pyrogallate of potash; and its presence may be tested for by (2) its power of causing a glowing splinter to burst into flame, (3) its causing de-oxygenated and therefore

decolorised methylene blue to resume its blue colour, (4) the production of white fumes of phosphorus oxide from phosphorus.

For volumetric experiments, **carbon dioxide** is best absorbed by **caustic potash,** and **oxygen** by **pyrogallate of potash.** A 1 in 3 solution of caustic potash (1 gram KHO to every 3 c.c. water) will absorb at least thirty times its volume of carbon dioxide in a few minutes if well shaken up with the gaseous mixture to be analysed; the solid sticks weigh roughly 1 gram to the centimetre, so it is only necessary to measure off the length of stick required in making up the 1 in 3 solution. For absorption of oxygen, dissolve 1 gram of pyrogallic acid and 5 grams potash in every 30 c.c. of water; the solution should be made up just before use, by mixing equal volumes of (*a*) 1 in 15 solution of pyrogallic acid in water and (*b*) 5 in 15 solution of caustic potash in water; the pyrogallate of potash solution thus made will absorb about ten times its own volume of oxygen.

234. Ganong's Photosynthometer. —For the quantitative study of the two gases concerned in photosynthesis it is necessary to keep a leafy shoot, or single leaves, or an entire plant, in a closed chamber, expose the apparatus to light, and analyse the enclosed gases to determine the increase of the oxygen (20 per cent.) and the diminution of the carbon dioxide (0 004 per cent.) that were present in the air at the beginning of the experiment

Since the percentage of carbon dioxide in the atmosphere is so small, and plants will thrive for a time in air containing a much larger percentage, up to 10 per cent. or even more, we can add a selected percentage of this gas to the air in the vessel by means of a generator (or a Sparklet siphon charged without addition of water)

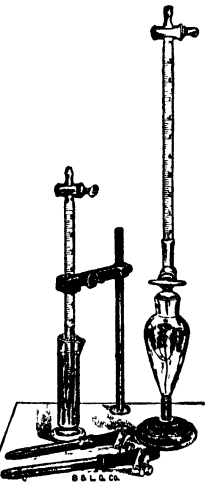


Fig 53 —Ganong's Photosynthometer

Prof. Ganong's Photosynthometer, large enough for a shoot of a small-leaved plant, is shown in Fig 53. It is supplied by the Bausch and Lomb Optical Company, with full instructions for use.

Since in most land-plants the leaves are chiefly or wholly on the lower surface, it is sufficient for rough purposes to use a screen that shall allow free access and exit of gases on the lower side, and the "Normal Light Screens" devised by Prof. Ganong, and supplied by the Bausch and Lomb Optical Company, are constructed on this principle.

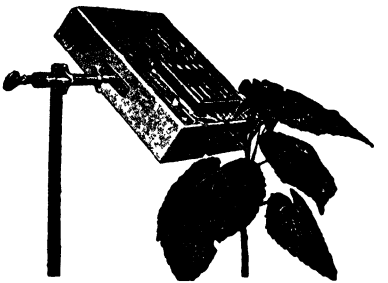


Fig. 54.—Ganong's Light Screen (large form).

The larger screen (Fig. 54) is especially useful; in the figure it is shown fitted with five tubes of coloured liquid (see § 245). The screen consists of a box adjustable for height and angle, black inside, adapted to take a fairly large leaf. It is separated lengthwise into two compartments, with a middle space for petiole and midrib The bottoms of the compartments are largely open but provided with diaphragms so that air can enter freely but direct light cannot. Movable gratings of threads hold the leaf against the glass cover, which may carry tinfoil cut with any desired pattern and gummed to its lower side, or which may be replaced by a 5 × 4 photographic negative (see § 239).

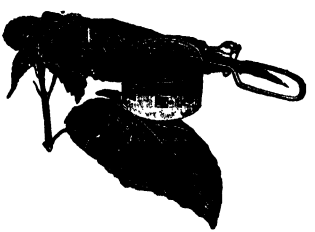


Fig 55.—Ganong's Light Screen (small form).

The smaller screen (Fig. 55) consists of a spring clip holding a glass disk against the upper side of the leaf, which is supported below by a grating of threads stretched across the top of a ventilated dark-box, the glass being removable from the clip so that a tinfoil sheet cut into any pattern may be gummed to its lower surface.

238. Warmth required for Photosynthesis.—It is easy to udy the **influence of temperature upon photosynthesis** by)tmg the results of warming or cooling the air in contact with the aves of land-plants, or the water in which aquatic plants are aced, setting up in each case a control experiment in which the ants are subjected to the ordinary temperature

(1) Place a starch-free leaf (or a whole plant with starch-free aves) of Tropaeolum, Fuchsia, or Primrose, in a jar kept cold by e, expose to light, and after an hour or two test with iodine. Set) a control experiment along with this, with everything the same .cept that no ice is used

(2) Time the rate of bubbling of oxygen from a shoot of Elodea other submerged aquatic. Drop pieces of ice into the water, ad the temperature, and note that the bubbling becomes slower

239. The **Influence of Light Intensity on Photo-nthesis** may be demonstrated, roughly at any rate, by ich experiments as the following:—

(1) Cover half of a starch-freed leaf with a piece of irly thin white paper, or a piece of ground glass, and pose to light; after a few hours, remove the paper or ass and decolorise and test the leaf with iodine.

(2) Instead of using a tinfoil stencil, cover the upper le of a starch-freed leaf with a photographic negative, fit a 5 × 4 negative into the light-screen shown in g. 54. After exposure to light, test with iodine; a starch print" is obtained, in which the lightest parts of e negative show up darkest in the "starch print," and *ce versa.*

(3) Place some healthy cut branches of Elodea or other ater-plant under water, and select one which gives a good ream of oxygen-bubbles (fairly rapid and constant) from cut end. Count the time required for, say, ten bubbles be given off, and repeat the counting several times till u get a fairly constant result. Then remove the jar into e shade, or cover it with a sheet of thin white paper to aken the light, and take times as before, noting the ange in the rate of bubbling.

(4) Set the jar containing the water-plant under a box en at one side, and throw light on the plant from this le by placing a lamp at different distances from it, noting e distances and the rates of bubbling. Bring the lamp

into such a position that bubbles begin to come off, and count the rate, when it becomes fairly constant, bring the lamp to half this distance from the plant and count again Part of the effect, however, is due to the heat given out by the lamp, a flat-sided bottle vessel of water (kept cold by constant renewal) should be used as a screen to absorb the heat

240. Non-starchy Leaves.—If a variety of plants be tested for starch-formation by photosynthesis, it is found that in many cases the amount of starch present, even under the most favourable conditions, is small, while others produce no starch at all. Most non-starchy leaves produce relatively large quantities of sugar, and there is in general an inverse ratio between the amounts of sugar and of starch produced by the leaves of different plants

On the afternoon of a warm bright day collect leaves of Onion and of Sunflower (or other starch-leaved plant) (1) Test an Onion leaf with iodine, no starch is formed by this plant Cut a second Onion leaf into pieces and boil with Fehling's solution, abundant sugar is present, as shown by the copper oxide precipitated (2) With a meat-juice press crush separately the chopped-up leaves of the two plants, in each case measure the volume of juice obtained, boil it, let it cool, replace the water lost in boiling, and filter. Now determine the volumes of juice required for the reduction of 20 c.c. of Fehling's solution; a very small amount of Onion juice is sufficient, but a much larger amount of Sunflower juice is required.

Keep an Onion plant in darkness for two days; cut off portions of several leaves, noting either their fresh weight or their total length, press out the juice, and determine the volume of juice required to reduce 20 c c. of Fehling Now expose the plant to light for several hours; cut off an equal fresh weight, or total length, of leaves, press out the juice, and determine its reducing power with Fehling as before. The result will show that the Onion leaf produces sugar by photosynthesis. Now place the plant in darkness again, and after a day test the reducing power of the juice; the sugar content of the leaf is diminished,

part of the sugar has migrated and been used up in respiration

Keep an Onion plant in darkness for two days; then cut out parts of the leaves, measure the area of the pieces, kill with steam and dry them, and record their dry weight Expose the plant to light, and after several hours take an equal area of leaf, and in the same way find its dry-weight Note that increase in weight occurs in starchless as well as in starch-forming leaves as the result of photosynthesis

241. Starchy and Non-starchy Leaves. — Experiments have shown that on the whole the variations in the capacity for producing starch as a photosynthesis product are characteristic of certain families Very large quantities of starch occur in the leaves of Solanaceae and Papilionaceae; large quantities in Papaveraceae, Crassulaceae, Geraniaceae, Labiatae, etc., moderate amounts in Caryophyllaceae, Ranunculaceae, etc , very little in many Gentianaceae and Iridaceae; and none at all in Allium, Scilla, and various other Liliaceae, also in many Amaryllidaceae and Orchidaceae.

Even in the plants that are richest in starch, it can be proved that starch is not the first product of photosynthesis; when water-plants are exposed to light, the giving-off of oxygen (which accompanies the assimilation of carbon dioxide) begins almost *instantly*, though starch only appears after an interval of several minutes. If a starch-leaved plant (*e.g.* Sunflower or Bean) is kept in darkness until sugar, as well as starch, has disappeared from the leaves, and the plant is placed in the light, it is found that the appearance of starch is preceded by that of sugar.

The difference between starchless leaves (*e g* Onion) and starchy leaves simply arises from the fact that in the former the sugar produced is stored as such, while in the latter the sugar produced is partly converted into starch It is easy to prove that even in normally starchless leaves starch may be produced if sugar is present in sufficient concentration, which can be attained by (1) separating the leaves from the stem, and thus preventing the translocation of the sugar; (2) increasing the amount of carbon dioxide

supply, and thus causing an increase in assimilative activity; or (3) supplying sugar from outside. Both in starchy and starchless leaves, the formation of starch from sugar can be effected in darkness, and that this process is not dependent upon chlorophyll, but can be carried on by leucoplasts, is shown by the fact that colourless parts can make starch from sugar

242. Formation of Starch from Sugar by normally Starchless Leaves.—Cut leaves of Iris germanica into pieces, say 10 cm. long. Test one or two pieces with iodine; this plant does not form starch in its leaves Float some pieces in a dish containing 20 per cent. cane sugar solution, others in a dish of water. Set both dishes in darkness, covered with a sheet of glass raised slightly so as to allow access of air From day to day cut off pieces and test them with iodine; after about a week the leaves supplied with sugar will be found to contain starch, while those supplied only with water will remain starchless

243. Formation of Starch from Sugar by normally Starchy Leaves.—Keep a plant of Tropaeolum or Tobacco in darkness for two days, so that a piece cut from a leaf shows no starch. Cut the rest of this leaf into two; place one half in sugar solution and the other in water, as in the preceding experiment, and keep both in darkness. After several days starch appears in the sugar-supplied piece of leaf, but not in the other.

Another method is to place water-plants (*e g* Elodea, Callitriche, or Duckweed) in two jars of water, adding cane sugar (about 5 per cent.) to one jar Set the two jars in darkness, and after about a week note that the starch-supplied plants contain starch, while those in water are starchless and unhealthy in appearance if not dead

Glycerine (5 per cent. solution) may be used instead of sugar in these experiments, but it is more difficult to keep the glycerine culture free from moulds In any case, it is advisable to add to the culture a few drops of carbolic acid, or thymol, or eucalyptus oil, as an antiseptic

244. Formation of Starch from Sugar by Colourless Leaves.—That chlorophyll is not necessary for this "chemosynthetic" form of starch-production is readily shown by using, instead of green leaves, the white flowers of various plants—those of Phlox answer well, but others should be tried. Test the leaves first with iodine, to ensure that no starch is already present; it is of course unnecessary to treat the petals with alcohol, but they should be boiled in water before applying the iodine test. Float some of the flowers in water, others in 5 per cent sugar solution, keep some in darkness, expose others to light. In both cases note that in a few days the sugar-supplied flowers contain abundant starch, while those in water are still starchless

245. Which Light Rays are concerned in Photosynthesis?—We may investigate this by comparing the effects of exposing plants to light of different colours—*i e* allowing only certain rays to fall upon the leaves It is usually found that the rays at the red end of the spectrum are more active than any of the rest in promoting photosynthesis, and that for most plants the curve obtained when the results of experiments are plotted on squared paper shows two "humps" or maxima, a higher one in the orange and a lower in the blue, with the lowest intermediate part (minimum) in the green.

It must be noted, however, that the experiments usually made on this topic are open to various objections; some of these objections are mentioned in connection with the following experiments, but the greatest are (1) the extreme difficulty in obtaining spectroscopically pure colour screens, (2) the different heating effect of the different colours as transmitted through screens.

(*a*) A rough comparison may be made by setting a starch-free plant, or a leaf with its stalk dipping into a bottle of water, in a box, one of whose sides is replaced by a sheet of *red* glass, another in a box with a side of *yellow* glass, another with *green* glass, another with *blue* glass. After several hours' exposure to light, test each for starch with iodine solution. However, coloured glass is probably never pure, in the sense of allowing only rays of one colour to pass through it. This can easily be seen by testing coloured glass

with a spectroscope (an excellent direct-vision spectroscope can be had for 25s.), or with a lantern and prism.

(*b*) A better method is to use coloured solutions (which should be examined with the spectroscope to see which rays they absorb and transmit) in flat bottles, instead of using coloured glass, in the preceding experiment. "Aniline scarlet" dye may be used for the *red*; a solution of potassium dichromate for *orange-yellow*; a mixture of ammoniacal copper sulphate and potassium dichromate for *green*; and ammoniacal copper sulphate solution for *blue*

(*c*) In Fig. 54 the light-screen is shown fitted with a series of five corked glass vials, four of them containing the red, orange, green, and blue solutions just mentioned, and the fifth filled with water as a control Between the vials tinfoil is placed, so that on five strips of the starch-freed leaf there fall rays of red, orange-yellow, green, blue, and white light respectively

(*d*) Another method is to use a pair of double-walled bell-jars One is filled with watery solution of potassium dichromate, which allows the red, orange, and yellow rays to pass but absorbs the rest. The other is filled with watery solution of copper sulphate to which ammonia has been added, this solution transmits blue and violet but absorbs the rays of the red end of the spectrum In this way we can at any rate divide the spectrum into a red-end half and a blue-end half A starch-freed leaf or leafy shoot in a bottle of water, or a pot plant, is set below each bell-jar, the edges of which should rest on a damp cloth or on sawdust, so as to exclude white light. Set both jars in diffuse light, and after several hours test each leaf for starch Put a thermometer under each jar, and note the temperature registered in each case

(*e*) A makeshift double-walled jar can be fitted up as follows. Get two large wide-mouthed jars, and two narrower jars each of which can be placed inside one of the large jars Partly fill one large jar with potassium dichromate solution, the other with ammoniacal copper sulphate solution; into each lower the smaller jar, placing in the latter some shot or stones to sink it in the solution. Then place in each of the small jars a starch-freed Tropaeolum leaf in a test-tube of water, and securely seal the necks of the jars Home-made double-walled jars of this kind answer almost as well as those sold by dealers in glass apparatus.

246. Chlorophyll essential for Photosynthesis.—Some of our experiments have suggested that only green (chlorophyll-containing) tissues, organs, or plants are able to use the carbon dioxide of the air and to manufacture carbohydrates.

(*a*) Keep in darkness, until the leaves are starch-free, some plant, or a cut shoot, which has variegated leaves

(*i.e* in which some portions of the leaf are devoid of chlorophyll); the Japanese Maple, or variegated kinds of Ivy, Coleus, Abutilon, or Geranium, answer well. Sketch each leaf before it is decolorised and tested, and observe that only the green parts produce starch

(*b*) Get five wide-mouthed bottles, with tightly fitting corks. Wash each bottle out with water, to keep the air inside it moist, and label them *A*, *B*, *C*, *D*, *E* Leave *A* empty, to serve as a check or "control" Into *B* and *C* put some living green leaves; into *D*, some green leaves which have been killed by boiling; into *E*, some pieces of living wood cut from a branch, or some roots, or mushrooms, or any other living but *not green* tissue Charge the bottles with carbon dioxide by breathing into each several times. Another plan is to pour into each jar some "plain soda-water" from a syphon (a convenient method is to use a Sparklet syphon, charging it without adding soda); the "soda-water" is of course simply water charged with carbon dioxide. Cork each bottle tightly, smearing the edges of the corks with vaseline. Place bottle *B* in the dark, the others in the light, for a whole day Then test each bottle for carbon dioxide by pouring in a little lime-water and seeing whether it turns milky.

Try the experiments several times, and record your results, with the inferences to be drawn from them. If carefully carried out, these experiments will show (1) that living green leaves absorb carbon dioxide from the air in sunlight; (2) that they do not absorb it in darkness; (3) that dead leaves do not absorb carbon dioxide, (4) that living but not green parts of plants do not absorb it.

(*c*) Repeat the observations on the giving-off of oxygen by water-plants, but put into the water, along with the water-plant, pieces of living roots and of mushrooms. Do these living but not green tissues give off oxygen? If any gas-bubbles escape from them, do they come off in light only, or in darkness as well?

247. The **General Properties of Chlorophyll** should be studied, with special reference to its absorption spectrum. The best materials for this purpose are thin, fairly young, clear-green leaves, a good typical spectrum is given by alcoholic extract of leaves of Grasses, Primrose, Tropaeolum, etc. Fine fluorescence is shown by extract of leaves of Ivy and Cineraria

(*a*) Almost any leaves may be used, and it would be interesting to compare, with the spectroscope, chlorophyll from the leaves of various plants, including those with

yellowish-green leaves Leathery leaves and those of Grasses should be chopped up, being boiled in water and steeped in alcohol The extraction is best carried on in darkness; the leaves may be left in a covered dish of alcohol overnight, filter, and place the filtered extract in corked bottles. A wedge-shaped bottle ("indigo prism") should be used, by its means one can examine different thicknesses of the solution

(*b*) Note the colour of the solution on holding the bottle up to the light, and on holding it against a black surface · it is green by transmitted light, red by reflected light If a spectroscope is not available, obtain a continuous spectrum on a screen by fastening on the lens of an optical lantern a card with a vertical slit, and holding a prism in the path of the light Hold a test-tube of alcoholic chlorophyll-solution against the slit, and notice that the colours in several parts of the spectrum are replaced by dark bands. The most prominent dark band appears in the red part, but if the solution is strong bands will also be seen in other regions of the spectrum. Hence chlorophyll absorbs certain light-rays, allowing the rest to pass through it, and we may conclude that these absorbed rays in some way supply the energy which is needed in carrying on the work of photosynthesis

Our experiments in § 245 show, roughly, the relation between the dark bands of the chlorophyll spectrum and the light energy used in photosynthesis

(*c*) Place some leaf-extract in a test-tube, dilute with a few drops of water, then add benzol, shake, and allow to settle. The benzol, which floats above the alcohol, dissolves out a bluish-green colouring-matter, leaving a yellow substance dissolved in the alcohol. These two pigments present in the extract can also be separated by using ether or olive oil instead of benzol. Find out, by using the spectroscope, or lantern and prism, which light-rays each of these substances absorbs.

(*d*) Fill three test-tubes with leaf-extract, cork them, and place *A* in sunlight, *B* in diffused light, *C* in darkness. Carefully boil some extract in a fourth test-tube (*D*), cork

the tube, and place it with *A* in sunlight. Notice, after a day's exposure, that *A* becomes brown, *C* is unchanged, while *B* and *D* are only slightly changed; the absence of oxygen in *D* hinders the destructive effect of light.

(*e*) Add some 10 per cent solution of copper sulphate to some leaf-extract in two test-tubes; a copper compound is produced which is not red by reflected light, and which is not destroyed by light. Verify the latter point by placing one tube in sunlight, the other in darkness, in each case with a tube of ordinary leaf-extract for comparison.

248. Conditions essential for Formation of Chlorophyll.—We have seen that seedlings grown in darkness have no chlorophyll, their leaves are yellow, owing to the presence of etiolin in the plastids That iron is essential is shown by means of water cultures (§ 221)

(*a*) Grow seedlings, *e g* Cress or Mustard, in darkness, then place some of them in a good light, close to a window, and note the time required for the production of a distinct green colour. Place the others in a dark part of the room, and when they have become green test the leaves for starch. These observations will show that (*a*) a green tinge, due to formation of chlorophyll, may be developed in an hour, or less, in good light; (*b*) light too weak for photosynthesis is strong enough for the production of chlorophyll.

(*b*) Sow in the same pot or box some seeds of Pine and of Bean or Pea, keeping them in darkness, and compare the colour of the Pine-seedlings with that of the others

(*c*) Place some etiolated seedlings (Cress, Mustard, Bean, etc.) in a bottle or small glass jar, cover with a glass plate, and set it in a larger jar half filled with water. Keep the water at 30° C. In a similar apparatus keep some of the seedlings in cool water, or water kept at 10° C., by adding bits of ice from time to time. Compare the depth of the green colour developed in the two sets of seedlings after an hour or two of exposure to light.

(*d*) To show that oxygen is necessary for the formation of chlorophyll, fill a test-tube with mercury, or with boiled and cooled water, invert it in water, and pass under its rim some etiolated Mustard seedlings. Though exposed to light, the seedlings do not become green, owing to lack of oxygen. Another method is to place heavier seedlings—*e g* Bean, Pea—in a glass jar and cover it with water. In each case similar etiolated seedlings should be placed on wet blotting-paper at the bottom of a jar, whose mouth must of course be left open.

III Respiration.

249. Respiration.—The organic products of photosynthesis, and of the further metabolic processes starting from the photosynthate, in autotrophic plants sooner or later disappear owing to (1) absorption by animals or parasitic plants, or (2) processes of decay, or (3) respiration. We have studied the loss in dry weight undergone by seedlings grown in darkness, and it could easily be proved by experiments that a similar loss occurs in all living plants, whether they are actually growing or merely maintaining life Since the disappearing material cannot be destroyed, it must escape from the plant in the form of gas Our experiments on seedlings showed that the loss is mainly a loss of carbon, escaping in the form of carbon dioxide, and this giving-off of carbon dioxide is accompanied by absorption of oxygen

250. Respiroscopes and Respirometers.—For the simple demonstration of respiration, it is only necessary to test the gas in a vessel in which active tissues have been enclosed for some time and compare its composition with that of the atmospheric air originally present A respiroscope consists of any gas-tight chamber connected with a tube in which the identity of the gas may be ascertained by a visible test with a gas-absorbing reagent. A respirometer is simply a similar apparatus adapted by graduation for the quantitative analysis of the gas in the chamber

251. Respiroscope Experiments.—Various forms of respiroscope may be put together easily from ordinary laboratory apparatus

(*a*) To show that considerable volumes of carbon dioxide are rapidly produced in the germination of seeds, half fill a large glass jar with soaked Peas and fit the cork with a twice bent tube leading into a narrow-necked bottle or large test-tube containing lime- or baryta-water. Set in a fairly warm place and note the copious white precipitate (calcium or barium carbonate) produced by the escaping carbon dioxide As a control, place an equal volume of the reagent in a second bottle or tube of the same size As a second control, set up a similar apparatus containing soaked Peas which have been boiled; to prevent the growth of Bacteria, cover the killed Peas with 10 per cent formalin

(*b*) Repeat the preceding experiment, but this time lower into the jar a smaller jar or tube containing baryta-water or caustic potash, and let the bent tube dip into water coloured with red ink. As the reagent absorbs the carbon dioxide produced, the coloured water rises in the outside tube

(*c*) Fit a wide jar with a cork bored by two holes Cork the wide mouth of a thistle-tube, and push the tube through one of the holes until it nearly reaches the bottom of the jar. Through the other hole pass a short glass tube connected by rubber tubing, carrying a clip, with a J-shaped tube the short arm of which is drawn out in a flame to a fine capillary point. On the bottom of the jar place wet blotting-paper and some Peas or Wheat grains that have been allowed to germinate until the roots are about 1 cm. long

Then dip the J-tube to the bottom of a tall narrow jar or large test-tube filled with lime- or baryta-water, and clip the rubber tubing. After two or three days, loosen the clip, uncork the thistle-tube and pour water into it so as to drive the gas out of the jar through the J-tube, from the fine opening of which it escapes into the reagent, causing a precipitate Set up a similar apparatus, but without the seeds, as a control.

(*d*) About one-fourth fill a cylindrical jar with lime- or baryta-water, then push into the jar, well above the liquid, a piece of gauze to support some germinating seeds and blotting-paper, and cork tightly. During several days note the gradual whitening of the reagent, which should be gently shaken from time to time Set up a similar apparatus without the seeds, as a control

(*e*) A striking and roughly quantitative experiment may be arranged as follows, taking the composition of air as 20 per cent. oxygen and 80 per cent nitrogen Get three similar J-tubes, to graduate one tube—and from it the others—into fifths, cork the short arm, fill the tube with water, pour the water into a measuring glass, and as each fifth part of the water is poured back into the tube mark the level by a file scratch Into the short arm of each tube place six soaked Wheat seeds, with a wad of wet cotton to keep them moist, and cork tightly. Take three narrow jars or large test-tubes, a little wider than the J-tubes, and place in them (*A*) caustic potash, (*B*) pyrogallate of potash, (*C*) water; into each dip the long arm of one of the inverted J-tubes. If test-tubes are used for the reagents, support them in a stand

In *B* the reagent (pyrogallate) quickly rises in the tube to about the first fifths-mark, and the seeds germinate very little; in *A* the reagent (potash) rises gradually and to the same extent, while the seeds germinate quite well; in *C* the water hardly rises at all, though germination occurs as in *A*. What has happened in each case, and what inferences may be drawn from the observed facts?

(*f*) Suspend three healthy laurel leaves by threads from the well-fitting cork of a large bottle containing lime-water, and expose them to bright light. After several hours the lime-water is still comparatively clear Cover the bottle with black cloth, and in a few hours the lime-water will become quite milky, owing to the respiration being no longer masked by the re-assimilation of the carbon dioxide it produces.

(*g*) Place some green leaves in a glass jar (Fig 56), through which a slow current of air is passed This air is deprived of its carbon dioxide by the potash contained in the U-tube, so that the lime-water or baryta-water in both bottles remains clear so long as the leaves are exposed to sunlight or very bright daylight, whereas if the

bell-jar is covered with a black cloth, the liquid in (a) soon becomes turbid and milky.

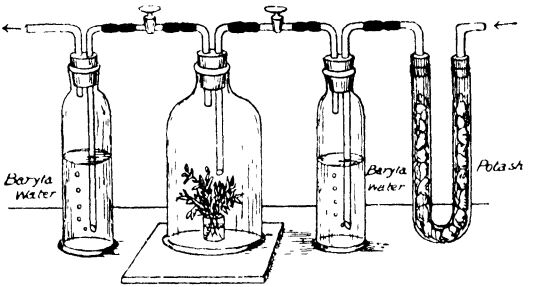


Fig 56 —The Arrows show the direction of the Current of Air, which is drawn through by attaching an "Aspirator" at the left of the Apparatus The two pieces of glass tubing fitted with stop-cocks may be dispensed with and clips used on the rubber tubing (shown black)

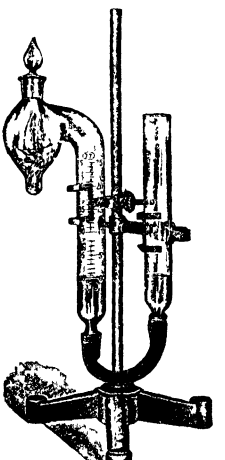


Fig 57 —Ganong's Respirometer.

In Fig 56 the plant used is covered with a bell-jar standing on a glass plate, its rim being smeared with plasticine and vaseline to make the junction air-tight.

252. Respirometer.—Almost any of the respiroscopes described may be used as respirometers, for qualitative analysis of the gases exchanged in respiration The tube leading from the chamber should dip into mercury (for rough purposes water may be used, though it will of course absorb the gases—especially carbon dioxide—to some extent); allowance must be made for the volume occupied by the seeds; the volumes of gas absorbed by the reagents (potash solution and pyrogallate solution) must be measured with corrections for temperature, etc

In using Ganong's Respirometer (Fig 57), supplied by the Bausch and Lomb Optical Company, ten Oats or Barley grains are germinated till the roots are visible (2 to 4 mm long), their volume is found by immersion in water in a small glass measure, and they are put in the oval chamber which has a water-bulb for a measured small volume of water to keep the seedlings moist The seeds and water placed in the chamber

occupy 2 c.c , the volume allowed for them in the graduation of the tube connected with the chamber; this tube is joined by rubber tubing to the reservoir tube, which has index marks 25 c.c. apart. Pour mercury into the reservoir-tube until it stands level at the 100 c.c. mark in the graduated tube and at the lowest index mark of the reservoir itself; the stopper is inserted with its air-opening matching that of the neck, and then twisted, so that the chamber is sealed without compression of the air.

After three or four days of growth in a shaded place, the reservoir tube is slipped off under water, allowing the mercury to run out; then the rubber tube is clipped and the graduated tube slipped first under potash and then under pyrogallate solution, to measure the volume of carbon dioxide produced and that of any oxygen left over in the apparatus, which originally contained 100 c c of air

253. The Respiratory Equation.—Weigh two sets, each of ten seeds, of Oats or Barley. Soak one set (*A*) for twelve hours and place it in the chamber of a respirometer, with potash in the measuring-tube; when the potash stops rising, measure the volume of the carbon dioxide Dry the two sets of seeds, using the percentage of moisture in *B* to determine the original dry weight of *A*, from which calculate the loss of dry substance by respiration in *A* From the molecular weights in the equation

$$C_6H_{10}O_5 + 6O_2 = 6CO_2 + 5H_2O$$

calculate the relation between the starch used up (loss of dry weight) and the carbon dioxide formed, and see whether they agree with or approximate to the formula.

Since starch is converted into sugar in germination, and sugar is usually the first complex product of photosynthesis, we may regard the respiratory equation as the reverse of the photosynthetic equation and express the two by the reversible equation

$$C_6H_{12}O_6 + 6O_2 \rightleftarrows 6CO_2 + 6H_2O.$$

But it must be remembered that (1) this equation is largely conventional; (2) it expresses only the end result and not the complex intermediate stages; (3) the volumes of gas absorbed and evolved in respiration are by no means always equal, as the equation would imply.

254. Respiratory Ratio in Peas and Beans.—With a suitable respirometer ascertain the respiratory ratio (relation between carbon dioxide released and oxygen absorbed) in the case of Peas or Beans In these and various other seeds the respiratory ratio is considerably more than unity—that is, much more carbon dioxide is released than oxygen is absorbed, and such seeds can even release carbon dioxide in the absence of an oxygen supply

255. Respiratory Ratio in Oily Seeds.—Set up two similar respirometers. Weigh out equal quantities of (A) starchy seeds like Wheat or Oats, (B) oily seeds—*e g.* Linseed or Hemp; after soaking the seeds place them in the two vessels, set side by side for comparison. When oily seeds are ripening (after fertilisation) the supplies of sugar they receive are changed to oil, and in this process more carbon dioxide is given off than oxygen is absorbed—about five times as much, for instance, in Castor Oil.

When the seed germinates the oils are changed back into sugar, and for some time the amount of carbon dioxide given out is considerably less than that of the oxygen absorbed In an experiment with Linseed, the ratio $\frac{CO_2}{O_2}$ for the first six days was found to be 0 3, 0 35, 0 4, 0 5, 0 6, 0 7. As the conversion of oils into sugar continues, the ratio gradually approaches 1 Also, during the first few days of germination, oily seeds show an increase in dry weight—explained by the fact that carbohydrates contain more oxygen than oils do

256. Respiration of Succulents —Set up two respirometer experiments; in *A* use as material leaves of Sedum, Crassula, Rochea, or fleshy shoots of Cacti, cut from the plant at the end of a warm day—the material may be chopped into pieces; in *B* place leaves of some non-fleshy plant, *e g* Sunflower Keep the apparatus in darkness overnight; in the morning note that the fleshy plants have absorbed oxygen without giving out as much carbon dioxide, so that the air in the chamber diminishes in volume.

In fleshy plants organic acids are produced by respiration in such quantities that the formation of carbon dioxide may be stopped altogether for a time. The amount of acid in the leaves increases at night; the accumulation of these would injure the plant if continued, but after a time the plant begins normal respiration, giving off carbon dioxide, which in sunlight is at once assimilated, oxygen being set free Hence in the morning, when assimilation begins, fleshy plants give out more oxygen than they absorb carbon dioxide, and they will continue to give out oxygen in air freed from carbon dioxide

Succulent plants reduce their rate of transpiration, on account of their xerophytic habitat, by having few stomata and small air-

spaces, and this means a reduction in the rate of gaseous exchange and therefore in that of photosynthesis. Hence they avoid, by their peculiar mode of respiration, the loss of carbon that takes place in ordinary plants by the setting free of carbon dioxide into the air

The acidity of equal-sized leaves, or pieces of leaf, chopped up and extracted with water, should be tested and compared by titration (1) in the evening, (2) on the following morning

257. The **intensity of respiration** varies greatly in different species, in different plants of the same species, in different organs of the same plant, and in the same plant or organ at different times. A rough comparison may be made with some form of respirometer, taking the volume or weight of the plant material used as a basis. Note that flowers and opening buds, like germinating seeds, respire much more actively than fully-grown roots, leaves, etc.

258. Heat Released in Respiration.—Just as the dissociation of carbon dioxide in photosynthesis converts kinetic energy, or energy of motion, into potential energy, or energy of position, so in respiration there is a release of energy—that is, a conversion of potential energy into kinetic energy. Some of this released energy appears in the form of heat, and this may be demonstrated by thermometric observations of respiring tissues, in comparison with similar but non-respiring tissues.

(1) For experiments on the production of heat in respiration we require a more or less perfect non-conducting chamber, in which we place, along with the seeds or other materials used, a dish or bottle containing potash to absorb the carbon dioxide and thus promote respiration and growth, and a thermometer as accurate and sensitive as possible. In each case, also, we require a control experiment similar in every way, except that the plant material placed in it is killed with boiling water and then placed in water containing 5 per cent formalin, or 10 per cent. corrosive sublimate, to prevent decay or fermentation by micro-organisms—processes in which heat is released

The best form of non-conducting chamber is a Dewar bulb consisting of two concentric bulbs with a vacuum between, or two "Thermos" or vacuum flasks answer

well for experiments with small seeds. Along with the seeds insert in each case a small bottle containing a piece of potash stick, covering the mouth of this bottle with wire netting; insert the thermometer and pack the space between it and the neck of the vessel with cotton-wool

(2) In the absence of a better form of non-conducting chamber, we may either (*a*) line a funnel with filter-paper, fill it with the seeds, and support it with the tube dipping in a bottle of potash, insert a thermometer, and cover the whole with a bell-jar; or (*b*) place in a tumbler a flat dish of potash, cover this with wire gauze, and insert the seeds and a thermometer; or (*c*) place the seeds in a small flower-pot, set this in a larger pot with wool or other packing between the two pots, cover the mouths of the pots with wire gauze, invert the apparatus over a saucer with potash stick, and insert a thermometer through the holes in the two pots; or (*d*) put potash stick in a beaker, then wire gauze, then seeds, then a cork bored for the thermometer, and place the beaker inside a larger beaker lined with wool.

In each case it is advisable to set two controls—in one place seeds boiled and treated with antiseptic (formalin or corrosive sublimate), in the other wet sawdust instead of the seeds. Opening buds, flowers, and flower-heads should also be used; a remarkably large rise in temperature is observed in the case of the opening inflorescence of Arum and other Aroids

259. "Intramolecular" Respiration.—In some of the preceding experiments it was noticed that the amount of carbon dioxide set free is much greater than that of the oxygen absorbed—*e.g.* in germinating Peas. This suggests that in such cases there might be a release of carbon dioxide even in the absence of a supply of free oxygen, and it is easy to test this by placing such seeds in "an anaerobic culture vessel"—*i.e.* a vessel from which oxygen is excluded, though other conditions are favourable for respiration Such a vessel may be either (1) a short tube filled with mercury and inverted over mercury, the seeds being passed up to the closed end of the tube; (2) a similar tube over 76 cm long and therefore with a Torricellian vacuum, in which the seeds are placed; (3) a seed-containing chamber filled with pure hydrogen, which is afterwards analysed for carbon dioxide.

(*a*) Soak some Peas in water (previously boiled) until the seed coat can be removed without damaging the embryo—the coats are removed to avoid introducing air with the seeds. Fill a test-tube with mercury and invert it in a dish of mercury, clamping it in a stand; then pass three or four peeled Peas into the tube; plunge them with forceps under the mercury, shake them free of air, and release them under the tube, when they will rise to the top. With a bent tube introduce a few cubic centimetres of previously boiled water into the tube to keep the Peas moist. In about three days, when the gas produced has ceased to push down the mercury column, introduce a little more water if necessary, then with forceps slip a piece of solid potash into it; the potash solution thus formed absorbs the gas (carbon dioxide) and the mercury rises to the top of the tube again. To get a more accurate quantitative result, use a graduated tube, and before introducing the seeds tie to each a fine wire (or sew a thread through the seed and tie it in a loop) longer than the tube, so that the seeds can be withdrawn before introducing the potash at the end of the experiment. Set up a control with Peas killed by boiling and soaked in antiseptic. See Appendix III., Fig. 117.

(*b*) Pass a few peeled Peas or Beans into the Torricellian vacuum at the top of a tube 100 cm. long and 1·5 cm. diameter, filled with mercury and inverted in mercury; the "vacuum" contains mercury vapour but no air. To prevent the adhesion of air-bubbles to the tube while filling it, pour the mercury, by a funnel with its end finely drawn out in a flame, through a narrower tube extending to the bottom of the tube to be filled. When the tube is inverted and the seeds introduced, note (1) the temperature, (2) the barometer reading, and (3) the length of the mercury column in the tube—if the tube is not graduated, gum paper strips to the tube at the upper level of the mercury and at the place where the tube touches the mercury in the dish. At the end of, say, 24 hours, gum a strip at the upper level of the mercury, and measure the volume of carbon dioxide that has been produced—in an ungraduated tube, this is easily done by running mercury from a burette up to the top mark on the tube. From the data given in Physics text-books, correct this volume for temperature, pressure, etc.

260. Comparison of Normal and "Intramolecular" (Anaerobic) Respiration.—In general, the amount of carbon dioxide produced in "intramolecular" respiration is much less than that formed in normal respiration, though in some cases—*e.g.* germinating Broad Beans—the two are about equal. Alongside the barometer tube used in § 259 *b* set up a similar tube containing the same number of soaked seeds, pushing the seeds up to the closed end of the tube and holding them in position by pushing in either a piece of coiled wire or a bored cork (in either case with an attached wire long enough to withdraw the obstacle at the end of the experiment).

If we now remove 20 c.c. of the air in the tube, the mercury will rise in it to that extent. To do this, fit a flask with a rubber stopper carrying a bent tube with a piece of rubber tubing on its free end; warm the flask, close the tubing with a clip, introduce it into the end of the barometer tube, release the clip—as the air cools in the flask the latter sucks air from the tube and the mercury rises. Compare the volumes of carbon dioxide produced in the two experiments—by intramolecular and by normal respiration respectively.

If experiments on intramolecular respiration are allowed to continue for several days, the rate at which carbon dioxide is produced falls off, whereas normal respiration becomes increased as germination advances. Plants undergoing intramolecular respiration grow badly, and soon pass into a pathological or unhealthy condition. Moreover, this form of respiration is accompanied by the production of various substances in addition to carbon dioxide, and among these is alcohol, as may be proved by the following experiment

261. Production of Alcohol by "Intramolecular" Respiration.—In order to show that alcohol is produced in the higher plants by intramolecular respiration, just as is the case with Yeast (§ 411) growing in a sugar solution, and thus to demonstrate the relation between intramolecular respiration and alcoholic fermentation, we proceed as follows:—Lay a quantity of Peas for a minute in 0·1 per cent. solution of corrosive sublimate to kill any Yeast-cells that may be clinging to them, rinse them with distilled water that has been boiled for several minutes, and place them in some of this sterilised water to soak for two days, to start germination. Nearly fill a large flask with the Peas, and fit it with a rubber stopper through which passes a twice-bent tube with its longer arm dipping into mercury

After the free oxygen present is used up, intramolecular respiration begins, but if the apparatus is left for three or four weeks the evolution of gas will have ceased, the seeds being now dead. On turning them out of the flask, the Peas in contact with the air will undergo decomposition, and if a few are sown they will fail to grow. Since Pea seedlings under these conditions may produce in the three or four weeks no less than 5 per cent. of their dry weight of alcohol, the latter may be detected by smell, especially if the Peas are rubbed up with water in a mortar. The presence of alcohol may be demonstrated by adding some water to the grown-up Peas and distilling off the alcohol.

CHAPTER VI.

TRANSPIRATION.

262. Transpiration.—Various simple observations show that water-vapour is given off by leaves and other green organs, and that a current of water (transpiration current) passes from the roots to the transpiring organs The root absorbs a very dilute solution of salts, in order to obtain the essential elements for growth, and the excess of water is got rid of by evaporation from the leaves; but transpiration is a process of evaporation controlled by the living protoplasm of the plant

(*a*) Pull up a Broad Bean seedling and place it on a dry piece of glass; cover it with a tumbler, and note that (*a*) the leaves and stem of the seedling become limp or "wilted," (*b*) drops of water collect on the inside of the tumbler owing to condensation of the vapour given off by the leaves

(*b*) Get three similar narrow-necked bottles filled with water, and two cut leafy shoots of about the same size Put a leafy shoot into one bottle; into the second a shoot deprived of its leaves; and leave the third as a control. After some hours' exposure to light, compare the amounts of water left in each bottle: which bottle loses most water, and which least? Repeat the experiment, but put the bottles in darkness, and note how the amount of loss of water is affected

(*c*) Fix a long-stalked leaf, or a cut leafy shoot, in a card, passing the leaf-stalk (or the stem) through a hole in the middle of the card and sealing it up with putty or plasticine. Place several cards, each with a leaf or shoot

fixed into it, over tumblers half filled with water, and over each of these tumblers invert a dry empty tumbler, resting on the card. Notice the drops of water formed on the inside of each empty tumbler, by condensation of the water-vapour given off by the leaves.

(*d*) To demonstrate transpiration from the leaves of a rooted plant, push a leaf of a pot plant into a test-tube, rolling the leaf up if necessary; plug the mouth of the tube with cotton-wool, and note the moisture that condenses on the inside of the tube. Another plan is to clip or cement a small watch-glass on a large leaf, so as to form a chamber in which moisture may condense

263. The Water Channels in Root, Stem, and Leaf.—By various simple methods we can demonstrate that the transpiration current passes along the xylem of the vascular bundles.

(*a*) Get leaves with broad thin blades and long stalks, *e g.* Lesser Celandine, Tropaeolum, Violet—in winter the radical leaves of Garlic Mustard are useful for transpiration experiments, or seedlings can be used. Place them in narrow-necked bottles containing red ink, and note that in a short time the ink runs up in the stalk and colours the veins in the leaf-blade. Good results are obtained by using a shoot with white flowers, the veins in the petals being coloured by the ink; or by using transparent stems, *e g.* Balsam, and noting how the vascular bundles of the stem are stained by the ink.

(*b*) To demonstrate the path of the water in a woody stem, cut two similar leafy twigs, and from a place towards the base of each cut a ring of tissue, in one case (*A*) including only the soft outer tissue as far in as the sticky cambium, but in the other case (*B*) including also the outer portion of the wood. In each case cover the injured part with vaseline, and set the two twigs in water; note that in *A* the leaves remain fresh, while in *B* they soon become wilted. Two other twigs similarly treated should be set in red ink; after some time see whether the ink has reached the leaves.

(*c*) Set a Broad Bean seedling in a bottle, with its root dipping into some red ink. After a time the veins of the leaflets become coloured; now cut across (1) the portion of the root which was above the ink, (2) the stem, (3) a petiole, and note the red-stained vascular strands

(*d*) To show that the water travels in the xylem vessels, it is necessary to use pigments which will stain the lignified walls, and to examine microscopically thin sections of the stem after it has absorbed the coloured water. The transparent stems of Balsam are perhaps best, but shoots of Sunflower, Broad Bean, etc., will answer Place the cut shoot in a bottle of water coloured with safranin or eosin, and after an hour (or as soon as the colour has appeared in the transparent stem of Balsam, or in the leaves of other plants) cut thin transverse sections, and note that the xylem of the vascular bundles is stained

(*e*) Set a cut leafy shoot of Lime or other woody plant in water for some time, then transfer it to water coloured with safranin or eosin. After a few hours cut thin sections, and note that only the vessels and tracheids are stained

(*f*) Repeat the preceding experiment with fairly thick branches of various trees and shrubs, and note that in some cases only the outer part of the wood is stained, the inner portion no longer conducting water.

N B —In all experiments with coloured water, in order to ascertain the exact paths of water-conduction, the experiment must not be continued too long, otherwise the stain will diffuse into other tissues than those actually concerned in conduction.

264. The **Use of Coloured Solutions,** to measure the rate of the transpiration current, is open to objections. It is easy to prove that the pigment is arrested by the walls, while the water passes onwards. Pour some weak watery solution of eosin into a tall jar, and fasten a strip of filter- or blotting-paper to the cork or to a rod placed over the mouth, so that the lower end of the paper just dips into the solution. After a short time note that the paper is wetted above the point reached by the pigment itself.

265. The Use of Solutions of Salts of metals that give a characteristic colour, or bright line spectrum, when placed in a spirit or Bunsen flame and examined with a spectroscope, is a much better method Make up a litre of 2 per cent. solution of lithium nitrate, and use some of this instead of the coloured water in the preceding experiment; cut off the uppermost portion of the paper that becomes wetted, hold it in a flame, and with the spectroscope note the characteristic red lithium line This solution may be used for measuring the rate of the transpiration current. If pot plants are used, set the plant in good light and for two days do not water the soil; then saturate the soil with the solution, set the plant in light, and at intervals of about fifteen minutes pick off a leaf, or cut off a small piece of leaf (rinsing the scissors in clean water or under a tap and drying them each time they are used), hold it in the flame, and note the red lithium line with the spectroscope Maize plants raised in culture solution answer well; transfer them to the lithium nitrate solution at the beginning of the experiment. By this method it has been found that the height reached by the solution in an hour may be as much as 40 cm. in Maize, 60 cm. in Sunflower, 80 cm. in rooted Willow cuttings, 120 cm in Tobacco

266. Does Water ascend in the Walls or in the Cavities of the Xylem Vessels?—To answer this question, we may either (1) block the vessels of a stem with wax or gelatine, or (2) strongly compress the stem in a vice so as to narrow considerably the lumina of the vessels

Uproot two well-grown Bean seedlings, or other plants, and let them get somewhat wilted. Have ready three dishes containing (*a*) a mixture of lampblack and warmed wax or cocoa-butter melting at 33° to 35° C —a temperature which will not injure the plant; (*b*) water warmed to the same temperature; (*c*) cold water. Place one seedling (*A*) in the warm water, the other (*B*) in the melted wax; in each case cut across the stem under the liquid, and note that in *B* the blackened wax enters the vessels owing to negative pressure (see § 269), then transfer both plants to the cold water. This will harden the wax in *B*, with a razor pare a thin layer from the cut end of the stem, to get rid of the surplus wax and ensure that the walls as well as the blocked lumina are exposed to the water. After some time note that *B* becomes more wilted than before, while *A* recovers from its wilted condition.

Repeat the experiment, colouring the cold water with eosin, and note that in *A* the veins after a time become tinged, while this does not happen in *B* If the blocking of the vessels in *B* cannot be seen clearly, use an additional plant, and cut across the stem to see that the wax has actually entered

Another plan is to use 20 per cent. gelatine which melts in water at about 33° and stiffens in cold water at about 20° C., blacken the gelatine, by mixing it with Indian ink, or colour it with eosin

267. Mobility of Water in Wood.—It is interesting to compare the ease with which water moves through the wood of Coniferous and Dicotyledonous trees in different directions In experiments with longitudinal cylinders of Coniferous wood, it is as well to close up the protoxylem vessels by turning a knife-point in the pith so as to form a small cavity at each end of the piece, and filling up this cavity by applying a little melted shellac or other cement.

(*a*) From a Pine twig (Fir or Yew may be used) cut pieces about 20 cm. long and 1 or 2 cm diameter; remove the outer tissue from the wood, and smooth the cut ends with a sharp knife Place the pieces in water for an hour to soak the wood, then remove one piece and wipe the ends dry, no water escapes, because that would require the entrance of air, which does not readily pass through wet membranes. Hold the piece vertically, and with a brush place some water on the upper end, the drop disappears, while a corresponding drop appears on the lower end. Invert the piece, wipe dry, and repeat the experiment.

(*b*) That small pressure is sufficient to set in motion the threads of water in the wood is also shown as follows Join a longitudinal Pine-wood cylinder by rubber tubing to the shorter arm of a J-tube, and pour water in at the other end; water escapes from the upper cut surface until the pressure is equalised. Another plan is to slip a long piece of rubber tubing over one end of the wood, fill the

tubing with water, and (holding the wood vertical) raise the tubing and note the appearance of water on the upper surface as soon as the water-level in the tube equals that of the upper end of the wood

(*c*) The pressure required to force water through the wood in different directions may be applied by means of a column of water in a long straight tube, or by mercury in a J-tube. Get three cylinders of Pine wood of the same dimensions—say, 5 cm. long and 2 cm. in diameter—but cut in different planes, so that one is longitudinal, the second radial, the third tangential, a turner will prepare cylinders like this very cheaply, and they must then be soaked well in water Experiment with each cylinder in turn as follows

Join the cylinder by rubber tubing to a vertical glass tube about 1 metre long (or use several shorter pieces joined up by rubber tubing, if a long enough tube is not available), join a funnel to the upper end of the tube, and support the funnel in a retort-stand on a shelf. Place a clip on the rubber tubing just above the wood cylinder, and set below the latter a graduated vessel to catch the water that passes through the wood Pour clean water into the funnel until the whole apparatus is filled, then release the clip and note how long it takes for, say, 100 c.c. to pass through.

(*d*) If in an experiment like the preceding we use an emulsion—*i e.* water containing fine insoluble particles in suspension—we shall be able to determine that the pits in the tracheids of Coniferous wood are closed, hence the water of the transpiration current must filter through them; and that vessels are, on the other hand, continuous tubes, though of limited length

Prepare longitudinal cylinders of Pine wood (*A*) and of the wood of a Dicotyledonous tree (*B*); in each case compare cylinders of different lengths—*e g* 2 cm., 10 cm., 20 cm. Soak the cylinders in water Stir some vermilion or cinnabar into distilled water, and filter it through blotting-paper; examine a drop of the filtrate with the microscope, and note the numerous suspended particles in it Force

the emulsion through the cylinders, using it instead of the water in the preceding experiment.

In the case of Pine or other Coniferous wood, colourless water passes from the lower end of the wood In the case of Dicotyledonous wood, containing vessels, the particles pass through—unless the cylinder is longer than the longest vessels in it In each case examine the wood to see whether it is coloured throughout or whether the colour is confined to the young outer wood; also cut longitudinal sections and note with the microscope the distribution of the particles

(*e*) Another method, which gives interesting data as to the length of the vessels in Dicotyledonous wood, is to suck through a cut stem first a mixture of 1 part "dialysed iron" (oxychloride of iron, which is a colloid and incapable of diffusing through membranes) and 3 parts water, and then a solution of ammonia—which produces with the iron salt a red-brown precipitate

Cut a piece about 10 cm. long from the middle of a twig of Alder or Lime about eight years old (other trees may be tried), holding the stem under water Join the upper end of the twig by strong rubber tubing to a piece of glass tube, and join this in turn to a gently-acting aspirator. Let the lower end of the stem dip into diluted iron, and note that the fluid that passes through the stem is colourless instead of brown if none of the iron has traversed the stem. After an hour, transfer the lower end of the stem to a solution of ammonia, and apply suction again until the water that passes through smells strongly of ammonia. Examine sections with the microscope and note the distribution of the precipitate formed.

268. Sucking Force of Transpiring Leaves.—Connect a leafy shoot—*e g.* a Willow twig—by rubber tubing to a straight glass tube about 20 cm long. Fill the tube with water and dip it into mercury in a dish. As the water is used up by the leaves, mercury enters the tube and may rise several centimetres in a few hours.

269. Negative Pressure in the Water Channels.—In the stem of an actively transpiring plant there is often a partial vacuum in the water-conducting channels of the xylem. Starting with a plant in which these channels (vessels or tracheids or both) are filled with water, if the plant does not receive a good supply the water gradually disappears from the cavities of these xylem elements, so that they will contain moist rarefied air, thus leading to "negative pressure" If the stem is cut across under liquid this at once rushes into the vessels until the normal (atmospheric) pressure is equalised; if the stem is cut in air, the air of course enters the opened vessels, to equalise the atmospheric pressure.

In connection with negative pressure, it is to be noted that the presence of air in the vessels retards the transpiration current, when the air is under ordinary pressure; also that, although the membranes of the vessels (and tracheids) are very permeable to water, they are much less permeable to air when moist, but when dry they allow air to pass readily through them. Negative pressure is also of practical importance—in experiments on transpiration the stem or petiole should be cut under water, not in the air

It is easy to demonstrate the existence of negative pressure in any shoot in which the leaves are transpiring and the root is not absorbing enough water to replace that lost by transpiration

(*a*) Pull up two Bean seedlings, and let them lie on the table until somewhat wilted; then place each seedling with the lower part of the shoot in red ink, and cut across the stem under the ink. That the ink at once rushes up in the xylem of the bundles is seen by immediately slitting the stem of one seedling longitudinally, and cutting transverse sections at different heights in the other seedling. For comparison, pull up a third seedling which has been well watered and is not at all wilted, treat it in the same way, and note that in this case the ink travels slowly up the stem.

(*b*) Of two similar herbaceous plants in pots, keep one unwatered and in a dry place, the other being kept moist

by ample watering under a bell-jar Cut a shoot of each under (1) eosin solution or red ink, (2) mercury, (3) melted cocoa-butter coloured with lampblack If a plant with a very transparent stem (*e g* Balsam) is used, note the height to which the liquid runs by simply holding the shoot up to the light, if the stem is not transparent enough for this, at once slit it longitudinally, or cut transverse sections at different levels, and examine with the microscope, to see how far the coloured liquid has reached in the vessels

(*c*) Let a number of different plants become partially dry and wilted, and cut from each plant two shoots, one under water and the other in air. Place both with their cut ends in water, and note which one remains wilted and which one becomes fresh and flaccid.

270. Influence of External Conditions on Transpiration (Weighing Experiments).—Various forms of apparatus may be used for this inquiry—experiments with Potometers are given later As might be expected, transpiration is influenced largely by the same factors as those controlling ordinary evaporation—temperature, humidity of the air, movement of the air

In **weighing experiments** the simplest plan is to set a cut shoot in a bottle of water, then pour in olive oil to form a layer on the water and prevent direct evaporation, and weigh the whole apparatus. Or pot plants may be enclosed with rubber sheeting, to prevent evaporation from pot and soil, leaving only the shoot uncovered. Another plan is to cover the soil with a divided disc of cork previously soaked in and smeared with wax, and to wrap rubber sheeting over the pot; to water the plant during the experiment, fit a corked tube or thistle-tube into a hole in the cork disc.

