

PRACTICAL BOTANY

FOR

ADVANCED LEVEL AND
INTERMEDIATE STUDENTS

BY

C. J. WALLIS, M.A.(Cantab.)

*Principal, Eaton and Wallis, Tutors.
Formerly Master-in-Charge of Biology, University College
School and Biology Master, Highgate School*

A

LABORATORY MANUAL

Covering the Syllabuses in *Botany* of the General Certificate of
Education (Advanced Level) and other Examinations of similar
standard.

FIFTH EDITION



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FOREWORD TO THE FIFTH EDITION

WHEN revising my *Practical Biology* for its Fourth Edition, it was decided to publish it in two separate volumes under the titles *Practical Zoology* and *Practical Botany* to provide for the needs of students taking only one of these subjects at Advanced Level in the General Certificate of Education and to publish a separate book, *Advanced Level Practical Biology*, for those taking this subject in "A" Level and First M.B.

In the latest revision, extra types required by new Examination Syllabuses, additional descriptive detail and many extra practical directions have been added. At the same time the original object of *giving adequate instructions, illustrated where desirable, but avoiding long and tedious reading* of facts which can be learned from text-books has been maintained. Many of the illustrations have been re-drawn and a number of additional ones inserted.

The book has been divided into appropriate parts as follows:—

- | | |
|--|---|
| Part I. Microscopical Technique | Part III. Biochemistry |
| Part II. Morphology, Cytology
and Histology | Part IV. Physiology
Part V. Genetics |

As stated in the Preface, it is essentially a laboratory manual and must be used in conjunction with text-books of botany. In choosing the material for the book I have endeavoured to cover the syllabuses in Botany of the various Examining Boards for the General Certificate of Education and other Examinations of similar standard. At the same time I have not confined the text to these particular requirements.

In the Introduction I have given general instructions for practical work and for the keeping of practical notebooks and a list of apparatus and instruments required. Additional notes precede the text in each part of the book which are peculiar to that part. The Introduction also contains a summary of the characteristics of living organisms, the differences between plants and animals and the principles of plant classification. The appendices contain information which it is hoped may be of some use to those in charge of biological laboratories while some of it may, in fact, prove helpful to students themselves.

I am very grateful to those critics, teachers and students who kindly made constructive criticisms and offered useful suggestions for improvement of the previous Edition and I have, as far as possible, taken advantage of them.

Once again I must record my indebtedness to my publishers and to Mr. Owen R. Evans in particular for the very considerable help given in the production of this Edition, and to Mr. Frank Price for his inestimable help in preparing my drawings for the block-maker. Finally I should like to express my gratitude to my wife for her assistance in reading the proofs and with the tedious process of preparing the index.

C.J.W.

August 1966.

PREFACE

THE original book, published in 1935, was entitled *Practical Biology for Medical Students*. During the course of new editions this was considerably enlarged in material and scope in order to provide for students taking Botany and Zoology and division into two separate books, *Practical Botany* and *Practical Zoology* (under which titles they are now published) became necessary. It was realised that there was much in these books which was unsuitable or unnecessary for those studying the subject Biology and it was therefore decided to publish a new book under the title *Advanced Level Practical Biology*, which would provide for their needs.

Quite apart from the fact that practical classes are often unavoidably large, making it difficult for a great deal of attention to be given to the students, it is desirable that they should learn by discovering things for themselves, provided they are guided along the right lines. By this method they absorb facts more easily and learn to work and think along scientific lines. This is evident even in the smaller groups one takes in a tutor's practice in which the students work individually and not necessarily as a class and in which there is adequate time to devote to each student.

In all my books an attempt has been made to give *sufficient directions* to enable the student to proceed with his practical work with a minimum of assistance from the demonstrator, at the same time *avoiding the inclusion of elaborate and unnecessary details which make the reading long and tedious* and which should, in any case, be learned from text-books. The book is essentially a laboratory manual.

When writing the original book I had much pleasure in expressing my gratitude to Sir Frederick Gowland Hopkins, O.M., M.B., F.R.S., Professor of Biochemistry in the University of Cambridge, to Professor A. G. Tansley, M.A., F.R.S., Sherardian Professor of Botany in the University of Oxford and to Dr. L. A. Borradaile, M.A., Sc.D., Lecturer in Zoology in the University of Cambridge, for kindly reading through the manuscripts of the Biochemistry, Plant Biology and Animal Biology sections respectively, and for many helpful suggestions; also to Professor Tansley for allowing me to adapt some of the experiments on crystalloids and colloids from his "Elements of Plant Biology" and my own records of his practical course at Cambridge.

I was also deeply indebted to Dr. J. H. Woodger, D.Sc., Reader in Biology in the University of London and Lecturer in the Middlesex Hospital Medical School, for reading through the proofs of the

entire First Edition and of the vertebrate types of the Second; and to Dr. C. L. Foster, M.Sc., Ph.D., also of the Middlesex Hospital Medical School, for reading through the proofs of the complete Second Edition. In the course of these readings they made several invaluable suggestions, the majority of which I was glad to adopt.

I gratefully appreciate the courtesy of the authors and publishers of certain text-books for permission to use or adapt illustrations (acknowledged in each instance) from those books.

Lastly, I should like to acknowledge my indebtedness to my publishers, and particularly to the late Mrs. G. Fielding, the late Mr. L. B. Cavender and Mr. Owen R. Evans for the assistance they have given me in the production of the various editions of the book.

LONDON, W.

C. J. WALLIS.

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WM. HEINEMANN MEDICAL BOOKS LTD., London. *Text Book of Bacteriology for Dental Students*. Bulleid Edition.

W. WATSON & SONS LTD., Barnet, Herts, for the illustrations of the microscope.

INTRODUCTION

I. GENERAL DIRECTIONS FOR PRACTICAL WORK

(1) Cleanliness, tidiness and accuracy are of the utmost importance. See that all your instruments are sharp and that all apparatus is clean before beginning your work. **To sharpen a scalpel** an oilstone is necessary. *Put a drop of oil on the stone and push the scalpel, edge foremost, obliquely across the stone. Then turn the scalpel over and repeat the movement with the other edge. Alternatively, a circular movement may be made, edge foremost, as before. Repeat until the blade is sharp. Then draw it once or twice away from the edge to remove the burr.* For sharpening of section cutting razors see p. 13. Scissors must be sharpened by an expert. *Never use scalpels, scissors or razors for any purpose other than that for which they are intended.*

(2) Read the directions carefully.

(3) Wash, clean, dry and put away all your instruments, apparatus, etc., when you have finished with them.

(4) In the case of *microscopical preparations*, it is advisable to compare your own slides with permanent slides. Always put the permanent slides back *in their proper places* in the trays or boxes; otherwise you (and others) will have difficulty in finding them on a future occasion.

(5) Examination of museum specimens is often very helpful.

(6) Read the appropriate subject in your text-book *before* you start any practical work.

(7) Finally throughout your studies, try to realise the co-relation between the structure (morphology) and functions (physiology) of the various organs and systems.

II. INSTRUMENTS AND APPARATUS REQUIRED

By each Student

(1) A set of *dissecting instruments* in a case or cloth roll as follows:—

1 small *scalpel* (1 or 1½ in. blade)

1 pair of small *forceps* (4½ in.)

2 or 3 *mounted needles*

1 *camel-hair brush* or *section lifter*

1 flat and hollow ground *razor* for section cutting.

(2) Large-page practical *note-books* or *files*, with plain pages. The Elementary Biochemistry and the Plant Physiology can be conveniently kept together in separate parts of the file or in another book, preferably with alternate ruled and unruled pages.

(3) A *hand-lens* (unless supplied by the laboratory).

(4) A *microscope* (unless supplied by the laboratory). See below.

(5) The necessary *drawing materials* and *coloured pencils*.

(6) At least one *white coat* is desirable unless an old jacket is kept for laboratory work.

By the Laboratory

In addition to the usual laboratory apparatus, the following will be needed:—

(1) *Microscopes* (unless provided by the students) with $\frac{2}{3}$ and $\frac{1}{6}$ in. objectives, on a double or triple nose-piece, and No. 1 ($\times 5$) and No. 3 ($\times 8$) or No. 4 ($\times 10$) eyepieces. A few better instruments fitted with sub-stage condensers, Nos. 2 ($\times 6$) and 4 ($\times 10$) eyepieces, and a $\frac{1}{12}$ -in. O.I. objective are also desirable. A blue glass disc to fit below the condenser giving a daylight effect is an advantage when using artificial light. *All must be kept covered when not in use.*

(2) *Microscope lamps*, preferably fitted with daylight bulbs.

(3) *Lenses*. Watchmaker's lenses clamped in small retort stands serve well as mounted lenses. Better hand lenses should also be provided.

(4) A *dissecting microscope*. This is useful when low magnifications are desirable and both hands are free.

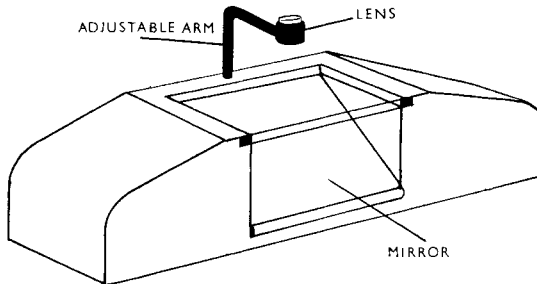


FIG. 1. A Simple Dissecting Microscope.

(5) A *turntable* for ringing slides is useful but not essential.

(6) Soft *cloths* (e.g., chamois leather) for lenses and objectives.

(7) *Stains* and *Reagents*. See Appendix 1.

(8) The *Apparatus* and *accessories* mentioned in the text. Much of this can be made or adapted from other pieces of apparatus at small cost.

III. CHARACTERISTICS OF LIVING ORGANISMS

The features peculiar to living organisms by which they may be distinguished from non-living objects may be summarised as follows:—

- (1) All living organisms are largely composed of a living substance called **protoplasm**.
- (2) They all require substances to provide energy for the performance of their life processes and materials for growth and repair. These substances may have to be synthesised from simple inorganic compounds or organic materials may have to be broken down in order that they may be assimilated into the protoplasm. The complete process is included in **nutrition**.
- (3) **Growth** is effected by the absorption and assimilation of material different from those of which the organism is composed. This is known as growth by *intussusception*.
- (4) In order that energy may be available for all the life processes it must be released from energy-providing compounds and the breaking down of these compounds for this purpose is **respiration**.
- (5) During the performance of the various metabolic processes which the organism performs, waste products which are of no further use to the organism and which may, in fact, be harmful to it, are produced. The elimination of these waste products of metabolism from the organism is known as **excretion**.
- (6) External stimuli produce responses in organisms. This is called **irritability**.
- (7) Irritability usually produces **movement**. This may be to enable the organism to perform its life functions or it may be in order to obtain food. It may be rapid or slow and it may entail movement of the entirely organism (locomotion) or of only a part.
- (8) **Reproduction** of new individuals similar to the organisms which produce them is necessary in order to perpetuate the race.

IV. CLASSIFICATION OF ORGANISMS (TAXONOMY)

Owing to differences in structure and mode of life, living organisms are classified into two **Kingdoms**, *Plantae* and *Animalia*.

There are many exceptions to these differences and it is perhaps difficult to assign some of the simplest organisms to one kingdom or the other. This fact, however, simply emphasises the unity of life.

The main **differences between the two kingdoms** may be summarised as follows:—

Plants	Animals
1. Nutrition is <i>holophytic</i> . Inorganic food is synthesised into protoplasm and organic compounds to provide energy.	1. Nutrition is <i>holozoic</i> . Food is organic and consists of plants or other animals or both.
2. <i>Chlorophyll</i> is necessary for this method of nutrition and occurs in plant cells.	2. Chlorophyll is not found in animal cells.
3. <i>Cellulose</i> is found in the cell walls.	3. Cellulose is never found in animal cells and cell walls are normally absent.
4. Plants are stationary.	4. Animals are motile.
5. They continue to grow throughout their lives.	5. Growth ceases after a certain limited period.
6. Growth is localised.	6. Growth is uniform throughout the body.
7. Plants are branched and have a large surface area.	7. Animals are more compact, have a definite shape and are unbranched.

These differences between the two kingdoms apply to all but the simplest plants and animals.

It should be noted that the *Fungi* and *Bacteria*, usually classified as non-green plants, could well be considered as forming an entirely separate kingdom.

CLASSIFICATION OF PLANTS

The **Plant Kingdom (Plantae)** is classified firstly into **Divisions**—*Thallophyta*, *Bryophyta*, *Pteridophyta* and *Spermatophyta*. These are divided into **Sub-Divisions**, the Sub-Divisions into **Classes**, the Classes into **Orders** and the Orders into **Families**. Plants in the same family are classified into **Genera** and the varieties found in each genus into **Species**. There are also Sub-Classes and Sub-Orders and all the non-seed bearing plants are still sometimes known as *Cryptogamia*. Flowering Plants constitute the Division *Spermatophyta* (formerly known as *Phanerogamia*).

Other and more recent names have been introduced for the various Divisions, etc., of the Plant Kingdom but the scheme in more universal and current use here is adopted in this book.

BINOMIAL NOMENCLATURE

Every plant is given two names, its generic name and a specific name. Thus the creeping buttercup is *Ranunculus repens* while the bulbous buttercup is *R. bulbosus*. This is known as the **binomial nomenclature**.

PART I

MICROSCOPICAL TECHNIQUE

I. THE MICROSCOPE

(a) Description

The microscope is a delicately adjusted scientific instrument and *must be handled with care.*

It consists of the following parts (see Fig. 2):—

- (i) **The Stand.** This is made up of a heavy **foot** which carries an inclinable **limb** or **arm**, bearing the **body-tube**. The body-tube can be raised or lowered by the **coarse adjustment** which works by a rack and pinion arrangement and by the **fine adjustment**

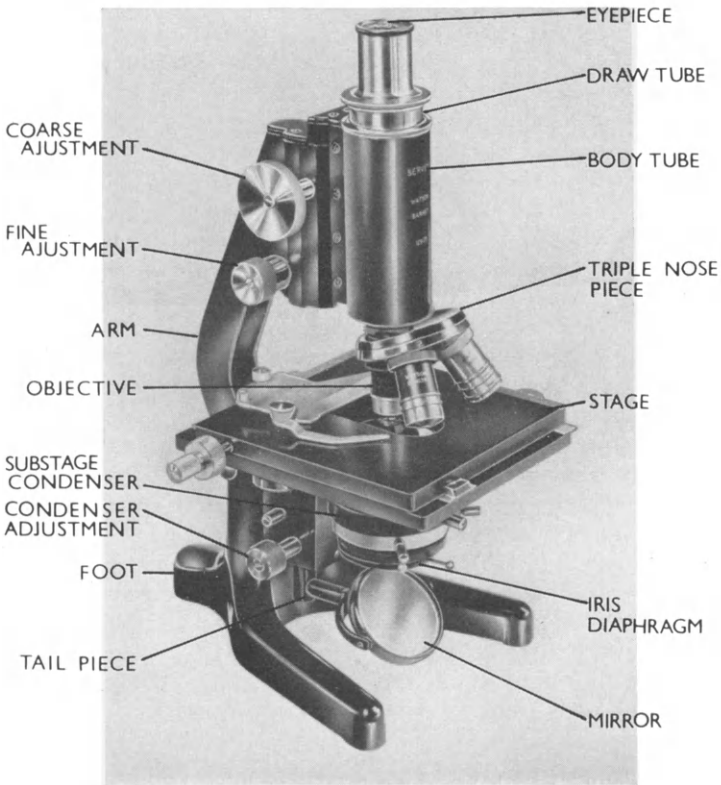


FIG. 2. The Microscope.

for more accurate focussing. Both are controlled by milled heads. Most modern microscopes are made with a tube length of 160 mm. This may be increased by raising the **draw-tube**, thus giving greater magnification. The draw-tube is usually graduated. A **nose-piece** (which may be *single, double or triple*) at the bottom of the tube carries the magnifying lenses or **objectives**. The arm also carries the **stage** on which the slides to be examined are placed and kept in position by springs. An attachable **mechanical stage** provided with vernier scales for moving the slides can be fitted. It is a luxury for ordinary use but a necessity for advanced work. In the more expensive instruments, it is built in. The **tailpiece**, into which the mirror is fitted, is on the lower part of the arm.

- (ii) **The Optical Parts.** These consist of the objectives, the eyepieces, the mirror and the sub-stage condenser. The **objectives** are small tubes containing a combination of lenses. Those in common use have a focal length of $\frac{2}{3}$ in. (16 mm.) (low power) and $\frac{1}{6}$ in. (4 mm.) (high power). A $\frac{1}{1\frac{1}{2}}$ in. (2 mm.) oil immersion lens is used in bacteriological, cytological, and other work requiring a much higher magnification. The $\frac{2}{3}$ in. and $\frac{1}{6}$ in. objectives are used dry, but when using the $\frac{1}{1\frac{1}{2}}$ in. O.I. objective, a drop of cedar wood oil (of practically the same refractive index as the glass) is put on the coverslip and the objective focussed into it. This increases the illumination. The high power objectives are focussed slightly nearer the object than the $\frac{2}{3}$ in. objective, and the distance between the objective and the slide is called the **working distance**. In most modern microscopes, once the object has been focussed with the low power, it is almost in focus when the high power objective has been swung into position, about one turn of the fine adjustment being all that is necessary to get it sharply into focus. The **resolving power** of a lens or its power to define detail depends on what is known as its **numerical aperture**. This is constant for any one lens and the higher it is, the greater the resolving power though the working distance is decreased. Good resolution is obtained with a $\frac{2}{3}$ in. objective of N.A. of about 0.28, with a $\frac{1}{6}$ in. objective of N.A. of about 0.7 and with a $\frac{1}{1\frac{1}{2}}$ in. O.I. objective of N.A. of 1.25 to 1.28. Makers always state the N.A. of their objectives. It should be noted that lenses have a curved field and consequently when, under the high power, the object is focussed in the centre of the field, it is only *this* part which is in sharp focus. Flatness of field is only possible with low power objectives. The magnified

images produced by the objectives are further magnified by the **eyepieces** which fit into the top of the draw-tube. The magnification of these lenses is marked on them, thus $\times 4$, $\times 5$, $\times 6$, $\times 8$, $\times 10$, $\times 15$, or they may be numbered: No. 0 ($= \times 4$), No. 1 ($= \times 5$), No. 2 ($= \times 6$), No. 3 ($= \times 8$), No. 4 ($= \times 10$) and No. 6 ($= \times 15$). A table of magnifications with different objective and eyepiece combinations is provided by the makers with each instrument. **Binocular eyepieces** are often fitted to the more expensive microscopes. The **mirror** is concave on one side for use when the substage condenser is not in use and plane on the other side for use with the condenser. The **condenser** fits into the underside of the stage, or into a **substage**, and can usually be swung out when not required. It is focussed either by a spiral focussing arrangement or by rack and

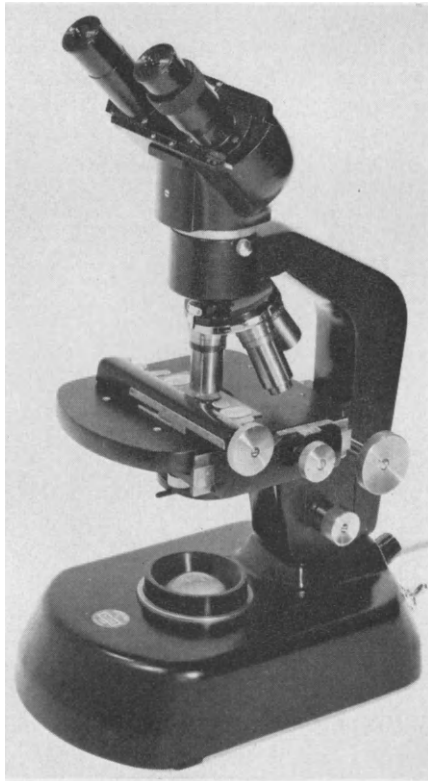


FIG. 3. Binocular microscope with built in lamp.

pinion. It increases the illumination when high power objectives are in use but it may be used with the low power also. Accurate centering is essential; this can be attained by means of **centering screws**. The amount of light passing through can be varied by the **iris diaphragm** which is fitted at the base of the condenser.

(b) Magnification

The approximate magnification is found from the formula—

$$m = \frac{l}{f} \times e$$

where m = magnification.

l = length of body tube (usually 160 mm.).

e = magnification of eyepiece.

f = focal length of objective.

As a very *approximate* guide it may be assumed that the—

$\frac{3}{8}$ in. objective magnifies 10 times,

the $\frac{1}{6}$ in. objective magnifies 40 times,

and the $\frac{1}{2}$ in. O.I. objective magnifies 95 times.

These magnifications should be multiplied by the magnification of the eyepiece.

A greater magnification can be obtained by raising the draw-tube and the new magnification can then be calculated from the formula

$$M_2 = \frac{m_1 \times l}{160}$$

where M_2 = final magnification.

m_1 = magnification for 160 mm. tube.

l = total length of tube in mm.

160 = length of tube in mm. for which makers calculated m_1 (*i.e.*, length without draw-tube extended).

(c) Measurement

The unit of length under the microscope is 0.001 mm., and is known as 1 micron (μ).^{*} Measurement is made by means of an **eyepiece micrometer** and a **Ramsden's eyepiece**, but this is not required of the elementary student.

(d) Use and Care

- (i) Always lift the instrument by the arm.
- (ii) Never allow any liquids to get on to the lenses or stage and keep them free from dust. Do not touch the instrument with wet fingers.

^{*} = $\frac{1}{25,000}$ in. (approx.)

- (iii) See that the slide and coverslip are *clean* and *dry*.
- (iv) Always examine an object as follows:
 - (a) with the naked eye if visible, otherwise with a hand lens.
 - (b) with the $\frac{2}{3}$ in. objective.
 - (c) with the $\frac{1}{6}$ in. objective.

In some cases, high power magnification will not be required while in others low power is inadequate.

- (v) Never use the high power unless the object is covered with a coverslip.
- (vi) Illuminate with the plane mirror when a substage condenser is used and with the concave mirror when it is not used.
- (vii) **Focussing.** With the *low* power bring the object clearly into view with the *coarse* adjustment and then focus accurately with the *fine* adjustment.

In using the *high* power, first see that the object is in the centre of the field and accurately focussed under the low power, then, if, as is usually the case, a nose-piece is fitted, swing the high power objective into position without touching the coarse adjustment. About one turn of the fine adjustment will then bring the object into sharp focus. If the instrument has no nose-piece, with the head at the side of the microscope, slowly lower the tube with the coarse adjustment until the objective is close to the slide: then carefully focus *upwards*, using the fine adjustment as before. You will be less likely to break the slide or damage the objective if this method is used.

- (viii) If a substage condenser is used, it must be accurately focussed. This is usually the case with the Abbé condenser and similar types when the upper lens of the condenser is almost touching the under side of the slide.
- (ix) Use the microscope with *both* eyes open (it is only a matter of practice) and get accustomed to using either eye.
- (x) Have your note-book on the right of the instrument.
- (xi) Study the object carefully before beginning to draw it.
- (xii) After using a $\frac{1}{12}$ in. O.I. objective, carefully clean off the oil from the objective and the slide with xylene on a clean soft cloth or blotting paper. Dry with a soft chamois leather.
- (xiii) Clean the outsides of the lenses, if dusty, with a piece of clean soft chamois leather. *Never take an objective to pieces.* Dirt which will not wipe off with a cloth can generally be removed by wiping the lens with a soft cloth dipped in

alcohol. To locate dust specks visible when looking through the microscope, rotate the eyepiece. If the specks also rotate, it is obvious that they are there. Move the slide and if the specks move too, they are on the coverslip. Otherwise they will be on the condenser, the mirror or the objective.

- (xiv) Always keep the microscope covered when not in use and always keep an eyepiece in the microscope to prevent dust getting into the tube-draw and thus on the inside of the objectives. Dust is the worst enemy of the microscope.

II. THE PREPARATION OF MICROSCOPICAL SLIDES

Objects to be examined under the microscope are usually mounted on glass **slides** measuring 3×1 in., and covered by a circular or square **coverslip**. Unless specimens are very small and thin, it is generally necessary to cut **sections**.

Minute organisms, tissues or sections may simply be mounted in water or dilute glycerine, etc., covered and examined (**temporary mounts**), but it is often necessary to use **stains** to show up certain structures; and, again, it is sometimes desirable to make a **permanent mount**.* In this case, the object must be subjected to certain processes as follows:—

- (1) Killing, Fixing and Hardening.
- (2) Staining.
- (3) Dehydrating.
- (4) Clearing.
- (5) Mounting.

The reagents and stains are put into watch glasses and the object is transferred from one to another by means of a section-lifter.

(1) SECTION CUTTING

Sections may be **transverse** or **longitudinal (radial or tangential)**, depending on the symmetry of the object from which they are cut).

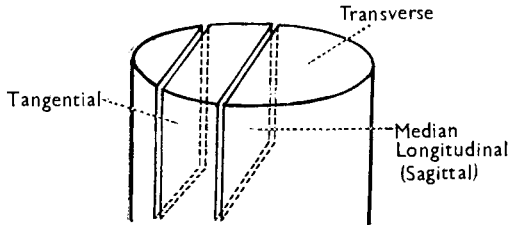


FIG. 4. Kinds of Sections.

* Several permanent mounts of plant tissues should be made. Suitable reagents and stains are given in the text.

For section cutting a *sharp section-cutting razor*, hollow-ground on one side and flat-ground on the other, is necessary.

To Sharpen a Razor

(1) Stropping

Suspend the razor strop from a hook on the wall and, holding it taut with one hand, draw the razor blade backwards and forwards,

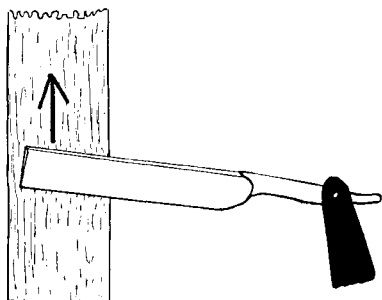


FIG. 5. Stropping a Razor.

away from the edge, several times on the rougher side of the strop, keeping the blade flat on the strop. Repeat the process several times on the smoother side of the strop. Always strop the razor before and after section cutting.

(2) Honing

Periodically, stropping is insufficient and a razor requires sharpening on a stone. Put a small quantity of oil on an oil-stone

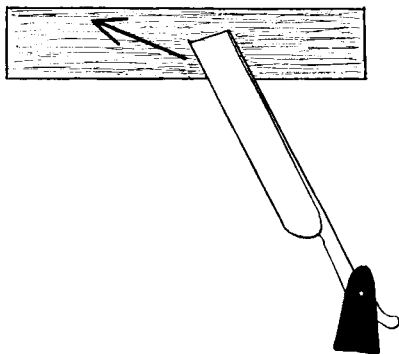


FIG. 6. Honing a Razor.

and draw the razor, *edge foremost*, obliquely across the stone, keeping the blade flat on the stone. Then turn the other edge to the stone and repeat the movement. This should be done several times. Then draw it once or twice away from the edge to remove the burr.

Cutting Sections

Objects from which sections are to be cut should be held between the thumb and forefinger or between the tips of the fingers of the left hand, or, if slender, between pieces of pith held in a similar manner.

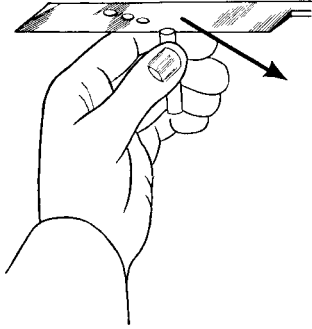


FIG. 7. Hand Section-cutting.

(Alternatively they can be held in a **hand microtome**, but in most types of this instrument it is first necessary to **embed** the tissue in paraffin wax in order to give it rigidity. This is afterwards dissolved out of the sections. The use of a microtome is more necessary for softer material than it is for the more rigid tissues like woody stem. The most perfect sections are cut by a **bench microtome**.) It is sometimes helpful to rest the hand against the edge of the bench.

Thoroughly moisten the tissue and the razor with dilute alcohol (30 or 50 per cent.) or with water with fresh tissues and keep them moist. (This prevents sections from drying and air bubbles from appearing in the mounted specimen.) Holding the razor between the thumb and forefinger of the right hand with the remaining three fingers alongside the latter and, resting the blade on the forefinger of the left hand, make a long oblique stroke. (See Fig. 7.)

The sections must be thin and of uniform thickness. Ideally, they should be one cell thick. Several should be cut and considerable practice will be required.

Transfer the sections by a camel's hair brush to a watch glass containing a little of the liquid in which the material has been stored.

Use of the Hand Microtome

(1) Embedding in Wax

- (i) First fix, dehydrate and clear the material in bulk by one of the methods described below, using small pieces of material about 1 cm. cube and allowing very much longer in each reagent than is necessary for sections.
- (ii) Then melt some paraffin wax* in a suitable receptacle such as the small evaporating basin with a handle known as a casserole. Wax of melting point about 48° C. should be used

* See Appendix 1 (7).

as a higher temperature may affect the material. Place the material in the molten wax, kept at a constant temperature which must be more than one or two degrees above its melting point and leave it there for two hours or more. This will replace the clearing agent by molten wax and will thoroughly **impregnate** the material with it.

- (iii) *Pour some of the wax into the well of the hand microtome (if it is of suitable type, otherwise an **embedding mould** must be used) and transfer the impregnated material to it with forceps, adjusting it in the required position by a mounted needle. Pour in more molten wax to cover the material. Now cause the wax to set quickly (to prevent it crystallising) by placing the bottom of the microtome (or mould) in cold water and by gently blowing on the surface until a skin is formed. Then lower completely into cold water.*
- (iv) *Cut sections with a dry razor. The thickness of the sections can be adjusted by rotating the milled head at the base of the instrument. In the better types of hand microtome, this is usually graduated so that one division represents 10 μ .*

(2) Subsequent Treatment

- (i) *Place the sections in xylene to dissolve out the wax.*
- (ii) *Transfer the sections to absolute alcohol to remove the xylene.*
- (iii) *Hydrate the sections by placing them for a couple of minutes in alcohols of decreasing concentration (absolute, 90, 70, 50 and 30 per cent.) down to the concentration of the solvent in the stain to be used. If the solvent is water, put the sections in distilled water after the 30 per cent. alcohol.*
- (iv) *Stain, dehydrate, clear and mount as described below.*

(2) KILLING, FIXING AND HARDENING*

The organism or tissue is exposed in the living state to a killing or fixing agent. This (i) **kills** the tissues and at the same time **fixes** them so that their histological form does not alter; (ii) prepares the tissue for subsequent treatment with stain and (iii) **hardens** it for section cutting. The tissue must be thoroughly washed in a suitable medium afterwards.

There is a large number of Fixing Agents of which the most important are tabulated below with their uses and washing media.

Tissues may be fixed in bulk or as sections. Immerse the material in several times its own volume of the fixing agent for at least fifteen minutes and preferably, in most cases, for considerably longer.

* Material is often supplied to students already fixed by the laboratory.

Thoroughly wash out the fixative in the washing medium stated. Use of the wrong medium will cause changes (e.g., precipitates) which will affect the subsequent treatment and results.

Hardening and Fixing Agents*

The Fixing Agents in more general use are shown in **thick type**.

Fixing Agent	Washing Medium
Alcohol (ethyl) 70%	70% alcohol.
Bouin's fluid	50 and 70% alcohol.
Chromo-acetic	Water, then 70% alcohol.
Flemming's solution	Water.
Formalin-alcohol	70% alcohol.
Mercuric chloride	Iodine-alcohol. Decolourise with sodium thiosulphate.
Picric acid	50 and 70% alcohol.

(3) STAINING

Staining is the colouring of tissues and structures by the addition of solutions of dyes and its object is to show up tissues and structures which would otherwise be imperfectly seen. Tissues may be stained in bulk but it is better to cut sections first.

Solutions of stains are made up in water or alcohol and the solvent must be known before proceeding with the next process (dehydration). Some stains are acid, some basic, and others are neutral. These terms do not refer to the reactions of the solutions but to the coloured radicles. Consequently, while some stain the nucleus (basic stains), others stain the cytoplasm (acidic stains) or cell contents, but most nuclear stains also stain the cytoplasm to a lesser extent. The converse is also true in some cases. There are **general stains** for ordinary use as opposed to **specific stains** which stain certain tissues only. Owing to this specific nature of some stains, it is possible to use two or even three different stains on the same sections in order to differentiate the tissues more clearly. This is called **Counter-staining** (or **Double** or **Triple staining**). A list of the commoner stains and the uses to which they are put is given below.

Methods of Staining†

There are two methods of staining:—

- (i) **Progressive staining** in which the tissue is left in the stain until it has reached the required depth of colour.

* See Appendix I (1) for their preparation.

† Detailed examples of single and double staining are given at the end of this chapter.

- (ii) **Regressive staining** in which the tissue is intentionally over-stain and then decolorised (**differentiated**) to the required depth of colour.

The beginner should use the method of progressive staining as it is a little difficult for him to judge when the required degree of differentiation has been reached. When he has reached some degree of success with this method, he can try the method of regressive staining.

(1) Single Staining*

The sections are placed in the stain and left there until they are stained to the required depth (*progressive staining*). This can be ascertained by placing the watch glass on the stage of the microscope and examining with the low power. Normally, sections take only two or three minutes. Excess of stain must afterwards be removed by placing the section in the solvent used for the stain. This will not remove the stain from the tissues. If a section is overstained or if the method of regressive staining is used, the degree of staining can be lessened by placing it in **acid alcohol**.† This is called **differentiation** because it differentiates the extent to which the various parts of the tissue are stained. Differentiation should be watched under the low power of the microscope as when staining, and when it is complete the tissue should be washed in 70 per cent. alcohol.

(2) Double Staining*

The sections are stained with one stain at a time, the excess being removed as in single staining before placing the section in the second stain. It may be necessary to differentiate and even to dehydrate before adding the second stain (as when using Safranin and Light Green in Clove Oil for plant stems).

Stains

Temporary stains are in *italics*.
Good general stains are in **thick type**.

Stain‡	Solvent	Use
Aniline blue	Alcohol.	Sieve plates—blue.
Aniline (Cotton) blue	Lacto-phenol.	Fungi.
<i>Aniline sulphate</i> (or <i>hydrochloride</i>)	<i>Water.</i>	<i>Lignin—yellow.</i>
Bismarck brown	Alcohol.	Cellulose and nucleus— brown.
Carbol-fuchsin	Alcohol.	Bacteria. Bacteria and Fungi—red.

* Detailed examples of single and double staining are given at the end of this chapter.

† See Appendix I (4).

‡ See Appendix I (3).

Stain*	Solvent	Use
Congo red	Water.	Fungal hyphae—red.
Eosin Y.	Water or alcohol.	Cytoplasm—pink. Cellulose walls—red.
Gentian violet	Water (or alcohol).	Nucleus—violet.
Haemalum	Water.	Nucleus—blue.
Haematoxylin (Delafield)	Alcohol.	Nucleus—blue.
Iodine	Aqueous.	Starch—blue.
Light green	Alcohol or clove oil.	Cellulose—green.
Methylene blue	Alcohol.	Nucleus—blue. Bacteria. Lignin—red.
<i>Phloroglucin and conc. hydrochloric acid</i>	Alcohol.	
Safranin	Alcohol.	Lignin—red. Suberin—red. Nucleus—red.
<i>Schultze's solution (chlor-zinc-iodine)</i>	Water.	Cellulose—blue (or violet). Starch—blue. Proteins—yellow. Lignin—yellow. Fats—red.
<i>Sudan III</i>	Alcohol.	

Double Staining

- { Safranin.
- { Light Green.
- { Safranin.
- { Haematoxylin or Haemalum.
- { Haematoxylin or Haemalum.
- { Eosin.

(4) DEHYDRATION

Owing to the fact that water is immiscible with the oil used for clearing on the one hand and with the solvent of the mounting medium (usually xylene) on the other, it is essential that all traces of water should be removed from the tissue. This removal of water is usually effected by means of ethyl alcohol and is known as **dehydration**. Now, if the sections were placed directly into absolute alcohol, the cells would lose their water so quickly that they would shrink and their shape would be altered. This is avoided by adopting the following method:—

Place the sections in solutions of ethyl alcohol of gradually increasing concentration† for one or two minutes. The concentrations generally used are:—

30 per cent. alcohol.	70 per cent. alcohol.
50 " "	90 " "
Absolute alcohol.	

* See Appendix I (3).

† See Appendix I (4).

Start with the concentration next above that in which the sections were last placed, i.e., if the stain was aqueous, begin with 30 per cent. alcohol, but if it was alcoholic, begin with 70 per cent. alcohol.

To ensure complete dehydration, finally transfer the sections to a second watch glass of absolute alcohol. Avoid breathing on the absolute alcohol in the watch glass and in the bottle; otherwise it will no longer be absolute. It is an advantage, therefore, to cover the watch glass with a second one inverted over it.

In place of alcohol, a substance known as "Cellosolve" (Ethylene glycol monoethyl ether) can be used for dehydrating sections. This mixes with water, alcohol, oil of cloves and xylene and there is no need to use varying concentrations, as it does not cause shrinkage or alteration in shape: nor is it necessary to clear after using it. It dehydrates rapidly and because of this it is not recommended for animal tissues as it may cause distortion.

Place the sections in cellosolve for about a minute and then mount direct.

(5) CLEARING

If alcohol has been used as the dehydrating agent, it must now be removed. This process is called **clearing** as it also renders the tissues transparent. For permanent preparations the best results are obtained by using *oil of cloves* for plant tissues. Xylene can be used but it has a tendency to cause shrinkage. Clove oil may remove safranin from tissues stained with it and counter staining with light green may displace safranin. In this case it is necessary to restain with safranin after going down through the alcohols as far as 70 per cent. and then completing the dehydration. For delicate Algae and Fungi, it is best to use *lacto-phenol* as the clearing agent.

Leave the sections in the clearing agent until they are transparent. This usually takes two or three minutes. If there is any sign of a white film around the sections, this indicates incomplete dehydration and they should be returned to absolute alcohol and cleared again.*

(6) MOUNTING

For final examination under the microscope and for preservation, the sections must now be **mounted** in a suitable medium† of about the same refractive index as crown glass (1.5). The following may be used:—

(1) Temporary Mount

Glycerine . . . 50 per cent. aq. (R.I. = about 1.39–1.34)

* See Appendix I (5).

† See Appendix I (6).

(2) Permanent Mounts

The best Mounting Medium for general use is *Canada Balsam* dissolved in xylene. (R.I. = about 1.524.)

Euparal (R.I. = about 1.4) can be used, and in this case it does not matter if dehydration is not complete.

Place a drop of *Canada Balsam* on the centre of a clean slide. Transfer the section to the balsam with a camel's-hair brush. Cover with a clean coverslip by resting the coverslip against the finger and levering it down with a mounted needle (Fig. 8). This will prevent the



FIG. 8. Levering down the Coverslip.

entrance of air bubbles. There should be no air bubbles in the balsam. If any appear, they can generally be removed by gently warming the slide over a very small flame. A white film in the balsam indicates incomplete dehydration. Label the slide and leave it to dry. The coverslip may be ringed but this is not by any means essential for ordinary purposes.

In labelling slides, state the name of the organism from which the tissue was obtained, the name of the object or tissue and, if it is a section, whether it is L.S. or T.S. It is also an advantage for future reference to add the name of the fixing agent and stain used.

Delicate algae and fungi can be mounted in *lacto-phenol*.* In this case it is necessary to ring the slide (see para. 7 below).

(7) RINGING

Place the slide on a turntable (Fig. 9) and fix it in position by the springs provided.

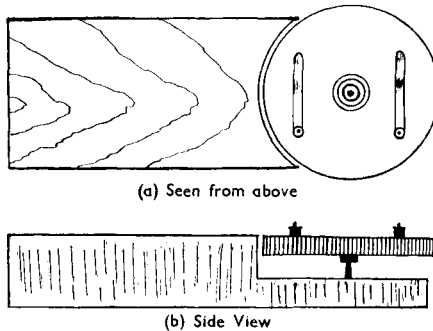


FIG. 9. Turntable for Ringing Slides.

* This is also a clearing agent for these objects.

Dip a brush in the ringing cement and start the turntable revolving rapidly by applying the finger to the milled edge. Apply the brush to the edge of the coverslip, covering it with a thin layer of the cement. Allow this to dry and repeat the process until a sufficiently thick ring is obtained.

(8) IRRIGATION

Minute organisms often require treatment with fluids while still living. This is done on the slide by the process of **irrigation**.

Having made a temporary mount of the object, place a drop of the irrigating fluid against one edge of the coverslip and a small piece of filter paper against the opposite edge. This gradually withdraws the fluid in which the object is mounted and the irrigating fluid enters underneath the coverslip to take its place.

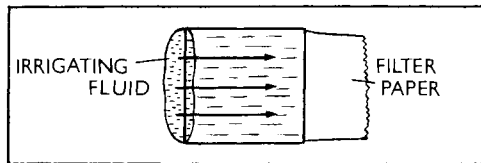


FIG. 10. Irrigation.

EXAMPLES OF METHODS OF MAKING PERMANENT STAINED PREPARATIONS

In the following examples, the times stated must not be taken too rigidly. Some sections will take longer than others to stain. Examination of the tissues under the low power of the microscope will show when they are suitably stained. Again, sections should be left in the washing fluid until no further stain comes out and in the clearing agent until they are transparent.

These methods are suitable for sections of root and stem and for plant tissues generally.*

(i) Single Staining with Delafield's Haematoxylin

- (1) Cut sections into 50% alcohol.
- (2) Delafield's Haematoxylin, undiluted (to stain) about 5 mins.
- (3) Distilled water (to remove excess stain) 1 min.
- (4) Acid alcohol (to differentiate) 3 mins.
- (5) Tap-water (to "blue" Haematoxylin) 2 mins.
- (6) 70% Alcohol (to dehydrate) 2 mins.

* It should be ascertained whether Double Staining is required or permitted in any particular examination syllabus.

- (7) 90% Alcohol (to dehydrate) 2 mins.
- (8) Absolute Alcohol 2 mins.
- (9) Absolute Alcohol 1 min.
- (10) Clove oil (to clear) until transparent . about 2 mins.
- (11) Mount in Canada balsam.

(ii) **Single and *Double Staining with Safranin and Light Green**

- (1) Cut sections into 50% Alcohol.
- (2) Safranin (stains lignin) 5–10 mins.
- (3) 70% Alcohol (to remove excess Safranin and Dehydrate) 2 mins.
- (4) 90% Alcohol (to dehydrate) 2 mins.
- (5) Absolute Alcohol 2 mins.
- (6) Absolute Alcohol 1 min.
- (7) Light Green in Clove Oil (stains cellulose) . 1–3 mins.
Do not leave sections in light green too long or the safranin will be entirely displaced. Should this occur, go down through absolute 90 and 70% alcohol and restain with safranin. Then repeat the dehydration.
- (8) Clove Oil (to clear. Also removes excess of light green) until transparent . . . about 2 mins.
- (9) Mount in Canada Balsam.

For Single Staining simply omit (2) or (7) as the case may be.

(iii) ***Double Staining with Safranin and Delafield's Haematoxylin**

- (1) Cut sections into 50% Alcohol.
- (2) Safranin (stains lignin) 5–10 mins.
- (3) 50 per cent. Alcohol (to remove excess safranin) 2 mins.
- (4) Delafield's Haematoxylin (stains cellulose) . 3 mins.
- (5) Distilled water (to remove excess Haematoxylin) 1 min.
- (6) Acid Alcohol (to differentiate) 3 mins.
- (7) Tap-water (to "blue" Haematoxylin) . . . 2 mins.
- (8) 70% Alcohol (to dehydrate) 2 mins.
- (9) 90% Alcohol 2 mins.
- (10) Absolute Alcohol 2 mins.
- (11) Absolute Alcohol 1 min.
- (12) Clove Oil (to clear) until transparent . about 2 mins.
- (13) Mount in Canada Balsam.

* It should be ascertained whether Double Staining is required or permitted in any particular examination syllabus.

PART II

MORPHOLOGY, CYTOLOGY AND HISTOLOGY

INTRODUCTORY NOTES

Reference should be made to the General Directions for Practical Work in the Introduction and, where necessary, to Part I (Microscopical Technique).

(1) Personal examination of plants is the only way in which to learn their structure, physiology and life-history. Examination should be made of the structure, growth, development, reproduction and seasonal conditions of *living* plants in the botanical garden, in the countryside and in the laboratory.

An accurate knowledge of the living plant can only be obtained, however, by a more detailed study under the hand lens and microscope, and by the performing of physiological experiments.

(2) **Microscopic Plants** should be examined (i) living, if possible, under the low and high powers, (ii) killed and stained.*

(3) **Larger Plants** should first be examined externally, alive if possible, a lens being used when necessary. This study should include examination of modifications of plant organs. Then a microscopical study of the cells (**cytology**) and tissues (**histology**) of plant organs must be made.*

(4) **Entire Specimens and Microscopical Preparations.**

(i) Print the name of the plant and the structure or organs on top; state whether it is an entire specimen, and which view.

If it is a microscopical preparation, state whether it is a longitudinal or transverse section or entire specimen, and whether it is as seen under the low or high power.

(ii) Write notes of any special directions, *e.g.*, method of staining.

(iii) Then make a **drawing** or **diagram**, roughly to scale. Examine the object carefully before you begin to draw it. *Draw only what you see* and draw on a *large* scale, showing all the necessary details.

(iv) PRINT the names in BLOCK CAPITALS *horizontally* and join them to the corresponding parts by straight lines. Avoid crossing these lines over one another. (The use of letters and a key at the bottom is not desirable.)

* See Part I for detailed instructions in microscopical technique.

(v) When you have a complicated microscopical structure to draw, make a **diagram** (or plan) of the whole structure under the *low power* and detailed **drawings** of small samples of each tissue under the *high power*, all suitably named.

(vi) All drawings and diagrams should be drawn in pencil and should be *large*. The use of shading and colours should be kept down to a minimum.

FIG. 11. **Lower Power Diagram.**
(Part of T.S. Stem).

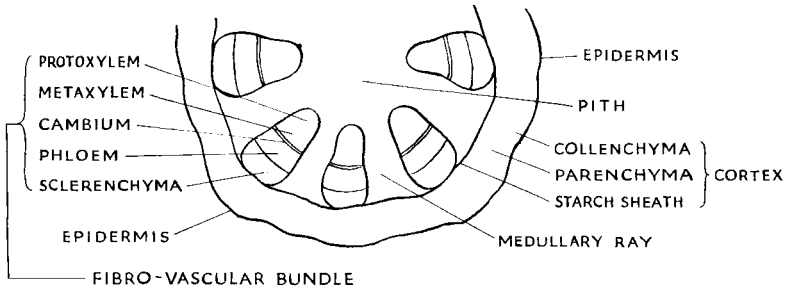
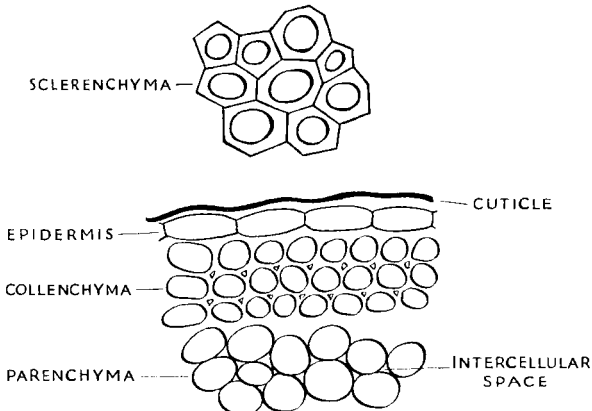
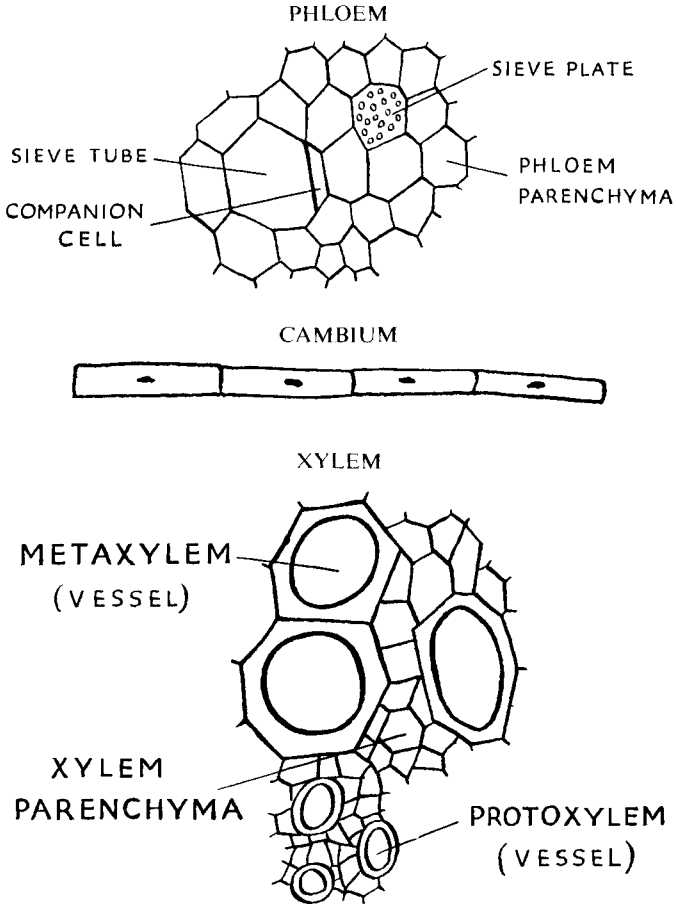


FIG. 12. **High Power Drawings of Tissues Indicated in Low Power Diagram.**
Walls thickened with lignin.



Walls thickened with cellulose, particularly at the corners.
Thin cellulose walls with many intercellular spaces.



Walls thickened and lignified. No protoplasmic contents.

(vii) Never copy drawings or diagrams from textbooks. By doing so, you learn very little and are therefore wasting valuable time. Draw only what you see in nature. *The illustrations in this book are intended solely to help the student to find and identify the various structures and tissues. Some of the figures are diagrammatic or semi-diagrammatic, though this is not so in all cases. This is intentional.*

(5) The names of the structures given in **thick type** in the text should be put in the drawings or diagrams (*provided they can be seen in the specimen*), but *all* the structures mentioned in the text may not be seen or identified. Draw exactly what you see and make *large* drawings of your specimens.

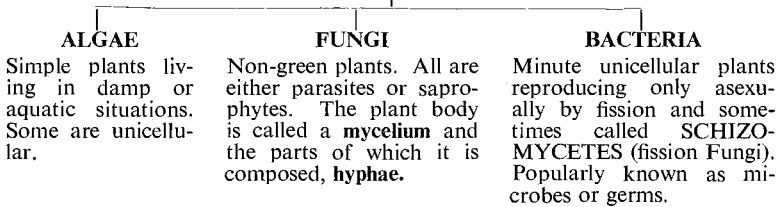
(6) The plants in this section are arranged in their systematic positions. The order in which they are studied will depend, of course, on the time of the year in which they are available.

(7) If Families of Plants have to be studied, a separate part of the file or note-book should be kept for them and a *Flora* will be necessary.

DIVISION THALLOPHYTA

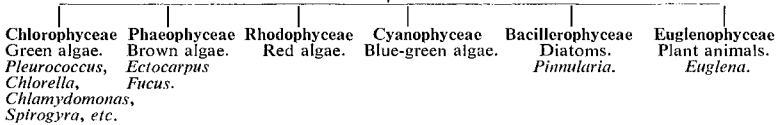
Plants without differentiation into roots, stems and leaves. The plant body is called a **thallus**.

SUB-DIVISIONS



ALGAE

CLASSES



CLASS

CHLOROPHYCEAE

PLEUROCOCCUS (PROTOCOCCUS) VIRIDIS

(1) Mount a little of the green "powder", **Pleurococcus**, in water. Cover.

Under *low power* note that the plant is spherical and non-cellular and that though it does occur singly, it is found more frequently in groups (often in more than one plane) due to rapid reproduction by fission. The walls in contact are usually flattened.

(2) Under *high power*, note the colourless **wall**, green lobed **chloroplast**, and the colourless **cytoplasm**. Look for the **nucleus** in the centre (it is difficult to see), and for cell division.

CHLORELLA

This organism lives in stagnant water. *Zoochlorella* lives in the endodermal cells of the animal *Hydra viridis* and *Zooxanthella* in *H. fusca*.

Mount a drop of water containing *Chlorella*. Cover. Also examine a stained preparation under high power.

Note the ovoid shape of the organism which is enclosed in a cellulose **wall**. Inside is a U-shaped **chloroplast** in contact with the

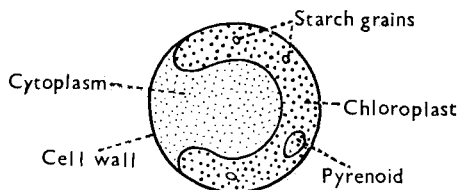


FIG. 13. *Chlorella*.

wall. In older plants the chloroplast may extend almost round the entire wall. A large **pyrenoid** is found inside the chloroplast. In the stained specimen, a small **nucleus** will also be visible.

HAEMATOCOCCUS (SPHAERELLA) PLUVIALIS

Mount a drop of the water containing living *Haematococcus*. Cover.

Under *high power*, note the plant is ovoid, non-cellular, green or red in colour, with a cellulose **wall** and colourless **cytoplasm** separated from the wall by a mucilaginous layer traversed by protoplasmic threads. In the centre is a green centrally placed reticulate **chloroplast** containing one or more **pyrenoids**. The **nucleus** is central. Cavities or **vacuoles** may be present in the protoplasm. Look for two **flagella**, delicate protoplasmic threads projecting from the anterior end (difficult to see), and the red **pigment-spot** (or **eye-spot**). The cell may contain a red pigment, haematochrome, particularly if encysted.

CHLAMYDOMONAS

Mount a drop of the water containing living *Chlamydomonas*. Cover. Also examine a stained preparation. Under *high power* and a prepared slide under the $\frac{1}{12}$ O.I. objective, note the ovoid non-cellular plant with cellulose **wall**. The small projection at the anterior end is called the **beak**. Inside the wall is a green cup-shaped **chloroplast**,

containing a glistening **pyrenoid** (containing protein and probably also a centre of starch formation, at the rounded posterior end. Colourless **cytoplasm** occupies the central part of the organism between the arms of the chloroplast. The **nucleus** (visible only in stained specimens)

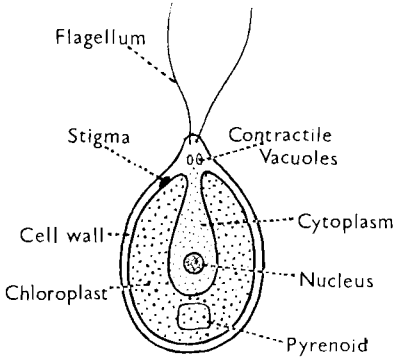


FIG. 14. *Chlamydomonas*.

lies in the cytoplasm. At the anterior end are two **contractile vacuoles** and a laterally placed red **pigment spot** or **stigma**. Look for two delicate whip-like protoplasmic strands, **flagella**, projecting from the pointed end (they are not always visible). Examine the movement of the cells and look for cells in the **palmella stage** in which the asexually formed **zoospores** have themselves divided while still in the parent cell wall.

PANDORINA

Examine a drop of water containing living **Pandorina** or a prepared slide. Note the spherical **coenobium** (colony) consisting of sixteen **cells** of structure similar to that of *Chlamydomonas* but rather pyramidal-shaped due to their being pressed close to one another.

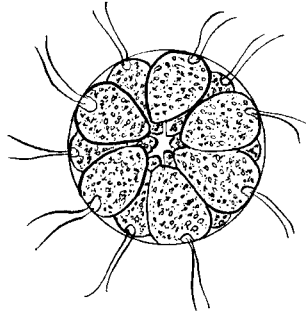


FIG. 15. *Pandorina*.

Daughter coenobia are formed asexually by each cell dividing to form sixteen zoospores which form a new colony, and also sexually by the formation of sixteen or thirty-two similar **gametes (isogametes)** from each cell. The coenobium contains no soma.

EUDORINA

Examine a drop of water containing living **Eudorina** or a prepared slide. Note the spherical **coenobium** containing thirty-two or sixty-four cells (according to the species) similar in structure to *Chlamydomonas*, though more spherical. The colony is larger than that of *Pandorina* and the cells more loosely packed. Daughter colonies are formed asexually. Male and female gametes are usually found in

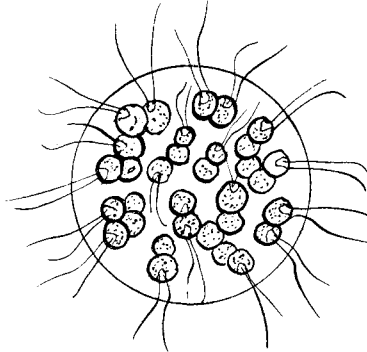


FIG. 16. *Eudorina*.

separate colonies. The **spermatozoids** (male) are elongated, bi-flagellated and devoid of chlorophyll. The **oospheres** are larger spherical cells. There is slight differentiation into soma and germ cells in this coenobium. (In *Pleodorina*, which is similar to *Eudorina* but larger, this differentiation is more advanced.)

VOLVOX

(1) Mount a drop of water containing living **Volvox** on a cavity slide. Cover. Examine under low and high power.

In *V. globator*, the larger species, the cells are more closely packed than in *V. aureus*. Note the large spherical **coenobium** containing several hundreds or thousands of *Chlamydomonas*-like cells (there may be as many as 20,000 in *V. globator* and up to 4,000 in *V. aureus*) on the surface of the sphere, the centre of which is mucilaginous. These cells are connected together by protoplasmic strands which may or may not be visible. They are more slender in *V. aureus*. The **somatic cells** preponderate and each is a rounded cell containing cytoplasm, nucleus and chloroplast and is enclosed in the space bridged by protoplasmic strands. The flagella project outside the colony. Look for **gonidia** (which reproduce asexually) large rounded

cells with large nuclei, and the **germ cells**, which are of two kinds: (a) tiny biciliate cigar-shaped male gametes, **antherozoids or spermatozoids**, devoid of chlorophyll and developing in **antheridia**, in plates

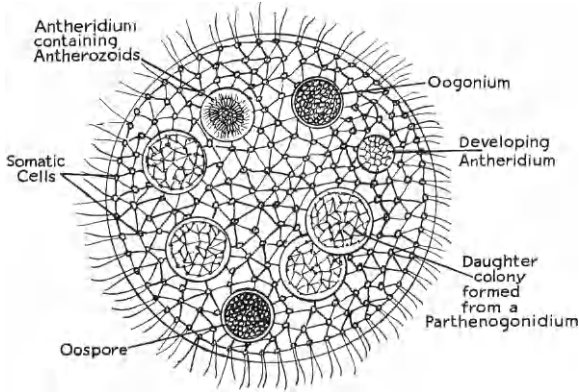


FIG. 17. **Volvox.**

or spheres, and (b) large spherical female gametes, **oospheres**, without cilia and containing **chlorophyll** and developing in spherical **oogonia**. Look for **oospores** formed by fusion of gametes. The germ cells may be produced in the same (*dioecious*) or different (*monoecious*) colonies. There is a distinctly advanced division of labour in this type with clear differentiation into *soma* and *germ cells*.

(2) Stain a colony with methylene blue by irrigation. Examine.

SPIROGYRA

(1) Mount a few filaments of **Spirogyra** in water, separating the filaments by mounted needles. Cover.

Under *low power* note that the multicellular **filament** is an unbranched thallus of identical cylindrical **cells**. Under *high power* note the **cell wall** with **cytoplasmic lining**, the **nucleus** containing a **nucleolus** and suspended by **protoplasmic strands** (or **bridles**). The spirally-wound **chloroplast** has serrated edges and contains a number of **pyrenoids**. The rest of the cell is **vacuole**. Look also for cell walls developing transversely across the cells, thus making the chloroplast appear continuous from cell to cell. The plant is morphologically multicellular and shows division of labour; physiologically it is unicellular. There is no differentiation into *soma* and *germ cells*.

(2) Examine cells showing stages in **sexual reproduction**.

Look for **conjugation tubes** in various stages of formation joining two adjacent cells, usually in separate filaments (scalariform conjugation), **male gametes** (contracted cell contents) in various positions

and **female gametes** (they are isogamous) and the empty cells which have lost their (male) gametes. Large brown oval **zygospores** formed by conjugation will also be seen.

(3) *Decolourise a filament in warm alcohol. Mount in Schultze's solution.*

The cell wall is turned blue (cellulose), the pyrenoids brown, and the starch grains which are seen around them, blue.

(4) *Decolourise a filament in warm alcohol. Stain with safranin or haematoxylin. Mount in dilute glycerine.*

The nucleus and bridges will be more easily seen.

OEDOGENIUM

(1) *Mount some filaments of Oedogonium in water. Cover.*

Under *low power* observe that the plant is composed of unbranched filaments. All the cells are not identical as in *Spirogyra* as there are specialised **gametangia** which give rise to gametes (these are *germ cells*) apart from the vegetative cells incapable of reproduction (these are the *soma*).

Under *high power*, note the **cell-wall**, the **nucleus**, the irregular **chloroplast** in the form of a network (or reticulum) containing **pyrenoids** and lying in the centre of the cytoplasm. Look for **cap-cells** with a series of ring-like caps at their upper ends.

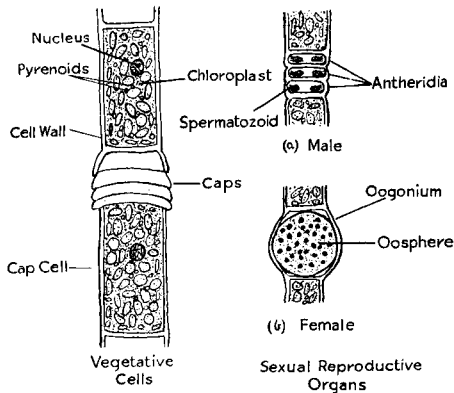


FIG. 18. Oedogonium.

(2) *Examine a slide showing asexual reproduction.*

Note the spherical **zoosporangium** from which is set free a single multinucleate pear-shaped **zoospore** with a colourless **beak** at its anterior end and chlorophyll elsewhere.

(3) *Examine a slide showing sexual reproduction.*

Note the male **antheridia**, small cells the contents of which divide to give rise to two motile male gametes, **spermatozoids** (or **antherozoids**) like miniature zoospores but almost devoid of chlorophyll, and the female **oogonia**, large rounded cells in which the single female gametes or **oosphere** which contains chlorophyll, develops. Thus there is heterogamy. In some species, the antheridia are developed on small filaments, all the cells of which produce spermatozoids: these filaments are called **dwarf males** and are joined to female filaments. It is therefore *dioecious*. In other species, antheridia and oogonia develop on separate plants and these are therefore *monoecious*.

VAUCHERIA

(1) *Mount some filaments of Vaucheria in water. Cover and examine under low and high power.*

In most species, the filaments are branched. There is no division into cells: the filaments are non-septate but multinucleate, *i.e.*, it is *coenocytic*. Note the numerous discoid **chloroplasts**, devoid of pyrenoids, in the **cytoplasm** lining the **cell wall** and the continuous central **vacuole**. The nuclei are difficult to see.

(2) *Examine a slide showing asexual reproduction.*

Note the club-shaped **zoosporangium** at the tip of the filament and separated from it by a septum, from which develop the ovoid, ciliated and multinucleate **zoospores** which contain chloroplasts.

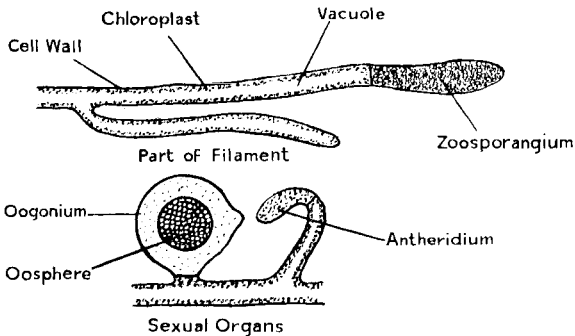


FIG. 19. Vaucheria.

(3) *Examine a slide showing sexual reproduction.*

On a lateral branch of the filament, note the long curved **antheridium** in the free end of which, separated from the filament by a septum, the biciliate **spermatozoids** develop. The large spherical

oogonium, also on a lateral branch of the filament from which it is separated by a septum, contains the female gamete or **oosphere**. Note the **beak** on the end of the oogonium which, in a later stage, is perforated. *Look for oospores.*

CLADOPHORA

Mount some filaments of Cladophora in water. Cover and examine under low power. Also examine a stained preparation.

The filament, which is branched, is composed of long cells and the branches originate near the upper ends of the cells. If the lower end of a filament is present, it will be seen to consist of many septate branches which serve as a means of attachment.

Examine a cell of Cladophora under the high power.

A diffuse, irregularly-shaped **chloroplast** will be seen occupying the greater part of the cell which contains many **nuclei** and is therefore *coenocytic*. Many **pyrenoids** will also be seen in the chloroplast.

Ovoid zoospores each with two flagella may be formed in and liberated from any of the cells of the filament and these cells are therefore **zoosporangia** when so doing. In a similar way, isogametes may be formed in any of the cells which then function as **gametangia**.

CLASS

EUGLENOPHYCEAE

EUGLENA

Euglena may be classified as a green *Alga* and thus as for *Pleurococcus* or as a *Protozoon* belonging to the CLASS *Mastigophora* (Protozoa having a pellicle and one or more flagella. Reproduction asexual). It lives in ditch and puddle water.

As this green organism can be considered as an alga or as a protozoon, it is, in fact, a "plant-animal". Saprozoic nutrition does occur in *Euglena* when holophytic nutrition is impossible.

(1) *Mount a drop of the water containing Euglena viridis. Cover.*

Under *low and high power* note the noncellular green organisms moving in all directions and *examine the method of movement. By using the iris diaphragm*, movement of the water due to the flagellum may be seen.

(2) *Irrigate with iodine. Also examine a prepared slide. Under high power note that the organism is spindle-shaped with a blunt anterior end bearing a single **flagellum** and a pointed posterior end. An elastic **pellicle** encloses a clear **ectoplasm** and a granular **endoplasm**. At the anterior end is a **contractile vacuole** communicating with the exterior by the so-called **gullet** (or **cytopharynx**). The **endoplasm***

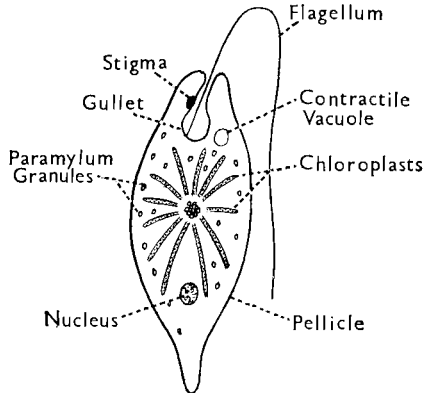


FIG. 20. *Euglena*.

contains elongated **chloroplasts** radiating from a centre, **paramylum granules**, a large **nucleus** containing a **nucleolus** (karyosome) towards the posterior end and a red **pigment-spot** (or **stigma**) anteriorly. *Look for stages in asexual reproduction by binary fission.*

CLASS
PHAEOPHYCEAE
ECTOCARPUS

This is a simple marine filamentous brown alga and the filaments are branched. If the lower end of the plant is examined it will be seen to have a disc-like holdfast.

*Examine a slide of **Ectocarpus**.*

Note that the filament is composed of similar cells except for the apical cell (if present) which is elongated into a hair-like structure. In each cell is a centrally placed **nucleus** suspended by **cytoplasmic strands** and one large and several small **chromatophores**. Reproductive structures appear on branches of the main filament. If the plant is a **sporophyte** unicellular ovoid **sporangia** which are unilocular may

be present. Zoospores, which develop into gametophytic plants, are produced in these sporangia. If the plant is a **gametophyte**, **gametangia** may be found. These are ovoid, pointed multilocular structures and are borne on basal cells as branches of the main filament. They are larger than the sporangia and in them, the gametes develop.

FUCUS

THE BROWN SEAWEED

(1) *Examine with the naked eye* **Fucus serratus**, **F. vesiculosus** or **F. spiralis**.

The former has serrated edges and *F. vesiculosus* bears small bladders to give it buoyancy. Note the colour and that the body or **thallus** consists of a somewhat cylindrical stalk, the **stipe**, with a disc-like base, the **hapteron** or **holdfast**, and an expanded, branched, flattened, upper portion, the **frond**. Note also the thickened **midrib** which runs up the centre of the lobes of the thallus, inconspicuous in the newer **apical growing point**. Observe the swollen reproductive areas known as **receptacles** in the outer parts of some of the lobes of the frond, and on their surfaces small papillae, which are the entrances to the internally placed **conceptacles**.

(2) *Cut a transverse section of the frond of Fucus. Mount in dilute glycerine.*

Under *low power*, note the external mucilaginous **cuticle** covering the outer layer of cells, elongated at right angles to the surface and known as the **palisade layer** or **meristoderm**. This layer contains **phaeoplasts** in which the green chlorophyll is masked by the brown *fucoxanthin*. Below this is the **cortex**, a four- or five-layered compact region of larger polygonal cells containing fewer phaeoplasts. In the centre is the **medulla** composed of filaments of cells mostly devoid of phaeoplasts and loosely embedded in a mucilaginous matrix.

(3) *Examine a L.S. of the frond in the apical region, passing accurately through the growing point.*

Note the **apical cell** which divides longitudinally into two, each new cell developing into a new frond. This kind of branching is known as *dichotomy*.

(4) *Cut and examine a T.S. of the frond through the male and female conceptacles under the low and high powers.*

In *F. spiralis* the male and female gametes are developed in the same conceptacles on the same plant (*monoecious*) but in *F. serratus* and *F. vesiculosus* male and female conceptacles are borne on separate plants (*dioecious*).

Note the orange male and dark green female conceptacles containing numerous unbranched hairs called **paraphyses**. In the male conceptacle on branched hairs are ovoid **antheridia** in which **antherozoids** (or **spermatozoids**) develop. Each antherozoid contains one or

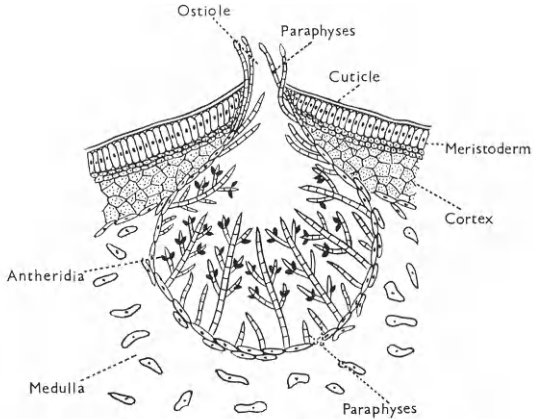


FIG. 21. *Fucus*. T.S. Conceptacle—male.

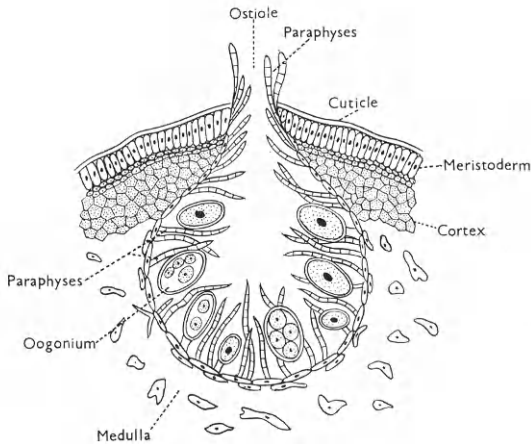


FIG. 22. *Fucus*. T.S. Conceptacle—female.

more orange chromatophores. In the **female conceptacle** are **oogonia**, each composed of an ovoid or spherical **oogonial cell** from which eight **oospheres** develop and a lower **stalk cell** which attaches it to the wall of the conceptacle.

CLASS
BACILLAROPHYCEAE

PINNULARIA

DIATOMS are *Algae* and are non-cellular plants with a wall of transparent siliceous material. They contain a yellow pigment, diatomin, in addition to chlorophyll. They mostly live in freshwater though some are marine.

Examine a slide of the diatom **Pinnularia**

The plant is bilaterally symmetrical and consists of two **valves**. Each valve is composed of silicified ornamented walls and one valve overlaps the other at the edges; these overlapping edges are known as the **mantles**. This will be seen in what is known as the *girdle view* (side-view) since the connecting edges constitute the girdle. Here they all appear to be rectangular. When the *valve view* (surface-view) is examined it will be seen that the plant is rather ellipsoid, though the sides are somewhat flattened. The delicate ornamentation of the wall will be clearly seen in this view. The **nucleus** is centrally situated and is suspended by **cytoplasmic strands** which reach to the cytoplasmic lining of the wall. Two large chloroplasts, or rather **chromatophores** since they contain additional pigments such as the yellow diatomin, each with a **pyrenoid**, lie in the cytoplasm. A slit known as the **raphe** runs along the centre of each valve connecting three **nodules**, one at each end and one in the centre.

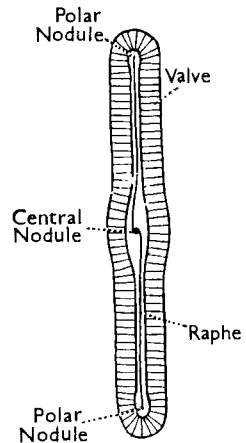


FIG. 23. *Pinnularia*.

It is worth while examining other slides of diatoms, particularly those of selected diatoms, when the beautiful effects of their structure will be seen.

FUNGI

CLASSES

PHYCOMYCETES

Algae-like fungi, generally with non-septate hyphae and sexual reproduction.

Mucor, Pythium, Peronospora, Albugo, Phytophthora, etc.

LICHENES

Fungi and Algae in symbiotic association.

ASCOMYCETES

Form spores called **ascospores** within elongated sporangia called **asci**.

Saccharomyces, Eurotium, Penicillium, Monilia etc.

BASIDIOMYCETES

Develop a club-shaped **basidium** from which four **basidiospores** arise, budding from different points.

Puccinia, Psalliotia

CLASS
PHYCOMYCETES

Phycomycetes are sometimes divided into two Sub-Classes—*Zygomycetes* (no antheridia or oogonia. Isogametes which give rise to zygospores) and *Oomycetes* (Antheridia and oogonia are developed, though asexual zoospores are readily formed).

MUCOR

(1) *Expose a piece of wet bread to the air for about an hour. By this time spores of the white mould **Mucor mucedo**, a saprophyte, will have settled upon it from the air. Cover with a small bell-jar to exclude dust. Examine a few days later with a lens.*

Note the aerial part of the filamentous **mycelium** composed of a number of white threads, **hyphae**. Some (the older ones) bear black knobs on their ends: these are **sporangia**.

(2) *Soak some of the mycelium in alcohol to remove air bubbles and mount in dilute glycerine.*

Under *high power* note that the mycelium is composed of non-septate, multinucleate (coenocytic) branching tubes called **hyphae**, the aerial branches of which are known as **sporangiophores** because they bear spherical **sporangia** at their tips. These contain numerous **spores**. By careful focussing it will be seen that the end of the hypha bulges into the sporangium: this is the **columella**.

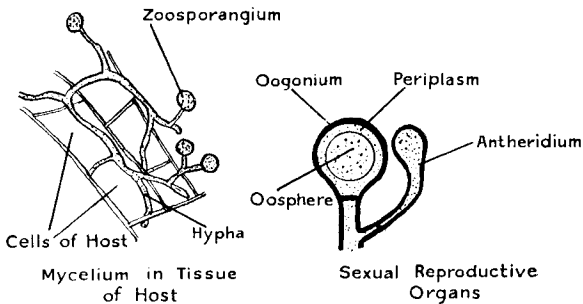
(3) *Examine a prepared slide showing sexual reproduction.*

Look for **gametophores**, hyphae, the apical parts of which are known as **gametangia** and which are cut off by a septum and contain multinucleate gametes. Look for conjugation between them. There is no differentiation into male and female gametes as the difference between them is a purely physiological one and the mycelia producing them are referred to as + and — strains; this is known as **heterothallism**. Zygospores can be produced only by fusion between different strains. Note the large, black, rough **zygospores**, formed by fusion of the gametes.

Your slide may show **germinating zygospores**, each with a **promycelium**, a slender hypha bearing a **sporangium** at its tip but these are usually formed only after a period of rest.

PYTHIUM

(1) *Examine with a lens a seedling (e.g., cress or broad bean) which is “damping off”, i.e., infected by **Pythium de Baryanum** owing to the plant having germinated in very moist and crowded conditions.*

FIG. 24. *Pythium*.

This fungus is a *facultative parasite*, i.e., it can continue to live saprophytically for a time after it has killed its host.

Note the aerial **hyphae** which bear **zoosporangia** arising from the mycelium in the tissues of the seedling.

(2) *Examine a prepared slide of the mycelium* on a host.

Note the non-septate coenocytic **mycelium** which penetrates between and into the cells of the host.

Note also the **zoosporangia** which germinate into new mycelia and the contents of which divide to form **zoospores**, each bearing two laterally placed **flagella**.

(3) *Examine a slide showing sexual reproduction* which occurs in the older cultures.

Note the spherical (female) **oogonium** on a hypha (not necessarily at the tip) cut off by a **septum** and containing a uninucleate **oosphere** surrounded by **periplasm** in which are several nuclei. The club-shaped (male) **antheridium** may be on the same or an adjacent hypha and contains the uninucleate **male gamete** surrounded by **periplasm**.

On another slide look for stages in conjugation, showing **fertilisation tubes**, **zygotes** and **oospores** (zygotes with resistant coats). Examine **germinating oospores** if present, each producing a **hypha** and a **zoosporangium**.

PERONOSPORA

This mildew is an *obligatory parasite* (i.e., solely a parasite) living within the tissues of the host and is a *selective parasite*, i.e., it can attack only a specific host such as turnips, beet and cabbages.

(1) *Examine with a lens the leaves of a plant infected with Peronospora* (if available).

(2) *Examine a prepared slide of the mycelium of Peronospora in a leaf*.

Note the branched **mycelium**, the **hyphae** of which penetrate *between* the cells of the leaf; and the **haustoria**, short branches of those hyphae which penetrate *into* the cells and then branch considerably.