A still better method is to use the "aluminium shells" supplied by the Bausch and Lomb Optical Company (Fig. 58); the rubber roof (made by cutting a hole with a cork-borer in the middle of a piece of rubber sheet, cutting a slit from the hole to the margin of the piece, placing

the sheet round the stem and sealing the stretched and overlapped cut edges with rubber cement) is held by an aluminium band in a groove below the edge of the shell Use a plant with large leaf-surface but grown in a small pot so as not to be too heavy for the balance.



Fig. 58 —A Pot Plant, enclosed in an Aluminium Shell, for Transpiration experiments

(*a*) To test the influence of **light**, determine and compare the loss of weight, by transpiration, in successive equal periods in (1) direct sunlight, (2) in diffuse light, (3) in darkness

(*b*) To test the influence of **atmospheric humidity**, set the apparatus for equal periods (1) in moist air—place it below a bell-jar on a wet cloth, along with a dish of water, (2) in dry air—replace the wet cloth by a dry cloth, and the dish of water by a dry dish containing dry calcium chloride

(*c*) Determine the influence of **temperature** by setting the apparatus in a warm place, a cool place, and a cold place. Warmth may be supplied by placing under the bell-jar a dish of dry sand, and after noting the loss in weight during an hour, taking out the dish of sand, heating the sand, and placing it again under the bell-jar. To cool the air, set the apparatus in a large flower-pot, fill this with sawdust up to the edge of the pot containing the the plant, and above this place chopped ice, and cover with a bell-jar.

271. Influence of Water-Supply Conditions on Transpiration.—In the weighing experiments just described, we have studied only the conditions that surround the transpiring leaves, but transpiration is of

course affected by factors which bear upon the absorbing roots. The chief of these are (1) the quantity of water available; (2) the temperature of the soil, (3) the soluble substances present in the soil In each of the following experiments use the weighing method, with pot plants in which pot and soil are covered—*e g* with an aluminium shell

(*a*) Determine the loss of weight by transpiration of two similar plants, one of which (*A*) is supplied each day with as much water as has been lost during the preceding twenty-four hours, while the other (*B*) is left entirely unwatered

(*b*) Put a thermometer in the soil with a pot plant. For a day determine its transpiration loss under normal conditions. Then immerse the pot in a vessel of chopped ice, with a felt pad or other packing of non-conducting material to prevent the ice from cooling the leaves. Determine the loss of weight in three hours, after removing the pot from the ice and wiping it dry. Leave the plant for an hour to recover, then place it in a vessel of water heated by a spirit-lamp or Bunsen until the soil is at 35° C.; keep it at this temperature for three hours, and find the transpiration loss for that time

(*c*) Determine the daily loss of a pot plant for two days, and then water the soil on three successive days with 1 5 per cent., 1 per cent., and 0 5 per cent. of potassium nitrate, and observe the effect on the amount transpired each day.

272. Transpiration checked by Bloom, Cuticle, and Cork.—By simple weighing experiments we can demonstrate the importance of these coverings in reducing transpiration from leaves, etc.

(*a*) Select three apples of about equal size, well covered with waxy bloom Rub one (*A*) with a cloth dipped in warm water, so as to remove the bloom (water at 35° C. will not injure the cells); peel the second (*B*); and leave the third (*C*) untouched. Weigh the three apples, place

them together in a dry place, weigh them at intervals daily for several days, and note the loss in weight in each case, due to loss of water by transpiration; *B* loses most water, but *A* loses much more than *C* (which loses very little) Cut and examine with microscope a vertical section of the peel from *B*, and note especially the cutinised epidermis

(*b*) That the waxy bloom on many leaves limits the rate of transpiration is easily shown. Take two leaves of Ficus (India Rubber), and to the petiole of each fasten a piece of wire with a loop; from one leaf (*A*) wipe off the bloom, using a cloth and warm water, and leave *B* untouched Weigh the two leaves, hang them up, weigh them two or three times daily, and note that *A* loses more than *B*.

(*c*) Take two potatoes of equal size. Peel one (*A*) so as to remove the corky layer; weigh the two, place them together in a dry place, weigh daily, and note that *A* loses much more water than *B*. Examine thin vertical sections of the peel, and note the cork layer at the surface

(*d*) The slight loss of water from unpeeled Potatoes is chiefly accounted for by the presence of lenticels Cut two similar short pieces from a twig of Horse Chestnut or other tree showing conspicuous and not too crowded lenticels. With wax seal up in *A* the lenticels, and in *B* patches of cork corresponding in size to the lenticels sealed in *A*. Weigh both pieces, put them in a dry place, and after twenty-four hours note that *A* has lost less water than *B*.

273. Cuticular and Stomatal Transpiration.— Though various simple experiments show that very little of the water lost by a leaf passes through the cutinised epidermis, especially when the cuticle is impregnated with or covered by waxy substances, it is equally easy to prove that the cuticle is not absolutely impermeable to water

(*a*) Cut off a Begonia or Ficus leaf (or other leaf with a thick cuticle and no stomata on the upper surface), and lay it in a dish together with a glass slide or a watch-glass.

Slightly moisten a handful of salt by adding a drop or two of water, sprinkle some of the salt over the upper surface of the leaf and also over the glass side, cover the dish, and note that the salt on the leaf soon becomes deliquescent, attracting water out of the leaf tissue through the cuticle, while the salt on the slide remains comparatively dry—in both cases, of course, the salt absorbs some water also from the air Instead of salt we may use dry calcium chloride, which readily deliquesces, or dry cobalt nitrate, which on being dried (*e g.* by heating) is blue, but turns red when moist; or copper sulphate, which is white when dried but blue when moist.

(*b*) Take four similar Begonia or Ficus leaves Make an easily melted mixture of wax and olive oil, and with this cover the upper side of *A*, the lower side of *B*, both sides of *C*, leaving *D* untouched In each case fasten a wire or thread, 10 cm. long, into the stalk of the leaf, forming a loop, smear the cut end of the stalk. When the wax has cooled and set, weigh the four leaves, hang them up, and after some hours of exposure to light weigh them again, and compare the loss in each case

(*c*) **Cobalt Paper Method.**—Soak pieces of filter-paper, or thin white blotting-paper, in 5 per cent solution of cobalt nitrate (or chloride), and dry them, when the papers turn blue, Take two of the dry cobalt papers, two dry sheets of glass, and a leaf (wiped dry with a soft cloth, if necessary), and arrange them thus:—Glass, paper, leaf, paper, glass. Sheets of mica are perhaps better than glass. Fasten the whole together with clips, and note which surface of the leaf causes the paper to redden or even turn white (owing to transpiration) the more rapidly. Various leaves should be tried, especially those in which the stomata are all or mostly on the lower surface—*e g.* Ivy, Willow, Phaseolus, Lilac. Test the sensitiveness of the papers to moisture by breathing on a dry paper, and seeing whether it changes from blue to red or faint pink, or nearly white

Prepare a number of cobalt papers for further experiments; if put aside, they will probably lose their blue

colour, but this can be restored by drying them again over a flame.

274. Transpiration influenced by Opening and Closing of Stomata.—As may be proved by simple experiments, light accelerates transpiration. No doubt this is chiefly due to the heating effect of the light rays entering the leaf, but in many cases at any rate the acceleration is increased by the widening of the stomata under the influence of light. The stomata of different plants do not, however, always react in the same way to light; in Lime and various other plants they open widely only in direct sunlight, while they close in diffuse light; on the other hand, in Willow they remain open in diffuse light. Another important factor in the movements of stomata is the vapour tension of the air—abundance of water vapour in the air retards transpiration, for purely physical reasons, but at the same time it usually causes the stomata to open widely, on the other hand, in dry air evaporation is increased, but in many plants transpiration is reduced because the stomata close when wilting begins. Here again there are exceptions—in many plants (especially those growing in marshes and shady places) the wilting leaf has its stomata wide open and therefore continues to give off water and soon becomes shrivelled, when the air is dry and the water-supply is curtailed or stopped

(*a*) Cut leafy twigs of Lime and of Willow, and set each in a bottle of water, covering the water with a layer of olive oil; weigh the whole apparatus in each case, expose to bright light for two hours, and weigh again. From a leaf of each plant tear or shave off a piece of the lower epidermis, mount in water, and note that the stomata are widely open; also test a leaf of each plant with cobalt paper. Now set both plants in not too bright diffuse light, and after two hours weigh again; note that the Lime has lost very little water, while the Willow has transpired vigorously; examine a piece of epidermis in each case, and note that in Lime the stomata are closed while in Willow they are still wide open—also apply the cobalt paper test, comparing the rate of change of colour in the two cases. In winter, use the common indoor plants Cyperus alternifolius (which behaves like Willow), and Aspidistra or Ficus (which behave like Lime) for these experiments.

(*b*) Set two cut twigs of Lime in water ; expose one (*A*) to direct sunlight, the other (*B*) to weak diffuse light. After an hour, apply the cobalt test to a few leaves of each, and note the marked difference in the rate of reddening of the paper in contact with the lower surface in the two cases

(*c*) Weigh cut twigs of Willow and of Lime, as nearly as possible similar in weight, also in size and number of leaves Lay the two twigs together on a dry table or shelf Weigh them at intervals during two days ; note that the Willow transpires much more actively than the Lime, and that its leaves become quite withered and dry at a time when the Lime leaves are still fairly fresh. Test a wilting leaf of each plant with cobalt papers—the stomata are closed in Lime, but open even in the withering leaf of Willow

(*d*) Take a pot plant of Aspidistra or Ficus, cover the pot and soil with sheet rubber, and put the plant in bright light for half an hour ; weigh it, then set it back in direct light for two hours, and weigh again—note the loss by transpiration Now set the plant in weak diffuse light for half an hour, weigh it, put it back in the diffuse light for two hours, and weigh again—note the greatly reduced loss by transpiration in weak as compared with strong light Repeat the experiment in the reverse way—putting the plant first in weak and then in strong light

(*e*) Set a Tropaeolum plant or a Bean seedling in a dark place, or under a cover, overnight ; in the morning pick off a leaf and test with cobalt paper, noting the time required for reddening, then set the plant in the light, and in half an hour test another leaf in the same way—or pick off a leaf at intervals of five minutes and test them with the cobalt paper, to ascertain whether the stomata are open Remove the plant to darkness again, and at intervals test the leaves with cobalt paper, to ascertain whether the stomata have closed

(*f*) Cut off a Tropaeolum or other thin leaf and let it lie on the table until somewhat withered ; then cut off a fresh leaf from the plant, and test the two leaves together with a cobalt paper. The stomata in this plant close on wilting Let the two leaves be exposed to bright light ; the wilted leaf does not wither any further even after some hours, while the fresh leaf dries up rapidly because it does not close its stomata.

(*g*) Get fresh shoots of Alisma or Menyanthes, and let a cut leaf of one of these plants remain on the table, along with a cut leaf of Tropaeolum, until the leaves are withered and half-dried Then apply the cobalt paper test, and note that there is still vigorous transpiration from the wilted leaf of the marsh plant, in which the stomata remain open, while in Tropaeolum this is not the case owing to the rapid closure of the stomata on wilting.

(*h*) That salt-solution causes the stomata to close may be shown, without using the microscope, by means of cobalt paper Water

a Bean or other seedling in a pot with 0 5 per cent solution of common salt for a few days. Test with cobalt paper leaves from this seedling (*A*) and leaves from a seedling (*B*) watered in the ordinary way. The leaves of *A* redden the paper much more slowly than those of *B* A *weak* salt-solution actually keeps cut or dug-up plants fresh ; plants sent by post will wilt much less if sprinkled with a weak solution of salt

275. Relation between Transpiration and Absorption.—This may be determined roughly by means of apparatus like that of Fig. 59. As the plant in the jar absorbs water, the water column in the narrow graduated tube (covered with a layer of oil to prevent evaporation) sinks, measuring the volume absorbed ; the amount of water transpired is simply measured by weighing the whole apparatus. It is best to use a rooted plant (*e.g* Bean or Maize seedling grown in culture solution), though a cut shoot (*e g* a Willow twig) will serve to show that the amounts absorbed and transpired are roughly equal By increasing the temperature of the air, transpiration may be increased until the plant loses more water than it absorbs , if transpiration is then diminished by covering the apparatus with a bell-jar, more water will be absorbed than is transpired.

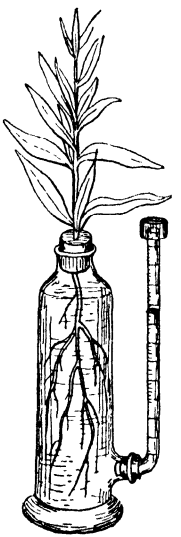


Fig 59.—Apparatus for estimating the Absorption of Water by the Roots, and the Loss by Transpiration from the Leaves

An apparatus of this kind merges into the forms called Potometers

276. Potometers.—By making the graduated tube in Fig. 59 very narrow, and sealing into the larger vessel a large and actively transpiring plant, we could measure with greater accuracy and convenience the passage of the water through the plant. This is the principle of the **potometer,** of which almost endless forms have been devised.

(*a*) A fairly good simple potometer may be made as follows. Fit a wide-mouthed bottle with a rubber stopper having two holes; through one hole fit tightly the stem of a cut shoot or the stalk of a large leaf, and through the other hole one end of a twice bent narrow glass tube—the straight horizontal part of which should be at least 20 cm long The other end of this tube dips into a dish of water supported on two or more blocks of wood or other objects that can be slipped from under it when desired Fill the bottle to the brim with boiled water, so that when the stopper (carrying the plant and the glass tube) is forced into the neck enough water will pass into the narrow tube to fill it—if not, fill the tube with water before forcing in the stopper, keeping the water from running out by placing a finger on the end. When about to take a reading, remove the dish of water, let a short air-bubble enter the open end of the tube, and time the passage of the bubble along the tube

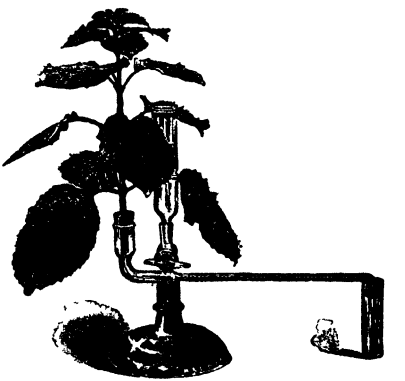


Fig 60 - Ganong's Potometer

The obvious disadvantage of this makeshift potometer is the necessity for taking out the plant-carrying stopper at the beginning of each experiment, in order to pour more water into the jar. See Appendix III, Fig 118

(*b*) One of the best forms of potometer is that shown in Fig. 60 In this instrument, belonging to Prof Ganong's "Normal" set, and supplied by the Bausch and Lomb Optical Company, the shoot chamber is made small, so that the water may quickly take the temperature of its surroundings and not vitiate the readings by volume changes; the record tube is horizontal, so as to prevent buoyant rise of the air-bubble used as the index of movement of the water The record tube is calibrated as well as graduated in c.c, so that transpiration may be determined absolutely as well as relatively; and it has a small lateral air-opening, readily closed by a sliding piece of rubber tubing The reservoir of water, which can be used either to supply a reserve to the plant or to drive the air-bubble back to the starting-point, is connected with the rest of the apparatus by a stop-cock, and is made removable to permit the use of the potometer with a supported bell-jar as shown in Fig. 61

To start an experiment, close the air-opening on the record tube,

fill the reservoir and the shoot-chamber with boiled-out water, cut the shoot under water and fix it into the rubber stopper, sealing with soft wax, then push the stopper, with the lower end of the shoot projecting, into the chamber neck; open the air-hole to let the record tube fill, close the stop-cock, and the apparatus is ready. Transpiration at once draws upon the water, so that air enters the air-hole; the bent end of the tube is then placed in a vial of boiled water, after an air-bubble has been admitted to act as an index. When the index bubble reaches the other end of the record tube, open the stop-cock so as to drive the bubble back again. When no observation is being made, close the air-hole and open the stop cock, when the plant will be supplied from the reservoir tube

277. Potometer Experiments.—The potometer affords an extremely neat and effective method for demonstrating and measuring the rate of the transpiration current in the same plant under different external conditions. In making comparative readings we may either observe the distance travelled by the index bubble in a given time, or the time required for the bubble to travel a given distance

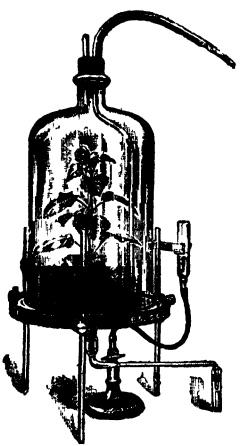


Fig 61.—Ganong's Potometer, fitted into a supported Bell-jar

(*a*) The influence of varying external conditions may be determined by simply carrying the potometer into different positions and taking readings, for instance, (1) in a warm position and in a cool position; (2) in bright light, in diffuse light, in shade, and in darkness; (3) under a bell-jar or glass-sided box with air dried by calcium chloride, or kept moist by means of a dish of water; (4) in still air and in a draught. In each case allow a few minutes under the new conditions for adjustment to temperature, etc.

(*b*) For more exact experiments the shoot should be passed through a plate into a supported tubulated bell-jar, the glass stopper of which is replaced by a rubber one carrying inlet and outlet tubes (Fig 61); a thermometer should be placed in the jar.

In this apparatus the external conditions may be varied one at a time—(1) light, by shading in various ways; (2) humidity, by drawing air, by means of an aspirator, through calcium chloride U-tubes, or through wet sponge, into the chamber, (3) temperature, by drawing the air through a glass tube heated by a spirit or Bunsen flame, or cooled by ice or cold water

(*c*) Many of the transpiration experiments already given should be repeated with the potometer, and various others may be made For instance, leaves of a shoot fitted into a potometer should be smeared with wax and vaseline on their upper or lower surfaces or both, the effect of this treatment on the rate of the current being noted. To imitate the effect of a hairy covering, tie cotton-wool over the leaves with thread—vary the experiment by covering the upper surface, lower surface, or both surfaces—and note the slowing down of the current. The diminution of transpiration by the rolling-up of leaves—either temporary as in Psamma, or permanent as in Erica or Empetrum—may be demonstrated by rolling up each of the flat thin leaves of a shoot fixed in the potometer, to form a tube with the lower epidermis on the inside, and tying it with thread; note the reduced rate of the transpiration current.

278. "Root Pressure."—Evaporation from the leaves will tend to suck up fresh supplies of water to replace that removed by the air in the form of vapour—this upward suction is easily proved Is water also *forced* upwards by the root? It is not easy to test the individual absorbing root-hairs and rootlets, but we can ascertain the collective action of these organs as shown by the forcing of water upwards from root to stem

(*a*) The exudation of sap forced up by the root may be demonstrated as follows. Cut across the stem of a vigorous pot plant, at about 5 cm. above the soil. Over the stump slip a tight piece of strong rubber tubing, with about 2 cm. projecting, and into this insert a tight cork with a slender bent glass tube leading into a graduated vessel containing some oil to prevent evaporation. Keep the soil watered, and measure the amount of water exuded. Suitable

plants for root-pressure experiments are Fuchsia, Begonia, Marguerite, Phaseolus

(*b*) A simple method of demonstrating exudation is the following Cut across the stem of a Phaseolus seedling, or a small pot plant, tie a bit of rubber tubing about 10 cm long over the stump, and fill this with water coloured with red ink Then insert in the rubber tube a capillary glass tube, and support this horizontally—*e g.* by means of a cleft stick placed in the soil Pinch the rubber tubing so as to force some water out of the open end of the fine glass tube, and absorb it with blotting-paper; then release the rubber tube so that air is drawn into the glass tube. Watch the advance of the coloured water along the glass tube, from the open end of which it falls in drops

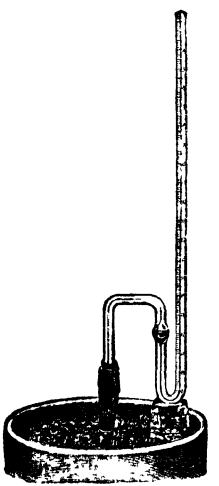


Fig 62 —Manometer, for demonstration of Root Pressure

(*c*) That the sap exudes under pressure may be simply demonstrated by joining to the cut stump, by means of rubber tubing, a long straight glass tube of the same diameter as the stem, keeping this tube vertical by lashing it to a stick placed in the soil or fixing it to a retort-stand. Fill up the tube with water to a point about 5 cm above the rubber tubing, marking the place with a file-scratch or a strip of gummed paper, and pour in a little olive oil to prevent evaporation of the water.

(*d*) In order to **measure,** roughly at any rate, the pressure set up during exudation—that is, root-pressure—it is necessary to use a manometer or pressure-gauge instead of an ordinary glass tube. An excellent form of manometer, supplied by the Bausch and Lomb Optical Company, is shown in Fig 62.

Clean and dry the gauge with alcohol; hold it vertically with the shorter limb in boiled (air-free) water; through a piece of thick rubber tubing slipped over the end of the long limb pour mercury

until it fills the gauge from end to end ; air is then admitted above and the mercury allowed to find its natural level ; then put the rubber tubing on again, with a clip at its free end, and pinch it below the clip, at once releasing it so that the air column is forced down round the bend to the bulb and allowed to spring back, when a part of the mercury will be forced out and replaced by water, which should fill rather less than half of the bulb ; then remove the clipped tubing, seal the end of the glass tube with a drop of hot shellac applied with a knife, and stand the gauge in the water for two hours to let the plug of shellac harden.

Join the short limb to the cut stem stump by means of thick rubber tubing and wire or string, and note the exact height of the mercury column at the beginning of the experiment. The pressure developed is calculated by Boyle's Law

279. Escape of Liquid Water from Leaves.—This process of "guttation" is shown commonly by plants on a cool evening following a hot day When the stomata close at night, water is still absorbed by the roots—in this way a plant that had become somewhat wilted on a hot day recovers its turgidity at night, and drops of water may be forced out of the leaves, usually through special non-motile stomata (water-pores) on the edges of the leaves In Grasses the water escapes between the ridges on the upper side of the leaf, and in seedlings at any rate from the tip of the leaf.

(1) Cover various growing plants with a bell-jar overnight, and look for water-drops excreted by the water-stomates The plants should be growing in pots, and the following will usually give good results .—Fuchsia, Tropaeolum, London Pride (a Saxifrage, with chalk-glands). In a cut twig of Cherry, set in water and kept under a bell-jar, drops of water are seen oozing from the glands on the leaf stalk

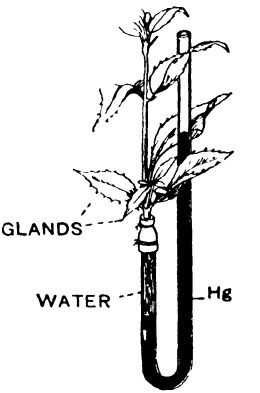


Fig 63 —Mode of demonstrating the Excretion of Fluid Water from Leaves

(2) Cover seedlings of Wheat or Maize with a bell-jar, and note the excretion of water from the tips of the young leaves.

(3) Fix a cut piece of a Fuchsia into the short limb of a J-tube, as shown in Fig. 63 Pour some water into the tube and then pour in mercury. Drops of water are caused to escape from the " water glands" on the teeth of the leaf-margin. A water-gland is a mass of tissue on the end of a vein, communicating with the water-stomates on the leaf-teeth.

280. Root Absorption: Corrosive Action of Roots.—It is readily shown that roots are able to bring into solution substances which are insoluble in pure water, that roots have an acid reaction, and that they at any rate give out carbon dioxide which dissolves in water to form carbonic acid.

(1) Half fill a small flower-pot with wet sand or fine soil, insert a flat slab of marble with the upper surface polished; fill up the pot with sand, and plant a soaked Bean or other seed so that when the roots reach the marble they will grow over it horizontally. After about ten days, remove the marble, rinse it with water, and note the lines of corrosion where the root and its branches have removed the polish from the surface.

(2) Grow seedlings with their roots resting on blue litmus paper wetted with distilled water, and note the change in colour where the roots touch the paper. Another plan is to use gelatine solution (1 part sheet gelatine to 5 parts water) coloured with litmus solution made blue with lime-water. Place some of this gelatine in a saucer, set in it some germinated Peas or Beans with radicle 3 to 6 cm long, and note the change in colour (blue to red) of the gelatine around the roots Or place the gelatine in the tube of a funnel stoppered with a small cork, support the funnel, insert a Pea seedling with its roots in the gelatine, add cotton soaked in distilled water to keep the seed moist and cover the funnel with a glass sheet.

(3) To show that roots give out carbon dioxide, which on being dissolved in water yields acid, grow seedlings for a short time with their roots dipping into lime-water; set up a control experiment with a jar containing lime-water but no plant.

281. Root Absorption: de Saussure's Law.—The roots of a plant placed in solutions of salts do not necessarily absorb the water and the salt in the same proportion. This is called de Saussure's Law, though he only observed the special case in which the root was placed in a relatively strong solution and absorbed less than the due proportion of the salt and more than that of the water.

(1) Place any rooted plant—*e g* a Maize seedling raised in culture solution—for a day in distilled water, and then transfer it to a jar

containing 0 1 per cent solution of potassium chloride in distilled water. After several days (about 6) measure the volume of solution left in the jar, then evaporate it to dryness and weigh the residue of salt; or analyse it volumetrically by titration with decinormal silver nitrate, using potassium chromate as indicator. Calculate the proportion of salt left in the solution, and note that it is about twice as great as in the original solution.

(2) Pull up and rinse in water three similar Bean seedlings germinated in moist sawdust. Get ready three small jars, each holding about 120 c c ; into each pour 50 c c. of water, gum a strip of paper at the water level, throw the water out, then fit each jar with a bored cork, and into the hole pass a seedling. Into *A* pour 100 c.c. of 0 25 per cent. solution of potassium nitrate in distilled water; into *B*, 100 c c. of 0 05 per cent. solution; into *C*, 100 c c. of 0 025 per cent. solution. Place the three jars in the light, and in each case when the level of the solution falls to the 50 c.c. mark, take out the plant, rinse its root in a little distilled water, and add this water to the solution ; then pour the solution into a weighed evaporating dish or a flask, boil to dryness, and weigh again. From the data thus gained, we find that the plant absorbs from the 0 25 per cent solution relatively much water and little salt, the solution left being more concentrated than that originally offered to the root. On the other hand, with weaker solutions the plant absorbs relatively more salt than water, so that the remaining portion of the solution is more dilute than that originally present.

CHAPTER VII.

MOVEMENT IN PLANTS.

I Phototropism (Heliotropism).

282. Positive Phototropism.—A familiar example of tropistic movement is the turning of shoots towards the light, as shown by plants growing near a window. Ordinary erect (orthotropic) shoots grow towards the source of unilateral illumination, the stem tending to place its axis parallel with the direction of the light (positive phototropism), while the leaves place their surfaces at right angles to it (diaphototropism).

(*a*) Grow seedlings of Bean, Wheat, Sunflower, etc., in darkness the shoots are erect. Now place them in front of a window, or in some other position where the light falls on them mainly from one side, and note the changed direction of growth of the shoots. When marked curvature has taken place, turn the seedlings round again, through 180°, and note the result.

(*b*) Place in each compartment of a phototropic or dark chamber a pot plant, or some seedlings, or a cut shoot in a bottle of water. Make one compartment light-tight, but allow light to enter the other through a slit in the side Try various plants. A very rapid reaction is given by seedlings of Tare (Vetch) and of various Grasses (especially Millet, Italian Millet, and Canary Grass) that have been germinated in darkness; Phaseolus shoots are less sensitive, while cut young shoots of Elder, etc., react rather slowly.

283. Dark Chamber for Phototropism Experiments.—Make a "phototropic chamber," large enough to contain pot plants

(*a*) Get a box of wood or stout cardboard, with one side open; the size should be about 40 × 40 × 20 cm Insert a vertical partition to divide the box into two equal compartments (each 20 cm. square and 40 cm high); paint the whole of the inside black The amount and direction of the light falling on the plants can be further regulated by placing over the open side a sheet of white paper (for diffuse light), or a sheet of black paper or cloth or card with a vertical slit cut out of it.

(*b*) For various experiments, all that is required is a cardboard box painted black inside and with a vertical slit cut in one side (the side which is to face the light). Set the pot of seedlings on a plate or shallow box of wet sand, and let the rim of the dark-box rest on the sand so as to exclude light from below

284. Region of Phototropic Curvature.—Grow Bean seedlings in darkness when the epicotyl is 4 or 5 cm long mark it with ink lines at intervals of 5 mm , starting from the apex. Expose to one-sided light, and note that the curvature takes place in the actively growing upper region

285. Curvature and Turgidity.—Cut off the plumules of (*A*) a few Bean seedlings that have been exposed to one-sided light only until the plumule has bent slightly, (*B*) similar seedlings exposed for 24 hours. Put the two lots in strong (15 or 20 per cent) salt solution, in two saucers. In *A* the curvature is diminished by plasmolysis, because the difference in osmotic pressure between the convex and concave sides has not yet been completely fixed by growth, in *B* the curvature is not affected by plasmolysis, since it has now become fixed by growth

286. Transmission of Stimulus.—Germinate seeds of Wheat or Oats in darkness, in pots filled to the rim with soil. When the shoots are about 2 cm. long expose them to one-sided light Examine them at frequent

intervals, and note that the curvature begins at the tip and proceeds downwards; the upper region, as it continues to lean forwards, becomes straightened; eventually (usually after about six hours) the curvature is found at the base, with which the upper part of the shoot forms an angle of 60 to 90°.

287. Perception of Stimulus.—Take a number of Oat seedlings, germinated in darkness until the shoot is 2 to 3 cm long Model on the tapering point of a lead-pencil a number of small conical caps of tinfoil, about 5 mm. long and just large enough to fit closely over the tip of the young shoot. Note that the curvature begins at the top of the unshaded part, proceeding downwards more slowly than when the whole seedling is illuminated, and remains comparatively flat (10° to 40°). This experiment should be made simultaneously with the preceding one; in both cases fill the pot with soil right to the rim, to prevent any shading of the shoot when placed in the chamber with light entering by the lateral slit.

Seedlings of Millet (Sorghum) and Italian Millet (Setaria italica) are more sensitive than Oat seedlings, and should be used for various experiments on phototropism.

288. Perception and Transmission.—Grow Oat or Wheat seedlings in darkness, in pots not quite filled with soil (to within, say, 2 cm. from the rim). When the shoot is about 1 5 cm. long, cover the seedling with sand or fine soil so that it is almost buried, with only 2 or 3 mm of the tip projecting. Expose to one-sided illumination, and note the result. No light can enter the soil (beyond a depth of perhaps 2 or 3 mm), yet the basal part of the shoot curves; evidently the heliotropic stimulus has been transmitted to this part from the exposed tip

289. Negative Phototropism may be demonstrated in the roots of some plants. Sow a few Mustard seeds on muslin tied over three tumblers of water. Set the tumblers in darkness: the shoots grow straight upwards, the roots straight downwards. When the seedlings are well grown, keep one set (*A*) in darkness; put *B* in a position where

it will get the light evenly all round, put *C* in a chamber so that it gets light only from one side, through a vertical slit. After a few days note the directions of growth of roots and shoots in the three cases

290. Influence of Light Intensity on Nature of Response.—That the same organ may differ in its heliotropic response according to the intensity of the light may be shown as follows. Get ready three pots of darkness-grown Mustard seedlings, each covered by a cardboard chamber with a vertical slit in one side Place *A* at a distance of several yards from a window; place *B* close to a window but in diffuse light; expose *C* to direct sunlight, turning the cover from time to time so that direct sunlight shall enter the slit. After several days compare the three sets of seedlings. Both *A* and *B* curve towards the light, while *C* (exposed to strong sunlight) shows little or no curvature. If very strong light from a lantern is focussed (passing through a layer of alum solution to minimise the heating effect) on a seedling, the shoot will show *negative* heliotropic curvature If Cress or Mustard seedlings are planted in a row in a pot or box, which is placed at an angle of 45° to the beam from the lantern, so that one seedling stands nearest the focus of the lens and the rest are more and more remote from it, the seedling nearest the focus undergoes negative curvature, the next ones remain straight, and the farthest ones show positive curvature

291. Diaheliotropism is readily demonstrated. Dig up a White Dead Nettle plant, set it erect in a pot, and tie the stem firmly to a vertical stick so that it cannot curve. Set the plant near a window, placed so that one row of leaves faces the window, *i e* if the window faces north, the four rows of leaves will face N, S, E., W After a day or two note the positions assumed by the leaves in the four rows In the N. and S. rows the petiole simply curves—downwards in the former case, upwards in the latter In the E. and W. rows, however, the petiole undergoes twisting, in order to bring the leaf blade into the right position.

II Geotropism

292. Geotropism of Root and Shoot.—Grow Bean seedlings in a glass-sided box (§ 170). When the root has produced a number of side roots, mark on the glass the positions of a few of these, also of the main root Then tilt the box up at an angle of 45° and fix it in this position, setting it in darkness From day to day note the change in the direction of growth of (1) the main root, (2) the side roots, (3) the shoot

293. "Moist Chambers" for Geotropism Experiments.—Various other simple methods may be used to demonstrate the fundamental facts of geotropism, using seedlings. For apparatus all that is needed is a receptacle in which the seedlings are given a supply of water, saturated air, and aeration daily, the apparatus should be set in the dark, to eliminate the influence of light on the direction of growth

(*a*) For small seeds, *e.g.* Wheat, place between two sheets of glass a sheet of wet blotting-paper, put the seeds between paper and glass, in different positions, put additional bits of paper at the corners to prevent too great pressure on the seeds, and clamp the glasses and papers together with clips. When the seedlings have grown, tilt the apparatus up at different angles, and note the directions of growth of the roots.

(*b*) Pin Bean seedlings, with root horizontal, to the underside of the cork of a glass jar containing some water, or pin them to the upper side of a cork, set in a saucer of water, covering all with a bell-glass

(*c*) Instead of a glass-sided box, a glass funnel may be filled with moist sawdust or sphagnum and supported in a bottle or jar, the seeds being planted close to the glass in the funnel; or a germination jar (§ 169) may be used.

294. Region of Geotropic Curvature in Root.—Take some Bean seedlings with roots about 5 cm. long, and mark the root of each with transverse Indian-ink

lines 2 mm. apart, starting from the tip Place the seedlings horizontally in a moist chamber or glass-sided box, and after a day or two measure the distances between the marks, the root having curved downwards. The region of greatest curvature corresponds to that of greatest growth in length, the curvature first becomes evident in the second zone from the tip, appearing later in the zones farther back, and the most active curvature is usually in the third zone.

295. Oxygen necessary for Geotropic Curvature.—(*a*) Take several Bean seedlings with roots 3 or 4 cm long, and pin them, with the radicles vertical, to the lower sides of the corks of two jars (*A*, *B*), using long pins. Fill *A* with water, and push the cork in tightly; put a little water in *B*, to keep the air moist, and cork loosely or leave open; then place each jar on its side, so that the roots lie horizontally, and prop them in this position The roots in *A* make no curvature, or very little, owing to lack of oxygen; those in *B* make the usual downward curvature

(*b*) Pin in a number of seedlings horizontally to a strip of wood, set the strip vertical in a bottle half filled with water that has been boiled and cooled, so that half of the seedlings are in the water and the rest above it, and put wet cotton-wool above the mouth of the jar on either side of the strip of wood In the lower (submerged) seedlings, the roots continue to grow horizontally, but in those above the water and therefore in damp air the roots soon curve downwards.

296. Effect of Removal of Root-tip.—(*a*) Take twelve Bean seedlings with roots 2 to 3 cm. long, and mark each root with a transverse Indian-ink line at 1 5 cm from the tip. Amputate the tips of the roots in all except three of the seedlings by making a transverse cut with a razor; from three of the roots remove 1 mm, from other three roots remove 2 mm., and from the remaining three roots remove 3 mm Lay the twelve seedlings in moist sawdust or sphagnum, with the roots horizontal. Note that the three intact roots grow more actively than the nine amputated roots, and make the normal downward curvature. If the decapitated roots make curvatures, these will be in various directions—sideways or even

upwards more often than downwards Hence the removal of the root-tip at any rate interferes with the normal downward geotropic curvature.

(*b*) The preceding experiment is not conclusive, but it suggests further simple experiments. Take twelve Bean seedlings with roots about 2 cm. long, and lay them horizontally in moist sawdust or sphagnum. After an hour, leave two of the seedlings undisturbed (*A*); turn two of them round so that the root points vertically downwards (*B*); remove the others (*C*) and cut off their root-tips (at 2 mm. from apex), then place them vertically. In *A* the root curves downwards; in both *B* and *C* the root curves sideways (towards the side that was downwards while the root was laid horizontally). These results show that (1) the root on being shifted from the horizontal to the vertical position proceeds at first to carry out the curvature induced in it while it was horizontal; (2) removal of the root-tip makes no difference to the carrying-out of this induced curvature

297. Apogeotropism.—The **apogeotropic (negatively geotropic) curvature** of the shoot will have been noticed in the preceding experiments, as contrasted with the positively geotropic curvature of the radicle of seedlings It may be demonstrated in various ways. (1) Lay a pot of seedlings, or a potted plant, on its side; or invert the pot, after securing the soil from falling out (2) Fix a cut shoot in the split or bored cork of a bottle or test-tube filled with water and laid horizontally. (3) Fix a shoot into a sloping bank of wet sand in a box; one end of the box may be replaced by a glass sheet, so that the changes in position of the shoot may be readily traced on the glass.

298. Region of Geotropic Curvature in Stem.—Mark the epicotyl of a Bean seedling, or the hypocotyl of a Sunflower or Castor Oil seedling, at intervals of 10 mm., starting from the tip. After twelve hours of horizontality, note the form of the curved stem, and measure the distances between the marks The strongest curvature takes place in the region of greatest growth. Later, however, when the stem has become erect, the greatest curvature is at the base of the growing region, and it continues until the upper part of the stem is carried beyond the vertical, to which it returns at a still later stage

299. Apogeotropism in Grass "Nodes."—In the stems of most plants the power of apogeotropic curvature is confined to the uppermost internodes, but in Grasses the tissue in the swollen "nodes" (really the swollen bases of the leaf-sheaths) remains capable of growth-curvature for a long time, after the internodes have become mature and rigid. Cut from the flowering stem of Rye, Barley, or other Cereal or Grass, a number of pieces about 10 cm. long, each with a "node" at the middle, and set them in order side by side, horizontally, in the sand box After a day note that the pieces have curved upwards, the younger pieces curving more vigorously than the older ones. The free internode above the node remains straight; curvature is confined to the node itself

300. Measurement of Curvature (Grass "Nodes").—Cut a piece of Grass stem with a node, and mark the latter on two opposite sides with an ink line or dot at each end of the node, measuring the distance between the two marks (say 2 to 3 mm) Stick the piece horizontally, so that one of the marked sides is above and the other below. After a day, when the node has bent, measure again, and note that the lower side of the node has grown greatly in length, while the upper side has shortened

301. "After-effect."—(1) Fit a Bean-shoot into a bottle or tube of water, using a bored or split cork and sealing with plasticine, and let the shoot project horizontally. Stick a pin or needle into the free end of the shoot and set up beside it a foot-rule, note the position of the index-pin on the scale. After half an hour (the shoot will have made little or no upward movement in that time) turn the bottle round through 180°, taking care to keep the free end at the same point on the scale. The shoot soon begins to curve *downwards*, then it comes to rest, and finally it curves *upwards*—showing that there is an interval between (1) the *perception* of the stimulus and (2) the visible *response* made by the shoot.

(2) What happens if a shoot, laid horizontally, is fixed so that it cannot curve upwards? Lay a few Bean seedlings on moist sawdust and keep the shoots down with a piece of thick glass, or fix them to a sheet of cork by means of crossed pins, and set them in moist air for about six hours. Then remove the glass or the pins: the shoot will quickly bend upwards. How can you explain this result?

302. Diageotropism and Exotropism of Rootlets.—As seen in § 292, lateral roots of the first order take up a definite angle—a few degrees below the horizontal position—to which they return when the plant is tilted. The rootlets of the second and higher orders grow in various directions; instead of being geotropic they are **exotropic,** tending to grow away from their parent root.

(*a*) Instead of tilting up the glass-sided box through 45°, turn it upside down, after tying cloth or wire-gauze over the soil (or in any other way preventing it from falling out); note that the secondary roots grow downwards until they have reached their original angle below the horizon. After two or three days, turn the box round into its original position, and note the further curvature made by these roots in order to resume their original direction of growth, thus becoming S-shaped.

(*b*) Grow a Bean seedling in the glass-sided box until it has a well-developed root system with diageotropic secondary roots and exotropic tertiary roots. Then cut off the terminal portion of the primary root, at from 2 to 4 cm. behind the tip. Note that (*a*) one of the young secondary roots curves downwards and behaves like the primary root—forming a new "tap-root" or "leader"; (*b*) the branches borne by this "promoted" root are diageotropic instead of exotropic, and these in turn bear exotropic rootlets.

303. Diageotropism in Stem Branches.—Take four potted plants of any kind, which have several lateral branches. In each case note the positions of the branches

with reference to the stem, and tie the stem to a vertical stick. Place the plants in darkness, *A* laid horizontally, *B* at an angle of 45° above the horizon, *C* at 45° below the horizon, and *D* upside down After several days note any changes in the direction of growth of the branches.

304. Diageotropism in Leaves.—Tie the stem of a potted plant to a vertical stick, secure the soil against falling out, invert the pot, and keep the plant in darkness for several days Note how the leaves move into the normal position, the petiole or base of the leaf-blade usually curves so as to turn the tip of the blade towards the stem, but this inward curvature is followed by a twisting movement—if the petiole is very short or absent, only the twisting movement (geo-torsion) occurs.

305. Diageotropism and other Orientation Movements in Flowers.—(*a*) Take four specimens of a single-flowered Narcissus in which the perianth tube is horizontal and at right angles to the flowering stem Cut off each flowering stem a few inches below the flower, and stick it through the bored cork of a test-tube filled with water. Fix the four tubes so that the perianth-tube of *A* faces vertically downwards, that of *B* at 45° above the horizon; that of *C* 45° below the horizon, that of *D* vertically upwards In which of the four does the flower-stalk curve so as to bring the perianth-tube into the horizontal position?

(*b*) Bend down the inflorescence of Monkshood (the plant should be dug up and placed in a flower-pot, set in darkness during the experiment) so that the terminal portion with its flower-buds points vertically downwards, and secure it in this position Note that the stalk of each flower curves upwards so as to bring the hood-sepal once more uppermost. But this bending movement causes the flower to face the axis of the inflorescence, and the flower-stalk now undergoes torsion so that the flower comes to face outwards. The first movement (bending) is geotropic, but the second (twisting) is evidently autonomous.

(*c*) The labellum of Orchis is posterior, but by torsion of the ovary during the unfolding of the bud it is brought to the anterior position That this torsion is induced by gravitation is shown by the fact that if the plant is rotated on a clinostat (§ 306) the flower opens in the "inverted" position The torsion can also be prevented by the following method. Cut off the flowering stem of Orchis, and bend down the inflorescence so that it points vertically downwards: the young flowers do not undergo torsion

III. Clinostat Experiments

306. The **Clinostat** (or **Klinostat**), a most important instrument in the study of geotropism and phototropism, consists essentially of a driving mechanism which causes the rotation of a rod (axis, or spindle) carrying a plant-holder In the simpler forms the driving mechanism consists of a clock, and the plant-holder is a disk to which may be attached a small pot or a wire cage, or some other arrangement in which the plant is contained or to which it can be fixed. The rotating rod may be either placed horizontally (plane of rotation vertical) or vertically (plane of rotation horizontal), or in an inclined position. The ordinary form of clinostat gives continuous rotation, but for some purposes an intermittent clinostat is used.

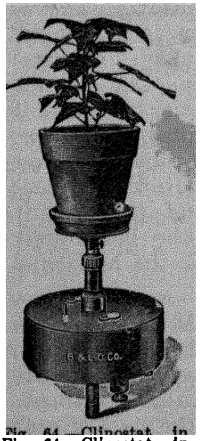


Fig. 64.—Clinostat, in vertical position as used for experiments on Phototropism

By means of the clinostat we can eliminate the influence of either gravitation or lateral illumination, or both, thus preventing geotropic or heliotropic curvatures. If the axis is vertical (Fig. 64), the effect is to remove the directive influence of light; if it is horizontal (Fig. 65), the directive action of gravity is eliminated. In the horizontal position, the clinostat may be used for both

purposes simultaneously; if the axis is parallel with the light rays, the plant (also horizontal) is subject to light

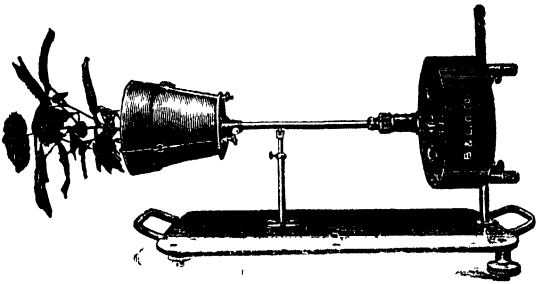


Fig 65 —Clinostat, supported in horizontal position.

stimulation but not to gravity stimulation; if the axis is at right angles to the light rays, the plant will show neither geotropic nor heliotropic curvature. See also Appendix III., Fig 119

307. Elimination of Phototropism.—Germinate seeds (*e g* Cress, Mustard, Sunflower) in two small pots (*A*, *B*) in darkness. When the erect shoots are 2 or 3 cm. long, wind up the clinostat and place it, with the axis vertical, in a dark box (heliotropic chamber) with the opening facing the window. Fix *A* to the plant-holder, so that the seedlings will be carried round in a horizontal plane; place *B* in the box beside the clinostat. In a few hours the seedlings in *B* will show a marked curvature towards the light, while those in *A* will be growing quite erect and continue to grow erect so long as they are rotated.

308. Elimination of Geotropism in Shoots.—Place the clinostat horizontal, and fix the plant-pot horizontally so that the seedlings will be rotated in a vertical plane. To prevent heliotropic curvature, cover the whole with a box, or put it in a dark room. As long as the clinostat is going, the shoots will grow straight forwards horizontally, but if the clock is allowed to stop the shoots will soon curve upwards.

309. Elimination of Geotropism in Roots.—To one side of a flat circular cork (*A*) fix several Bean or Pea seedlings in different positions, with two pins through each seed to keep it in place; between the seeds pin to the cork pieces of wet cotton-wool, some of which project beyond the margin of the cork Prepare a second cork (*B*) in the same way, with the same number of seedlings fixed in as nearly as possible the same positions With the clinostat axis horizontal, fasten *A* to the holder so that it is vertical; set a dish of water below, so that the projecting bits of cotton-wool will dip into the water as the cork revolves. Set the cork *B* vertical so that it also dips into water, some at least of the radicles should lie horizontally.

After a day or two, note that the radicles of the seedlings on the fixed cork are growing downwards, curving where necessary to do this, but the radicles of the seedlings on the clinostat simply grow straight on and undergo no curvature, so that they still point in different directions.

310. Elimination of Geotropism and Phototropism.—Place the clinostat so that the axis is parallel to the plane of the window, and fix a pot of seedlings horizontally to the holder. After a day or two note that the shoots do not curve, but grow quite straight—in this position the effects of both gravitation and light on the shoots are eliminated

311. "Rectipetality."—Fix a pot of darkness-grown seedlings to the clinostat, placed horizontally and set in a dark chamber. Do not start the clock until the seedlings have remained horizontal long enough to show a distinct apogeotropic curvature; then start it, and after a day or so note that the curvature has disappeared owing to autotropism (rectipetality)

312. Reaction Time and Presentation Time.—The time during which a root or shoot must be kept horizontal until bending begins is called the (geotropic) **reaction time**; determine this for the radicles and shoots of several seedlings—in the Bean radicle it is about 80 minutes

If a plant is laid horizontally for a time, and then removed from the geotropic stimulus by being rotated on a clinostat, whether or not it will make an "after-effect" curvature depends upon the period during which it was horizontal; determine for various seedlings this **presentation time**, or minimum period of horizontality sufficient to produce a reaction—in the Bean radicle it is about 20 minutes, in the Bean plumule about 7 minutes. But this does not mean that the root or shoot requires so long a period to *perceive* the stimulus; experiments with the intermittent clinostat show that **perception time** is so short as to be practically instantaneous. It would obviously be disadvantageous if the plant were to respond to every momentary stimulus it might receive in nature.

IV. Hydrotropism.

313. Hydrotropism.—The following experiments demonstrate the positively hydrotropic curvature of roots towards the moister part of the soil, showing that if the moisture of the soil is not evenly distributed the root will turn aside from its normal downward vertical course

(*a*) Grow seeds of Pea, Sunflower, etc., in wet sawdust in a sieve, or in a box with the bottom replaced by wire gauze, and hang the sieve or box in an oblique position or tilt it by putting a support under one end The roots grow down through the gauze into the air, but they soon curve and grow upwards again into the wet sawdust—this may be repeated several times, so that the roots become threaded through the meshes

(*b*) Take two glass jars or tumblers or beakers Place some water in *A* (about a quarter full); keep *B* dry, and in it place some calcium chloride (to keep the air dry). Tie over each a piece of coarse muslin, on the muslin place wet sawdust or sphagnum, with some seeds (Sunflower, Mustard, etc.), and cover all with a large jar or bell-glass. In *A* the roots on emerging from the muslin do not grow back into the wet sawdust (as they do in *B*), but grow down into the moist air.

(*c*) Fill with water a porous pot, of the kind used in electric batteries, and securely cork it. Soak a strip of flannel in water, and tie it lengthwise over the pot, putting

a number of soaked seeds (*e g* Sunflower) between the cloth and the surface of the pot. Hang the pot up horizontally by means of two loops of string, in such a way that the zone of seeds is horizontal. After a few days note that the roots grow down and follow the curved surface of the pot, instead of leaving it in order to grow vertically downwards. If the porous pot is suspended in a vessel containing some water, however, the roots grow vertically downwards, instead of following the curved surface of the pot.

(*d*) In the middle of a box of not too wet soil place a flower-pot, first plugging the hole in the bottom by a cork Put some soaked seeds in the soil around the pot, but do not water the soil, pour water into the pot, however, and fill it up daily as the water evaporates. After a few days remove a seedling carefully, and note that its root has curved towards the pot of water

V. Experiments with Twining Stems.

314. Material for Study.—Many experiments on twining can be made with the Scarlet Runner, but other twiners should also be used, *e.g* Hop, Convolvulus; potted plants are required for most of the experiments.

Sow Runner seeds in pots of garden soil; as the seedlings grow up, leave only the strongest one in each pot Note that the first few internodes of the stem grow erect and firm, but the later ones begin to bend so that the tip of the shoot nods to one side and becomes horizontal or even directed a little downwards Get ready several vigorously growing plants.

315. Revolving Movement of Stem Tip.—Take a plant in which the upper part of the shoot hangs over for a few inches. Tie the lower part of the stem to a stick placed in the soil, set the pot on a sheet of paper and record the position of the tip of the shoot.

This may be done in several ways ·—(1) by drawing lines on the paper radiating from the centre of the pot, so as to

show the direction in which the stem-tip points; (2) by using a plumb-line (a string with a weight tied at one end) and marking the spot on the paper below the stem-tip; (3) by fixing a sheet of glass above the plant and marking on it the position of the stem-tip Whichever plan is used,



Fig 66 —Twining Plants. I, Convolvulus, II, Hop.

record the time when each observation is made, and find out how long it takes for the stem-tip to swing round through a complete circle. In which direction does the shoot revolve—with the hands of a clock[1] or in the opposite direction?

316. Influence of Temperature on Rate of Revolution.—Compare the times taken by the same plant to make a complete revolution when kept first in a warm

[1] The terms "with the sun" and "against the sun" are sometimes used instead of "clockwise" and "anti-clockwise." The plant (placed between sun and observer) points successively to East, South, and West in revolving "with the sun"; this occurs in the Hop (Fig 66, II) and Honeysuckle The plant points successively to West, South, and East in going "against the sun," *i.e.* in the anti-clockwise direction; this occurs in most climbers, *e.g.* Scarlet Runner, Convolvulus.

place and then in a cold place, or *vice versa* At 33° C a Runner plant revolved in 2 hours 20 minutes, while at 24° C. the plant took 3 hours 25 minutes to revolve

317. Influence of Light Direction on Rate of Movement.—Place the plant near a window, so that the plane of curvature of the upper part is at right angles to that of the window, and the shoot tip faces you Note that the movement towards the light (the first half of the revolution) is more rapid than that away from the light (the second half of the revolution)

318. Revolution causes Twisting of Stem.—Mark an ink line along the convex side of the stem, and watch what happens during a revolution; place the plant as in the preceding experiment. If the shoot tip faces north to begin with, at quarter revolution it will face west and the ink line will be on the left side of the stem; therefore the zone of most active growth (indicated by the convex side) has shifted 90° to the right, while the stem tip has described a horizontal arc of 90° to the left. At half revolution, the line will be on the concave side of the stem, and so on until, when the revolution is complete, it regains its original position, and has then described a spiral

319. Tightening of Coils around Support.—Place a vertical stick near the plant in one of the pots. Note that the revolving stem on touching the stick begins to revolve in a narrower circle, twining round the stick. Later, the coils become more closely applied to the stick, also becoming steeper. The twining stem continues to grow for some time after coiling; it cannot straighten itself completely, because of the support which stands in the way, but this growth tightens the clasp of the stem on the support

320. Free Coiling of Stem-tip.—Examine a vigorous Runner plant growing in the open, and note that the shoot tips which happen to project beyond the support do not show distinct spiral coils. Cut off several of these free tips, about 5 cm long, as nearly straight as possible, and set each in a test-tube of water, placing the tubes in a

moist chamber of some kind so that the air about the cut shoots is kept saturated. Keep some of the cut shoots in darkness, others in the light. After two or three days, note that in both cases the shoot has made several free spirals, the lower (older) being steeper than the upper (younger) In vigorously growing intact plants these coils would have become more or less completely smoothed out owing to growth after coiling, but in cut shoots the coils persist because the vigour of the plant is greatly reduced

321. Influence of Thickness of Support.—Place supports of different thicknesses beside different Runner plants in separate pots. All except the thinnest supports can be made by rolling paper or cardboard into tubes and tying them at intervals with string, for thinner supports, use wooden sticks; for the thinnest, stretched strings, tied to the base of the stem below and to some convenient support above, *e g* a shelf, or a ⅂-shape made of two pieces of wood (the long upright piece lashed to the pot)

Note that the Runner will not twine round a support more than about 10 cm in diameter (probably because the support is not curved enough to enable the stem to hold on while the growing free end swings round the support), but it twines readily round a very thin support It has already been noted that the upper coils made round a support are relatively flat, while the lower ones are steeper owing to the fact that growth occurs after coiling. Note that the thinner the support the steeper are the coils, *i.e.* the greater is the erecting of the stem, with thick supports, the application of the older coils to the support takes place early, the erecting of the internodes soon stops, and the permanent coils are therefore less steep.