(3) *Examine a slide showing asexual reproduction on a leaf.*

Note the branching **conidiophores** which grow up through the stomata of the leaf from the intracellular mycelium inside and which bear **conidia** (spores formed by budding off in chains) each joined to the hypha by a thin, short **sterigma**.

(4) *Examine a slide showing sexual reproduction.*

Note spherical female **oogonium** on the tip of a hypha and the narrow **antheridium** on lateral branches of the same or another hypha. Look for **stages in conjugation**, showing **fertilisation tubes** and **oospores (zygotes)**.

ALBUGO (CYSTOPUS)

An *obligatory parasite* (i.e. solely a parasite) which is *selective*, one species attacking Shepherd's Purse. It causes what is called "white rust".

(1) *Examine, if possible, a plant infected with Cystopus.*

It will be most readily seen on the peduncle (i.e., the stalk of the inflorescence).

(2) *Examine a prepared slide of the mycelium of Cystopus in an infected plant.*

The **mycelium** will be found in the *intercellular spaces* of the host plant. It is unbranched and non-septate. On the mycelium will be seen a number of round structures penetrating *into* the cells; these are **haustoria**.

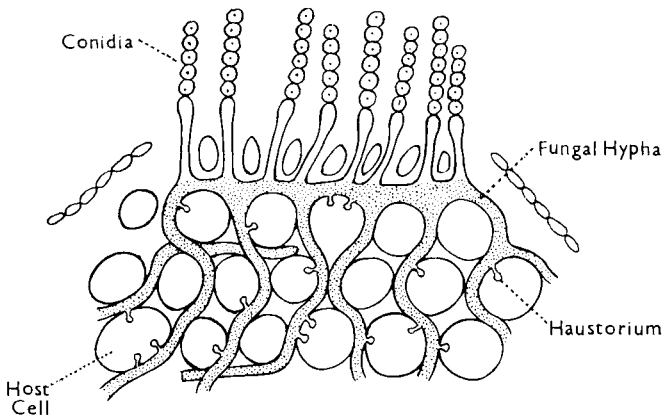


FIG. 25. Albugo on Host.

(3) *Examine a slide showing asexual reproduction.*

The tips of the hyphae near the surface are separated off by a septum. These are either **conidiophores** since **conidia** bud off from them in chains and penetrate through the epidermis of the host or **sporangiophores** which give rise to **zoospores** in water.

(4) *Examine a slide showing sexual reproduction.*

Note the spherical female **oogonia** on the hyphae, each containing an **oosphere**, and the somewhat club-shaped **antheridia** in which the **male gametes** develop. Fertilisation takes place between them and from the thick-walled **oospore**, which later becomes multinucleate, **zoospores** are liberated.

PHYTOPHTHORA INFESTANS

This *obligatory parasite* is the cause of *Potato Blight*. It attacks the leaves which develop brown patches, wither and die. Later it may spread down the stem into the tubers causing them to rot.

Examine a prepared slide of a leaf infected with Phytophthora infestans.

The **mycelium** which is composed of non-septate **hyphae** (arising from zoospores which settle on the leaf surface) enters the leaves of the host through stomata or by penetrating through epidermal cells. In the intercellular spaces of the leaf, the hyphae develop finer

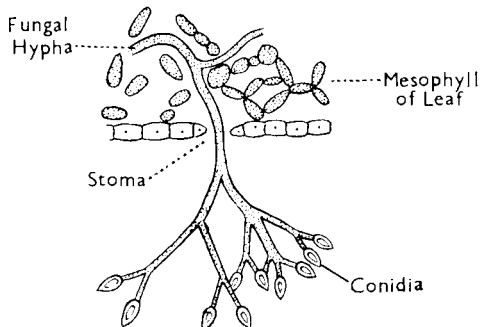


FIG. 26. *Phytophthora Infestans* on Leaf of Potato.

branches, **haustoria**, which enter the cells. It is when these cells die that the brown patches appear on the leaves.

Hyphae also grow out through the stomata of the leaves. These hyphae branch and develop **sporangia** and are therefore known as

sporangiohores. Conidia are set free in dry conditions and zoospores when conditions are wet and it is these which cause infection of the leaf.

(Some species of *Phytophthora* also reproduce sexually, branches of the hyphae giving rise to antheridia and oogonia, the zygote producing a short mycelium from which a conidiophore develops.)

In addition to the types given above, the Class *Phycomycetes* includes *Phytophthora parasiticae* which attacks cabbages, while other species infect maize, beet, cauliflower, etc., *Saprolegnia*, the cause of "fish fungus" and *Microsporon*, one of the causes of ringworm. These Fungi are therefore of considerable economic importance.

CLASS ASCOMYCETES

The Fungi in this group includes *Yeast*, *Penicillium* and *Trichophyton*, one of the causes of ringworm.

SACCHAROMYCES

YEAST

(1) *Mount some baker's yeast (S. cerevisiae) in a drop of water and examine under the high power.*

Note that the mycelium consists of isolated ovoid cells each with a **cell wall** containing granular **cytoplasm** with a large centrally placed **vacuole**. Lying at the side of this is an indistinct vacuolated **nucleus** traversed by a network of threads which may be composed of chromatin and containing a volutin granule often referred to as a **nucleolus**. Volutin granules occur in the cytoplasm together with glycogen. The formation of **buds** may be seen (gemmation), often in groups or chains.

(2) *Smear some yeast on a coverslip. Dry by gentle heat. Stain by immersion in methylene blue for about a minute. Wash carefully. Invert the coverslip in dilute glycerine on a slide. Examine under the high power or, better, $\frac{1}{12}$ in. O.I. objective.*

The nuclear structure can now be more clearly seen.

(3) *Mount some yeast in Schultze's solution on a slide.*

Observe the effect on the cell wall.

(4) *Mount some yeast in iodine.*

Note the reddish-brown colour in the cytoplasm showing glycogen.

(5) *Examine a prepared slide showing ascospores.*

These are formed under adverse conditions in certain species and the **sporangium** containing **ascospores** may be regarded as an **ascus**.

(6) *Investigate the fermentation of sugar by yeast. (Part IV, Physiology, Experiment 6, p. 152.)*

PENICILLIUM

Penicillium glaucum is another saprophyte which will develop on bread. It is a blue green mould and must not be confused with the green mould Eurotium (Aspergillus). *P. digitatum* attacks oranges, *P. expansum* destroys apples during winter storage while other species live on cheese (*P. camemberti* and *P. roqueforti*). The **antibiotic Penicillin** is extracted from *P. notatum* and *P. chrysogenum*.

Antibiotics are substances produced by living organisms such as Fungi which are used to prevent the growth (**bacteriostatic**) or to kill off (**bactericidal**) organisms which cause disease. *Streptomycin*, *aureomycin*, *chloromycetin* and *sigmamycin* are further examples.

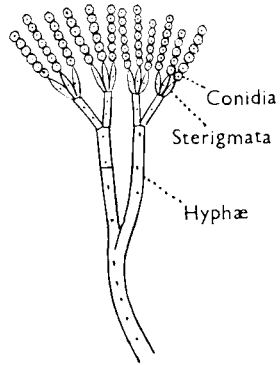


FIG. 27. **Penicillium.**

(1) *Mount some **Penicillium** in dilute glycerine.*

Under *high power* note that the **mycelium** is septate. The aerial **hyphae (conidiophores)** bear branched finger-like processes called **sterigmata** on which develop chains of tiny spores or **conidia** (so-called because they are formed by budding off in chains).

(2) *Examine a slide of sexual reproduction.*

The sexual process occurs rarely in this plant. Short lateral branches develop on the hyphae and these bear the sexual organs. The male **antheridia** are unicellular and multi-nucleate and they grow and coil round the outside of the female **archicarp**, which arises from the same hypha. It is composed of a multi-cellular **stalk** which bears an **ascogonium** on the free end of which is a unicellular **trichogyne**. In some species the archicarp is surrounded by **sterile nutritive hyphae**. This structure is known as an **ascocarp** or **perithecium**.

Inside this structure division takes place (actual fusion between the antheridial and ascogonial nuclei is rare) and a club-shaped **ascus** is formed, eight **ascospores** developing inside it.

EUROTIIUM (ASPERGILLUS)

This is an olive green *saprophyte* which will grow on damp bread in addition to Mucor. It also grows on fruit and vegetables if damp.

(1) *Examine some of the **mycelium of Eurotium** from a culture on bread.*

Under the *high power*, note that it is composed of branched septate **hyphae**. It is coenocytic. Some of the aerial hyphae are swollen at the tip and radiating out from the tip is a series of short processes called **sterigmata** from each of which bud off a chain of multi-nucleate spores called **conidia**. These hyphae are called **conidiophores**.

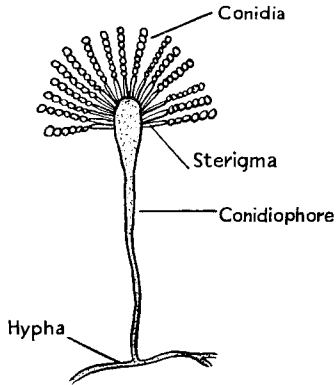


FIG. 28. *Eurotium*.

(2) *Examine a slide showing perithecia and asci.*

Each **peritheceum** is a sexual body composed of a closely coiled hypha, the **ascogonium** or **archicarp** (female) around which, another hypha, the **antheridium** (male) has grown, the whole being surrounded by a number of nutritive hyphae so that the whole structure has become a solid mass.

Union between ascogonium and antheridium is comparatively rare. Usually the latter withers while the former divides and branches, each branch developing a sporangium or **ascus** containing eight **ascospores**.

ERYSIPHE

A parasitic fungus or *mildew* common on cereal plants is **Erysiphe graminis**, the mycelium of which gives a white downy appearance to the leaves of the host as it spreads over the surface.

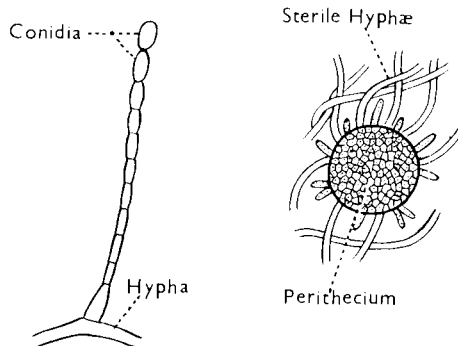


FIG. 29. *Erysiphe*.

(1) *Examine a slide of Erysiphe on a leaf.*

Note the branching **mycelium**. The hyphae do not penetrate into

the tissues of the leaf though outgrowths of the hyphae, **haustoria**, enter into the epidermal cells.

(2) *Examine a slide showing asexual reproduction.*

Note the short unbranched septate **conidiophores** from which large spores called **conidia** are budded off.

(3) *Examine a slide showing sexual reproduction.*

Short branches develop on separate hyphae and these bear either a slender uninucleate **antheridium** or a larger ovoid uninucleate **ascogonium**. From their bases, sterile nutritive hyphae arise which entwine over the sexual organs and enclose them forming a spherical structure called a **perithecium**; this later becomes brown. It is doubtful if union between the nuclei of the antheridium and ascogonium takes place but the latter divides to form sporangia called **asci** in which two, four and sometimes eight **ascospores** develop.

MONILIA FRUCTIGENA

This is the conidial stage of a parasitic fungus, **Sclerotinia fructigena**, and is the cause of "Brown Rot" in apples. This conidial stage is the only stage found in this country. The fungus can continue to live saprophytically after the host has died and is therefore a *facultative parasite*.

Examine a slide of Monilia fructigena on its host.

There are branched septate **hyphae** between the cells of the host and branched **conidiophores** will be seen on the surface of the host arising from these hyphae. The conidiophores are composed of chains of **conidia**.

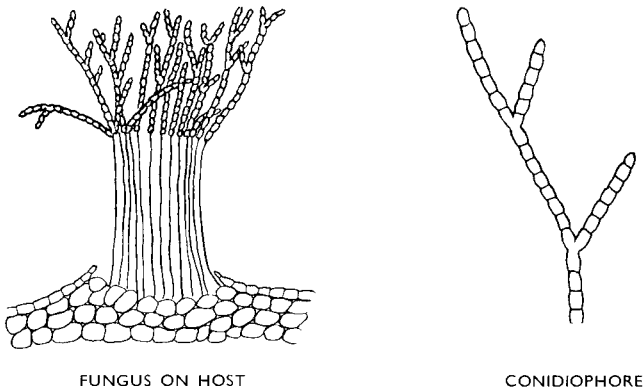


FIG. 30. *Monilia Fructigena*.

CLASS
BASIDIOMYCETES

This group includes mushrooms and toadstools and *Puccinia graminis*, the cause of black rust of wheat.

PUCCINIA

This fungus is an *obligatory parasite* on such plants as wheat, barley and oats and is the cause of the disease known as "Rust". *P. graminis* causes the disease in these plants *P. graminis tritici* affecting wheat, *P. graminis secalis* barley and *P. graminis avenae* oats. Other species affect other plants. *P. graminis tritici* has two hosts, barberry and wheat.

(1) *Examine a slide of P. graminis tritici on barberry leaf.*

Note the branched septate **hyphae** in the intercellular spaces of the host with small **haustoria** entering the cells and on the upper side of the leaf the cluster of tightly packed hyphae enclosed in flask-shaped cavities, the **spermogonia**. Some of the hyphae penetrate the upper epidermis. These are known as **receptive hyphae**. Inside the spermogonia are short hyphae which bud off minute spores called **spermatia**.

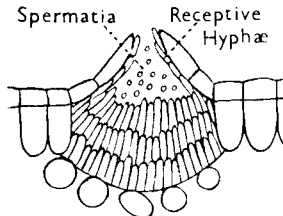


FIG. 31. *Puccinia*. Spermogonium.

The spermogonia and therefore the spermatia are of two types, + and —. They are therefore *heterothallic*. The spermatia are transferred to receptive hyphae of opposite types by insects. New hyphae develop and aggregate together to form **aecidia**.

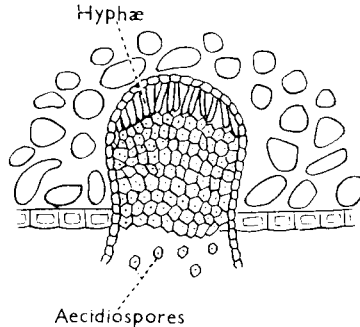


FIG. 32. *Puccinia*. Aecidium.

On the lower surface of the leaf of the host note these orange **aecidia**, each composed of collections of septate hyphae composed of cells, each having two nuclei enclosed in a cavity by tightly packed hyphae. The aecidia grow and rupture the epidermis. **Aecidiospores** are budded off from the hyphae in the aecidia. These are set free by the wind and may alight on the leaves of wheat when they germinate producing slender hyphae which enter the leaf through its stomata. In the mesophyll these hyphae, which are septate, each cell being bi-nucleate, ramify through the intercellular spaces and produce a mycelium.

(2) *Examine a slide of uredospores on a leaf of wheat.*

Clusters of erect hyphae which develop from the mycelium end in yellowish brown **uredospores** which are also bi-nucleate. If the wind disperses these uredospores on to the leaves of other wheat plants, they germinate hyphae which enter the leaves through the stomata and give rise to further uredospores in that plant. Later in the Summer a different kind of spore is produced and these are called **teleutospores**.

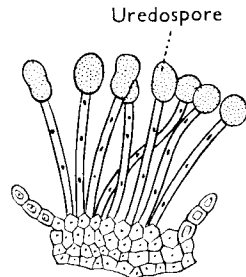


FIG. 33. *Puccinia*.
Uredospores.

Teleutospore

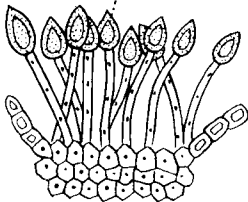


FIG. 34. *Puccinia*.
Teleutospores.

(3) *Examine a slide showing teleutospores on a leaf of wheat.*

These are found in clusters on the free ends of hyphae and are black: hence the name "black rust". Each consists of two cells enclosed in a thick wall and the nuclei of the two cells fuse. In the Spring these **teleutospores** germinate hyphae which form a **basidium** which develops a small **sterigma** the tip of which swells to form a **basidiospore**. When these basidiospores alight on the leaf of a barberry plant they germinate a new mycelium which produces spermatogonia.

PSALLIOTA (AGARICUS)

THE MUSHROOM

Psalliota (Agaricus) campestris, the mushroom, belongs to the **Agaricaceae** or Gill-fungi and is a *saprophyte*.

(1) *Examine some mushroom "spawn" (manured loam containing the mycelium).*

Note the white filamentous hyphae of the **mycelium**. *Tease some of these out and examine in water.* The **hyphae** are septate but there are many **nuclei** between the **septa** (coenocytic).

(2) *Examine the fructification.*

This is the part of the mushroom above ground. It develops from the mycelium and is the reproductive part of the plant. Reproduction is entirely asexual. The fructification is umbrella-shaped consisting of a large upper part, the **pileus**, which is convex on its upper surface, while the lower surface bears a number of vertical plates, pink when young and chocolate coloured when mature, radiating from the centre to the periphery. These are the **gills** (or **lamellae**). The pileus is borne on a stalk, the **stipe**, around which, in a mature plant, is the **annulus**, the remains of the **velum**, a membrane which, in the young plant, encloses the gill-chamber.

(3) *Take a fully-grown specimen and cut across the stipe just below the pileus. Place the pileus, gills downwards, on a piece of white paper. A few hours later carefully lift the pileus.*

Note the rows of black spores corresponding in position with the gills. It is on the gills that the basidia which bear the spores are situated.

(4) *Cut longitudinal and transverse sections of the stipe and mount in dilute glycerine.*

It is composed of long, branching, septate hyphae closely interwoven and more densely arranged towards the outside than in the centre.

(5) *Cut a tangential vertical section of the pileus, passing transversely through the gills. Mount in dilute glycerine.*

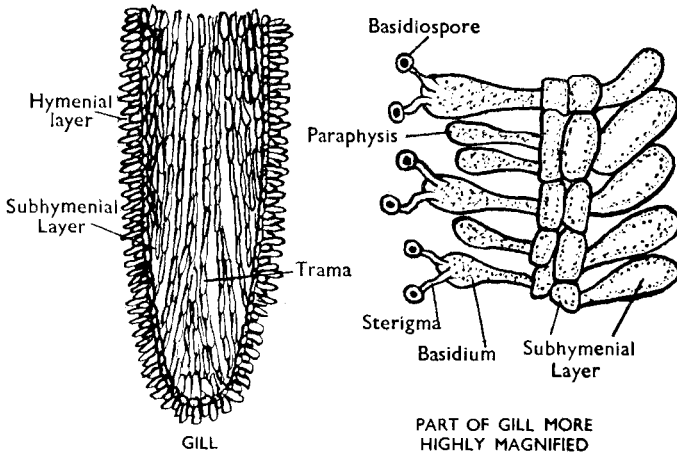


FIG. 35. *Psalliota*. Structure of Gill.

The tissue of the pileus is similar to that of the stipe, the loosely woven hyphae running vertically down the centres of the lamellae to form a tissue called the **trama**.

(6) *Examine a section of a gill under the high power.*

Note the more closely packed layer of hyphae, the **subhymenial layer** enclosing the **trama** and, outside the subhymenial layer, a row of stouter elongated cells forming the **hymenial layer**. Some of these cells are club-shaped **basidia** which bear two or four peg-like branches called **sterigmata**, the tips of which are swollen to form **basidiospores** each containing a nucleus. Other cells of this layer are narrower and shorter than the basidia and are the sterile **paraphyses**.

CLASS

LICHENES

A LICHEN—PUCCINIA

Lichens, which consist of a fungus and an alga living *symbiotically*, live on rocks, the bark of trees and the soil. There are three kinds—**crustaceous lichens** in which the thallus is in the form of an incrustation, **foliaceous lichens** with a flattened thallus and **fruticose lichens** which have a branched filamentous thallus. The fungus is, in almost all cases, one of the Ascomycetes and the Algae are usually green and so belong to the Chlorophyceae.

(1) *Examine a lichen plant* such as Dog's Tooth Lichen (*Peltigera canina*) or *Cladonia furcata*.

In this the thallus is flat and of a dull green colour when wet though rather greyish when dry. Underneath the thallus are hairlike **rhizoids** or **rhizines** (rhizoid-like hyphae) and, near the margin of the thallus flat, brown, rounded structures may be present. These are covered with layers of **asci** from which **ascospores** are liberated, though these are unable to germinate unless they come into contact with Algae, when a new lichen plant will develop.

(2) *Examine a transverse section through the thallus of a lichen.*

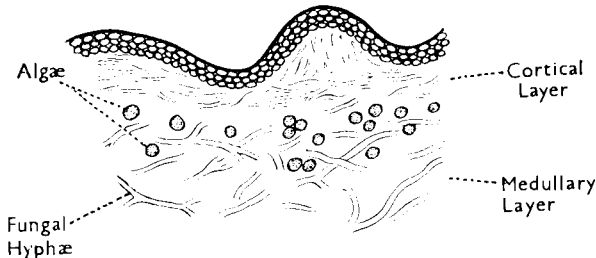


FIG. 36. Lichen. T.S. Thallus.

Note the **upper cortical layer** and the **lower cortical layer**, each composed of fungal hyphae closely woven. In between these two regions is the **medullary layer** in which the **fungal hyphae** are loosely arranged and amongst them are groups of unicellular green **Algae**, particularly towards the upper surface of the layer.

These algal cells divide and in due course become loosely entwined by fungal hyphae thus forming somewhat compact bodies known as **soredia**. On the rupture of the thallus these are set free and give rise to new lichen plants.

BACTERIA

Bacteria are unicellular or non-cellular non-green plants. Owing to their method of nutrition and lack of chlorophyll and their method of reproduction, they may be called **Schizomycetes** (Fission Fungi).

They were discovered by **van Leeuwenhoek** in the 17th Century (he also discovered Protozoa) but he regarded them as animals as the bacteria he examined were motile. It was **Louis Pasteur**, however, who discovered the part they play in putrefaction and disease and **Joseph Lister** who applied this knowledge to surgery while one of the earliest pioneers in the science of bacteriology was **Robert Koch**.

Owing to their extremely small size, the study of the structure of bacteria is difficult. They appear to be non-nucleated but chromatin granules are scattered throughout the protoplast which is enclosed in a definite wall and certain definite shapes can be recognised. The majority are colourless but some species contain pigments. Some are **flagellated** and therefore motile but in other cases any mobility is due to Brownian Movement. Reproduction is by **binary fission**. Under favourable circumstances this can take place every twenty minutes to half an hour and a single bacterium dividing once every thirty minutes could give rise to 2^{47} (= 140,000,000,000,000) in the course of 24 hours. Fortunately such favourable conditions never arise owing to lack of sufficient nutriment for the bacteria and unfavourable external conditions produced by their own metabolism and, in some cases, due to sunlight in which they are unable to thrive. In spite of this, however, the number of bacteria in favourable habitats is enormous. Resistant **spores** are formed by some bacilli but these are resting forms which enable the bacteria to survive adverse conditions rather than reproductive bodies. They may be **endospores** formed within the bacterium (*e.g.*, *B. Anthracis*) or formed externally, **exospores**, such as is the case with *B. tetanus*, known as the "drumstick bacillus" as they are formed at one end. These spores are very resistant to extremes of temperature such as exposure to boiling water for several hours. They are equally resistant to very low temperatures. However, the chemical substances known as **antiseptics** are fatal to them. The majority of bacteria thrive at the temperatures most favourable to other organisms, *i.e.*, between 30° and 37°C. and are killed by higher temperatures such as 60°-70°C. for a few minutes. On the other hand there are some which need high temperatures for growth such as those living in manure heaps.

Respiration may be aerobic or anaerobic, some being entirely aerobic (**obligatory aerobes**), others solely anaerobic (**obligatory anaerobes**) while still other species can use either method (**facultative anaerobes**).

A few are **autotrophic**, building up their protoplasm by the assimilation of inorganic materials as is the case with the nitrifying bacteria (*B. nitrobacter* and *B. nitrosomonas*) in the soil. The sulphur bacteria such as the filamentous *Beggiota* oxidise sulphur compounds. This behaviour is known as **chemosynthesis** and the energy liberated by the oxidation is used by the bacteria in building up their organic compounds. The majority of bacteria, however, are **heterotrophic** and are either **saprophytic**, obtaining their nutrition from non-living organic matter, or **parasitic**, obtaining it from another living organism, the pH of the food medium affecting their growth. Parasitic bacteria responsible for communicable diseases are said to be **pathogenic**. Poisonous **toxins** produced by these bacteria are responsible for the symptoms of the diseases they cause while toxins produced by some saprophytic bacteria are the cause of such conditions as food-poisoning which results from the activities of *Clostridium botulinum*.

Owing to their minuteness identification of individual types of bacteria is often possible only by their physiological characteristics such as their reaction to specific stains, whether they form spores, whether they are saprophytic or parasitic or autotrophic and by their nutritional requirements.

Certain definite shapes can be recognised. These are as follows:—

Bacilli—rod-shaped

Cocci—spherical

Micrococci—occurring singly

Diplococci—grouping in pairs

Staphylococci—grouping in bunches

Streptococci—grouping in chains

Sarcina—grouping in three dimensions of space, forming “packets” like minute cubes

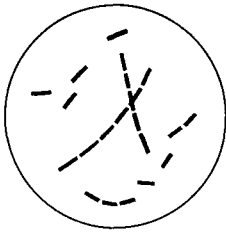
Spirilla—twisted

Vibrios—comma-shaped.

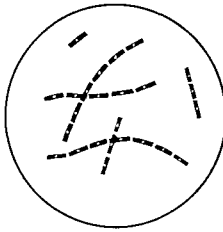
The longest Bacilli are about $20\mu^*$ and there is a great deal of variety in both size and shape. The smallest cocci are around 0.1μ in diameter. Sometimes large numbers occur together in a sticky medium and this is known as the **zoogloea condition**.

The extreme importance of bacteria in the lives of plants and animals, including man, will be obvious. It may be added at this point that many processes definitely beneficial to man are caused by bacteria. Examples of these are the making of butter, the ripening of cheese, the making of vinegar, the tanning of leather and sewage disposal, quite apart from the effect of soil bacteria on plants on which man depends for food.

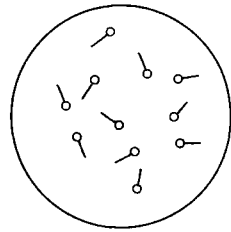
* $\mu = 0.001$ mm.



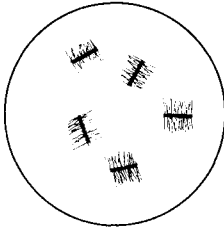
Bacilli



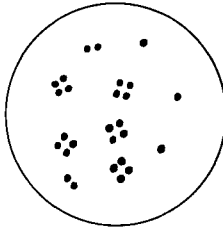
Bacilli with Endospores



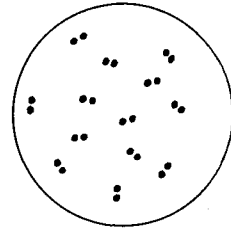
Bacilli with Exospores



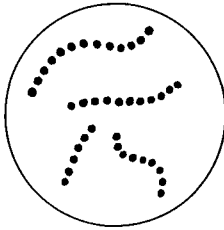
Flagellated Bacilli



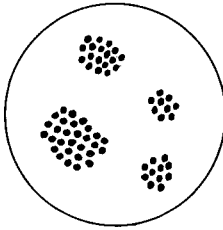
Micrococci



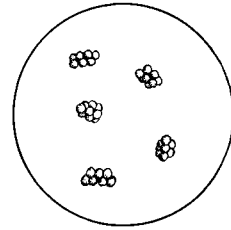
Diplococci



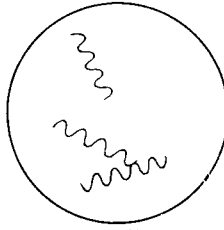
Streptococci



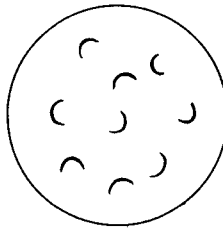
Staphylococci



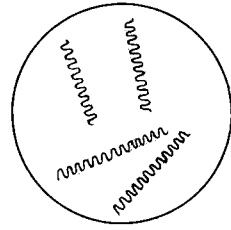
Sarcina



Spirilla

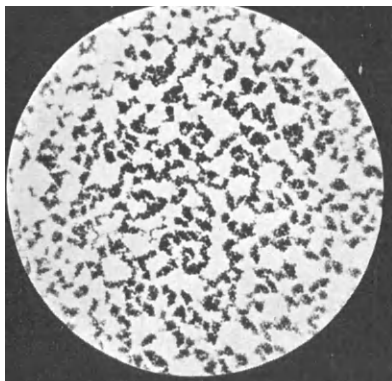


Vibrios

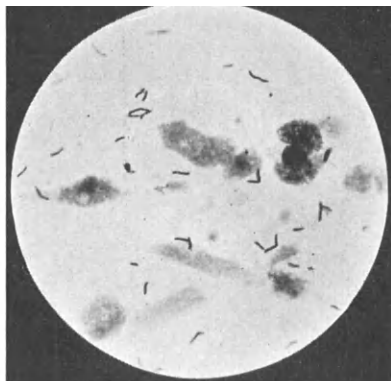


Spirochætes

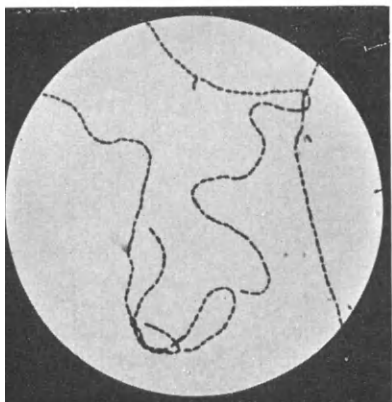
FIG. 37. Bacteria. Shapes.



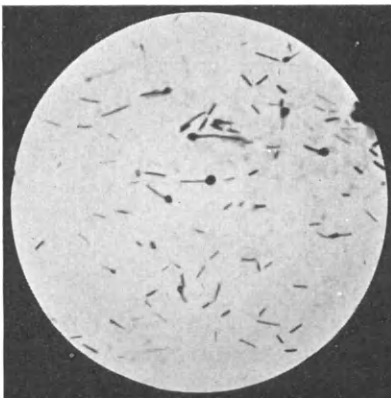
Staphylococcus pyogenes aureus.



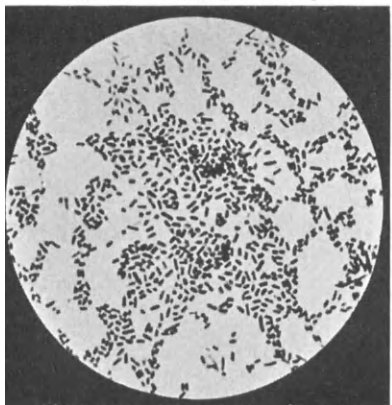
Bacillus tuberculosis.



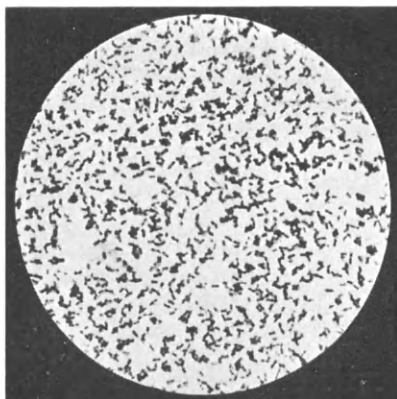
Streptococcus pyogenes longus.



B. tetani.



B. coli communis.



Pneumococcus.

FIG. 38. Forms of Bacteria.

(Bulleid "Textbook of Bacteriology for Dental Students." London: Heinemann.)

ELEMENTARY BACTERIOLOGY

Bacterial cultures can be grown on suitable **culture media** such as *nutrient agar* under suitable conditions of temperature and the colonies are visible to the naked eye.

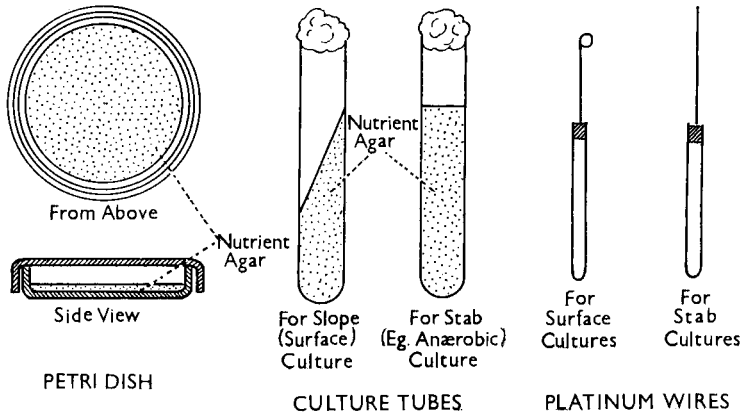


FIG. 39. Bacteriological Apparatus.

GRAM'S TEST. A standard bacteriological test stain is Gram's Iodine (see *Appendix I(A)(3) Microscopical stains*) and bacteria fall into two groups, those which retain a stain after treatment and those which do not. The former are said to be **Gram-positive** and the latter **Gram-negative**. The test is performed as follows:—

Stain the smear with aqueous Gentian (Crystal) Violet. Drain off excess of the stain (do NOT wash it off). Add a few drops of Gram's Iodine and leave for about two minutes. The smear should be a blackish-purple colour. Destain with 95 per cent. or absolute alcohol until the colour is no longer discharged. The smear should now be faintly violet. Then wash with water. Now counterstain with aqueous fuchsin for half a minute and wash out excess of stain with water. If a permanent mount is required, dehydrate, clear and mount in balsam in the usual way; otherwise, cover and examine straight away, using a 12 in. O.I. objective.

If the violet colour has been retained the bacteria are Gram-positive; if it has been removed they are Gram-negative.

(1) Aerobic Bacteria

Place a slice of potato in a fairly deep vessel of water such as a beaker or gas jar and leave it for several days. Then remove a drop of

the liquid from the surface of the water with a pipette. Put it on a slide and cover. Examine under the high power.

Note the motile rod-shaped **bacilli (B. mesentericus)**. These are aerobic bacteria.

Stain with iodine by irrigation.

(2) **Anaerobic Bacteria**

Carefully remove a drop of the liquid from the deeper layer of the water near the surface of the potato by means of a tube or pipette, by regulating your finger on the pipette, and treat as in (1).

Note the motile rod-shaped bacteria (**Clostridium butyricum**). These are anaerobic bacteria.

Stain with iodine by irrigation.

(3) **Bacillus subtilis**

Prepare some hay infusion by pouring hot water on to some hay and allowing it to stand for several days. Filter. Place a drop of the liquid on a slide and examine under the high power.

Note the comparatively large motile bacilli (**B. subtilis**).

(4) **Bacteria in Air**

Show the presence of bacteria in air as follows: Melt some sterile nutrient agar by immersing the tube in hot water. Remove the sterile cotton-wool plug if the medium is in a bacteriological test-tube and pour the contents into a petri dish, previously sterilised by heating in an oven at 150°–200°C. for half an hour. It will remain sterile as long as the cover is left on. Then leave the nutrient medium exposed to the air for an hour. Cover and leave for a few days at 18°–20°C. (average room temperature).*

Observe the colonies of bacteria which develop. Distinguish from any moulds which may also grow on the agar.

(5) **Bacteria in Water**

(i) *Show the presence of bacteria in river, pond or puddle water as follows: Melt some nutrient agar, remove the cotton-wool plug if present, add a few drops of water with a sterile pipette. Set the cotton-wool plug on fire* and replace in the tube with forceps while the wool is still burning. Shake without wetting the cotton-wool. Then remove the plug and pour the contents into a sterilised petri dish and cover. Leave for several days at room temperature and examine the colonies of bacteria which grow on the culture medium.*

A slant culture may be made in the tube instead of in a petri dish if preferred.

* Nutrient Media is often supplied in small phials instead of being in bacteriological test tubes plugged with cotton-wool. If this is the case, the procedure of firing the cotton-wool does not, of course, apply. The caps should be loosened slightly before melting the agar.

(ii) Show the **absence of bacteria in boiled river, pond or puddle water** by following the procedure in (i), but using water which has been boiled for a quarter of an hour and allowed to cool in a sterile flask plugged with burning cotton-wool.

(iii) Test a sample of **tap water** for bacteria by the method used in (i).

(6) **Bacteria in the Soil**

(i) Show the **presence of bacteria in the soil**, following the procedure in (5), transferring a few grains of soil with forceps sterilised in the flame.

(ii) Show the **absence of bacteria in baked soil** following the procedure in (5) but inserting a little soil which has been previously heated strongly in a crucible for half an hour and allowed to cool with the lid on.

(7) **Bacteria in Milk**

Using the method given in (5) above, test for the presence of bacteria in (i) **pasteurised* milk** (ii) **unpasteurised milk** (if available) (iii) **milk which has been allowed to stand exposed to the air for several days** (iv) **milk from (iii) which has been boiled once** (v) **milk from (iii) which has been boiled on three consecutive days**. This flask or tube should be plugged with fired (sterile) cotton-wool in the meantime.

(8) **Bacteria on the Skin**

Remove the cover from a petri dish containing nutrient agar and run your finger over the surface and replace the lid. Examine a few days later.

Colonies of bacteria should be seen growing on the agar.

(9) **Bacteria Carried by Flies**

Using forceps, place the body of a freshly killed fly on nutrient agar in a petri dish or remove its legs and put them on the agar. Cover the dish and leave for a few days.

Bacterial colonies will be seen growing on the agar where the legs or body have been in contact with it. (Fungal growths may also appear.)

(10) **Bacteria in the Mouth**

Gently scrap the surface of one of your teeth and transfer the scraping to a drop of water on a slide. Stain with methylene blue. Cover and examine with a $\frac{1}{12}$ in. O.I. objective.

Saprophytic bacteria—bacilli, cocci, spirilla and vibrios may be found.

(11) **To Prepare a Slide of Bacteria**

Make a smear on a slide from one of the cultures grown in one of the previous experiments. To transfer the material use a platinum

* **Pasteurisation** is a process used for sterilising milk to free it from pathogenic bacteria. The milk is raised to 62°–65°C for 30 minutes and then rapidly cooled. Such milk must not contain more than 100,000 bacteria per millilitre.

needle previously sterilised in the flame (it will cool almost immediately). Apply it to the material and stroke the surface of the agar with it then transfer to a slide or a coverslip and, holding another slide or coverslip in contact with one edge of the material at an angle of about 45° , draw the second slide or coverslip over the first to produce a film (smear) of even thickness. Dry the smear with gentle heat. Stain with methylene blue or carbol-fuchsin by immersing the slide or coverslip in the stain. Cover and examine with a $\frac{1}{12}$ in. O.I. objective or make a permanent mount.

(12) Bacterial Cultures

(i) Saprophytic Bacteria

Examine some cultures of various Saprophytic bacteria:—

E.g., *B. phosphorescens*, *Chromo-bacterium violaceum*, *B. prodigiosus*, *Photo-bacterium phosphoreum*, *Sarcina lutea*.

(ii) Pathogenic Bacteria

Examine preserved killed cultures of various pathogenic bacteria (if available) such as the following:—

Staphylococcus pyogenes, *Bacillus typhosus*.

(13) Microscopical Examination of Permanent Mounts of Bacteria

Examine prepared slides of bacteria with a $\frac{1}{12}$ in. O.I. objective:—

Bacilli, micrococci, diplococci, staphylococci, streptococci, sarcina, spirilla and vibrios:—

(i) Saprophytic bacteria such as the following, using a $\frac{1}{12}$ in. O.I. objective:—

(a) **B. nitrosomonas**, **B. nitrobacter**, **Azotobacter chroococcum**, **Clostridium pastorianum**.

(b) A T.S. through the nodules on the root of a leguminous plant, such as **Broad Bean** (*Vicia faba*) or **Clover** (*Trifolium*), using a $\frac{1}{12}$ in. O.I. lens.

Inside the **nodules** (larger in *Vicia* than in *Trifolium*) flagellated bacteria, *B. radicola* (*Rhizobium leguminosarum*) will be seen. These bacteria live in the soil as spherical cocci, develop flagella and penetrate into the roots of leguminous plants forming nodules in which they reproduce. In this form they are known as **bacteroids**. The bacteria benefit by absorbing carbohydrates from the roots of the plant and the plant receives nitrogen compounds which these *nitrogen-fixing bacteria* synthesise from the nitrogen in the soil atmosphere. Thus this is a case of *symbiosis*.

(ii) Pathogenic bacteria such as the following, using a $\frac{1}{12}$ in. O.I. objective:—

B. tuberculosis (tuberculosis), *B. anthracis* (anthrax), *B. (Corynebacterium) diphtheriae* (diphtheria), *B. tetanus* (tetanus or lockjaw), *Diplococcus pneumoniae* (pneumonia), *Staphylococcus pyogenes* (pus),

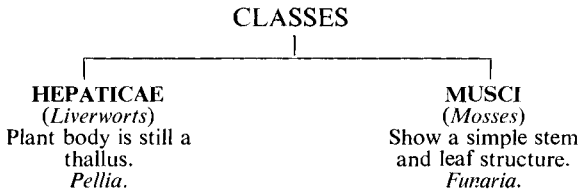
Streptococcus pyogenes (in pus), *Vibrio cholerae* (cholera), *Spirochaete recurrentis* (relapsing fever), *Spirochaete (Treponema) pallida* (syphilis). Look for **endospores** in *B. anthracis* and for **exospores** in *B. tetanus*.

VIRUSES

Viruses are ultra-microscopic and some have been seen under the electron microscope, their sizes varying between 6μ and $400m\mu^*$, one of the largest being *Vaccinia*, the smallpox virus. They have now been studied in some detail and they appear to be either spherical or rod shaped. Diseases such as influenza, the common cold, poliomyelitis, smallpox, myxamytosis, rabies, fowl pest, tobacco mosaic disease and leaf roll in potatoes are all caused by viruses. There are some which cause **lysis** (disintegration) of bacteria and these are known as **bacteriophages**; they are differently shaped from the others and are larger. Viruses reproduce only in the presence of living matter. The practical study of viruses (**virology**) is clearly beyond the scope of this work.

DIVISION BRYOPHYTA

Moisture and shade-loving terrestrial plants bearing hair-like rhizoids for anchorage and for transporting water. There is an "alteration of generations." The plant bearing sexual organs is called the **gametophyte**, a spore-forming generation, the **sporophyte**, developing on it.



CLASS HEPATICAE

PELLIA THE LIVERWORT

(1) *Examine the liverwort plant entire under a hand lens.*

Note the small green **thallus**, branching dichotomously and notched at the tips and bearing slender unicellular **rhizoids** on its under surface. Look for **sporogonia**, capsules borne on stalks, in various stages of development. These represent the sporophyte generation; the plant itself is the gametophyte.

(2) *Examine a T.S. of the Thallus.*

Under *low power* note that it consists of **parenchymatous cells** (cells of approximately the same length and width) containing numerous

* $m\mu = 0.000001$ mm.

chloroplasts, particularly near the surface. The centre of the thallus is thicker and has the appearance of a midrib. There is *no* vascular or conducting tissue.

(3) *Examine a L.S. of the Thallus through Archegonia and Antheridia.*

Under *high power* note flask-shaped (female) **archegonia** at the apex of the thallus on the upper surface, each consisting of a wide basal **venter** and a long **neck** and borne on a short stalk on a pad of tissue, the **receptacle**. The **involucre** arches over the archegonia and

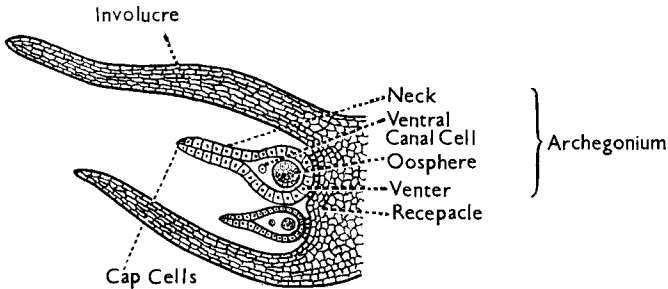


FIG. 40. *Pellia*. L.S. Thallus showing Archegonia.

serves to protect them. In the venter note the spherical **oosphere** surmounted by a small **ventral-canal cell** and in the neck the row of **neck-canal cells**, also **cap-cells** at the tip of the neck.

When the oosphere is ripe, the neck-canal cells disintegrate and mucilage thus produced bursts open the cap-cells.

Note the rounded (male) **antheridia** also on the upper surface of the thallus near the thickened centre, each borne on a **stalk** and protected by a wall consisting of a single layer of cells and formed

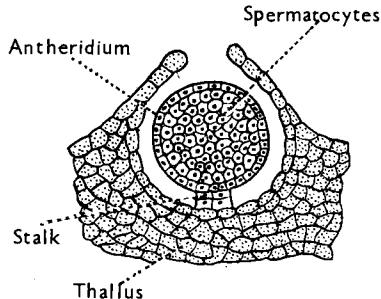


FIG. 41. *Pellia*. L.S. Antheridium.

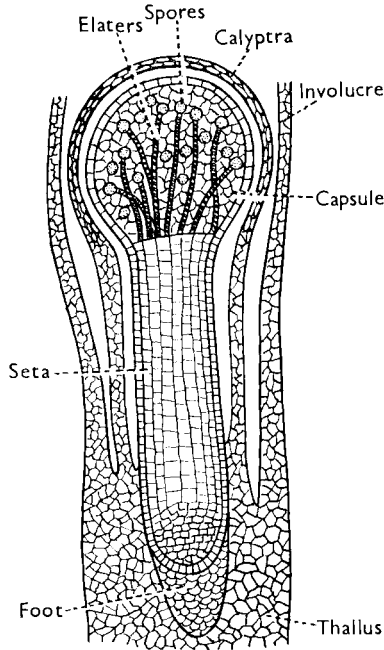


FIG. 42. *Peltia*. L.S. Sporogonium.

from the thallus so that it is enclosed in a cavity. Inside the capsule note the **spermatocytes** which develop into spiral spermatozoids each bearing two long cilia at one end.

(4) *Examine a L.S. of a sporogonium.*

This develops from the zygote.

Under *high power* note the spherical **capsule**, borne on a **stalk** or **seta** and surrounded by a sheath, the **calyptra**, which is the developed archegonial venter. Below this is the spatulate **foot**, an absorbing organ embedded in the tissue of the thallus. Note also that the capsule wall is three or more cells thick and that inside it are long thin cells with spiral thickening, the **elaters**, and numerous **spores**.

By elongation of the seta, the capsule is forced through the calyptra and under dry conditions its wall bursts open and the elaters, being hygroscopic, eject the spores. From these a short filament or **protonema** germinates; this eventually develops into a new gametophyte.

Under *low power* note that the capsule divides into four **valves** and the **spores** are set free.

CLASS
MUSCI
FUNARIA
THE MOSS

Mosses show an advance on some liverworts in that the plant body is differentiated into simple stems and leaves, though it has no roots, rhizoids still being present, but these are multicellular and have oblique septa. The moss plant is the gametophys.

(1) *Examine the Moss plant entire under a hand lens.*

Note the **stem** bearing small oval, sessile **leaves** arranged spirally, each with a definite midrib. Slender multicellular **rhizoids** with oblique septa give the plant anchorage and absorb water from the soil. Growth is effected by an apical cell. Note also the **sporogonia**, each consisting of a **capsule** borne on a stalk or **seta**.

(2) *Examine a T.S. of the stem.*

Under *low power* note the outer layer or **epidermis** enclosing the many-layered **cortex**, containing **chloroplasts**, the cells being thick-walled towards the epidermis and thin-walled towards the **conducting strand** in the centre.

(3) *Examine a T.S. of the leaf.*

Under *low power* note the single layer of thick-walled cells containing **chloroplasts**, and the many-celled **midrib**, where there is **conducting tissue**.

(4) *Examine a L.S. through a main shoot bearing Antheridia.*

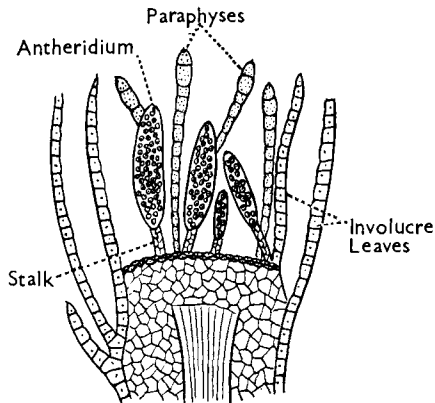


FIG. 43. **Funaria. Antheridia.**

Under *low power* note the club-shaped **antheridia** borne on multi-cellular stalks and containing numerous **spermatocytes**. These develop into biciliate spermatozoids. The antheridial wall is composed of a single layer of cells. On either side will be seen the sterile **paraphyses**, club-shaped or pointed and containing **chloroplasts**.

(5) *Examine a L.S. through a branch shoot bearing Archegonia.*

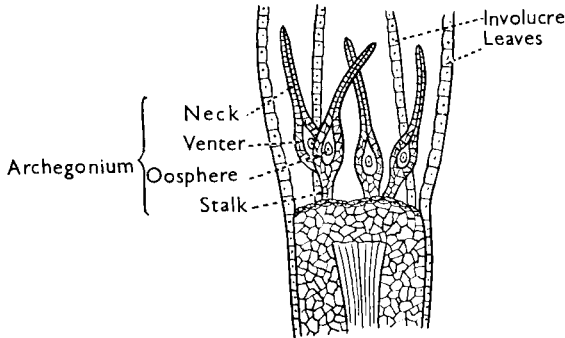


FIG. 44. *Funaria*. Archegonia.

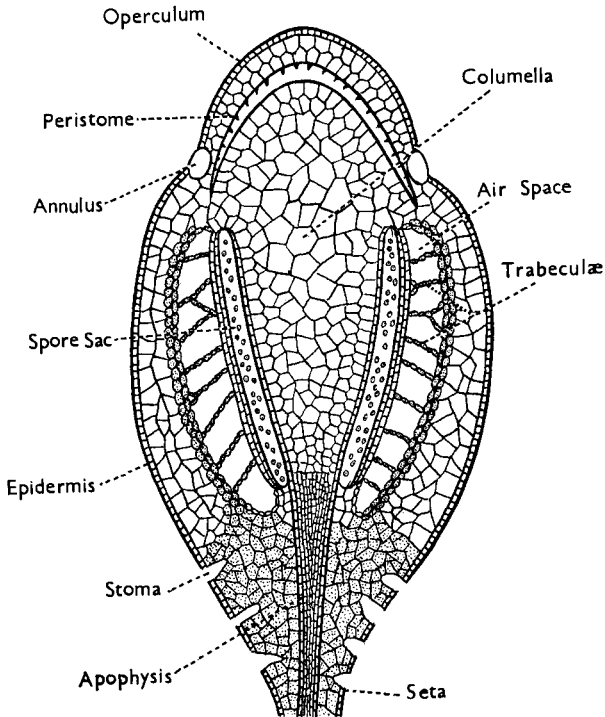
Under *low power* note the dilated **venter** at the base containing a central **oosphere**, above which is a single **ventral-canal cell** and an elongated **neck** with **cap-cells** at the tip and containing a row of **neck-canal cells**. Each archegonium is borne on a well developed **stalk** and an **involucre** protects the group.

(6) *Examine a L.S. through a mature Sporogonium.*

This is more complicated than that of the liverwort and contains no elaters. The calyptra falls off when the capsule is mature.

Under *low power* note the point of attachment or **foot** embedded in the tissue of the gametophyte, the multicellular stalk or **seta** and the elongated **capsule**. The basal part of the capsule is called the **apophysis**, the thick-walled **epidermis** or outer layer of which bears pores called **stomata**. Beneath the epidermis are parenchymatous cells containing chloroplasts. The thick multi-cellular capsule wall surrounds an **air space** traversed by strands of cells, **trabeculae**. Inside this is the **spore sac**, which surrounds the central **columella**. The apex of the capsule is covered by a row of cells, the **operculum**, borne on a ring of cells, the **annulus**. Inside the operculum note the toothed **peristome**.

When the capsule is ripe, the annulus ruptures and the operculum is cast off. The teeth of the peristome are hygroscopic and their consequent movement assists in the ejection of the spores.

FIG. 45. *Funaria*. L.S. Sporogonium.(7) *Examine germinating spores.*

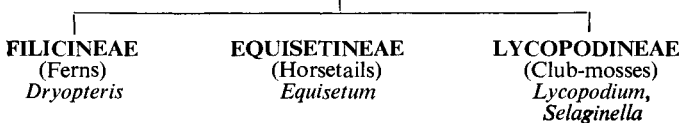
Under *low power*, note the two germ-tubes developing at opposite ends, one forming a **rhizoid** and the other the branched **protonema** containing chloroplasts which gives rise to a new moss plant.

DIVISION

PTERIDOPHYTA

Plants showing a distinct root, stem and leaf structure and further adaptation to a terrestrial life. There is a definite "alternation of generations," the sporophyte generation being well developed and the gametophyte reduced and on a separate plant. Vascular tissue is well developed, too. The plants are therefore called vascular cryptogams.

CLASSES



CLASS
FILICINEAE

This is the largest group and includes the male or shield fern (*Dryopteris*, *Aspidium* or *Nephrodium*), the hart's tongue fern and the bracken fern. The plant is the sporophyte.

DRYOPTERIS FILIX-MAS

THE FERN

(1) *Examine the male fern entire (Sporophyte).*

Note the short, thick **rhizome** (underground stem) which is almost erect and covered with **leaf-bases**. The large spirally arranged **compound leaves** or **fronds** consist of a **rachis** or stalk, bearing numerous green leaflets or **pinnae** on either side (*bipinnate*) subdivided into lobes or **pinnules**, the lower part of the rachis being covered by brown scales called **ramenta**. The multicellular fibrous **adventitious roots** are developed between the bases of the leaves.

(2) *Examine the under surface of a pinna on an older leaf.*

Note the kidney-shaped structures borne over the veins (green or brown according to age). These are **indusia** and contain groups of sporangia called **sori**.

Leaves bearing sori are called **sporophylls** and those without them **trophophylls**.

(3) *Examine or cut a T.S. of the rhizome, stain with haematoxylin or safranin and mount in dil. glycerine or make a permanent preparation and mount in Canada balsam.*

Under the *low power* note the **vascular bundles** or **meristemes** arranged in a ring and embedded in ground-tissue, which is chiefly parenchyma (cells of approximately the same length and width) and in which a number of bundles passing to the leaves may be seen. The parenchyma surrounding the bundles is called the **cortex**.

(4) Under the *high power* examine a **vascular bundle** and note the **metaxylem**, large empty cells and the smaller **protoxylem** cells with **xylem parenchyma**. This is surrounded by the **phloem** with **phloem parenchyma** and small **protophloem** cells lie outside the phloem. The **pericycle** is a single layer outside the protophloem and the **endodermis** is a double layer outside the pericycle (in parts it may be single). *Stain a separate section with iodine* and note the starch (stained blue) in the pericycle and in the inner of the two layers of the endodermis.

A T.S. of the rhizome of the bracken fern (*Pteridium aquilinum*) shows a slightly different arrangement. Here note the outer ring of **sclerenchyma** (thick-walled cells) and the **parenchyma** in which are two series of **vascular bundles** with an incomplete ring of **sclerenchyma** between them, the inner bundles being larger and fewer. The endodermis is only one cell thick.

(5) *Examine a longitudinal section of the rhizome.*

Note the thickening of the **protoxylem** is **spiral** and that of the **metaxylem** is **scalariform** (in bands, transversely placed) and that the xylem is composed solely of **tracheids**, elongated cells end on to one another, the end walls remaining.

(6) *Examine or cut a transverse section of the root.*

Note the outer **piliferous layer**, the parenchymatous **cortex**, the inner portion of which is sclerenchymatous, the **endodermis** and the many-layered **pericycle** composed of thin-walled cells. The central **stele** is composed of (a) two **protoxylem** groups (and therefore said to be *diarch*) outside the **metaxylem** which is in the centre and composed of larger cells and (b) **phloem**, alternating with the protoxylem.

(7) *Examine or cut a T.S. of a pinnule through a sorus.*

Note that the leaf is composed of an **upper epidermis** containing chloroplasts, a **lower epidermis** containing **stomata** and, between them, the **mesophyll**, composed of **palisade tissue** containing numerous **chloroplasts** above and beneath it, **spongy tissue**, also containing chloroplasts but with intercellular spaces. The **sporangia**, composed of oval flattened capsules on multi-cellular stalks develop on a cushion of tissue, the **placenta**. The sporangial group

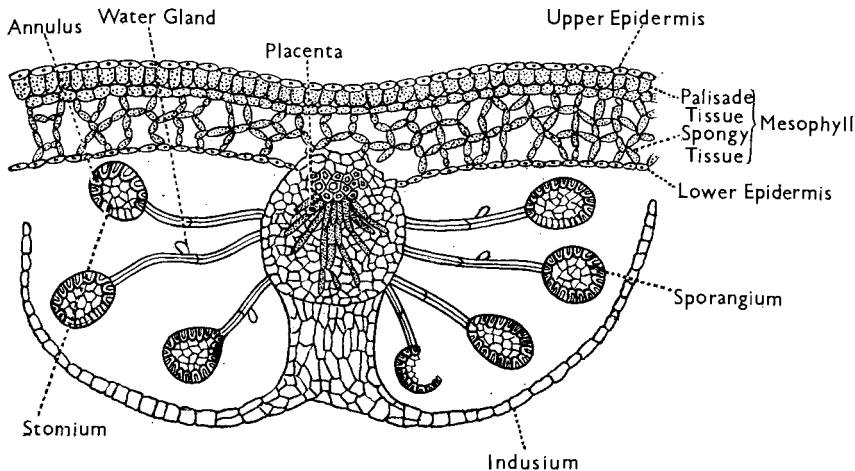


FIG. 46. *Dryopteris*. T.S. Pinnule through a Sorus.

is called a **sorus**. The outer wall of the sporangium consists of thickened cells forming the **annulus** except on one side, where it ruptures later, the **stomium**. Note the **spores** (usually 48 formed from 12 spore mother cells) which may have escaped from the sporangia.

The spores are all the same size and the plant is therefore said to be *homosporous*. **Water glands** occur on the sporangial stalks. The sorus is protected by a layer of tissue called the **indusium**, which arises from the placenta and arches over the sporangia.

(8) Examine a **prothallus** (Gametophyte) with a hand lens and under the low power.

Note that it is a thin heart-shaped, plate-like structure composed of parenchymatous cells, with a thickened region, the **cushion**, in the centre. On the lower surface you will see long brown unicellular **rhizoids**, the female **archegonia** near the centre of the cushion and the male **antheridia** towards the more pointed end.

(9) Examine a T.S. of the **prothallus through antheridia**.

Under *high power* note spherical **antheridia** containing developing **spermatocytes**, which give rise to spermatozoids (or antherozoids).

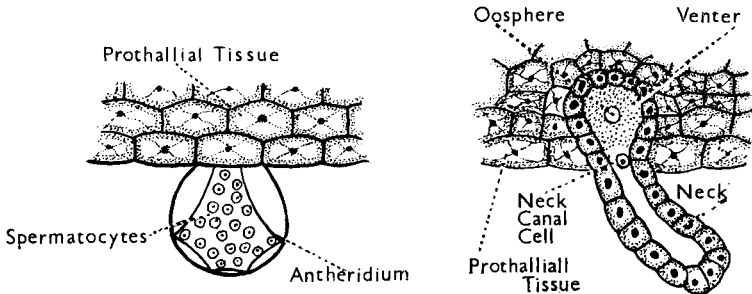


FIG. 47. *Dryopteris*. Sexual Organs.

(10) Examine a T.S. of the **prothallus through archegonia**.

Note the flask-shaped **archegonia**, each consisting of (a) a wide basal part, the **venter** embedded in the tissue of the prothallus, containing one **oosphere** or **ovum** and (b) a narrow bent **neck** shorter than that found in the Bryophyta. Note also the **ventral canal cell** where the neck and venter join and the multinucleate **neck-canal cell** above it in the neck itself.

CLASS LYCOPODINEAE

The two most important club-mosses are *Lycopodium* and *Selaginella*. The former is homosporous and the latter is heterosporous.

LYCOPODIUM

(1) Examine the plant *Lycopodium clavatum* (Sporophyte.)

The creeping **stem** branches dichotomously and bears simple linear **leaves** spirally arranged. **Adventitious roots** arise from the

stem where it branches. Some of the branches are erect and bear fewer and smaller leaves at their free ends where there are **strobili** of closely packed **sporophylls**.

(2) *Cut a T.S. of the stem, stain with haematoxylin or safranin and examine under the low power.*

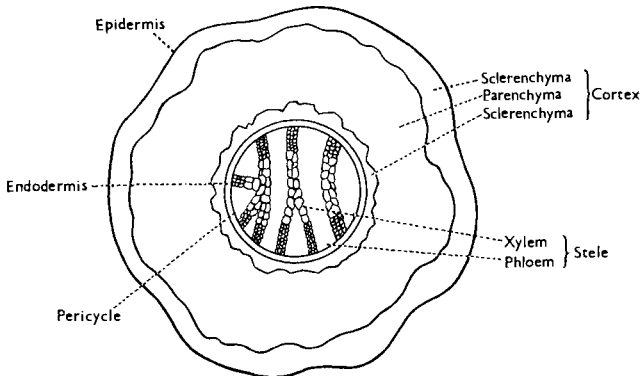


FIG. 48. *Lycopodium*. T.S. Stem.

Note the **epidermis** surrounding the stem inside which is the **cortex**. This is composed of sclerenchyma* externally and parenchyma* internally with further sclerenchyma inside this. The **pericycle** surrounds the **stele** which is in the centre. This consists of four more or less parallel bands of xylem and the outer ones will be seen to branch. The **protoxylem** is outside the **metaxylem** and is therefore said to be *exarch*. It is composed of tracheids. The **phloem** lies between the bands of **xylem**.

(3) *Cut a L.S. through the centre of the strobilus.*

The **sporangia** are large, contain numerous spores, and are situated on the upper surfaces of the spirally arranged **sporophylls**. The spores produced by these sporangia are identical in size. The plant is therefore *homosporous*.

(4) *Examine a T.S. of the prothallus (Gametophyte).*

The **antheridia** and **archegonia** are similar in structure to those of the fern but are more deeply seated in the tissue of the prothallus.

SELAGINELLA

(1) *Examine the plant **Selaginella kraussiana** entire (Sporophyte).*

This, too, has a creeping **stem** which branches dichotomously and bears small simple **leaves** placed in four rows. The leaves in the two

* See p. 68.

rows on the lower surface are larger than those on the upper and they are arranged so that a large leaf is opposite a small one. At the base of each leaf is a small membranous **ligule** on the upper surface. At the points where the stem branches are root-like structures called **rhizophores** from which **adventitious roots** arise. On the free ends of erect vegetative branches are **sporophylls** aggregated together to form **strobili** (or **cones**).

(2) *Cut a T.S. of the stem, stain with haematoxylin or safranin and examine under the low power.*

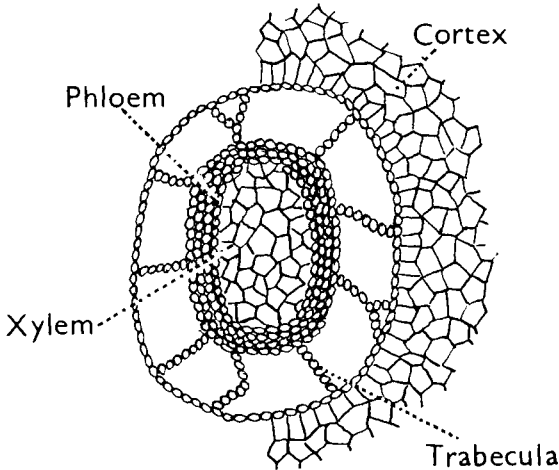


FIG. 49. *Selaginella*. T.S. Monostelic Stem.

Note the **epidermis** enclosing the many layered parenchymatous **cortex**. Inside this is an **air-space** traversed by delicate strands of cells called **trabeculae** which connect the cortex to the **pericycle**. In the centre is the **stele**. This is composed of **xylem** surrounded by **phloem**. There is one group of **protoxylem** towards the outside of the **metaxylem**: it is therefore **exarch**. Two or more steles may be present according to the species.

(3) *Examine a L.S. through the centre of the Strobilus.*

In the axils of the sporophylls are stalked spherical sporangia which are of two kinds, **microsporangia**, which contain a large number of **microspores** and **megasporangia** which contain four large **megaspores**. The microsporangia are more concentrated towards the upper end.

The leaves bearing the sporangia are known as **microsporophylls** and **megasporophylls** respectively. As spores of different sizes are produced, the plant is **heterosporous**.

Each microspore gives rise to a microprothallus which bears an antheridium. The megaspore develops into a megaprothallus on which are several archegonia. These sexual organs are similar to those of the fern but the prothallial tissue is more reduced. The megaspore begins to germinate while still in the sporangium.

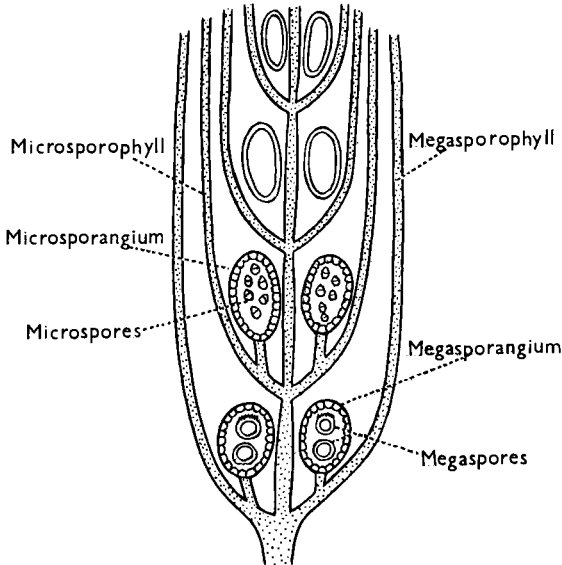


FIG. 50. *Selaginella*. L.S. strobilus.

DIVISION SPERMATOPHYTA

Seed-bearing Plants

These are plants in which there are two separate and very much reduced gametophytes which are borne on the sporophyte. The sporogenous tissue is also much reduced while vegetative tissue is increased. The sporophylls are of two kinds and are aggregated together to form flowers from which seeds develop. There is a still greater adaptation to terrestrial life and a more highly developed vascular system. Instead of the ciliated sperms, a pollen tube germinates from the pollen grain (microspore) to carry the sperm to the egg, thus eliminating the necessity for water for fertilisation. (There are some primitive seed-bearing plants which have ciliated sperms, *e.g.*, *Cycas*.) Thus Spermatophytes are flowering plants or seed-bearing plants.

CLASSES

GYMNOSPERMAE
(Seeds exposed)
(*Most evergreens*)

ANGIOSPERMAE
(Seeds enclosed)
All the common flowering plants

CYTOLOGY AND HISTOLOGY

A **cell** is defined as a unit of protoplasmic matter (*protoplast*) containing a nucleus. In plant cells the protoplast is enclosed in a non-living *cell-wall*. The nucleus consists of *nucleoplasm* enclosed in a *nuclear membrane* and the rest of the protoplast is called *cytoplasm*. There are differences in form and in the cell-contents of plant cells, according to the functions they have to perform, each being adapted to a particular function. Cells of similar form and function are aggregated into **tissues**. Simple organisms such as the simple Algae not differentiated into cell units are therefore best described as non-cellular.

The study of cells is known as *Cytology* and that of tissues as *Histology*.

MITOSIS IN PLANT CELLS

Mitosis comprises the series of complicated structural changes which occur in the nucleus prior to its division. (In the formation of gametes the process is modified in order to halve the chromosome number: this is known as **Meiosis**).

Under high power (or $\frac{1}{12}$ in. O.I.) *examine a slide showing mitosis in a L.S. of the root-tip of allium or other plant. Search for different stages of mitosis in different nuclei. You may not succeed in finding them all.*

(i) **The Prophase.** The apparent chromatin network of the **resting nucleus interphase** actually consists of **chromosomes*** and these split longitudinally into **chromatids**. The double nature is not visible at this stage and the apparent continuous spiral is an artefact. It is due to the chromosomes being coiled round each other. Meanwhile the nuclear membrane and nucleolus disappear.

(ii) **The Metaphase.** A spindle develops. The chromosomes clearly show their double structure and the chromatids arrange themselves on the equator of the spindle at points on them known as **centromeres**.

(iii) **The Anaphase.** The chromatids begin to move towards the opposite poles of the nucleus, guided by the spindle and begin pulled into V-shaped loops in the process.

(iv) **The Telophase.** The chromatids continue to move to opposite poles and a new nucleus is formed at each pole by passing through the changes of the prophase in reverse, the chromatids becoming the new chromosomes and these become less visible.

Cell division follows. In plant cells, a delicate cell-plate forms between the two nuclei and this becomes a **middle lamella** by deposition in it of pectin and calcium pectate. Cellulose is then deposited on each side of the middle lamella and thus a new **cell wall** is formed. Two new cells are thus formed, each with its own nucleus containing the original number of chromosomes.

* The shape and number of chromosomes varies with different plants but is constant for any one species. In the higher plants the number varies between 6 and 65 pairs. In *Cheiranthus*, for example, there are 7 pairs, in *Primula* 9 pairs and in *Triticum* 21 pairs.

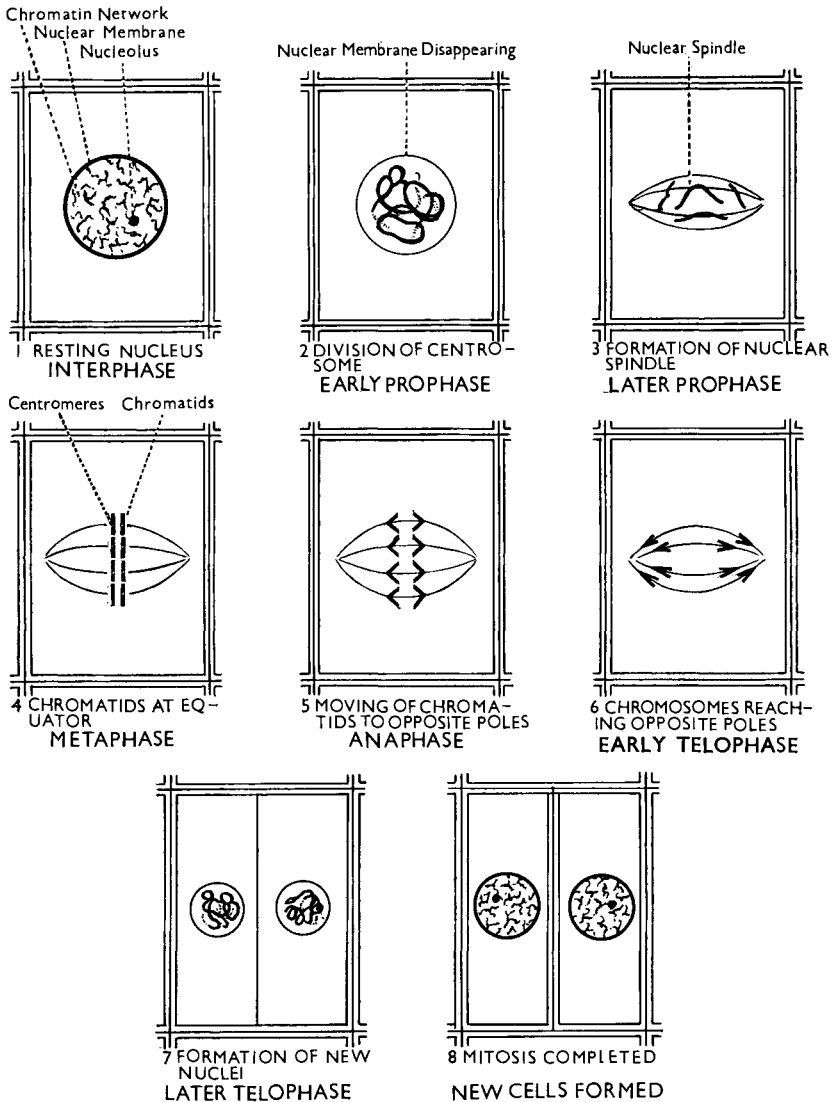


FIG. 51. Mitosis in Plant Cells.

Meristematic Tissues

Meristematic tissue is composed of cells capable of division and shows *mitosis*. It may be apical or intercalary.

(1) **Primary Meristem** will be seen in the apical growing points of the root and stem and in the fascicular cambium. It consists of cells which have retained their capability of division.

(2) **Secondary Meristem** will be seen in cork cambium in the woody stem. This is formed from permanent tissue cells which have become meristematic later in the life of the organ in which they are situated.

Permanent Tissues

The cells of permanent tissues are incapable of further division.

(1) **GROUND TISSUE. Thin-walled Parenchyma.** This is composed of oval or round cells with thin cell walls and small intercellular spaces. It is seen in the cortex and pith of the stem and root and in the mesophyll of the leaf of the Gymnosperm and Angiosperm as well as in some of the Cryptogams.

Examine a T.S. of an herbaceous dicotyledonous root or stem.

The cells in the wide region inside the external exodermis (the cortex) of the root and in the centre of the stem (pith) are composed of **thin-walled parenchyma**.

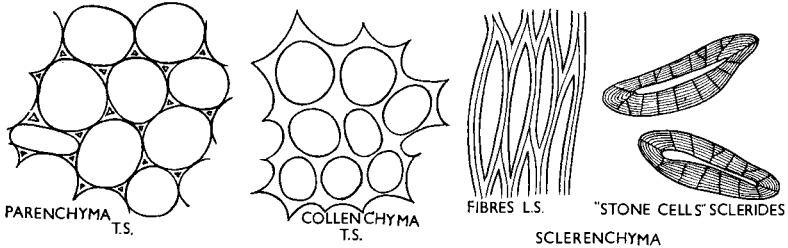


FIG. 52. Spermatophyta. Ground Tissue and Supporting Tissue.

(2) **MECHANICAL OR SUPPORTING TISSUE. Thick-walled Parenchyma.** This consists of parenchymatous cells the walls of which are thickened either with (i) cellulose, particularly at the angles of the cells (**collenchyma**) seen in the outer part of the cortex of the stem, or with (ii) lignin as in **wood-parenchyma**. When the whole of the inner wall of the cell is thickened with lignin the tissue is called **sclerenchyma**. This may be in the form of long narrow and usually pointed **fibres** or much shorter and generally non-pointed **sclerides**. This is often found external to the phloem in stems. Elongated spindle-shaped cells, usually thickened, are also found. The walls may be lignified as in **wood fibres**, or unlignified as in **phloem fibres**.

(i) *Mount a T.S. of an herbaceous dicotyledonous stem in Schultze's solution.*

In the outer cortical region immediately inside the epidermis, the cells are thickened with cellulose, particularly at their corners and the cell walls are stained blue. This is **collenchyma**.

(ii) *Examine a L.S. of the stem of the sunflower (*Helianthus annuus*) stained with safranin and light green. Sclerenchymatous fibres will be seen in the first red tissue working inwards from the epidermis.*

(iii) *Cut a section of the fleshy part of the fruit of the pear (*Pyrus communis*) near the core. Stain with aniline sulphate or chloride and mount in dilute glycerin or mount direct in Schultze's Solution.*

In the parenchymatous tissue are so-called *stone cells*. These are **sclerides** and are stained yellow both by the aniline dye and by the Schultze's solution.

(3) **SUBERISED TISSUE.** The brick-like cells of *cork* in the walls of which suberin has been deposited form this tissue. It is seen in stems showing secondary growth.

Examine a T.S. of a woody stem or cut a thin T.S. of a bottle cork. In the stem brick-like **cork cells** will be seen immediately inside the external bark. Cork cells are impregnated with suberin and this is **suberised tissue**.

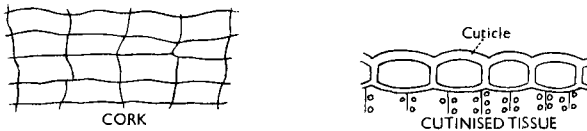


FIG. 53. Spermatophyta Suberised and Cutinised Tissue.

(4) **CUTINISED TISSUE.** This occurs in the thickened outer cell walls of the epidermal cells of the leaf, where a layer of waxy cutin has been deposited.

Examine a T.S. of a leaf.

Note the layer of **cutinised tissue** forming a continuous **cuticle** along the outside walls of the external layer of cells (the epidermis).

(5) **VASCULAR OR CONDUCTING TISSUE.** These are tissues modified for the conduction of water, sap, etc.—the essential tissues of **xylem** (wood) and **phloem** (bast).

(i) **Xylem Vessels** are formed from cells end on to one another. The walls are thickened and lignified, the end walls have disappeared forming wooden tubes and the protoplasmic contents have been lost. The thickening may be **annular** (in rings), **spiral**, **reticulate** (an irregular network), or **pitted** (the thickening complete except in places known as **pits**). When these pits are elongated like the rungs of a ladder, the thickening is said to be **scalariform**. The protoxylem (the first xylem to develop from primary meristem) usually has spiral or annular thickening, and the metaxylem (formed later) reticulate or pitted thickening.

Examine a L.S. of a dicotyledonous stem stained with safranin and light green.

Annular and **spiral thickening** will be seen in the vessels of the inner region of the xylem towards the centre (protoxylem). In the vessels in the outer region of the xylem (metaxylem) **reticulate** and **pitted thickening** will be seen. Both are stained red.

(ii) **Tracheids** are developed from single cells and are thickened, lignified elongated cells in which the end walls have not disappeared.

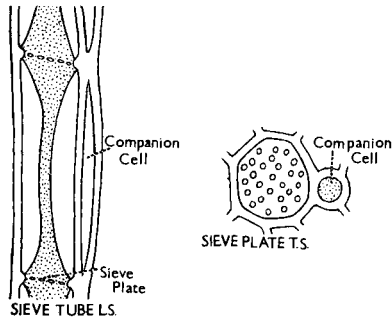
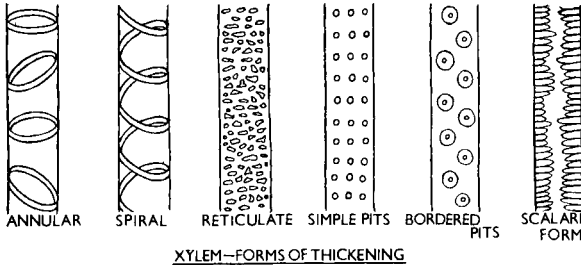


FIG. 54. Spermatophyta. Vascular Tissue.