322. Change from thick to thin Support.—Tie a Runner to the support 3 cm. in diameter, and when the stem has made a few coils round it replace the support by a much thinner one, say 5 mm in diameter. Note carefully the appearance of the coils, making a sketch. In a few days at least the younger of the coils formed round

the thicker support become steeper, and thus become closely applied to the thin support, the younger internodes have continued their growth and have therefore become raised. The older coils do not show this change, because the growth of the older internodes has ended

323. Effect of Inversion of Plant on already formed Coils.—Take a plant which has made a few coils round a support, turn the pot upside down and support it in this position, and place it in darkness. The younger parts of the stem soon begin to unwind from the support, and the end of the stem directs itself upwards. Clearly this is because each growing zone of the stem has a tendency to grow in a left-handed ascending spiral, so that when the plant is inverted the concave side of the stem (turned towards the support) soon becomes convex, and thus the growing parts become unwound

324. Inclined and Horizontal Supports.—Try the effect of setting the stick in an inclined position, in one pot at 30° from the vertical, in another at 45°, in another at 30° above the horizontal, and lay a fourth pot plant horizontally. Note that the Runner, like most other twiners, cannot climb up a stick set at more than 45° from the vertical

325. Persistence of Torsion after Disappearance of Coils.—Take a pot plant which has made several coils round a stick, and fasten just below its tip a thread which runs over a pulley and carries a small weight, enough to keep the stem from bending over—1 gram will generally do. Make fine ink dots along the stem at short distances from each other Watch the plant during three or four days, and note that (1) free coils are formed by the upper part of the stem, but these later disappear owing to the straightening of the internodes; (2) the ink marks are no longer in a straight line, but on a spiral ascending from left to right. The experiment shows (1) that twining is not due to contact, (2) that even when the free coils of the stem disappear with growth the stem still shows torsion.

326. Smooth and Rough Supports.—Will the Runner climb up a very smooth support? Use a piece of glass tubing as a support; the stem will twine round the glass, but the coils formed are not so steep as when a rough support is used For comparison, set up a plant with a stick of the same diameter as the glass tube

327. Behaviour of Twiners on the Clinostat.—(1) Tie the lower part of a Runner seedling, which is about to begin twining, to a stick so that only the apical part (a few cm) is free. Rotate horizontally revolution does not occur. (2) Rotate horizontally on the clinostat a Runner which has already made several coils, and note that the youngest parts of the stem become loosened from the support; the youngest turns unwind, and the shoot straightens out. A twining plant when rotated on the clinostat behaves in the same way as an ordinary shoot, hence the power of twining is dependent upon geotropism The straightening out of the coils already made is evidently due to internal causes, and forms an example of autotropism (rectipetality)

VI. Experiments with Tendrils

328. Thigmotropism is a general term applied to response to contact and to mechanical shocks of various kinds. The responses made by tendrils and such organs as Sundew tentacles to contact stimuli are usually distinguished as a special case under the term **haptotropism.** Other thigmotropic responses are shown by the leaves of Mimosa and other "sensitive" plants, the stamens of Barberry, Centaurea, etc , the stigmas of Mimulus, etc

329. Material for Experiments with Tendrils.—Some simple general experiments may be made with the tendrils of Garden or Sweet Pea plants raised in pots or boxes. Note that (1) the young tendrils are slightly hooked at the tip; (2) coiling results on stroking with a pencil or stick the more sensitive apical region of the tendril; (3) coiling is caused by a small loop of thread attached to the tendril tip; (4) the tendrils will coil around supports placed at any angle whatever; (5) the

sticks or other supports used must not be very thick, since the tendrils cannot coil around a thick support

But since Pea tendrils make somewhat slow responses to stimuli, obtain if possible plants of Sicyos angulatus, Cyclanthera explodens, or Echinocystis lobata—all belonging to the Cucurbitaceae and easily raised from seed, other members of the same family are White Bryony—which answers fairly well—and (with much less sensitive tendrils) Cucumber and Marrow. In some species of Passion-flower the tendrils are sensitive enough for most experiments; those of Vine are much less so

330. Growth of Tendril before Contact.—In Sicyos, for instance, note that the tendrils as they develop from the bud are rolled up spirally, the convex side being the morphologically lower side; in a few days the tendril straightens out, performing meanwhile revolving movements; when these movements cease, the tendril elongates rapidly, growth being greatest in the lower half of the tendril and amounting to about 50 per cent or even more per day for three to five days; then for a few days the tendril grows slowly; then one-sided growth begins, the upper side growing more rapidly than the lower and thus causing the formation of a spiral—the concave side being now the morphologically lower side of the tendril. Carefully observe all these points; mark the tendrils with ink lines into zones and note the rate of growth daily.

331. Localisation of Responsiveness.—Rub a tendril gently at different points with a thin stick, and note that it is most irritable near the free end and on the lower side (which is slightly concave in the young tendril ready to attach itself); if the upper side is rubbed, even in this terminal region, no curvature takes place.

332. The Response to Stimulation.—Rub the inside of the terminal slightly hooked portion of a young tendril with a pencil or stick; the tendril soon shows a distinct curve, and forms a complete ring in a time varying according to the species and the external conditions—about six

seconds in Cyclanthera, thirty seconds in Sicyos, one to two minutes in Bryonia. Stimulate the tendril more strongly—*e g* by drawing it between the fingers: it becomes rolled up more completely After slight stimulation —just sufficient for the formation of a complete ring—the tendril soon begins to straighten again, though the undoing of the curvature takes considerably longer than its formation, *e g* about 25 to 30 minutes in Cyclanthera.

333. Distinction between Sensitiveness and Responsiveness.—Show by experiments that (1) if the reacting side of a tendril is touched with a stick at two places, say 1 to 2 cm apart, two curvatures result, the region between remaining straight; (2) if the tendril is stimulated first on the upper side and then on the lower side, no curvature takes place provided the two stimuli be equal; (3) if a part of the upper side and at the same time the whole of the lower side be stimulated, curvature occurs only at the place on the lower side which lies opposite the unstimulated regions of the upper side From these results it follows that the apparently insensitive upper side is really as sensitive as the lower, but that stimulation of the upper side either produces no visible result, or else simply inhibits curvature on the lower side

334. Tendrils respond only to Stimulation by Solids.—Prove by experiments that (1) extremely small and light objects, like very small pieces of thread, cause curvature when placed on the tip of a tendril; (2) a shoot of Sicyos, etc., may be violently shaken, yet only slight responses are made so long as the tendrils are not allowed to come into contact with any obstacle; (3) a jet of water may be directed against the reacting region by means of a syringe or wash-bottle or by holding the tendril under a running tap, without causing curvature; (4) water into which a little chalk has been stirred causes stimulation at once Hence neither friction with the air nor the falling of rain will act as stimuli, and a tendril can apparently distinguish between liquids and solids, even when the latter are present as small suspended particles in water.

335. Tendrils irresponsive to Stimulation with Gelatine.—Make a 15 per cent. solution of gelatine in hot water in a test-tube, and dip into it two glass rods, or pieces of glass tubing, so as to coat thickly a length of about 5 cm of each When the gelatine sets, touch the convex side of a tendril with one rod so as to hold the tendril (the gelatine being slightly sticky), and with the other rod rub the lower side in the reacting region. No curvature takes place—evidently the tendril cannot distinguish gelatine from a liquid. Now rub with the uncoated part of the rod, curvature takes place.

336. Growth in Upper and Lower Sides of Tendril.—Carefully mark, in each case with two ink dots or transverse lines, a zone on (1) the convex, (2) the concave side of a slightly hooked young tendril, and measure the distance between the marks in each case, after allowing the tendril to straighten should the act of marking cause curvature Stimulate the tendril strongly, and note that the curvature is due to the great growth in length of the upper side; the marks on the lower side come a little closer together The neutral line lies below the middle (axial) line of the tendril, and is close to the concave side The whole movement following stimulation is complex, since the curvature is not effected by simple contraction of the stimulated side, but by acceleration of growth, which is greatest at the spot on the convex side directly opposite that on the concave side where the stimulus has been applied Perception, transmission, and reaction follow each other much more rapidly than in any of the tropistic movements studied so far

Soon after the completion of the curvature, growth ceases, and then the tendril begins to straighten; the convex side remains unaltered, while growth occurs on the concave side—less vigorously than that which took place on the convex side during curvature, but still showing a marked acceleration of growth as compared with that of an unstimulated tendril This straightening is evidently a case of autotropism

337. How the Tendril clasps its Support—So far we have been studying the effects of a *temporary* contact stimulus. When the tendril rubs against a fixed support it curves, and thus new parts come into contact with the support.

(*a*) If the support is of the right thickness, tension arises which exerts pressure on the support—to observe this, use a roll of paper as a support This pressure does not act as a stimulus, but the reverse curvature which appears causes the loosening of the coil, and there is set up

a fresh stimulus which again induces incurving and brings about a permanent spiral coiling round the support.

(*b*) When the spiral on the support becomes slack, curvature takes place in the part of the tendril just below the support, so that this part comes into contact with and surrounds the support, pushing in front of it the previously-formed but now loose coils. To demonstrate this, when a tendril has made a single coil round the support, make ink-marks on (1) the tip of the tendril, (2) the part of the tendril vertically below this, (3) the support at the point corresponding to (2), and (4) a point on the tendril at a distance of 1 or 2 cm from the support. After a few hours the point marked at (4) will be found to be in contact with the support.

338. Changes in Tendril after Attachment.—After the completion of the permanent coiling, growth in length stops, and there appears not only in the coils but also in the rest of the tendril a number of changes.

(*a*) A spiral twisting occurs in the basal region, whereby the stem is drawn closer to the support. This spiral changes its direction at least once, and that this reversal is due to purely mechanical causes may be demonstrated by fixing a strip of Dandelion stalk at both ends and placing it in water, or by trying to produce a spiral coiling in a piece of rubber tubing fixed at both ends

(*b*) As a rule, marked secondary thickening, accompanied by the development of sclerenchyma, appears not only in the part clasping the support, but also in the basal portion of the tendril. Compare transverse sections of (1) a tendril which has not yet clasped a support, (2) a tendril of the same plant after having made several coils round a support.

339. Tendrils with sticky Pads.—Observations should be made on the **Self-clinging Virginian Creeper,** which differs from most species of Ampelopsis (usually merged in **Vitis,** to which the Vine belongs) in that its branched tendrils become attached by means of sticky pads at the tips of the branches.

(*a*) Place a pot plant in a box with the open side facing the light; the leaves turn towards the light, while the tendrils turn away from the light towards the back of the box.

(*b*) Turn the plant round through 180°; the leaves and tendrils again curve as before—the tendrils show marked negative heliotropism

(*c*) Set in the pot a flat strip of wood, close to the plant, and note that the tendrils spread out on coming in contact with the wood, the tips swelling to form sticky discs which adhere to the wood. For the first day or two the tendrils remain thin and weak, but later they become thicker and stronger, and some force is needed to tear them from the support Moreover, they contract spirally after becoming attached, though before contact they do not revolve in the manner typical of tendrils

340. Tendrils with Hooks.—Get pot plants of Cobaea scandens, or grow plants from seed. The tendrils replace the upper leaflets of the compound leaf, each tendril being branched and representing not only the midrib but also the veins of a leaflet. At the tips there are hooks, which enable the tendril to catch on to supports.

Note that the tendrils show revolving movements before becoming attached; they are very sensitive to friction, bending over towards the rubbed side, and straightening themselves again in about half an hour; a tendril will coil around a thin support in about ten minutes, though unable to coil round a thick support

VII. Experiments with Mimosa.

341. Specimens of the Sensitive Plant (Mimosa pudica) may be raised from seed, even with a cool greenhouse. Note the alternately arranged compound (bipinnate) leaves, each leaf consisting of a main stalk, from the top of which diverge four secondary stalks, each bearing numerous leaflets in pairs. The pulvini are at the base of (1) the main or primary stalk, (2) each of the secondary stalks, (3) each of the leaflets.

At the large basal primary pulvinus the movements are in a vertical plane, raising or lowering the whole leaf; the movements of the four secondary pulvini cause the approximation or separation of the four secondary stalks; while

the movements of the pulvini at the bases of the leaflets cause the latter to move upwards (so as to bring their upper surfaces in contact) or to spread out horizontally. The leaves of Mimosa perform movements as the result of (1) shock or contact, (2) changes of temperature and illumination.

342, Day and Night Positions (Fig. 67).—Note that during the day the main stalk is directed upwards, making with the stem an angle of about 60°; the secondary stalks diverge, the two lower standing at right angles to the main stalk, the two upper forming an angle of about 60° with each other; and the leaflets spread out horizontally, forming angles of about 90° with the secondary stalks in the same plane. At night the primary stalk bends downwards through about 90°; the four secondary stalks bend forwards, so as to place themselves almost parallel with the axis of the main stalk, the leaflets bend upwards, coming together in pairs with their upper faces and also twisting slightly so as to form an acute angle forwards with the secondary stalk, the basal leaflets overlapping the apical ones like tiles on a roof.

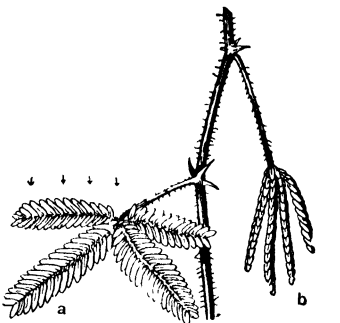


Fig 67.—Leaves of Mimosa Pudica (the Sensitive Plant).
a, Expanded day position; b, Drooping folded night position.

343, Effects of General Mechanical Stimulation.—Shake a Mimosa plant: the leaves rapidly assume the "night" position. After a short time they regain the normal "day" position; in fact, as soon as the main stalk reaches the position of maximum depression, it begins to rise again, and in 10 to 15 minutes the original position is regained. Shake the plant continuously for several minutes: the leaves become insensible to shock, and resume

their normal position while the shaking is continued, but in 5 to 15 minutes after the shaking has stopped the leaves become sensitive again.

344. Sensitiveness of Lower Side of Pulvinus.—With a pencil or thin stick tap or rub the upper surface of the large pulvinus at the base of the petiole: at first there is no response, even to vigorous stimulation, but if it is continued a response is eventually obtained. Now gently tap or rub the lower side of the pulvinus: an immediate response is made to even a slight stimulus.

345. Effect of Repeated Stimuli.—With a light piece of wood strike the lower side of the main pulvinus repeatedly, at intervals of half a minute for about 5 minutes. On leaving the plant to itself the leaf rises, but at first it does not respond to a stimulus, though it soon regains its irritability. If the blows are applied more frequently—about ten per minute—the stalk falls at first but afterwards rises (in spite of the continued blows) and is then insensible even to stronger stimuli for some time.

346. Heat as a Stimulus.—Hold a lighted match below the tip of one of the four secondary stalks, and note the successive closing of the leaflets of this stalk; then the stimulus travels in the opposite direction from the bases of the other three secondary stalks towards their tips; finally the main stalk sinks, and if the stimulus is continued the neighbouring leaves are also affected.

347. Irritant vapours, like ammonia, act as a stimulus on Mimosa leaves. Set a plant under a bell-glass, along with a watch-glass containing a little ammonia. Note the movements of the leaves, and after a few minutes remove the bell-glass and the ammonia, to prevent the latter from injuring the plant

348. Effects of Anaesthetics.—The leaves of Mimosa are rendered insensible by chloroform and other anaesthetics. Stimulate a leaf so that the leaflets close up, then

cut off the leaf and set it in a bottle of water. Place this in a dish containing a little chloroform, or ether; cover the whole with a bell-glass and set it in strong light. In a few minutes the leaflets expand, but they will not now respond to stimuli—try the effect of a lighted match, striking the leaflets, etc. Remove the chloroform, and note that the anaesthesia soon passes off, the leaf recovering its power of reacting to stimuli.

349. Effect of continued Darkness.—We have seen that continued stimulation causes loss of power to react to stimuli. Take two pot plants of which *A* is to be kept under normal conditions of illumination, being set in a good light during the daytime. Set *B* on a plate of wet sand or sawdust, cover it with a box to exclude light, and keep it at a temperature of about 20° C. For a few days the leaves perform periodic movements, expanding during the day and closing at night, though in constant darkness, but in about five days these movements stop.

Now expose the plant to light for a few minutes, then replace it in darkness: no movements occur—the plant has passed into a state of darkness-rigor, and in this state it is no longer sensitive even to mechanical stimuli. Now set both plants in front of a window for a few hours, then place them both in darkness: the leaves of *A* close up, those of *B* remain expanded. After about half an hour, set both plants in the light again for the rest of the day: *B* gradually recovers its phototonus, or power to react to changes in illumination, but its sensitiveness to mechanical stimulation does not return until later

350. Mechanism of Movement in Mimosa.—Careful observation has shown that the upper half of the pulvinus shows a slight increase in volume during the downward movement of the petiole, while the lower half shows a marked decrease in volume. As the movement is made just as well when the epidermis is removed, and the passive veins need not be considered, this reduction in volume must be due to contraction of the parenchyma on the lower side of the pulvinus.

Cut across the petiole a little above the pulvinus, and set the plant in saturated air under a bell-glass to recover; after stimulation, liquid is seen to ooze from the cut surface, having been

secreted by the parenchyma of the lower half of the pulvinus. Moreover, at the moment of stimulation this part becomes dark coloured, in the same way as a leaf that has been injected with water under the receiver of an air-pump ; water has entered the intercellular air-spaces

This excretion of water from the pulvinus cells might be due to either (1) increase in the elasticity of the cell-walls, (2) decrease in the osmotic pressure of the cells. At any rate, in the movement there is a decrease in expansive power on the lower side of the pulvinus ; expansion on the upper side is due simply to the removal of the opposing pressure below, and the weight of the leaf helps to compress the lower side. But that the weight of the leaf is not necessary for the carrying out of the movement is readily seen ; place a plant horizontally or vertically, and note that in both cases the sensitive side of the pulvinus contracts in response to stimulation.

VIII. Experiments with Sundew.

351. Material for Study.—Dig up a number of Sundew plants, with the peat about their roots, and grow them in pots of wet sphagnum. Note the arrangement of the leaves in a rosette, the rounded or oblong form of the blade, the tentacles borne on the slightly concave upper surface of the blade—the central tentacles erect and short-stalked, those at the margin long-stalked and bent outwards. Examine a tentacle with the microscope, and note that a vein runs up the centre of the stalk, ending in a mass of spirally thickened cells in the centre of the glandular head, the outer (epidermal) cells of which are columnar in form.

352. Responses to various Stimuli.—The tentacles react both to mechanical (contact) and chemical stimuli. In their responses to mechanical stimuli the tentacles agree closely with tendrils

(*a*) Strike a gland with a pencil : a single tap produces no movement. Watch the tentacle for about a minute, then tap it again several times, and note that movement results.

(*b*) Place on a gland a small particle of sand or gravel : movement occurs.

(*c*) Fill a pipette or syringe with clear water, and let drops fall on a leaf for about a minute: no movements occur

(*d*) Fill the tube with water made milky with powdered chalk, instead of clean tap-water: movement occurs this time. Another method is to cut off a few leaves and place some in clear water, others in chalk-containing water, in watch-glasses; after a few minutes the leaves in the chalky water show bending of the tentacles, those in the pure water do not.

(*e*) Strike or rub the gland of a tentacle with a gelatine-coated rod: no movement occurs

353. Mode of Curvature of the Tentacles.—Stimulate a tentacle with a piece of meat, or in some other way, and carefully watch (using a lens) the process of curvature Bending may begin after 10 to 15 seconds, and a distinct curvature, visible to the naked eye, may be seen in less than a minute. The marginal tentacles will sometimes curve through 270° in an hour. Note that the curvature is confined to the base of the stalk, which bends sharply, while the upper part remains straight and is carried over passively.

354. Chemical stimuli generally act more vigorously than mechanical stimuli, as shown by the greater rapidity of the movement and the longer duration of the curvature. Many substances (some useful, some injurious, some indifferent) in solution act as stimuli. The tentacles will respond to a very dilute (0·02 per cent.) solution of ammonium phosphate. In experiments on chemical stimulation, a simple plan is to place a cut Sundew-leaf in a watch-glass containing the dissolved substance

355. Transmission of Stimulus.—(*a*) Put a very small fragment of raw meat on the centre of a leaf; in about 24 hours nearly all the tentacles will have bent inwards. Note that the short central ones, on which the meat was placed, remain erect, but the stimulus is transmitted from them outwards, so as to induce inward curvature of the outer tentacles.

(*b*) Place a fragment of meat on one of the long outer tentacles. The latter bends inwards so as to carry the

meat to the middle of the leaf; then a stimulus is apparently sent to the other peripheral tentacles, which begin to curve inwards. Hence we can here distinguish between movement caused by a direct stimulus and movement resulting from a transmitted stimulus, and transmission can only be effected by means of the central tentacles.

(*c*) Place two fragments of meat half-way between the centre and the margin, at two opposite points: half of the tentacles bend towards one centre of stimulation, and half towards the other

356. Direct and Indirect Stimulation.—Cut off the glands of some of the marginal tentacles of a leaf, then place a piece of meat on one of the remaining marginal tentacles; the results show that stimuli are transmitted from the tentacles in the centre to those on the outside, and that the stimulus acts on the motile portion of the tentacle from below (a decapitated tentacle, though not directly sensitive, reacts to a transmitted stimulus)

When a tentacle is indirectly stimulated, the stimulus is transmitted from above downwards to the motile region; in indirect stimulation, the transmission is from below upwards. In the former case it is always the outer side of the tentacle which becomes convex (nastic curvature); in the latter case the curvature is tropistic, being determined by the direction from which the stimulus comes.

IX. Haptotropism (Contact Irritability) of Stamens and Stigmas.

357. Stamens of Berberis.—Examine flowers of Barberry, and note that there are several series of perianth leaves, arranged in whorls of three. The inner six petals have a nectary at their base; each of the six stamens is slightly attached at the base to one of these six petals. The anthers open by two lateral valves near the top of the anther.

In the open flower, the stamens and petals are spread

out, the anther of each stamen lying within the concave upper portion of one of the petals. If the filament is irritated it moves inwards, bringing the anther close to the stigma. When a bee pokes its proboscis into the flower, to reach the nectary at the base of each petal, the stamen moves inwards, dusting the bee's head with the pollen.

(*a*) Touch or stroke, with a mounted needle, different parts of the stamen, and note that the anther is not sensitive Localise the sensitive region, by touching or stroking different parts of the outer and the inner surfaces of the filament in different stamens. Note that after the stamen has moved inwards it at once moves outwards again, and in a few minutes has regained its original position.

(*b*) Pull off some of the petals; some of the stamens will come away with a petal Place each specimen (petal and stamen) on wet blotting-paper under a watch-glass for about ten minutes, to recover irritability. Then touch different parts of the stamen, using a lens or dissecting stand, in order to locate the exact region of irritability.

(*c*) Carefully detach a few open flowers, holding by a forceps just below the flower and cutting with scissors just below the forceps. Test the irritability of each flower by touching one of the stamens. Then place the flowers floating in a watch-glass of water Put the watch-glass under a bell-jar or large inverted beaker, along with another watch-glass containing a few drops of chloroform

Note that the flowers may be left for about ten minutes in this atmosphere without injury. On removing the bell-glass and the chloroform, note that the stamens are now quite insensible to touches.

Now leave the flowers exposed to fresh air, and test from time to time. In from fifteen to thirty minutes the stamens will have recovered their irritability.

(*d*) Repeat the preceding experiment, but this time touch each stamen immediately before placing the flower under the bell-jar. Note that the stamens are able to recover their normal position in spite of the chloroform

358. Stamens of Centaurea.—Of special interest are the movements of the stamens in Centaurea, *e g.* the Corn-flower. Examine the flower-head and note in the central flowers the five anthers joined to form a tube, through which the style grows after the flower opens

(*a*) Choose a flower which has just opened and in which some pollen is exposed on the top of the anther-tube, though the style has not yet protruded. With a camel-hair brush rapidly wipe off the pollen, and note the extrusion of a worm-like mass of pollen from

the tube; this is due to the contraction of the filaments pulling down the anther tube

(*b*) Poke a mounted needle into the corolla-tube of a similar flower, and note that the anther-tube is not merely pulled down, but turns towards the side on which the stimulus has been applied to the filaments

(*c*) Dissect out a number of similar flowers, and put them on pieces of wet blotting-paper; carefully slit down the corolla-tube in each case and open it out so that the filaments may be seen Invert a tumbler over the flowers, and leave them for ten or fifteen minutes to recover. In the rest position each filament is curved with the convex side outwards. Touch a filament with the needle, and note that it contracts and becomes straightened. As in Barberry, there is no transmission of the stimulus from one filament to the next.

359. Stigma of Mimulus.—Examine the flower of Mimulus, and note that the stigma consists of two diverging flat lobes, which on being irritated on the inner surface close up.

(*a*) Use the commonly cultivated Mimulus cardinalis for experiments. Note that (1) in the unopened flower bud the stigma lobes are pressed together; that (2) the anthers of the two longer stamens dehisce first, before the flower opens; that (3) when the corolla expands the two shorter stamens open; that (4) then the stigma lobes begin to diverge, and in three or four hours show a divergence of about 90°; that (5) after five to six days, during which the irritability of the stigmas diminishes, they become spirally rolled up (the inner surface remaining convex), and then wither; and that (6) although the lobes are irritable as soon as the flower opens, the stigma lobes do not close completely on being stimulated, only reaching their complete irritability after about six hours; and that (7) the lobes have completely lost their irritability after about six days.

(*b*) Touch the inner surface of one of the lobes with a pencil or needle, and note that the closing occurs at once; after five to eight minutes they begin to diverge, and in ten to fifteen minutes have reached their original position. Note that the outer surface of the lobes is quite insensitive; at any rate, no closure takes place when they are stimulated

(*c*) Ascertain whether or not the stimulus is transmitted from one lobe to the other; if so, we should expect that if one lobe is prevented from moving, a stimulus applied to it would cause movement of the other lobe. Cement, in one flower, the upper lobe, and in another flower the lower lobe, to the corolla, by means of seccotine (or of mastic dissolved in ether), and note that the stimulus is transmitted from the fixed lobe to the free lobe in both cases.

(*d*) Note carefully that (1) if the stimulus has been due to touch with a pencil or needle, or the placing on the stigma of pollen from a quite different plant (*e.g.* Foxglove, Snapdragon, Plantain, or any other plant from which pollen can be obtained and scraped on to the Mimulus stigma), the lobes open and do not close again; and that (2) if pollen of Mimulus itself is placed on the stigma, though the lobes open again as usual, after from two to three hours a second closing occurs, and this lasts for about twenty minutes if a small amount of pollen has been used—if much pollen is applied, so as to cover the inner faces of the lobes, the second closing is permanent.

(*e*) Make experiments to show that (1) the question whether the lobes shall remain closed or will reopen depends on the quantity of pollen applied (whether of the same or of an alien species) to the stigma; (2) dry powdered starch or dry sand will cause prolonged closure; (3) withdrawal of water from the stigma tissue, by placing the stigma in salt solution, causes prolonged closing. The results will show that, while the instantaneous first closing movement is due to contact irritability, the permanent closure results from the prevention of the automatic reopening movement, and is evidently due to (at least in part) absorption of water from the stigma tissue by the pollen-grains and their tubes

The sole advantage of these remarkable movements of the stigma lobes (which occur in species of Martynia and Torenia as well as in Mimulus) appears to be that the germination of the pollen is favoured by the formation of a "moist chamber" in which the pollen can germinate more rapidly; when pollen is placed carefully on the stigma, shock being avoided so that no movement results and the lobes remain open, the pollen grains germinate much more slowly. The lobes remain closed only if the pollen grains and the germinating pollen-tubes can by abstraction of water prevent the return of the original osmotic pressure in the stigma tissue and the consequent reversal of the primary closing movement, until a sufficient number of pollen-tubes have penetrated the conducting tissue and so disorganised it (by some chemical process) that reopening is made impossible.

X. "Nyctitropic" Movements.

360. In many plants the foliage and floral leaves assume in the evening positions other than those they occupy by day. The movements concerned have been called nyctitropic, though as a rule they are not tropistic movements but *nastic* movements, and the stimulus is the *alternation* of light and darkness. Similar movements may also be

caused by changes in temperature—in nature, of course, increased light is usually accompanied by rise of temperature and diminished light by fall of temperature.

Some flowers respond especially to changes in temperature (*e.g.* Tulip and Crocus); others only, or especially, to changes in light; others again only when light and temperature are altered at the same time.

361. Temperature Effects in Tulip and Crocus Flowers.—Use pot plants, or cut flowers set in bottles of water. In the morning bring a closed flower from outside, or from a cold place indoors at about 10° or 12° C., into a warm room at about 20° C., and note that the flower soon begins to open. Some Tulip plants were kept at 12° C. from 5 p.m. until about noon next day, and then transferred to 18° C.; during the first hour the flowers opened, but during the second they closed again (owing to autotropism).

362. After-effects of Temperature Changes.—Take a closed Tulip or Crocus flower in the morning and with seccotine or shellac fix a thin piece of wood to the middle of the outer side of (*a*) one of the outer perianth segments, (*b*) the opposite inner segment, so that 2 or 3 cm of the stick projects above the flower. Fix a scale horizontally in such a way that the distance between the tips of the two pointers can be read on the scale.

On bringing the plant into a warmer place (say from 12° to 20° C.), in about five minutes the movements of the pointers against the scale show the beginning of the opening movement. Replace the flower in a temperature of about 12°; it continues for a time to open, and then begins to close. Before closing is complete, bring the flower once more into the higher temperature, and note that it continues for a time to close, and then begins to open

363. Opening and Closing of Composite Flower-heads.—Various Compositae may be used for experiments on the opening and closing movements of floral leaves. Cut off a Daisy with the flower-head open, fix it in a bottle of water, and place it in darkness. Note the time required

for a distinct closing movement; this may be done by marking two opposite ray-flowers with a spot of ink and measuring the horizontal distance between the tips of these flowers before and after placing the plant in darkness.

364. Effect of continued Darkness.—Keep some Daisies in darkness for several days, using either dug-up plants in pots, or cut flower-heads; the darkening may be effected by covering some Daisies with an inverted flower-pot with the hole plugged. After two days, the heads open again, though not fully, and then remain in this condition, showing that the alternation of light and darkness is necessary for the continuance of sleep movement. In the case of Goatsbeard, the inflorescence opens again after being kept about nine hours in darkness.

365. Effects of Temperature and Light Changes.—The flower-heads of Daisy are sensitive to temperature as well as to light, but their responses to temperature are feeble as compared with those made by Tulip and Crocus flowers. If closed Daisy heads are brought indoors at night, they do not open, though the rise in temperature may be as much as 15° C.; nor does a corresponding fall in temperature make the open head close during the day. But if in the morning the closed heads are warmed through 15° they will open, and if at evening the open heads are cooled through 15° they will close.

366. Sleep Movements of Non-pulvinate Leaves.—In many plants the young growing leaves perform sleep movements, but these become less and less marked as the leaf grows older. In other cases the fully grown leaves retain the power of performing sleep movements, and these leaves are distinguished by having a pulvinus or motile organ.

In the movements of non-pulvinate leaves, the day position is more or less horizontal, and the night position vertical, the movements being due to curvature of either the petiole or the base of the blade. The leaves may sink at night, *e.g* Balsam, Hop, Polygonum convolvulus; or they may rise and stand erect, *e.g* Chenopodium, Polygonum aviculare, Stellaria, Linum, Mirabilis. In both cases

the leaves pass in the evening from a horizontal to a vertical position. The cotyledons of some seedlings, *e.g* Radish, spread out during the day and close up at night. Observations should be made on all these plants

367. The Movements of Pulvinate Leaves are of greater interest. In the leaves of various plants (especially Leguminosae and Oxalidaceae), movements occur which depend not upon growth but simply on unequal osmotic pressure on the opposite sides of the swollen leaf-base (pulvinus). We have seen that tropistic curvature is frequently due in the first instance to increased turgescence of the convex side of the curving organ, this being followed by growth in length of that side. In the movements of pulvini there is no permanent elongation of the convex side, *i e.* no growth occurs.

The movements of the pulvinate leaves of Mimosa have already been studied in detail.

Examine the leaves of Wood Sorrel, Clover, Phaseolus (French Bean, Scarlet Runner). Note that in Phaseolus a pulvinus is present not only at the base of the petiole, but also at the base of each leaflet. Study the day and night positions of the leaves of these plants, as well as of others showing sleep movements, *e.g.* False Acacia (Robinia).

368. Experiments with Clover.—Note that by day the three leaflets are spread out horizontally from the top of the stalk; at night the two basal leaflets rotate until they stand in the vertical plane, then they swing round till their upper surfaces come together, and finally the end leaflet rotates upwards through 180° and comes down like a roof over the edges of the other two leaflets.

(*a*) On a bright day, cover with a flower-pot or dark-box a Clover plant growing in the open, or one dug up and set in moist soil in a saucer; note that in about half an hour the leaves have assumed a night position.

(*b*) Keep a Clover plant in darkness for a week, and note that the leaves ultimately assume a position resembling the day position, except that the leaflets are more drooping.

369. Experiments with Wood Sorrel.—Note that by day the three leaflets spread out horizontally, as in Clover; at night they droop so that their midribs touch the leaf-stalk, while each leaflet becomes folded along the middle.

(*a*) Repeat the experiments given for Clover.

(*b*) The leaves of Wood Sorrel respond to mechanical stimulation, though not nearly so sensitive as Mimosa; shake a plant with its leaves in day position, and note that the leaflets droop, though repeated or prolonged shaking may be required

(*c*) Rub the lower surface of a pulvinus; as compared with Mimosa, the Oxalis leaflets take a long time (often about an hour) to recover, and the stimulus is apparently not transmitted from one leaflet to another.

(*d*) Try the effect of striking a leaflet; repeated blows are usually required to cause drooping.

370. General Experiments with Phaseolus.—Note that in Phaseolus, in the evening the petiole rises, while the leaflets move downwards; in the morning the petiole sinks, and the leaflets rise and become nearly horizontal, though in direct sunlight they usually droop to some extent.

(*a*) Place a plant in darkness, and note that periodic "after-effect" movements occur for several days; then the leaves become "darkness-rigid" and are expanded horizontally. On being again exposed to normal illumination, phototonus is regained rapidly by the younger leaves, more slowly and less completely by the older ones.

(*b*) Place in darkness three pot plants of Phaseolus; set *A* in the normal erect position, *B* horizontally, *C* inverted Note especially that in *B* and *C* curvature and torsion occur, which eventually bring the leaves into their normal position

371. Influence of Gravitation.—On the morning of a summer day, take two pot plants of Phaseolus, and note the angles made (1) between the stalk of each of the two primary leaves and the stem, (2) between the blade of each primary leaf and its stalk, (3) between the stalks of two or three of the trifoliate leaves and the stem, (4) between the leaflets and the petiole of these trifoliate leaves.

Invert one plant, and examine it from time to time. Note that the blades of the leaves rise considerably in six to eight hours, while the petioles also rise but more slowly. Instead of measuring the angles, it will suffice to invert the erect control plant (*A*) from time to time and hold it alongside the plant (*B*) that is being kept inverted, comparing the positions of the leaves and leaflets in the two plants.

Next morning, bring *B* back into the upright position, and note that the leaves resume their normal positions in the course of the day.

372. Influence of Gravitation and of Darkness.—Now invert the plant *B* of the preceding experiment, and keep it inverted for four or five days. The leaves show periodic sleep movements, but the day and night positions are now in the reverse direction with reference to the stem—that is, the movements retain their relation to the direction of gravitation. Now turn the plant into the upright position, and note that the leaves either regain their normal position very slowly or not at all—geotropic curvature of the pulvini has been fixed by growth.

373. Autonyctitropic and Geonyctitropic Movements.—From the preceding experiment, and from the fact that the "sleep" movements of Phaseolus cease when the plant is rotated (with its stem horizontal) on the clinostat, it is clear that we have here not a "nyctitropic" but a geotropic movement—the plant shows different geotropic reactions according to whether it is exposed to light or to darkness. Similar changes in the geotropic reaction are seen when rhizomes and roots are exposed to light. In Mimosa and most other plants showing sleep movements, these movements retain their original direction after continual rotation on the clinostat for many days. Hence we can distinguish between *autonyctitropic* and *geonyctitropic* "sleep" movements.

374. Structure of Pulvinus of Phaseolus.—In a transverse section from the middle of the petiole of Phaseolus, note the ring of vascular bundles, enclosing the central pith and surrounded by a narrow zone of cortex which is largely collenchyma. In transverse sections of a pulvinus, note that the bundles are collected towards the centre, while there is a broad zone of thin-walled cortex parenchyma. The movements of the petiole (and of each leaflet) are due to changes in the turgescence of the parenchyma on the upper and lower sides of the pulvinus. An increase in turgescence on one side, or a decrease on the other side, or both these changes occurring together, will result in elongation of one side and contraction of the other, the vascular tissue bending passively and undergoing no change in length.

CHAPTER VIII.

ALGAE.

Chlamydomonas.

375. The greenish, or sometimes reddish, colour of standing rain-water (in tubs, gutters, ponds, puddles, and ditches) is due to various minute organisms, among which are species of Chlamydomonas and Haematococcus (Sphaerella). These two genera are readily distinguished, though closely allied

(*a*) Mount a drop of water containing actively motile Chlamydomonas. All that can be seen with the low power is a number of minute green specks swimming rapidly through the water in all directions. If possible, find one which is stationary, and with the high power note that the plant is **unicellular,** consisting of a pear-shaped cell, in which may be distinguished (1) a thin but distinct **cell-wall;** (2) two fine threads (**cilia**) at the pointed end—these may be still seen waving from side to side; (3) a bell-shaped **chloroplast,** open towards the anterior pointed end of the cell; (4) a small bright speck, the **pyrenoid,** lying in the chloroplast at the broad end of the cell; (5) the **nucleus,** lying in the protoplasm within the chloroplast, at the centre of the cell; (6) two minute clear vesicles **(contractile vacuoles)** at the pointed end of the cell; (7) a red dot of pigment (**"eye-spot"**) lying at one side just behind the vacuoles.

(*b*) Place a drop of chlor-zinc-iodine on a slide, add a drop of water containing Chlamydomonas, cover, and with the high power note (1) that the reagent has killed and

stained the protoplasm, (2) the nucleus, (3) the starch collected at the pyrenoid, (4) the cilia, (5) the cell-wall

(*c*) To some fresh *Chlamydomonas* add a drop of alcohol; this kills the cell and extracts the chlorophyll.

(*d*) In the scum-like deposit sometimes found in water containing *Chlamydomonas*, the plant may be seen in what has been called its "palmelloid" condition; many other simple Algae, however, pass at times into this condition. The plant comes to rest, and loses its cilia, eye-spot, and contractile vacuoles; the cell-wall becomes swollen and mucilaginous, and in the mucilage thus formed the cells undergo active division, and thus multiply rapidly. Then, with the return of favourable conditions for active motile life, the cells escape, and the plant becomes motile again.

(*e*) Some of the *Chlamydomonas* cells may also be seen to have come to rest, withdrawn their cilia, and divided into four daughter-cells, or **zoogonidia**, which later are set free, each acquiring a pair of cilia, and form independent plants like the parent. This is a simple example of asexual reproduction.

(*f*) The different species of *Chlamydomonas* differ considerably in structure and in their modes of sexual reproduction. The chloroplast, instead of being simply bell-shaped, may be cut up into lobes; there may be four instead of two cilia. The greatest differences, however, are seen in the sexual reproduction, which may be effected by (1) the conjugation in pairs of equal-sized biciliate **zoogametes**, wall-less (in rare cases walled) cells produced by repeated division (to the number of as many as 32) of an ordinary cell and set free into the water, the **zygote** formed by fusion often acquiring a thick coat and resting before passing into the motile state again; (2) the conjugation of smaller biciliate gametes (**microgametes**) with larger ones (**megagametes**); or (3) the fertilisation of a large walled cell by a smaller one. That is to say, we find in this genus a gradual transition from isogamy to heterogamy, the gametes being in the former case similar in size and in the second case dissimilar.

(*g*) It has been shown that the plant only reproduces asexually when cultivated in Knop's solution, but when transferred from this to distilled water it soon produces zoogametes, which fuse in pairs to form zygotes. If a single zoogamete is isolated in some distilled water it perishes; but if it be isolated in culture solution it will give rise to a new individual—*i.e.* it will behave in the same way as a zoogonidium. Such experiments, which are more readily performed with larger Algae, and also with Fungi, show that the

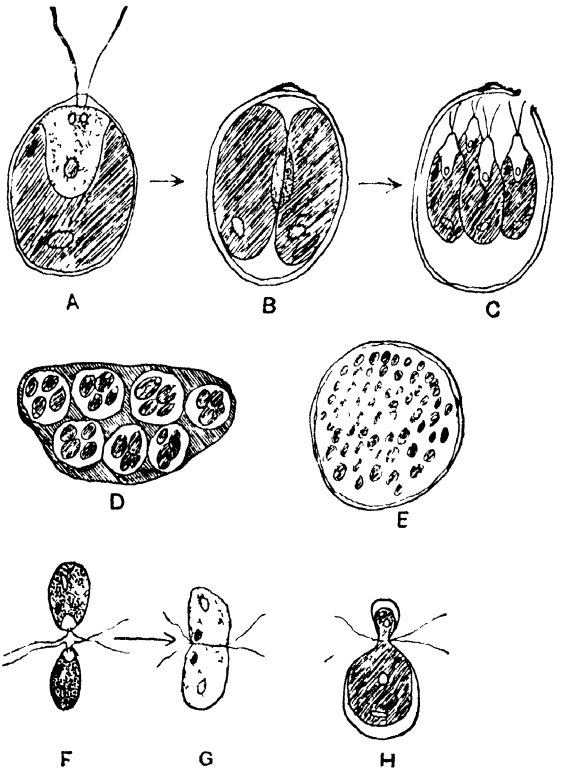


Fig. 67*a*.—*Chlamydomonas*. A, Swimming cell; B, Resting cell, showing division into two; C, Formation of four zoospores; D, Palmella stage; E, Formation of sixty-four gametes; F and G, Two stages in isogamy; H, Heterogamy in *C. monadina*, showing a small (male) and a large (female) gamete.

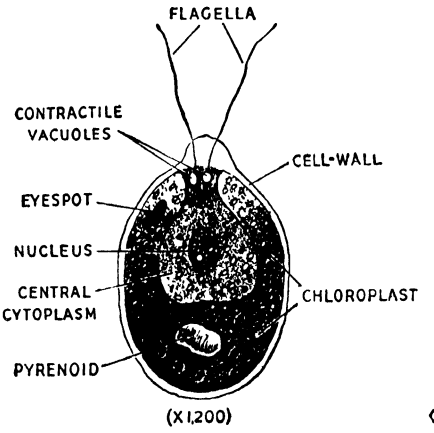


Fig 67*b* —*Chlamydomonas.* A motile cell.

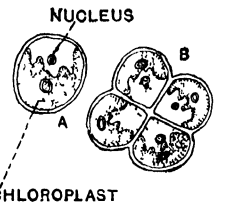


Fig 67*c* —*Pleurococcus*

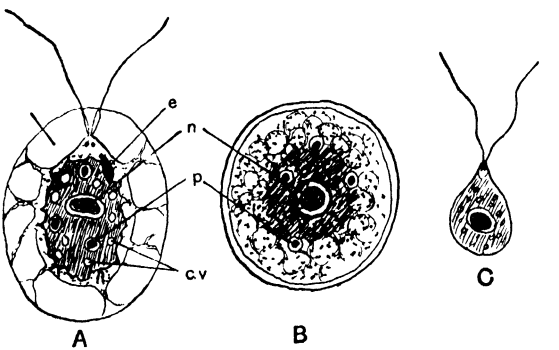


Fig 67*d* —*Sphaerella.* A, Motile cell; B, Resting cell; C, Gamete n, nucleus; p, pyrenoid; c v., contractile vacuoles; e, eye-spot, m, mucilaginous layer Cytoplasm dotted in A. chloroplast line-shaded

life-history of various Algae and Fungi may be controlled and modified at will by changing the conditions under which they grow, especially as regards nutrition. They show also, in many cases at any rate, that *starvation* may in itself induce sexual reproduction.

HAEMATOCOCCUS (= SPHAERELLA).

376. Sphaerella (Haematococcus) also occurs in rain-water in gutters or puddles, in ditches, bog-pools, etc., often giving the water a green or red colour from its abundance Like Chlamydomonas, it is unicellular, with an ovoid body, a bell-shaped chloroplast, and two cilia. The chloroplast, however, is often flecked with red pigment, and the cell has a curious and characteristic appearance owing to the position of the cell-wall. The wall stands out from the rest of the cell—the protoplast—so that a space is left between the wall and the rest of the cell. This space is traversed by fine protoplasmic threads, and through it pass the two cilia on their way out through the wall at the anterior end of the cell.

(*a*) Mount in chlor-zinc-iodine, which will stain the **cell-wall,** the **starch,** the general **protoplasm,** the **cilia,** the **protoplasmic threads** crossing the space, and the **nucleus.**

(*b*) Some *Sphaerella* cells may be found which have passed into a resting condition, and have become rounded, covered by a thickened cell-wall, and had the chlorophyll largely replaced by red pigment. The general life-history is much the same as in Chlamydomonas. In the resting condition, the cell may divide into two, four, or eight daughter-cells (**zoogonidia**) which acquire cilia, escape from the mother-cell, and become new plants. In sexual reproduction, the cell divides into a larger number of **zoogametes** (32 to 64), naked biciliate cells like the zoogonidia, but conjugating in pairs to form a **zygote** which acquires a thick wall and passes for a time into a resting-stage.

PLEUROCOCCUS

377. *Pleurococcus vulgaris* is far commoner than either of the two preceding plants, and is, in fact, one of the commonest forms of plant known. It gives rise to the green powdery deposit on wooden fences, walls, and tree trunks.

Scrape a little of the green powder from a piece of tree bark on to a slide, mount in water, and note the cells, which are either isolated or, more often, associated in groups or packets Unless the material has been soaked in water beforehand, it will probably be difficult to see clearly on account of air-bubbles; to remove these, add a little alcohol. From the appearance of the cell-walls it is easy to see that the packets are temporary aggregates or colonies of cells which do not immediately separate after division and the formation of walls, but which gradually split apart as division continues In a single cell note (1) the well-marked **cell-wall**; (2) the **chloroplast,** which is lobed and perforated so as to present the deceptive appearance of a number of separate chloroplasts; (3) the colourless vacuolated **protoplasm, nucleus;** and (4) a **pyrenoid** (not always present).

In order to see the parts of the cell more clearly, mount some material in iodine, and some in chlor-zinc-iodine; also steep some material in alcohol, to remove the chlorophyll, and then treat it with these reagents Starch grains will probably be seen in the chloroplast

In addition to the simple vegetative multiplication due to the dissociation of the loosely connected colonies of cells, reproduction in *Pleurococcus* is brought about by (1) the formation of resting cells (**gonidia**) either directly or after a few divisions of the cell, (2) the formation of biciliate **zoogonidia,** (3) the conjugation of isogamous biciliate **zoogametes.**

SPIROGYRA

378. Occurrence.—One or other of the various species of Spirogyra may be found at almost any time of year, but as a rule they are (at least in the south of England) most abundant in spring and early summer; plants may be found in the vegetative condition throughout the winter, while conjugation occurs chiefly from April to June.

Spirogyra grows commonly in low-lying quiet waters, as large flocculent green mats covering the surface in ponds

and ditches and consisting of unbranched filaments which are slippery to the touch and are often frothy owing to entangled gas-bubbles. The filaments vary greatly in thickness (from about 0·01 mm. to 0 15 mm. in the British species), but in the larger species it is easy to see with a pocket lens the characteristic spiral chromatophores in the cylindrical cells of the filament.

379. Culture Methods.—Some trouble is necessary in order to keep Spirogyra in healthy growth indoors—the smaller kinds keep better than the larger ones, as a rule. Metals are very injurious to Spirogyra, and it is better to use rain-water instead of either tap-water or distilled water in making up the culture solution. Knop solution (§ 184) of 0·1 or 0 2 strength may be used, but the following formula gives better results with Spirogyra:—ammonium nitrate, 0 5 gr.; potassium dihydrogen phosphate, 0 2 gr.; magnesium sulphate, 0 2 gr.; calcium chloride, 0·1 gr.; ferric chloride, a trace; water, 1 litre If it is necessary to use tap-water, let the tap run for several minutes before taking what is required for the culture.

Use a small quantity of material—a mass of Spirogyra the size of one's finger is enough to place in 5 litres (about a gallon) of the culture solution. Either keep the culture in a large glass jar or aquarium, or place the material in a number of large beakers—set each beaker in a flower-pot containing moist sand, so that the sand reaches nearly to the rim of the beaker, cover with a glass sheet, and keep in a cool room out of direct sunlight but in good diffuse light To obtain filaments free from starch (in order better to see the pyrenoids), place a culture in a shaded place for a day or two

In the culture solution the filaments will usually show vigorous growth and cell-division. If after a week the filaments are transferred to pure water and kept in bright sunlight, conjugation will probably begin in about three days. Material which has been kept in ordinary water or culture solution may also be induced to conjugate by transference to 2 per cent. cane sugar solution.

380. Structure of living Spirogyra Cell (Fig. 68).—Place some filaments on a slide, with water, examine with the low power, select those which are thickest or have few or loosely coiled chromatophores (and which will therefore show the details of structure most clearly), and with a brush transfer the selected filaments to a watch-glass or saucer of water. With scissors cut these filaments into pieces which can be covered by a cover-glass, and mount them in water.

With the low power, select a suitable filament, move the slide so that this filament crosses the centre of the field, put on the high power, and study carefully the appearances successively presented by the cell-walls and cell-contents

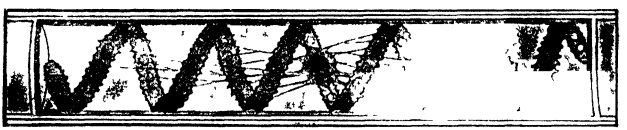


Fig 68 —SPIROGYRA. Part of a Filament, showing the Structure of one of the Cells

on focussing with the fine adjustment, beginning with the upper surface and turning the micrometer screw until the middle is reached (giving an "optical section" of the cell) and finally the lower surface. Sketch a portion of the filament, including at least one complete cell, as seen (*a*) in upper surface view, (*b*) in optical section. Note

(1) The outer or **longitudinal cell-wall,** smooth and colourless and often covered by a layer of mucilage.

(2) The disc-like **transverse walls,** continuous with the outer wall and dividing the filament into a row of cylindrical cells.

(3) The thin continuous film ("primordial utricle") of colourless fine-grained **protoplasm** lying within the cell-wall.

(4) Embedded in this film and running spirally round the cell, the green band-like **chromatophore**—there may be several chromatophores in each cell.

(5) The edges of the chromatophore are usually serrated, while along the middle there is a series of conspicuous rounded bodies, each consisting of a central **pyrenoid** surrounded by small strongly refractive **starch-grains.**

(6) The large central cell-cavity or **vacuole,** containing the colourless cell-sap.

(7) The **nucleus,** a strongly refractive body, usually lens-shaped, lying in the centre of the cell and surrounded by a layer of protoplasm from which proceed fine radiating protoplasmic threads.

381. Cell treated with Iodine.—Treat filaments with iodine—either remove the cover-glass, add a drop of iodine, and mount in water after washing off the superfluous iodine with water, or irrigate with iodine and then with water—and compare with fresh filaments. Note that the starch-masses around the pyrenoids are stained dusky purple or almost black; the protoplasmic contents are stained brown; the nucleus is more deeply stained than the general protoplasm, and the **nucleolus** contained in the nucleus still more deeply; the threads radiating from the protoplasm around the nucleus are branched, and may be traced outwards to the pyrenoids close to which they end.

382. Decolorised Cells.—Place some filaments in alcohol (strong methylated); the chlorophyll is dissolved out of the chromatophores and the alcohol becomes green. Rinse a decolorised filament in water, treat with iodine, and note that the starch and protein contents are stained as in fresh material; the chromatophores are seen to be specialised band-like portions of the protoplasm

To see the pyrenoids clearly, decolorise and treat with iodine filaments that have been kept in shade or darkness until starch-free. To stain the cell-wall as well as the cell-contents, either use alcohol material, or filaments preserved in formalin; or (better) place fresh material in 400 c.c. of water with 1 gram chromic acid and 4 c.c. glacial acetic acid, leave in this fixing solution overnight, then wash by steeping in water for a few hours (changing the water two or three times), and stain with safranin or haematoxylin or aniline blue, or safranin followed by one of the other stains

383. Plasmolysis.—Alcohol and various other reagents cause the cell-contents to separate more or less completely from the cell-wall, leaving a space between the protoplasm film and the wall. In this state the cell is **plasmolysed;** alcohol, iodine, etc., not only

plasmolyse but also kill the cell, hence in plasmolysis experiments we use reagents which cause plasmolysis without also causing death. Irrigate a fresh specimen, while watching it under the high power, with some 2 or 3 per cent salt solution; as the solution passes through the cell-wall and water escapes by diffusion (osmosis) from the cell-sap in the vacuole, the protoplasm film is forced inwards, and the cell-contents become rounded off. Now draw water through; as this replaces the salt solution, the cells return to the normal turgid condition.

Place some fresh material in very strong salt solution; or simply put some dry salt on a slide, add enough water to dissolve it, and add some fresh filaments. Marked plasmolysis occurs, the cell-contents becoming rounded off to form a ball, while the cell-wall itself becomes folded and crumpled owing to the sudden and complete collapse by plasmolysis.

384. Conjugation (Fig. 69).—Examine material showing stages in conjugation; either collect fresh conjugating material (chiefly found in spring and early summer) or try to induce conjugation in material kept indoors (§ 379). Note that

(1) The cells of two opposite filaments put out rounded **projections** of their lateral walls.

(2) These processes meet and fuse at the tips, so that the two opposed cells become continuous by a transverse **conjugation tube.**

(3) Meanwhile, the contents of both cells have become rounded off, forming the **gametes,** this rounding-off occurring earlier and being more marked in one cell (male) than in the other (female)—usually all the cells of one filament are "male" and all those of the other filament "female."

(4) The contents of the male cell pass over through the conjugation tube and fuse with those of the female cell, to form the egg-shaped or rounded **zygote.**

(5) The zygote acquires a thick wall, its contents become brownish, and it is now a **zygospore.**

Keep conjugated filaments in a vessel of water, and observe the development of the zygospores from time to time; note that the thick coat is stratified, the outer and inner layers being colourless while the middle layers become brown, also that the zygospore at first contains

starch which is later replaced by oil-drops, the chlorophyll being more or less replaced by red pigment.

These filaments may also show, if kept under observation, that when the zygospore germinates the thick outer

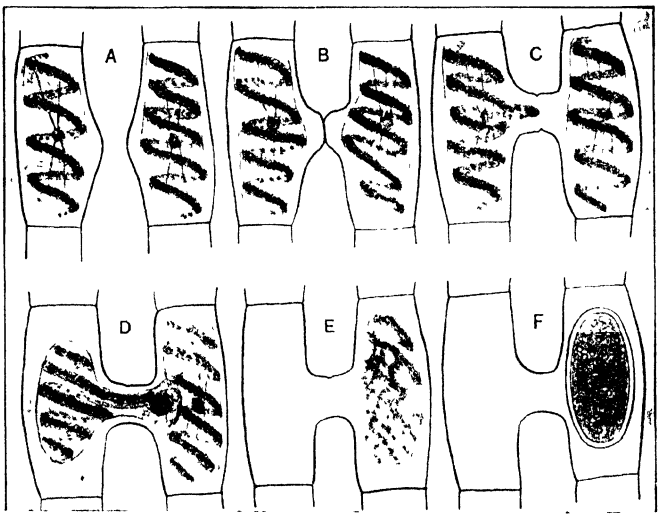


Fig 69 —SPIROGYRA Stages in conjugation See § 384.

coats are ruptured, and the protoplasm (covered by the thin inner coat) grows out as a tube divided into two cells, of which the upper is green and developes into the filament, while the lower cell (covered by the spore-coat) is colourless and soon disappears

VAUCHERIA.

385. Occurrence.—Most of the species of Vaucheria grow in fresh water, or on moist soil or mud in ditches, but some live in the sea or in brackish water in estuaries and salt marshes. A convenient species for study is V.

sessilis, which can be found all the year round on the soil in pots in greenhouses. Another species, V. terrestris, is sometimes found along with V. sessilis, or in similar places; in the former species the sexual organs are in groups (usually an antheridium with an oogonium on either side) on a common stalk, while in V. sessilis the antheridia and oogonia are seated separately on ordinary branches of the thallus.

Another species, V. geminata, may be found in spring in ponds and ditches; it usually fruits during April and May, and may then be lost sight of until late summer, when the oospores germinate. Specimens of Vaucheria found in running water are usually sterile.

386. Culture Methods.—The development of **zoogonidia** may be observed in Vaucheria material which has been transferred from damp soil to water, placed in saucers. If no zoogonidia appear normally, their production may be induced by either of the following methods, each of which should be tried:—

(1) Cultivate the plant in 0·3 per cent. Knop solution for a week, then transfer it to distilled or tap water.

(2) Cultivate the plant in 0 3 per cent. Knop solution for a week, then place it in darkness.

(3) Cultivate the plant in 2 per cent. cane sugar solution in darkness—if no zoogonidia appear, add more sugar to make the solution stronger, up to 4 or 5 per cent., and after keeping in the light for a few days put the culture in darkness again.

When once induced in one or other of these ways, the production of zoogonidia may continue for two or three weeks.

The formation of **sexual organs** in V. sessilis, which is usually found in greenhouses in the vegetative condition, may be induced by placing the plant in 3 per cent. cane sugar solution and exposing the culture to sunlight. Bright light or a temperature above 15° C. will usually check the formation of zoogonidia, while sexual organs are not formed in weak light or in darkness. Again, the

plant may be kept indefinitely in the sterile or vegetative condition either by exposure to bright light in 0 5 per cent. Knop solution which is renewed frequently, or in weak light if this solution is changed very seldom—only when a whitish scum appears on the surface

387. Structure of Thallus.—Place some fresh Vaucheria on a slide, mount in water, tease out the felted mass gently with needles, and with low power note that:

(1) The thallus consists of fairly stout green cylindrical branching **unseptate filaments**, about 0 1 mm. diameter in the larger species.

(2) The **growing tips** of the filaments are colourless and transparent.

(3) The **branching,** which may occur sparingly at long intervals, is monopodial.

(4) Some branches may develop as colourless **rhizoids** which enter the soil—these are well seen in young plants arising from zoogonidia on germination.

If no reproductive organs are present, treat the material as directed in § 386, in order to induce the development of these organs. Place some of the material in alcohol, to extract the chlorophyll.

Examine with the high power portions of fresh material mounted in water; also get ready preparations treated with iodine, irrigated with salt solution, treated with alkannin (or mounted in water and covered for some time with a slice of alkanna root). Also decolorise some material with alcohol, and treat as directed for Spirogyra; a good fixing solution for Vaucheria consists of 1 gram chromic acid and 8 c.c. glacial acetic acid, dissolved in 800 c.c. water.

In these preparations, note (1) the outer **cell-wall;** (2) the layer of **protoplasm** lining the wall; (3) the central **vacuole** running through the entire filament. Embedded in the protoplasm layer are (4) numerous oval or spindle-shaped **chloroplasts,** some of which may be seen in process of division; (5) numerous small **nuclei**—well seen in decolorised and stained specimens; (6) bright

refractive **oil-drops,** stained red by alkanna. No starch is present; the filaments are normally devoid of cross-walls except where a reproductive organ (zoogonidangium, antheridium, oogonium) is formed.

388. Asexual Reproduction by means of Zoogonidia.—If material which is producing zoogonidia be placed in saucers or other vessels and observed from time to time, numerous **young plants** will be seen, some floating on the surface, others attached to the saucer; these have arisen from germinating zoogonidia.

To watch the development of zoogonidia, keep the culture in darkness and examine next morning. Some filaments are seen, even with the naked eye or a lens, to have swollen and dark-green tips. Mount some of these filaments in water; in some cases there will be seen simply the swollen tip, with dense contents surrounding the vacuole, in others the tip will be cut off by a transverse wall, and in others again the **zoogonidangium** thus formed will be empty.

Place some of this material in alcohol, and stain it when decolorised. Note that after the formation of the septum which cuts off the **gonidangium,** the contents become rounded off to form an ovoid or nearly spherical mass, with transparent outer (ectoplasm) and densely granular inner (endoplasm) protoplasmic layers, and a large central vacuole; the ectoplasm may be radially striated, and staining shows that it contains numerous nuclei, while the denser endoplasm contains chloroplasts The rounded off **zoogonidium** finally escapes by rupture of the gonidangium wall near the tip, swims through the water with a rotating movement, and after a short motile period of ten to fifteen minutes it settles down, acquires a wall, and germinates to form a new plant, sending out a green filament and a colourless one (rhizoid).

Watch a zoogonidium which is moving slowly or coming to rest, and note the numerous **cilia;** add iodine, which kills the zoogonidium, and note that there is no cell-wall, and that the cilia are arranged in pairs—the insertion of each pair is exactly opposite one of the nuclei in the clear

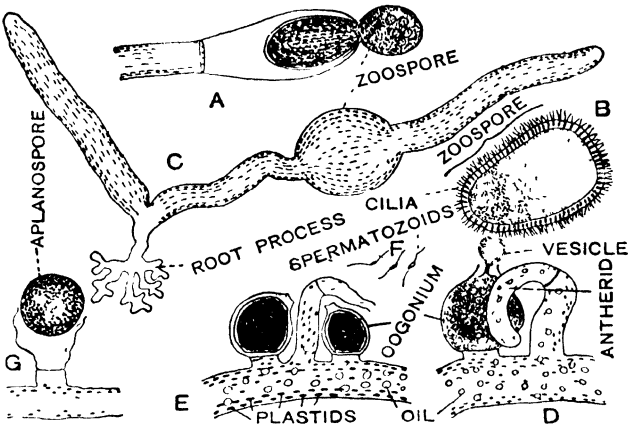


Fig 69*a*.—*Vaucheria* A, B, The zoospore, C, Young plant formed from zoospore, D, E, F, Sexual organs

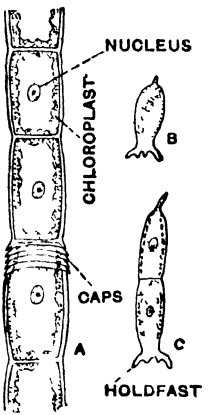


Fig 69*b*.—*Oedogonium* A, Part of filament, B, C Young plants developed from zoogonidia

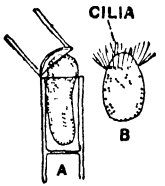


Fig 69*c* —*Oedogonium* A, Zoospore escaping, B, Zoospore

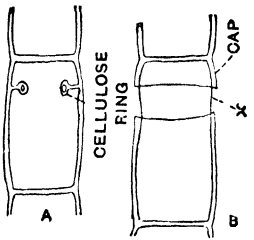


Fig 69*d* — *Oedogonium* Method of cell growth (X = intercalated membrane)

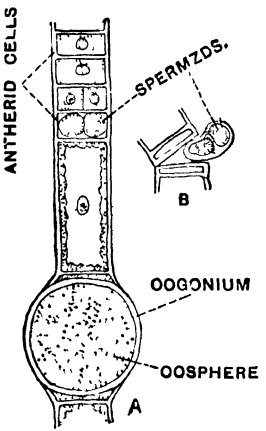


Fig 69*e* —*Oedogonium* Sexual Reproduction (Monoecious species)

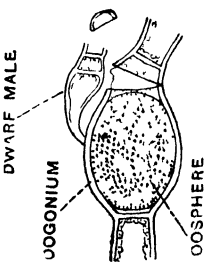


Fig 69*f* —*Oedogonium* Dioecious nannandrous species Dwarf male on Oogonium

ectoplasm. Treat with 2 or 3 per cent. salt solution a zoogonidium which has come to rest; plasmolysis of the contents reveals the presence of a thin wall.

389. Sexual Reproductive Organs.—In fresh or preserved material with sexual organs, note that the antheridium in a branch is continuous with the contents of the ordinary filament, while the upper portion or **antheridium** proper is cut off by a transverse wall and is usually curved. The mature antheridium is almost colourless and contains numerous nuclei—each of which becomes surrounded by protoplasm and forms a biciliate **antherozoid,** the tip of the antheridium opening by a pore to let the antherozoids escape. The mature **oogonium** is also separated from the rest of the thallus by a wall; the single **oosphere** contains chloroplasts and oil-drops, except at a clear place ("receptive spot") opposite the pointed beak where a pore is formed to admit the antherozoids.

OEDOGONIUM.

390. Oedogonium includes a large number of species, which differ very little in vegetative structure, but show well-marked differences in the distribution of the sexual organs and in the life history. Oedogonium is easily distinguished from other freshwater Algae, even when sterile, by the structure of the cells of the unbranched filaments—especially by the peculiar "caps" of the cell-wall.

In rather less than half the known species, the life history is somewhat complicated by the presence of "dwarf male plants"—these species are said to be **dioecious and nannandrous;** most of the remaining species do not show these dwarf males, and are **monoecious and macrandrous;** the rest are dioecious but without dwarf males, and are called **dioecious and macrandrous.** In addition to antheridia and oogonia, Oedogonium shows asexual reproduction by means of zoogonidia.

A curious character of the genus is that all the motile reproductive cells agree in having at the anterior clear end a circle or crown of cilia. This is also the case in another genus, Bulbochaete, which resembles Oedogonium in most respects and like it grows in fresh water, but which consists of **branching** filaments.

The plant is attached at one end when young, and in those species which grow in running water this condition remains throughout life, the plant being attached to stones and other objects in streams Most of the species, however, grow in quiet waters, especially in ponds and ditches, either attached to water-plants, twigs, etc, or floating freely on the surface in masses which somewhat resemble those of Spirogyra, but are not so slippery. Like various other Algae without a thick mucilage coat, Oedogonium is often covered with Diatoms and other epiphytes.

391. Culture of Oedogonium.—If only sterile material can be obtained, attempts should be made to induce it to form sexual and asexual reproductive organs.

(*a*) Keep plants in weak (0·1 or 0 2 per cent.) Knop's solution in a cold place, the water being chilled from 6° to 0° by addition of ice from time to time, and then bring the culture into a temperature of 15° or 16°. In a day or two abundant **zoogonidia** may be produced, as the result of this treatment

(*b*) **Sexual organs** may be produced if plants are placed in plenty of water, in bright light, at the ordinary room temperature—15° to 20°.

Light does not seem, as a rule, to have any influence on the production of zoogonidia, but it is necessary for the formation of sexual organs.

392. Structure of Thallus.—Mount Oedogonium threads in water. If it is an attached species (often forming a fuzzy covering on water-plants) and has been scraped carefully from the substratum, note the basal colourless **attachment disc**—consisting of finger-like outgrowths of the lowest cell.

Note that the entire filament consists of **a single row** of cells. Here and there a cell may show, at its upper end, a series of **parallel transverse marks**—the "caps" characteristic of Oedogonium. Each cell contains a parietal **chloroplast** having the form of a network with large meshes—what appear at first sight to be individual chloroplasts are thickenings of the network. Associated with the chloroplast are **pyrenoids** and **starch grains**. Note also the **protoplasm** layer lining the wall; the fairly large **nucleus**, with a distinct nucleolus; the central **vacuole** of cell-sap.

Some of these points can be made clearer by (1) plasmolysing a filament with salt solution; (2) treating with iodine; (3) treating with chlor-zinc-iodine; (4) declorising with alcohol and staining with various reagents and stains.

393. Reproduction.—It is difficult to make a successful series of observations from which to piece together the somewhat complicated **life cycle** of Oedogonium. An attempt should at least be made to observe the zoogonidia and the young plants formed by their germination; the antheridia; the oogonia in different stages; and the dwarf male plants.

394. The **zoogonidia** are formed singly from ordinary cells, the contents of this cell (**zoogonidangium**) contract and escape (by the formation of a transverse rupture of the wall) as a pear-shaped zoogonidium with a circle of cilia at its clear narrow anterior end. After a motile period, the zoogonidium becomes attached by the clear anterior end, forms a cell-wall, and grows into a new filament; the young plants may be found on the sides of the vessel containing the material with zoogonidia, or on glass slides dipped into or suspended in the water, or attached to plants or stones in ponds, etc., with mature Oedogonium plants.

395. Oogonia may be formed from any of the cells, either singly or in series one above another; the contents of the oogonium (recognised by its large size and swollen form) round off to form an **oosphere**, containing abundant chlorophyll except at the clear receptive spot which faces the part of the wall where the opening will be formed—the opening may take the form of a circular split or a pore (at top, base, or middle of oogonium), or a lid may be detached.

396. In the "macrandrous" species, whether monoecious or dioecious, the **antheridia** appear as short disc-like cells, usually in a series; the contents of each usually divide to form two **antherozoids**, which have very little chlorophyll and are much smaller than the zoogonidia, but resemble them in their ciliation, and are set free in the same way, ultimately fertilising an oosphere.

397. In the "nannandrous" species the antherozoids are formed in a curiously roundabout way. Short cells are formed in the (female) filaments, either singly or in chains, and from each of these **androgonidangia** there is produced an **androgonidium**, intermediate in size between zoogonidium and an antherozoid. The androgonidium swims about, and then settles by its clear ciliated end either on an oogonium or on a cell near one, acquires a cell-wall, and grows into a small male filament (**dwarf male plant**), consisting usually of a basal vegetative (attaching) cell supporting one or two antheridial cells, each of the latter producing an antherozoid which is set free and finally enters an oogonium.

398. The fertilised oosphere, or **oospore**, acquires a wall which becomes thickened, the contents become yellow or red, the starch changes to oil, and after a resting period the outer wall bursts and either grows out at once to form a filament (new plant), or (more often) its contents divide into four portions which are set free as motile **zoospores** resembling zoogonidia in form and ciliation When the zoospore germinates it often gives rise to an asexual filament which produces zoogonidia, and so on for several asexual generations before a sexual plant is formed; or a sexual plant may be formed at once

Fucus.

399. General Characters.—Two species of Fucus are easily distinguished among the Brown Algae which grow on the coast between the tide marks. Fucus serratus has toothed margins; while in F. vesiculosus the margin of the thallus is entire, and along the middle there are conspicuous air bladders, often in pairs side by side.

In both cases note (1) the flattened irregular **attachment disc**, firmly fixed to rock or stone; (2) the cylindrical lower portion or "**stem**" formed—as seen on comparison with young plants—by the thickening of (3) the **midrib** and decay of (4) the thinner lateral portions or **wings** which are distinguishable in the flattened upper part of the mature plant; (5) the repeated **branching** of the thallus, especially

in the flat upper region; (6) the growing tips of the ordinary sterile branches, showing a notch at the apex, or two notches where forking has occurred; (7) the oblong and thickened tips, or **receptacles,** of the fertile branches, studded with (8) the projecting wart-like **conceptacles** —flask-like cavities, each opening by a pore (ostiole) from which some hairs (**paraphyses**) may be seen projecting

If mature, it is easy to distinguish, on separate plants in these two species of Fucus, the yellow or orange male conceptacles containing the **antheridia;** and the dark-green female conceptacles containing the **oogonia.** Also note, scattered over the thin lateral wings in various parts of the thallus, the small **sterile conceptacles,** which contain only hairs

400. Material for Study.—For part of the work on Fucus it is essential to have fresh plants. Those residing inland should get specimens sent to them from the coast, on arrival the specimens should be placed in sea-water, or sea-salt solution. Fresh material may be kept alive in sea-water, or in solution of Tidman's Sea Salt (5 oz. to a gallon of tap-water).

For study of the general habit of the plants, Fucus material may be allowed to dry; when required for use, soak the dry specimens in water until they become soft and flexible—the same material can be used repeatedly in this way.

For microscopic work on the thallus structure use material that has been hardened by being preserved in either formalin or in 70 per cent. alcohol, or (better) placed in 70 per cent. alcohol after being left for 24 hours in fixing fluid consisting of 1 gram chromic acid and 0 4 c c. glacial acetic acid to 400 c.c. of sea-water and then rinsed well in sea-water.

To make the brittle alcohol-preserved material easier to cut, place the pieces in mixture of 1 part glycerine and 3 parts alcohol for 24 hours, or in glycerine for a shorter time, or in equal parts alcohol and glycerine for a longer time (2 or 3 days).

401. Mucilaginous character of Thallus.—Cut across a fresh thallus, and note the slimy mucilage that oozes out or is easily squeezed out. With a dry razor, cut thin sections across a piece of thallus, held in pith; the sections become twisted, since the outer tissues expand and the inner tissues contract. Evidently in the intact thallus the two tissues are in unequal conditions of tension, the outer being compressed by the inner and the inner stretched by the outer. Mount some sections in tap-water, they swell up greatly and become more distorted. Mount some in sea-water; the swelling is much less marked, hence fresh material should be examined in sea-water.

402. Structure of Thallus (Fig. 70).—From the upper region of the thallus, where the wings are well developed, cut (*a*) horizontal sections, parallel to surface

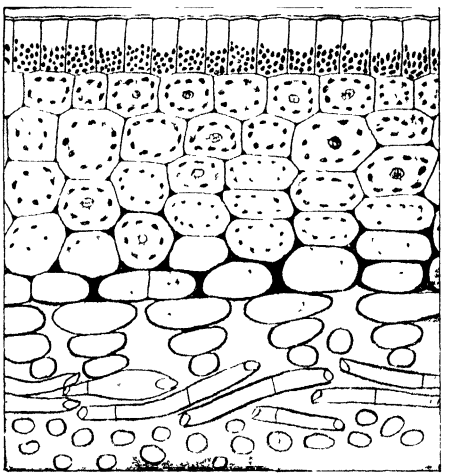


Fig 70.—Fucus. Part of a Transverse Section of the Thallus. See § 402.

of thallus, at different depths; (*b*) transverse sections, (*c*) median longitudinal sections of midrib and of wing. Mount the sections in glycerine, and note that

(1) The superficial cells, forming the limiting or **epidermoid layer,** are in surface view rectangular or polygonal, and arranged in longitudinal rows, but in T. S. and L. S of thallus are prismatic and vertically elongated

(2) Below this layer come wider cells, increasing in size as we pass towards the interior of the thallus—these cells, with the epidermoid layer, form the **cortex.**

(3) The internal tissue or **medulla** consists of elongated cells joined end to end to form filaments which run parallel to each other and to the long axis of the midrib and are embedded in mucilage.

(4) The medulla of the midrib is continued at each side into that of the wings, where the filaments form a loose network.

(5) The cells of the cortex and medulla have pitted walls, the cross-walls in the medulla filaments resembling sieve-tubes in appearance.

(6) All the cells contain protoplasm and a nucleus, also rounded **chromatophores,** which are abundant in the outer cortex and scanty in the medulla

403. Structure of Air Bladder.—In sections passing through an air bladder of Fucus vesiculosus, note that the wall of the air-filled cavity consists of a cortical tissue, with a lining of medullary tissue which is loose and disorganised. Cut across a young bladder: it contains a network of filaments like that seen in the wings, but this is torn as the jelly is replaced by air during the growth and expansion of the bladder.

404. Growth in Thickness of Midrib.—In T. S. and L. S. through successively older parts of the thallus, trace the processes which lead to **growth in thickness** of the midrib, accompanied by disappearance of the wings. As the thallus grows older, the outer cells of the midrib cease to divide, and this tissue is thrown off. The inner cortex remains active, increasing in bulk by division of the cells, and also producing finger-like prolongations which grow into the medulla, dividing by transverse walls and undergoing branching; these new filaments may be distinguished from the old ones among which they are intruded by their smaller diameter and lighter-coloured contents.

405. Sterile Conceptacles.—In sections that include one or more of these structures, note the flask-like cavity, surrounded by the inner cortex and opening on the surface of a conical projecting

rim by a round hole, through which protrude hairs which spring from the tissue lining the cavity, the latter also containing mucilage ; the young conceptacle is closed, and the hairs are seen to arise from single cells of the lining tissue ; in successively older regions of the thallus the conceptacles become closed again, the projecting portions of the long hairs dying off, while the opening of the cavity is obliterated by bundles of shorter unicellular hairs which grow from the lining tissue, as well as by the bases of the long hairs and by brown mucilage.

406. Sexual Organs and Cells (Figs. 71, 72).—Before making sections of the conceptacles, study the free sexual organs (**antheridia** and **oogonia**) and sexual cells (**antherozoids** and **oospheres**).

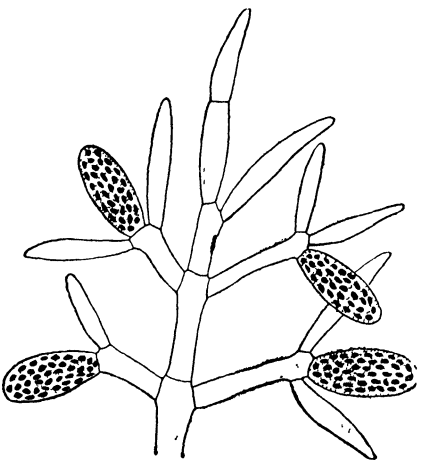


Fig. 71.—FUCUS. Four Antheridia, borne on one of the branched hairs in a Male Conceptacle.

Place fresh fertile plants in a large vessel of sea-water or sea-salt solution, and after about six hours hang up the plants or lay them in a dry place for about six hours; note that drops of mucilage ooze from the fertile branch tips—orange coloured in the male plant, green in the female plant.

Mount in sea-water some of the orange slime, and note that it contains numerous ellipsoid antheridia; each **antheridium** contains numerous antherozoids, and each antherozoid is a pear-shaped cell with a bright orange chromatophore. On watching an antheridium in water, it

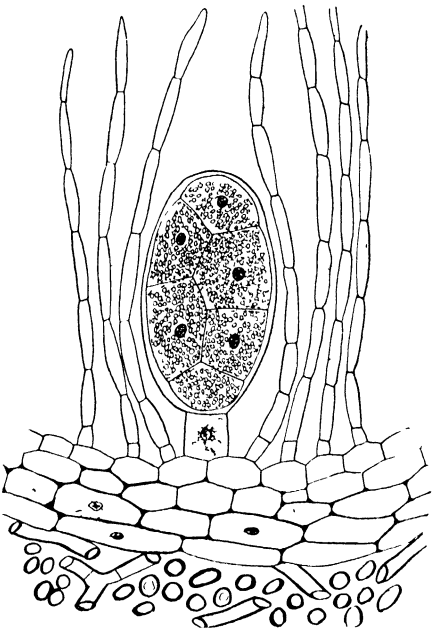


Fig 72 —Fucus An Oogonium, seated on its Stalk-cell, with Paraphyses, as seen in Section of a Female Conceptacle

may be seen to dehisce; the outer layer (**extine**) of the wall bursts open at one end, the mucilaginous inner layer (**intine**) swells up and disappears, and the **antherozoids** are set free as motile bodies with two laterally inserted cilia—easily seen on treatment with iodine, which kills and stains the antherozoids.

Examine in the same way the green slime from a female plant, and note that it contains numerous oogonia, each **oogonium** containing eight **oospheres**; the firm extine and mucilaginous intine of the oogonium are easily distinguished, and frequently at one end the stalk-cell remains attached to the oogonium. In the dehiscence of the oogonium, the extine bursts at the apex and the intine protrudes; the extine shrinks backwards, exposing more of the intine which then swells and disappears, while the oospheres, which have meanwhile become rounded off (in the intact oogonium they are pressed against each other and therefore polygonal), are set free as naked spherical masses of protoplasm, containing chromatophores and a central nucleus.

Interesting permanent preparations of the developing sexual organs, showing the numerous nuclei in the maturing antheridium (which when young has a single nucleus) and the eight nuclei in the maturing oogonium (which also begins with a single nucleus), may be made as follows. Cut a fertile branch into pieces, each including only a few conceptacles, and stain them in bulk by placing them in borax carmine for 24 hours; then place them in acid alcohol (2 drops strong hydrochloric in 50 c.c. of 70 per cent. alcohol) until they become clear red; then place them successively in 70 per cent. alcohol and in absolute alcohol—about an hour in each; then mount them in a drop of clove oil on a slide, tease out with needles the contents of the conceptacles—sufficiently to show (1) the branching shrub-like hairs bearing the antheridia, (2) the oogonia with the adjacent paraphyses and lining tissue—and mount in balsam.

407. Fertilisation.—Mix some orange slime and some green slime in sea-water in watch-glasses. Also place a drop of each on a slide and note that the antherozoids approach the motionless oosphere and swarm around it, giving it a rotating movement if present in large numbers.

On keeping the mixed fluids in sea-water, note in a few days that the oospore, which acquires a cell-wall after fertilisation has occurred, becomes pear-shaped and divides by a transverse wall, the fixed lower cell forming the hold-fast while the upper produces the rest of the thallus.

Young Fucus plants of different ages may also be seen on rocks and stones, forming velvety olive-brown patches,

the younger ones being club-shaped and fixed by the narrow end, while the free end usually shows a tuft of hairs arising from a depression (in which lies the growing-point) at the apex of the thallus.

408. Sections of Conceptacles (Fig. 73) —Cut a good number of transverse sections through fertile branch tips, and mount in glycerine. In T. S. of a **male branch** note (1) the flask-like or nearly spherical form of the con-

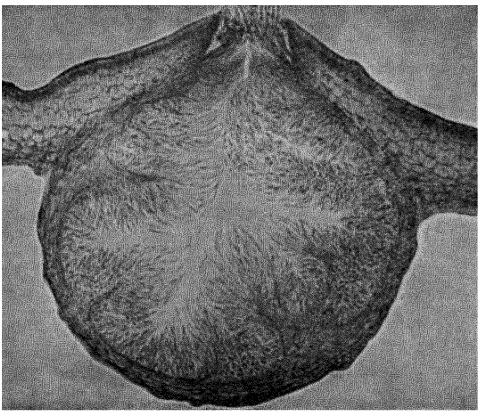


Fig 73.—Fucus. Transverse Section of a Male Conceptacle See § 408.

ceptacles; (2) the raised **pore** by which the conceptacle cavity opens on the surface of the branch—naturally, only a few conceptacles, if any, will show this narrow pore cut through; (3) the hairs or **paraphyses** which arise from the wall of the cavity, and of which the upper ones protrude through the pore; (4) the densely granular ellipsoid **antheridia,** borne on branching hairs, (5) the small-celled **lining tissue** of the cavity, which merges towards the outside of the branch into (6) the compact **cortex,** and towards the interior into (7) the loose **medulla** of the branch.

In similar sections of a female branch, note (1) that the female conceptacles resemble the male in form and position; (2) the paraphyses are all unbranched or only slightly branched; (3) the **oogonia** are large and ovoid or rounded, with a thick wall—their contents may have divided into two, four, or eight **oospheres,**—and (4) each oogonium is carried on a unicellular stalk.

CHAPTER IX.

FUNGI AND LICHENS.

Brewery Yeast (Saccharomyces Cerevisiae).

409. Structure of Yeast Cell.—Before starting experiments with Yeast, examine its structure, in the **resting condition,** as follows:—

(*a*) Place a little dry Yeast on a slide, add a drop of **water,** and stir it up with a needle, cover, and examine. With the low power, note the extremely small size of the rounded or ovoid cells. With high power, note (1) the thin **cell-wall,** (2) protoplasm, often showing bright clear dots (**oil-drops**), and (3) the central **vacuole.**

(*b*) Treat preparations with (1) iodine, (2) chlor-zinc-iodine, (3) potash; note that there is no starch, that the cell-wall does not give the reactions of cellulose, that the protoplasm is stained brown by iodine, and that the wall is made clearer by the disorganising action of potash on the protoplasm.

(*c*) Stain some Yeast with haematoxylin; then press on the cover-glass, and look for cells which have been crushed —these will show the empty ruptured wall and the extruded contents

410. Pasteur Solution.—To prepare a stock of Pasteur culture solution for Yeast and other Fungi, weigh out, powder, and thoroughly mix the following salts:—

Ammonium tartrate $(NH_4)_2C_4H_4O_6$	50 grams
Potassium phosphate, KH_2PO_4 . .	10 „
Calcium phosphate, $Ca_3(PO_4)_2$	1 gram
Magnesium sulphate, $MgSO_4$ . . .	1 „

A small quantity of yeast may be bought for a few pence at most bakers' shops

Dissolve the powdered mixture in water, as required for use in the proportion of 2 grams to 100 c.c of water, and add 16 grams of cane sugar.

411. Alcoholic Fermentation.—About two-thirds fill a fairly large flask with Pasteur solution and add some Yeast that has been stirred up with water to form a paste.

(*a*) Plug the neck of the flask lightly with cotton-wool, and set it in a warm place; note that the liquid becomes cloudy and frothy, bubbles are given off, and the liquid after a time smells of alcohol.

(*b*) Now fit the flask with a bored cork through which passes a tube bent like a J, with the longer arm dipping into a vessel of baryta-water (or lime-water); note the evolution of bubbles of carbon dioxide.

(*c*) Now replace the J-tube in the cork of the flask by a straight narrow tube about 30 inches long, not allowing its lower end to dip into the liquid; heat the flask over a Bunsen or spirit lamp, fixing it in a retort-stand, and note that after a time the alcohol-vapour given off can be lighted at the upper end of the tube, burning with the characteristic blue flame seen in a spirit lamp.

(*d*) Repeat the preceding experiments with a flask of Yeast and Pasteur solution which has been **boiled** for five minutes; no fermentation takes place, and no alcohol or carbon dioxide are formed, because the Yeast cells have been killed.

(*e*) Get ready a series of six jars or tumblers, fitted with covers or corks. In *A* put water; in all the others, Pasteur solution; add to each a tablespoonful of thin Yeast paste (or simply a bit of dry Yeast). Place *B* in darkness, keep the others in the light in the ordinary way. Place *C* in a temperature of 0° C. or very little above it; *D* at the ordinary room temperature; *E* at a high temperature, about 35° C.; and boil the Yeast and the Pasteur solution for *F* in a flask for five minutes before pouring it into the jar.

Note that the growth of the Yeast (as judged by the cloudiness and frothiness of the liquid) is arrested by a very low temperature and is increased by warmth; the liquid in *F* does not become frothy or smell of alcohol, the Yeast having been killed by boiling; in *A* there is very little growth, any that does occur being due to the fact that the Yeast placed in the water contains enough food to last for a short time.

412. Budding of Yeast.—Examine a drop of culture solution containing actively growing Yeast, and note that many of the cells are joined together in chains, often branched, which have evidently been formed by a process of **budding,** since the cells at the free end of each chain are the smallest

In order to watch the actual budding process, place a very little dry Yeast in a hanging drop of Pasteur's fluid in a moist chamber slide (§ 18), and examine it from time to time; note that the larger cells are evidently putting out little projections, which may grow until they reach the size of the parent cell, and that instead of being at once detached the buds may in turn produce other buds, and these yet others, until a chain is formed.

Since the original cell may produce buds at two or more points of its surface, radiating colonies may be formed, but the outer cells of the chains become abstricted, each growing larger when free and budding in the same way as its parent, and so on.

413. Spore Formation.—Under certain conditions the cells of Yeast may produce **resting-spores,** the protoplasm of the cell dividing into (usually) four portions which become rounded off and acquire a thick wall, so that they resist drought, and are on account of their minute size readily wafted about in the air. The production of abundant spores may be readily induced by one of the following methods:—

(1) Set aside a culture of Yeast in Pasteur's fluid with sugar; after a few weeks spores will appear. (2) Spread

some actively budding Yeast on a slab of plaster-of-Paris, made by pouring the plaster mixed with water into a greased vessel or on a piece of wood. (3) Spread some active Yeast on a slice of Potato, and keep it under a bell-glass

Pythium Debaryanum.

414. Material for Study.—Sow seeds of Common Cress (Lepidium sativum) thickly in a pot of wet sawdust or loose soil, or on muslin stretched across a tumbler of water, cover with a glass plate or bell-glass, and keep the seedlings thoroughly wet and in a saturated atmosphere.

Note that in a few days the seedlings become weakened and fall over; a thick web of fungus-threads appears binding the seedlings together; and finally they become completely decayed. Quite early they show a pale sickly appearance, the hypocotyl becomes constricted and softened, and bending occurs here.

The fungus, which begins by attacking the live seedling as a *parasite*, kills it and then thrives as a *saprophyte* on the decaying tissues of the dead seedling; the spores of Pythium are present, along with those of Bacteria and countless Fungi, in the air, and these spores produce threads which penetrate the hypocotyl and ramify in the tissue of the seedling.

415. Structure of Thallus and Gonidangia.—Mount some of the infected seedlings in water, and note

(1) That the tissues are disorganised and yellowish at the base of the hypocotyl, where the seedling has collapsed owing to the attack of the Fungus

(2) The colourless branching fungus-threads (**hyphae**) running along the surface of the seedling, entering either by a stoma or by boring through the epidermal cells.

(3) The hyphae running through the intercellular spaces inside the seedling, or through the cells themselves.

(4) The unseptate or **coenocytic** structure of the hyphae, *i.e.* the absence of cross-walls, as in Vaucheria.

(5) The presence of numerous **nuclei** and of **oil-drops** in the hyphae.

(6) The **gonidiophores,** which are formed by the ends of certain hyphae swelling up to form a **gonidangium** which is cut off by a cross-wall—or the gonidangia may be intercalary and marked off by two cross-walls

416 Development and Germination of Gonidangium.—The **development of the gonidangium** may often be traced if portions of infected seedlings are placed in water in a watch-glass and examined day by day. If a piece of material showing gonidangia is placed along with a healthy Cress seedling in a watch-glass of water, the **germination** of the **gonidangia** may also be observed. The ends of some of the hyphae, growing out from the infected seedling, swell up and become densely granular; after the formation of the cross-wall, the portion of the hypha immediately below is seen to be partly emptied of its protoplasmic contents.

When the gonidangium germinates, it may either (1) send out a protruding vesicle into which the contents pass, these dividing to form numerous **zoogonidia** (so small that it is hard to say whether they have two cilia or a single cilium) which swim about and on reaching a host-plant put out a hypha to enter it; or (2) act as a **gonidium** and germinate directly, putting out a hypha.

It is usually stated that the direct germination of the gonidangium, without the formation of zoogonidia, indicates partial adaptation to subaerial life-conditions, but as a matter of fact this type of germination occurs freely in cultures made in water

417. Antheridium, Oogonium, and Oospore.—The **sexual organs** should be looked for in material that has already produced gonidangia, on placing it in a watch-glass or a larger vessel of water. The **oogonium** arises as a terminal (or sometimes intercalary) swelling on a hypha, at first resembling a gonidangium, and is cut off by a cross-wall as a spherical cell containing a single **oosphere.** The **antheridium** arises as a lateral branch, often on the same hypha a little below the oogonium, and its tip is cut off by a transverse wall.

If material is obtained, the process of **fertilisation** may be followed in a hanging drop of water (Ward's tube or moist-chamber slide); the tip of the antheridium comes into contact with the oogonium and puts out a short tube

which pierces the oogonium-wall, the male nucleus passing through the ruptured tip of this "fertilising tube" into the oosphere. The **oospore** secretes a thick wall, lying freely inside the oogonium, and its contents are densely granular and oily.

MUCOR.

418. Material for Study.—Mucor is the common "black mould" which appears in about a week on damp bread kept under a bell-glass. Various other Fungi may appear in addition, but Mucor is easily recognised by the outgrowth from the fluffy white **mycelium**, after a few days, of the erect **gonidiophores**, each bearing at its tip a small black head—the **gonidangium.**

To prevent the bread from becoming too wet and mushy, set a tumbler inverted in a plate of water, place on the tumbler a piece of bread that has been allowed to get rather stale by exposure to the air for a day or two, and cover the whole with a bell-glass.

419. Mycelium and Gonidiophores.—Pick up with needles, or with a knife-point, some of the bread on which Mucor is growing, tease it out gently in water on a slide, and note the following :—

(*a*) The branched **mycelium**, consisting of thick primary filaments (**hyphae**), which give off thinner branches, these again branching repeatedly and ramifying through the bread and becoming finer as branching proceeds.

(*b*) The absence of transverse walls, the hyphae being unseptate (**coenocytic**). Sometimes, however, septa are found in the hyphae, especially in old cultures which have produced gonidangia.

(*c*) The thick straight unbranched **gonidiophores**, each ending in a spherical **gonidangium.** Many of the older gonidangia will have burst open.

(*d*) To make out the structure of the hyphae of the

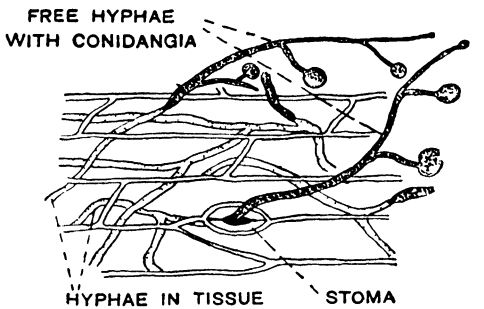


Fig 73a —*Pythium* Epidermis of a plant attacked by the fungus

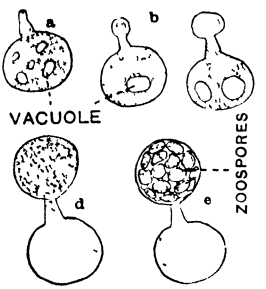


Fig 73b —Zoosporangium of *Pythium*. Stages in the production of zoospores

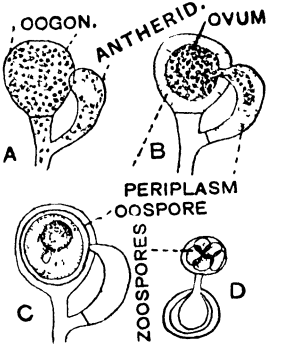


Fig 73c —Sexual Reproduction in *Pythium* B, Fertilisation, D, Germination of oospore.

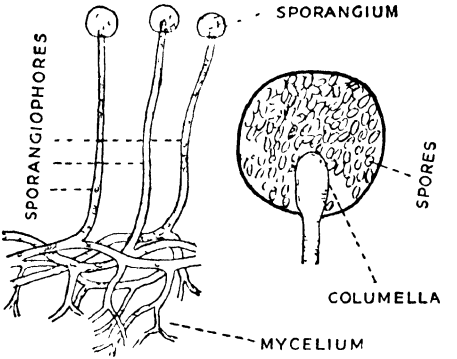


Fig 73*d* —*Mucor* A, A portion of a mycelium bearing sporangiophores, B, A single sporangium

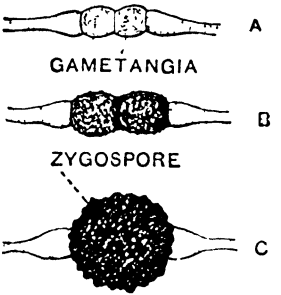


Fig 73*e* —The Process of Conjugation in *Mucor*.

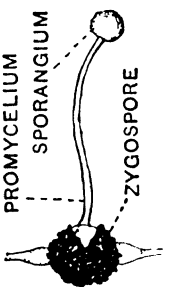


Fig 73*f* —Germination of the Zygospore of *Mucor*

mycelium, treat preparations of fresh Mucor with (1) salt solution, which will cause plasmolysis and make the protoplasmic lining visible; (2) iodine, which stains the protoplasm brown—note that the hyphae contain no starch

(*e*) The numerous small **nuclei** in the hyphae can be demonstrated on staining, with haematoxylin, material that has been fixed with alcohol or picric acid or chromo-acetic acid (1 gram chromic acid and 2 c c. glacial acetic to 200 c c. water).

420. Structure and Development of Gonidangium.—Examine gonidiophores before their tips have begun to turn black. Note that (1) the end of the gonidiophore becomes swollen up and pear-shaped; (2) a cross-wall is formed below the swelling, cutting off the **gonidangium**; (3) the latter now enlarges and becomes spherical; (4) the cross-wall bulges upwards into the cavity of the gonidangium, forming the **columella.**

Carefully seize with forceps a number of mature gonidiophores, a little below the gonidangia themselves, cut them off with scissors, and mount in alcohol. Note, in an undamaged gonidangium, (1) the thin **wall,** often covered externally by an incrustation of minute radiating needle-like calcium oxalate **crystals**—not always present; (2) the dense contents, consisting of the **gonidia**; (3) the clear place at the base of the gonidangium, corresponding to the position of the columella

421. Dehiscence of Gonidangium.—While watching a gonidangium mounted in alcohol, place a drop of water at one side of the cover-glass, and draw it through with filter- or blotting-paper. Note the sudden dehiscence of the gonidangium, the outer wall being broken into fragments and the ovoid gonidia escaping along with **mucilage.** Note also that the columella is left as an ovoid or nearly spherical swelling of the top of the gonidiophore, often surrounded at its base by a fringe representing the lowest portion of the gonidangium wall

422. Germination of Gonidium.—To follow the germination of the gonidia, make hanging-drop cultures in a moist-chamber slide (§ 18). Boil some French plums or prunes in water to make a dilute decoction of the juice (five prunes to 100 c.c. of decoction); boil the juice in order to sterilise it and to prevent the growth of other Fungi, and place a drop of it on a cover-glass. Moisten a needle with the boiled juice, touch a ripe gonidangium with the needle-point, and dip the latter in the drop on the cover, the object being to place in the drop as few gonidia as possible. Invert the cover and watch the germination: the gonidium puts out a hypha, which branches repeatedly, the branches spreading out radially in all directions.

In these culture experiments, all the apparatus used must be sterilised as thoroughly as possible, the prune juice or other nutrient medium by boiling, the needle by heating in a spirit-lamp or Bunsen flame and allowing to cool, the moist-chamber slide and cover by placing them in boiling water for a short time.

Instead of prune juice, Pasteur's fluid (§ 410), or a decoction of horse dung, may be used for Mucor. Cultures should also be made in agar or gelatine, mixed with prune juice and with cane sugar and placed in Petri dishes (shallow glass dishes with slightly wider glass covers fitting over them)—in each case all the utensils and nutrient media must be sterilised by exposing them to a temperature of 100° C. for at least half an hour, or to a higher temperature for a shorter time.

423. The "Torula" or **Yeast-condition** of Mucor can be induced by making a culture of gonidia (or of a portion of mycelium) submerged in cane-sugar solution or in Pasteur's solution to which sugar has been added. The hyphae become divided up by cross-walls into cells (**gemmae**) which proceed to undergo **budding** in the same way as Yeast cells, and like Yeast set up **alcoholic fermentation**, alcohol and carbon dioxide being produced by decomposition of sugar. On being exposed to the air again, by filtering off the turbid liquid and keeping the residue under ordinary culture, the plant may pass into its normal condition and produce gonidangia as usual

424. Sexual Reproduction in Sporodinia.—It is often difficult to obtain material showing the **sexual organs** of Mucor, nor is there any method for inducing their formation by cultivating the plant on special culture media, or at altered temperatures. Sometimes one does succeed by making several cultures and mixing the mycelia, for instance by sowing on one piece of bread spores from several isolated cultures.

The conjugating hyphae belong to different strains of Mucor mycelia, which we may simply call "male" and "female", when male and female mycelia come together zygospores are formed, and any given mycelium produces gonidia which give rise to mycelia of the same nature as the parent mycelium

This apparently applies to the majority of the Mucoraceae, but in some forms, *e.g.* Sporodinia grandis, zygospores are produced by the conjugation of hyphae of the same mycelium. In Mucor, the conjugating hyphae are formed on the portion of the mycelium which ramifies through the substratum, but in Sporodinia they are formed on erect aerial hyphae.

Sporodinia grandis grows as a parasite on several of the larger fleshy toadstools (Hymenomycetes), such as Boletus (a pore toadstool), as a greyish fluffy mycelium, on which the reddish zygospores can be seen with the naked eye It is common, and readily found in late summer and autumn.

Sporodinia can easily be cultivated indoors, and some interesting observations can be made on it. Pour some water into a wide-mouthed jar, and put filter- or blotting-paper round the inside of the jar, so as to keep the sides moist, then place a small beaker or dish, without any water, in the bottom of the jar; in the beaker place a small bit of bread moistened with prune juice, and infect the bread with Sporodinia gonidia or a piece of the mycelium itself—sections of Carrot root may be used with advantage instead of bread. Cover the jar with a sheet of glass, and examine the culture from time to time; the zygospores appear in a few days.

Note that the gonidiophores of Sporodinia differ from those of Mucor in being dichotomously branched, each branch ending in a small gonidangium.

The zygospores are formed by conjugation between erect hyphae. Where two hyphae are close together, there arises from each an outgrowth, like the conjugating tubes of Spirogyra, and these become swollen at the ends. When the swollen tips come into contact, each tip is cut off by a cross-wall from the rest of the tube (which is called the

"suspensor") to form a **gamete**—more strictly, a gametangium or coenogamete, since here, as in other Mucoraceae, the "gamete" is multinucleate. The double wall between the "gametes" is absorbed, and a **zygote** is formed—the male and female nuclei fuse in pairs

The wall of the zygote, or fused gametangia, becomes thickened, and the contents of the zygote become rounded off and acquire a thick wall to form the **zygospore,** the contents of which become dense and oily

The ripe zygospore wall shows (1) the original thin wall of the fused gametangia, (2) the dark-coloured warty epispore, (3) the thicker and more transparent endospore. After zygospores have been formed, numerous cross-walls appear in the hyphae of the mycelium. Sometimes the two gametes do not come into contact, but each gamete may still develop into a zygospore-like structure—an "azygospore."

Keep zygospores under observation in water in autumn, and note that on germination the epispore bursts open, and the contents, covered by the endospore, grow out to form hyphae which build up a mycelium, if this is cultivated, conidiophores may be seen to develop, completing the life cycle of the plant.

EUROTIUM.

425. Material for Study.—Eurotium or Aspergillus herbariorum or Aspergillus glaucus (names given by different botanists to the same plant) is the "green mould" which grows so commonly on bread, preserves, fruit, cheese, etc. It often occurs along with, or is replaced by, the still commoner "blue mould" (Penicillium).

In damp bread kept under a bell-glass for a few days the usual succession of moulds is (1) Mucor with its long black tipped conidiophores; (2) the bluish Penicillium with short conidiophores like miniature paint-brushes; (3) Eurotium, rather like Penicillium but with taller conidiophores bearing a globular cluster of chains of conidia.

426. Mycelium and Conidiophores (Fig. 74)—Examine a piece of bread, or some apricot jam, bearing **conidiophores** of Eurotium; shake some of these gently

with a needle, and note the conidia which are easily detached and float in the air as a fine cloud—the conidiophore remains after the shaking, and the conidia are evidently not enclosed in a conidangium, as was the case in Mucor

Mount some of the material in a drop of water, and note (1) the **mycelium,** consisting of **septate hyphae,** with the cross-walls at rather long intervals; (2) the stout non-septate **conidiophores,** each with a dense terminal cluster of **conidia** —some of which will have become detached and will be seen in the water. The roughly spherical clusters are not easy to make out, especially as the projecting conidia entangle air between them: draw under the cover-glass some alcohol, or mount a fresh portion in a mixture of water and alcohol—the commotion set up by the mixing of the water and alcohol will detach some of the conidia and also clear away the air-bubbles

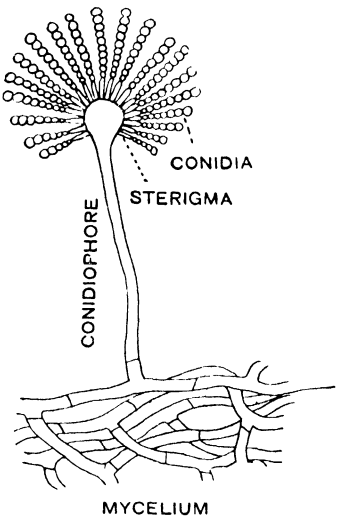


Fig 74.—EUROTIUM Part of the Mycelium, with a Conidiophore

Now note that the head of the conidiophore is swollen up (but not cut off by a cross-wall) and bears numerous **chains** of conidia; with the high power, note that the head gives off peg-like radiating outgrowths, the **sterigmata,** and that each sterigma has budded off a chain of conidia, the smallest (youngest) conidia being at the base of the chain and the largest (oldest) ones at the end The terminal conidia are being continually abstricted as they become rounded off, and therefore very loosely attached to the next ones below in the chain.

427. Development of Conidia.—Examine young portions of the mycelium, which have not yet become coloured by the ripening conidia

Note that (1) the conidiophore arises as an unbranched stout hypha, close to a transverse wall on a mycelial hypha and containing distinct granular vacuolated protoplasm and numerous nuclei; (2) the free end of the conidiophore swells up, but is not cut off; (3) from the enlarging head there grow out numerous papillae, the sterigmata, (4) each sterigma elongates, and then becomes skittle-shaped, an oval or spherical conidium being budded off from its tip, (5) after the first conidium has grown in size, a second is abstricted from the sterigma, just below it, in the same way, and so on until a chain is formed. Before each budding occurs, nuclei pass into the developing conidium, which when mature has about four nuclei; the conidium has a distinct but rather thin wall, which contains the greenish pigment (*not* chlorophyll) that gives Eurotium its colour.

428. Germination of Conidia.—Boil some prune or plum decoction, and make a hanging-drop preparation with a few conidia, as directed for Mucor (§ 422); the germination and the formation of the mycelium take place as in Mucor, except that in Eurotium the mycelium hyphae are from an early stage onwards septate owing to the numerous cross-walls formed.

429. Structure and Development of Ascocarp.—To observe the ascus-fruits, or ascocarps, keep some bread dry for a month or more; when ripe the ascocarps are easily seen with naked eye or lens as yellow spherical structures. Some of the stages in the development of the ascocarp may be seen on examining some Eurotium which has already produced conidiophores; mount in alcohol, add water, and tease out with needles.

Two simple methods may be used to induce the formation of sexual organs and ascocarps. (1) Transfer some conidia to a piece of bread soaked in 40 per cent. cane-sugar solution in prune juice, and keep at about 30° C.,

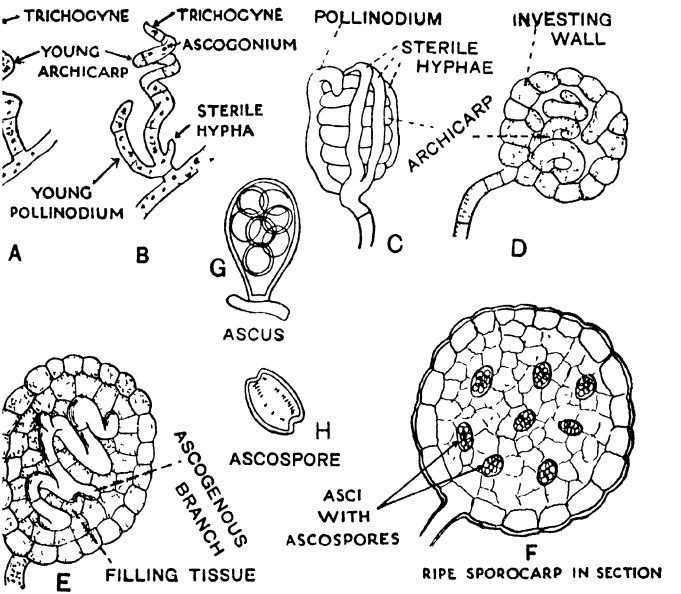


Fig 74*a* —*Eurotium* Development of Sporocarp and Ascospores

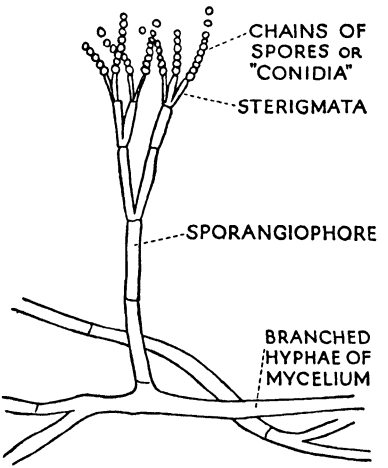


Fig. 74b —*Penicillium.*

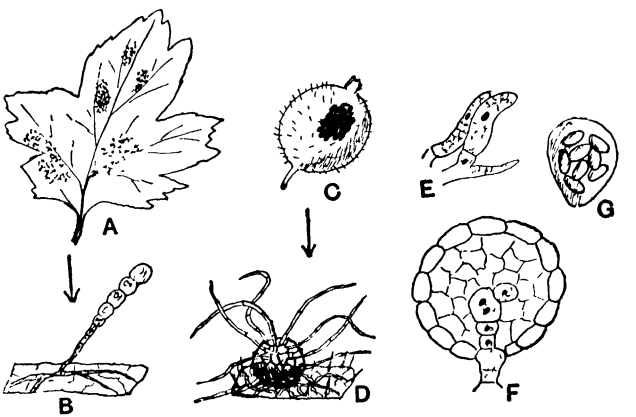


Fig 74c —*Sphaerotheca mors-uvae* (American Gooseberry Mildew) A and B, Conidial (summer) stage, on gooseberry leaf; C and D, Ascus (autumn) stage, on gooseberry, E, Antheridium and ascogonium coming together; F, Sporo carp in section; G, ripe Ascus, containing eight ascospores.

starting to examine after four days (2) Transfer some conidia to gelatine in sterilised Petri dishes. A good medium is 5 per cent. gelatine made up with prune decoction and 40 per cent. cane sugar In this medium cultures can be kept for a long time, and will fruit readily a few days after raising the temperature of the culture to 20° C.