The thickening is similar to that of vessels. The Pteridophyta and Coniferales have their secondary xylem exclusively of tracheids.

Examine a L.S. of a rhizome of a fern stained with safranin.

The **tracheids** will be seen as long slender cells with **scalariform thickening** in the centre of the vascular bundles. The tracheids are stained red.

(iii) **Sieve-Tubes** are long, thin tubes composed of long unligified cells end on to one another. The end walls are thickened and perforated, forming the **sieve-plates**. Sieve-plates also occur on the side walls of the sieve-tubes of many woody plants. The protoplasmic contents

line the tubes but the nuclei have disappeared. These are the essential elements of **phloem**. Associated with the sieve-tubes in Angiosperms are thin-walled elongated cells known as **companion cells**.

Re-examine the L.S. and T.S. of a dicotyledonous stem.

In the L.S., the **phloem** will be seen stained green external to the red-stained xylem. It consists of long, slender **sieve-tubes** with thin side walls of cellulose. The end walls which occur at intervals are thickened with cellulose and perforated; these are **sieve-plates**. The smaller thin-walled elongated cells alongside the sieve-tubes are the **companion cells**.

In the T.S. the perforated nature of the **sieve-plates** will be seen quite clearly.

(6) **GLANDULAR TISSUE** occurs in various structures and is responsible for the production of secretions and, in some cases, for excretion.

(i) **Resin ducts** surrounded by secretory cells occur in the stem of the pine and sunflower.

*Examine a T.S. of the stem of the pine (*Pinus sylvestris*) or sunflower (*Helianthus annus*).*

Resin canals (or **ducts**) lined by a single layer of **secretory cells** (resin cells) will be seen in the cortex.

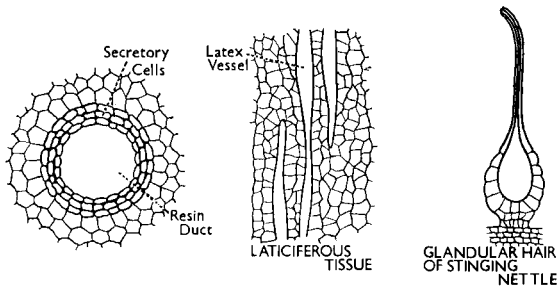


FIG. 55. Spermatophyta. Glandular Tissue.

(ii) **Laticiferous tissue** can be seen, for example, in the Dandelion. It consists of branching and anastomosing **vessels** with somewhat thickened cellulose cell walls, lined by protoplasm and containing a milky fluid, **latex**.* In the Spurge family (*Euphorbiaceae*) the laticiferous tubes are branched but not anastomosed.

*Examine a L.S. of the root of the dandelion (*Taraxacum officinale*) or of the stem of the Spurge (*Euphorbia*).*

* Rubber is a latex and opium the dried latex of the opium poppy.

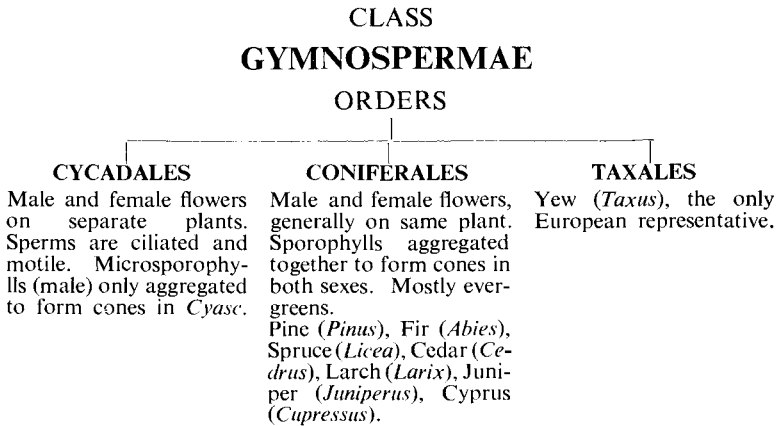
Latex vessels will be seen as branching and anastomosing tubes forming a network in the cortex.

(iii) **Nectaries**, which secrete a sugary substance called nectar, will be seen in the flower.

(iv) **Glandular hairs** are epidermal structures, and can be seen on the scale leaves of the Winter bud of the horse-chestnut. They secrete the gum or resin. The glandular hairs on the leaf of the Sundew secrete enzymes to digest insects.

*Examine a T.S. of the hairs on the leaf of a stinging nettle (*Urtica dioica*).*

Pointed tapering **glandular hairs** are situated on the surface of the leaf with bulbous bases attached to the epidermis. Glandular cells in the hair secrete the substances which cause the stinging sensation in the skin when punctured by the hairs.



CYCADALES

CYCAS

Cycas revoluta is an Asiatic plant. It must therefore be examined. (i) in a greenhouse in a botanical garden or (ii) as preserved material.

(1) *Examine the living tree* (if possible).

There is a short thick woody stem, usually devoid of branches, crowned by large pinnate leaves. Note the resemblance to the fern sporophyte.

(2) *Examine a preserved male cone.*

Note the spirally arranged **microsporophylls** bearing numerous **microspurgia** on their lower surfaces.

(3) *Examine preserved female sporophylls.*

Note the spirally arranged **megasporephylls** also pinnately divided but not aggregated into cones, each covered with brown hairs and devoid of chlorophyll. From two to eight **megasporangia** are situated on the edges of the basal parts of the sporophylls.

(4) *Examine a prepared slide of the sperms.*

Note their oval shape and the numerous cilia concentrated towards the apical end which make these male gametes motile.

CONIFERALES

PINUS

THE SCOTS PINE

Pinus sylvestris is the Scots Pine. The tree, which is an evergreen and can live in quite poor soil, is the sporophyte.

(1) *Examine the living tree* if possible.

Note the upright **stem** (trunk) with a reddish-brown, scaly bark, bearing lateral branches which arise in whorls. **Dwarf-shoots** arise on the ordinary branches (**long shoots**). **Scale leaves** grow on both long shoots and dwarf shoots and needle-shaped **foliage leaves** on the dwarf shoots and amongst them **cones** may be seen. The long shoots bear scale leaves only. Dwarf shoots with their green leaves are known as **spurs**. The tree may attain a height of a hundred feet.

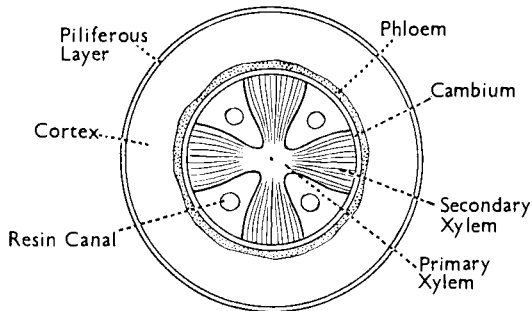
The Root

(2) *Examine a T.S. of a very young root of Pinus.*

The single outer layer of cells is the **piliferous layer** though it is usually devoid of root-hairs. Inside this is a many-layered **cortex**, the innermost layer of which is the **endodermis**. Immediately below this is a many-layered **pericycle**, from the second layer of which lateral roots arise. The stele is composed of two (*diarch*) or three (*triarch*) Y-shaped **xylem** bundles with the **protoxylem** on the arms of the Y and thus external to the larger **metaxylem**. Between the arms of the Y a **resin canal** will be seen. In the very young root the **phloem** alternates with the xylem. There is no pith in the centre.

(3) *Examine a T.S. of an older root*

Secondary growth takes place early, the **primary xylem** lying in the centre and surrounded by **secondary xylem** in which **resin ducts** will be found. External to the secondary xylem is the **cambium** (from which it was formed) and outside the cambium is the other vascular tissue to which it gives rise, namely the **secondary phloem**. This is a

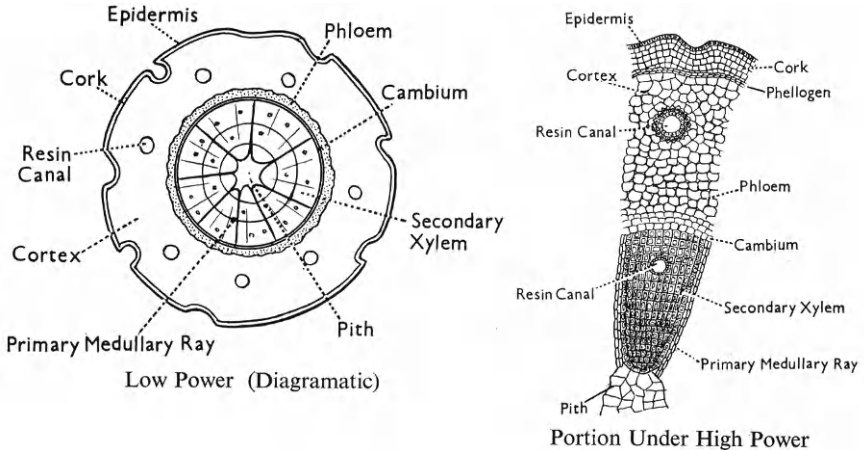
FIG. 56. *Pinus*. T.S. Root.

narrow layer of thin sieve tubes and the **primary phloem** lies outside this.

The Stem

(4) *Examine a T.S. of a three- or four-year-old stem. Stain with aniline hydrochloride (or sulphate) and mount in glycerine. Examine under low power.*

In a young stem there is an epidermis with its outer walls thickened to form a cuticle but in an older stem, the outermost layer is **bark** and, immediately underneath, it is a layer of **cork** composed of

FIG. 57. *Pinus*. T.S. Stem.

several rows of cells. This arises from the **phellogen**, a secondary meristem immediately beneath it. Inside this is the **cortex**, containing a ring of **resin canals**. In the centre of a young stem is the **stele** composed of **vascular bundles** with the phloem external to the

xylem from which it is separated by **cambium**, and the centre of the stem is occupied by pith. In the three- or four-year-old stem, however, secondary growth has taken place and **annual rings** of **secondary xylem** will be seen inside the **cambium**, immediately outside which is **secondary phloem**. The **primary xylem** will be found inside the annual rings protruding into the pith and the **primary phloem** outside the secondary phloem. Small resin canals will be seen in the xylem.

(5) *Examine the above section under the high power.*

Find the structures seen under the low power and observe the following further details.

The **resin canals** are lined by **secretory cells**. The **phloem** consists of **sieve-tubes**—**sieve plates** may be visible—and some **phloem parenchyma**. (There are no companion cells as will be seen in Angiosperms.) The **xylem** is composed of **tracheids** and some **wood parenchyma** and small **resin canals** will be found in it. The annual rings are composed of large thin-walled tracheids which form the **Spring (early) wood** and, outside them, small thick-walled tracheids form the **Autumn (late) wood**. Radiating inwards from the cortex are the medullary rays which traverse the phloem. Some, the **primary medullary rays**, reach from pith to cortex while others, the **secondary**, go only part of the way.

Stain a section with iodine or Schultze's solution.

Note the starch grains, stained blue, in some of the medullary rays.

(6) *Examine a radial L.S. of the stem.*

Under *low power* find the structures seen in the transverse section. Under *high power* note the **sieve plates** in the walls of the **sieve tubes** and the single row of **bordered pits** seen in surface view in the **tracheids**. Annular and spiral thickening occur in the **protoxylem**, which is innermost, and **bordered pits** in the **metaxylem**. The marginal

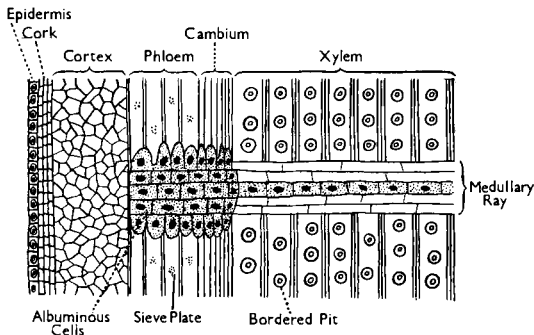


FIG. 58. *Pinus*. R.L.S. Stem.

parts of the **medullary rays** where they pass through the xylem are lignified (**tracheids**) and contain starch grains, and the parts which pass through the secondary phloem have in addition to the ordinary parenchymatous cells, cells having albuminous contents and therefore known as **albuminous cells**.

Stain a section with aniline hydrochloride (or sulphate) and note which parts are lignified.

(7) *Examine a **tangential L.S.** of the stem.*

Under *low power* observe the structures seen in the R.L.S. above.

Note also the **bordered pits** in section and the **medullary rays** across which, of course, the section cuts.

Under *high power* examine the detailed structure of a **bordered pit** as seen in this view.

The Leaf

(8) *Cut a **T.S.** of a leaf, stain with aniline hydrochloride (or sulphate) and mount in glycerine.*

Under *low power*, note the **upper epidermis** and **lower epidermis** composed of thick-walled cells with a thick **cuticle**. **Stomata** will be seen sunk below these layers, a characteristic feature of the xerophytic state. Inside the epidermis are a few layers of sclerenchymatous cells, except below the stomata, constituting the **hypodermis**, and

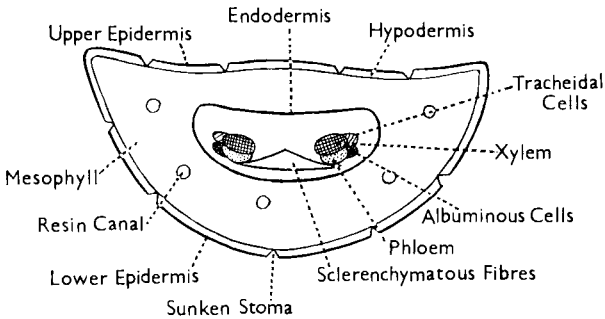


FIG. 59. *Pinus*. T.S. Leaf. Low Power Diagram.

inside this again is the **mesophyll**. This tissue is composed of thin-walled parenchymatous cells, the walls of which are infolded. **Chloroplasts** and starch grains are present in the cells. Between some of these are **intercellular spaces** and in its outer regions, this tissue contains **resin canals**.

The centre of the leaf is occupied by vascular tissue surrounded by a many-layered **pericycle** and a definite single-layered **endodermis**. There are two vascular bundles, the **xylem** being on the upper side

with the **phloem** beneath. The tissue in which these are embedded is of two kinds—**parenchyma** and **transfusion tissue**. The latter is composed of (a) **tracheidal cells** with bordered pits in their lignified walls and (b) **albuminous cells** containing starch and protein grains and having cellulose walls. (This tissue is characteristic of Gymnosperms.) Beneath and between the two vascular bundles, **sclerenchymatous fibres** will be seen.

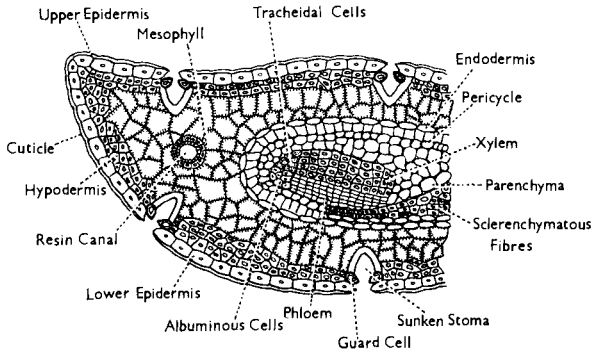


FIG. 60. *Pinus*. T.S. Leaf. High Power Drawing.

Mount a section in Schultze's solution.

Note the **starch** and **protein grains** in the **albuminous cells** of the transfusion tissue and the **cellulose cell walls**.

The Cone

(9) *Examine a branch bearing male cones (Microsporangiate).*

These are numerous and are in clusters in the axils of **scale leaves** at the base of long shoots.

(10) *Examine a male cone entire and a L.S.*

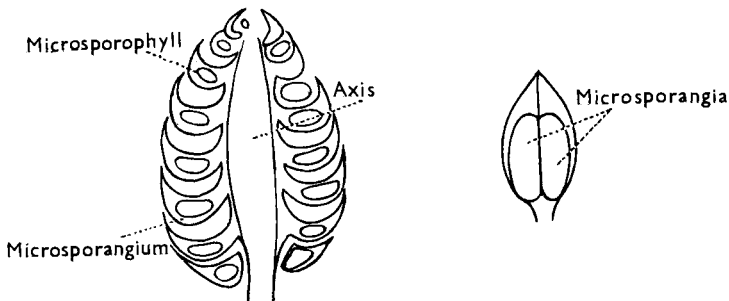


FIG. 61. *Pinus*. Male Cone.

It is ovoid and consists of **microsporophylls (stamens)** arranged spirally on a central **axis**. Each sporophyll is a small short-stalked scale bearing two **microsporangia (pollen sacs)** on the lower side.

(11) *Examine a L.S. of a microsporophyll in the L.S. of the male cone.*

Note that it contains **microspores (pollen grains)**.

(12) *Examine a branch bearing female cones (Megasporangiate).*

These are situated on the ends of long shoots and are of three kinds: (i) small green **first-year cones** on the end of the long shoot; (ii) large green **second-year cones** on the end of the previous year's shoot and therefore at the base of the present year's shoot which is continuous with it and (iii) still larger **third-year cones**, which are situated on the end of the shoot of the year before last. These cones are brown and woody and their scales are separated, thus exposing and liberating the seeds.

(13) *Examine a First-year Female Cone.*

It consists of a central axis on which is a number of spirally arranged **megasporophylls**.

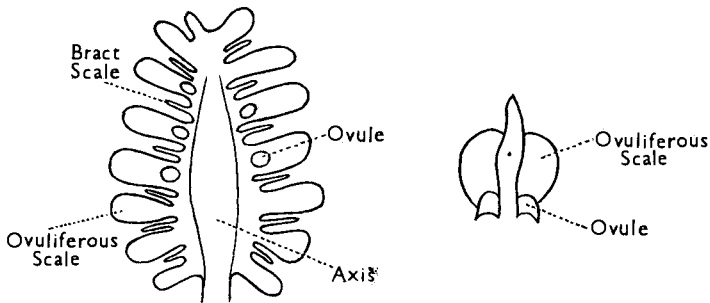


FIG. 62. Pinus. Female Cone.

Remove a **megasporophyll** and examine with a hand lens. Each is composed of a lower **bract scale** (visible only in young cones) with a thicker **ovuliferous scale** above on the upper surface of which is a pair of **ovules (megasporangia)** close to the axis.

(14) *Examine a L.S. of the ovule in a First-year Female Cone.*

Each ovule has one coat or **integument** and this encloses a mass of tissue, the **nucellus**, in which is a large cell, the **megaspore (embryo-sac)**. There is a gap in the integument called the **micropyle**, which faces the axis.

(15) *Examine a Second-year Female Cone.*

Note that the scales and ovules are larger and thicker. Pollination occurs in the first year and the pollen grains remain in the female

cone for about twelve months, during which time they complete their development. Fertilisation then takes place.

(16) *Examine a L.S. of the ovule in a Second-year Female Cone.*

The embryo-sac is now filled with a compact tissue, the **female prothallus**, which becomes the endosperm of the seed and at the micropylar end are two or more **archegonia**, each having a short neck and each containing a large **oosphere** and, if not fertilised, a small **ventral canal cell**.

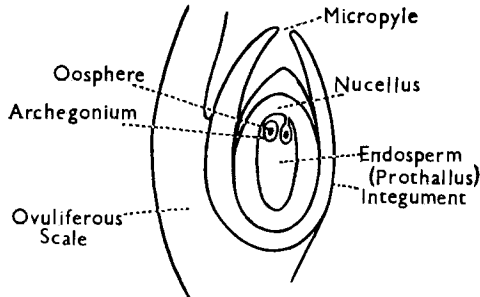


FIG. 63. *Pinus*. L.S. Mature ovule.

The oosphere is fertilised by one of the two male gametes which are liberated when the pollen tube, which germinates from the pollen grain, enters an archegonium. The fertilised oosphere then develops a thick wall and becomes an oospore. The pollen tube is necessary to carry the non-motile male gametes to the oosphere.

(17) *Examine a Third-year Female Cone.*

The cone is now mature and the scales are hard and woody and separated from one another. Two **winged seeds** are situated on the upper sides of the scales unless they have already been blown away. As many embryos as there were archegonia in each ovule may be formed (polyembryony) but normally only one matures.

The Seed

(18) *Examine a seed of Pinus.*

It consists externally of a thick, hard, brown seed coat or **testa** (developed from the integument of the ovule) with a thin **wing** attached and with a **micropyle** at one end.

Remove the testa.

Note the **endosperm** (often covered by the membranous remains of the nucellus) in which lies the embryo composed of a **radicle** at the micropylar end, continuous with which is the **hypocotyl** and a small **plumule** surrounded by a ring of several **cotyledons**.

TAXUS

THE YEW

Taxus baccata, the Yew, is another evergreen and is dioecious.

(1) *Examine the living tree* (if possible).

Note the scaly reddish-brown bark, the upright main branches bearing spirally arranged narrow pointed foliage leaves and the horizontal branches bearing similar leaves arranged laterally in two rows (compare with *Pinus*).

The Root

(2) *Examine a T.S. of a root.*

The **stele** is diarch, the **pericycle** is many-layered outside the **phloem** but only one- or two-layered outside the **xylem**. Secondary growth takes place in older roots.

The Stem

(3) *Examine a T.S. of a stem.*

The structure is similar to that of *Pinus* but lacks resin ducts.

The Leaf

(4) *Examine a T.S. of a leaf.*

The leaf is convex on its upper surface and concave on the lower. There is an **upper epidermis** which is devoid of stomata. The **mesophyll** consists of **palisade cells** above and a **spongy layer** below and is devoid of resin ducts. There is no hypodermis (unlike *Pinus*). The vascular bundle in the centre consists of **xylem** above and **phloem** beneath. The **lower epidermis** contains a number of **stomata**.

The Cone

(5) *Examine a male cone.*

The male flowers develop in the axils of the leaves of the previous year on the under side.

Each cone consists of a short **axis** bearing brown **scale leaves** at the base and ten **microsporophylls (stamens)** above. Each microsporophyll is peltate (shield shaped) and bears on its head five to ten **microsporangia (pollen sacs)** which hang down.

(6) *Examine a female cone.*

The female flowers develop in a position similar to that in the male. There is only one ovule in each flower, borne on a short lateral shoot just below the apex and protected by **scale-leaves**.

(7) *Examine a L.S. of an ovule.*

Note the general nutritive tissue, the **nucellus**, surrounded by a single **integument** with its **micropyle** at the upper end. The **megaspore (embryo-sac)** is towards the lower end which is surrounded by the **aril**, a ring-like structure (seen here in section) which develops into a red cup in which the seed is formed (unlike *Pinus*, in a single season).

The Seed(8) *Examine a seed of Taxus.*

It is endospermic but, unlike *Pinus*, it has only two **cotyledons**. As already stated it is enclosed in the bright red cup-like **aril**.

CLASS

ANGIOSPERMAE

SUB-CLASSES

MONOCOTYLEDONS

Seeds have one cotyledon.
Many vascular bundles in stem, scattered in the ground tissue. Larger number of protoxylem groups than in dicotyledons. Bundles closed. No cambium. Venation parallel. No distinct calyx and corolla in flower.

DICOTYLEDONS

Seeds have two cotyledons.
Vascular bundles arranged in a ring near the outside in the stem. Bundles open. Limited number (maximum five) in root. Cambium between xylem and phloem. Venation reticulate. Definite calyx and corolla found in many families.

THE ROOT(1) **ROOT SYSTEMS****Tap Roots**

These are elongated radicles.

(1) *Examine the root of the broad bean or pea.*

Note the **primary root** which is the elongation of the radicle, **secondary roots**, borne on the primary root and the **root-hairs** growing on both. (This is the Tap Root System and is characteristic of dicotyledonous plants.)

(2) *Examine the swollen roots of carrot (conical), radish (fusiform) and turnip (napiform).* These so-called tap-roots are really hypocotyls and are swollen with food store. (Typical of biennials.)

Fibrous Roots

(1) *Examine the roots of grass.*

The primary root ceases to grow and there is no main root as such as it branches considerably and a large number of roots grow from the base of the stem. (This is the Fibrous Root System and is characteristic of monocotyledonous plants, though it is also found in a number of dicotyledons.)

(2) *Examine the tuberous roots of Dahlia or Lesser Celandine.*

Note the swellings, **root-tubers**, on the fibrous roots.

Test for inulin in Dahlia and for starch in Lesser Celandine (see p. 18).

(3) *Examine the aerial roots of ivy.* These are **adventitious roots** which develop on the stem and are climbing organs. Adventitious roots are roots which develop from any part of the plant other than the radicle.

(2) THE GENERAL STRUCTURE OF THE ROOT

Mount a carefully washed root of cress (Lepidium) in water.

Under *low power* note the **root-cap** (or **calyptra**) protecting the root tip and, some distance behind, the **root-hairs**. Note also the clear outer tissue, the **cortex**, surrounding the darker **vascular cylinder** in the centre.

(3) THE HISTOLOGY OF THE ROOT

DICOTYLEDONOUS ROOTS

(i) Apical Meristem

Examine a L.S. of the root tip of the broad bean.

Under *low power* note the **root-cap** (or **calyptra**), protecting the tip and behind it the **calyptragen** which gives rise to it. Behind this is an outer layer, the **tunica**, which develops into the protoderm giving rise to the epidermis, and, internal to this, the **corpus** composed of **ground meristem** (which gives rise to the cortex) and **procambium** from which the xylem and phloem develop. Cells showing mitosis will be seen in the apical meristem.

(ii) Transverse Section of Young Dicotyledonous Root in the Root-hair Region

Cut a T.S. of the root of a broad bean, buttercup, sunflower or other dicotyledonous seedling in the root hair region. Mount in Schultze's solution and observe results of staining.

(1) Under *low power* draw a diagram showing the **piliferous layer** (epidermis), on the outside, a single row of cells some of which are elongated as **root hairs**. Next comes the **cortex**, composed of thin-walled, rather rounded cells with intercellular spaces at the corners (**parenchyma**) several layers in thickness. The innermost layer of the cortex is the single-layered **endodermis**, with thickened cell walls and the next layer is the **pericycle** and is usually single (sometimes double though it may not be continuous). In the centre is the **stele** or vascular cylinder, consisting of alternating single **bundles** of (i) **xylem**, roughly triangular with small **protoxylem** at the apex (nearest the endodermis) and larger **metaxylem** towards the centre, and (ii) **phloem**. The **pith** (when present) is the parenchymatous ground tissue in the centre. In many roots this is absent and the xylem reaches to the centre. *Count the number of xylem groups.* (Dicotyledonous roots vary normally from two to five and are described as di-, tri-, tetr-, or pentarch.)

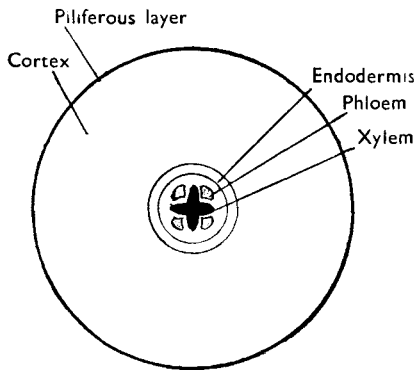


FIG. 64. T.S. Dicotyledonous Root.
Low Power Diagram.

(2) Under *high power* make a drawing* of a wedge or portion of the root showing all the tissues, viz. **piliferous layer**, cells with **root hairs**, **cortical parenchyma**, **endodermis**, **pericycle**, **protoxylem** and **metaxylem** and **phloem** with **sieve plates** (perforated transverse walls) and narrow **companion cells**. The cells of the endodermis opposite the protoxylem are unthickened and are the **passage cells**.

* See pp. 24 seq.

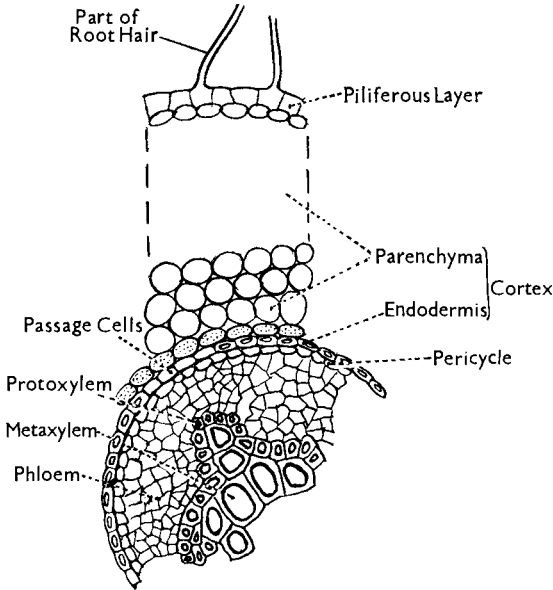


FIG. 65. T.S. Portion of Dicotyledonous Root.
High Power Drawing.

(3) *Stain with safranin or haematoxylin and make a permanent preparation.** The safranin stains the lignin and the haematoxylin the cellulose walls.

(4) *Examine a transverse section of a broad bean root through a lateral root.*

Note that the **secondary roots** arise from the **pericycle** usually opposite the protoxylem.

(iii) Secondary Growth

Transverse Section of Old Dicotyledonous Root

Cut a T.S. of an old root in the region where the lateral roots are found. Mount as before.

(1) Under *low power* note **epidermis**, **cortex**, **endodermis** and **pericycle**. The **cambium**, at first a wavy band of meristematic cells outside the primary xylem and inside the primary phloem gives rise to **secondary xylem** (inside) and **secondary phloem** (outside). If the secondary growth is well developed, the **secondary xylem** will be seen to occupy the greater part of the root between the **primary xylem** bundles but is transversed by parenchymatous **medullary rays**

* See Part I. Microscopical Technique, pp. 16 seq.

radiating out from the end of the primary xylem bundles. The **secondary phloem** is continuous around the now circular ring of **cambium** except where it is cut by the medullary rays. The **primary phloem** is external to the secondary phloem and is in its original position relative to the primary xylem. The cortex and endodermis are no longer visible and the piliferous layer has disappeared with the development of a protective layer of **cork** (formed from the **cork cambium** or **phellogen**, a secondary meristem derived from the pericycle) with **bark** external to it.

(2) *Cut and stain a transverse section of an old root either singly with safranin or haematoxylin or by double staining with safranin and haematoxylin or methyl green and haematoxylin or safranin and light green and make a permanent preparation.*

MONOCOTYLEDONOUS ROOTS

Transverse Section of Monocotyledonous Root

*Cut a T.S. of an old root of maize (*Zea mais*), wheat (*Triticum*) or onion (*Allium*).*

Note the large number of **vascular bundles** (polyarch). Xylem and **phloem** again alternate, the large **metaxylem** being internal to the much smaller **protoxylem**. The **endodermis** is clearly defined and both radial and tangential walls of most of its cells are thickened,

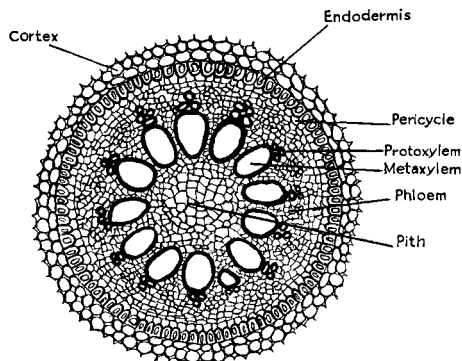


FIG. 66. T.S. Monocotyledonous Root—Central Portion.

the others remaining unthickened for the passage of water and are the **passage cells**. **Pericycle**, **cortex**, and **piliferous layer** are present. There is no cambium and no secondary growth. In the centre is **pith**.

Make a low power diagram of the root and a high power drawing of a vascular bundle.

THE STEM

(1) FORMS OF STEM

The Herbaceous Stem

Examine the stem of the **broad bean, sunflower** or other annual or biennial plant. It is green and comparatively soft though rigid and **erect**. The places where the leaves are attached are called **nodes**, and the spaces between, **internodes**. The angles between the upper part of the stem and the leaves are called the **axils** of the leaves.

The Woody Stem

(1) Examine the **winter twig of the horse-chestnut** (*Aesculus hippocastanum*). It is brown, hard (woody) and **erect**.

Note the **terminal bud** covered with **scale leaves** and, further down, the **ring-scars** formed by the falling of scale leaves of former terminal buds. *Mark the growth of each year in your sketch.* Note the **lateral** or **axillary buds** on the sides of the stem and the horse-shoe like **leaf-scars**. The oval brown scars dotted over the surface are the **lenticels**.

(2) Examine and draw **twigs of sycamore, ash, elm, lilac and lime**.

Note the parts as in (1) and examine the method of branching.

When growth in length takes place from the terminal bud, lateral branches developing from the main axis, branching is said to be **monopodial** (or **racemose**). On the other hand, when growth is continued by a lateral bud instead of the terminal bud, the branching is said to be **sympodial** (or **cymose**). In some cases branching is **dichasial** (or **dichotomous**) in which growth is continued by two lateral buds.

(2) THE BUD

(1) Cut a **L.S. of the terminal bud of a horse-chestnut**.

Note the short **axis**, covered by overlapping **leaves** with short internodes and protected by thick sticky **scale-leaves (bud scales)** which also overlap and are tightly packed.

(2) Place a **horse-chestnut (or other) twig in water and leave in a warm room**.

Note the opening out and eventual dropping of the **bud scales**, the **elongation of the axis** and the development of the **foliage leaves** in the terminal bud.

(3) MODIFICATIONS OF THE STEM

(i) Runner

Examine the **creeping buttercup** (*Ranunculus repens*) or **strawberry plant** (*Fragaria vesca*).

Slender branches, arising in the axils of the leaves trail along the ground. These **runners** are **weak stems** which at intervals bear buds which develop adventitious roots and leaves, *i.e.*, give rise to new plants.

(ii) Offset

Examine the **house-leek** (*Sempervivum tectorum*). Note the short, stout, runner-like offsets which turn up at the ends producing new plants.

(iii) Stolon

Examine the **stolon** of the **blackberry** (*Rubus fruticosus*), **gooseberry** (*Ribes grossularia*) or **currant** (*Ribes rubrum*).

These are long branches which bend down, reach the soil and develop adventitious roots. A new shoot develops from an axillary bud at the node.

(iv) Thorns (or Stem Spines)

Examine the **stem** of **hawthorn** (*Crataegus oxyacantha*) or **gorse** (*Ulex europaeus*).

The ends of some branches arising in the axils of leaves are developed into sharp-pointed **thorns**, the growth of the stem being arrested by the absence of a terminal bud which is replaced by a thorn. These are modified complete stems.

(v) Prickles (or Hooks)

Examine the **stem** of a **raspberry** (*Rubus idaeus*) or **blackberry** (*Rubus fruticosus*). Prickles are found at various places on the stem. These are modified hairs.

(vi) Cladodes (or Phylloclades)

Examine the **stem** of **butcher's broom** (*Ruscus aculeatus*).

It is flattened, leaf-like and green and bears buds in the axil of a scale leaf half way up the midrib. These **cladodes** arise in the axils of leaves which are reduced to mere scales.

(vii) Stem Tendrils

Examine the **white bryony stem** (*Bryonia dioica*).

Some of the branches, arising in the axils of the leaves, are modified into slender coiled **tendrils** to assist in climbing.

(viii) Rhizome

Examine the **rhizome of lily of the valley** (*Convallaria majalis*), **Solomon's Seal** (*Polygonatum multiflorum*) or **Iris** (*Iris pseudacorus*).

It is a thickened **underground stem** growing horizontally, rich in food store and bearing a **terminal bud** from which the aerial shoot develops. It is covered by brown **scale leaves**. (Note the **shoot**, if present, in place of the terminal bud.) Growth is sympodial and is continued by the **axillary bud** just behind the apex. Note the circular **scars of previous shoots** and the **adventitious roots**. The rhizomes of couch-grass (*Agropyrum repens*) and sedge (*Carex*) are slender.

(ix) Bulb

(1) Examine a **bulb of the tulip** (*Tulipa*), **hyacinth** (*Hyacinthus*), **lily** (*Lilium*), or **daffodil** (*Narcissus pseudonarcissus*). It is a condensed **underground shoot**.

Note the membranous **protective scale leaves** and **adventitious roots**.

(2) Cut a L.S. through the centre of the **bulb**.

Note the condensed **stem** at the base, to which **adventitious roots** are attached, the **flowering shoot** continuous upwards with this, and the overlapping **fleshy scale leaves** surrounding the shoot and growing from the stem. **Buds**, which form new bulbs, may be present in the axils of the fleshy scale leaves.

(x) Corm

(1) Examine a **corm of the Crocus** (*Crocus vernus*) or **Gladiolus** (*Gladiolus communis*). This is also an **underground stem**.

Note the membranous **protective scale leaves**, **adventitious roots** and (if present) shrivelled **remains of last year's corm** at the base of the present one.

(2) Cut a L.S. through the centre of the **corm of Crocus or Gladiolus**.

Note the large solid **present year's corm**, the **flowering shoot**, which develops from a **bud**, the **adventitious roots**, and (if present) the **remains of last year's corm** (or **scar** left by it at the base). Look for the **beginning of next year's corm** at the base of the stem of the flowering shoot. Some corms develop **contractile roots**.

(xi) Tuber

Examine the **potato** (*Solanum tuberosum*) or the **Jerusalem artichoke** (*Helianthus tuberosus*), another **underground stem**.

Note the three **buds** in the axils of the **scale-leaves** ("eyes") and the **point of attachment to the stem**. The brown coating on the

tuber is a thin layer of cork: lenticels are present. These **stem tubers** develop on the ends of underground branches or slender rhizomes.

(4) THE HISTOLOGY OF THE STEM

DICOTYLEDONOUS STEMS

(i) Apical Meristem

Examine a L.S. of the apex of a dicotyledonous stem.

The meristematic tissue is composed of an outer layer, the **tunica**, from which the protoderm develops (and it is from this that the epidermis arises), and the inner core or **corpus**, the **procambium** in which is the source of the xylem, phloem and fibres, while its **ground meristem** gives rise to the cortex and pith. Cells showing mitosis occur in this meristematic tissue.

(ii) Transverse Section of Young Dicotyledonous Stem

Cut a T.S. of the young stem of a sunflower (Helianthus annus). Mount sections separately in Schultze's solution and aniline sulphate.