In suitable material showing the **sexual organs** note (1) that thin branches arise from the mycelium; (2) that the first of these hyphae to be formed in a group is **coiled** like a corkscrew—this is the **archicarp** or female hypha, (3) that other hyphae arise below the archicarp and grow up to form a loose envelope around it—one of these, the **antheridium,** becomes applied by its tip to the apex of the coiled archicarp, while the sterile hyphae form the **sheath** of the fruit.

In a mature **ascocarp,** treated with potash to make it more transparent and mounted in glycerine, note (1) the **wall** or sheath of the fruit, consisting of a single layer of cells, (2) the ovoid sacs or **asci** within, each ascus containing eight **ascospores.**

To see the **asci** and **spores** better, mount an ascocarp in glycerine, crush it by pressing on the cover-glass, and note (1) the ruptured ascocarp wall; (2) the asci and the spores, the latter being ovoid when young and having when old a peculiar form—notched at each end, like two biconvex lenses fused together; (3) the nutritive tissue—found only in young ascocarps, but absorbed when the latter is mature. The ascospores contain about eight nuclei; they germinate like the conidia.

Penicillium.

430. Penicillium, found on all sorts of organic substances, from bread and jam to old boots and dried-up ink, is the commonest of the Moulds.

The mycelium is easily cultivated in a watch-glass of Pasteur's solution to which conidia are transferred from an infected slice of bread. The mycelia soon appear as floating white patches, which as they grow in area become

first pale blue and then dull green, the colour changes starting at the centre of the patch and spreading to the outside. From the floating mycelium there arise erect hyphae which develop into conidiophores, and submerged hyphae which grow vertically down into the liquid.

The mycelium has the same structure as in Eurotium, but the conidiophores are repeatedly branched, the parallel branches being arranged in a brush and each ending in a chain of conidia formed by basipetal abstriction as in Eurotium.

The ascocarps, which are rarely met with, are formed in much the same way as in Eurotium. In Penicillium, however, both sexual organs are spirally coiled round each other, one of them later giving off ascogenous hyphae. The enveloping hyphae form a densely interwoven firm mass of tissue, the outer layers of which are yellow and the inner (containing the ascogenous hyphae) colourless, and the hard and relatively large ascocarp undergoes a resting period of about two months. Ultimately the inner tissue of the mass is used up by the developing asci, and the spores escape by the breaking up of the hard brittle rind.

Sphaerotheca.

431. Allied to the Eurotium and Penicillium section of Ascomycetes is an interesting group, the Erysipheae, including the **Mildews** which are parasitic on the leaves of various plants, *e.g.* Hop, Rose, and various other wild and cultivated flowering plants.

Sphaerotheca, species of which grow on Hop and Rose, is one of the simplest forms of Mildew. The mycelium of Mildews is peculiar in that it creeps over the surface of the infected leaf, forming a web of intercrossing threads the hyphae, which send in short processes, the suckers or **haustoria,** into the epidermal cells of the leaf. The hyphae are single rows of cells, each containing a single nucleus.

During the summer, the mycelium sends up erect **conidiophores,** each of which buds off at its tip a chain

of **conidia**—behaving exactly like a single sterigma of a Eurotium conidiophore on a large scale. The mealy white appearance to which the Mildews owe their name is due chiefly to these conidiophores, which produce countless conidia and cause the parasite to spread rapidly from leaf to leaf, and from plant to plant, until very often the health and even the life of the "host" plant are endangered—the Hop Mildew (Sphaerotheca Castagnei) sometimes causes great loss to the hop-growers.

In autumn, or in late summer, should a drought follow a spell of wet weather, the fungus produces small **ascocarps**, developed in practically the same way as those of Eurotium, but having a much simpler structure. The **antheridium** and **archicarp** (oogonium) arise on separate branches, where these happen to cross each other, and the whole process has been fully worked out. Both these organs grow out from the parent hypha as a short branch, and are cut off by a wall. The oogonial branch enlarges, without division of its single nucleus, but the male branch divides into two superposed uninucleate cells, the lower and longer one being merely a stalk-cell and the upper shorter one the actual antheridium. The two organs fuse at their tips, the male nucleus fuses with the oogonium nucleus, to form a **zygote** nucleus; meanwhile from the cell below the oogonium there grow out numerous hyphae which form a **sheath** as in Eurotium.

The zygote (fertilised oogonium) now divides into a lower (stalk-) cell and an upper cell, each with one nucleus; the stalk-cell develops no further, but the upper cell divides into a row of cells, all except the penultimate one (second from the top) having one nucleus. The penultimate cell has two nuclei, which now fuse, and this cell simply enlarges and becomes the solitary **ascus** of the ascocarp, the fusion nucleus dividing into eight nuclei, around which the protoplasm collects to form the eight **ascospores**. From the tissue of the sheath or envelope there arise (1) internal cells forming a nutritive tissue as in Eurotium, (2) external septate hyphae or **appendages**—in some Mildews allied to Sphaerotheca, these hair-like outgrowths or appendages of the ascocarp are hooked at

the ends, or have much-branched ends, or have a large swelling at the base.

The ascocarps are just visible to the naked eye as black dots on the diseased leaf; they remain on the dead leaves during the winter, and when germination occurs in spring the ascus absorbs water, swells, bursts the sheath and its own wall, and sets free the ascospores which infect the young Hop shoots. In most of the Mildews each ascocarp produces several asci, as in the case of Eurotium.

Mushroom (Agaricus Campestris).

432. Mycelium, etc.—The **Common Mushroom** grows in open well-manured fields usually from June to the end of September; its stalk is white, short, and usually quite solid; its cap is dry and cottony above, the radiating gills on the underside of the cap are closely set, not running down on to the stalk; the gills are at first white, but later turn pink, and finally brown; the flesh of cap and stalk is white, but soon turns reddish-brown on exposure to the air when cut or broken

Get a piece of Mushroom "spawn," which is sold in pressed blocks by seedsmen, and note its fibrous peat-like texture. Put a small piece on a slide in water, tease it out with needles, and note (1) the **hyphae** are largely bound together into bands or bundles, from which single hyphae are here and there given off; (2) many of the hyphae are encrusted with rod-like **crystals,** consisting of calcium oxalate—test with acids The "spawn" consists of a mixture of dung and loamy soil, permeated by the resting mycelium, the bands of hyphae being visible to the naked eye—their whiteness is largely due to the encrustation of crystals.

Get a large flower-pot, or a box with holes bored in the bottom for drainage, and place in it first some stones and gravel, then some soil (good garden soil, mixed with cow dung), then some broken-up "spawn," then a few inches of soil on the top. Keep in darkness in a warm place, and sprinkle with water daily. In a few weeks the growing

mycelium will permeate the soil, and on the surface there will appear the white-rounded or egg-shaped masses which develop into the "mushroom" themselves, *i.e.* the spore-producing organs of the plant, the mycelium being the vegetative portion.

Place some of the mycelium-containing soil in water, to remove as much as possible of the soil from the mycelium, tease out a piece of the latter on a slide, and note that the hyphae branch irregularly, have cross-walls here and there, and are sometimes covered with calcium oxalate crystals, as in the resting mycelium; look for the rounded growing tips of the hyphae

433. Development of Gonidiophore.—Pick or wash the soil from a part of the mycelium on which young mushrooms of different sizes are seen; trace the connection between these and the mycelium, and the stages in their development. Note that—

(1) The young mushroom arises from the mycelium

(2) It is at first a rounded or ovoid mass, consisting of uniform solid tissue, as seen on cutting it longitudinally.

(3) Later it becomes differentiated into a narrower lower portion (**stalk**) and dilated upper portion (**cap**).

(4) Later still, as the cap expands, a ring-like cavity (**gill-chamber**) is seen running horizontally in the tissue

(5) The roof of the chamber is seen to bear numerous white radiating vertical plates (**gills**), as shown on making horizontal and tangential longitudinal sections.

(6) Later still, the cap extends further, the tissue forming the floor of the gill-chamber is ruptured, and the gills are now exposed,

(7) The stalk meanwhile grows in length, carrying up the cap, the gills turn brown, and the place where the rupture occurred is marked by

(8) The **annulus,** a ring of tissue on the stalk, and by

(9) A corresponding ragged fringe on the edge of the cap.

434. "Spore Print."—In the fully-grown mushroom note that the gills do not all reach from the edge of the cap to the top of the stalk; some extend only a part of

this distance. Cut across the stalk, just below the cap, and lay the cap with the gills downwards on a sheet of white paper. After a few hours, note that the spores fall out in the usual way and collect in ridge-like heaps, forming lines corresponding to the gills. If the paper has been moistened with diluted gum, the "spore print" thus obtained can be kept as a permanent specimen. Coprinus gives very neat spore-prints; other toadstools should also be tried

435. Structure of Gonidiophore.—It is difficult to cut good sections from fresh material Harden mushrooms, both young and mature, by placing them—cutting both the stalk and the cap into pieces—in 1 per cent. chromic acid for a day, rinsing with water, and placing them successively (for a day in each case) in 50 per cent., 70 per cent, and strong alcohol. This treatment will make the tissues firm and easy to section.

In transverse and longitudinal sections of the **stalk**, mounted in glycerine, note (1) the whole tissue consists of long branching septate hyphae, closely interwoven; (2) the central hyphae are relatively narrow and loosely arranged, the peripheral hyphae thick and closely packed.

In a **tangential vertical section of the cap** (Fig. 75), cutting the **gills** at right angles, note that (1) the tissue of the cap itself resembles that of the stalk; (2) the looser central tissue passes down into the middle of each **gill**; (3) this central tissue ("trama") of the gill consists of hyphae which run longitudinally downwards, and curve outwards to form (4) the **sub-hymenial layer** of short closely-packed cells and, beyond this layer, (5) the palisade-like **hymenium,** consisting of elongated club-shaped and closely packed cells of two kinds—viz. (6) the more slender **paraphyses** with rounded ends, and (7) the stouter and longer **basidia**—(8) each basidium bears on its free end two small peg-like outgrowths, **sterigmata,** each sterigma budding off a single **basidiospore.** Each basidium is exhausted after producing its two spores, and it develops no more; the bare sterigmata can be seen after the spores have fallen off.

For **surface view of gill,** mount a piece of a gill on a dry slide, and note (1) the **basidia** with rounded ends, bearing two spores each; (2) the bare rounded ends of the **young basidia** which have not yet formed spores and

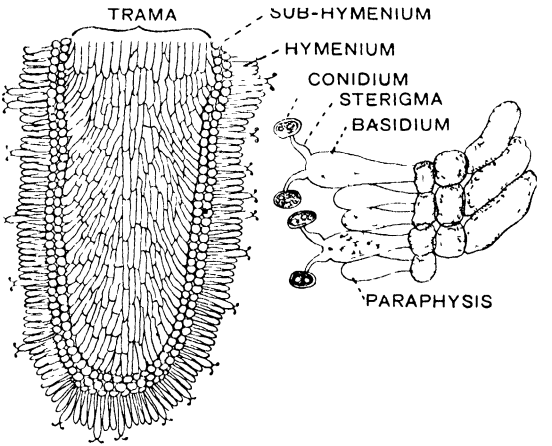


Fig 75 —AGARICUS. Section across one of the Gills The diagram to the right represents the Hymenium and Sub hymenium more highly magnified. (Four Conidia should have been shown on each Basidium)

of the **old basidia** from which the spores have fallen; (3) the **paraphyses,** narrower than the mature basidia; (4) the brown coloured **spores,** the basidia and paraphyses being colourless like the general tissue of the gill.

PUCCINIA GRAMINIS.

436. Uredospores on Wheat Plant.—Examine "rusted" plants of Wheat in summer, showing the reddish orange elongated spots on the leaves and stems With a lens note that these spots are cracks or slits from which an orange yellow powder is shed or can easily be scraped.

Scrape off some of the "rust," and note the numerous uredospores, each consisting of an ovoid cell with a thick outer coat (covered with fine spines when mature); the inner layer is thinner; the spore contents are coloured with drops of orange or yellow oily matter. Note the four pits or thin spots, situated at regular intervals round the equator of the spore—at each of these spots the endospore is interrupted so that the cell-contents are in contact with the exospore, the latter being also thinner at these spots than elsewhere.

Examine transverse sections of Wheat stem, leaf-sheath, or leaf, bearing patches of uredospores. Note (1) the patch corresponds to a region between two of the hard bundles below the epidermis; (2) the epidermis is broken through at each side of the patch; (3) the mycelium of the Fungus consists of slender threads traversing the soft parenchyma tissue and forming a denser layer just below the patch; (4) each uredospore is borne on a slender stalk forming an outgrowth of the mycelium

437. Teleutospores on Wheat (Fig. 76).—Later in the year, from July onwards, note that the "rust" patches

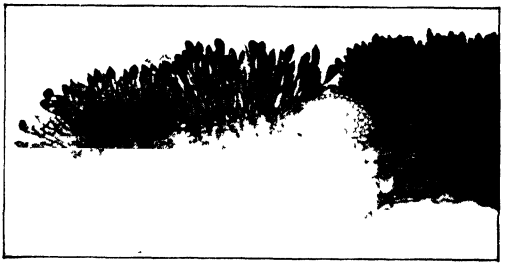


Fig. 76.—PUCCINIA. Part of a Transverse Section of Wheat Leaf, with Teleutospores of Puccinia.

become blackish instead of orange, especially on the stems and leaf-sheaths. This is due to the fact that the mycelium is now producing spores of another kind instead

of uredospores Scrape one of these dark patches, and note the dark brown spindle-shaped teleutospores, often showing at one end part of the slender stalk on which the spore was borne; the two cells have thick walls, showing two layers (exospore and endospore). Note the pit in the wall of each cell of the teleutospore—in the upper cell the pit is at the apex, in the lower it is at one side just below the cross-wall separating the two cells.

Also examine sections across the patch; both uredospores and teleutospores may be seen, since the two kinds of spores are produced by the same mycelium.

438. Germination of the Uredospores.—Place uredospores in a hanging drop of water or of Pasteur's solution, and examine each day until germination occurs. Note that a hypha may grow out from either or both of the pits or germ-pores. Also try to infect Wheat plants, as follows:—Grow Wheat in pots of soil out of doors, to get healthy young plants with leaves about 10 cm long; bring in fresh Wheat leaves well covered with the rusty patches, and tie two of the young Wheat leaves together with the infected leaf between them, so that the three are in close contact for some length; cover the plants with a bell-jar, keep them moist, and each day examine tangential sections of the epidermis for germinating uredospores, sending a germ-tube in through a stoma

439. Germination of the Teleutospores.—The uredospores usually germinate promptly in summer, though they can last through the winter; but the teleutospores are essentially resting spores which germinate in the following spring. They may be induced to germinate in autumn, but it is better to tie together in a bundle Wheat straw (stems) bearing teleutospore sori, leave the bundle outdoors all winter, and in spring (March or April) cut off small portions of stem with teleutospores on them and place these in water in a watch-glass, keeping them under a bell-glass and examining with the microscope daily until germination occurs.

Also scrape teleutospores from the patches into water or Pasteur's solution in moist-chamber slides. Note that the exospore of one or both cells of the teleutospore bursts, and the endospore-covered contents grow out as a hypha (**promycelium** or basidium) which divides by cross-walls into a row of four or five cells, each of these (except the long basal one) then putting out a short hypha which swells up at the tip and cuts off a single **sporidium** or basidiospore.

440. Infection of the Barberry.—The sporidia produced by the germination of the teleutospore do not germinate on the Wheat, but infect the leaves of Barberry.

(*a*) Cut twigs of Barberry in spring, when the buds are unfolding; remove some young leaves, place them on wet blotting-paper, put a drop of water on each leaf and add a few teleutospores (from a batch which have shown signs of germination, if possible); after a full day, cut tangential sections from a leaf, so as to obtain the epidermis, where the teleutospores were sown, and look for the sporidia, which may be seen putting out a hypha. This hypha is able to eat its way into the leaf (by secreting cytase and other enzymes), and therefore does not need to make use of a stoma in order to infect the leaf.

(*b*) Also try infecting in this way some young leaves on a Barberry twig placed in Knop's solution in a large jar

(*c*) In these plants, or in Barberry bushes growing under natural conditions, note that the leaves often show in spring swollen discoloured patches due to the growth of the mycelium produced by the entrance of the hypha (germ-tube) emitted by the germinating sporidium.

441. Aecidia on Barberry.—Examine a Barberry leaf showing these blotches. At some points on the underside of the leaf the cup-like aecidia may be seen with naked eye or lens, while at other places the yellowish blotches will be seen as swellings—young aecidia which have not yet burst through the epidermis of the leaf. An open aecidium has the form of a cup with a ragged and outwardly curved margin; its yellow contents are the aecidiospores. On the upper side of the leaf look for much smaller projections, appearing as minute pointed warts—these are the spermogonia, better seen in section.

442. T. S. of Barberry Leaf, with Aecidia and Spermogonia (Fig. 77).—Cut transverse sections of Barberry leaf showing groups of aecidia; mount in glycerine, and note (1) the **mycelium** of the Puccinia, the hyphae of which ramify through the intercellular spaces and, especially in the spongy lower mesophyll, are so closely packed that the mesophyll cells may be widely separated from each other and appear embedded in a dense matrix of mycelium; (2) an **aecidium**, cut through the middle and therefore appearing U-shaped, containing

closely packed parallel chains of **aecidiospores**; (3) the **wall** of the aecidium.

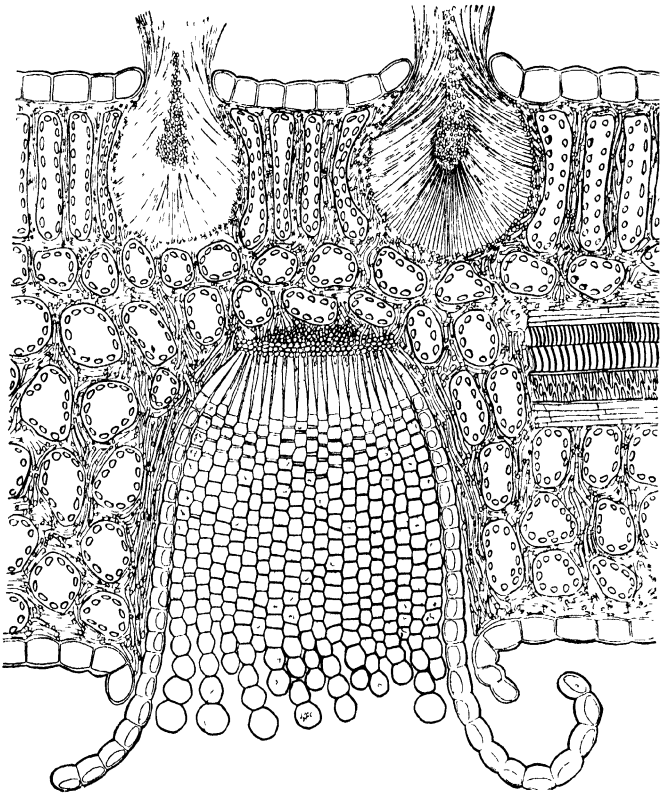


Fig. 77.—PUCCINIA. Part of a Transverse Section of Barberry Leaf infected by the Aecidium Stage of Puccinia. Note the Mycelium (in the Mesophyle), the two Spermogonia (above), and the Aecidium (below).

Note also the **spermogonia,** chiefly on the upper side of the leaf; each spermogonium resembles a miniature Fucus conceptacle, and contains a dense mass of fine filaments.

443. Structure of Aecidium.—Examine carefully with high power a **young aecidium,** which has not yet burst through the epidermis, and compare its structure with that of the **mature aecidium.**

In the mature aecidium note (1) the dense hyphae at the base of the aecidium; (2) the layer of closely packed parallel **rod-like hyphae** above this; (3) the row of **aecidiospores** produced in basipetal sequence by abstriction from each of these rod-like hyphae; (4) in each row the orange-coloured thick-walled spores, of polygonal (hexagonal in section) form owing to the close packing of the rows; (5) the presence of small much flattened **interstitial cells** alternating with the spores in each row; (6) the outer **wall** of the aecidium, consisting of a layer of cells with very thick cell-walls (the outer wall especially thick and striated)—this layer evidently corresponds to sterilised rows of aecidiospores.

Mount in water some loose aecidiospores, and note the (usually six) thin places or **pits** ("germ-pores") in the cell-wall

444. Structure of Spermogonium.—In a **spermogonium,** with the high power, note (1) in the lower portion the closely packed parallel rod-like hyphae or sterigmata, converging to the centre of the flask-like cavity; (2) the upper hyphae or paraphyses projecting in a tuft from the raised apical pore; (3) the numerous small ovoid cells or spermatia abstricted from the sterigmata

445. Culture of Aecidiospores and Spermatia.—Remove some aecidiospores from a ripe aecidium, place them in a drop of water on a Wheat seedling, keep moist by placing it on wet blotting-paper under a bell-glass. After two or three days, cut tangential sections of the epidermis, and look for germinating spores, putting out a hypha which enters the leaf through a stoma. If this is not seen in a few days, try another lot, since germination normally occurs within two or three days

Tease spermatia from a spermogonium, and grow them in a hanging drop of Pasteur's solution or a weak sugar solution; they may germinate, put out hyphae, and buds like Yeast cells, but they do not continue to grow for long, nor are they capable of infecting either host (Barberry or Wheat).

446. Aecidia, etc., of other Uredineae.—The aecidium stage on the Barberry is not essential for the vigorous development of Wheat Rust, and is in fact very rarely found. Where Barberry bushes are rare or absent in a district, use the aecidia of other Uredineae. These may be found, especially in spring and early summer, on Buttercups, Lesser Celandine, Violet, Coltsfoot, Stinging Nettle, Docks, etc

Also examine the uredospores and teleutospores of the Rusts which attack such plants as Mallow, Hollyhock, Chrysanthemum, etc.

The teleutospores of the Rust (Puccinia arenariae) found on Chickweed and other Caryophyllaceae, *e g.* Sweet William, germinate as soon as ripe, hence they serve admirably for the study of the stages which may not be readily observed in Wheat Rust.

Xanthoria Parietina.

447. General Characters.—This Lichen (which was formerly placed in the genera Parmelia and Physcia) is very common on roofs, old walls, trees, etc, often forming large brilliant orange patches.

Examine a patch, and note (1) that the **thallus** is leaf-like or foliaceous, smooth, bright yellow above but pale and whitish below; (2) the irregular branching lobes at the margin of the thallus, which tends to assume a more or less circular outline; (3) that the margins are free and can be raised from the substratum by means of a knife, without damaging any tissue, but elsewhere it is firmly attached by (4) whitish processes called **rhizines**; (5) on the upper side, the small cup-like organs or **apothecia,** each apothecium being about 3 or 4 mm. in diameter and bright orange in colour; (6) that the thallus is brittle when dry, but when moist or after being soaked in water it becomes soft though leathery in texture.

448. T. S. of Thallus (Fig. 78).—Cut transverse sections across a part not bearing apothecia; place the sections in water and note that they swell. Mount some sections in glycerine and note

(1) The upper yellow limiting or **epidermoid layer,** not sharply marked off from

(2) The upper **cortex** of densely aggregated hyphae, forming a pseudo-parenchyma tissue of cells with thick swollen walls and scanty contents

(3) The colour in the outer layers is due to crystalline **yellow granules** deposited between the hyphae and also on the free upper surface of the thallus.

(4) The broad **medullary zone** of loosely interwoven hyphae.

(5) The large green **Alga cells** ("gonidia"), either iso-

lated or in packets, having distinct cell-walls and green contents (chlorophyll). The Algae are confined to a layer

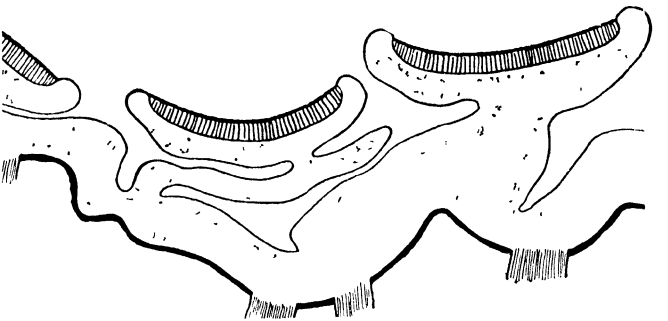


Fig. 78.—XANTHORIA Part of a Transverse Section, showing general structure of Thallus The position of the nests of Alga cells is shown by dots, note the three Apothecia (above) and the Rhizines (below)

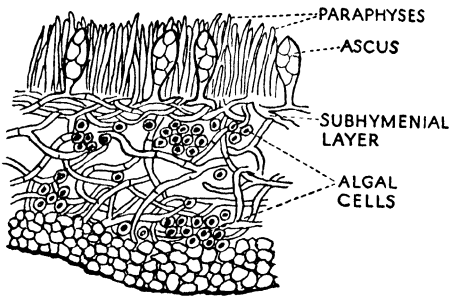


Fig. 78a T S *Lichen apothecium*

below the compact upper cortex, sometimes called the "gonidial layer," though the term "gonidia" is unnecessary as well as misleading, and the Alga in this case is

called Cystococcus humicola, probably allied to Pleurococcus. Note also

(6) The still looser lower portion of the medulla, passing downwards into

(7) The lower cortical zone, resembling the upper in structure but colourless

(8) The **rhizines** or rhizoids—strands of hyphae arising from the lower cortex and fixing the thallus to the substratum.

449. Treat sections with potash, or apply potash to the upper side of the thallus in one spot and to the lower side in another, and note that the tissues become reddish or purplish. Also place some pieces of thallus in a test-tube, add potash and warm; the thallus changes from yellow to red or purple, this colour passing into the potash solution. Neutralise by adding acetic acid, the colour disappears, but may reappear on again adding potash. The pigment of our type resembles litmus, which is obtained from various Lichens.

450. Vertical Section of Apothecium (Fig. 78).—Cut transverse sections of the thallus, passing through some apothecia; mount in glycerine.

Note (1) the general structure of the thallus, as already described; (2) the shallow cup-like form of the mature **apothecium,** the central portion of the upper surface being only slightly concave or plane or even slightly convex, (3) the raised **rim** of thallus tissue around the margin of the apothecium; (4) the continuation of the **Alga layer** into the marginal rim and also below (5) the **hymenium** or hymenial layer consisting of closely packed vertical parallel outgrowths of two kinds—viz. (6) the clear **paraphyses** or sterile hyphae with thickened yellowish ends, and (7) the shorter and thicker club-shaped **asci;** each ascus contains when mature eight ovoid **spores,** some of which may be seen lying free on the surface of the hymenium; (8) the **sub-hymenial layer,** just below the paraphyses and asci, consisting of densely packed hyphal tissue and passing below into the looser tissue containing the groups of Alga cells in its meshes (see § 448)

Treat some sections with (*a*) iodine, (*b*) chlor-zinc-iodine, (*c*) warm water, note the results in each case

451. Examine some **ascospores** with the high power, and note that each spore is two-celled, with a peculiar structure—the two rounded cells are at the two poles of the spore, and they are connected by a protoplasmic strand, the wall of the spore being of great thickness except at the two ends.

452. Spermogonium and Spermatia.—In some of the sections the spermogonia (very similar to those of Puccinia) may be seen; but our type does not produce spermogonia freely, and these organs are better seen in the so-called "Iceland Moss" (Cetraria), which can be bought dried from a druggist, or in the "Reindeer Moss" (Cladina), which grows commonly on heaths. In Cetraria, cut sections passing through the little marginal teeth of the thallus, in Cladina, cut sections of the drooping tips of the erect much-branched thallus. Note that the spermogonia are flask-like cavities containing numerous converging fungal hyphae from which are abstracted the small unicellular spermatia.

453. Soredia.—Our type is usually fertile, with abundant apothecia, but some specimens may be found with few or no apothecia, and these are likely to bear **soredia.** Remove these by scraping the upper surface of the thallus on to a drop of water; or moisten the thallus and press it on a slide The soredia are rounded bodies, each soredium consisting of Fungal hyphae enclosing a few Alga-cells. Soredia can be obtained in great numbers on various species of Cladonia—the so-called "Trumpet mosses" which are very common; the whole "trumpet" or stalked cup-like structure (podetium) is often covered with a greyish-green powder consisting of soredia, while the apothecia form brown or in one species ("red cup-moss" or "matches") scarlet outgrowths on the margin of the cup.

Collema Pulposum

454. General Characters.—The family to which Collema belongs is distinguished from the majority of other Lichens in that (1) the thallus is extremely gelatinous; (2) the form of the thallus is determined by the Alga, not by the Fungus; (3) the thallus is homoiomerous, *i.e.* the Alga and Fungus are distributed uniformly through

the thallus, the Alga not being restricted to a definite zone as in the heteromerous Lichens, (4) the thallus is much folded, owing to inequalities in growth and to the interrelation between the Fungus and the Alga; (5) the thallus contains Nostoc as the Alga constituent, and therefore has a bluish tint, varying from greyish blue to almost black; (6) soredia are rarely produced—owing doubtless to the difficulty of enclosing the Algal jelly by the hyphae

455. Structure of Nostoc.—In order to understand the structure of Collema, that of Nostoc should be examined. The species of Nostoc grow in rounded, or flattened and often lobed, olive-green gelatinous masses, either on wet ground, among mosses, on stones in streams, or floating in the water of streams and ditches. The plant is soft and gelatinous when wet, brittle when dry. Mount a small specimen of Nostoc, or a section of a large one, in water, and note the irregularly contorted filaments which are embedded in the gelatinous **matrix**; in each filament note (1) the single row of rounded cells with **bluish-green** contents, interrupted at intervals by (2) larger cells, the **heterocysts,** with thicker walls and transparent colourless contents.

456. Structure of Collema.—Now examine some species of Collema, of which C pulposum is one of the commonest, growing on moist soil, stones, old walls, and among Mosses; another species grows on tree trunks in damp woods, with a thin dark-coloured thallus. Like Nostoc itself, Collema is thin and brittle when dry, soft and pulpy and gelatinous when wet. If fertile, it is easily distinguished from Nostoc by the apothecia.

Cut sections, and note (1) the chains of blue-green Nostoc cells, with heterocysts at intervals; (2) the transparent gelatinous matrix; (3) the branching colourless septate narrow Fungus hyphae; (4) that though the thallus is more nearly "homoiomerous" than in any other Lichens, the Nostoc chains are more abundant towards the upper surface; (5) there is no definite cortex on the surface —a cortex is present, however, in other Collemaceae, which

otherwise resemble Collema; (6) the plant is loosely attached to the substratum by rhizines consisting chiefly of single rows of cells. The **apothecium** has the same general structure as in Physcia, but the spores show several cross-walls and also usually longitudinal divisions, and have thin walls.

457. Apothecium of Discomycetes.—Since the Fungus in the great majority of Lichens is of the Ascomycetous type, it is advisable to study some Ascomycete, like Peziza or Ascobolus, with special reference to the structure of the ascocarp.

In Eurotium and Sphaerotheca, which have already been studied, the ascocarp is a closed case or cleistothecium, but in many other Ascomycetes it is either a cup-like apothecium (Discomycetes), or a flask-like perithecium with a pore (Pyrenomycetes). In a fair number of Ascomycetes, a process of fertilisation has been found to precede the formation of the ascocarp, and in some cases male cells (**spermatia**) are produced in **spermogonia**, like those of Puccinia in form, while the **ascogonium** has a filamentous outgrowth (**trichogyne**) which receives the male cell.

In many Ascomycetes the ascocarp is developed without a fertilisation process; either an oogonium is formed which produces the ascogenous hyphae without being fertilised, or there may be no trace of an oogonium at all.

Exactly the same applies to Lichens In a few cases, an oogonium —consisting of a coiled lower portion embedded in the thallus, and a straight upper portion (trichogyne) which protrudes from the surface—is fertilised by a spermatium, which is carried by rain-water to the trichogyne and adheres to it. This has been seen, for instance, in species of Collema and Physcia. In other cases there are oogonia with projecting trichogyne, but fertilisation has not been observed; in others there are oogonia without a trichogyne —spermatia may or may not be produced, but no fertilisation occurs, the ascocarp arising directly from the unfertilised oogonium in others; again, no oogonium has been found, and the ascogenous hyphae apparently arise from ordinary vegetative hyphae.

In any case, the ascocarp of Lichens resembles that of the higher Ascomycetes, the ascogenous hyphae ending in a palisade-like layer of asci, between which there grow up sterile hyphae (paraphyses), the two together forming the hymenium. The paraphyses are usually gelatinous, and serve to keep the asci moist, besides assisting in the dispersal of the ascopores. The spores, usually eight in each ascus (but sometimes six, four, two, or one), are

typically unicellular, but often become divided up so as to be multicellular when ripe (this also occurs in many Ascomycetes). In the apothecia of both Ascomycetes and Lichens, the spores are often forcibly thrown out to a distance, by the pressure due to the swelling of the asci and paraphases, acting against the firmer rim of the apothecium.

Cut vertical sections of the apothecium of Ascobolus or Peziza. The former occurs on horse or cow dung kept for a few weeks under a bell-glass; various species of Peziza (often red or orange-coloured) occur on rotten twigs or dead wood, P. stercorea on cow dung. Note (1) the densely interwoven hyphae, forming on the lower side a compact tissue from which attaching and absorbing hyphae run into the substratum; (2) the **hymenium,** consisting of long narrow **paraphyses** and thicker **asci,** each ascus with eight spores; (3) the compact **sub-hymenial layer** below the hymenium

CHAPTER X.

PELLIA AND FUNARIA.

Pellia.

458. General Characters.—Of the three British species, Pellia epiphylla—the commonest and most widely distributed—is easily distinguished: it is monoecious (the antheridia and archegonia are borne on the same plant), while the other two species are dioecious. Other differences between the three species are given in § 469.

Pellia grows in spreading patches in moist places, especially by the sides of streams. Examine the plants at different times of year, remove patches, together with some of the soil, and cultivate them in dishes indoors, keeping them moist and partially covering them with glass sheets

In making a seasonal study of Pellia, note the following points: late summer and autumn—developing sporogonia; winter—ripening and ripe sporogonia, resting thallus-branches at apex; spring—elongation of seta, dehiscence of capsule, dispersal of spores, branching of thallus, development of sexual organs; early summer—mature sexual organs, fertilisation, early development of sporogonium. At each stage preserve specimens in alcohol or formalin, for microscopical examination, in jars labelled with date of collection.

Isolate as much as possible of a single thallus from the overlapping and matted branches that make up a patch of Pellia, rinse in water to remove the soil from the lower surface, and note

(*a*) The smooth upper surface, wavy margin, and mode of branching of the flat green **thallus.**

(*b*) The median thickened portion or **midrib,** passing on either side into the thin lateral portions or **wings.**

(*c*) At the anterior end of each branch the **notch** in which lies the apical growing-point.

(*d*) The unbranched **rhizoids** springing from the projecting underside of the midrib

In fertile plants, note, according to the time of year,

(*e*) The **antheridial cavities,** wart-like projections scattered over the upper side of the midrib, each with a small **pore** at its apex—slit the cavity open, to see the small spherical **antheridium** which it contains.

(*f*) The **archegonial cavity** near the anterior end of a branch, forming a pocket open in front and extending backwards into the tissue of the midrib, the opening of the pocket protected by

(*g*) A flap (**involucre**) of thallus-tissue projecting over it from behind—slit the cavity open, to see the group of hair-like archegonia springing from its closed posterior end.

(*h*) The **sporogonium,** which may be in one of the following stages—(1) in process of development, enclosed in the calyptra, (2) ripe and showing the dark-coloured spherical capsule lying just within or slightly projecting from the opening of the cavity, with the seta still very short, (3) the ripe capsule carried up by elongation of the seta to a height of as much as 8 or 10 cm., (4) the dehiscence of the capsule by splitting of its wall into four valves which open outwards and downwards, exposing the mop-like tuft of elaters and spores, (5) collapse of the seta shortly after the dispersal of the spores, and the persistence for some time of the four capsule-valves and the tuft (elaterophore) of fixed elaters

459. Structure of Thallus.—(*a*) Cut **transverse sections** of the thallus at different places. Some of the sections should include antheridial cavities (§ 460). Note (1) the form of the section, with the **midrib** projecting below and thinning out to a single layer of cells at the

margin of the **wings**; (2) the almost uniform structure of the tissues, except that the lower cells of the midrib are devoid of the **chloroplasts** which are present most abundantly in the wings and the upper layers of the midrib; (3) the **thickened bands** on the walls of the inner cells of the midrib—seen better in longitudinal

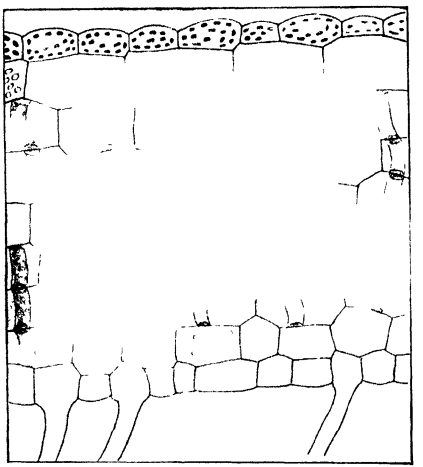


Fig. 79.—PELLIA. Part of Longitudinal Section of Thallus, showing the band-like thickenings on the walls of the inner cells

sections; (4) the **rhizoids**, each an unseptate outgrowth of one of the cells of the lowest layer of the midrib.

(*b*) In **longitudinal sections** through the midrib—some of the sections should traverse an archegonial cavity (§ 460)—note (1) the tissues as seen in T. S.; (2) the vertical yellow or brown **thickenings** on the internal cells, the bands running vertically in the tissue (Fig. 79). In both transverse and longitudinal sections, note that the cells of the internal tissue contain starch grains, and that the rhizoids are unicellular though their free ends may be branched slightly.

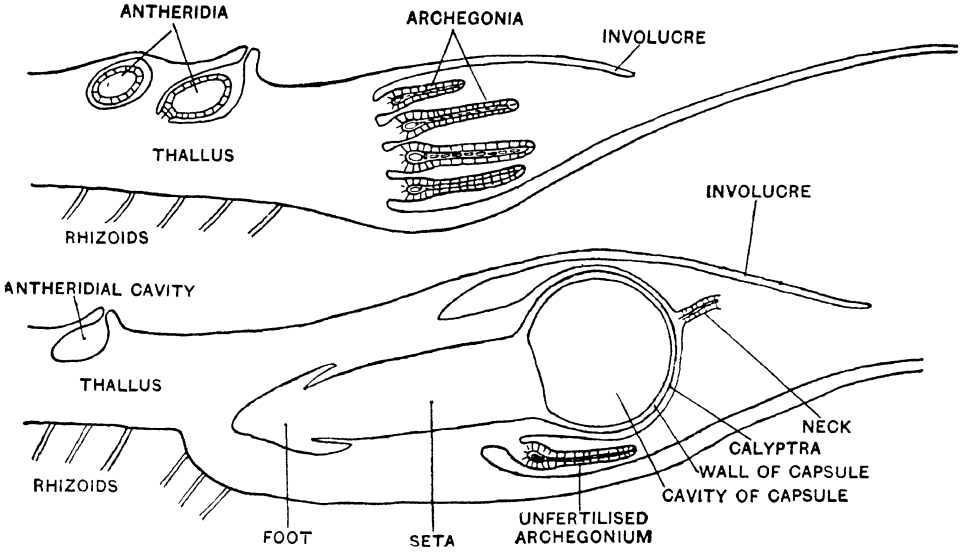


Fig 80 —PELLIA Longitudinal Sections of Thallus with Sexual Organs (upper figure) and with Ripe Sporogonium (lower figure)

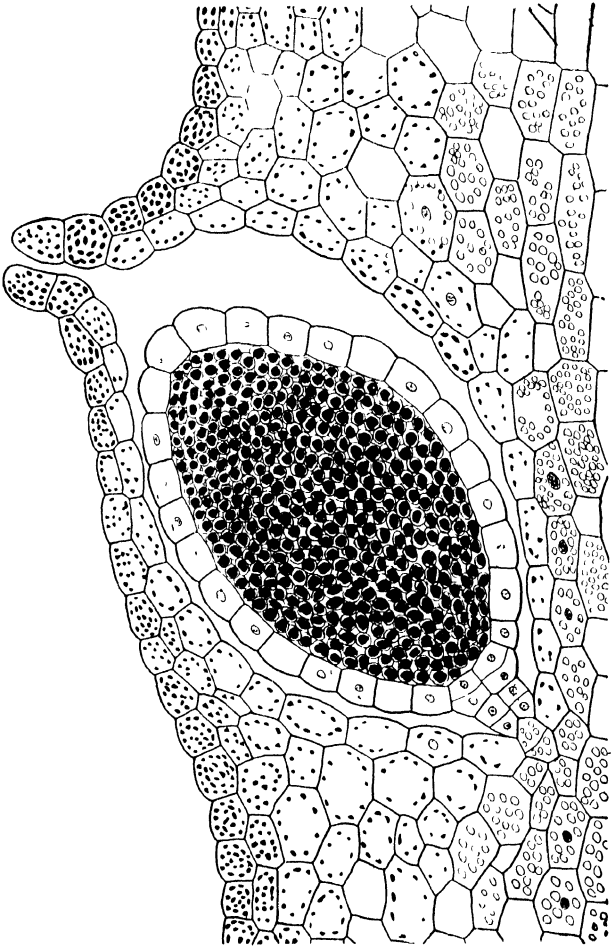


Fig 81.—Pellia. Part of a Longitudinal Section, showing an antheridial cavity with a nearly ripe Antheridium

460. Antheridium and Archegonium (Figs 80, 81).—In sections (transverse and longitudinal) passing through an **antheridial cavity,** note that the **antheridium** is nearly spherical, with a very short **stalk,** and is seated in a **flask-like depression** in the upper tissue of the thallus, which has grown up over the antheridium but has left a narrow **pore** at the top; the antheridium has a single-layered **wall,** and (unless dehiscence has already occurred) contains very numerous small **sperm-cells** which produce the antherozoids.

In longitudinal sections passing through an **archegonial cavity,** note the **archegonia,** which spring at right angles from the convex vertical hinder end of the cavity and are therefore horizontal in position. In a well-grown archegonium, note (1) the short thick **stalk;** (2) the dilated **venter,** containing the spherical **oosphere** and the **ventral canal-cell** above it; (3) the long **neck,** consisting of a single layer of cells and an axial row of **neck-canal-cells.** Between the archegonia note the short mucilage-hairs or **paraphyses,** usually two-celled

461. L. S. of Sporogonium in situ (Figs. 80, 82).—(*a*) In longitudinal sections through a plant with a **nearly ripe sporogonium,** note (1) the tissues of the **thallus;** (2) the **unfertilised archegonia** and the **paraphyses,** at the posterior end of the cavity and around the base of (3) the **calyptra** or enlarged venter of the fertilised archegonium, which is several cells thick and bears the **withered neck** near its apex; (4) the **sporogonium,** covered anteriorly by the calyptra and projecting posteriorly into the thallus tissue.

(*b*) In the **sporogonium** itself, note (1) the **seta,** consisting of regular longitudinal rows of very short cells filled with small starch-grains—test with iodine; (2) the **foot** or haustorium, conical in form and having its edge produced around the base of the seta like a collar—thus increasing the surface for absorption from the thallus-tissue; (3) the **capsule,** nearly spherical.

(*c*) In the **capsule** note (1) the **outer wall layer,**

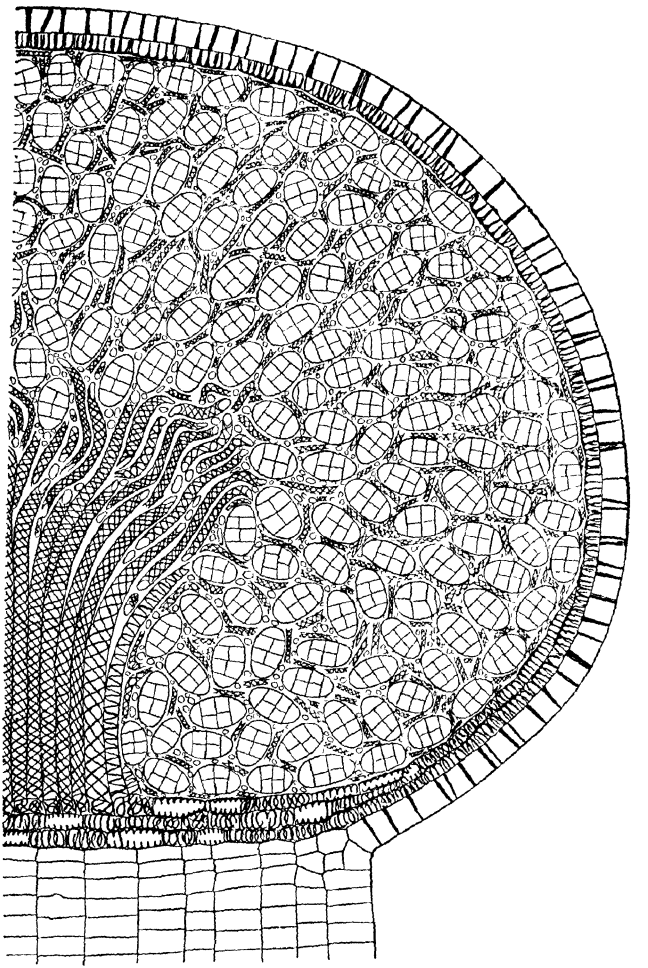


Fig. 82.—PELLIA. Part of a Longitudinal Section through ripe Sporogonium, showing half of the Capsule and the uppermost portion of the Seta.

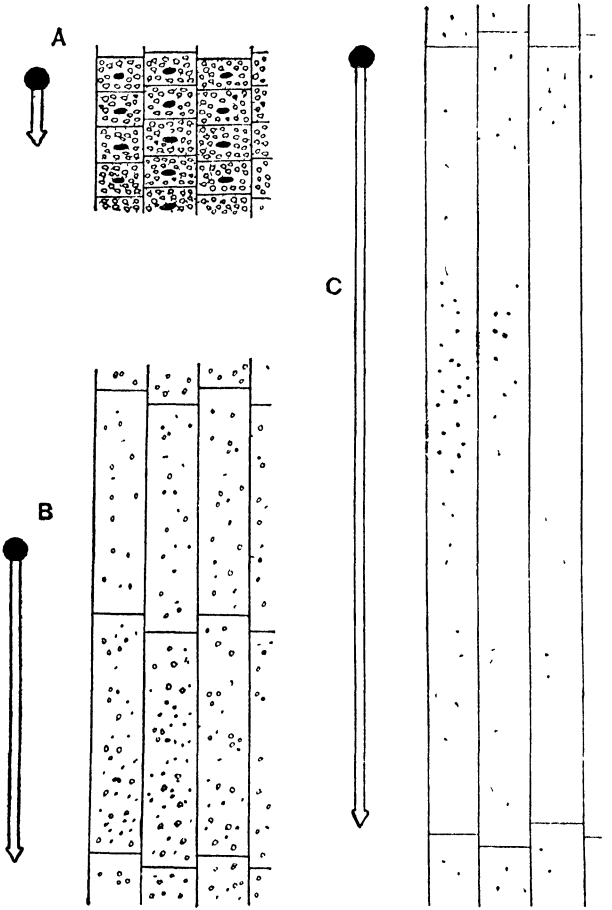


Fig 88.—PELLIA. Elongation of Seta due to growth in length of its cells. A, resting stage, B C, stages in elongation. In A, B, and C the entire Sporogonium is shown diagrammatically on the left, and part of the Seta on the right, the magnification being uniform in the three drawings.

consisting of large cells nearly square in section, with radial thickening bands; (2) the **inner wall layer,** the cells flattened and bearing numerous semi-annular thickening bands; (3) the **elaterophore**—a tuft of spirally thickened filaments (**fixed elaters**) springing from the top of the seta and radiating into the cavity of the capsule, which contains (4) the **free elaters** mingled with (5) the **spores.**

462. Structure of Sporogonium (Figs 82-85).—Mount in water and examine (1) an entire capsule removed from its pocket and either ruptured by being pressed between two slides, or teased open with needles; (2) a ripe capsule which has been carried up by the elongation of the seta and has dehisced; (3) a capsule valve with its outer surface uppermost In these preparations note

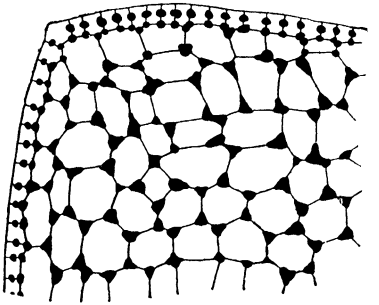


Fig 84.—PELLIA The tip of a Capsule Valve, seen from the outer side, showing the rod-like thickenings on the outer layer of cells

(*a*) The enormous **elongation** undergone by the cells of the **seta,** which in a few days will grow to about 40 times its original length, the cells losing their starch grains in the process

(*b*) The **elaterophore,** a bundle of stout elaters fixed by their lower ends to the top of the seta and consisting of single elongated cells containing from one to three spiral fibres.

(*c*) The **free elaters,** with spiral fibres like the fixed elaters but with both ends free and pointed—these free elaters are twisted irregularly and are mingled with

(*d*) The **spores,** which (except in a quite young capsule) are no longer unicellular but have germinated to form

ovoid cell-masses—these usually have a single cell at each end, while the middle part is divided by longitudinal as well as transverse walls, the cells containing chloroplasts.

(*e*) The **outer capsule-wall layer,** consisting in surface view of polygonal cells with brown rod-like

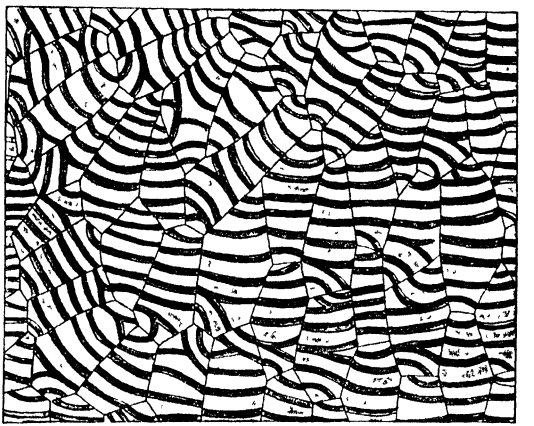


Fig. 85.—PELLIA A portion of the Capsule Wall, seen from within, showing the fibrous bands on the inner layer of cells.

thickenings on the vertical walls, chiefly at the corners between adjacent cells.

(*f*) The **inner capsule-wall layer,** consisting of polygonal cells with numerous half-ring thickenings.

463 Dehiscence Lines in Capsule-wall (Fig. 86).—With a razor cut off the upper half of a nearly ripe capsule, and transfer it to a drop of water on a slide, with the concave side upwards; with a brush remove the spores and elaters, then turn the piece over, flatten it under a cover-glass, and examine the convex (outer) surface.

Note the four **dehiscence lines,** marking out the valves by which the wall would have split at dehiscence of

the capsule; each line is bordered by somewhat narrow clear cells, and along the line itself the cell-walls have no thickenings At the apex of the capsule, the four lines do not all meet in a point, but are in pairs, each pair meeting

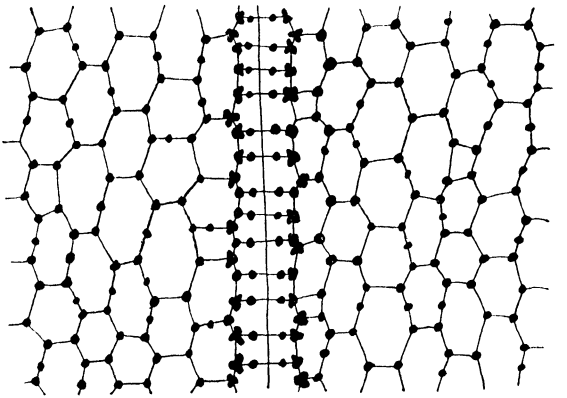
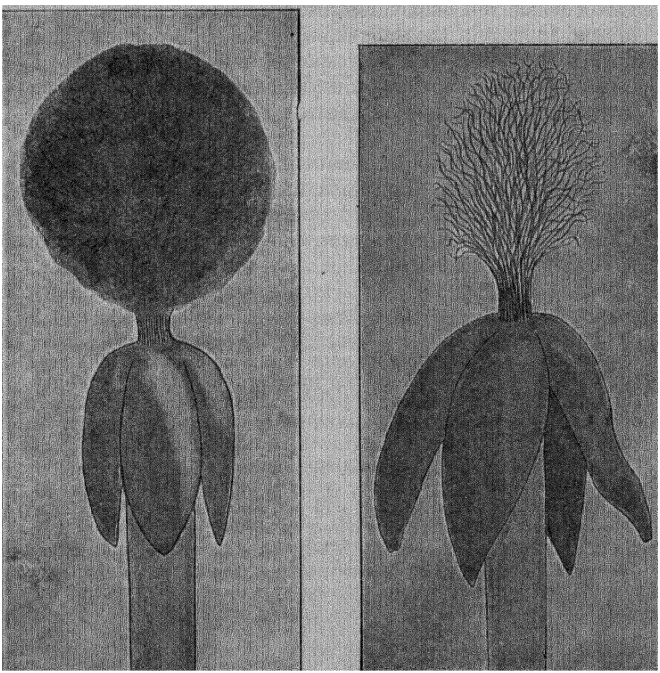


Fig 86.—Pellia Part of the Capsule Wall, seen from inner side, showing a portion of one of the Dehiscence Lines

at one end of a line over the apex; hence two of the valves are longer and have truncated tips, while the alternate two are slightly shorter and have pointed tips—but there is some variation in the course of these dehiscence lines and therefore in the form and size of the four valves.

464. Further Studies on the Sporogonium.—The development of the sporogonium may be traced by (1) cutting longitudinal sections passing through cavities with fertilised archegonia; (2) dissecting out the entire sporogonium when it is large enough; (3) teasing out the capsule in water and examining the capsule-wall, spores, and elaters. The archegonia are fertilised in early summer; the development of the sporogonium proceeds during summer and autumn, and by late autumn all the parts are differentiated.

About July the division of the **spore mother-cells** may be seen—each mother-cell becomes deeply four-lobed when the nucleus divides, one of the four daughter nuclei passing into each lobe, which is then separated off to form



Figs 87, 88.—PELLIA Two stages in the Dehiscence of the Capsule and the Dispersal of the Spores.

a spore; by November the spores have separated from each other, and at once begin to germinate, while the elaters and capsule-wall soon show their characteristic thickenings, laid down at first as cellulose bands at first colourless but later turning brown.

During winter the capsule, covered by the calyptra, is seen projecting from the mouth of the cavity; in spring (March or April) the seta—hitherto only about 3 mm. long—suddenly lengthens, breaking through the calyptra and carrying up the capsule; the wall splits into four valves which roll back through 180° and hang downwards around the top of the seta, exposing the elaterophore which holds together the mass of spores and free elaters for a time; this mass expands on drying, the elaters performing hygroscopic wriggling movements and thus loosening the spores (see Figs. 87, 88)

The spores readily germinate if sown on moist tiles, or in Knop culture solution in a moist chamber; as a rule, the first rhizoid grows from one end of the ovoid mass, while the other end produces the growing-point of the young thallus

465. Other Species of Pellia.—Pellia calycina, which occurs chiefly in chalky soils, often along with P. epiphylla, is dioecious; the thallus is usually concave, with raised margins, and is green, the midrib not dark coloured; the internal cells have no band-like thickenings; the antheridia are fewer and more sparingly scattered over the male thallus; the mouth of the archegonial cavity is surrounded by a tubular outgrowth of thallus tissue, which is longer than (and therefore completely encloses) the calyptra; the capsule has no ring-fibres in the cells; the spores are smaller, and the free elaters shorter, thicker, and not so contorted; the elaterophore consists of about 100 very long slender threads

P. Neesiana, which occurs chiefly beside mountain streams, resembles P epiphylla in the structure of the thallus and of the capsule, but is dioecious, and the outgrowth at the mouth of the archegonial cavity is a short collar instead of a mere scale.

FUNARIA.

466. General Characters.—Funaria hygrometrica may be found at almost any time of year in patches, often extensive and yellowish green in colour, on waste ground, soil that has been burnt, old cinder heaps and paths, etc., the plants being sometimes so closely matted together as to be difficult to separate without damage to the lower parts. Cut out portions of the patches, with the soil, and

cultivate them in saucers or shallow pots, to obtain various stages in development.

Wash part of a patch in water, to remove the soil, and carefully separate the plants Note that each plant is erect and about 1 to 3 cm. high (apart from the sporogonium, which may be 4 to 7 cm. long).

From its lower end the stem gives off numerous branching brown **rhizoids,** the rhizoid systems of the individual plants being usually densely interwoven The **leaves** are spirally arranged on the stem, the lower ones smaller and scattered, the upper larger and more crowded at the top of the stem

The plants are **monoecious,** the main axis ending in a " male flower," consisting of a group of **antheridia** surrounded by a rosette of spreading leaves, while a branch arising from near the base, but eventually growing higher than the " male flower," bears the **archegonia** and later the **sporogonium.** The patch usually shows female axes with sporogonia of different ages; the ripe sporogonium consists of an obliquely pear-shaped **capsule** borne on a slender curved and twisted **seta** and, until nearly ripe, capped by a membranous conical **calyptra.**

467. Rhizoids.—Mount in water an entire Funaria plant, and with a brush or a needle carefully spread or tease out the felted mass of rhizoids. Note that (1) each rhizoid springs from a superficial cell on the lower part of the stem; (2) the rhizoid consists of a branching row of cells; (3) the transverse walls are oblique—inclined in various planes—and curved; (4) each branch arises immediately behind one of the transverse walls; (5) the red or brown colour of the rhizoids is due to the walls, not to the contents.

468. Surface View of Leaf of Funaria.—Mount in water a few leaves, detached from the stem—lay a female plant in water on a slide and scrape off some of the uppermost leaves with a needle.

Note that the leaves are sessile, with oval or oblong outline, almost entire margin, concave upper surface,

pointed tip; the leaf consists of a central cylindrical **midrib** and a single-layered **wing** on either side; the cells of the midrib are long and narrow, those of the wings polygonal.

Examine carefully the **chloroplasts** in the cells of the wing—look for stages in the multiplication of the chloroplasts by median constriction and **division.** Treat a leaf with iodine, and note the small **starch-grains** inside the chloroplasts.

469. Sections of Stem and Leaf of Funaria.—Hold several stems in pith, and cut transverse and longitudinal sections. Note in the **stem** (1) the peripheral tissue with brown cell-walls; (2) the **central strand** of long narrow colourless cells with thin walls. In T. S. of **leaf,** note the single-layered wing on either side of the midrib; in the midrib (1) a sheath of green cells on the surface above and below, (2) an inner sheath of narrower cells with thicker walls, (3) a **central strand** of narrow thin-walled cells In favourable longitudinal sections of stem, note that the "leaf-races" pass downwards in the outer tissue but do not directly join the central strand of the stem.

470. Male "Flower"; Antheridia (Fig 89).—Cut off a male shoot, including the star-like male "flower," and tease this out in water so as to isolate the antheridia; cut longitudinal sections of another flower, held in pith.

Note (1) the leaves forming a spreading cup around the central portion of the "flower," which consists of (2) **antheridia,** oblong sacs inserted on a short stalk, the sac having an outer layer of flattened cells containing chloroplasts which later turn red or brown, and a dense central mass of sperm-cells; (3) the **paraphyses,** mixed with the antheridia and consisting of a single row of cells —the uppermost cells greatly enlarged—and containing abundant chloroplasts

Antheridia of different ages may be found. Look for the large clear **cap-cell** which is thrown off when the ripe antheridium dehisces. Some of the antheridia may be empty, having opened to let the **antherozoids** escape by the pore thus formed at the apex In examining a ripe

antheridium, the sperm-cells (antherozoid mother-cells) may be seen to escape in a mass from the burst apex, and the spirally coiled biciliate antherozoids may be seen swimming about on being set free.

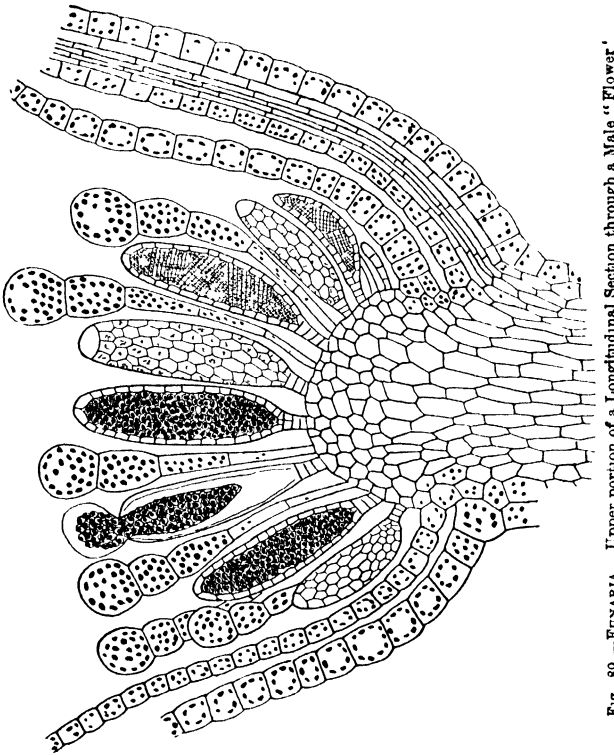


Fig 89.—Funaria. Upper portion of a Longitudinal Section through a Male "Flower" showing Stem, Leaves, Antheridia, and Paraphyses

In L. S. of the "flower" note the convex expanded apex of the stem, on which stand the closely packed vertical antheridia and the paraphyses

471. Male Flowers of Mnium.—Instead of, or in addition to, the male shoots of Funaria, examine those of Mnium hornum, a very common Moss found in shady moist places in woods, in dark-green patches, often about 5 cm high, with star-like male flowers which are very conspicuous in spring and early summer. In Mnium, the paraphyses have no swollen cells at the top, and the dehiscence cap of the antheridium consists of a group of cells.

472. Archegonia.—Examine some of the plants on which no sporogonia can be seen, and cut off the bud-like tips of the shoots, which are likely to show archegonia, tease up some of these in water, make longitudinal sections of others, and look for the **archegonia,** which are seated on the apex of the stem and are enclosed by the uppermost leaves, and accompanied by **paraphyses.**

In an archegonium note the relatively long and thick **stalk,** the slightly enlarged **venter** consisting of two layers of cells, and the long **neck;** the structure is essentially the same as in Pellia If the organ is not too old, the central series of cells may be seen—the **oosphere** and **ventral canal-cell** in the venter, the **neck canal-cells** in the long neck, if it is ripe and has opened, the canal-cells will have disappeared, leaving only the oosphere (or the embryo sporophyte developed from the fertilised oosphere) in the venter.

473. Stages in Development of Sporogonium.—Examine with the low power a series of female shoots showing different stages in the growth of the sporogonium The withered archegonium neck can be seen at the top of the **calyptra** (enlarged venter of fertilised archegonium), which is swollen at the base. Treat with potash, or cut longitudinal sections, to see the **young sporogonium** as an elongated rod with pointed upper and lower ends, the upper part closely invested by the narrow upper portion of the calyptra, while towards the base it is separated from the calyptra by a liquid-containing space (seen externally as a swelling), and its pointed lower end is plunged in the tissue of the stem.

Lay in water on a slide a plant with a young sporogonium, and with the fingers or a needle carefully remove

the calyptra from the upper end of the young rod-like sporogonium, then pull the lower end of the latter out of the stem tissue. If the upper end of the sporogonium breaks off and is left behind in the narrow upper portion of the calyptra, try again until you get the sporogonium isolated without damage. Note that the upper portion is green, while the sharply pointed lower end is colourless or reddish. Examine the upper end with the high power; in surface view the cells are seen to be arranged in regular transverse rows; focus carefully, and note the well-defined **apical cell,** from which segments are cut off by walls parallel to its two sloping sides, and the very regular arrangement of the tissues for some distance below the apical cell.

Remove the calyptra from successively older sporogonia, and note that at a relatively late stage the sporogonium shows differentiation into capsule and seta, in the form of a thickened zone between the two—this thickening forms the apophysis, at first thicker than the capsule itself Before this, however, the enlarged lower portion of the calyptra ceases to keep pace with the elongating sporogonium, and is ruptured by a circular rent near the base, the upper part being carried up as a pointed cap on top of the sporogonium

474. Operculum, Peristome, Annulus (Figs. 90-94). —In nearly ripe capsules, note that the convex lid or **operculum** which covers the apex is oblique—sometimes nearly parallel to the long axis of the capsule.

(*a*) Remove the operculum, and note that its outer layer consists of thick-walled cells running in spiral lines from the rim to the raised central point. The removal of the operculum brings to view the sixteen curved teeth of the outer peristome. With a razor, cut off the peristomes of several capsules, mount some with the upper surface uppermost, others with the lower surface uppermost

(*b*) Note that the peristome consists of two series of curved narrow triangular plates or teeth, sixteen teeth in each series; the teeth of the **outer peristome** are directly

superposed on those of the inner; the outer teeth are red and have thick transverse bars—these teeth are twisted spirally to the left as seen from above, and their tips are

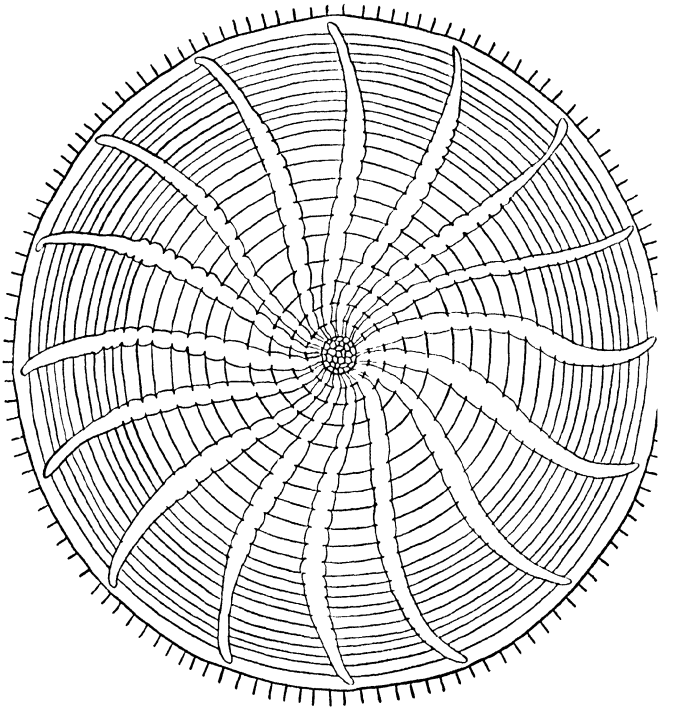


Fig. 90.—FUNARIA. Outer Peristome, seen from above, showing the sixteen curved teeth with their tips joined to a small central disc of tissue.

joined to a small central **disc** of tissue; the **inner teeth** are almost colourless and are shorter than the outer, but of the same general shape—at their bases they are directly under the outer teeth, but towards the centre of the capsule mouth they curve so that they occupy the widest parts of the slits between the outer teeth.

(*c*) In dry weather the whole peristome moves upwards, and the slits between the outer teeth become wider; while

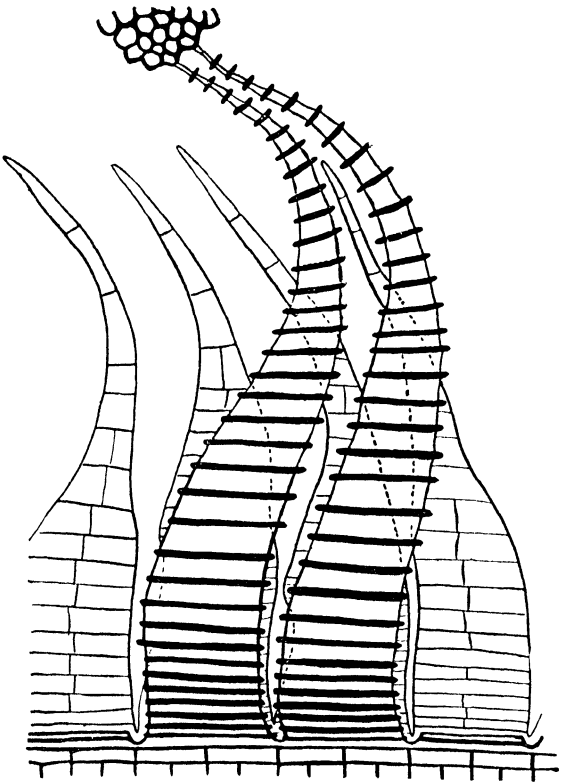


Fig 91.—FUNARIA. A portion of the Peristome from above, showing two of the outer (exostome) teeth and four of the inner (endostome) teeth

in moist air the peristome moves downwards, and the slits between these teeth become closed. This can be readily seen on removing the peristome from a ripe capsule,

mounting it on a dry slide, and breathing on it, then letting it dry again. Note also that the seta of the ripe capsule is wavy and twisted; put a drop of water on the ripe capsule, so that it can run down the seta—the latter becomes untwisted, swinging round and of course carrying

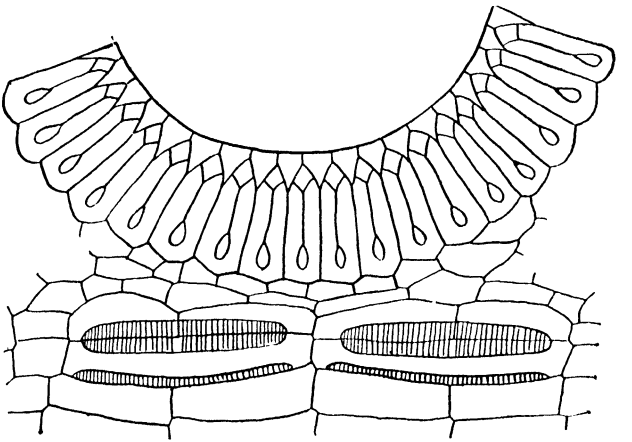


Fig. 92.—FUNARIA. Part of a Transverse Section of the Capsule, showing a portion of the Annulus (above) and two pairs of Peristome Teeth (below)

the capsule round with it The effect of sprinkling a little water over a patch of fruiting plants is often very striking, as the seta dries again, its movements are reversed.

(*d*) Note that the operculum is very readily detached from a quite ripe capsule Around the rim of the operculum, there are several rows of radially elongated and narrow cells; these are part of the **annulus,** a ring of tissue which separates the operculum from the rest of the capsule-wall. If fruiting plants are kept under observation, the annulus may be seen to separate from the ripe capsule as a strip of tissue which curls up with the concave side outwards, leaving the operculum free to fall off.

475. L. S. of Capsule (Figs. 93, 94) —Select sporogonia in which the peristome and operculum are still of a pale yellow colour, cut across the seta near the top, and then cut thin longitudinal sections of the capsule; also cut a series of transverse sections at different points, for comparison

In a **median longitudinal section,** note first the **seta,** expanding above to form the **apophysis,** which passes gradually into the capsule proper (the "sporangium") which is separated by a constriction (in which lies the **annulus**) from the **operculum.** The whole structure is somewhat complex, and a large drawing should be made showing the following details.

(*a*) The **spore-sac,** in a strictly median longitudinal section, is almost U-shaped, but broken through at the base by the lower portion of

(*b*) the **columella,** which also extends upwards into the concave inner portion of the operculum; outside of the spore-sac, which surrounds the columella, there are two or three layers of cells, forming the inner wall of

(*c*) the **air-space,** which is traversed by filaments (**trabeculae**) consisting of long narrow cells joined externally to the inner surface of

(*d*) the **capsule wall** (or sporangium wall), the latter consists in this region of two or three layers of cells covered by a distinct **epidermis**—the outer layers form compact colourless tissue, while the inner cells are loosely arranged and contain chloroplasts. Below the end of the spore-sac this colourless tissue of the capsule-wall thins out, while the inner green tissue increases in thickness, forming in

(*e*) the **apophysis** a broad zone of spongy green tissue, around the compact central tissue which is continuous below with

(*f*) the central **strand** of the seta. Trace the epidermis downwards to the apophysis, where the green tissue lies directly within it, and note

(*g*) the **stomata,** which will be examined presently in surface-view. At the lower end of the spore-sac, where the

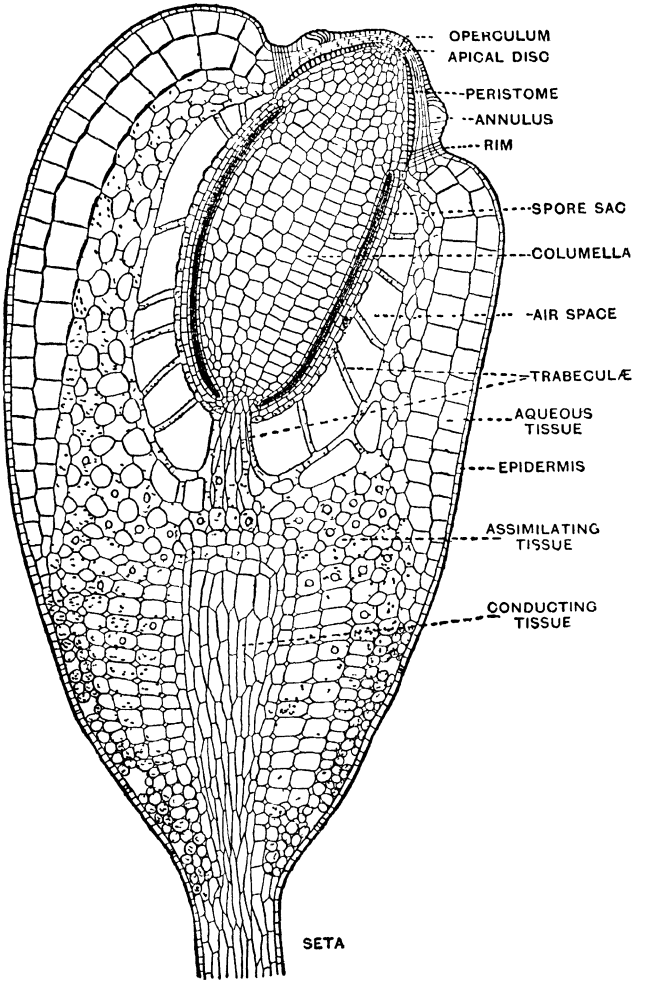


Fig 93.—FUNARIA Longitudinal Section of Capsule.

sac is, so to speak, perforated by the columella, the latter passes into a bundle of green cell-rows resembling the trabeculae and joined on to the inner apophysis tissue

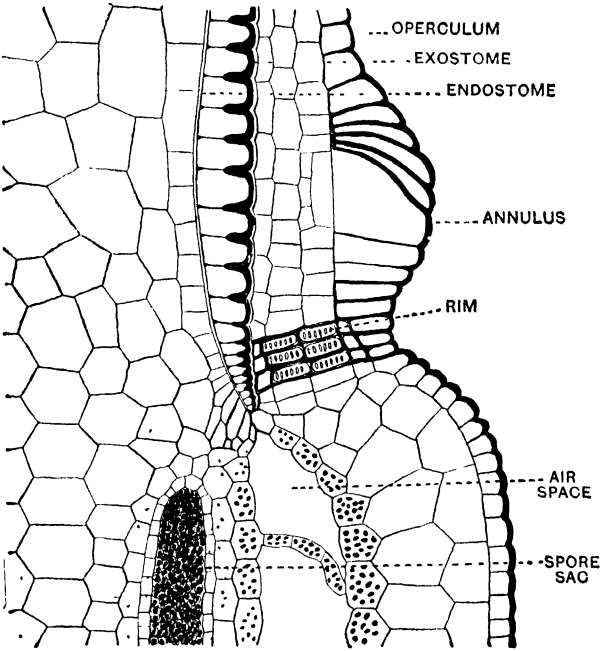


Fig 94.—FUNARIA. A portion of Fig. 81, enlarged to show details of the tissues in the region of the Annulus

Now examine carefully the *upper* part of the capsule, and note

(*h*) the **operculum,** consisting of an outer layer (epidermis) of cells with thickened outer walls, and three layers of small thin-walled cells;

(*i*) the **peristome,** appearing as a curved layer of cells with the vertical and outer walls strongly thickened. Trace

the peristome downwards, and note that just above the upper limit of the air-space there is

(*j*) a **rim** consisting of two or three layers of cells which are elongated radially and have pitted walls. These layers of cells join the peristome to the epidermis of the capsule-wall, reaching the latter at the constriction which separates the operculum from the rest of the capsule. When the operculum is detached, the layers form the rim of the open capsule. Just above this rim tissue is

(*k*) the **annulus,** consisting of about five superposed layers of epidermal cells, distinguished from the general epidermis by their greater radial depth; the upper annulus cells are narrow and thick-walled, but the two lowest layers (the annulus proper) have thinner walls and are swollen

476. Stomata on the Apophysis (Fig. 95) —Cut tangential sections from the surface, and note that although each stoma is formed in the usual way—by division of an epidermal cell and splitting of the division-wall—the two guard-cells have joined at their ends, so that the mature stoma is surrounded by a single continuous guard-cell.

477. T. S. of Capsule.—Examine **transverse sections** taken at different levels through capsules of different ages, and compare the structures with the description in § 475

478. The Capsule as Assimilating Organ.—That the capsule of Funaria is well adapted for carrying on photosynthesis can be shown by steeping a number of unripe capsules in alcohol in a corked tube, and comparing the depth of the colour of the extracted chlorophyll with that obtained on placing pieces of the foliage-leaf of a flowering-plant, *e g.* Sunflower or Nasturtium, in an equal quantity of alcohol in another tube. A Funaria capsule contains as much chlorophyll as about fourteen Funaria leaves, and in this respect is equal to nearly 5 square mm of a Sunflower leaf, and more than equal to the chlorophyll-content of the rest of a well-grown leafy Funaria shoot.

479. Protonema, Buds, Bulbils.—Sow spores from ripe capsules on moist soil, bricks, or tiles, kept under a bell-glass, and note that after a few days green threads

appear. These constitute the protonema, the filaments consisting of a branching row of long cells containing chloroplasts and separated by transverse cross-walls.

The branches, each of which arises just behind a cross-wall, may grow into (1) green filaments with colourless

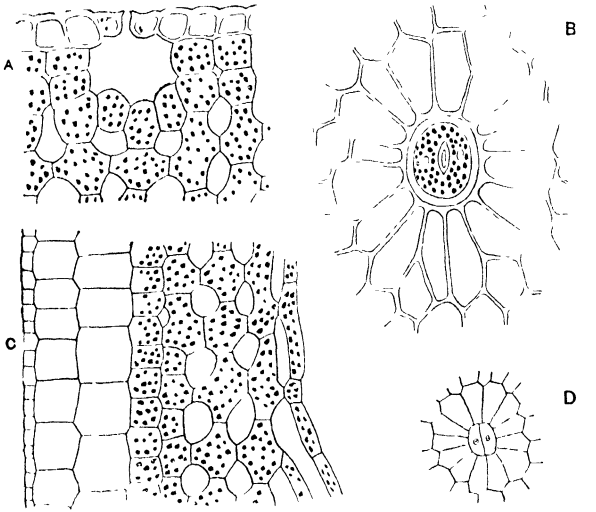


Fig 95.—FUNARIA Structure of Capsule Wall and Apophysis A, part of a transverse section of the Apophysis, showing a stoma with underlying spongy green tissue, B, surface view of Stoma, C, part of a longitudinal section of Capsule-wall, showing epidermis, colourless aqueous tissue, spongy green tissue, D, Stoma on apophysis of a young capsule, in surface view

walls (ordinary protonema); or (2) thinner filaments with brownish walls, oblique cross-walls, and no chlorophyll (rhizoids); or (3) buds from which arise young Moss shoots; or (4) small pear-shaped "bulbils" or "tubers."

Protonema can also be obtained (1) from rhizoids, by turning a Funaria sod upside down and keeping it moist under a bell-glass; (2) from detached leaves and pieces of stem, treated in the same way; (3) from paraphyses; (4) from the wall of the antheridium; (5) from cut pieces of the sporogonial seta.

CHAPTER XI.

MALE FERN, LYCOPODIUM, SELAGINELLA.

Male Fern (Lastrea Filix-mas).

480. Male Shield Fern (Lastrea filix-mas) is common in wood and hedgerows, and is easily distinguished from other woodland Ferns by its robust growth, massive rhizome, its rosette of large compound leaves, and the kidney-shaped scales (indusia) scattered on the underside of the leaf. Examine the plant at different times of the year

481. General Characters of Sporophyte —The short and stout **rhizome,** obliquely ascending or nearly erect, is covered by the leaves and the remains of leaves, and also by the numerous roots, so that the actual stem-surface cannot be seen. Starting from the oldest part of the plant, note the following general characters:—

(*a*) The crowded **leaf-bases**—the stumpy remains of the leaves of former years—covered by brown **scale-hairs** (**ramenta**); the leaves die down in autumn, but are not cut off by an abscise layer, hence their withered and hardened bases remain on the stem.

(*b*) The **mature leaves** of the current year, pinnately compound, each consisting of a main **leaf-stalk** with two ridges along its sides, and numerous **leaflets** given off by the main stalk in two lateral rows corresponding to the ridges. The leaflets are again divided more or less deeply into lobes with toothed edges; each lobe has a midrib, giving off finer veins which undergo repeated forking and

end blindly—this **forked venation** is characteristic of Fern leaves. The leaf-stalk bears scattered scale-hairs, which are also sometimes present on the main veins of the leaflets.

(*c*) In summer the mature leaf bears, on its lower side, the **sori**—projections arranged in two rows on each of the lobes of the leaflets; the sori are at first light green, but later turn brown. Each **sorus** consists of a collection of small stalked bodies, the **sporangia** or spore-cases, covered by a white kidney-shaped membrane or **indusium.** Pick off the indusium with your forceps, and note that it has a stalk arising in the midst of the sporangia; on removing the sporangia themselves, note that they spring from a small cushion (**placenta**) seated on one of the veins.

(*d*) The **young leaves**—which will unfold next spring and the next again, and are covered with the brown scale-hairs—lying within the expanded leaves, at the growing apex of the stem; a rosette of leaves is formed each year, but each leaf takes two years to develop. Each young leaf is rolled up lengthwise like a watch-spring (**circinate vernation,** characteristic of Fern leaves), owing to the greater growth of the lower (and outer) side of the leaf, each leaflet is rolled up in the same way. Carefully remove the brown scales from successively younger leaves; in the first year only the stalk is (as a rule) developed, the blade being formed later.

(*e*) The **buds,** which occur at the base of some of the leaves; and the hard wiry **roots,** also arising from the bases of the leaves close to their junction with the stem The roots are (except at the tips) dark brown or almost black, and much branched; the branching is monopodial, the branches arising in acropetal succession.

482. General Anatomy of Rhizome.—With a knife or razor, pare down the leaf-bases on the older part of the shoot, for about three inches, also remove the roots; then cut off this portion and set it to steep in dilute (about 10 per cent.) hydrochloric acid (for § 483).

(*a*) On the cut transverse surface, note that the actual stem portion is relatively small, the greater part of the thickness being made up by the leaf-bases; the **ground-tissue** contains starch and gum Test the ground-tissue with iodine, then cut a fresh surface and apply aniline sulphate, which will bring out clearly the **vascular bundles,** or, more correctly, **meristeles,** arranged in a ring both in the stem itself and in each leaf-base.

(*b*) Next, cut the upper portion of the shoot in halves by a slice passing through the centre as nearly as possible, including the growing tip, and note that the thickness of the stem is practically uniform throughout its length. The large central bundles run, on the whole, longitudinally, but have an uneven course By scraping away the ground tissue, you can see that these bundles form a network, and that smaller bundles run from the large central stem-bundles into the bases of the leaves

(*c*) The course and arrangement of the two sets of bundles can be made out rather better by cutting off tangential slices, starting from the outside; note that each mesh of the **central network** corresponds to the insertion of a leaf, and that the bundles passing out into the leaf come from the margin of the mesh. Each mesh is therefore called a "leaf-gap" or **foliar gap.**

483. "Vascular Skeleton." —Prepare a "vascular skeleton" of the stem, as follows.—With a blunt instrument or a hard brush, clear away the ground tissue from the piece of stem that has been soaked (or boiled for a short time) in dilute acid. The skeleton resembles a piece of wire netting rolled up into a tube, the leaf-bundles arising as branches from the larger central bundles of the tube at the edges of the diamond-shaped meshes or leaf-gaps.

484. T. S. of Fern Stem (Fig. 96).—Owing to the bulky nature of the stem and the curved course of the bundles in it, the microscopic structure of the bundles is more easily made out by cutting sections of the leaf-stock. Cut **transverse sections** of the **stem** (or leaf-stalk), mount some unstained in glycerine, others treated with iodine, others treated with aniline sulphate.

(*a*) With the low power note (1) the **epidermis,** a single layer of narrow cells with thick dark-brown outer walls; (2) the **scale-hairs,** each consisting of a single-layered plate of cells arising from the epidermis; (3) the **sclerenchyma,** a band of compact tissue with thick yellow lignified and pitted walls, separated by (4) a layer of compact **outer parenchyma** with thick but colourless walls from (5) the large **central parenchyma** in which

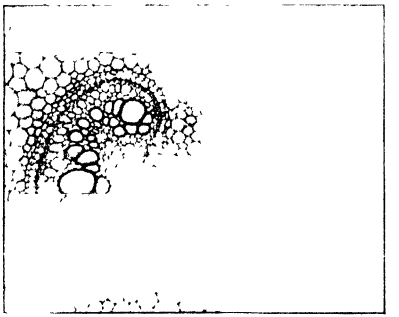


Fig. 90. Fern. Part of Transverse Section of Stem, showing two of the Vascular Strands

(6) the **vascular bundles** are embedded. The tissues 3, 4, and 5 constitute the **ground tissue;** in 5 the cells are separated by intercellular spaces (in which short **glandular hairs** sometimes occur), have thin pitted cellulose walls, and contain abundant starch grains.

(*b*) In a single **vascular bundle,** note (1) the sharply marked **endodermis,** consisting of a single layer of narrow cells with thick brown or yellow walls, surrounding the proper vascular tissue; the latter consisting of (2) **pericycle,** usually a single layer at the two ends of the bundle and a double layer at the sides, its cells polygonal with cellulose walls and starchy as well as protoplasmic contents; (3) **phloem,** surrounding (4) the **xylem** which occupies the centre of the bundle. The phloem consists of **sieve-tubes,** polygonal in cross section, apparently empty

but with a thin protoplasmic lining, and "conjunctive" **parenchyma,** the latter mingled with the sieve-tubes and having thin walls and dense protoplasmic contents. The **xylem** consists of (i) **vessels,** varying in width, with thick lignified walls; and (ii) "conjunctive" **parenchyma** like that of the phloem

485. L. S. of Fern Stem (Fig. 97) —Cut **longitudinal sections** of the **stem** (or leaf-stalk); treat them in

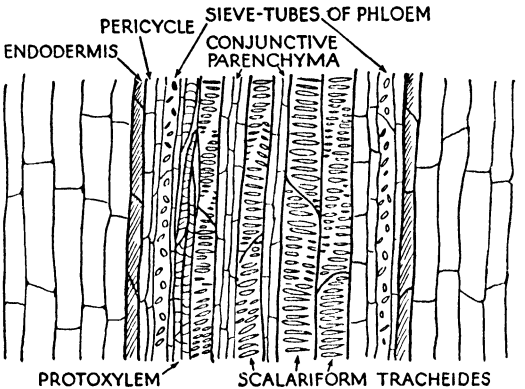


Fig 97 —Fern Part of a Longitudinal Section of Stem, passing through a Vascular Strand.

the same way as the transverse sections, and note the following tissues (compare with the transverse sections and the slides of macerated tissues) :—

(1) The **epidermis.**

(2) The parenchymatous **ground tissue.**

(3) The **phloem,** consisting of **sieve-tubes** with pointed ends and with **sieve-areas** (perforated by small pores) on the lateral walls; the areas being of irregular outline and separated by thicker portions of cell-wall. These thickened parts give the walls a beaded appearance as seen in longitudinal sections.

(4) The large **scalariform vessels**, with tapering ends and ladder-like thickening of the side walls. This appearance is due to the very regular transversely elongated **bordered pits.**

(5) The smaller tracheids of the **protoxylem,** with spiral fibrous thickenings on their walls.

(6) The narrow square-ended cells of the endodermis, the pericycle, and the conjunctive parenchyma of the xylem and phloem.

(7) The large short square-ended or polygonal cells of the general ground tissue, separated by intercellular spaces.

486. Macerated Tissues of Fern Stem.—Cut out a few pieces of stem, or longitudinal sections including portions of the vascular skeleton, warm them in a test-tube with nitric acid and potassium chlorate (§ 120), rinse in water, and tease out the tissues by means of needles. Examine the macerated tissue, noting the forms of the **isolated tissue elements** and the markings on their walls, for comparison with their appearance in sections.

487. T. S. of Root.—Cut transverse sections of a root, held in pith. Note—

(1) The **piliferous layer,** some cells of which grow out to form **root-hairs.** Remains of these may be seen on the old root, or the hairs themselves may be found on carefully digging up a plant, washing the roots gently to free them from soil, and examining the young tips.

(2) The thin-walled **outer cortex.**

(3) The sclerenchymatous **inner cortex,** which forms a dense thick ring around the **vascular cylinder.** The latter is surrounded by

(4) The **endodermis,** a single layer of rather flattened cells—the dot-like markings due to the thickening bands on the radial walls are not easily seen—and

(5) The **pericycle,** partly single- and partly double-layered

(6) Two strands of **phloem,** one on either side of

(7) The plate-like strand of **xylem,** which has the small **protoxylem** elements at either end and is therefore **diarch.**

488. Structure of Leaf.—Cut **transverse sections** of barren **leaflet,** held in pith. Note (1) the **upper epidermis,** a single layer of cells containing chloroplasts;

(2) the **mesophyll,** practically uniform and consisting of more or less branched and star-shaped cells, separated by large intercellular **air-spaces;** (3) the **lower epidermis;** (4) **stomata,** present in the lower epidermis but very rarely in the upper; (5) **vascular bundles,** embedded in the mesophyll, each bundle surrounded by a conspicuous sheath (endodermis) and having the xylem nearer the upper side of the leaf, instead of in the centre of the bundle, as in the stem This almost collateral structure of the leaf-bundles can be well seen in sections of the leaf-stalk, especially in the upper part.

Remove strips of the epidermis from (1) the upper and (2) the lower side of the leaflet, in both cases note the very wavy vertical walls of the epidermal cells, which have chloroplasts, in the lower epidermis note the stomata, with two **guard-cells.**

489. Sorus and Sporangia (Fig 98).—Cut several transverse sections of leaflets with **sori.** In one that has passed through the centre of a sorus, note, in addition to the structure of the leaflet, as given above, the following:— (1) the small cushion-like **placenta,** a mass of tissue seated on a **vein** which sends a branch into it; (2) the stalked **indusium** arising from the end of the placenta and spreading out like an umbrella over (3) the stalked brown oval **sporangia.**

Also cut off several sori from a leaf, tease in water, cover, examine Note the simple structure of the **indusium.**

In both these preparations, note that sporangia in different stages of development occur in the same sorus. The mature sporangium consists of a fairly long **stalk** (composed of either two or three rows of cells, and often bearing a pear-shaped one-celled **gland** supported on a long slender cell) and a **capsule** having the form of an oval biconvex lens.

The **wall** of the capsule is one cell thick, and the cells round the edge, starting from the stalk and going over the top of the sporangium half-way down the other side, are large and thick-walled, forming the **ring** (*annulus*); each

cell of the ring is thickened chiefly on its inner and lateral walls, the outer wall being much thinner. The annulus is not a complete ring, being replaced at its end by broad flat cells (this region is sometimes called the **stomium**, since dehiscence of the sporangium-wall begins here); the rest of the cells forming the wall are flat and thin-walled.

Inside the ripe sporangium there are numerous (usually forty-eight) brown unicellular **spores**; each spore is kidney-shaped, and when ripe has a two-layered wall, the outer layer being thick and cutinised.

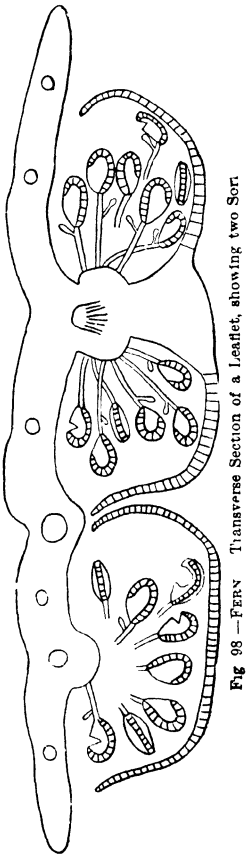


Fig. 98.—Fern. Transverse Section of a Leaflet, showing two Sori.

490. Dehiscence of Sporangium.—Mount some ripe but still intact sporangia in water and, while watching them under the microscope, place a drop of glycerine at one edge of the cover-glass and draw it through by means of a piece of filter-paper or blotting-paper placed at the opposite edge; watch the bursting of the sporangia. The explosion can be caused by any other method for withdrawing water from the ripe sporangia; for instance, mount some sporangia on a dry slide, then warm the slide gently, and quickly observe it again under the microscope.

Dehiscence is due to contraction of the cells of the ring as these cells lose water, the thinner outer wall bulges inwards; this pulls the radial walls together, until the

strain thus set up makes the ring straighten itself out and become curved in the opposite direction, the sporangium-wall being torn open below the end of the ring.

491. The development of the Fern sporangium can be readily followed in sections through young sori, since in the Male Fern (as in all the commoner British Ferns) sporangia of all ages are mixed together in the same sorus. The fertile leaf of the **Hartstongue** is especially useful for this purpose, since successive sections can be made across the long sori.

The superficial cell of the placenta which is about to form a sporangium grows out and divides into an upper **capsule-forming cell** and a lower **stalk-forming cell.** The former divides by three curved intersecting walls, cutting out an inner pyramidal cell (apex pointing downwards) from three outer cells, then the inner cell divides by a curved transverse wall, so that we have now a central pyramidal cell enclosed by four outer cells, which are to form the **wall** of the capsule. The stalk-cell divides by both transverse and longitudinal walls, giving rise to two or three rows of cells.

Returning to the capsule, the central cell now divides by four walls parallel to those first formed, so that within the **wall-layer** there is now a **tapetum,** or tapetal layer, of four cells, which undergo further division and are ultimately used up in the nutrition of the spore-forming cells The latter are produced by repeated divisions of the central cell (**archesporial cell** or **archesporium**) to form a mass of (usually twelve in Male Fern) **spore-mother-cells,** each of which divides later into four **spores,** after the nucleus has divided into four

The wall-forming cells divide only by vertical walls, hence the wall remains one layer of cells, but the tapetum usually divides into two layers; eventually the spore-mother-cells separate from each other, they float in mucilaginous liquid formed by disintegration of the tapetal cells

In Hartstongue each of the (usually sixteen) spherical mother-cells divides by walls between the four nuclei (formed by division of the mother-cell nucleus) in such a way that each spore is tetrahedral, with three flattened sides (where it was in contact with its three sister-spores) meeting at a point (the centre of the mother-cell) and a curved outer side; in Male Fern the mother-cell is divided by walls at right angles, so that each young spore is the quarter, or quadrant, of a sphere, but later it becomes bean- or kidney-shaped.

492. Cultivation of Fern Prothalli.—Failure of prothallus cultures is generally due to invasion by fungi, but this can be largely obviated by sterilising the vessels and the soil used. Collect the spores on pieces of paper, or in envelopes, by simply cutting off fertile leaves and allowing them to dry on the paper, which will

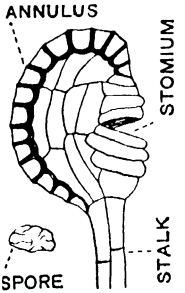


Fig 98a —Sporangium and Spore of Fern

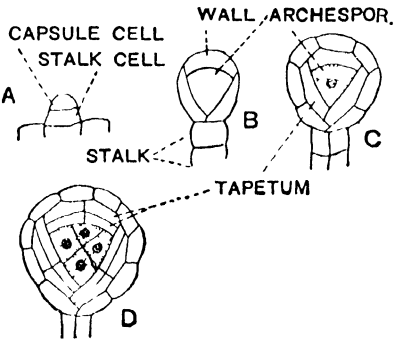


Fig 98b —Development of the Sporangium of the Fern

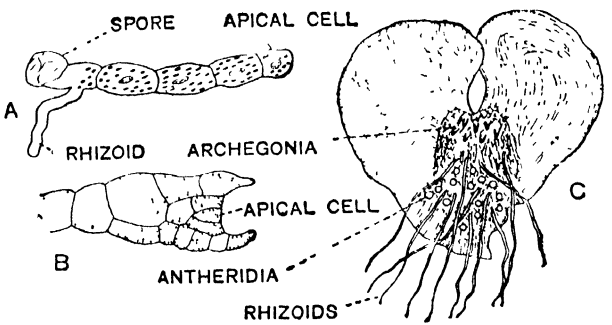


Fig 98c.—Germination of Spore and Development of Prothallus of Fern

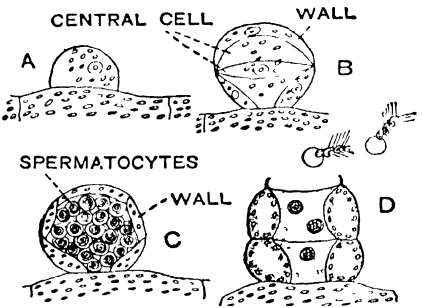


Fig 98*d* —Development of Antheridium of Fern

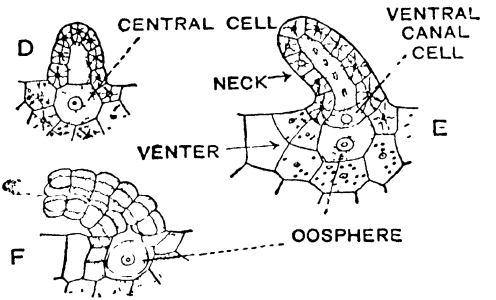


Fig 98*e*.—Development of Archegonium of Fern

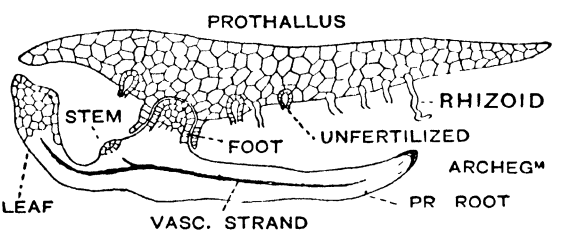


Fig 98*f* —Embryo of Fern attached to Prothallus (Longitudinal section)

soon be covered by the spores set free by the bursting of the sporangia.

Get some flower-pots or shallow seed-pans, some lumps of peat or leaf-mould, and some glass sheets or bell-jars. If you use flower-pots, half fill the pot with gravel and then put in enough peat to fill the pot to an inch from the top; if seed-pans are used, simply stand the lump of peat in the middle of the pan The vessels and soil can be sterilised either by baking them in a hot oven for a few hours, or by steeping them in boiling water; if you bake the soil afterwards, moisten it and the pot or pan

Shake some spores over the soil, and cover with the glass; the latter keeps the soil moist, since the evaporated water condenses and runs back into the vessel, but the glass should be removed now and then to renew the air. Set the vessels out of direct sunlight, the early germination of the spores is hastened by gentle warmth

Another method is to sow the spores on previously heated or scalded bits of brick or tile sloping into water in a dish, instead of using soil. Do not sow the spores too thickly, and do not water them from above.

In a few weeks you will see greenish threads creeping over the soil, with here and there a small green disk After a month or two, you may see a few small leaves appearing on the prothalli. If the prothalli are thickly crowded, thin them out, as one does with seedlings

493. Development of the Fern Prothallus.—For the early stages in germination of the spore, pick up, with needle or knife-point, soil on which spores have been sown, as soon as young prothalli can be seen, or place spores in a hanging drop of water or Knop solution in a moist chamber.

Note (1) the bursting of the thick outer spore-coat; (2) the outgrowth of the spore-contents, covered by the inner coat, to form a short green filament; (3) the early formation of a colourless undivided rhizoid from the base of the green thread; (4) the formation of transverse walls in the elongating green thread, giving rise to a row of cells; (5) the setting-in of oblique walls at the free tip of the thread, forming a two-sided or wedge-shaped apical cell from which segments are cut off right and left; (6) the more rapid growth of the tissue at either side of the apical cell, causing the latter to occupy a notch and the prothallus to become heart-shaped; (7) the formation of further rhizoids from the underside of the young prothallus.

494. General Characters of Prothallus.—Note the naked-eye features of a large well-grown **prothallus**—its flattened form, its kidney- or heart-like outline, its green colour, and its size (generally about a quarter of an inch in diameter); the smooth upper side, with the notch at the

anterior end, in which lies the growing-point; the thickened median portion, or **cushion,** and the thin lateral expansion or wing on either side; the **rhizoids,** springing from the underside, chiefly from the cushion. Note that the prothallus is not usually pressed quite closely to the soil, but is tilted upwards in front, so that under moist conditions there is a water-holding space between the underside of the prothallus and the soil; if the prothalli are crowded, they slope steeply upwards in front, especially if grown in a rather deep vessel

495. Prothallus with Sexual Organs (Fig. 99).—Mount a number of prothalli in water, with the lower surface uppermost, and examine with the microscope.



Fig. 99.—FERN. A Prothallus, seen from lower side.

Note the general features, and the following additional points:—

(1) The polygonal thin-walled cells of the lateral **wings,** each containing numerous **chloroplasts** in addition to protoplasm, nucleus, and cell-sap.

(2) The several-layered **cushion,** consisting of similar cells—those on the underside of the cushion usually contain relatively few chloroplasts, and some have grown out to form

(3) Long colourless unicellular **rhizoids,** sometimes branched at the free end.

(4) The **growing-point,** occupying the notch and now consisting of a series of initial cells which undergo active growth and division.

(5) The **antheridia,** seen in surface view as circular bodies occupying the hinder (older) portion of the prothallus and occurring on both the cushion and the lateral wings.

(6) The **archegonia,** occurring on the cushion at the front of the prothallus (a little behind the growing-point) and showing their necks as little finger-like projections—the older archegonia are conspicuous owing to their brown colour.

496. Antheridium and Archegonium.—Examine the sexual organs more closely with the high power.

(*a*) **Antheridium.**—In surface view, note the central group of **antherozoid mother-cells,** small spherical bodies, surrounded by the antheridium wall which may be seen as either a ring of cells or as a single ring-like cell; the whole antheridium is smaller than the prothallus cell on which it is seated. In the small and more or less filamentous (and often branched) prothalli, which may bear only antheridia, the latter may be seen in **side view,** and the **wall** can be made out as consisting of two superposed ring-like cells with a dome-like **cap-cell** on the top.

(*b*) **Archegonium.**—In surface view, note the four rows of cells of which the **neck** is composed, and the **neck-canal;** by focussing more deeply, the **canal-cells** and the **oosphere** may be seen in an archegonium which has not yet opened; in an older archegonium that has been fertilised, the **embryo** may be seen by focussing down to the embedded **venter.**

497. L. S. of Prothallus (Fig. 100).—Cut median longitudinal sections of an alcohol-hardened prothallus, held in pith; only those sections passing through the cushion are required, but with care several useful sections may be

obtained from each prothallus cut. Note especially the **archegonia,** and try to make out the **axial series** of cells —the elongated **neck canal-cell** (sometimes divided into two), the small rounded **ventral canal-cell** below this, and the large rounded **egg-cell** embedded in the cushion tissue; the **neck,** consisting of a single layer of cells. In the **antheridia,** note the single-layered wall and the central mass of antherozoid mother-cells.

498. Young Sporophyte.—With lens, or low power of microscope, examine prothalli with young Fern-plant attached. Note that the young plant grows out from the



Fig. 100.—FERN. Part of a Longitudinal Section of a Prothallus, showing Antheridia.

underside of the prothallus, near the notched anterior end, and consists of the following parts:—(1) the first **root,** which grows down into the soil; (2) the first leaf (**cotyledon**), which turns upwards through the notch of the prothallus and consists of a relatively long stalk and a simple or lobed blade; (3) the **foot,** a projecting body buried in the tissue of the prothallus; (4) the growing tip of the young **stem,** lying in the angle between the base of the cotyledon and the foot and showing several developing leaves. In very young plants, note that the primary root is usually the first part to break out through the sheath (**calyptra**) formed by the prothallus tissue surrounding the embryo.

In **older plants,** note (1) the additional roots that grow out from the stem; (2) the later leaves, which are succes-

sively larger and more lobed, (3) the gradual decay of the prothallus, as the young Fern-plant obtains its own food by means of its roots and leaves and therefore becomes independent of the food-supply it had at first drawn from the prothallus by means of the foot.

These points may be clearly seen in specimens decolorised with alcohol, and made transparent with chloral hydrate, potash, or some other clearing reagent.

Also cut longitudinal sections of prothalli with young Fern plants, and note the relative positions of cotyledon, stem, root, and foot.

LYCOPODIUM.

499. General Characters.—The **Common Club Moss** (Lycopodium clavatum), the largest as well as commonest of the five British species, grows chiefly on heaths, moors, and mountain-sides Note

(*a*) The creeping **stem,** tough, flexible, and much branched—some of the branches creeping, others ascending and growing erect

(*b*) The **roots,** arising from the lower side of the stem—often one root at each point of branching, but also at other parts of the stem—and showing more or less distinct **dichotomy** (forked branching)

(*c*) The small simple lance-shaped **leaves,** crowded and overlapping on the creeping stem and branches, arranged spirally or in whorls or in both ways, each leaf having a finely toothed margin and ending in a long hair-point which curves in towards the stem—at the ends of the branches all the leaves tend to curve strongly upwards

(*d*) The erect branches which are slender below (owing to the leaves being here small, scattered, and closely pressed to the stem), but at the top, after branching into two or three, expand again into the cylindrical or club-like **cones.**

(*e*) The **sporangia,** kidney-shaped and almost sessile

capsules, one seated on the base of each of the broad spirally arranged leaves **(sporophylls)** of the cone—the ripe sporangia is yellow and opens by a transverse slit.

500. Structure of Stem.—In **transverse sections** of the **stem** note (1) the **epidermis,** with thick cutinised outer walls; (2) the sclerenchymatous **outer cortex;** (3) the thin-walled parenchymatous **middle cortex;** (4) the sclerenchymatous **inner cortex;** (5) the **leaf-trace bundles,** lying here and there in the cortex, through which they pass from stem-stele to leaf; (6) the **"bundle-sheath,"** several layers of thin-walled cells (the outer layers with cutinised or corky walls—on placing a drop of sulphuric acid on a section, these cells remain unaffected after the other tissues have lost their clear outlines); (7) the **xylem** and the **phloem,** arranged in more or less horizontal alternating bands, though there are often connections between the xylem-bands towards the centre of the stele.

With the high power note, in the xylem, the small protoxylem tracheids at the outside (the edges of the bands); in the phloem, the narrow thick-walled protophloems, alternating with the protoxylems. In both xylem and phloem the main part of each band consists of wide elements.

In **radial longitudinal sections** of the stem note (1) the **leaf-bases;** (2) the **epidermis,** the three zones of the **cortex,** and the **bundle-sheath;** (3) **leaf-trace bundles,** running down obliquely from leaf to central cylinder; (4) the **spiral** (protoxylem) **tracheids** and the wide **scalariform tracheids;** (5) the **phloem** cells, some narrow and others wide—the latter are the sieve-tubes, but when mature they are almost empty and the small sieve-plates are difficult to make out.

501. Structure of Leaf.—In sections taken across the leafy stem, some **leaves** will be found cut transversely; note the triangular outline of the section (the longest side being upwards), the epidermis with stomates on both upper and lower sides (examine entire leaves in surface view), the spongy mesophyll, and the small central vascular bundle. In transverse sections of a **root,** note the general resemblance to the stem, but the smaller number of xylems and phloems, the former being often irregular and fused together.

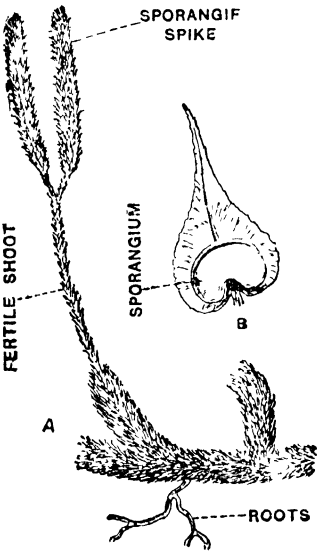


Fig 100a.—A, Part of *Lycopodium clavatum*, showing stem, leaves, roots, and sporangiferous spikes; B, A sporophyll and sporangium in surface view

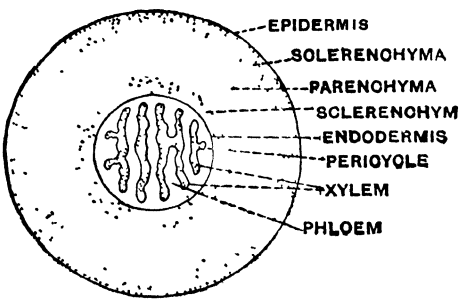
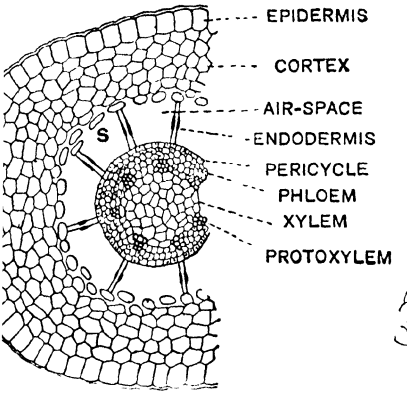


Fig 100b.—Transverse Section of Stem of *Lycopodium annotinum*. (Diagrammatic.)