(a) Under low power note the outermost layer, the **epidermis**, a single row of cells with multicellular **hairs**, then the narrow but many-layered **cortex**, the innermost row of which form the **endo-dermis**, also known as the **starch sheath**. Inside this is the ring of **vascular bundles** constituting the **stele**, each bundle having **phloem** on the *outside* and **xylem** on the *inside* on the same radius (and

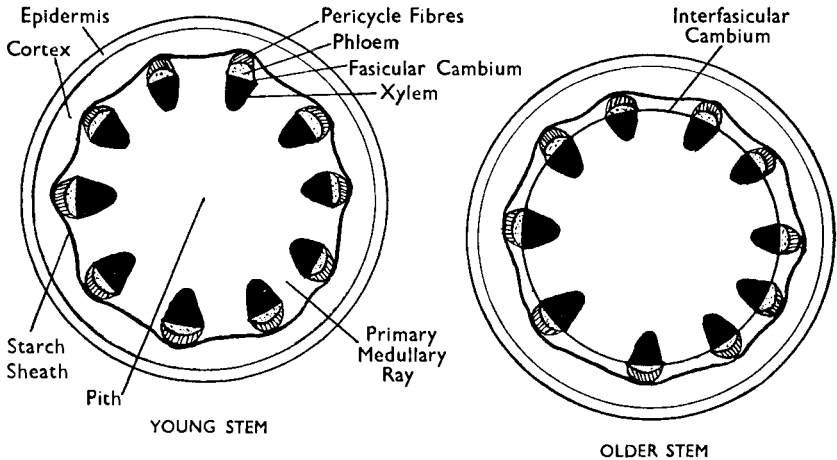


Fig. 67. T.S. Dicotyledonous Stem. Under low power. (Diagrammatic).

therefore said to be *collateral*), the two separated by a primary meristem several cells thick, the **fascicular cambium** (the bundle is therefore said to be *open*). Look for the **inter-fascicular cambium**, a secondary meristem continuous with the fascicular cambium between the bundles, and the group of **pericycle fibres** (sclerenchyma) outside the phloem. The thin-walled **parenchyma** is prolonged between the bundles as the **primary medullary rays** and in the centre as the **pith**.

Note also the numerous **resin ducts** (each surrounded by gland cells) in the cortical parenchyma.

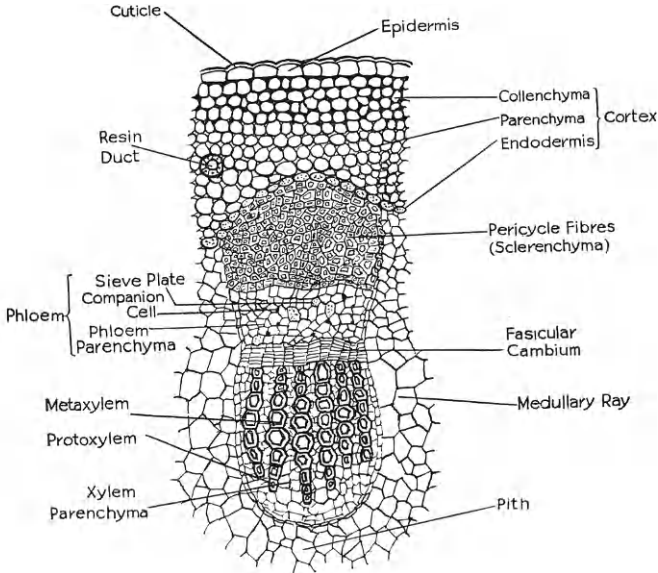


FIG. 68. T.S. Portion of Dicotyledonous Stem. Under high power.

(b) Under *high power* note that the **epidermis** has thickened cutinised outer walls forming a **cuticle** and bears a few multicellular **hairs**. The **cortex** is composed externally of **collenchyma**, cells with cellulose thickening at the corners, and internally of thin-walled **parenchyma** with intercellular spaces. The **endodermis** or **starch sheath** is a single layer and contains starch grains. Inside this are the **pericycle fibres**, the **phloem** composed of **sieve tubes with sieve plates**, **companion cells** and **phloem parenchyma**. Next comes the thin-walled **cambium** followed by the large **metaxylem** (vessels), the smaller **protoxylem** (tracheids and vessels) being internal. (Compare with the root.) The **xylem parenchyma** is composed of small cells with cellulose walls.

(c) *Stain a section singly with haematoxylin or safranin or doubly with safranin and light green and make a permanent preparation.*

(iii) Longitudinal Section of a Young Dicotyledonous Stem

Examine a L.S. of a young sunflower stem.

(a) Under *low power* note the structure as in the T.S.

(b) Under *high power* note particularly the **endodermis**, the **pericycle**, the **phloem** (composed of **sieve tubes** with **sieve plates** and **companion cells**), the **cambium**, the **metaxylem** (composed of **pitted vessels**, **wood fibres** and **wood parenchyma**) and the **protoxylem** (composed of **annular** and **spiral vessels**). *Examine the various forms of thickening in the xylem.*

(iv) Transverse Section of Vegetable Marrow Stem

Cut a T.S. of the stem of a vegetable marrow (Cucurbita). Mount sections separately in Schultze's solution and aniline sulphate.

(a) Under *low power* note the **epidermis**, the **collenchyma** and **parenchyma** of the **cortex**, the **endodermis** and **sclerenchyma**, the wide **pericycle** and the two rings of **bicollateral vascular bundles** each composed of **outer phloem**, **cambium** and **xylem** followed by **inner phloem**. The bundles in the outer ring are smaller than those in the inner ring with which they alternate. Note also the **parenchyma** in which the bundles are embedded and the **pith**.

(b) *Examine a vascular bundle* under *high power* and note the **outer phloem** with **sieve tubes**, **sieve plates** and **companion cells**, the **outer cambium**, the wide outer **metaxylem vessels** and narrower inner **protoxylem**, the **xylem parenchyma**, and the **inner phloem** with **sieve plates**.

(v) Longitudinal Section of Vegetable Marrow Stem

Examine a radial longitudinal section of the Stem of Vegetable Marrow.

(a) Under *low power* note the structures as in T.S.

(b) Under *high power* examine a **vascular bundle** and note the **outer phloem** with **sieve tubes**, **sieve plates** and **companion cells**, the long narrow cells of the **cambium**, the large outer **pitted vessels** of the **metaxylem** and the **spiral** and **annular vessels** of the **protoxylem** and the **inner phloem**.

(vi) Secondary Growth

(a) Transverse Section of a Woody Stem

Examine a T.S. of a three-year-old stem of lime (Tilia europaea). Under *low and high power* note the ruptured **epidermis**, the **cork**

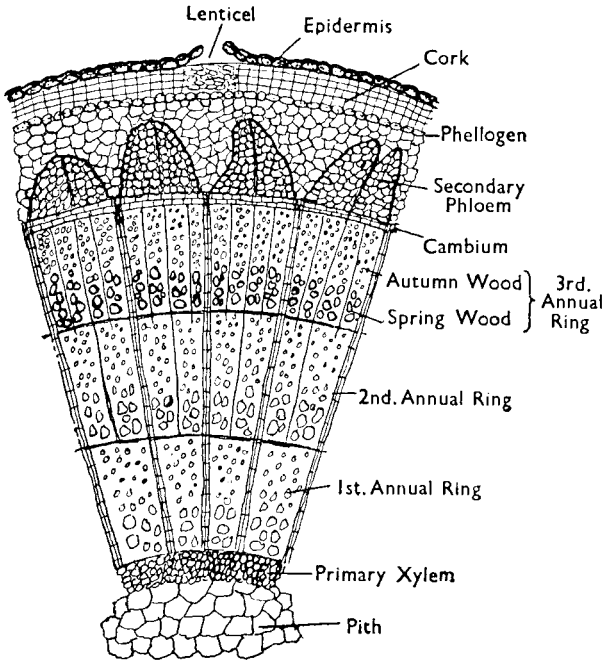


FIG. 69. T.S. Part of Three Years Old Woody Stem. Under low power.

consisting of rows of flattened cells and the **phellogen** or **cork cambium** inside this. Next comes the **pheloderm** or secondary cortex, followed by the **cortex** (parenchyma). Cork, phellogen and pheloderm are collectively known as **periderm**. The **secondary phloem** is divided into wedges roughly triangular in shape due to

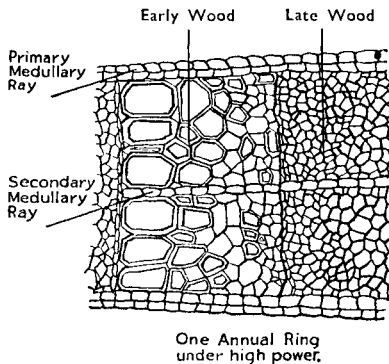


FIG. 70. Annual Ring. Under high power.

the widening of the medullary rays and contains some sclerenchymatous fibres. Outside the secondary phloem is the **primary phloem** and next to the former is the single ring of **cambium**. The **secondary xylem** is composed of three **annual rings**, apparent rings caused by the juxtaposition of small well thickened elements of **late wood (Autumn wood)** externally and large and less thickened elements of **early wood (Spring wood)** internally. The **primary xylem** is in the centre projecting into the **pith**. Note the radiating **primary medullary rays** running from pith to cortex and the shorter **secondary medullary rays** between them, both produced by the cambium.

Draw a portion of an annual ring under high power.

(b) Lenticels

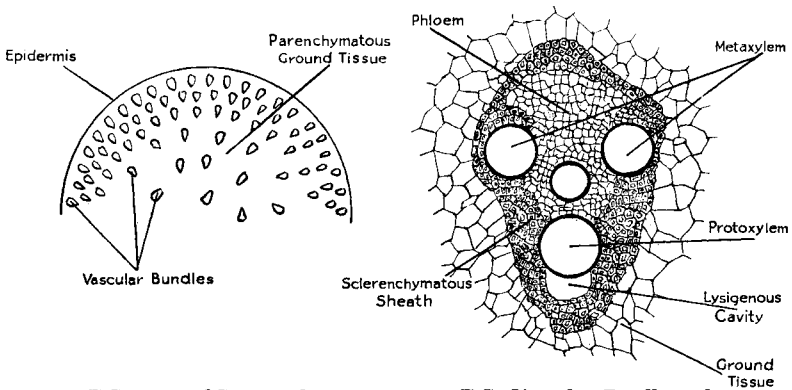
Examine a T.S. of a stem through a lenticel under high power. Note the broken **epidermis**, the **cork**, the **loosely packed cork cells** in the **lenticel** and the compact ones beyond it, the **phellogen**, **phelloderm** and **cortex**.

MONOCOTYLEDONOUS STEMS

(i) Transverse Section of Monocotyledonous Stem

Cut a T.S. of the stem of maize (Zea mais).

(a) Under *low power* note the **epidermis** with **stomata**, and the **vascular bundles** scattered throughout the **parenchymatous ground tissue** but more concentrated and smaller near the periphery.



T.S. Part of Stem under Low Power.

T.S. Vascular Bundle under High Power.

FIG. 71. T.S. Monocotyledonous Stem.

(b) *Examine a vascular bundle under high power.* Note the Y-shaped xylem with the two large **metaxylem** vessels outside forming the arms of the Y and the **protoxylem** inside. The **phloem** is between

the arms and the bundle and is collateral. The bundles also contain parenchyma and are almost completely enclosed in a **sclerenchymatous sheath**. There is no cambium and the bundle is said to be closed. A **lysigenous cavity** may be present if the innermost xylem has broken down.

(ii) Longitudinal Section of Monocotyledonous Stem

Examine a L.S. of the stem of maize.

Under *low power* note the structures as in T.S. The **metaxylem** consists of **pitted vessels** and the **protoxylem** contains **annular** and **spiral vessels**.

THE FOLIAGE LEAF

(1) FORMS OF LEAF

(i) Simple Leaves

(1) *Examine the leaf of the garden pea (*Pisum sativum*) attached to the stem.*

Note the leaf blade or **lamina**, the margin of which is **entire**, the stalk or **petiole**, the **leaf base** or point of attachment to the stem and the **stipules**, membranous outgrowths at the base of the petiole (this leaf is therefore said to be **stipulate**).

(2) *Compare with the leaf of the sunflower (*Helianthus annuus*).*

No stipules are present (and the leaf is said to be **exstipulate**).

(3) *Examine the sycamore leaf (*Acer pseudo platanus*).*

Note the lobing or indentation of the margin, which does not, however, divide the lamina into separate leaflets. Like the two just examined, it is a **simple leaf**.

(ii) Compound Leaves

(1) *Examine the leaf of the horse-chestnut (*Aesculus hippocastanum*).* The lamina is divided into separate **leaflets** borne on a common stalk, thereby making it a **compound leaf**. It is **palmate** (*i.e.*, the leaflets all join the petiole at the same point).

(2) *Examine the leaf of the ash (*Fraxinus excelsior*).*

This is another **compound leaf** but it is **pinnate** (*i.e.*, feather-like). The leaflets are borne on either side of the petiole.

(2) VENATION

(i) Reticulate Venation

*Examine the arrangement of the veins in the leaf of privet (*Ligustrum vulgare*) or other dicotyledonous plant.*

Note the single **midrib** with an irregular network of veins on each side. This is characteristic of dicotyledons and is known as *pinnate venation*. In leaves in which there are several main veins it is called *palmate venation*.

(ii) Parallel Venation

Examine the leaf of **Iris, tulip** or other monocotyledonous plant.

The veins are parallel and there is no midrib. Cross-connections between the parallel veins occur at intervals. (This is characteristic of monocotyledons.)

(3) PHYLLOTAXIS

Examine the arrangement of the leaves on the stems of the following:—

(1) **Broad Bean**. The leaves are **alternate**, there being only one at each node, and the phyllotaxis is **spiral**.

(2) **Dead Nettle**. There are two opposite leaves at each node which are **decussate**, *i.e.* at right angles to the pair above and below. The phyllotaxis is said to be **cyclic** or **whorled**.

Represent the **phyllotaxis** in each of the above cases by a fraction as follows:—

Trace round the stem from one node to the next above in a clockwise direction until you have reached the node vertically above the one from which you started. This may be done with a piece of cotton. Count the number of times you encircle the stem and make this the numerator; count also the number of internodes passed and make this the denominator.

(4) MODIFICATIONS OF THE LEAF

(i) Leaf Tendrils

Examine a well-grown **garden pea** (*Pisum sativum*).

Its **weak stem** climbs by means of stringy threads, **tendrils**, which are the modified tips of leaves. In some plants whole leaves are modified into tendrils, *e.g.* yellow vetchling (*Lathyrus aphaca*).

(ii) Leaf Spines

Examine the leaves of **holly** (*Ilex aquifolium*) and **gorse** (*Ulex europaeus*).

In the former the leaf margin, and in the latter the entire leaf, is modified into spines.

(iii) Phyllodes

Examine the preserved phyllodes of an Acacia.

The lamina is absent (unless it is a young seedling, when leaflets may be seen at the distal end of the petiole) and the petiole is flattened vertically thus exposing only the inner edges to the sun. This plant grows in open tropical country.

(iv) Insectivorous Plants

In these green plants, insects are trapped and digested and the products of digestion absorbed, thus augmenting the ordinary processes of holophytic nutrition.

The Pitcher Plant (*Nepenthes*)

This grows in tropical forests but it can be purchased in this country.

Examine the pitcher plant.

The leaves are modified into **phyllodes** and the ends of these phyllodes are modified into what are known as **pitchers** from their shape. In some species these are very large. The surface of the pitcher is splotched with red and purple patches which attract insects. The open entrance to the pitcher is partially closed by a small lid. Downwardly projecting hooks surround the mouth of the pitcher and glands secrete a watery solution containing the enzyme pepsin. This liquid collects in the pitcher and when flies enter it, their escape is prevented by the hooks and they drown in the liquid and are digested by the enzyme. The products of digestion are then absorbed into the walls of the pitcher.

The Sundew (*Drosera*)

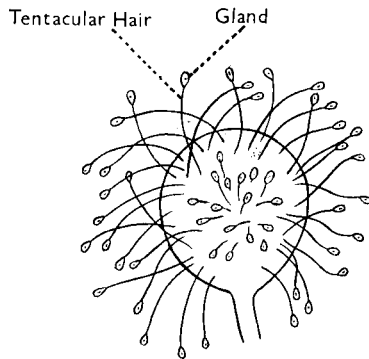
This is commonly found growing on peaty soil which is damp and in bogs.

Examine a Sundew plant.

The circular **lamina** of the leaves is covered with long, tentacle-like **hairs**. Glands on the tips of these hairs secrete a watery mucilaginous substance (which glistens in the sun—hence the name “sundew”) and when flies settle on the leaves, they are unable to escape as the tentacles are then stimulated to bend over and enclose them. The flies are then digested and the products of digestion absorbed.

*Examine a slide of the leaf of **Drosera**.*

Note the **tentacle-like hairs** on the leaf which bears **glands** on their tips.

FIG. 72. *Drosera* Leaf.

(5) HISTOLOGY OF THE LEAF

(i) Transverse Section of the Leaf

Examine a T.S. of the lamina of the leaf of sunflower or other dicotyledonous plant.

(1) Under *low power* note the **upper epidermis**, a single layer of cells with the outer walls thickened forming a protective **cuticle**, the middle layer or **mesophyll** composed of (i) the **palisade layer** above, one or more rows of vertically elongated cells containing numerous **chloroplasts**, with smaller **collecting cells** (not always distinguishable) beneath them and (ii) the **spongy layer** below, several rows of somewhat rounded cells (also containing chloroplasts), loosely packed and with abundant **intercellular spaces**.

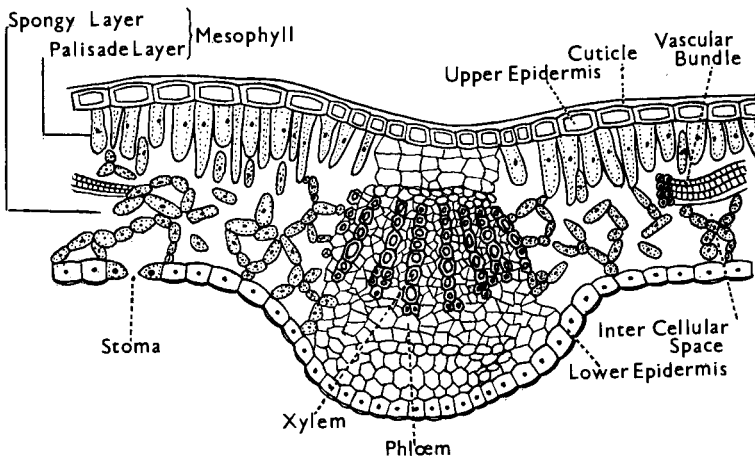


FIG. 73. T.S. Part of Dicotyledonous Leaf.

Between these layers will be seen the veins or **vascular bundles** (in transverse section in the midrib and elsewhere in transverse, oblique or longitudinal section) with the **xylem above** and **phloem below**. The **lower epidermis** with **cuticle** is a single layer of cells with numerous **stomatal pores**, leading to the intercellular spaces of the spongy layer and each protected by two **guard cells**: each of these structures is a **stoma**.

(2) Under *high power* examine the **palisade cells**, cells of the **spongy layer**, **epidermal cells** and the **stomata**.

(ii) Surface View of Stomata

Macerate a leaf in 10 per cent. caustic potash for about ten minutes, cut round the edge and carefully separate the three layers. Then mount the lower epidermis in dilute glycerine. Examine the surface view of the epidermis.

Under *high power* note the **epidermal cells** and the **stomata** (**pores with guard cells**, the only epidermal cells to contain chloroplasts).

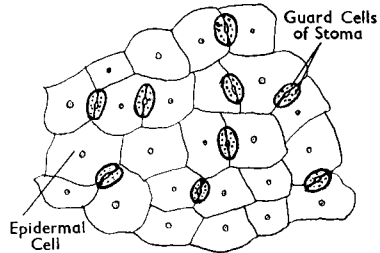


FIG. 74. Epidermis of Leaf.
Surface view.

THE INFLORESCENCE

Examine and draw diagrams of the arrangement of the flowers on the peduncle (mother axis) in such examples as those given below:—

Racemose Inflorescence

The peduncle continues to grow, bearing flowers along its length.

(1) **Raceme.** *Lily of the Valley, Bluebell* or *Foxglove*. The elongated peduncle bears **stalked flowers**.

(2) **Panicle.** *Oats*. This is a compound raceme: the branches are branches of the peduncle, each being a raceme. Note the elongation of the **peduncle** which bears **stalked flowers**.

(3) **Umbel.** *Cherry*. The **flowers** are stalked but appear to be given off at the same point owing to the undeveloped **peduncle**.

(4) **Compound Umbel.** *Parsley, parsnip*. Similar to the Umbel but the branches are branches of the peduncle, each bearing pedicels (flower stalks).

(5) **Corymb.** *Candytuft*. All the **flowers** are on the same level owing to the elongation of the lower **pedicels** while the **peduncle** is less elongated.

(6) **Spike.** *Plaintain.* Similar to the raceme, but the **flowers** on the **peduncle** are **sessile**, *i.e.*, without stalks.

(7) **Capitulum.** *Daisy, Dandelion, Sunflower.* The closely packed **sessile flowers** or **florets** are all on the same level on a reduced **peduncle**. The so-called “flowers” of the Daisy, Dandelion and Sunflower are really inflorescences.

Cymose Inflorescence

There is no elongation of the **peduncle** after it has given off one or two **daughter axes** each bearing a **flower**.

Monochasial Cyme. *Buttercup.* A single daughter axis is formed.

Dichasial Cyme. *Christmas Rose.* Two daughter axes are formed.

Note. Mixed inflorescences, partly racemose and partly cymose, are very common.

THE FLOWER

The flower is the reproductive organ of the plant. The outer floral leaves are accessory structures concerned with the mechanism of pollination and show great diversity of form and colour in different plants. The essential organs of reproduction are inside these floral leaves.

Five types are taken to illustrate floral structure.

(i) RANUNCULUS

(1) *Examine and draw the flower of the buttercup.*

It belongs to the Family *Ranunculaceae*, and is composed of floral leaves of various kinds borne on a stalk, the **pedicel**. Examine the floral leaves. There is an outer whorl of *five free* green **sepals** constituting the **calyx**; inside this is the **corolla** composed of *five free* yellow **petals**. Next comes the **androecium** consisting of an *indefinite* number of *free* **stamens** (microsporophylls) while the **gynaecium** is in the centre and is made up of an *indefinite* number of *free* **carpels** (megasporophylls). The flower is symmetrical about any plane and is described as follows:—

Hermaphrodite (stamens and carpels present), *polysepalous* (sepals *free*), *polypetalous* (petals *free*), the androecium *indefinite* (many stamens) and *polyandrous* (stamens *free*), the gynaecium *indefinite* (many carpels) and *apocarpous* (carpels *free*), *actinomorphic* or *regular* (*radially symmetrical*.)

Write a full description of the flower.

(2) Remove all the floral leaves carefully. Note that they are inserted on the **receptacle** (as the top of the pedicel is called) which is somewhat convex. Note also that the outer whorls of floral leaves are inserted *in order below* the gynaecium. This is known as the **hypogynous** arrangement and the ovary is said to be **superior**.

(3) **Floral formula.**

Write the floral formula.

The following abbreviations or symbols are used in writing floral formulae:—

- ♂ = staminate (male) flower } Usually unnecessary as subsequent parts of the
 ♀ = pistillate* (female) flower } formula indicate this.
 ♂♀ = hermaphrodite flower }
 † or † = zygomorphic (bilaterally symmetrical).
 ⊕ = actinomorphic (radially symmetrical).
 K = Calyx.
 C = Corolla.
 P = Perianth (*i.e.*, calyx + corolla, when it is impossible to distinguish them into two whorls).
 A = Androecium.
 G₊ = Gynaecium, with a superior ovary (*hypogynous*).
 G₋ = Gynaecium, with an inferior ovary (*epigynous*).
 G = Gynaecium, with the floral leaves *perigynous*.
 A number after the above letters = that number of parts.
 If the parts are in separate whorls, the numbers in the whorl are joined by +. If the number varies, the two extreme numbers are joined by —.
 ∞ after the above letters = an indefinite number of parts.
 () round a number = cohesion (floral leaves of the same whorl fused).
 ⌒ above parts = adhesion (fusion between floral leaves of different whorls).

The omission of brackets indicates that the parts are free.

Examples:

(1) Foxglove (*Digitalis*).

↑ K(5) C(5) A 4 G (2)

= Zygomorphic; 5 sepals, fused (*gamosepalous*); 5 petals, fused (*gamopetalous*); 4 stamens free (*polyandrous*) and fused with the petals (*epipetalous*), (= adhesion); 2 carpels, fused (*syncarpous*), superior ovary (*hypogynous*).

(2) Wallflower (*Cheiranthus*).

⊕ K2 + 2 C4 A2 + 4 G (2).

= Actinomorphic; sepals free (*polysepalous*) in two whorls of 2; 4 free petals (*polypetalous*); stamens free (*polyandrous*) in two whorls, one of 2 and the other of 4; 2 fused carpels (*syncarpous*), superior ovary (*hypogynous*).

*The gynaecium was formerly known as the pistil.

(4) Floral Diagram.

Draw the floral diagram. This is a diagrammatic ground plan of the entire flower.

Draw lightly a circle about 3 in. in diameter. Indicate the posterior position (main axis) at the top by o or + and the bracts in their appropriate positions outside the circle. Now draw the correct number of sepals on the circle in their proper positions and the petals, stamens and carpels inside. Cohesion and adhesion should be represented by joining the parts, otherwise they are left unjoined. The symbols generally used are those shown in the example below: the gynaecium is represented by its appearance in a transverse section.

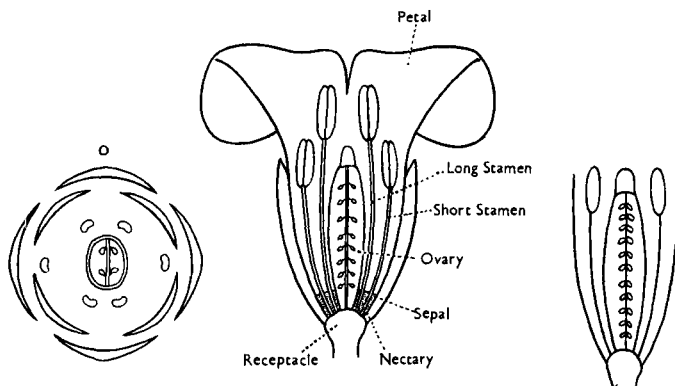


FIG. 75. Floral Diagram, Longitudinal Half-Flower and L.S. Flower of Wallflower (*Cheiranthus*).

(5) Longitudinal Half-Flower

Cut the flower in half longitudinally and draw it to scale. Name the parts.

(6) Longitudinal Section

Draw a longitudinal section to scale, working from the inside.

(7) Sepal

Remove a sepal and draw under a hand lens.

(8) Petal

Remove a petal and draw under a hand lens.

Note the nectary at the base.

(9) Stamen

Remove a stamen and draw under a hand lens. Note the stalk or filament bearing the anther composed of two lobes.

(10) Structure of Anther

(a) Examine a T.S. of a mature anther.

Under *low power* note that each **lobe** is divided into two **pollen sacs** (microsporangia) containing **pollen grains** (microspores) and joined by the **connective** in which the **vascular bundle** will be seen.

(b) Compare with this the T.S. of a **dehisced anther**.

(11) **Carpel**

Remove a **carpel** and examine under a hand lens.

Note the wide basal portion or **ovary**, prolonged above as the slender **style** at the tip of which is the **stigma**.

(12) *Structure of Carpel*

Examine a T.S. of an **ovary**.

Under the *low power* note the **wall of the carpel**, the **mid-rib**, the **vascular bundle** and the cushion or **placenta** to which is attached the **ovule** (megasporangium).

(ii) **PISUM**

(1) Examine the **flower of the garden pea** (*Pisum sativum*) or **broad bean** (*Vicia faba*).

It belongs to the Family *Leguminosae*. Examine the *symmetry of the flower*. Note that it is symmetrical about one plane only. It is therefore said to be *bilaterally symmetrical*, *zygomorphic* or *irregular*. Note the number of **sepals** and whether they are free or fused. The odd sepal is anterior. In the **corolla**, count the number of **petals** and note whether they are free or fused. The anterior petals are loosely joined and form the **carina** or **keel**; the large posterior petal is the **vexillum** or **standard**, and the smaller lateral petals are called **alae** or **wing petals**. Note the number of **stamens** either all fused together by the filaments (**monadelphous**) or with the posterior stamen free (**diadelphous**). This is sometimes referred to as a staminal tube. Lastly, note that the **gynaecium** consists of one carpel (*mono-carpellary*) and that the **ovary** is **superior**.

Write a **description of the flower**.

(2) Cut and draw (a) a **longitudinal half** and (b) a **longitudinal section** in the antero-posterior plane.

(3) Draw the **floral diagram**.

(4) Write the **floral formula**.

(iii) **LILIUM**

(1) Examine the **flower of the Bluebell** (*Endymion nonscriptus*) or **Lily** (*Lilium*).

Both belong to the Family *Liliaceae*. Examine the **perianth** and note the number of parts and whether they are fused or free; note also the nature of the perianth. Examine the **androecium** and count the number of **stamens** and note how they are arranged and whether

there is any cohesion or adhesion. *Note the number of carpels and whether they are free or fused in the gynaecium.* The **ovary** is *superior*.

Write a description of the flower.

(2) *Cut and draw (a) a longitudinal half and (b) a longitudinal section.*

(3) *Draw the floral diagram.*

(4) *Write the floral formula.*

(iv) **HELIANTHUS**

(1) *Examine the capitulum of the Sunflower (*Helianthus*) or Ox-Eye Daisy (*Chrysanthemum leucanthemum*).*

This is characteristic of the Family *Compositae*. It is really an inflorescence consisting of a large number of miniature flowers called *florets* on the flattened surface of the head of the **peduncle**, but is sometimes known as a *composite flower*. There is no calyx but there is an **involucre** of small green **bracts** on the outside. The florets are of two kinds: (i) Large **ray florets** externally, yellow in the sunflower and white in the daisy; these are female and one of their important functions is to attract insects. (ii) **disc florets**, internally and yellow in both flowers; these are hermaphrodite. Each floret arises in the axil of a bract and the oldest are on the outside.

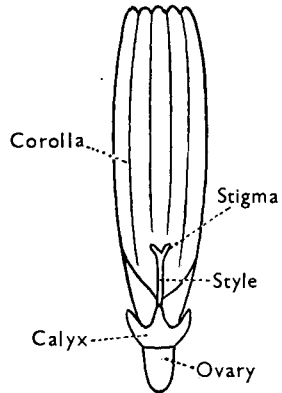


FIG. 76. Ray Floret.

(2) *Examine one of the older ray florets.*

The **corolla** is large and shaped like a strap and therefore said to be *ligulate*. Four ridges along its surface show it to be composed of five fused petals. Below this is a **corolla tube** from the inside of which emerges a single **style** terminating in two **stigmas**, showing that the gynaecium is composed of two fused carpels. Beneath the corolla tube is a single **ovary** which is therefore inferior. This is known as the **epigynous** arrangement.

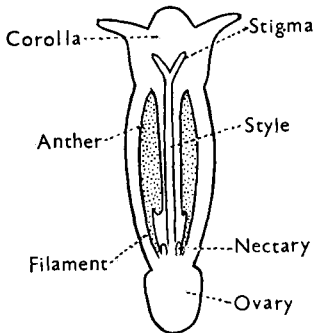


FIG. 77. Disc Floret.

(3) *Examine a disc floret.*

The **corolla**, which is smaller than that of the ray floret, is also composed of five fused petals as can be seen by an examination of the free edge. These petals form a tube and the floret is

consequently said to be *tubular*. Inside this tube is the **androecium**, consisting of five small epipetalous **stamens**, the anthers of which are fused to form a tube. In the centre is the bicarpellary **gynaecium** which is syncarpous and which bears a single **style** which grows up through the anther tube, after which the two **stigmas** open out as in the ray florets. The **ovary** is again inferior and contains a single ovule.

Write a description of the flower.

(4) *Draw (a) a longitudinal section of the capitulum (b) a longitudinal half and (c) a longitudinal section of a ray floret and (d) of a disc floret.*

(5) *Draw the floral diagram for a ray floret and for a disc floret.*

(6) *Write the floral formula for a ray floret and for a disc floret.*

(v) POA

(1) *Examine the inflorescence of Meadow Grass (Poa).*

This is typical of the Family *Gramineae*. Other Grasses show a similar structure with modifications.

The inflorescence which is on a long stem well above the leaves is racemose and bears sessile flowers. It is known as a **spikelet**. Each spikelet is pointed and is enclosed in scale-like bracts called **glumes** arranged alternately on opposite sides of the axis. The lower ones are **sterile glumes** but the upper ones contain florets.

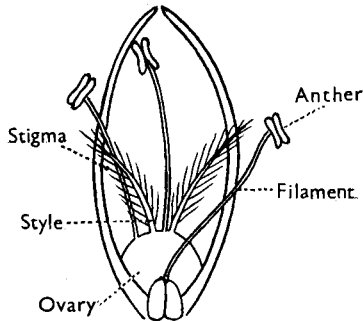


FIG. 75. Meadow Grass. Flower.

(2) *Remove a floret and examine with a hand lens.*

It arises in the axil of a small scale leaf known as a **flowering glume** (or **lemma**). At the base are two minute scales (which may be rudiments of perianth leaves) called **lodicules** and the axis of the flower bears a scaly **palea** on the opposite side to the lemma. Each

floret contains three **stamens** with long filaments and a single **carpel** which has a feathery **stigma**. The ovary contains a single ovule and is superior.

- Write a **description of the flower.**
- (3) Draw a **longitudinal section.**
- (4) Draw a **floral diagram.**
- (5) Write the **floral formula.**

THE GERMINATION OF POLLEN

(1) Mount and examine some **pollen grains** from a flower in sugar solution (see Part III, Physiology Chemotropism Experiment 10, p. 161).

Under *high power*, note the outer wall (**exine** or **extine**), the inner wall (**intine**) and the granular **cytoplasm**.

(2) After leaving for the time prescribed in Experiment 10 examine the **germinated pollen grains**.

Note the **pollen-tube** which has grown out of the grain by the bursting of the intine through the exine.

(3) Examine a prepared slide of stained **germinating pollen grains**.

Note the two male **gametes** and the larger **vegetative (or pollen-tube) nucleus** in the **pollen-tube**.

After pollination, the pollen tube grows and enters the ovule by the micropyle. One gamete fuses with the oosphere, the other with the secondary (or primary endosperm) nucleus, forming the **endosperm nucleus**. The vegetative nucleus (tube nucleus) has by this time disorganised.

STRUCTURE OF THE OVULE

Examine a **L.S. of an ovule**.

Under *high power* note two outer coats or **integuments** which arise from the base or **chalaza**, and which completely invest the ovule

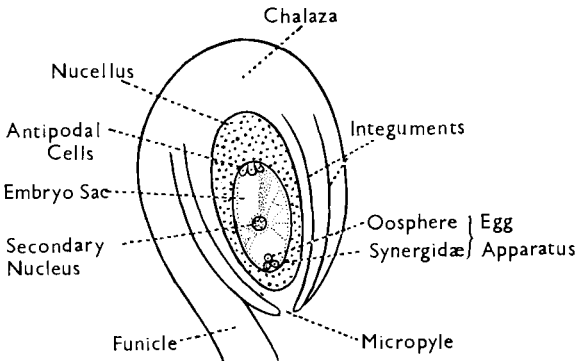


FIG. 76. L.S. Anatropous Ovule.

except at the apex (opposite end to the chalaza), the gap left here is the **micropyle**. The parenchymatous tissue inside the integuments is the **nucellus** (this, strictly speaking, is the megasporangium). At the micropylar end is a large cell, the **megaspore** (often referred to as the **embryo sac**) containing vacuolated cytoplasm, and in its centre is the large **secondary nucleus** (also called the **primary endosperm nucleus**). In the chalazal end of the embryo sac are three **antipodal cells** and in its micropylar end a large cell, the egg cell or **oosphere**, accompanied by two smaller cells, the **synergidae** (the three cells constitute **egg apparatus**). Note the **vascular bundle** running up the stalk or **funicle** from the placenta to the nucellus.

When the ovule is curved over so that the micropyle and funicle are at the same end and there is some fusion between ovule and funicle, it is said to be **anatropous**. This is the commonest form. An ovule curved over without fusion is said to be **campylotropous**. Less frequently found is the straight ovule with the micropyle and funicle at opposite ends and this is called an **orthotropous ovule**.

THE DEVELOPMENT OF THE EMBRYO

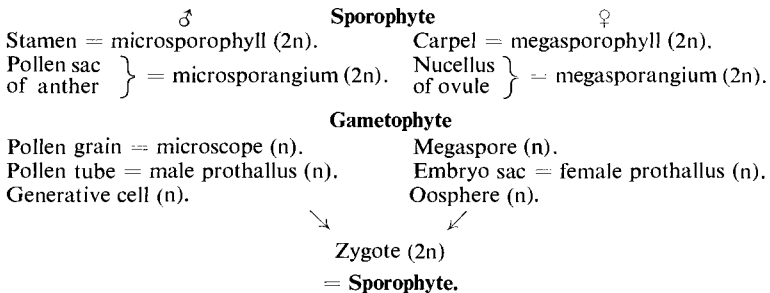
Examine the L.S. of ovules after fertilisation.

Under *high power* look for various **stages in the development of the embryo**. The following may be seen: Division of the **zygote** (fertilised oosphere), with the disappearance of the **synergidae**, into an upper **embryo cell** and a lower cell, which by division forms a chain of cells, the **suspensor**. The endosperm nucleus divides to form a nutritive tissue, the **endosperm**. In more developed ovules look for the division of the embryo cell forming the embryo.

The integuments become the seed-coat or **testa** which encloses the (one or two) **cotyledons**, the **plumule** and **radicle** and the **endosperm** (if present). Thus the **ovule** becomes the **seed**. The ovary wall becomes the fruit wall or **pericarp**.

Note on the Flower

The Flowering Plant is the **sporophyte** (2n chromosomes).



THE FRUIT

SIMPLE FRUITS

(Developed from a single flower with a monocarpellary or syncarpous gynaecium)

Examine and draw the following fruits.

(a) DRY

(1) One-seeded Indehiscent Fruits

(1) Achene

Examine the fruit of the sunflower (or a single achene from that of the buttercup (an aggregate fruit)). It is formed from a superior ovary. Note the hard, **leathery** nature of the fruit wall or **pericarp**. (Characteristic of *Ranunculaceae*.)

(2) Samara (Winged Achene)

Examine the fruit of the elm (*Ulmus*) or *ash* (*Fraxinus excelsior*). Note the **wing** attached to the **achene**.

(3) Cypsela

These are formed from inferior ovaries.

(i) *Examine the fruit of the dandelion* (*Taraxacum officinale*) or **Thistle** (*Cirsium*).

Note that the achenial fruit is crowned by a hairy tuft or **pappus**. (This is equivalent to the calyx.)

(ii) *Examine the fruit of Clematis*.

The style remains as a hairy structure.

(4) Caryopsis

Examine the fruit of oats (*Avena*).

It is similar to the achene but the pericarp and testa are fused together. In this case a bract and bracteole remaining from the flower enclose the fruit. (Characteristic of *Gramineae*.)

(5) Nut

Examine the acorn, beech or hazel nut.

The pericarp is hard and woody.

(2) Many-seeded Dehiscent Fruits

(1) Capsule

(i) *Examine the capsule of the poppy* (*Papaver*).*

It is formed from a polycarpellary, syncarpous gynaecium. Note the **pores** at the top, through which the seeds escape.

* The common field poppy is *P. rhoeas*. The Opium poppy, from the latex of which, obtained by incising the unripe capsules, opium is extracted, is *P. somniferum*. Opium contains the alkaloids papaverine, morphine and codeine.

(ii) *Examine the capsule of the chickweed (Stellaria media).*

It dehisces about half way down into **teeth** (twice the number of carpels).

(2) **Legume**

Examine the pod of the broad bean, pea or lupin (Lupinus).