00c —Stem of *Selaginella spinosa* (Transverse section)

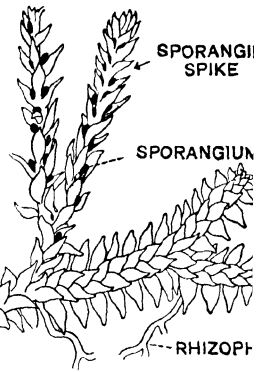
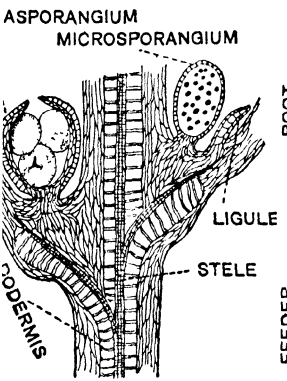


Fig 100*d* —*Selaginella helvetica*



g 100*e* —Part of "Sporangiferous Spike" of *Selaginella* (Longitudinal section.)

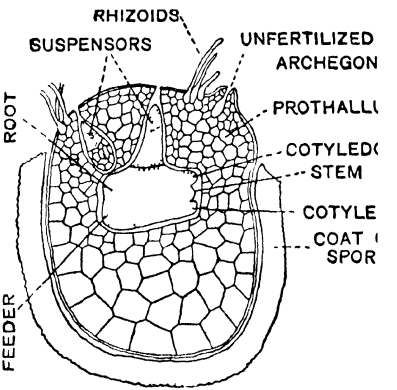


Fig 100*f* —Female Prothallus of *Selaginella* (Longitudinal section) An old stage, showing two developing embryos.

502. L. S. of Cone.—Cut radial longitudinal sections through a **cone,** and note

(*a*) The structure of the **axis,** resembling that of the stem, with a bundle running out to each sporophyll.

(*b*) The division of each **sporophyll** into a horizontal lower portion, with a short downwardly-directed flap at the end and a much longer upwardly-directed portion—the sporophyll is therefore somewhat *peltate*, and in some other species of Lycopodium the downward portion of the expansion or lamina is much larger than in our type.

(*c*) The **sporangium,** inserted by a short stalk on the horizontal basal part of the sporophyll, above the vein—the sporangium is nearly circular in section, but into the cavity there projects upwards a rounded **pad** of tissue, making the cavity kidney-shaped in outline

(*d*) The sporangium **wall,** consisting of an outer layer of large cells and one or two inner layers of small cells (disorganised in ripe sporangium)—at one point on the top of the sporangium but towards the outer side, the cells are narrower, marking the line of **dehiscence.**

503. Structure of Sporangium Wall.—In surface view, the cells of the mature but not yet ruptured sporangium-wall are seen to be elongated and to have thickened lignified wavy radial walls, but along the dehiscence-line the cells are nearly cubical, with straight radial walls; the two rows of cubical cells have their tangential as well as their radial walls lignified, as may be seen on treating the preparation with aniline sulphate or with phloroglucin

504. Spores.—Examine the spores with the high power; they are very small, roundish tetrahedral in form, and covered with a network of minute projections—the latter cause the spores to be unwettable by water, owing to the entangled layer of air covering them.

SELAGINELLA.

505. Nearly all the species of Selaginella are tropical, mostly growing in wet forests and including epiphytes and climbers, but a few are xerophytes. The simplest type is that seen in the single and rare British species,

S spinosa, where the leaves are all alike and are spirally arranged, but most of the species correspond to the type described below. The species most commonly cultivated are S. Martensii and S Kraussiana; if possible, obtain specimens of both.

506. General External Characters.—In a good-sized portion of one of the above exotic species, obtained from a greenhouse, note—

(*a*) The profuse **branching** of the relatively slender but wiry **stem,** which takes place in one plane and is *apparently* dichotomous (forking) but really monopodial.

(*b*) The small simple **leaves,** single-veined and more or less lance-shaped, arranged in **four rows** (two side rows and two dorsal rows)—closer inspection shows that the leaves are in pairs, each pair consisting of a larger lateral (strictly speaking, *ventral*) leaf and a smaller *dorsal* leaf opposite to it.

(*c*) The **roots,** arising singly at the points of branching of the stem—the first portion of the root is stiff, green or brownish, and unbranched, and is often termed the **rhizophore,** but on reaching the soil (over which the stem grows in a straggling and ascending manner) it divides into numerous slender white branches which are apparently dichotomous.

(*d*) The **cones,** which show radial symmetry, unlike the dorsiventral symmetry of the rest of the shoot, and bear spirally arranged leaves (**sporophylls**), forming four longitudinal rows and being all alike in size.

(*e*) The **sporangia,** of which there are two kinds, seated singly in the axils of the sporophylls, and seen on turning these down—the **megasporangia,** confined to the base of the cone, and each containing four large rounded **megaspores**—these cause the megasporangium to be lobed and therefore easily distinguished with the naked eye from the smaller and more nearly spherical **microsporangia,** which occupy the upper portion of the cone

and contain large numbers of the much smaller **microspores.**

(*f*) The **dehiscence** of the sporangia, by a slit running transversely across from side to side

507. Structure of Stem.—In transverse sections of the stem of S. Martensii, note (1) the **epidermis,** a layer of thick-walled cells, covered by cuticle; (2) the green **cortex,** the outermost part of which is usually compact, thick-walled, and lignified; (3) the **vascular cylinder** (stele), surrounded by an **air-space** which is bridged by **trabeculae** consisting of cell-rows (here and there a *single* elongated cell may be seen forming a trabecula).

In the **vascular cylinder,** note (1) the **pericycle,** a layer of rather large cells; (2) the **phloem,** forming a continuous band around (3) the solid central **xylem,** which is oval or spindle-like in cross section, with a group of small **protoxylem** tracheids at each end (the xylem is therefore *exarch* and *diarch*). With the high power, note that there is a ring-like transverse band on the innermost cell of each of the trabeculae consisting of a row of cells, or at the middle of the long unicellular trabeculae the trabeculae represent the **endodermis.**

In cross sections of the **young** parts of the stem, taken an inch or so behind the stem, the development of the air-space can be followed, and the trabecular tissue more readily made out; note also that the polar portions (**protoxylem**) of the wood are fully formed and lignified, while the large-celled middle portion is still thin-walled and contains protoplasm.

In **longitudinal sections** of the stem, note the tissues as described above; the xylem consists of spiral (protoxylem) and scalariform **tracheids,** as in Lycopodium, note the **leaf-traces,** one running into each leaf—the leaf bundle carries with it into the leaf a continuation of the air-cavity surrounding the central cylinder.

508. For comparison, examine sections of the **stems** of other species of Selaginella. In S. spinosa there is a single stele as in S. Martensii, but in the creeping lower portion of the stem the

protoxylem is at the *centre* of the xylem (the latter being therefore *endarch*), while in the higher erect portions the stele is exarch but is radially symmetrical and has *numerous* protoxylems all round the outside, instead of two only; this structure is obviously connected with the spiral arrangement of the leaves, since the leaf-bundles are given off at the protoxylems) In S. Kraussiana, the stem has *two* steles, which are joined up at the points of branching of the stem and then separate again; each stele has its own air-space, and they are evidently formed by division of a single stele. In other species there may be three or even more steles, each usually flattened and having a protoxylem at each end of the plate-like xylem

509. Structure of Leaf.—Carefully remove a few **leaves,** and note (1) the single **bundle;** (2) the toothed margin; (3) the small outgrowth (**ligule**)—often fan-like—at the base of the leaf on the upper side; (4) the presence of **single** large **chloroplasts** in the cells of the epidermis; (5) the **stomata** on the lower epidermis, near the middle line.

In transverse sections of the leaf, note especially (1) the **spongy mesophyll,** which is absent at the thin margins of the leaf (here the upper and lower epidermis layers come together); and (2) the small central **vein.**

510. Structure of Rhizophore and Root.—In transverse sections of (1) the "**rhizophore**" and of (2) the **roots** which arise from it, note that the general structure is similar to that of the stem, except that the air-cavity is either absent or represented only by small intercellular spaces between the cells of the endodermis, while the vascular cylinder has a central mass of xylem, with a *single* protoxylem (*monarch* structure) at one side where the phloem is interrupted and therefore forms in cross section an incomplete ring.

511. Structure of Sporangia and Spores.—Examine with the microscope (*a*) an **entire cone,** treated with potash to make it more transparent; (*b*) **microspores** and **megaspores,** isolated by teasing out or pressing open the sporangia; (*c*) **longitudinal sections** of a whole cone.

Note (1) the form and size of the two kinds of **spores** (diameter of megaspores many times greater than that of the microspores)—both kinds are tetrahedral and rounded, with three radiating lines on one side and spiny projections on the outer **coat,** which is very thick in the case of the megaspore; (2) the coherence of the microspores in fours (*tetrads*) until quite ripe; (3) the structure of the **sporangium wall,** which is three-layered, the outer layer of radially elongated thick-walled cells, middle layer of small flat cells, the inner layer (**tapetum,** which persists until spores are ripe) of elongated but thin-walled cells, (4) the **dehiscence line** in the sporangium wall, where the outer cells are shorter than elsewhere, (5) the ligule of each sporophyll, inserted just outside the short thick stalk.

512. Germination of Spores.—Get ripe spores of both kinds by drying a fresh plant on paper, and sow them together on moist soil or tiles. In a few weeks young Selaginella plants will be seen—note (1) the **hypocotyl,** bearing two **cotyledons** (each with a small ligule) at its end, and between them the young **shoot,** which soon branches; (2) the first **root,** at either side of which the second and third roots arise from the base of the hypocotyl; (3) the megaspore, to which the young plant is attached by a **foot** projecting from the base of the hypocotyl into the tissue of the **female prothallus** which fills the spore—the foot and prothallus can be seen by carefully picking off with needles the ruptured spore-coat and treating with potash.

513. Development of Prothalli and Sexual Organs.—A good deal can be made out by crushing the germinated spores under the cover-glass, or treating them with potash, or (in the case of the megaspores) dissecting off the thick outer coat with needles—all these methods should be tried.

(*a*) In the **microspore** the first division (which occurs before the spore leaves the sporangium) cuts off a small lens-like **male prothallial cell** from a much larger cell; the latter forms the **antheridium,** consisting of a single-layered **wall** and a central group of antherozoid-mother cells

(*b*) The formation of the **female prothallus** in the megaspore begins while the spore is in the sporangium and before it has reached its full size; the nucleus of the young spore (which lies at the apex—the point where the three radiating lines meet) divides repeatedly, and the apical lens-like portion of the prothallus tissue is formed first—this tissue is easily seen on warming some megaspores with potash, dissecting off the coat or pressing the spore under the cover-glass.

(*c*) In free germinated megaspores, the prothallus tissue enlarges, filling the cavity of the spore, ruptures the outer coat of the spore along the three radiating lines, protrudes from between the flaps of the burst coat, and produces several **archegonia** resembling those of the Male Fern but with much shorter neck; the marginal portion of the exposed prothallus tissue may become green and produce a few rhizoids from the superficial cells.

(*d*) In older prothalli treated with potash, note the contrast between the so-called apical tissue (that first formed) and the large-celled lower tissue formed later.

CHAPTER XII.

SCOTS PINE, YEW, CYCAS.

Scots Pine (Pinus silvestris).

514. General External Characters.—In studying the Scots Pine, at different times of year, note the following general features :—

(1) The "excurrent" habit, the long straight trunk passing right to the top of the tree and tapering out.

(2) The scaly bark, orange-coloured except at the base of the trunk, where it is darker, and the rough scaly surface of the younger bare twigs.

(3) The origin of the branches, in apparent whorls, from lateral buds, each of which arises in the axil of a scale-leaf at the end of each year's growth.

(4) The consequent indication of the age of the tree by the number of these apparent whorls—even in the lower portion the stumps of fallen branches may be seen in whorls

(5) The spiral arrangement of the scale-leaves on the twigs, showing that the branching is not in reality whorled.

(6) The appearance of a tree in which the main axis happens to have been injured—a lateral branch bends upwards and replaces it, forming a new "leader" and often giving the top of the tree a bayonet shape

(7) The short shoots ("dwarf shoots," or "foliar spurs," or "shoots of limited growth"), each arising in the axil of one of the brown scale-leaves borne on either the main stem or one of the ordinary branches ("long shoots" or "shoots of unlimited growth").

(8) The narrow twisted green foliage-leaves ("needles"), two borne on each dwarf-shoot or "bifoliar spur."

(9) The persistence of the foliage-leaves for a number of years, and their fall owing to the cutting-off (by an abscisslayer) of the whole dwarf-shoot.

(10) The scale-leaves borne on the lower portion of each dwarf-shoot, below the two "needles"

It will be noticed that the Pine produces two kinds of branches and two kinds of leaves; the ordinary branches or "long shoots," formed annually in an apparent circle, bear only scale-leaves; in the axil of each scale-leaf there arises a "dwarf shoot" which bears a number of scale-leaves and a pair of foliage-leaves.

Examine the **buds** in autumn or winter. Study with special care the opening of the buds and the early growth of the resulting young shoots, during May and June—this is very important, in order to gain a clear idea of the morphology of the plant, as well as for the study of the male and female cones.

During May and June, visit the trees at frequent intervals, or if this cannot be done at least weekly, bring in the cut twigs and set them in water, so as to be able to watch the various stages in the early growth of the shoots

515. Resting Buds of Pinus.—In examining a **twig in winter** (or in early spring), note that it shows (1) a **terminal resting-bud;** (2) a few, usually three or four, smaller **lateral resting-buds** just below the terminal bud; (3) one or sometimes more of the lateral buds may be replaced by a one-year-old **female cone,** green in colour; (4) each resting-bud is ovoid and pointed, and bears numerous brown **scale-leaves,** which are covered and stuck together by resin and are arranged spirally (this latter point will be seen more clearly when the bud is expanding later on).

Treat a resting-bud with alcohol, to remove the resin, detach some of the scale-leaves from it, and note that each scale-leaf has a thickened base and bears a small **bud** in its axil (excepting those at the very base of the resting-bud). The resting-bud of the Pine might be termed a compound bud, a "bud of buds," since most of the bud-scales have axillary buds.

516. Opening of the Buds.—Examine an **opening bud** in spring, and note that—

(1) The axis elongates, carrying up the spirally arranged scales

(2) The lowest scales, which are hard and dry and have no axillary buds, remain at the base of the young shoot into which the resting-bud is now developing

(3) Each of the other scales is at first green below and membranous above, with pointed tip and fringed edges, but as the resting-bud opens the base of the scale hardens and the upper part falls off

(4) Each of the axillary buds of the compound resting-bud develops into a dwarf-shoot, bearing about ten scale-leaves at the base, these forming a sheath around the two young foliage-leaves, which soon project beyond this sheath and become visible

(5) The two foliage-leaves of each young dwarf-shoot have convex outer surface and flattened inner surfaces, the latter being closely apposed at first, but later, as these leaves grow in length, they diverge from each other.

(6) In some cases, a larger or smaller number of the lower dwarf-shoots are replaced by the yellow egg-shaped male cones, which therefore appear just after the opening of the resting bud in early summer, and each of which corresponds in position to a dwarf-shoot.

As the young shoot of the current year, arising from the opening bud, grows in length, note at the top (1) the young **terminal bud** which will open next year; (2) the young **lateral buds** which will also open next year, (3) the **young female cone** or cones, each of which clearly corresponds to an ordinary branch or long-shoot, since it has the same position as a lateral resting-bud

517. T. S. Young Stem (Bud Axis).—To see the primary arrangement of the tissues in the stem, cut transverse sections of the axis of a bud, after removing some of the bud-scales; treat some of the sections with potash, others with chlor-zinc-iodine, others with aniline sulphate Note (1) the irregular outline of the section, due to the bases of the scale-leaves and dwarf-shoot buds, (2) the

ring of **vascular bundles,** separated from each other by (3) the **primary medullary rays,** bands of parenchyma continuous externally with (4) the parenchyma of the **cortex** and internally with (5) that of the **pith** In each bundle note the internal **xylem** consisting of regular radial rows of thick-walled lignified elements, and the external **phloem** with cellulose walls, with the **cambium** between them ; in the cortex, note the large **resin-ducts,** the cavity of each duct lined by a layer of small **epithelium** cells

518. T. S. of Current Year Stem.—In transverse sections of the current-year stem note—

(1) The wavy outline of the section, owing to the bases of the scale-leaves

(2) The **epidermis,** a layer of cells with the outer walls thickened and covered by a distinct **cuticle.**

(3) The **cortex,** consisting of more or less rounded cells with cellulose walls, some containing starch, others tannin, while the outer cells have chloroplasts.

(4) The **resin-ducts** in the cortex.

(5) The **cork** and **cork-cambium,** lying immediately below the epidermis, in regular radial rows

(6) The complete ring of **vascular tissue**—**phloem** and **xylem** separated by **cambium,** the originally separate bundles having become joined up by the **inter-fascicular cambium.**

(7) The wavy inner outline of the xylem, the portions projecting into the pith showing the position of the primary bundles and containing the **protoxylem.**

(8) The **pith,** consisting of parenchyma like that of the cortex but without resin-ducts

(9) The **medullary rays,** some extending right from cortex to pith through the whole vascular cylinder (**primary rays**), others passing only part of the distance inwards and outwards from the cambium (**secondary rays**).

(10) The **resin-ducts** in the secondary xylem

Keep the sections for comparison with those of the older stem.

Now cut **transverse, radial longitudinal,** and **tangential longitudinal** sections of a **three-year-old stem,** for detailed examination of the xylem, phloem, cambium, and rays Treat some sections with chlor-zinc-iodine, others with aniline sulphate; others might be stained with safranin and aniline blue and mounted in balsam

519. T. S. of Three-year-old Stem (Fig. 101) —In beginning to examine the **tranverse sections,** note that (1) the cells of the cortex show here and there signs of having grown in length tangentially and undergone divisions by radial walls—to accommodate the increased volume of the vascular cylinder; (2) the phloem has grown in thickness, but its outer portion has undergone distortion; (3) the xylem has grown greatly in thickness, and shows three layers (**annual rings**) owing to the sudden increase in radial diameter of the four-sided lignified elements (**tracheids**) formed in **spring** as compared with those formed in **autumn,** the latter lying immediately within the wide spring tracheids, (4) in each ring, starting from within, the tracheids show a gradual transition from the wider and thinner-walled tracheids of the **spring wood,** becoming more flattened and thicker-walled on passing outwards till the **autumn wood** is reached

Starting with the **cambium,** note that (1) the cambium cells are in very regular radial rows; (2) their walls are very thin, especially the tangential walls; (3) the cells contain abundant protoplasm and a nucleus, especially in the active middle region where the cells are radially narrowest; (4) the cells of the **medullary rays** in the cambium-zone are radially longer than the other cambium-cells

Now trace the ordinary and the ray cells of the cambium inwards into the wood (**xylem**), and note—

(1) The **developing tracheids** immediately within the cambium, with relatively thin walls; the inner layer (that next the cavity) still giving cellulose reactions, while the rest of the wall is more or less strongly lignified

(2) The protoplasmic contents of these youngest tracheids, gradually diminishing in quantity and disappearing on tracing a row of tracheids inwards.

(3) The increasing thickness of the tracheid walls and their complete lignification as they grow older.

(4) The **bordered pits** on the radial walls, each pit appearing in

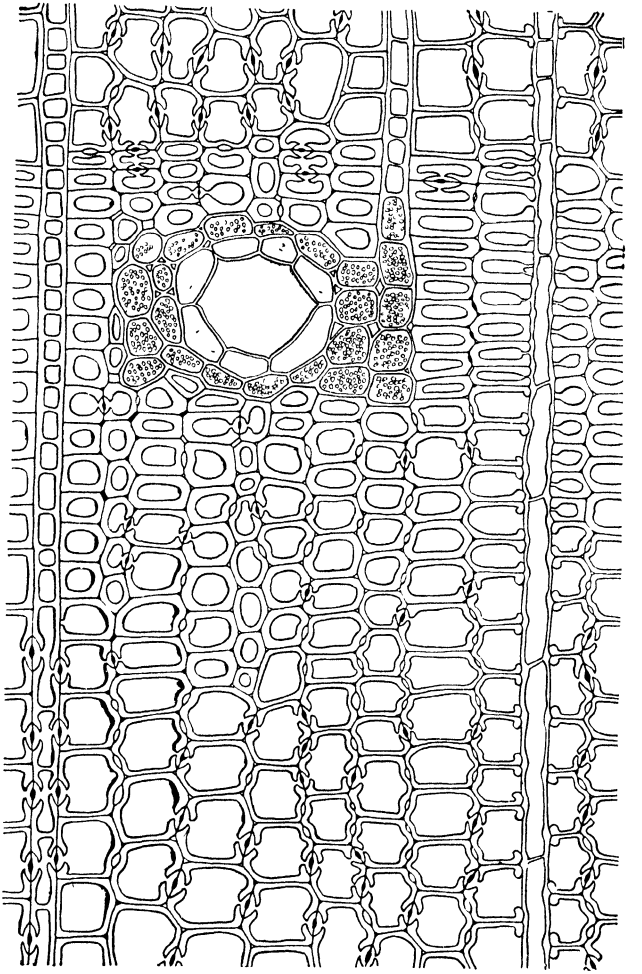


Fig 101.—PINUS Part of the Secondary Wood seen in a Transverse Section of a fairly old Stem For details see § 519

transverse section like a biconvex swelling of the wall. On close inspection some of the pits will show the biconvex lens-like **pit-cavity** across which there stretches the thin **pit membrane** with a thickening (**torus**) in the middle.

(5) The **rays,** consisting mostly of single rows of radially elongated cells with cellulose walls, and either containing protoplasm and nucleus, or appearing empty, the ray contains both kinds of cells, as will be better seen in longitudinal sections

(6) The **resin-ducts** of the wood, lined by epithelium and usually associated with a patch of **xylem-parenchyma** and often also with one of the rays; the ray may either be interrupted by the parenchyma which encloses the resin-duct, or the duct may be joined up to the ray by parenchyma

Next trace the cells of the cambium outwards into the **bast** (**phloem**), and note .—

(1) The phloem constituents are arranged in radial rows, quite as regular as those in the cambiun and xylem

(2) There is a gradual transition from cambium to phloem

(3) The cell-walls on the phloem side of the cambium become thicker, but are not lignified

(4) The **medullary rays** are continuous through cambium into xylem, and have protein and starchy contents

(5) The **sieve-tubes** are radially narrow, with finely-perforated **sieve-areas** on their radial walls.

(6) Here and there are large, and usually starch-containing, **phloem-parenchyma** cells

(7) The sieve-tubes lose their turgidity and become much crumpled and distorted and almost obliterated by the pressure of the cortex on which these older elements of the phloem abut; though the starch-containing phloem-parenchyma cells and those of the medullary rays may remain almost unaltered or even grow larger

(8) Sometimes the outer end of a ray, just outside the phloem, joins on to the parenchyma and epithelium around a resin-duct in the cortex.

520. In **Radial Longitudinal Sections of Three-year-old Stem** (Fig. 102), which must be cut very carefully so as to be exactly radial, note.—

(1) The outer tissues, consisting of epidermis, cork, and cortex parenchyma

(2) The **sieve-tubes,** elongated, with scanty contents, and showing here and there the **sieve-plates** which are confined to the radial walls; each plate is circular or oval, covered with **callus** (stained deeply by aniline blue) when young and functional, and shows a number of finely dotted **sieve-areas.**

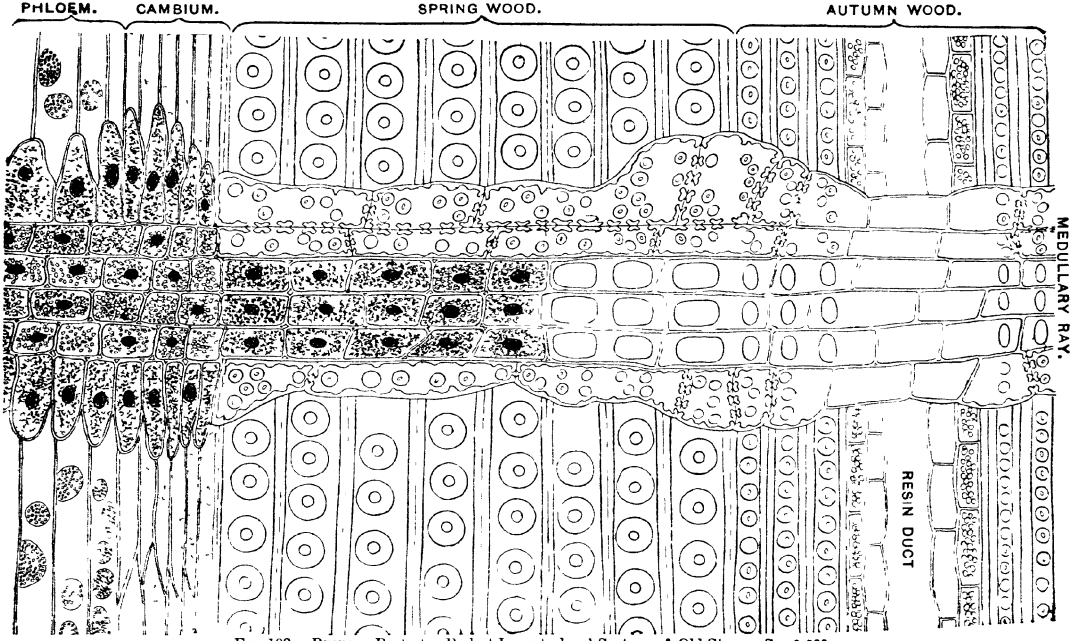


Fig 102 —PINUS. Part of a Radial Longitudinal Section of Old Stem See § 520.

(3) The **phloem parenchyma** cells, arranged in longitudinal rows and with abundant contents

(4) The brown phloem cells with crystals

(5) The phloem portions of the **medullary rays,** in which the middle cells (**"starch-cells"**) contain starch-grains, the upper and lower cells (**"albuminous cells"**) protoplasm but no starch as a rule; these cells have very large nuclei and are often elongated longitudinally and closely applied to the sieve-tubes.

(6) The **cambium,** consisting of long thin-walled cells with abundant protoplasm and long narrow nucleus.

(7) The **rays** running through the cambium and showing here the same features as in the phloem, but the upper and lower cells changing in character on reaching

(8) The **xylem,** where they apparently run across (in reality *between*) the tracheids and have their upper and lower cells empty (**"tracheidal cells"**) and the middle cells (**"starch cells"**) with protoplasm and usually also starch; some rays show only one of these two kinds of cells, or tracheidal cells may occur in the middle of the ray if the latter is rather deep.

(9) The **bordered-pit tracheids,** making up the bulk of the xylem, and seen as long narrow cells with oblique pointed ends, each bearing on the radial walls a single row of bordered pits, each pit appearing as two concentric circles

(10) The annual rings, shown by the narrower and thicker-walled autumn tracheids as contrasted with the wider and thinner-walled spring tracheids.

(11) The narrow spiral or annular **protoxylem tracheids**, lying nearest to

(12) The **pith,** which consists of parenchyma cells—some with cellulose pitted walls, protoplasms, and nucleus, others with lignified pitted walls but no contents.

521. Now cut successive **Tangential Longitudinal Sections** (Fig. 103) through (*A*) the **Phloem,** (*B*) the **Cambium,** (*C*) the **Xylem**—in each case, of course, only the *middle* portion of the section will be *strictly* tangential, *i.e.* at right angles to a radius of the stem

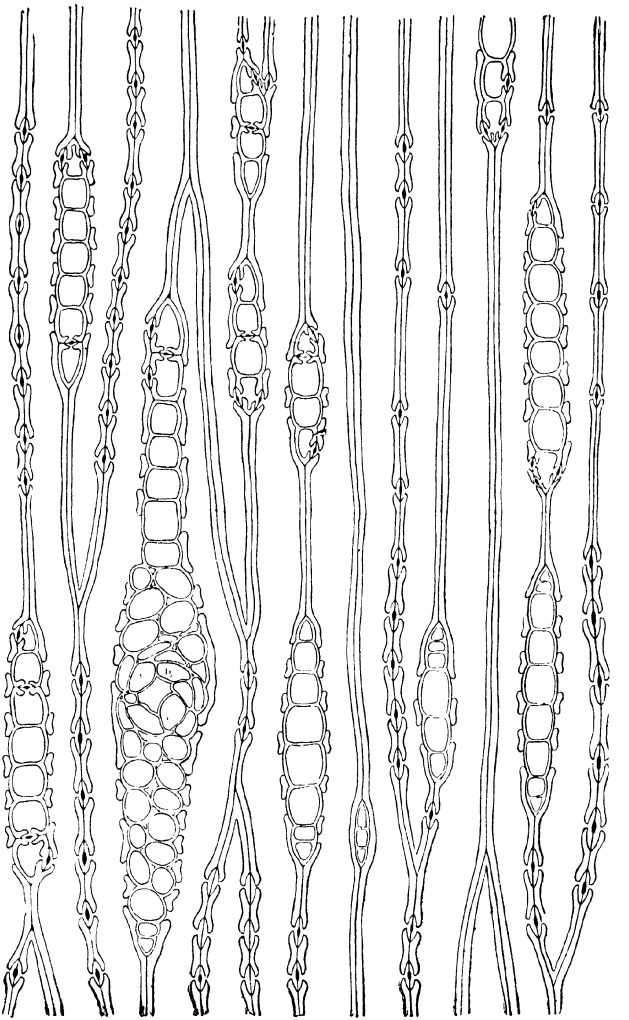


Fig 103 —PINUS. Part of a Tangential Longitudinal Section of the Secondary Wood of an Old Stem. See § 521

In *A* note (1) the **sieve-tubes,** in which the tangential walls, seen in surface view, are quite uniform, while the cut radial walls appear wavy owing to the **sieve-plates;** (2) the **phloem-parenchyma** cells arranged in longitudinal rows; (3) the brown crystal-containing cells; (4) the phloem **medullary rays,** seen as vertically elongated rows of cells, narrower above and below, all with dense contents

In *B* note (1) the ordinary **cambium cells,** elongated vertically, pointed above and below, thin-walled, with dense protoplasm and elongated nucleus; (2) the cells of the cambium **medullary rays,** resembling those of the rays seen in the phloem, and clearly formed by repeated transverse divisions of an ordinary cambium cell.

In *C* note (1) the very long **tracheids** with pointed ends, showing no, or very few, **bordered pits** in surface view on the tangential walls, but showing many cut longitudinally on the radial walls with the same appearance as in the transverse section of the stem, (2) the **medullary rays,** each tapering and pointed above and below, and therefore spindle-shaped (like a biconvex lens cut across).

The height of the ray varies from a single cell up to over a dozen cells, and in the larger rays there may be noted (3) minute intercellular **air-spaces,** (4) two or more cells abreast at the middle of the ray, (5) a **resin-duct** traversing the ray in the radial direction and therefore cut across in the tangential section of the stem, (6) a differentiation of the ray cells into "starch cells" and "tracheidal cells"

Note that in both radial and tangential longitudinal sections one or more **resin-ducts** may be seen cut longitudinally in the cortex and the secondary xylem; note the cavity of the duct, and the epithelium layer lining it

522. T. S. of Root (Young and Old).—Cut transverse sections of the **young root** of a Pine seedling, and note (1) the badly defined **piliferous layer,** which soon becomes disorganised—root-hairs are very seldom present; (2) the **cortex** parenchyma; (3) the **endodermis,** a layer of cells with folded radial walls; (4) the **pericycle,** which consists of several layers of parenchymatous cells, and which merges imperceptibly into the almost indistinguishable

phloem and the **conjunctive tissue.** Accordingly (5) the **xylem bundles** appear to be embedded in a mass of uniform tissue. (6) The xylem consists of from three to six radiating Y-shaped plates, and between the outwardly-directed fork of each Y (**pro toxylem**) there is a **resin-duct** lined by epithelium.

For transverse sections of successively **older roots,** use the roots of older seedlings. Note that (1) the cortex becomes brown and crushed, and is finally thrown off, owing to the activity of (2) the **cork cambium,** which arises in the pericycle and produces (3) a layer of **cork;** (4) **lenticels** may be seen in some sections. (5) A well-marked **cambium** is formed, beginning in the parenchyma within the phloems, spreading round the protoxylems (outside the resin-ducts), and producing internally (6) **secondary xylem**—at first in wedges, but later becoming continuous and showing **annual rings** as in the stem—and externally (7) a continuous band of **secondary phloem.**

523. T. S. and L. S. of Bifoliar Spur.—Get some **young dwarf-shoots** (bifoliar spurs) and (1) cut transverse sections across the lower part of the shoot, (2) cut off the two foliage-leaves close to their bases and cut longitudinal sections of the shoot so as to pass through the two leaves. In (1) note that the two leaves have their flat inner (morphologically upper) faces in contact, their outer (morphologically lower) faces being rounded, while each leaf shows a sharp ridge on each side at the junction of the two faces. In (2), if the section is exactly median, note the abortive growing tip of the axis between the bases of the two opposite foliage leaves.

524. T. S. of Foliage-leaf (Fig. 104).—Cut transverse sections from the middle of a leaf of the current year; mount some unstained; treat some with iodine, others with aniline sulphate. Note:—

(1) The semilunar outline of the section, with nearly flat upper side and convex lower side.

(2) The **epidermis,** a layer of thick-walled cells, covered by a thick **cuticle;** the cell-cavity is often nearly obliterated, being very small and star-shaped owing to fine pits which run out for some distance from the cavity; on heating a section in potash, the cuticle itself is dissolved, though it resists the action of sulphuric acid longer than the other layers.

(3) The **hypodermis,** with very thick and lignified walls, showing fine canals (pits); this layer is in places two or even more cells deep, especially at the two ridges.

(4) The sunken **stomata,** each stoma interrupting the epidermis and hypodermis and having its two **guard-cells**

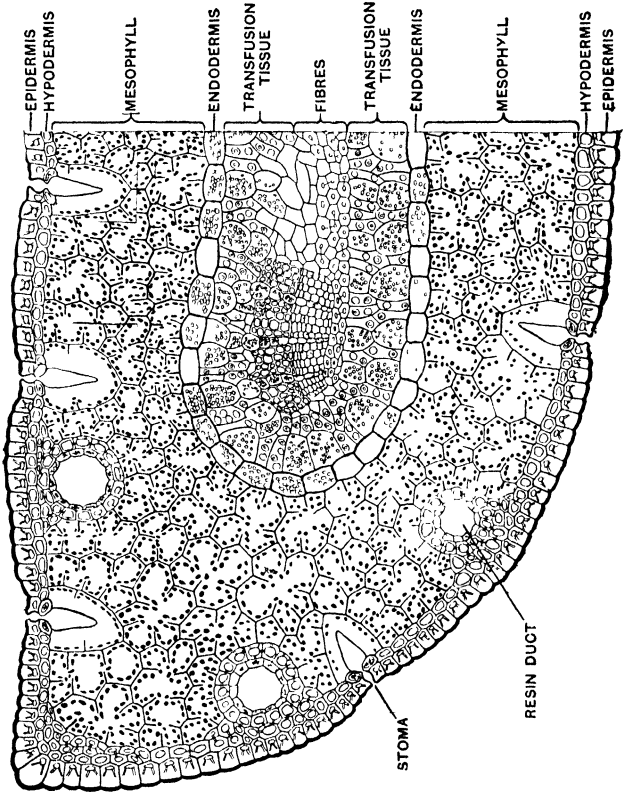


Fig. 104 —PINUS One-half of a Transverse Section of a Foliage-leaf
See § 521.

quite below the epidermis and above an **air-cavity** in the mesophyll.

(5) The **mesophyll,** consisting of rather large polygonal cells containing protoplasm, nucleus, and abundant chloroplasts and having their cellulose **walls infolded** to form projections into the cell-cavity; note the peculiar form (like a letter U in section) of the mesophyll cell or cells below a stoma, the concavity forming the air-space noted above.

(6) The **resin-passages** in the mesophyll, each passage lined by a layer of thin-walled **epithelium** surrounded by a **sclerenchyma sheath.**

(7) The bundle-sheath (**endodermis**) consisting of a single layer of cells.

(8) The many-layered colourless ground-tissue (**pericycle**) in which are embedded

(9) The **two vascular bundles,** each bundle having its **xylem** facing the flat upper side of the leaf and its **phloem** facing the convex lower side.

For the detailed structure of the **pericycle,** unstained sections, or sections stained only with iodine, are usually best. Note that the pericycle consists of two kinds of parenchyma cells, and an irregular band of **sclerenchyma** which lies below the phloem of each bundle and runs across between the two bundles The two kinds of parenchyma cells are (1) cells having cellulose walls and containing protoplasm, protein, and starch ("albuminous cells"), and (2) cells having lignified walls with bordered pits, and no contents ("tracheidal cells"); the two kinds are termed collectively the **transfusion tissue,** the former serving for the passage of elaborated organic food from mesophyll to phloem, and the latter for the passage of water and dissolved salts from xylem to mesophyll.

525. L. S. of Leaf.—Cut medium **longitudinal sections** through a foliage leaf, and note the various tissues described above. The mesophyll is usually seen to show a definite arrangement in fan-like groups of cells; the inner portion (abutting on the endodermis) is less compact than that below the hypodermis, air-spaces being present. Note the long lignified cells (fibres) of the hypodermis, the resin-duct sheaths, and the sclerotic layer below the bundles; the tracheids in the xylem of the bundle; the two kinds of cells in the transfusion tissue.

526. Male Cone (General Characters).—Examine the male cones which are seen on some of the elongating shoots, a number of them being grouped together at the base of the shoot. Note that each male cone arises in the axil of a scale-leaf, is ovoid and about 6 to 8 mm. long, and consists of an **axis** bearing a few basal scales and the numerous spirally arranged yellow **stamens**; each stamen consists of a very short **filament** and a flat scale-like **anther**; the anther ends in a narrow upturned **crest** and bears on its underside two **pollen-sacs** lying side by side Since the male cones are aggregated at the base of the shoot, and fall off after the pollen has been shed, the stem is left bare in this region—look for twigs with successive bare patches formed in this way, alternating with zones bearing the dwarf-shoots and ordinary branches.

527. Longitudinal Sections of Male Cone.—To study the development of the pollen-grains, male cones should be put into alcohol at intervals during spring, and the pollen-sacs teased out in water or glycerine on a slide.

In a **radial longitudinal section** of a cone—taken about end of May—note the **axis**, with **vascular bundles** giving off a single **bundle** to each of the **stamens**; each stamen shows on its underside one of the two **pollen-sacs**, and ends in an upturned **crest.**

Cut **a tangential longitudinal section** of the male cone; the stamens will be cut transversely, and the sections will fall away—in one of these sections, note (1) the two ripe **pollen-sacs** are separated by a vertical **partition** consisting of a few layers of collapsed cells; (2) this partition is thickened above and below, so as to be I-shaped in section, and in the tissue of the upper thickening there is embedded the small **vascular bundle**; (3) the whole stamen is covered by an **epidermis** layer, which is produced on the flank of each pollen-sac into a narrow **wing**; (4) along the lower side of each pollen-sac the epidermal cells are very small, diminishing in size on either side of the **dehiscence-line**; (5) within the epidermis, the cavity of each pollen-sac is lined by a few layers of flattened cells.

528. T. S. of Male Cone.—In a **transverse section** of the male cone, some of the stamens will be seen as T-shaped structures, with a pollen-sac on either side of the **T**.

Some of the sacs in these sections will show the structure of the **sac-wall**; also mount in glycerine a single stamen, and after noting the form of the stamen, flatten it under a cover-glass, and note that the wall of the pollen-sac shows numerous rod-like or U-shaped **fibres** in each epidermis cell.

529. Pollen-grains in various stages of development will have been seen in the sections, lying *in situ* in the pollen sacs. To trace the stages, tease out the contents of pollen-sacs from (*a*) very young cones still enclosed in the resting-bud in winter and early spring, (*b*) exposed cones taken at intervals during May and June Note that:—

(1) The pollen-grains are formed in fours (**tetrads**) from the mother-cells.

(2) The young grain is a spherical cell with a single nucleus.

(3) On two opposite points the outer cell-wall layer (*extine* or *exospore*) shows rounded **projections** containing sap and separating it at these points from the inner layer (*intine* or *endospore*) of the cell-wall.

(4) The two projections grow until each is about as large as the pollen-grain itself, and, as the cone dries, the sap in the projections is replaced by air, so that the grain now bears two rounded balloon-like vesicles or **air-bladders**. That they do contain air is easily seen on mounting in water some grains from a dry cone and noting the dark appearance due to the air, which is expelled from the vesicles on irrigation with alcohol.

(5) Each vesicle has its outer wall (extine) strengthened by a fine network of thickenings.

(6) The grain begins its **germination** before leaving the pollen-sac, two small cells being successively cut off, each by a watch-glass-like wall, from the convex end of the grain—that farthest from the vesicles.

(7) These two **male prothallus cells** are soon obliterated, or may be seen like cracks in the wall of the ripe grain, which shows at this part an **antheridial cell**, cut off by a curved wall from the larger cell which makes up the rest of the grain; this larger cell is often called the **vegetative cell**, or **tube-cell**, since it grows out later to form the **pollen-tube**.

530. Female Cone (General Characters).—Examine female cones of different ages. Note that in June cones are found showing three stages of development, in different

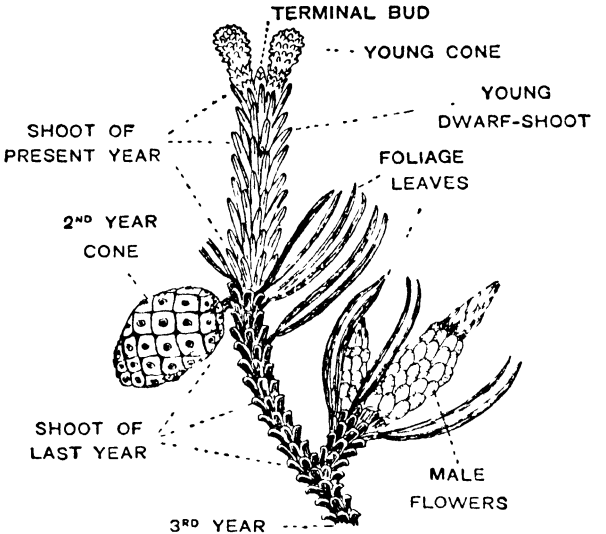


Fig 105 —Branch of *Pinus sylvestris* cut in May
Most of the older dwarf-shoots and foliage leaves removed

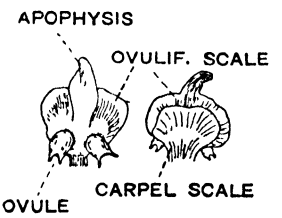


Fig 106 —Scales of Female Cone
From above and below (Carpel-scale = cover-scale),

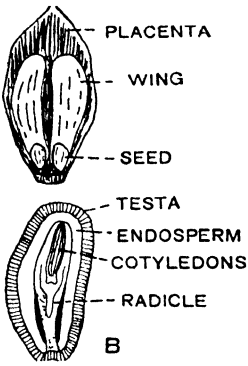


Fig 107 —Seed of *Pinus* A, Surface view, B, Longitudinal section.

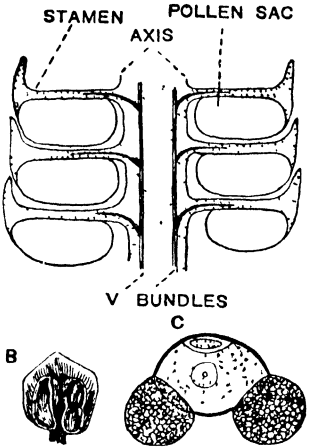


Fig 108 —Male Flower of *Pinus*
A, Part of a median longitudinal section (diagrammatic, B, Stamen (under surface); C, Pollen-grain (two-celled stage, highly magnified)

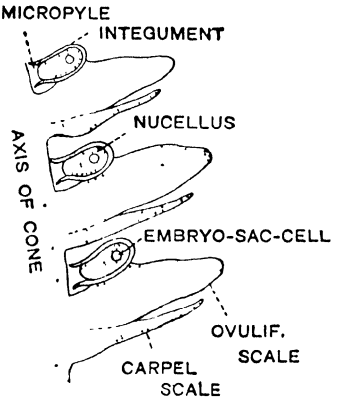


Fig 109 —Young Female Cone of *Pinus* (Part of a longitudinal section diagrammatic)

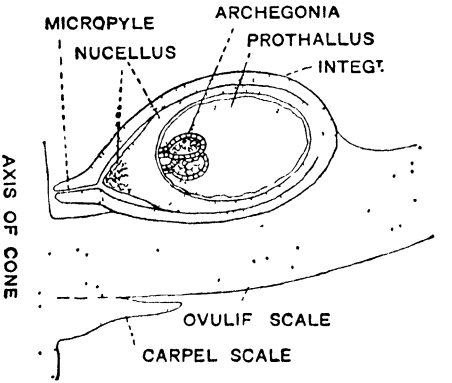


Fig 110 —Ovule of *Pinus* (Longitudinal section—about the time of fertilisation.)

positions on the twig:—(1) the small green cones at the tip of the current year's shoot; (2) the larger but still green and fairly soft cones, placed laterally at the top of last year's shoot; (3) still larger but brown and woody cones, placed laterally at the top of the shoot of the year before last and having the scales separated so that the seeds are exposed. In each case, split the cone longitudinally and sketch the cut surface, after noting and sketching the external characters.

(*a*) In the **young female cone,** note that (1) the cone-bud corresponds in position to a young long-shoot bud at the top of the young axis in May when the compound bud has opened; (2) instead of remaining small, as the young shoot-buds do in the first year, the young cone grows actively, and from being about 5 mm. long in May has by August grown much larger, though still green; (3) at first, when very small, the cone stands straight out in line with the main axis; (4) a little later its stalk curves backwards; (5) cut longitudinally, the cone shows an **axis** with numerous **scales,** bearing the **ovules** on their upper side close to the cone axis; (6) on dissection of the cone, each cone is seen to bear two ovules side by side, the flaring trumpet-like **micropyle** of each ovule facing the cone axis.

(*b*) Carefully remove a whole carpel and placental scale from the young cone, and treat with potash or eau de javelle or carbolic acid to clear it. (1) Mount it with the upper (ovule-bearing) side uppermost, and note (*a*) the outline of the placental scale, (*b*) its thickened outer portion, (*c*) the two ovules at its lower end, lying side by side, and each having the micropyle expanded laterally into little forceps-like processes. (2) Turn the preparation over, and on the lower side note (*a*) the form of the small thin carpel, (*b*) the large placental scale which grows beyond it.

(*c*) In the **one-year-old cone**—more correctly, **cone of second year**—note that (1) the cone remains closed during its first winter; (2) all the second season is taken up in the further growth of the cone; (3) the scales have become

harder and greatly thickened, especially at their outer ends; (4) the two ovules (now the **seeds**) on each scale have grown larger.

(*d*) In the **third-year cone,** note that (1) the scales have become brown and woody; (2) as the cone dries the scales gape asunder—starting at the top—so as to expose the ripe seeds, which are confined to the middle portion of the cone, the uppermost and lowest scales being sterile; (3) the **seeds,** two on the upper side of each fertile scale, each seed having a thin **wing;** (4) the seeds are easily detached from the scales and carried away by wind, leaving the empty cone on the tree—from which it usually falls later in the autumn or winter of the third year.

531. L.S. of Young Female Cone.—In radial longitudinal sections of the young current-year cone, note

(1) The relatively thick **axis,** bearing scales of two kinds—(2) small **bract scales** or **carpels** each having in its axil (3) a much larger and thicker **placental** or **ovuliferous scale,** which alone reaches the surface so as to be visible from the outside of the young cone. (4) On the upper surface of each placental scale, close to the axis, there may be seen one of the two **ovules** which lie side by side.

Note that in a section cutting an ovule in the middle, there are seen (1) the single **integument** several layers thick, with (2) the rather wide **micropyle** facing the axis, (3) the central mass of parenchyma tissue, **nucellus (megasporangium),** containing towards its lower end (4) the **embryo-sac cell** or **megaspore,** a large cell usually surrounded by a sheath of nucellus cells with dense contents. (5) If pollination has occurred, **pollen-grains** may be seen at the top of the nucellus, within the micropyle.

Note that the placental scales of the young cone ready for pollination are separated at their ends, to allow pollen to reach the ovules; after pollination, the scales grow thicker and close up, not opening again until the seeds are ripe and ready to be shed.

532. L. S. of Ovule with Archegonia.—Split open longitudinally, and dissect some of the ovuliferous scales from, a **second-year cone** taken in June Note that the scales and the ovules have grown considerably in size Cut off the upper part of the ovuliferous scale, above the ovules, embed an ovule in pith, and cut longitudinal sections through it. Note (1) the tissue of the **scale,** with **vascular bundles** and **resin-ducts;** (2) the **ovule,** showing (3) the **integument** with the **micropyle,** within which is (4) the **nucellus** and, within this again, (5) a mass of thin-walled tissue, the **endosperm** or **female prothallus** (formed by germination of the megaspore seen in the nucellus of the young ovule). (6) In the endosperm, near the upper (micropylar) end, there are seen (usually) two of the large **oospheres,** each containing numerous **vacuoles** and covered by a **layer of small cells** ("nutritive jacket").

In good sections, taken in just the right plane, note that the nucellus (whose lateral portions are greatly compressed and partially obliterated owing to the growth of the endosperm) forms a thick apical **cap** over the top of the endosperm; at the top of this nucellar cap, just below the micropyle, there is a cup-like depression, in which **pollen-grains** may be seen. The **pollen-tubes** proceeding from these grains traverse the tissue of the cap and reach the upper surface of the endosperm and there widen into a swelling (tip of tube). At the top of each oosphere there is a narrow **neck** continuous with those forming the "nutritive jacket" and consisting of two or three tiers of small cells—the jacket represents the venter of the **archegonium;** in some cases there may be seen the nucleus of the oosphere itself and at the top (just below the neck) a **ventral-canal-cell,** which disappears at fertilisation of the oosphere

533. Ripening Seed with Embryos.—Dissect some scales from the second year cone taken in August, for the study of the **ripening seeds;** since the integument or seed-coat is now rather hard, it may be better to dissect it off carefully with a knife-point or needles. After removing

the coat, treat the endosperm of one seed with potash to make it transparent, and place that of another in water and tease out with needles the numerous **embryos** which are embedded in the endosperm in a central cavity. Longitudinal sections should also be cut of the endosperm, to see the embryos *in situ*. Note the cylindrical or club-shaped **embryos,** each carried at the end of a twisted **suspensor** consisting of thin-walled transparent cells. In the largest embryo in each seed, note the parts named in the next paragraph (radicle, hypocotyl, cotyledons, plumule)

534. Structure of Seed.—Examine ripe seeds, and note:—

(1) The **wing** is readily detached from the seed itself.

(2) The thick and hard **seed-coat.**

(3) The **micropyle** at one end—demonstrated by dipping the dry seed into hot water

(4) The **endosperm,** seen on removal of the seed-coat—test for starch, proteids, oils, and also examine sections with microscope, noting results

(5) The membranous layer, usually reddish, covering the endosperm and representing the remains of the **nucellus.**

(6) The **embryo,** lying in an axial cavity in the endosperm, and having a long **hypocotyl.** This merges into the **radicle** at the micropyle end of the seed and at the other end bears a circle of narrow **cotyledons**—about six in Scots Pine—surrounding the small **plumule.** Cut soaked seeds open, some longitudinally and others transversely, and note that in most cases there is attached to the radicle a string-like **suspensor** greatly coiled up, in some seeds several suspensors may be found, each with an embryo at its free end—the end farthest from the micropyle.

535. Germination of Seed.—Sow soaked seeds, and note the stages in germination:—(1) the coat bursts, owing to swelling of endosperm and embryo; (2) the radicle grows out and curves downwards; (3) the hypocotyl elongates, carrying up the seed; (4) the

cotyledons lengthen and gradually escape from the seed-coat, after absorbing the endosperm, the empty coat then falling away; (5) after a time, the plumule grows up, bearing needle-like foliage-leaves Various stages in the growth of the seedlings may be found on collecting self-sown seedlings near the Pine trees.

The seedling shows two striking features (1) The cotyledons turn green in the absence of light—to test this, grow Pine seeds in darkness in the same pot with Bean or Pea seeds, and note that the latter give rise to etiolated chlorophyll-less seedlings, while the Pine seedling becomes green (2) The earliest foliage-leaves, borne by the plumule during the first year of its growth, are arranged singly and spirally on the stem, but later they give place to scale-leaves, in the axils of which arise the characteristic dwarf-shoots with paired foliage-leaves.

Yew (Taxus Baccata).

536. General External Characters.—In the Yew and its allies, the female "flower" consists simply of a single erect ovule borne at the end of an apparently simple axillary bud or short branch of the stem, so that there is no female cone. Yew also differs from Pinus in that the leaves are borne directly upon the ordinary branches or long-shoots, not on dwarf-shoots or spurs.

The following **general characters of the tree** should be noted:—

(1) Its usually "deliquescent" habit, owing to the fact that early in life some of the branches tend to grow erect and give the tree several "leaders" in place of a single leading or main shoot—this partly accounts for the deeply channelled and ridged character of the trunk, but the ribbing of very old Yew trunks is chiefly due to the upgrowth of strong erect shoots from the base, these encircling the trunk and fusing with it and with each other.

(2) Its extremely slow growth, as compared with that of Pines, connected with which is its ability to endure greater shade than almost any other Conifer

(3) The reddish-brown scaly bark, which comes off in thin flakes—also connected with the shade-enduring power of Yew.

(4) The very accommodating character of the tree, which can grow in any position and any soil, and its power (unusual in Conifers) of producing abundant buds, which adapts it so well for hedge-making and for cutting into ornamental shapes.

(5) The flat, narrow pointed, and spirally arranged leaves, each continuous at the base with a ridge running down the twig.

(6) The radiating arrangement of the leaves on the erect branches, and their two-rowed arrangement (owing to twisting of the short petioles) on the flanks of the horizontal and inclined branches.

(7) The small resting-buds, scaly but not resinous, arising in the axils of many of the leaves.

(8) The outgrowth of the buds at the ends of the twigs into branches, arranged chiefly on the flanks of horizontal and inclined shoots.

(9) The frequent formation of branches from hitherto dormant buds on older parts.

(10) The dark green upper side and lighter lower side of the leaf, which has a prominent midrib

(11) The persistence of the leaves for several years.

537. Structure of Stem.—In sections of the stem, note that though the general arrangement of the tissues is similar to that seen in Pinus, the Yew has **no resin-ducts** in any part of the plant, and the pericycle is sclerenchymatous.