It is formed from a monocarpellary gynaecium, and it dehisces along *both* edges or **sutures**. (Characteristic of *Leguminosae*.)

(3) **Follicle**

Examine the group of follicles of monkshood (Aconitum) or larkspur (Delphinium).

(These are **aggregate fruits**—single follicles are very rare.) It is formed from a monocarpellary gynaecium, and dehisces along *one* suture (the **ventral suture**) only.

(4) **Siliqua**

Examine the fruit of the wallflower (Cheiranthus).

It is developed from a bicarpellary gynaecium. The seeds are exposed on the **false septum**, a partition which stretches across a framework, the **replum**, when the fruit dehisces by the separation of the two **valves**.

(5) **Silicula**

Examine the fruit of Shepherd's Purse (Capsella) or Honesty (Lunaria).

This is really a shortened flattened siliqua.

(3) **Schizocarpic Fruits**

These are fruits which split into (usually) one-seeded, indehiscent, units called **mericarps**.

(1) **Lomentum**

Examine the fruit of the radish (Raphanus).

It splits transversely.

(2) **Double Samara**

Examine the fruit of the sycamore or maple.

The two samaras are fused.

(3) **Regma**

Examine the fruit of the geranium (Pelargonium).

The units are dehiscent and are called **cocci**. Each is one-seeded.

(4) **Cremocarp**

Examine the fruit of carrot (Daucus carota) or parsley (Pastinaca sativa).

It splits longitudinally into two hanging mericarps.

(5) Carcerulus

*Examine the fruit of white deadnettle (*Lamium album*).*

The two carpels develop a false septum forming four mericarps.

(b) SUCCULENT**(1) Drupe**

*Cut a longitudinal section of a cherry (*Prunus cerasus*) or plum (*Prunus domestica*).*

Note the skin or **epicarp**, the fleshy **mesocarp** and the stony **endocarp** (stone) which together constitute the **pericarp**. The seed (kernel) is inside the endocarp.

(2) Berry

*Cut a longitudinal and a transverse section of a gooseberry (*Ribes grossularia*), grape or tomato (*Solanum lycopersicum*).*

Note that the pericarp consists of a skin, the **epicarp**, and that both the **mesocarp** and the **endocarp** are fleshy. The seeds are embedded in this.

(3) Pome (Pseudo-Carp)

*Cut longitudinal and transverse sections of the false fruit of the apple (*Pyrus malus*) or pear (*Pyrus communis*).*

Note the leathery core or **pericarp**, which *cannot* be differentiated into epicarp, mesocarp and endocarp, in which are enclosed the seeds and which is surrounded by the **swollen fleshy receptacle**, the skin of which is the **epidermis**. For this reason the pome is a *pseudocarp* (false fruit).

AGGREGATE FRUITS

(Developed from a single flower with an apocarpous gynaecium)

These are collections or **etaerios** of simple fruitlets.

(1) Etaerio of Achenes

(i) *Examine the fruit of the buttercup (*Ranunculus*) or Wood Avens (*Geum*).* Note the collection of **achenes**. This is a dry fruit.

(ii) *Examine the strawberry (*Fragaria vesca*).* The **achenes** are pressed into the surface of a swollen, **fleshy receptacle**. This is, of course, a succulent fruit and is a *pseudocarp*.

(2) Etaerio of Drupes

*Examine the fruit of the blackberry (*Rubus fruticosus*) or raspberry (*Rubus idaeus*).* Each fruitlet is a tiny **drupe** or **drupel** and is therefore succulent.

(3) Etaerio of Follicles

This has already been seen in studying simple fruits.

COMPOSITE FRUITS

(Developed from an inflorescence)

There are few of these. *Fig, hop, pineapple and mulberry* are common examples.

THE SEED AND SEEDLING
DICOTYLEDONOUS SEEDS

Hypogeal**Non-Endospermic****Broad Bean (*Vicia faba*)**

(1) *Examine dry seeds and seeds which have been soaked in water for a day.*

Note the **testa** or seed coat, the **hilum**, a brown or black scar at one end, and the **micropyle**, a small pore at one end of the hilum. (*If the soaked seed is squeezed, water will ooze out of the micropyle, and if the dry seed is put into hot water, bubbles of air come out of it.*)

(2) *Remove the testa from a soaked seed.*

Note the two large white lobes, **cotyledons**, and the pointed **radicle** (embryonic primary root), protruding between the cotyledons, which fits into a small "pocket" in the testa just below the micropyle.

(3) *Separate the cotyledons by opening them out.*

Between the **cotyledons** is the **plumule** (embryonic shoot) which is continuous with the **radicle**. The part where the plumule and radicle join is the **hypocotyl**. These constitute the **embryo**.

Scarlet Runner (*Phaseolus multiflorus*)

Examine and compare with the Broad Bean seed.

Note the conspicuous micropyle.

Epigeal**(a) Non-Endospermic****Sunflower (*Helianthus*)**

This is really a one-seeded fruit (achene).

(1) *Examine externally.*

Note the **pericarp** and the point of attachment to the plant.

(2) *Remove the pericarp.*

Note the thin **testa**, easily peeled off the **embryo**, which consists of two flat oval **cotyledons**, the **radicle** and small **plumule** with an **hypocotyl** between at the pointed end of the seed.

(3) *Cut longitudinal sections and treat separately with Schultze's solution and Sudan III to ascertain the location of protein, starch and oil* (See p. 18).

Note the parts as in (2).

French Bean (*Phaseolus vulgaris*)

Examine and compare with the sunflower, scarlet runner and broad bean seeds. Note the conspicuous micropyle.

Vegetable Marrow (*Cucurbita*)

Examine and note the two cotyledons and the point of attachment to the plant.

(b) Endospermic**Castor Oil** (*Ricinus*)

(1) *Examine externally* and note the mottled **testa** bearing a small swelling, the **aril** (or **caruncle**) at one end.

(2) *Remove the testa and cut longitudinal sections and treat with Sudan III.*

Note the **embryo**, lying in a cavity in the centre and composed of two thin **cotyledons**, a small **plumule** between their bases and a **radicle** below this. You will also see the oily **endosperm**, almost completely surrounding the embryo, stained red by Sudan III.

(3) *Remove the testa and cut a transverse section.*

Note the **endosperm** and **cotyledons**.

MONOCOTYLEDONOUS SEEDS**Maize** (*Zea mais*)

This is really a one-seeded fruit (caryopsis).

(1) *Examine a maize grain externally.*

Note the **pericarp** to which the testa is inseparably fused and a light oval area on one side which marks the position of the embryo. Note also the point of attachment to plant.

(2) *Remove the coat (pericarp and testa).*

Note the **embryo** consisting of **plumule**, **radicle** and one **cotyledon**.

(3) *Cut a median longitudinal section at right angles to the broad surface.*

Note the **embryo**, *i.e.*, **plumule**, **radicle** and the shield-shaped **cotyledon** (or **scutellum**) above: also the large white **endosperm** to one side of the embryo.

Treat the section with Schultze's solution to ascertain the location of starch and protein in the grain.

Wheat (*Triticum*)

Examine this grain as for the maize grain and compare the two.

Onion (*Allium*)

(1) *Examine a soaked seed externally.*

Note the point of attachment to the plant.

(2) *Cut a longitudinal section.*

Note the curved **embryo**, *i.e.*, the **radicle** towards the pointed end, one **cotyledon** at the other end and the small **plumule** embedded in the cotyledon.

A PARASITIC ANGIOSPERM

DODDER

Cuscuta europoea

This is a flowering plant which is entirely parasitic and is therefore an *obligatory parasite*. There is a large number of species which attack different hosts, such as nettles, gorse, hops, etc. It belongs to the Family *Convolvulaceae*.

(1) *Examine a specimen of dodder (*C. europoea*) growing on a host.*

The yellowish twining **stem of the dodder** grows anti-clockwise round the **stem of the host** and the leaves are reduced to mere **scales** and are entirely devoid of chlorophyll. At intervals the stem is attached to that of the host by **haustoria**. In mid to late Summer spikes of pink **flowers** develop.

(2) *Examine a prepared slide of a T.S. stem of a plant with a dodder stem attached by haustoria.*

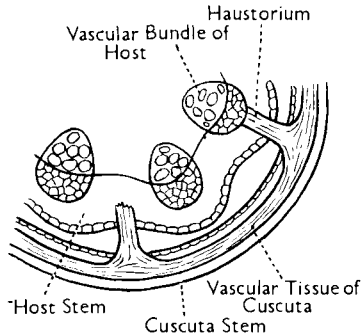


FIG. 80. *Cuscuta*. T.S. Stem of Infected Host.

The **haustoria** will be seen as structures growing from the stem of the parasite into that of the host. They penetrate into the stem and join up with the **vascular bundles of the host**, the xylem and phloem of the parasite fusing with the xylem and phloem of the host.

CLASSIFICATION OF ANGIOSPERMS

The **Classes** Monocotyledones and Dicotyledones are divided into groups called **Cohorts**, each composed of **Natural Orders** or **Families** of plants closely related and in many respects of similar structure. The different kinds of plants in the same family are classified in **Genera** and the varieties of plants of the same genus are classified in **Species**.

In order to identify and classify plants it is essential to use a good *Flora*, such as **Flora of the British Isles* by Clapham, Tutin and Warburg, *Concise Flora of Britain* by Makin, *Shorter British Flora* by Prime and Deacock or *Handbook of British Flora* by Bentham and Hooker (in two volumes, Vol. 2 consisting of illustrations). Some differences in nomenclature occur in these different *Flora*. *Drawings of British Plants* by S. Ross-Craig will also be found extremely helpful.

It is, of course, impossible to give details of the various Families here.

Specimens of the different Families will be available at different times of the year and a separate note-book or part of the file should be kept for these. It should be divided up, allotting sufficient space for several members of each Family. The specimens can then be drawn in the appropriate places as the material is obtained.

Under each Family the following should be entered up in the note-book:

- (1) Genus and Species.
- (2) A brief description of the plant (in note form).
- (3) Drawings of (i) the plant itself showing the inflorescence.
 (ii) the fruit (if available).
 (iii) the flower—(a) entire;
 (b) a half-flower; or
 a longitudinal section;
 (c) a member of each whorl
 (where desirable).
- (4) A floral diagram.
- (5) The floral formula.

* These are the *Flora* recognised by the Examining Bodies for "A" Level, G.C.E.

PART III

ELEMENTARY BIOCHEMISTRY

INTRODUCTORY NOTES

(1) The objects of the following series of experiments are (i) to give the student an insight into the nature of the chemical substances and reactions met with in the study of botany, (ii) to enable him to recognise these substances by simple tests and (iii) to help him to understand the nature of the various physiological processes met with in the study of the organism, experiments on which are given in Part IV.

(2) The student should work systematically through this part of the book, performing the experiments on crystalloids and colloids and on the chemical properties of organic substances and their detection in Plant tissues early in his studies. The experiments in small type may be omitted if desired or if time does not permit all the experiments to be performed. Records can be kept in the book or part of the file to be used for Physiology.

(3) The results of some experiments have been included to enable the student to check his own observations.

(4) In all cases, use *small* quantities of the substances to be tested and add *small* quantities of reagents unless otherwise stated.

Reference should be made to the General Directions for Practical Work in the Introduction.

BIOCHEMISTRY

Biochemistry is, as the term implies, the chemistry of the living organism. It includes not only the structure and properties of the substances which constitute the organism and which the organism itself produces, but also all the chemical processes, both anabolic and katabolic, which occur in its physiology.

The most important substances which enter into the composition and metabolism of the living organism may be summarised as follows:—

- (1) Inorganic compounds.
- (2) Proteins.
- (3) Carbohydrates.
- (4) Lipides.
- (5) Vitamins.
- (6) Enzymes.
- (7) Hormones.
- (8) Other organic substances such as Pigments, Excretory substances, etc.

I. THE PHYSICAL PROPERTIES OF ORGANIC COMPOUNDS

Substances which dissolve in liquids forming true solutions are called **crystalloids**. Substances which form with liquids a heterogeneous system intermediate between a true (molecular) solution and a suspension are called **colloids**.* A colloid **sol** thus consists of two parts: (i) the **disperse phase** composed of aggregates of molecules in constant motion (**Brownian Movement**) distributed in (ii) the **continuous phase** (or **dispersion medium**) which is, of course, liquid. If, on the addition of an electrolyte, the particles of the disperse phase coalesce or **coagulate** and are precipitated, the colloid is said to be **lyophobic**. (It is also called a **suspensoid**.) Colloids which are not precipitated in this way are said to be **lyophilic**. (They are also known as **emulsoids**.)

Water and solvents diffuse through semi-permeable membranes, the process being known as **osmosis**, and colloids are incapable of doing so. Crystalloids and colloids can therefore be separated by application of this property in the process of **dialysis**. Membranes which allow both the solute and solvent to pass through are said to be **permeable**. But those which allow the passage of the molecules of the solvent but not those of the solute are referred to as **semi-permeable**.

If a beam of light from a lantern is passed through a true solution and viewed from a position at right angles to the direction of the beam, the path of the rays cannot be traced through the solution, which appears clear. When the true solution is replaced by a colloid sol, however, the path of the beam becomes visible through the high power of a microscope owing to the scattering of the light by the particles. This is known as the **Tyndall effect** and is the principle of the **ultra-microscope**.

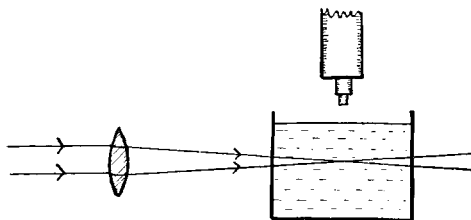


FIG. 81. **The Tyndall Effect.** Principle of the Ultra-microscope.

* The size of colloidal particles varies between $1\text{ m}\mu$ (millimicron) and 1μ (micron) ($\mu = 0.001\text{ mm}$. $\text{m}\mu = 0.000001\text{ mm}$ and $\mu\mu = \text{micromicron} = 0.00000001\text{ mm}$).

It should be noted that colloidal solutions of many substances such as gold and graphite can be prepared. It is therefore more accurate to speak of a substance as being in the colloid state.

CRYSTALLOIDS AND COLLOIDS

EXPERIMENT 1. *Cover some crystals of potassium dichromate with a small quantity of hot water in a test-tube and shake. Examine a drop of the solution under a microscope. It is clear and no crystals are visible. Allow the solution to cool. Crystals of the solute reappear. Potassium dichromate is a **crystalloid** and it forms a true **solution** with water. There is no expansion on solution.*

EXPERIMENT 2. *Add a little hot water to a small piece of gelatine in a test tube. The gelatine swells and disappears owing to the absorption of water, forming a **colloid sol**. Allow the sol to cool. It sets into a solid jelly. This is a **colloid gel**. Contraction takes place on solidifying owing to loss of water. Gelatine is a **reversible gel**.*

BROWNIAN MOVEMENT

EXPERIMENT 3. *To a drop of Indian ink in a test-tube, add water until you can see through the mixture: alternatively make a gamboge sol by rubbing a little gamboge under cold water. Filter. Place a drop of the liquid on a cavity slide and cover with a coverslip. Examine under the high power of the microscope. Fine particles can be seen: it is a **coarse suspensoid sol**. The particles are in constant motion bombarding one another and this is known as **Brownian Movement**.*

EXPERIMENT 4. *Examine a drop of aqueous Congo red sol under the microscope. It is clear, no particles being visible, but it is a **fine suspensoid sol**, the particles being ultra-microscopic.*

EXPERIMENT 5. *Examine (i) potassium dichromate solution, (ii) Indian ink sol, (iii) Congo red sol, illuminated by a Tyndall's Beam and compare the effects in the three cases.*

COAGULATION

EXPERIMENT 6. *Shake up some dried albumen with water. It forms an opalescent sol. Divide the sol into three parts. To (i) apply heat. To (ii) add a few drops of dilute acetic acid and then heat. Compare with (i). To (iii) add a saturated solution of ammonium sulphate.*

EXPERIMENT 7. *Make some starch sol by stirring some powdered starch with a little cold water and then adding boiling water and shaking. Divide into two parts. Allow (i) to cool. To (ii) add basic lead acetate solution.*

OSMOSIS

EXPERIMENT 8. *Cover the mouth of a thistle-funnel with a piece of parchment* or pig's bladder (a semi-permeable membrane) and tie it on securely round the rim. Carefully pour some sugar solution (which may*

* This is not a perfect semi-permeable membrane for sugar and water as it allows a small quantity of sugar to pass through it.

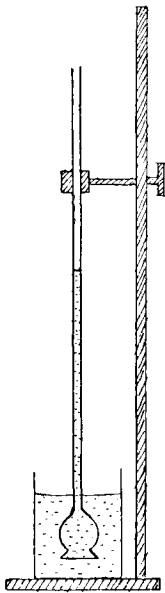


FIG. 82. Apparatus to demonstrate Osmosis.

be coloured) into the tube of the thistle-funnel until the liquid has risen a short distance up the tube. Clamp the tube in a retort stand with the mouth of the funnel in a dish of water. After a few minutes, mark the level of the liquid in the tube with gum-paper. Examine an hour or two later.

EXPERIMENT 9. Cut off the top and bottom of a potato tuber, leaving the skin on the sides, and scoop out a cavity at one end. Pour some sugar solution into the cavity and stand the tuber in a dish of water. Examine later and draw your conclusions. This demonstrates osmosis in living cells. Repeat the experiment with a tuber which has been boiled for a few minutes to kill the cells, and compare results.

DIALYSIS

EXPERIMENT 10. Put a mixture of sodium chloride solution and starch sol into a dialysing cylinder and float this in a large quantity of distilled water. Allow it to stand for several hours with frequent changes of water. Periodically test the water for salt by adding silver nitrate solution and for starch by adding iodine solution.

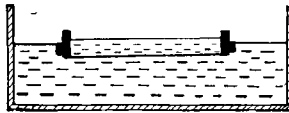


FIG. 83. Dialyser.

II. THE CHEMICAL PROPERTIES OF BIOCHEMICAL COMPOUNDS

To ascertain the Elements present in Organic Substances

The following experiments should be performed with proteins, carbohydrates and fats:—

Carbon and Hydrogen

EXPERIMENT 11. Mix about 0.5 gm. of the substance with 5 or 6 times its weight of cupric oxide previously heated in a crucible and allowed to cool in a desiccator. Place the mixture in a hard-glass test tube fitted with a delivery tube leading to a test tube of lime water. Heat the mixture. The evolution of carbon dioxide indicates the presence of **carbon**. If moisture appears on the cool upper part of the hard glass tube, test it with anhydrous copper sulphate. Water indicates the presence of **hydrogen**. (It is, of course, unnecessary to test for carbon if the substance is known to be organic.)

Nitrogen

EXPERIMENT 12. (Lassaigne Test.) Put about 0.5 gm. of the substance in a bulb ignition tube or small hard glass test tube. Add two small pellets of sodium. Heat in the bunsen flame for a few minutes, gently at first and afterwards more strongly. Then immediately plunge the tube while hot into distilled water in a mortar, grind it up and filter.* If nitrogen is present, sodium cyanide (NaCN) will have been formed. Test a portion of the filtrate as follows:—Add some freshly prepared ferrous sulphate solution and boil. (Sodium ferrocyanide is thus formed.) Acidify with conc. hydrochloric acid and add two or three drops of ferric chloride. A deep blue coloration or precipitate indicates the presence of **nitrogen**.

EXPERIMENT 13. Mix equal quantities of the substance to be tested and soda lime and heat in a hard glass test tube. Ammonia, recognised by its smell and by its turning red litmus paper blue, shows the presence of **nitrogen**. A negative result, however, is not conclusive.

Sulphur

EXPERIMENT 14. To a portion of the filtrate from Experiment 12, add some freshly prepared sodium nitroprusside. A violet colour indicates the presence of **sulphur**.

EXPERIMENT 15. To another portion of the filtrate from Experiment 12 add a few drops of acetic acid and then lead acetate solution. A black precipitate indicates **sulphur**.

Phosphorus

EXPERIMENT 16. Heat a portion of the filtrate from Experiment 12 with nitric acid in excess. Then add ammonium molybdate. A yellow precipitate indicates **phosphorus**.

Halogens

EXPERIMENT 17. Test portions of the filtrate from Experiment 12 as follows:—Acidify with nitric acid. Boil for a few minutes to decompose any cyanide present then add silver nitrate solution. A white precipitate indicates **chlorine**, a yellow precipitate indicates **bromine** or **iodine** (or both). To distinguish between bromine and iodine, add chlorine water to the acidified solution above. Bromine and iodine will be liberated and will colour the liquid brown. Add carbon disulphide or chloroform to this and the **bromine** will dissolve to give a brownish-red solution whereas **iodine** will give a violet solution, but if excess of chlorine water is added, the violet colour is destroyed.

Oxygen

There is no conclusive test of universal application for oxygen but if water is liberated when the dry substance is heated alone, it must obviously contain oxygen.

PROTEINS

As will have been seen in the previous experiments if they have been performed, proteins are compounds of carbon, oxygen, hydrogen and nitrogen. Most of them also contain sulphur and some contain phosphorus. Their molecules are of colloidal proportions and all have very complex molecules and are of high molecular weight. The protein molecule is composed of a combination of

* Retain some of this filtrate to test for sulphur, phosphorus and halogens.

amino-acids* by condensation. **Proteoses, peptones, peptides** and **amino-acids** are derivatives of proteins, formed in their synthesis and hydrolysis.

There are certain colour reactions given by proteins and these depend on the amino-acids present in the protein molecules. All give the "biuret" reaction (Experiment 18 below). This is due to the NH-CO group. Those containing tyrosine give the Xanthoproteic and Millon's reactions, those containing tryptophane give the test of that name and those containing cystine give the cystine (sulphur) test.

In the following tests, dried albumin can be used. *Prepare a solution of ovalbumin by shaking up a small quantity of the white powder with water.*

EXPERIMENT 18. "Biuret" Test: (a) *Add excess of sodium hydroxide solution and then a few drops of weak (1%) copper sulphate solution. A violet colour is produced. (A similar reaction is given by biuret, the substance obtained by heating urea: hence the name of this test, but proteins do not contain biuret.)* (b) *Perform the "biuret" test with an aqueous solution of peptone. A rose pink colour is obtained.*

EXPERIMENT 19. Xanthoproteic Test: *Add concentrated nitric acid. A white precipitate is obtained. Heat. It turns yellow. Cool under the tap and add excess of .88 ammonium hydroxide. It turns orange.*

EXPERIMENT 20. Millon's Test: *Add a few drops of Millon's reagent (this is a mixture of mercurous and mercuric nitrate in nitric acid). A white precipitate is obtained. Boil. It turns red.*

EXPERIMENT 21. Tryptophane Test: *Add excess of Glacial acetic acid which has been exposed to light (and therefore contains glyoxylic acid, on which the reaction depends). Then, by means of a thistle funnel or by sloping the tube, carefully add some concentrated sulphuric acid. A purple ring forms at the interface.*

EXPERIMENT 22. Arginine Test: *Add a few drops of sodium hydroxide followed by a few drops of alcoholic α -naphthol and then a few drops of sodium hypochlorite. A red colour is produced.*

EXPERIMENT 23. Cystine Test: *Add a few drops of lead acetate solution. A precipitate is obtained. Add sufficient sodium hydroxide solution to dissolve this and then boil. The solution turns dark brown (due to the formation of lead sulphide).*

* An amino-acid is an organic acid in which a hydrogen atom attached to the carbon atom is replaced by an amino group ($-\text{NH}_2$). Amino-acetic acid or glycine ($\text{CH}_2\text{NH}_2\text{COOH}$) is the simplest. Amino-propionic acid or alanine is $\text{CH}_3\text{CH.NH}_2\text{COOH}$. Tyrosine and cystine are further examples.

EXPERIMENT 24. **Molisch Test:** *Add a few drops of alcoholic α -naphthol and shake. Then carefully pour in a little concentrated sulphuric acid through a thistle funnel or by sloping the tube. A violet ring forms at the interface. This reaction is due to the presence of a carbohydrate group in the protein molecule and is really a carbohydrate test.*

CARBOHYDRATES

It will already have been seen that carbohydrates are compounds of carbon, hydrogen and oxygen. The hydrogen and oxygen are in the same proportion as in water and the general formula is $C_m(H_2O)_n$. They are classified as follows:—

- (1) **Monosaccharides:** Simple sugars of general formula $C_nH_{2n}O_n$.
- (2) **Disaccharides:** Sugars formed by the condensation of two hexose (monosaccharide) groups and therefore of formula $C_{12}H_{22}O_{11}$.
 $2C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}$.
- (3) **Polysaccharides:** Non-sugars of general formula $(C_6H_{10}O_5)_n$.

General Tests for Carbohydrates

All carbohydrates on boiling with concentrated hydrochloric acid yield substances called **furfurals** which can be identified by colour reactions such as by the addition of alcoholic thymol or α -naphthol.

EXPERIMENT 25. **α -Naphthol (Molisch) Test:** *Add a few drops of an alcoholic solution of α -naphthol to a solution of a carbohydrate. Then, by means of a thistle funnel or by sloping the tube, carefully pour in a little conc. sulphuric acid. A violet colour is formed at the junction of the liquids.*

EXPERIMENT 26. **Thymol Test:** *To a small quantity of a solution of a carbohydrate (or to a solid insoluble one) add a few drops of alcoholic thymol and excess of concn. hydrochloric acid. Boil for a couple of minutes, shaking periodically. A carmine colour is produced.*

Monosaccharides (Hexoses)

These are sugars with six carbon atoms in the molecule— $C_6H_{12}O_6$.

Glucose

EXPERIMENT 27. **Fehling's Test:** *Mix equal quantities of Fehling's solution A and B (see Appendix I) and add to some glucose solution. Boil. Note the red precipitate of cuprous oxide.**

EXPERIMENT 28. **Trommer's Test:** *Add a drop or two of 5 per cent. copper sulphate solution and a small quantity of caustic soda. The copper hydroxide formed redissolves. Boil. Note the red precipitate of cuprous oxide.*

* The reducing property of this and other sugars is due to the presence of an aldehyde ($-CHO$) radicle in the molecule.

EXPERIMENT 29. Barfoed's Test: *Add about 1 ml. of Barfoed's solution and boil.* A red precipitate of cuprous oxide is formed.

EXPERIMENT 30. Moore's Test: *Add a small quantity of caustic soda solution and boil.* A yellow and then a brown colour is produced due to the formation of a resin.

EXPERIMENT 31. *Boil some glucose solution with an equal quantity of conc. hydrochloric acid.* A black precipitate is obtained.

EXPERIMENT 32. Rapid Furfural Test: *Add a few drops of alcoholic α -naphthol and then a few millilitres of conc. hydrochloric acid. Boil.* Observe that a violet colour is produced only after prolonged boiling.

Fructose

EXPERIMENT 33. *Perform (a) Fehling's and (b) Barfoed's Tests with a solution of fructose.*

EXPERIMENT 34. *Boil with conc. hydrochloric acid.* A brownish-red colour is obtained. Compare with the result of this test with glucose.

EXPERIMENT 35. *Perform the Rapid Furfural Test (Expt. 32).* Note that a violet colour is produced immediately.

EXPERIMENT 36. Diphenylamine Test. *To a few millilitres of fructose solution add half the volume of conc. hydrochloric acid and a few drops of an alcoholic solution of diphenylamine. Boil for one or two minutes (no longer).* A deep blue colour develops.

Disaccharides*

Maltose

EXPERIMENT 37. *Carry out (a) Fehling's and (b) Barfoed's Tests with a solution of maltose.*

Sucrose

EXPERIMENT 38. *Perform (a) Fehling's and (b) Barfoed's Tests with a solution of sucrose.*

EXPERIMENT 39. *Hydrolyse some sucrose by boiling a solution with dilute hydrochloric or sulphuric acid for a few minutes. Then neutralise by adding some caustic soda solution and perform Fehling's Test.*

EXPERIMENT 40. *Boil some sucrose solution with conc. hydrochloric acid.* A red colour is obtained. Compare with the result of this test those with glucose and fructose.

EXPERIMENT 41. *Perform the Rapid Furfural Test with sucrose.* Observe the immediate formation of a violet colour.

Polysaccharides*

Starch (Amylum)

Perform the following tests with starch sol, prepared by making a paste of starch with cold water and then adding boiling water.

* Negative results will be obtained in some of the following experiments.

EXPERIMENT 42. *Add a few drops of dilute iodine solution and observe the effect. Heat and note the result. Cool under the tap and again note the result.*

EXPERIMENT 43. *Perform Fehling's Test.*

EXPERIMENT 44. (a) *Hydrolyse some starch by boiling with dilute sulphuric acid for a few minutes: neutralise by adding caustic soda solution (test with litmus paper).* (b) *Hydrolyse a separate portion of starch by adding a little of the enzyme diastase. Then divide each hydrolysed solution into two portions and to one add iodine solution and with the other perform Fehling's Test.*

Glycogen (Animal Starch)*

Make an aqueous solution of glycogen and perform the following tests:—

EXPERIMENT 45. *Add iodine solution. Observe the colour change.*

EXPERIMENT 46. *Perform Fehling's Test.*

EXPERIMENT 47. *Hydrolyse some glycogen by boiling for a few minutes with dilute hydrochloric or sulphuric acid. Neutralise by adding caustic soda (test with litmus paper) and then carry out Fehling's Test.*

Dextrin

EXPERIMENT 48. *Make an aqueous solution of α -dextrin. Add iodine solution and observe the result. Heat and note the effect. Allow to cool and again note what happens. Repeat the experiment with β -dextrine.*

Inulin (Dahlia Starch)

EXPERIMENT 49. *To an aqueous solution of inulin, add some iodine solution.*

EXPERIMENT 50. *Add a few drops of a saturated alcoholic solution of orcein followed by concentrated hydrochloric acid and then boil.*

EXPERIMENT 51. *Cut sections of a root tuber of Dahlia or of Dandelion root into 90 per cent. alcohol and leave for at least an hour. Then mount in dilute glycerine and examine under the microscope. Inulin which is insoluble in alcohol, separates out in spherical crystals.*

Cellulose

EXPERIMENT 52. *Put a few small pieces of filter-paper (almost pure cellulose) into Schweitzer's Reagent (Cuprammonia), prepared as follows:—Add sufficient ammonium hydroxide to a solution of copper sulphate to dissolve the precipitate of copper hydroxide formed at first.*

Leave the cellulose in the reagent for a while and note the effect.

* Occurs in some Fungi.

EXPERIMENT 53. Put some cotton-wool in strong iodine solution and after a few minutes' immersion, examine under the microscope. Then mount in 50 per cent. sulphuric acid and examine again.

EXPERIMENT 54. Mount some cotton-wool in Schultze's solution and note the effect on the cell walls.

Lignin

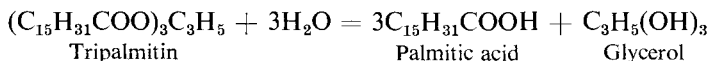
EXPERIMENT 55. Cut sections of a woody stem. Test and examine for lignin by immersion in (a) phloroglucin acidified with hydrochloric acid, (b) aniline sulphate (or hydrochloride). The wood (lignin) turns red in (a) and bright yellow in (b).

LIPIDES

Lipides are esters of higher members of a series of organic acids known as the Fatty Acids. They are compounds of carbon, hydrogen and oxygen but with a lower oxygen content than the carbohydrates. They are classified as follows:—

- (1) Simple lipides—fats, oils and waxes.
- (2) Complex lipides.
- (3) Lipide derivatives.

Fats and oils are esters of the polyhydric alcohol *glycerol*, $C_3H_5(OH)_3$ with higher fatty acids such as *stearic*, $C_{17}H_{35}COOH$ and *palmitic*, $C_{15}H_{31}COOH$, and with the unsaturated monobasic acid, *oleic* $C_{17}H_{33}COOH$. A fat differs from an oil in being solid whereas the latter is liquid at $20^\circ C$. On hydrolysis, a fat decomposes into glycerol and the fatty acid thus:—



The so-called "essential", "ethereal" or "volatile" oils such as oil of turpentine, oil of cloves, oil of lavender are not lipides though some of their reactions are similar.

Fats and Oils

EXPERIMENT 56. (a) Add some water to some olive oil in a test tube and shake. The emulsion formed is only temporary. (b) Add some caustic soda solution and shake again. The emulsion lasts longer but eventually the oil and water separate out.

EXPERIMENT 57. Add some ether to some olive oil in a test tube and shake. Note what happens. Then pour some of this liquid on to a piece of filter paper and examine again when the ether has evaporated.

EXPERIMENT 58. Add two drops of 1 per cent. solution of osmium tetroxide ("Osmic Acid") to a few drops of olive oil in a watch glass and observe the result.

EXPERIMENT 59. Saponification. (i) *Prepare an emulsion of olive oil in a boiling tube as in Experiment 58 (b). Place the tube in a beaker of boiling water and leave it there for about half an hour, shaking the tube periodically. The emulsion separates out into an upper layer of oil and a lower layer of caustic soda, but saponification takes place at the interface and the glycerol formed passes into the lower layer.*

(ii) *Pour off the liquid, carefully pouring away the unchanged oil, retaining the soap, and perform the following test for glycerol with (a) glycerol from the bottle and (b) the residual liquid:—add some solid sodium bisulphate and heat. Note the acrid smell of acrolein.*

(iii) *Now remove excess alkali from the small piece of soap left in the original test tube, by washing with water. Then dissolve the soap by boiling it in water. Divide the solution into three portions, (a), (b) and (c), and test for soap as follows:—*

To (a) add calcium chloride solution; (b) add lead acetate solution, and to (c) add a small quantity of solid sodium chloride. Repeat this with soap solution and compare results.

EXPERIMENT 60. Repeat Experiments 57 and 58 with some oil of turpentine, oil of cloves or other volatile oil.

TO TEST FOR THE PRESENCE OF PROTEINS, CARBOHYDRATES AND LIPIDES IN TISSUES AND FOOD-STUFFS

EXPERIMENT 61. Tests for proteins, carbohydrates and lipides should now be performed with various foodstuffs, seeds, fruits, underground stems, swollen tap-roots and leaves, and also with suitable solids or solutions provided by the laboratory. The following scheme is suggested as suitable for quick detection of the compounds present but any other convenient method can be adopted.

ANALYTICAL TABLE

To Test a Substance for Proteins, Carbohydrates and Fats.

TEST FOR PROTEIN 1. Add sodium hydroxide and a few drops of 1 per cent. copper sulphate (Biuret Test).	VIOLET COLOUR = Protein.
2. If Protein present confirm as follows:— Add Millon's Reagent.	WHITE PRECIPITATE. Heat IT TURNS RED.
TEST FOR CARBOHYDRATE 1. Add a few drops of α -naphthol. Then slowly add conc. sulphuric acid down sides of the test tube. (Molisch Test.)	VIOLET RING = Carbohydrate.
2. If Carbohydrate present. (i) Test for Monosaccharide* Add Barfoed's Reagent and heat.	RED PRECIPITATE = Monosaccharide. NO PRECIPITATE = Disaccharide or Polysaccharide.
(ii) (a) If Monosaccharide present. Confirm by adding Fehling's solution. Heat.	RED PRECIPITATE = Glucose or Fructose.
(b) If Monosaccharide absent. Test for Disaccharide. Perform Fehling's Test.	RED PRECIPITATE = Maltose.† NO PRECIPITATE = Sucrose or Polysaccharide.
(c) Test for Sucrose Add conc. hydrochloric acid and heat. Confirm by boiling with dilute sulphuric acid for a few minutes. Neutralise with sodium hydroxide (test with litmus paper) and then perform Fehling's Test.	IMMEDIATE VIOLET COLOUR = Sucrose. RED COLOUR = Sucrose (now hydrolysed to monosaccharide).
(iii) (a) Test for Polysaccharide Add iodine solution. (b) If inulin suspected. Add a few drops of Alcoholic Orcein and conc. hydrochloric acid. Boil.	BLUE COLOUR = Starch. RED COLOUR = Glycogen. NO CHANGE = Inulin.* RED COLOUR = Inulin.
TEST FOR FAT OR OIL (i) Add. 1 per cent. Osmium tetroxide ("osmic acid").	BLACK COLOUR after a few minutes = Fat.
(ii) If fat or oil present Add ether and shake. Pour solution on filter paper.	PERMANENT STAIN = Fatty oil. STAIN DISAPPEARS = Volatile oil.

* It should be remembered that both monosaccharide and disaccharide and/or polysaccharide may be present. If so, some confusion may result but subsequent tests should obviate this to some extent.

† Both lactose and maltose may be present but this is unlikely in simple examination tests as there is no simple specific test for maltose. If glucose is also present it will, of course, give a ppt. here, but it will have been identified already.

MICROCHEMICAL TESTS

EXPERIMENT 62. *Cut sections of various plant organs such as seeds and fruits and (a) perform the tests for proteins, sugars, starches and oils in test tubes or watch glasses or on microscopical slides: then examine the sections under the microscope. (b) Mount the sections in Schultze's solution. Proteins are turned yellow, starch blue, and cellulose blue or purple. (c) When oil is detected or suspected mount the sections in Sudan III. The oil is stained red.*

III. VITAMINS

Vitamins are complex chemical compounds of high molecular weight which are essential to the growth and maintenance of health of the vertebrate animal, though they are required in very minute quantities only. They occur in natural foods, the original source of most being green plants. Some are specific to certain animals. They may be termed *accessory food factors*. Some are fat-soluble, others are water-soluble. They are designated by letters and, when their chemical constitution is known, by appropriate chemical names.

The classification, sources and effects of the most important vitamins may be summarised as shown in the table of vitamins on p. 132.

Other vitamins have been discovered, some specific to certain animals, but not a great deal is known about them.

The vitamin content of foods is measured in what are known as *international units*. This is a different quantity for each vitamin and depends on minimum requirements. The daily requirement of Vitamin A is about 4,000 I.U. while that of Vitamin D is about 400 I.U. Halibut oil, one of the richest sources of Vitamin A contains 5,000,000 I.U. per gram, while cod-liver oil contains only 1/50 of that quantity.

There is little elementary practical work which can be conveniently done with vitamins.