538. Structure of Leaf.—In transverse sections of the leaf, note (1) the epidermis on the convex upper side of the leaf has no stomata; (2) there is **no hypodermis**; (3) the **mesophyll** is distinguished into upper **palisade tissue** and lower **spongy tissue**; (4) there is a single central **vascular bundle**, with xylem above and phloem below; (5) the epidermis on the concave lower side has numerous stomata

539. Structure of Root.—In transverse sections of the root, note (1) the diarch **xylem** plate, with protoxylem at each end; (2) the **phloem** groups, one on either side of the xylem plate; (3) the **pericycle**, one or two layers deep at the ends of the xylem

plate but widening out into several layers outside the phloems; (4) the **endodermis**, with one or two layers of similarly thickened cells outside it belonging to (5) the cortex parenchyma; (6) the **piliferous layer**, showing **root-hairs** in young root.

In an older root the process of **secondary thickening** is very easy to follow; the cambium arising on either side of the xylem plate (which is still recognisable in the centre of quite old roots) produces secondary xylem internally, and secondary phloem externally, the primary phloem becoming crushed; the cork cambium arises from the outermost layer of the pericycle, producing cork which cuts off all the outer tissues, the latter becoming organised and thrown off.

In a still older root, note the central primary xylem plate; the secondary wood showing annual rings; the cambium, the relatively narrow secondary phloem; the pericycle; the cork.

540. Male Flower.—The Yew is **dioecious**; pollination occurs in early spring (February or March), and the seed ripens in the same year.

Examine male flowers in early spring, and note (1) the small size, globular form, and yellow colour of the flower; (2) its origin in the axil of a leaf, on the underside of a twig produced in the previous season; (3) the short stalk-like lower part of the **axis**, bearing a number of brown convex **scales**, which protected the male bud during winter; (4) the spirally arranged **stamens**, 6 to 15 in number, on the upper part of the axis; (5) the **peltate** or umbrella-like form of the stamen, its pentagonal or hexagonal disc-like head bearing about six **pollen sacs** hanging around the stalk and fused together laterally.

541. L. S. of Male Flower.—In a longitudinal section of the male flower, note (1) the axis, with its ring of vascular bundles; (2) the peltate stamens, each showing, if cut through the middle, the stalk with a vascular bundle and the expanded head from which arises on either side one of the pollen-sacs; (3) the scales given off from the lower portion of the axis.

Note that the wall of each pollen-sac has a very distinct fibrous or dehiscence layer, with the U-shaped fibres on the cell-walls better developed than in Pinus; also that the pollen-grains have no vesicles.

When the male flower opens in spring, by the elongation of its axis and the thrusting of the stamens beyond the scales, the pollen-sacs on drying open in a curious manner—the wall of each sac splits at the base and along each side, and the outer portion of

the sac curls outwards, so that the stamen appears like an umbrella turned inside out, the pollen remaining in the pocket-like cavities until removed by the wind.

542. Female Flower.—The female flower is also formed from a bud arising in a similar position to that of the male, but the single ovule does not arise *directly* from the axis of this bud—it stands at the end of a very short branch arising just below the apex of the bud. The bud bears about ten spirally arranged scales, and in the axil of the uppermost scale there arises a small branch, which pushes aside the true apex; this little branch bears three pairs of opposite scales and ends in an ovule.

Cut longitudinal sections of the bud, which is easily recognised on the female tree by its position on the underside of the twig, and its ovoid and pointed shape, and try to make out the arrangement just described From the micropyle there oozes a drop of sticky liquid, by which the pollen-grains are caught when the flower opens.

543. L. S. of Ovule, etc.—In a median longitudinal section of the ovule, taken in March, note (1) the single **integument** with the **micropyle**; (2) the **nucellus**, forming the termination of the axis of the female shoot; (3) the **embryo sac** or **megaspore**, placed deeply in the nucellus; (4) the **aril**, a small ring-like outgrowth around the base of the integument, *i.e.* seen in section as a short projection on either side

In sections taken later in summer, note (1) the endosperm; (2) the archegonia; (3) the single embryo formed from the oospore; (4) the growth of the aril, which eventually protudes beyond the integument. The aril, at first green, forms a bright red fleshy cup, covered with waxy bloom, and invests the hard brown or purplish seed—giving a superficial resemblance to an acorn and its cup

The embryo has **two cotyledons**, which, like the spirally arranged leaves that succeed them in germination, resemble the ordinary foliage-leaves formed later

CYCAS.

544. Cycads.—Cycas and its allies, forming the **Cycadaceae,** are tropical and subtropical plants, most of the living members of this family being rare and very locally distributed, though in ancient times (especially in

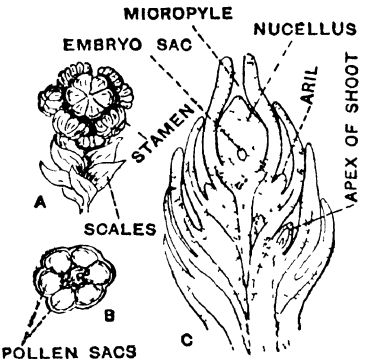


Fig 111 —Flowers of Yew

A Male Flower, B, Single stamen seen from below, C, Longitudinal section of bud containing female flower

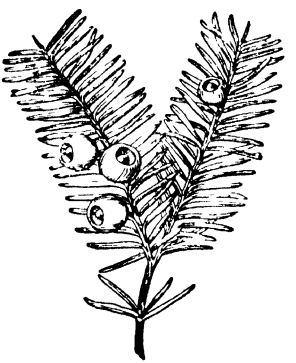


Fig 112 —Twig of Yew with "Fruits"

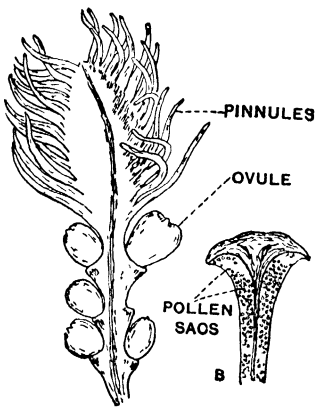


Fig 113 —Sporophylls of *Cycas revoluta*
A, Carpel, B, Stamen

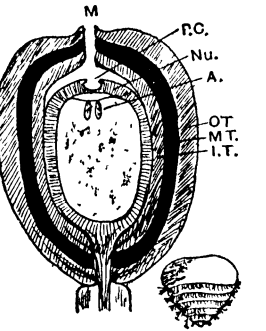


Fig 114 —Diagram of L V S through Ovule of *Cycas*, to the right a Mature Sperm
P C, Pollen chamber, Nu, Nucellus, A, Archegonia (two) lying in the female prothallus or gametophyte (endosperm), contained in the Embryo Sac, O T and I T, Outer and Inner fleshy layers of Testa, M T Middle stony layer of Testa

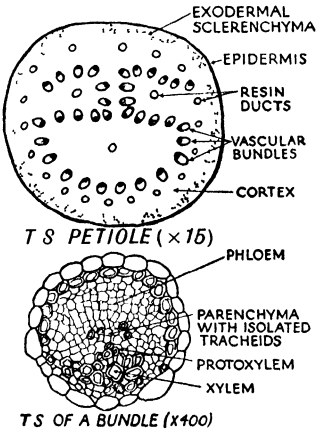


Fig 115 —*Cycas*

the Mesozoic Period), before Angiosperms had begun to form the dominant larger vegetation of the earth, Cycads were very abundant and very widely distributed. The Cycads are the lowest of the living Seed-Plants, and in some respects show marked affinities with the Ferns

545. General External Characters.—Cycas revoluta, a Japanese species, the pith of which yields a kind of sago, is often cultivated in hot-houses in this country, and large specimens may be seen at Kew and other botanic gardens; the leaves are sometimes used for memorial wreaths and may be obtained from florists.

In a large specimen, note

(1) The general resemblance of the plant to a Tree-Fern, various kinds of which can also be seen in botanic gardens.

(2) The relatively short, thick (up to nearly a yard in diameter in old plants), and usually unbranched **stem**, bearing at the top a rosette or crown of large **foliage-leaves.**

(3) The crowded **leaf-bases** and **scales** covering the bare lower part of the stem.

(4) The stout-leaf-stalk, bearing on either side a row of narrow leathery green leaflets (**pinnae**).

(5) The strong midrib running up the middle of each pinna, which is convex above and concave below (revolute).

(6) At the top of the stem, in the centre of the rosette of expanded leaves, there may be seen either

(7) A **bud** covered by scale-leaves, or

(8) Young foliage leaves in different stages of expansion, with each of the pinnae rolled up in a **circinate** manner (the main leaf-stalk itself is not rolled up), or

(9) A **male cone,** not unlike the seed-cone of a Pine, or

(10) A series of **carpels,** pinnate leaf-like structures covered with brown woolly hairs; the male cones and the carpels are borne on separate plants.

Each year, as a rule, the apex of the shoot produces first a series of scale-leaves (corresponding to leaf-bases with undeveloped blades), then a series of foliage-leaves (which were protected by the scales inside the bud)

In the case of a female plant, the carpels (megasporophylls) are clearly developed in place of several spirals, forming an apparent whorl, of ordinary leaves, for in the centre of the "rosette" of carpels the growing vegetative tip of the shoot may be seen, covered by scale leaves In this respect Cycas itself differs from, and is more primitive than, the other living Cycads, in which the carpels are borne in female cones very similar in form to the male cones.

546. Structure of Leaf.—The structure of the leaf of Cycas (and of other Cycads) is of great interest. Examine transverse sections of (1) petiole, (2) leaflet. In the **petiole** the bundles are arranged in a ring which on cross section resembles the Greek letter omega, Ω. These leaf-bundles, which are also seen in transverse section of a **leaflet**, are **mesarch**: on the outer side of each bundle (the lower side in the leaflet) there is phloem, then some parenchyma containing a number of tracheids, then the protoxylem forming the apex of a well-marked triangular xylem strand.

That is, the protoxylem is in the middle of the xylem, and the greater part of the xylem is **centripetal** (developed towards the centre of the petiole or the upper side of the leaflet), the **centrifugal xylem** (corresponding to the whole xylem in the stem or leaf of a Dicotyledon) being relatively small in amount

The cells of the spongy mesophyll of the leaf is modified on either side of the bundle, forming **transfusion tissue** which spreads out horizontally from the xylem and phloem of the bundle.

The **stomata** on the underside of the leaflet are peculiar; the guard-cells of each stoma are arched over by a cone-like outgrowth of the epidermis and cuticle.

547. Normal and Coralloid Roots of Cycas.—The root of Cycas resembles that of Yew in general structure, but sometimes rootlets grow up to the surface of the soil and branch in a coral-like manner. These "coralloid" roots usually show, at about the middle of the cortex, a

very conspicuous zone containing Nostoc chains; it would appear that the coralloid growth is due to hypertrophy set up by Bacteria, the Nostoc slipping in afterwards and inhabiting the middle cortical tissue as an endophyte—since these roots come to the light, the arrangement is probably a symbiosis

548. Stamens.—Each male flower (cone) consists of an axis bearing spirally arranged stamens.

Note that each stamen consists of a hard thick scale with an expanded head and bearing on its lower surface a large number of sessile egg-shaped **pollen-sacs (microsporangia)**, which are arranged more or less definitely in small groups (**sori**); the heads of the scales are hexagonal, and they fit closely together until the pollen is ripe, then they separate and each pollen-sac opens by a slit.

549. Carpels.—Note that each carpel (megasporophyll) is a pinnate leaf, much smaller than the foliage-leaves; the **leaflets** are only developed on the upper part of the leaf, and are narrow, thick, and woolly, while on the margins of the lower portion there are large **ovules** in place of leaflets

The **ovule**, which becomes very large before fertilisation, contains when mature a large mass of **endosperm (female prothallus)**, surrounded by a thin layer of **nucellus** tissue, which is covered by a thick **integument** consisting of a fleshy outer layer and a hard inner layer

APPENDIX I.

REAGENTS REQUIRED FOR MICROCHEMICAL AND PHYSIOLOGICAL EXPERIMENTS.

Acetic Acid.—A dilute (1 to 5 per cent.) aqueous solution of acetic acid (1) dissolves calcium carbonate with evolution of bubbles of carbon dioxide; (2) dissolves the globoids in protein grains, but does not affect crystals of calcium oxalate; (3) dissolves most ethereal oils, while most fatty oils are insoluble in it; (4) brings out clearly the nuclei of cells, and is for that purpose often used along with methyl green; (5) corrects the too great transparency often produced by the clearing action of potash. Stronger solutions, or the glacial acid, serve for (6) maceration of herbaceous organs, isolating the cells; (7) the clearing of dense growing-points, etc. (8) the preparation of various fixatives.

Alcohol.—(1) For dehydration of specimens to be mounted in Canada balsam, absolute alcohol is necessary. For most other purposes, ordinary methylated spirit will answer. This is used for (2) the solution of chlorophyll and other pigments, wax, ethereal oils, some fatty oils, resins, etc.; (3) the precipitation of sugars, inulin, proteins, asparagin, etc; (4) the fixation and hardening of tissues

If material becomes brown and discoloured in alcohol, it may be decolorised by placing it for a few days in 100 c.c of alcohol to which is added about 1 c.c of strong sulphuric acid and one or two crystals of potassium chlorate, and then transferring it to alcohol or to equal parts of alcohol, glycerine, and water.

Commercial alcohol (methylated spirit) is about 95 per cent alcohol. In making from this alcohols of different strengths, proceed as if it were absolute (100 per cent.) alcohol. If, however, it is desired to dehydrate the methylated spirit, so that it may be used as absolute alcohol, heat some copper sulphate in an iron pot (to drive off the water by crystallisation), place the powdered salt in a bottle, pour the 95 per cent. (methylated) alcohol in, and keep the bottle tightly stoppered.

Alkannin.—Use either the alcoholic solution, made from the roots of Alkanna tinctoria, or sections of the dry root itself, in making tests for (1) oils and resins, which are stained pink, (2) suberised and cutinised walls, also stained pink but often requiring the action to continue for some hours.

Ammonia may be used (1) as a clearing agent instead of potash, its action being less vigorous; (2) in the xanthoproteic test for proteins, which give a yellow colour with ammonia, deepening to orange on adding nitric acid

Ammonium Molybdate, as concentrated solution in a saturated solution of ammonium chloride, gives a yellow precipitate in tissue containing tannins.

Aniline Blue, generally used in alcoholic solution, is a good general stain, and is especially good for Algae and for nuclear structure and mitotic figures. It makes a good stain for cellulose walls when used along with safranin, which remains in the lignified walls, thus giving an effective double staining.

Aniline Oil may be used to dehydrate specimens to be mounted in balsam, since it will absorb about 4 per cent. of water, and may be kept dehydrated by placing in it a piece of solid potash, which is insoluble in the aniline oil. After treatment with aniline oil, the sections may be at once mounted in balsam.

Aniline Sulphate makes lignified walls yellow, leaving the other tissues unstained Make a saturated solution in water, filter, and add a few drops of sulphuric acid till the solution is distinctly acid in reaction. If aniline chloride is used, add hydrochloric instead of sulphuric acid to the solution

Asparagin.—Saturated solution in water is used as a test for asparagin precipitated in tissues by the action of alcohol; if the crystals consist of asparagin they will be unaffected, while crystals of other soluble substances would be dissolved by the asparagin solution.

Barfoed's Solution.—To 200 c.c. of 5 per cent. solution of neutral acetate of copper add 5 c.c. of 40 per cent. acetic acid When this solution is heated with glucose, red copper oxide is precipitated; no reaction is given with cane or malt sugar or with dextrin.

Barium Chloride is used to distinguish calcium oxalate from calcium sulphate. When the reagent is added, calcium oxalate if present is left unchanged, while a fine granular layer of barium sulphate is formed on crystals of calcium sulphate.

Baryta Water, or aqueous solution of barium hydrate, prepared by adding excess of the barium hydrate to water and filtering, is used in physiological experiments to absorb carbon dioxide, barium carbonate being formed as a white precipitate

Boracic Acid is used for mounting sections containing mucilaginous membranes. The sections are cut from dry material and placed in 10 per cent. solution of neutral lead acetate to harden the gummy layers, then they are stained with methyl blue, washed in water, and mounted in 2 per cent. solution of boracic acid.

Borax Carmine is especially useful for staining protein grains and the cells of Algae, also for differentiating cell-contents from cell-walls when the sections are afterwards stained with methyl green. Dissolve in 100 c c. of water 4 grams of borax, add 3 grams of carmine, which will dissolve on gently heating; add 80 c c of strong alcohol, and filter Preparations stained with carmine should be mounted in glycerine.

Calcium Chloride is used (1) solid and dried well by heating, for absorption of water in experiments on transpiration; (2) in strong aqueous solution for the clearing of growing-points, etc.

Calcium Nitrate is used (1) as an ingredient in Knop culture solution; (2) as a test for presence of oxalic acid, calcium oxalate being precipitated in crystals (3) To demonstrate the lamellae in starch grains, place sections in strong aqueous solution of methyl violet, then treat with dilute solution of calcium nitrate—the methyl violet is precipitated in the less dense lamellae of the grains

Canada Balsam, dissolved in xylol or benzole to form a syrup, is the best medium for making permanent mounts of sections, which must be previously dehydrated by means of absolute alcohol and then treated with oil of cloves, xylol, or cajeput oil If the balsam gets too thick, thin it with xylol; if too thin, thicken it by simply leaving the bottle open for some time to let the xylol evaporate off.

Callus Reagent (Russow's).—Mix equal volumes of chlor-zinc-iodine and of potassium iodide solution of iodine. This stains the callus of sieve-tubes deep brown.

Cane Sugar is used (1) as strong aqueous solution, along with sulphuric acid, as a test for proteins, giving a red colour; (2) in 5 to 20 per cent. solution as a nutrient medium for the growth of pollen-tubes, etc ; (3) in more dilute solution in which to mount living cells which are often injured by being mounted in water.

Carbolic Acid (Phenol) is used (1) in small quantities as an antiseptic, *e g.* in experiments on digestion of proteins; (2) to prevent growth of Fungi in glycerine or glycerine jelly; (3) as a clearing agent—sections, entire leaves, etc , after treatment with alcohol are placed in 3 parts of turpentine and 1 of carbolic acid, and soon become very transparent; (4) as a clearing agent, together with turpentine, before mounting alcohol-treated specimens in balsam; (5) along with hydrochloric acid, as a test for lignin—dissolve the carbolic acid in warm hydrochloric acid, and the solution will turn lignified walls green on being exposed to light.

Chloral Hydrate.—Dissolve 5 parts (in grams) of chloral hydrate in 2 parts (in c.c) of water. This solution is one of the best clearing agents for showing structure of leaves, crystals in tissues or whole leaves if not too thick, pollen grains, embryo in ovules and archegonia, etc.

Chloral Hydrate Carmine is useful for clearing pollen grains and staining their nuclei at the same time. Add 1 gram of carmine and 4 c c of strong hydrochloric acid to 30 c c of strong alcohol, and warm for 15 minutes on a water-bath. After cooling, add 25 grams of chloral hydrate, and filter the solution until clear

Chloral Hydrate Iodine is used to demonstrate the presence of starch in chloroplasts, or in any position where it is obscured by other substances. Dissolve 5 parts of chloral hydrate in 2 of water, and add enough powdered iodine to leave an excess undissolved after standing for some time; shake before using. Bleach leaves with alcohol and lay them in the solution for an hour or longer, to get the best results

Chloroform is used as a solvent for oils, rubber particles in latex, etc; as an anaesthetic in experiments on movements, irritability, etc.; and as an antiseptic in digestion experiments.

Chlor-zinc-iodine (Chloroiodide of Zinc, Schultze's Solution) is one of the most useful microchemical reagents for general work. It may be bought ready made, or it may be prepared as follows.—Dissolve 30 grams of zinc chloride, 5 grams of potassium iodide, and 1 gram of iodine in 14 c.c of water With this reagent, which should be kept in darkness, cellulose walls turn blue or violet, lignified walls yellow, cutinised and suberised walls yellow or brown, and proteins brown, while starch grains swell and turn blue.

Chlorophyll Solution, prepared as directed in § 247, in strong alcoholic solution, may be used to demonstrate suberised and cutinised walls Place sections of stems, etc, in the chlorophyll solution for an hour or so in darkness; the corky and cutinised walls are stained green, while the cellulose and lignified walls remain unstained. The solution will not keep, but should be freshly prepared when required

Chromic Acid.—(1) A saturated aqueous solution is used for maceration; thin pieces of the tissue are placed in it for a minute or two, then washed in water. (2) The strong solution dissolves cellulose and lignified walls, but cutinised walls resist its action (3) A 1 or 2 per cent. solution brings out the stratification of cell-walls clearly. (4) A 1 per cent. solution gives a brown precipitate with tannins. (5) The weak solution is used for killing and fixing tissues; the material should be well washed with water and dehydrated gradually in ascending series of alcohol (30, 50, 70, 90, and strong alcohol). (6) Flinty skeletons of Diatoms, flinty incrustations of Equisetum epidermis, etc, may be prepared by placing the material in strong sulphuric acid until it becomes black, then in 20 per cent. chromic acid for a few minutes, and washing in water.

Copper Acetate.—(1) Used, with iron sulphate, in detection of tannin, a brown precipitate being given. Place sections in

saturated aqueous solution of copper acetate for about a week, then on a slide with a drop of 2 per cent solution of iron sulphate for a few minutes; wash in water and in alcohol, and mount in glycerine (2) To demonstrate glucose in cells, lay the sections in alcoholic solution of copper acetate, mixed with equal volume of alcoholic solution of caustic soda and a little acetic acid, and bring to boiling on a water-bath. Glucose being insoluble in alcohol, the copper oxide indicating the presence of glucose is deposited in the cells containing this sugar. (3) To detect presence of resin, lay sections for a week in strong aqueous solution of copper acetate; the resin will be coloured bright green.

Copper Sulphate is used in the preparation of cuprammonia and of Fehling's solution and the Biuret test For the blue solution required in experiments with double-walled bell-jars, add ammonia to 10 per cent. solution of copper sulphate, until the precipitate first formed is redissolved

Corallin, dissolved in a saturated aqueous solution of sodium carbonate to form "corallin soda," is useful in staining the callus of sieve-tubes. It also gives a pink colour to starch grains and to lignified walls.

Cuprammonia is best freshly prepared when required, in one of the following ways.—(*a*) Put copper filings into a bottle with a ground-glass stopper, pour in enough strong ammonia to cover the filings, and shake gently. When the solution will dissolve cotton-wool, it is ready for use. (2) To a solution of copper sulphate in water add dilute caustic potash, collect the precipitate on a filter, and dissolve it in a little ammonia.

Dahlia.—(1) This stain may be used in very dilute aqueous solution—about 0 002 per cent.—to stain living nuclei, *e g.* if epidermis or hairs are placed in the solution for some hours (2) To demonstrate the structure of pyrenoids, fix the material in equal parts of 10 per cent solution of potassium ferricyanide and 50 per cent acetic acid, then stain with aqueous Dahlia solution, and treat with dilute potash to make the pyrenoids swell

Diastase may be bought ready prepared, either as powder or as extract of malt; it is also present in "liquor pancreaticus" and in "holadin." To prepare diastase, germinate Barley between pieces of wet blotting-paper until the shoot is 2 or 3 mm. long; then dry the Barley on a water-bath, powder it, and pour over 10 grams of the powder a litre of water containing 2 c.c. of chloroform, let stand for a day, filter, add a little chloroform, and keep the extract in a dark place in a stoppered bottle.

Diphenylamine is used to test for nitrates in plant tissues or in soils. Dissolve 0 05 gram of diphenylamine in 10 c.c. of strong sulphuric acid. The presence of nitrates is shown by a blue colour.

Eau de Javelle —To make this reagent, *either* (*a*) dissolve some chloride of lime in water, and to the filtered solution add a solution of potassium oxalate as long as a precipitate is formed, and filter, *or* (*b*) to 20 c c. of 20 per cent. solution of calcium chloride, add 100 c.c. of water, let stand for some hours, then add a solution of 15 grams of potassium carbonate in 100 c c. of water, and filter—if a film forms on the surface of this solution on exposure to the air, add a little more of the potassium carbonate solution and filter off the precipitate.

Eau de Javelle is used (1) for clearing growing-points and other dense tissues, by swelling and dissolving the cell contents—after treatment with the reagent, wash the sections with water, treat with dilute acetic acid to correct too great transparency, and mount in glycerine; (2) to extract lignin from sections of woody tissue, the cells then giving cellulose reactions, *e g.* violet colour with chlor-zinc-iodine; (3) to demonstrate starch grains included in chloroplasts, sections or whole leaves, etc, being treated with the reagent for an hour or more and then with iodine solution

Eosin.—(1) In dilute aqueous solution, eosin is a good general stain for protoplasmic cell contents and cellulose walls. (2) For staining protein grains, place small b ts of tissue, *e g* Castor Oil endosperm, in saturated alcoholic solution of picric acid for a day, rinse in alcohol; cut sections, stain with eosin, clear with clove oil, and mount in balsam—the ground substance of the protein grains should be red, the crystalloids yellow, the globoids colourless.

Fehling's Solution.—To make Solution *A*, dissolve 35 grams of copper sulphate in 200 c c. of water To make Solution *B* (to be kept in a separate bottle), dissolve 70 grams of Rochelle salt (sodium potassium tartrate) in 200 c c. of 10 per cent. caustic soda solution. Use equal volumes of Solution *A*, Solution *B*, and water. The object of the Rochelle salt is to prevent the precipitation of copper hydroxide by the action of soda on the copper sulphate.

Fuchsin, Acid —Dissolve 1 gram of fuchsin in 100 c c. of 50 per cent. alcohol. (1) Fuchsin is a good general stain, the different tissues taking different shades of red (2) An excellent double stain is obtained with fuchsin and methyl blue. Leave the sections in fuchsin overnight, rinse in water, transfer to methyl blue solution for a few minutes, rinse in weak alcohol, dehydrate with absolute alcohol, treat with clove oil or xylol, and mount in balsam (3) To stain crystalloids, fix the material in strong alcoholic solution of corrosive sublimate (mercuric chloride), place the sections for a day in fuchsin solution, dehydrate and pass through clove oil or xylol to balsam. (4) To stain crystalloids in leucoplasts, place the sections in fuchsin solution, rinse in strong alcoholic solution of picric acid, dehydrate and pass through clove oil or xylol to balsam (5) To stain leucoplasts and other plastids, fix the material in strong alcoholic corrosive sublimate for 24 hours, rinse in alcoholic solution of

iodine, and cut sections; place these in fuchsin for 24 hours, rinse in water, and mount in glycerine.

Glycerine, used for mounting, may be applied either pure or diluted with equal volume of water See § 15.

Glycerine Jelly, used for mounting, may be bought ready prepared. See § 16

Haematoxylin, Delafield's —This is perhaps the best general stain. It may be bought ready made up, or may be prepared as follows: Mix 4 c c of saturated alcoholic solution of haematoxylin crystals with 150 c c. of saturated aqueous solution of ammonia alum crystals. Let stand for a week exposed to light, filter, and mix the filtrate with 25 c c. of glycerine and 25 c.c of methylated alcohol. Let stand for a few hours, filter off any precipitate, and keep in a tightly stoppered bottle Precipitates are sometimes formed in specimens stained with haematoxylin, but these can be removed by rinsing with acid alcohol (5 drops of hydrochloric acid to 100 c c. of alcohol); then treat with strong alcohol, and with clove oil or xylol, and mount in balsam A good double stain is given if sections are placed in safranin for at least half an hour, washed in water, and placed for a minute or two in haematoxylin; lignified and suberised walls are stained red, cellulose walls purple.

Hoffmann's Blue is used in solution in 50 per cent. alcohol, with addition of a little acetic acid (1) It stains the protoplasmic cell contents and not the cell walls. (2) It stains the callus of sieve-tubes. (3) To show the continuity of protoplasm through pores in the walls, dissolve some dry Hoffmann's blue in strong sulphuric acid, place sections in this solution for about 15 minutes, then wash with water, and mount in glycerine.

Hydrochloric Acid has many uses, mostly in conjunction with other reagents. By itself, it turns lignified walls yellow.

Indian Ink.—The gelatinous sheaths of various Algae (*e g.* Spirogyra and other Conjugatae) may be shown up well by placing the Alga in water containing Indian ink.

Iodine has various applications in plant histology and microchemistry. Iodine solutions may be prepared in various ways. (*a*) Dilute iodine tincture with 5 to 10 times its volume of water. (*b*) Dissolve 1 gram of potassium iodide in a little water, dissolve crystals of iodine in this until a brown colour is given, and dilute with water A rather pale solution is sufficient to colour starch blue; to stain proteins and cell-walls, a stronger solution is required. (*c*) A mixture of equal parts of potassium-iodide iodine solution and glycerine often gives good results; the glycerine keeps the preparation from drying and also acts as a clearing agent. (*d*) To make phosphoric acid iodine, which stains cellulose violet, dissolve 1 gram of potassium iodide and 1 gram of iodine in 50 c c of strong aqueous solution of phosphoric acid.

The uses of ordinary iodine solution (either the diluted tincture or the potassium iodide solution) are various It stains starch blue, proteins brown, cellulose walls pale yellow, lignified and cutinised walls deeper yellow, and gums violet. Together with sulphuric acid, iodine makes cellulose walls blue or violet. See also **Chloral Hydrate** and **Chlor-zinc-iodine.**

Iodine Green is a useful general stain. (1) For instant fixation and staining of the nuclei of fresh material, use iodine green dissolved in 2 per cent acetic acid (2) Iodine green stains lignified walls, and can be used in conjunction with erythrosin or fuchsin in double staining

Iron Acetate is used as a test for tannin. The sections are placed in alcohol to remove the chlorophyll, if present, then in iron acetate solution ; a blue or green colour is produced by tannin.

Iron Chloride or **Iron Sulphate,** in aqueous solutions, are also used as tests for tannin, the colour produced varying from blue to green

Lead Acetate.—Make a saturated aqueous solution To make lead acetate papers, dip strips of filter paper into the solution ; on exposure to the action of sulphuretted hydrogen, the paper will turn black owing to formation of lead sulphide. To detect presence of sulphur in organic substances, heat with soda lime, and hold a lead acetate paper over mouth of tube.

Maceration.—Various reagents are used to isolate the cells of a tissue. (1) **Schultze's process** is perhaps the best where lignified tissues are present. Place a little strong nitric acid in a test-tube, add a crystal of potassium chlorate, heat to boiling, and drop in the sections ; when these turn white, pour the contents of the tube into a dish of water, and tease out the material on a slide. (2) **Mangin's process** :—place the sections for a day or two in a mixture of 3 volumes alcohol and 1 volume of hydrochloric acid, rinse them in water, place in 10 per cent. ammonia for 15 minutes, then mount the section in water and press on the cover-glass to force the cells apart. (3) **Chromic acid** is also used for maceration. Place the sections in concentrated aqueous solution for a minute or two, rinse in water, mount in water and press on the cover ; if the cells do not come apart, put the specimen for a longer time in the acid.

Methyl Blue, used in aqueous solution, is a good stain for cellulose walls, especially when used with safranin as a double stain. Stain with the safranin overnight, rinse in water, and then in acid alcohol, place in strong methyl blue solution for 15 minutes, treat with strong alcohol and pass through clove oil or xylol into balsam

Methylene Blue.—(1) A good stain for the nucleus, especially for cells filled with protein grains. (2) Cells containing tannin accumulate methylene blue from very dilute solutions, *e g* 1 part of

stain in 100,000 of water (3) The gelatinous sheaths of living Spirogyra and other Algae can be stained with dilute methylene blue without injuring the living protoplasm.

Methyl Green, in strong alcoholic solution, is a good general stain, especially useful for fresh material. (1) Alcohol-preserved material should be treated with the stain for 15 or 20 minutes, then washed with water and mounted in glycerine (2) Fresh material should be mounted in 2 per cent. acetic acid, to which a little of the stain has been added. The nuclei are simultaneously fixed and stained; wash with 1 per cent. acetic acid and mount in glycerine. The nuclei of Algae and Fungi are well brought out, being stained green or blue-green, while the protoplasm is unstained.

Methyl Violet.—(1) In strong aqueous solution, this is good for staining starch grains; if the grains are then treated with dilute calcium nitrate solution, the stain is deposited in the less dense layers of the grains. For (2) sieve-tubes and (3) lignified walls, dissolve dry methyl violet in strong sulphuric acid—the solution will be brownish green, but on adding water the violet colour appears; treat sections with this and wash with water—the cell-walls are made swollen and transparent, the protoplasm is deeply stained, sieve plates are brought out well, and lignified walls are usually stained bright yellow.

Millon's Reagent.—Dissolve 1 c.c. mercury in 9 c.c. strong nitric acid, and add 10 c.c. water. This reagent may be bought ready prepared, but it is better to make it up as required, since it acts best when fresh Proteins are stained brick-red—the reaction is hastened by heating.

Nigrosin.—See Picro-nigrosin.

Nitric Acid, generally in 5 or 10 per cent. solution, has a variety of uses. It (1) colours cutinised walls yellow; (2) colours proteins yellow—see xanthoproteic reaction, § 41,*c*; (3) causes swelling of cellulose and lignified walls; (4) dissolves crystals of calcium oxalate; (5) is used with potassium chlorate in maceration, and as a test for suberin.

Olive Oil is used (1) for experiments on oils and their emulsification and digestion—see §§ 85, 86—and (2) for mounting sections of oily seeds containing protein grains.

Orcin, dissolved in alcohol, is used as a test for inulin. Sections are soaked in the solution and then warmed with strong hydrochloric acid; an orange-red colour indicates presence of inulin.

Osmic Acid, used generally in 1 or 2 per cent. solution in water, serves for (1) fixing and hardening the protoplasm and nucleus; (2) staining oils black. Osmic acid darkens various organic substances, and is therefore by itself an unreliable test for oils. It is sold in sealed glass tubes containing one gram. To make up a 2 per cent. solution, place 10 c.c of water in a thoroughly clean bottle,

drop in the tube and break it by striking the bottle on the palm of the hand, then pour in the remaining 39 c c of water required. Osmic acid should be kept in the dark in a well stoppered bottle.

Phenol.—See **Carbolic Acid.**

Phloroglucin, used as a test for lignin (§ 79) and for inulin (§ 77) is rather expensive, but may be bought in the dilute solution required; hydrochloric acid should be added

Picric Acid.—(1) Saturated aqueous solution of picric acid is often used for fixing the cell contents, but it is difficult to wash it out—alcohol dissolves it better than water (2) To demonstrate the structure of protein grains, place the material in strong alcoholic solution of picric acid for several hours, rinse in alcohol, and stain for a few minutes in alcoholic solution of eosin (3) The chloroplasts and pyrenoids of Algae are simultaneously fixed and stained by placing the material in some strong solution of picric acid in 50 per cent. alcohol, to which has been added some acid fuchsin solution

Picric Aniline Blue.—For a rapid differentiating stain, add aniline blue to saturated picric acid solution in 50 per cent. alcohol, until the solution becomes blue-green. This mixture will stain cellulose walls and cell contents blue, while the lignified walls are stained yellow.

Picro-nigrosin is used for (1) simultaneous fixing and staining of delicate tissues; (2) staining leucoplasts and nuclei; (3) double staining modified and unmodified cell-walls; and (4) is especially good for filamentous Algae and Fungi. Dissolve nigrosin in concentrated aqueous or alcoholic solution of picric acid. The solution will need to act for 3 or 4 hours, or overnight. The alcoholic solution is best for material containing chlorophyll, which will be extracted by alcohol Nuclei and leucoplasts are stained steel-blue by the nigrosin.

Potash (Caustic Potash).—For general use, dissolve 5 grams of stick potash in 95 c c. of water. This solution serves (1) as a clearing agent—after clearing, the potash should be washed out with water and neutralised by adding some acetic acid; (2) to cause swelling of cell-walls and starch grains; (3) to dissolve inulin crystals, protein crystals, and most protein grains; (4) to saponify oils; (5) to make tannin-containing cells red Strong solution (50 per cent. is used as (6) a test for suberin; (7) a maceration fluid—boil the tissue in the solution for a few minutes, then pour into water, tease with needles, and mount in glycerine, adding acetic acid if the isolated cells are too transparent.

Potash, Acetate of.—Strong solution in water is used for mounting preparations of green tissues, green Algae, etc, since in this solution they keep their green colour for a long time.

Potassium Bichromate is used in dilute (1 to 5 per cent.) aqueous solution as (1) a test for tannin; (2) a fixing and hardening reagent; (3) a liquid allowing transmission of orange and red light, when placed in a double-walled bell-jar.

Potassium Chlorate is used, together with nitric acid, as a macerating fluid and a test for suberin.

Potassium Ferricyanide is used to demonstrate the structure of pyrenoids, especially in Algae. Place the specimen in a mixture of equal parts of 10 per cent aqueous solution of potassium ferricyanide, and 50 per cent. solution of acetic acid, then treat as described under Dahlia.

Safranin.—Make a saturated solution in alcohol, and dilute with equal volume of water This is a good general stain, and is also used along with haematoxylin, etc., in double staining. It gives good results with Spirogyra and other Algae. Place the material, after fixing with chromic acid or other fixative, in the safranin solution for several hours, then in 50 per cent alcohol, to which strong alcohol is added drop by drop so as to reduce the intensity of the colour; then transfer the specimen to dilute glycerine, or pass it through clove oil or xylol into balsam

Schultze's Maceration Fluid.—See Maceration.

Silver Nitrate —A 5 per cent. aqueous solution of silver nitrate is used to bring out the striations in fibres and in starch grains. (1) Sections containing fibres are allowed to dry, then placed in the solution for an hour and transferred to 1 per cent. solution of common salt; they are then placed in water and exposed to light for an hour, allowed to dry again, then moistened with strong alcohol and examined in clove oil. (2) Dry starch, or sections containing starch grains, are placed in the solution for an hour, then allowed to dry on a slide, then treated with 1 per cent solution and exposed to light for an hour

Sodium Chloride (Common Salt) is used (1) in 1 to 5 per cent. solution to induce plasmolysis; (2) in 10 per cent. solution as a solvent for protein crystals.

Sodium Salicylate, dissolved in an equal weight of water, is used as a clearing agent, and is almost as good as chloral hydrate. With the addition of iodine, this solution makes starch grains, included in tissues, swell and turn blue

Sulphuric Acid has a variety of uses. (1) The strong acid dissolves starch and cellulose, but suberised and cutinised walls resist its action; (2) it is used with cane sugar as a test for proteins—a red colour is given; (3) it dissolves crystals of calcium oxalate; (4) it causes cellulose walls, previously saturated with iodine solution, to become blue.

Turpentine is used as a clearing agent before mounting in balsam specimens previously dehydrated with absolute alcohol; for this purpose it may be either used alone or in conjunction with carbolic acid

Wax Mixture, for making joints in apparatus air-tight, may be made as follows:—Melt together 30 parts of beeswax, and 40 of vaseline; add to the mixture 15 parts of powdered resin, and stir. The hardness of the mixture may be modified by varying the proportions of beeswax and vaseline.

Xylol is used as a solvent for Canada balsam, and as an intermediary between absolute alcohol and balsam in the mounting of balsam preparations.

APPENDIX II.

PRACTICAL HINTS ON MICROTECHNICAL METHOD.

As the student works through the practical histology methods given in the book, he should carefully tabulate or summarise each method, as indicated below—preferably in a loose-leaf file, so that the pages can be re-arranged if necessary. Give each method one page, and keep a careful record of the name of each specimen to which you have found the method apply In this way a method of attack is gradually built up, so that when confronted in the examination room with an "unknown" you will keep certain definite rules in mind in making such microscopic preparations as will illustrate the nature or structure of the given specimen.

The following nine methods are suggested as a nucleus for the formation of a summary of methods.

METHOD I.

Mounting into Glycerine or Glycerine-jelly Direct, *e.g.* pine-pollen, spores and elaters of Pellia sporogonium, etc.

Formula = m.G. or m.G.-J.

(1) Tease out male Pine cone with two needles in small drop of water on slide

(2) Remove pieces, and drain off water, leaving pollen on slide.

(3) Add 1 drop of glycerine or warm glycerine-jelly.

(4) Mix pollen-grains into glycerine with needle.

(5) Gently lower polished cover-glass.

METHOD II.

Macerating and Mounting in Glycerine-jelly, *e.g.* leaf mesophyll.

Formula = mac. m G.-J.

(1) Boil leaf 5 minutes in 10 per cent. potash

(2) Tease out mesophyll in small drop of dilute glycerine on slide

(3) Remove lumps, and drain off glycerine.

(4) Add 1 drop of glycerine-jelly, and cover.

METHOD III

Staining with Iodine, and Mounting in Glycerine-jelly, *e.g.* T.S. broad bean cotyledon.

Formula = s.I.m G.-J.

(1) Cut thin sections of cotyledon into water in watch-glass.

(2) Pick out few best sections, and transfer to watch-glass of straw-coloured iodine-water—3 drops of tincture of iodine to a watch-glass of water.

(3) When stained—watch them under the microscope—transfer best section to slide, add 1 drop of glycerine-jelly, and cover

METHOD IV.

Fixing, Staining with Haematoxylin, and Mounting in Jelly, *e g.* fresh leaf-epidermis, sections of fresh stem, leaf, etc.

Formula = f Alc. s Hx. m G.-J.

(1) Peel off small pieces of epidermis, or cut thin sections of stem, and place in 95 per cent. alcohol, or in formalin-alcohol, for 15 minutes, in watch-glass.

(2) Transfer to watch-glass of distilled water (2 min).

(3) ,, ,, ,, ,, haematoxylin (2 min.).

(4) ,, ,, ,, ,, tap-water (1 min.).

(5) ,, ,, ,, ,, dilute glycerine (2 min.).

(6) Transfer to slide, and mount in jelly.

METHOD V.

Double-staining with Safranin and Haematoxylin and Mounting in Canada Balsam or Gurr's Medium, *e.g.* sections of fixed stem, leaf or root.

Formula = ss. Saf and Hx m.C.-B.

(1) Place material in absolute alcohol, 15 min., to stiffen it.

(2) Cut sections into distilled water, or 50 per cent. alcohol.

Transfer sections to watch-glass of :—

(3) Safranin, 10 min. (to stain lignin).

(4) 50 per cent. alcohol, 2 min. (to remove excess safranin).

(5) Delafield haematoxylin, 1 min (to stain cellulose walls).

(6) Tap-water, 1 min. (to remove excess haematoxylin).

(7) Acid-alcohol, 1 min. (to differentiate).

(8) Tap-water, 1 min (to "blue" the haematoxylin).

(9) 70 per cent. alcohol, 1 min. (to remove tap-water)

(10) Absolute alcohol, 2 min. (to dehydrate completely).

(11) Fresh absolute alcohol, 1 min (to dehydrate completely).

(12) Clove oil, or carbol-xylol, 1 min (to clear).

(13) Mount in canada balsam on slide; *or*, from stage (11), mount in Gurr's medium direct, omitting stage (12)

Stage (8) may usually be omitted, with no ill effects, in any case.

METHOD VI.

Counter-staining with Safranin and Light Green, and Mounting in Canada Balsam, *e g.* sections as for Method V.

Formula = ss. Saf. and L G. m C B

(1) Place material into absolute alcohol, 15 min.

(2) Cut sections into 50 per cent alcohol.

Transfer sections to watch-glass of —

(3) Safranin, 10 min. (to stain lignin)

(4) 70 per cent. alcohol, 1 min (to remove excess safranin)

(5) 90 per cent alcohol, 1 min (to remove 70 per cent. alcohol).

(6) Absolute alcohol, 2 min. (to dehydrate)

(7) Light green, 1 min. (to stain cellulose).

(8) Absolute alcohol, ½ min. (to remove excess of light green)

(9) Clove oil, or carbol-xylol, 1 min. (to clear).

(10) Mount in canada balsam on slide

Note —The light green stain is 2 gm of the powdered stain dissolved in a mixture of 50 c c. of absolute alcohol and 50 c c. of clove oil.

METHOD VII.

Staining with Haematoxylin, differentiating with Acid Alcohol, and Mounting in Canada Balsam, *e g* thick objects, whole mounts, or mitotic figures in sections.

Formula = ♯. Hx m C -B.

(1) Stain in haematoxylin, 5 min

(2) Tap water, 1 min.

(3) Acid-alcohol, 1 to 5 min. (till differentiated)

(4) Tap-water, 1 min. (to "blue" the haematoxylin).

(5) 70 per cent. alcohol, 1 min

(6) 90 per cent. alcohol, 1 min

(7) Absolute alcohol, 2 min.

(8) Absolute alcohol, 1 min.

(9) Xylol, 1 min

(10) Mount in canada balsam

Note.—Acid alcohol is 100 c.c. of 70 per cent alcohol, to which 3 c c. of strong hydrochloric acid have been added On differentiating, the stain washes out of nuclei last of all.

METHOD VIII.

For Bacteria. Gram Method.

(1) Make a thin smear, on slide, in small drop of distilled water, spreading it to about 1 sq. inch.

(2) Dry over flame (heat fixation).

(3) Aniline gentian violet (5 min.).

(4) Tap-water (to remove excess violet).

(5) Gram iodine, 2 min., until blackish.

(6) 90 per cent. alcohol, till no more colour lost.

(7) Counter-stain with weak carbolfuchsin ($\frac{1}{2}$ min.).

(8) Tap-water, $\frac{1}{4}$ min.

(9) Dry thoroughly.

(10) Put one drop of balsam or Gurr's medium on the dry, stained smear, and cover.

The above method is all done on the slide. Simple staining with carbol-methylene-blue, or night-blue also gives good results.

METHOD IX

Bulk Staining, *e g* sexual organs of Fucus

(1) Cut small pieces of Fucus, each with two or three conceptacles

(2) Place them in borax carmine, 24 hours.

(3) Then into acid alcohol, 15 min.

(4) ,, ,, 70 per cent. alcohol, 1 hour.

(5) ,, ,, absolute alcohol, 1 hour

(6) ,, ,, clove oil, and tease out with two needles.

(7) Mount in balsam.

THE FOLLOWING POINTS SHOULD BE CAREFULLY NOTED.—

Fixing.

(1) The formalin alcohol combination depends on the fact that the 70 per cent alcohol, being a quick penetrant, carries in the formalin, and fixes and hardens the tissues before there is any appreciable tendency to shrinkage or distortion It is good for everything except a critical study of nuclear structure.

(2) The theory of the chrome acetic mixtures is that, while the chromic acid has a tendency to produce contraction or plasmolysis, the acetic acid causes swelling. Hence, by varying the relative proportions of the two acids, a mixture can be found, by trial, which produces no distortion.

Cutting.

(1) It will be seen that most of the above methods demand the use of watch-glasses Six of these should be placed on a sheet of clean paper, so that their contents can be seen, and labelled

(2) A blunt razor gives thick sections, which start a whole chain of troubles. The delicate razor-edge is quickly blunted by dilute acids, and it is just as well to keep a new "Darwin" rustless safety-razor blade as a standard, in case you should suspect your sectioning-razor. A good plan is to use the same razor for shaving and for cutting sections—nothing short of a good shaving edge is of the slightest use.

(3) Carrot is better than pith for embedding leaves, Fucus, etc., for hand-sectioning. The carrot may be kept in chunks in methylated spirit. The top, horizontal surface of the piece of carrot should be slightly bevelled from behind, up to the slot in which the specimen is held.

Staining.

(1) The considerations which determine the best method for any given case are (1) whether a temporary or permanent preparation is desired, (2) the nature of the structures to be stained, and (3) the subsequent mounting. Thus, dilute iodine does not stain cellulose walls at all; while few iodine-stained preparations of any material will keep their colour more than a few days Haematoxylin stains cellulose walls, cytoplasm, and nuclei, but not starch grains Safranin stains lignin red, and starch-grains and cutin pink. Haematoxylin stands well in any of the ordinary mountants, but safranin usually washes out in glycerine-jelly in time.

The microchemical tests given in Chapter II. may be used to determine the nature of the substances present in any given specimen.

(2) Tissues already coloured yellow or brown in the unstained material are probably suberised, and will be difficult to stain by any of the ordinary methods. Living tissues also resist the stains.

(3) The test of any staining method is that it should bring out details of structure better than they can be seen in the unstained specimen. Overstaining may easily have the opposite effect.

Mounting.

(1) When transferring from absolute alcohol to xylol, lift the sections on a strip of clean, dry paper held in the right hand, transfer them quickly to a similar strip in the left hand, and hold them under the surface of the xylol; do not let them float about.

(2) If xylol causes shrivelling or curling, use cedar oil or clove oil instead.

(3) If sections go milky in xylol, they must go back into absolute alcohol again, at once, as they still contain water. The addition of about 5 grams of absolute phenol (carbolic acid crystals) to every 100 c.c. of xylol, helps to avoid this milkiness.

(4) Before mounting, wipe any excess of xylol off the slide with a clean Irish-linen handkerchief, try to estimate the correct amount of balsam, and let the cover down gently with a needle, or forceps—*not* with the fingers. More mountant may be added by placing a drop at the edge of the cover-glass, when it will suck under. Excess of mountant is not easily removed, and looks clumsy.

(5) On no account must preparations be allowed to dry up at *any* stage of the fixing, staining, and mounting procedure—except in certain special cases, *e.g.* bacteria.

(6) Generally speaking, balsam and Gurr's medium are the most satisfactory mountants. They give transparency to tissues, brightness to colours, and clearness to structures; also, they set hard. On the other hand, glycerine-jelly demands no previous dehydration of the specimen with alcohol, and is therefore indicated for quick mounting of large objects which are difficult to dehydrate, for small objects which may be lost in going up the alcohols, and for temporary mounts generally.

Finally, the finished preparation should be compared with some of the fresh, living material, mounted in its own fluid, or in water.

Of course, always glance at the preparation under the low power just before and after staining, and also just before mounting. If the microscope reveals defective staining, one can work backwards, down the steps into the stain again. It is usually pointless to mount more than one section on a slide, except in such cases as transverse, radial (longitudinal through the centre) and tangential (longitudinal away from the centre) sections of the same specimen.

METHOD X

Heidenhain's Iron Haematoxylin.

This method, although perhaps, more difficult than those previously mentioned, gives beautiful results in the case of well-fixed nuclear structures. Two separate solutions are used. Solution A, an iron mordant, is a $2\frac{1}{2}$ per cent. solution in boiled distilled water of ferric alum (ferric ammonium sulphate), the violet crystals of which must be dissolved in cold water. The solution slowly deteriorates. Solution B is a $\frac{1}{2}$ per cent. solution of ordinary haematoxylin crystals in distilled water. This solution is allowed to stand for a month to "ripen," and then remains in good condition for a further two months.

Treat sections with A for half an hour; then wash them with distilled water, and stain for half an hour in solution B. Then rinse with distilled water, and transfer back again into A, to differentiate, for 2–5 minutes. Then wash for 15 minutes in running tap-water, and dehydrate, clear and mount in balsam, in the usual way. Needless to say, the differentiation in this method, as always, should be controlled under the microscope.

APPENDIX III.

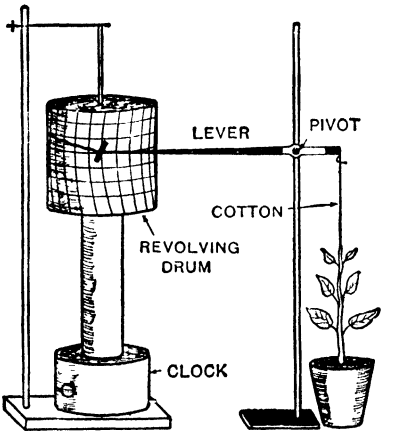


Fig 116.—Self-recording Auxanometer The tip of the lever touching the drum carries a pen-nib dipped in glycerine ink As the plant grows, so the line marked on the drum descends.

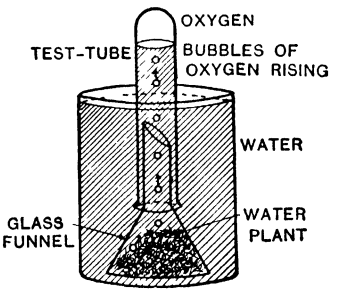


Fig 117 —Illustrating evolution of Oxygen during photosynthesis

Place some water plants, *e g* Water Starwort (*Callitriche*) or American Waterweed, under a glass funnel in a large glass vessel of water exposed to bright light. Observe the bubbles of gas given off When this gas is collected in the inverted test-tube full of water, it rekindles a glowing splint of wood, and is therefore chiefly oxygen The bubbling stops in the dark

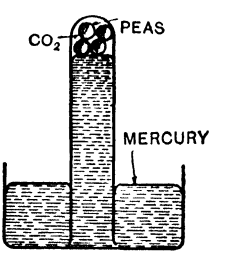


Fig 118 —Intramolecular Respiration of Germinating Peas

Soak six peas in water for a day or two, remove the coats, and pass the peas under the mouth of an inverted test-tube full of mercury, as shown. The peas float to the top In a day or so the test-tube will be half full of gas The gas is rapidly absorbed by a little strong potash solution introduced into the test-tube by a bent glass tube, and is therefore carbon dioxide.

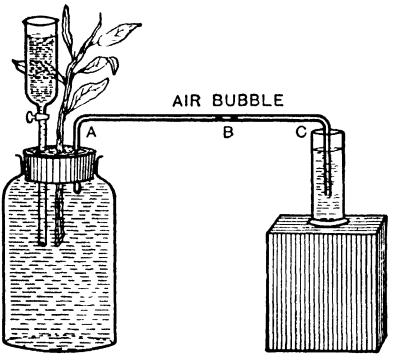


Fig 119 —Simple Potometer (after Farmer)
The rate at which the air-bubble at B is sucked towards A is a measure of the rate of transpiration of water vapour by the leafy shoot When the bubble gets to A, open the tap of the dropping-funnel, when it will move back to C again

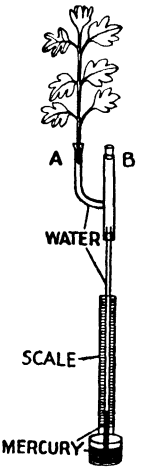


Fig 120 —To show Pull due to Transpiration

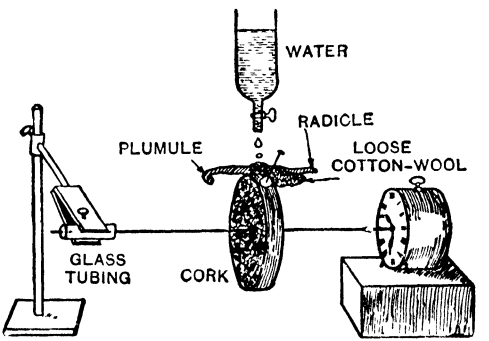


Fig 121 —A Simple Form of Klinostat The **Klinostat** consists essentially of a vertical plate or disc mounted on a horizontal axis A plant is attached to the disc so that its axis is horizontal, and the disc rotated *slowly* The normal influence of gravity is eliminated, as each side of the axis is, in turn, directed downwards It is found that stem and root grow in the directions in which they are placed

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