EXPERIMENT 63. **To demonstrate the presence of Vitamin A.**—*Dissolve some cod-liver oil or halibut oil in about five times its volume of chloroform, and to a drop of this add a drop or two of a saturated solution of antimony trichloride in chloroform. A bright blue colour develops.*

EXPERIMENT 64. **To demonstrate the presence of Vitamin C.**—*Add one drop of dichlorophenol indophenol to 1 ml. of lemon or other fruit juice. The pale blue solution turns pink and then fades owing to the reducing action of Vitamin C.*

TABLE OF VITAMINS

Letter	Name	Solubility	Source	Effect*	Remarks
A	Axerophthol	Fat	Liver oils of fish (e.g., cod, halibut), milk, eggs.	Promotes growth, prevents night-blindness and protects epithelia against infection.	Derivative of <i>catotene</i> (C ₄₀ H ₅₆) which is therefore known as <i>provitamin A</i> . Not destroyed by heat. Important are given
B	A Complex of several vitamins. About twelve are known. The most				
B ₁	Aneurin (Thiamin)	Water	Germ - layer of seeds, fruits and vegetables.	Anti-neuritic. Prevents beri-beri in man and polyneuritis in birds. Also concerned with release of energy from carbohydrates.	Removed by milling and therefore absent from white flour but present in whole meal flour. Absent from polished rice.
B ₂	Riboflavin	Water	Eggs, yeast, fruits, vegetables, liver, milk and cheese.	Anti-dermatitic. Necessary for cell metabolism.	
B ₃	Pantothenic Acid	Water		Necessary for growth in birds.	
B ₄		Water		Necessary for growth in rodents.	
B ₅		Water		Necessary for growth in birds.	
B ₆	Pyridoxine	Water		Prevents dermatitis.	
B ₇	Nicotinic Acid	Water		Concerned with growth and the prevention of pellagra.	
B ₁₂	Cyanocobalamin	Water		Necessary for the formation of blood corpuscles and used in the treatment of pernicious anaemia, also for growth and reproduction.	
C	Ascorbic Acid	Water	Fresh fruits and vegetables, particularly in citrus fruits and black currants.	Anti-infective. Anti-scorbutic. Deficiency causes scurvy.	Can be synthesised by mammals. Destroyed by heat and the presence of copper.
D ₂	Calciferol	Fat	Fish oils, egg yolk, butter.	Anti-rachitic, i.e., prevents rickets. Needed for bone and teeth.	Derivative of <i>ergosterol</i> (C ₂₇ H ₄₁ OH) in ergot and yeast. There are at least two other D vitamins. Can be synthesised by the animal in sunlight. Not destroyed by heat. Excess may have ill effects.
E		Fat	Green leaves, e.g., lettuce, wheat embryo, eggs, some plant and animal oils.	Necessary for fertility in women and certain lower animals, e.g., cows and rats. Deficiency causes sterility.	
H	Biotin	Water	Liver, milk, eggs, vegetables.	Prevents dermatitis.	
K	Phylloquinone	Fat	Green leaves, rose hips, strawberries, fish.	Necessary for the clotting of the blood. Deficiency in Haemophiliacs†	
P	Citrin	Water	Citrous fruits.	Necessary to enable blood capillaries to withstand pressure.	

* The symptoms of the deficiency diseases caused by lack or insufficiency of these vitamins can be found in a medical dictionary. Deficiency in the diet can be overcome by oral administration of vitamins in tablet form or by taking preparations containing them.

† As haemophilia is an inheritable disease presumably there is an inability to absorb vitamin K.

PART IV

PHYSIOLOGY

INTRODUCTORY NOTES

Experiments must be performed to investigate the physiology of the plant. There are innumerable methods of doing this, but the following experiments, most of which are best performed during the summer months, cover the chief physiological processes. It is not suggested that these experiments explain fully the details of the physiological processes. They are adequate, however, for the purpose for which they are intended.

Write the **object** of the experiment on the top of the page, then write a concise **method** or account of how the experiment was set up. Keep a record of any necessary readings and draw a *sectional diagram* of the apparatus, if any (*e.g.*, Fig. 96, p. 151). Lastly, when the experiment is finished, enter up the **result** together with any **observations** you have made and write a **conclusion**.

If an experiment has to be left for some time, carefully label it with the object (or number) of the experiment and keep a record of any other *relevant* information such as the name of the plant, date, time, temperature, barometer reading, stage of growth, etc.

Always set up a "control" experiment under opposite (or normal) conditions when practicable in order to show that the results you obtain are due to the conditions you have set up.

Reference should be made to the General Directions for Practical Work in the Introduction.

I. THE PASSAGE OF WATER THROUGH THE PLANT

(1) ABSORPTION

To demonstrate the Absorption of Water by the Roots

EXPERIMENT 1. (a) *Place a seedling with its root dipping into aqueous fuchsin solution. A few hours later, cut across the root and the stem.* Note that the red liquid has travelled up the vascular bundles.

(b) *Leave a seedling under similar conditions for a longer period.* Note that the fuchsin reaches the veins of the leaves.

To demonstrate Osmosis

EXPERIMENT 2. *To demonstrate the method by which the roots absorb water from the soil, perform the experiment to demonstrate the process of osmosis (Part III (Biochemistry) Experiments 8 and 9, p. 121 et seq.) already done.*

(2) ROOT PRESSURE

To measure Root Pressure

EXPERIMENT 1. Cut off the stem of a pot plant such as *Geranium* or *Fuchsia*, about 2 inches above the soil level. Wet the cut surface with water, then fit a piece of pressure tubing securely on the stump and fill it with water, continuing to do so until it is no longer absorbed. Make the joint water-tight by means of wire and wax mixture (see Appendix II under "Joints") or Chatterton's Compound. Then fit a root pressure manometer into the rubber tubing. If the type shown in Fig. 84B is used, pour water into the manometer tube through the side-tube (a), completely filling it. Then fit this tube with a rubber stopper, through which passes a short piece of glass tubing drawn out to a jet.

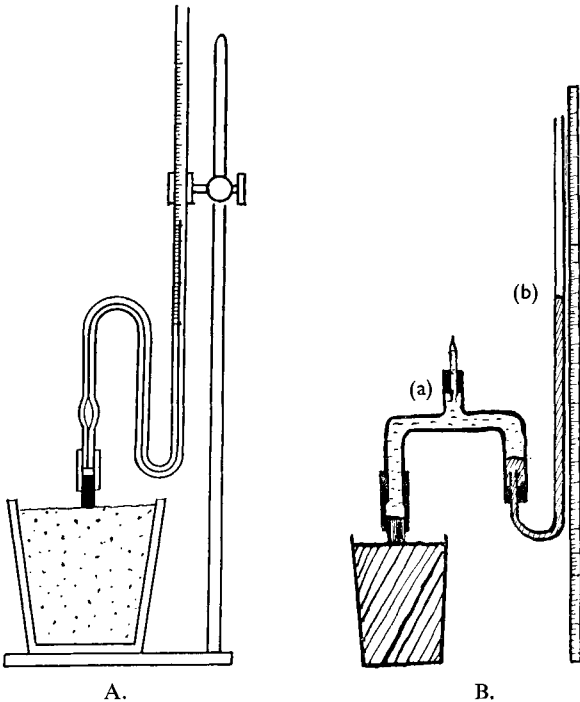


FIG. 84. Root Pressure Manometers.

This will prevent the leaving of an air bubble in the tube. Seal off the jet in the flame. Clamp the capillary tube (b) which contains mercury, and alongside it a metre scale, so that they are in a vertical position. Keep the plant well watered. Take a series of readings of the mercury

levels every few hours for a couple of days or so. In both types of manometer note the highest level reached. Root pressure = difference between mercury levels. Read the barometer and record your result in atmospheres.

(3) **TRANSPIRATION**

To demonstrate Transpiration and to measure the Rate of Transpiration

EXPERIMENT 4. Cut a slit in a piece of sheet rubber and pass it round the stem of a well-watered pot plant with well-developed leaves. Stick together the cut edges of the rubber sheet and put the pot in an aluminium container, fixing the edges of the sheet rubber under the metal band provided. Alternatively, make a sheet-rubber or cellophane bag to enclose the pot, tying it securely round the lower end of the stem. Weigh. Cover the plant with a bell jar. Weigh again later. Note the drops of water on the inside of the bell jar, formed by the condensation of the water vapour given off by the leaves. There is a loss in weight, due to the water loss by transpiration. Keep a record of the weight over several days under varying conditions of temperature, light and shade. Calculate the rate of transpiration per hour in each instance and compare results. Results can be tabulated as follows:—

External Conditions	Weight	Loss of Weight = Weight of Water Transpired	Time			Rate of Transpiration per hour
			t ₁	t ₂	Difference	

To find the Relation between Absorption and Transpiration

EXPERIMENT 5. Fit the neck of a chemical “drying-tower” with a one-holed cork which has been cut vertically in two, and the side-tube with a tube graduated in millilitres. Put a plant with a leafy shoot through the cork, fill the apparatus with water. Plug the graduated tube lightly with cotton-wool and make the joints of the cork air-tight with wax mixture or Chatterton’s Compound, to prevent evaporation. Weigh the apparatus and take the reading of the water level in the graduated tube. Repeat these measurements at intervals of several hours for about a couple of days or so. The loss of weight = the weight of water transpired, and the difference in level in the graduated tube represents the weight of water absorbed (1 ml. = 1 gram). Enter up your results as follows:—

Weight of apparatus	Reading of side-tube	Loss of weight = Weight of Water Transpired	Difference in side-tube readings \equiv Weight of Water Absorbed (1 ml. = 1 gm.).

Make a note of the atmospheric conditions and of the times at which the measurements are made. Compare the figures in the last two columns.

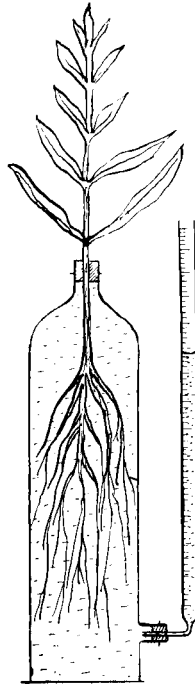


FIG. 85. Apparatus to show Relation between Absorption and Transpiration.

To Measure the Rate of Absorption and Transpiration

EXPERIMENT 6. Cut across the lower end of the stem of a leafy shoot under water and fix it through the vacant hole of a Farmer's potometer

filled with water. Make the joint air-tight with wax mixture or Chatterton's Compound if necessary. This potometer consists of a wide-necked bottle fitted with a three-holed cork. A tap-funnel passes through one hole, a piece of capillary tubing, bent for compactness, as shown in the diagram, through the second and the shoot is passed through the third. A scale is fixed alongside the capillary tube. Open the tap funnel and thus fill the capillary tube with water. Close the tap and, if not already provided, clamp a half-metre scale horizontally alongside the tube. As the leaves transpire, water will be absorbed by the cut end of the shoot and air will enter the open end of the tube. Note the position of the end of the water column on the scale and the time. When the water has travelled towards the other end of the tube, again record the position and time. If the tap is now opened, water will be forced back to the other end of the tube and the experiment can be repeated. The distance travelled by the water in the tube represents the rate of transpiration. Calculate the rate of transpiration per minute or per hour. The experiment should, if possible, be carried out (i) in sunlight, (ii) in shade, (iii) in a dry atmosphere, (iv) in a damp atmosphere, and (v) with (a) the upper surface and (b) the lower surface of the leaves covered with vaseline.

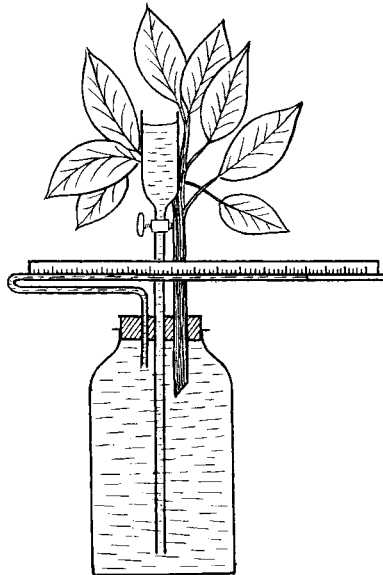


FIG. 86. Farmer's Potometer.

Enter up your results as follows:—

Atmospheric Conditions	Time			Tube Readings			Rate of Transpiration per min./hr.
	t_1	t_2	Difference	l_1	l_2	Difference	

It is assumed in this experiment that the weight of water absorbed = the weight of water transpired.

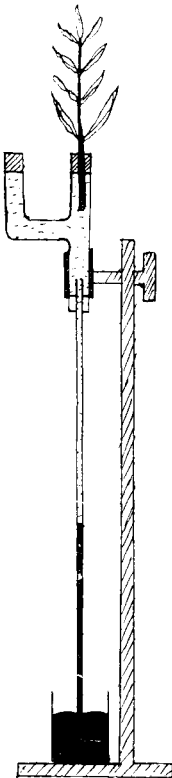


FIG. 87.
Apparatus to show Sucking Force of Transpiration.

Artificial atmospheric conditions can be produced in a bell jar through which a current of air is passed which has been previously passed through (1) calcium chloride, (2) water, (3) a heated iron tube, and (4) a freezing mixture. In these experiments the shoot should be fixed in the end of a piece of rubber tubing, the other end of which fits over a piece of glass tubing in the third hole of the cork. The bell jar can then be stood on a plate on a tripod at the side of the potometer. The tube containing the shoot of course passes through a hole in the plate, and all joints must be made air-tight with wax mixture or Chatterton's Compound.

To Compare the Extent of Transpiration from the two Surfaces of a Leaf

EXPERIMENT 7. Place a piece of dry cobalt chloride paper (prepared by soaking filter paper in a 5 per cent. aqueous solution of cobalt chloride and drying the paper, when it turns blue) on either side of a leaf still joined to the stem. Put a piece of glass outside the cobalt chloride paper on each side and clamp the sheets of glass together to protect the paper against atmospheric moisture and to support the weight. Note the change in colour of the cobalt chloride paper to pink, due to the giving off of water vapour, and compare the transpiration of the upper and lower epidermes as indicated by the extent and rate of the colour change.

(That the colour change is due to water can be shown by holding a piece of cobalt chloride paper in steam.)

To show the Sucking Force of Transpiration

EXPERIMENT 8. Cut a large leafy shoot from a plant under water and fix the stem in the upper end

of the straight limb of a Darwin's potometer tube and a piece of capillary tubing about 25 to 30 cm. in length through a cork in the lower end. Make the joints air-tight with wax mixture. Fill the apparatus with cold, previously boiled water through the side-tube, keeping the finger over the open end of the glass tubing. Then tightly cork the side-tube and test the apparatus for leakage. Stand the capillary tube in a dish of mercury and clamp it in position. Examine a few hours later. Note that the mercury is "drawn up" a considerable distance in the tube.

II. TURGIDITY AND PLASMOLYSIS

To Demonstrate Turgidity and Plasmolysis

EXPERIMENT 1. *Place a seedling on the bench.* Note that it becomes limp or flaccid, *i.e.*, it wilts. *Replace in water* and observe that it becomes rigid or **turgid** again.

EXPERIMENT 2. *Place a turgid bean seedling in 10 per cent. calcium chloride solution.* The water is drawn out of the cells of the plant due to exomosis and the contents shrink from the cell-wall. Consequently the plant wilts. This is called **plasmolysis**. *Replace the seedling in water.* It regains its turgidity and is **deplasmolysed**.

EXPERIMENT 3. *Split a turgid bean stem longitudinally into four strips.* Note that the strips bend outwards, due to release of tension in the outer cells, while the inner cells become more turgid and swell.

EXPERIMENT 4. *Cut thin sections of beetroot and examine under the microscope. Draw. Irrigate with 5 per cent. salt solution.* Observe the contraction of the coloured sap (which renders the protoplast visible) away from the cell-wall. *Draw:* this again is plasmolysis. *Now mount the section in water and observe* that the cells resume their original appearance, due to regained turgidity, *i.e.*, they are deplasmolysed.

To Ascertain the Concentration of (a) Potassium Nitrate, (b) Cane Sugar necessary to induce Plasmolysis

EXPERIMENT 5. *Prepare a molar solution (= gm.-molecular solution) of potassium nitrate by dissolving 10.1 gm. of potassium nitrate in 100 ml. of distilled water. Place the solution in a burette. Then make a 0.5 molar solution by diluting some of the original solution with an equal volume of distilled water from another burette. Similarly prepare 0.25 molar, 0.125 molar and 0.0625 molar solutions.*

Find which of these solutions causes plasmolysis in sections of beetroot by mounting sections in the solutions and examining under the microscope.

EXPERIMENT 6. Prepare a molar solution of sucrose by dissolving 34.2 gm. of sucrose in 100 ml. of distilled water. Prepare $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ and $\frac{1}{16}$ molar solutions as in Experiment 5 and find which of these solutions causes plasmolysis.

To Find the Osmotic Pressure of Cell Sap

EXPERIMENT 7. Now find the concentration of the solution of potassium nitrate or sucrose which just does not cause plasmolysis. This solution is isotonic with the cell sap, i.e., it has the same osmotic pressure. Find the temperature. Calculate the osmotic pressure of the cell sap as shown below.

Method of Calculation.

The gram-molecular weight of a non-electrolyte dissolved in a litre of water has an osmotic pressure of 22.4 atmospheres and the osmotic pressure of the solution isotonic with the cell sap can be calculated as follows:

Let C = Concentration of solution in gm. per litre

MW = Molecular weight of substance

Then Osmotic Pressure = $\frac{C \times 22.4}{MW}$ atmospheres.

In the case of electrolytes, when the solution is sufficiently dilute to ensure complete dissociation, the calculated osmotic pressure must be doubled when only two ions are involved (as in potassium nitrate).

To find the Temperature at which the Protoplasmic Membrane is Destroyed

EXPERIMENT 8. Cut a thin slice of beetroot. Wash away any liberated cell-sap (coloured). Suspend in a beaker of water and place a thermometer in the water. Heat gently and stir carefully. Observe the temperature at which the red sap passes into the water due to the protoplasmic membrane being killed and losing its semi-permeability.

III. THE SOIL

Soil is formed by the weathering of rocks which results in the formation of particles varying in diameter as follows:—(i) **Stones**—of large diameter and varying size (ii) **sand**—2.0–0.02 mm., particles between 2.0 and 0.2 mm. being called **coarse sand** and those between 0.2 and 0.02 mm. **fine sand** (iii) **silt**—0.02–0.002 mm. and (iv) **clay**—less than 0.002 mm.

The size of the particles largely determines the physical nature of the soil, such as its temperature, aeration, drainage and water-content and the most important of these mineral constituents are sand (almost pure silica) and clay (impure aluminium silicate). In addition to these, the soil contains **mineral salts** which, being soluble, serve as plant food, and **humus** which is organic and is derived from decayed plants. This, too, is a source of food for plants as

it is decomposed into mineral salts by bacteria which use it for their own nutrition. Humus also increases the water content of the soil and with the clay forms the soil particles which are known as **crumbs**. These absorb water and adsorb mineral salts. Between these crumbs is air forming the soil atmosphere. Finally **micro-organisms** inhabit the soil and they play an extremely important part in making the soil fertile. Fungi and animal organisms are also commonly present.

To make a Mechanical Analysis of the Soil

EXPERIMENT 1. *Either (a) take a tall jar and about a third fill it with soil. Add water until the jar is nearly full, cork it up and shake thoroughly. Then allow the jar to stand for several hours. The heavier stones settle to the bottom. Above this will be seen the sand, the silt, and on the top of this the clay. Humus may be seen floating on the surface of the water. Colloidal particles of clay will also be seen in suspension in the water.*

Or (b) Arrange two tall jars with outlet spouts near the tops at different levels on the bench, and a third in the sink, in such a way that the spout of each jar is above the jar below. Put some soil containing gravel and stones into the top jar and connect a piece of glass tubing to the water tap by rubber tubing. Put the glass tubing into the top jar and run a current of water into it, constantly stirring. When the washings are clear, turn off the water and examine the jars.

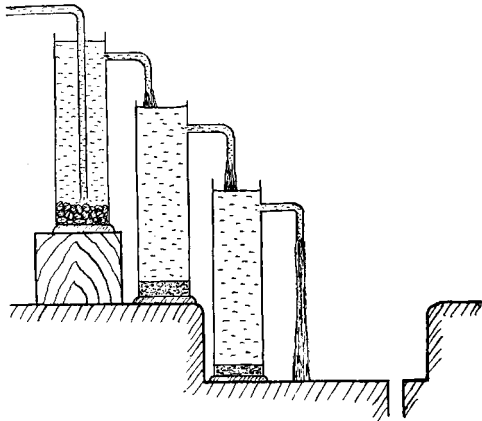


FIG. 88. Apparatus for the Mechanical Analysis of Soil.

The top jar contains gravel and stones, the middle one the lighter sand, and the bottom one the still lighter silt. Particles of humus may be seen floating on the water in the bottom jar but they will probably have been washed over into the sink.

To find the Percentage Weight of Water in a Sample of Soil

EXPERIMENT 2. *Counterbalance two pieces of filter paper on the pans of a balance. Put some soil on the paper on the left-hand pan and find its weight. Then put the soil and paper into a drying oven and later weigh again. Carry out a protective weighing and calculate the percentage loss of weight.*

To find the Percentage Weight of Humus in a Sample of Soil

EXPERIMENT 3. Weigh a crucible, then put in a little dried soil and weigh again. Heat the soil strongly for half an hour, to decompose the humus into volatile substances, by placing the crucible on a pipeclay triangle on a tripod. Allow it to cool, and find the weight of the crucible and contents. Carry out a protective weighing and calculate the percentage loss of weight.

To show the Presence of Soluble Matter in the Soil

EXPERIMENT 4. Vigorously shake up some soil with distilled water. Allow it to stand, and then filter, using a filter pump. Evaporate the filtrate to dryness, and see if there is any residue.

To compare the Permeability of Sand, Clay and Loam

EXPERIMENT 5. (a) **Permeability to Water.** (i) Place a perforated porcelain disc in a funnel and cover completely with a thick layer of wet sand. Put the funnel in a stand and place a beaker underneath.

Set up two similar experiments (ii) packed with wet clay and (iii) packed with wet loam. The samples should be equally wet and packed to the same degree of tightness. Now pour equal volumes of water on to the three samples and note the time.

Observe the extent to which the water passes through the samples and after a fixed time measure the volume of water in each beaker.

(b) Permeability to Air

Fix some rubber tubing on to the bottoms of the stems of the funnels used in the last experiment and fix the funnels in the tops of three burettes containing water up to fixed readings which should be just below the bottoms of the funnel stems. The funnels should be fixed in position by placing the rubber over the tops of the burettes. Place beakers under the burettes and open the clips, noting the time.

Observe the extent to which the water runs out of the burettes over a fixed time and take second readings of the burettes.

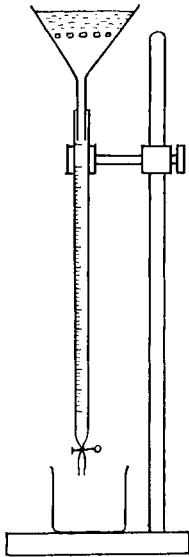


FIG. 89. Apparatus to show the permeability to air of sand, clay and loam.

To compare the Capillarity of Sand, Clay and Loam

EXPERIMENT 6. Fill three glass tubes, about 1 or 1.5 cm. diameter and about 30 cm. long, with dry sand, clay and garden soil respectively.

Plug the lower ends with cotton-wool, stand them in a vessel of water and clamp them in retort stands. The water will rise by capillarity. Compare the height to which it rises in the three tubes. Draw your conclusion.

To show the presence of Micro-organisms in the Soil

EXPERIMENT 7. Put a small quantity of lime-water into a small wide-necked flask. Suspend a muslin bag containing freshly dug soil in the flask and cork securely. Set up a similar flask but without soil or containing previously baked soil as a control.

The production of carbon dioxide, as indicated by the lime-water's turning milky, indicates respiration and, therefore, the presence of living organisms in the soil.

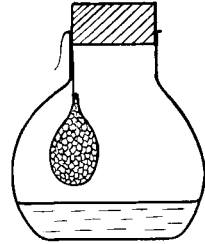


FIG. 90.
Apparatus to show presence of micro-organisms in soil.

IV. HOLOPHYTIC NUTRITION

Nutrition is necessary to an organism in order to provide the materials for growth and repair and for the acquisition of energy in order that the organism may be able to carry out its life processes.

Green plants synthesise their proteins and carbohydrates and, in some cases, fats or oils, from inorganic material and this is known as **holophytic nutrition**.

These foods may be stored in various organs of the plant and must be transported to other parts of the plant. This stored food may be in the form of protein or starch grains or oil in various plant organs. Before it can be utilised by the plant it must be changed into an absorbable form by the action of enzymes (biological catalysts) and transported to other parts of the plant.

(1) THE EFFECT OF DIFFERENT ELEMENTS ON PLANT GROWTH

To find the Effect on the Growth of the Plant of the different Elements in the soluble Mineral Salts in the Soil

WATER CULTURE EXPERIMENTS

The elements present in the plant can be ascertained by a chemical analysis of the ash obtained by the ignition of leaves or other plant organs.

To ascertain the effect of the different elements on the growth of a plant, plants should be grown in solutions of salts containing all the elements and in solutions devoid of one element.

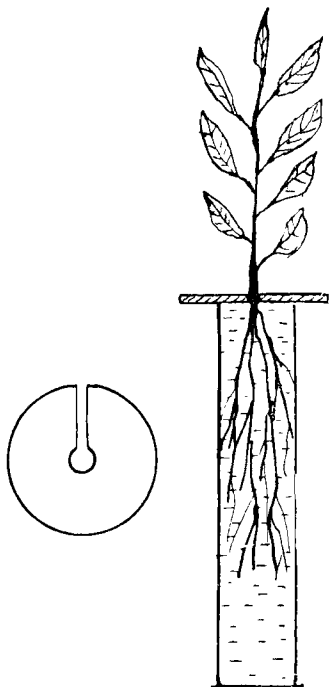


FIG. 91. Apparatus for Water-Culture Experiments.

- | | |
|-----------------------|--------------|
| (a) complete solution | (e) minus Fe |
| (b) minus Ca | (f) „ N |
| (c) „ K | (g) „ S |
| (d) „ Mg | (h) „ P |

and insert the plants. Set up a plant in distilled water as a “control”.

It is advisable to set up at least three of each. Examine the plants from day to day and add distilled water to replace that which has been lost by evaporation. Replace the solution once a fortnight. When necessary insert a stick through the second hole of the cover and lightly tie the plant to it. It is advisable to blow air through each day to supply the roots with oxygen.

Keep a record of the growth and development of the plants in each solution and deduce the influence of each element on the growth of the plant, tabulating your results.

(2) PHOTOSYNTHESIS

Photosynthesis may be defined simply as the building up of sugar from carbon dioxide and water by means of energy derived from sunlight with the aid of chlorophyll.

These solutions are called **culture solutions**. (These experiments also demonstrate the fact that roots absorb mineral salts from the soil, of course.)

EXPERIMENT 1. *Thoroughly wash twenty-seven jars, each of at least 350 ml. capacity, and each fitted with a cork or teak cover, which has a hole in the centre and a slit continuous with this to the edge. A second hole towards one side is useful for a stick for the support of the plant. Cover the jars with black paper to prevent algal growth.*

Take wheat or other seedlings with roots a few centimetres long, and as near the same stage of development as possible, and fix these in the holes in the covers, wedging them in position by a plug of cotton-wool. Then fill the jars with culture solutions (see Appendix I) as follows:—

This process is not by any means explained by the simple equation $6\text{CO}_2 + 6\text{H}_2\text{O} + \text{Energy} = \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O}$ as will have been discovered from the text book. A series of reactions is involved, of which one at least is independent of light energy. When the glucose formed has reached a sufficiently high degree of concentration, in most plants it is converted into starch for storage (other carbohydrates may also be formed). This starch is the first visible product of photosynthesis and its presence in a leaf previously devoid of it is therefore evidence of photosynthesis having taken place. At night the stored starch is reconverted into soluble sugars for translocation to other plant organs.

To test for Starch in Leaves

EXPERIMENT 1. Remove some leaves from various monocotyledonous and dicotyledonous plants which have been exposed to light. Dip them in boiling water and then transfer them to hot alcohol. The chlorophyll will dissolve in the alcohol so that the leaves become white if left in the alcohol for a sufficient length of time. Wash with water and to the decolourised leaves add iodine solution. A blue colour will indicate the presence of starch. The iodine can be removed from the cell walls and cytoplasm by the addition of benzene. This will leave untouched the blue colour produced by the starch (if present).

To see whether Starch is present in Leaves kept in the Dark

EXPERIMENT 2. Place a plant, in the leaves of which starch has been found to be present, in the dark for twenty-four hours. *Nasturtium* (*Tropaeolum*) answers well. Then remove some leaves, decolourise them and test for starch. Replace the plant in the light, and after a few hours again test a leaf for starch.

To see whether Light is necessary for Photosynthesis

EXPERIMENT 3. Take a nasturtium or other plant (previously kept in the dark overnight) and cover part of a leaf, on both sides, with

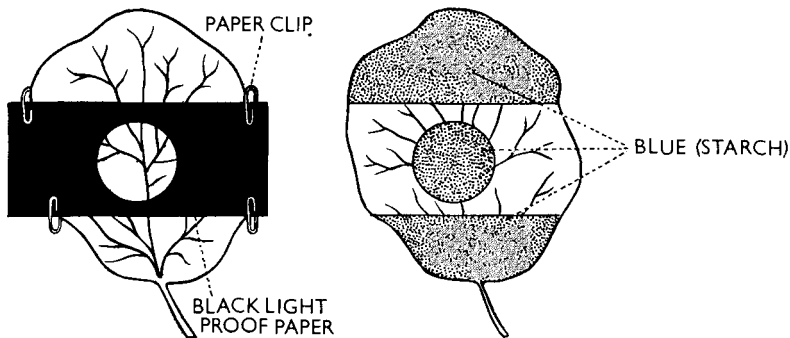


FIG. 92. Starch Print.

a piece of black (light-proof) paper, out of which a letter or figure has been cut. Stick or pin the two pieces of paper together with the cut portions over one another. Alternatively, cut a cork in half transversely and fix the two halves with the edges coincident on opposite sides of a leaf by pushing pins through them. After several hours remove the paper or cork, decolourise the leaf and test with iodine.

To see whether Chlorophyll is necessary for Photosynthesis

EXPERIMENT 4. Take some variegated leaves (e.g., Ivy, Geranium) and draw a diagram of the leaf showing the outlines of the variegated parts. Expose the leaves to light for a few hours, then decolourise and test for starch.

Observe where starch has been formed.

To find which Light Rays are used in Photosynthesis

EXPERIMENT 5. In order to do this it is necessary to expose the leaves (e.g., Nasturtium) to light of different colours. This can be done in dark chambers in which light enters from one side only and that through a coloured glass (or in double-walled bell jars containing solutions of the requisite colours).

By using orange glass, the red, orange and yellow rays only, and by using blue glass, the blue, indigo and violet rays only, will be allowed to enter.

After a few hours, remove the leaves, decolourise and test for starch. Compare results.

To see whether Light is necessary for the Formation of Chlorophyll

EXPERIMENT 6. Allow some cress or mustard seeds to germinate in the dark. Examine the leaves.

They are not green (i.e., they are devoid of chlorophyll) but yellow. This is due to the presence of a yellow pigment, *etiolin*.

(In the water culture experiments, it will have been seen that when the food of the plant lacks iron, the plant is *chlorotic*, i.e., no chlorophyll is developed.)

To see whether Carbon Dioxide is necessary for Photosynthesis

EXPERIMENT 7. Stand a pot plant (or some green leaves in a vessel of water), previously kept in the dark for a short time (so that the leaves become destarched), together with a small dish of caustic soda solution on a glass plate and cover with a bell jar. Fit the neck of the bell jar with a cork and double right-angle tube, on the outer limb of which is a U-tube containing soda-lime. Make all joints air-tight. The soda-lime absorbs the CO_2 from the air which enters and the NaOH absorbs the CO_2 formed in respiration. As a "control" set

up a similar experiment omitting the caustic soda and soda-lime. Expose the plants to sunlight and a few hours later remove a leaf from each, decolourise and test for starch.

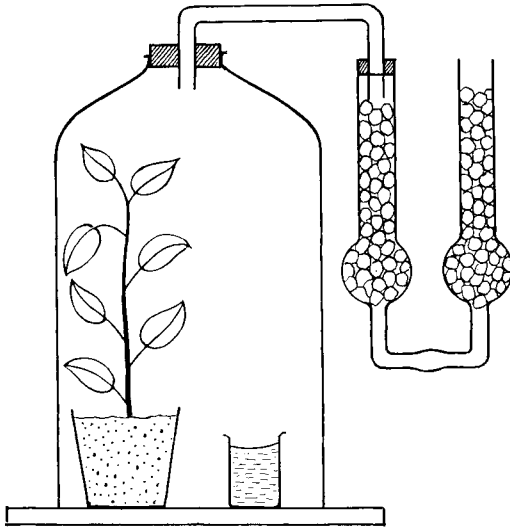


FIG. 93. Apparatus to demonstrate the necessity of Carbon Dioxide for Photosynthesis.

To show that Oxygen is given off in Photosynthesis

EXPERIMENT 8. Put some pond weed (e.g., *Elodea*) in some water in a beaker. Cover the weed with an inverted short-stemmed funnel and place a test-tube of water over the stem of the funnel. Clamp the test-tube in a retort stand and leave the plant exposed to sunlight for several hours. As a control set up a similar experiment and leave it in the dark.

A gas will collect in the test-tube in the experiment left in the light, bubbles rising from the leaves. When there is sufficient gas, test it with a glowing splint.

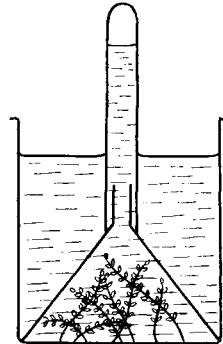


FIG. 94. Apparatus to show that Oxygen is given off in Photosynthesis.

V. PLANT PIGMENTS

Most of the pigments of plants are derived from a compound called pyrrole (C_4H_4NH) though some of the former are hydrocarbons.

CHLOROPHYLL

Chlorophyll is a compound of two pigments, **chlorophyll- α** ($C_{55}H_{72}O_5N_4Mg$) which is blue-green and **chlorophyll- β** ($C_{55}H_{70}O_6N_4Mg$) which is pure green. Associated always with chlorophyll are two other pigments, **carotene** ($C_{40}H_{56}$) and **xanthophyll** ($C_{40}H_{66}O_2$) which are orange and yellow respectively.

To prepare a Solution of Chlorophyll

EXPERIMENT 1. *Either (a) place some green leaves in hot water to kill them and to destroy the oxidising enzymes and then steep them in hot ethyl alcohol until a green solution of chlorophyll is obtained or (b) shake some dried leaves with acetone or ethyl alcohol until a green solution of the chlorophyll is obtained. The solution actually contains all four pigments.*

To make a Spectroscopic Examination of Chlorophyll

EXPERIMENT 2. *Put some chlorophyll solution from experiment 1 in a narrow flat-sided bottle and clamp it between a spectroscope and a source of light. Examine and draw the spectrum. Note the position of the absorption bands.*

To find the Effect of Bright Light on Chlorophyll

EXPERIMENT 3. *Put some chlorophyll solution into two test-tubes. Cork them and leave one (i) exposed to sunlight and the other (ii) in a dark cupboard. Examine twenty-four hours or so later.*

To separate Chlorophyll- α and Chlorophyll- β

EXPERIMENT 4. *Pour some of the solution prepared in Experiment 1 into twice its volume of petroleum ether and shake. Pour into a separating funnel and allow it to stand. Run off the lower layer and discard it. Shake the liquid left with methyl alcohol. This dissolves the chlorophyll- β and is pure green. The lower (etheral) layer contains the chlorophyll- α and is blue green.*

To separate Chlorophyll from Carotene and Xanthophyll

EXPERIMENT 5. *Shake some of the solution prepared in Experiment 4 with half its volume of a concentrated solution of potassium hydroxide in methyl alcohol in a separating funnel. Add a slight excess of water gradually and then some more petroleum ether. Shake and allow to stand. Two layers separate out. The upper layer contains carotene and xanthophyll and the lower aqueous layer contains the chlorophyll.*

To separate Carotene from Xanthophyll

EXPERIMENT 6. *Run off the lower layer from Experiment 5 and discard it. Shake the solution of the carotinoids with a little water and*

evaporate to a small volume. Add excess of petroleum ether and methyl alcohol. Shake well and allow to stand.

Two layers again separate out. The alcoholic layer contains xanthophyll and the ethereal layer contains the carotene.

ANTHOCYANINS AND ANTHOXANTHINS

Anthocyanins are responsible for the blue, red, purple and brown colours of flowers and the red colour of the beetroot. *Anthoxanthins* are the cause of the yellow colour in flowers and some vegetative organs.

To examine the Properties of Anthocyanins

EXPERIMENT 7. *Make a solution of the red anthocyanin of beetroot or of the blue anthocyanin of the flowers of Delphinium or Bluebell by steeping some small pieces of them in water or of the flower of Wallflower (brown), Antirrhinum (blue) or other suitable flower by steeping them in alcohol. Then perform the following experiments with the solution:—*

(i) *Add a few drops of caustic soda.* A green colour is produced (yellow in the case of beetroot).

(ii) *Add a few millilitres of hydrochloric acid.* A purple or red colour is obtained.

(iii) *Add a few drops of basic lead acetate solution.* A green or bluish-green colour (red in the case of beetroot) is obtained.

To Examine the Properties of Anthoxanthins

EXPERIMENT 8. *Prepare an alcoholic solution of an anthoxanthin by steeping buttercup flowers in boiling alcohol or an aqueous solution by steeping in boiling water. Then perform the following experiments with the solution:—*

(i) *Add a few millilitres of basic lead acetate solution.* A yellow precipitate of the lead salt is obtained.

(ii) *Add a few drops of ferric chloride solution.* A green or brown colour is produced.

VI. RESPIRATION

Respiration may be defined as the breaking down of sugar into simpler substances for the purpose of liberating energy for the organism.

To show that Germinating Seeds absorb Oxygen and give out Carbon Dioxide (Aërobic Respiration)

EXPERIMENT 1. *Take two flasks of equal capacity. Into one (A) put some soaked germinating pea seeds and into the other (B) put an*

equal quantity of pea seeds previously killed by boiling and add a little formalin to prevent decomposition. Place a small tube of caustic potash solution (which absorbs carbon dioxide) in each flask and fit each flask with a two-holed cork provided with a short piece of glass tubing fitted with a rubber tube and clip and a right-angle tube. Now connect a narrow U-tube containing a little coloured water between the right-angle tubes. See that all joints are air-tight and equalise the water levels in the limbs of the U-tube by opening the two clips. Then close the clips.

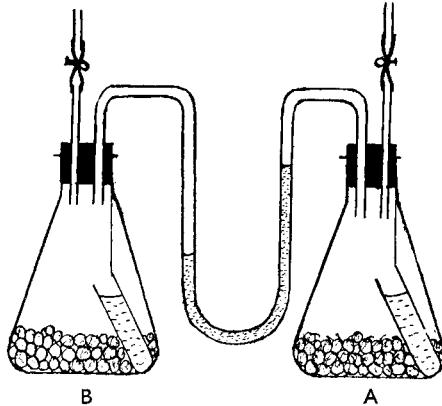


FIG. 95. Apparatus to demonstrate the Aërobic Respiration of Germinating Seeds.

Examine a little later. Note in which limb of the U-tube the water has risen, showing a reduction of pressure in that flask due to absorption of carbon dioxide. Remove the corks and insert a lighted taper into the flasks.

To demonstrate the Aërobic Respiration of the Green Plant

EXPERIMENT 2. Connect a U-tube of soda-lime or a Dreschel bottle of caustic potash solution to the inlet tube of a Dreschel bottle containing lime-water and connect the outlet tube to a short right-angle tube through the cork in the neck of a bell jar. Fit the bell jar with another longer right-angle tube and connect this to the inlet tube of a second Dreschel bottle of lime-water, the outlet tube of which is connected to a water pump fitted on the tap. Place a small pot plant or some leaves in a beaker of water on a glass plate and cover with the bell jar. Make all joints air-tight and set the pump working to draw air slowly through the apparatus.

As the air passes through the soda-lime or caustic potash the carbon dioxide will be absorbed and the lime-water in the first bottle will remain clear. The lime-water in the second bottle will also

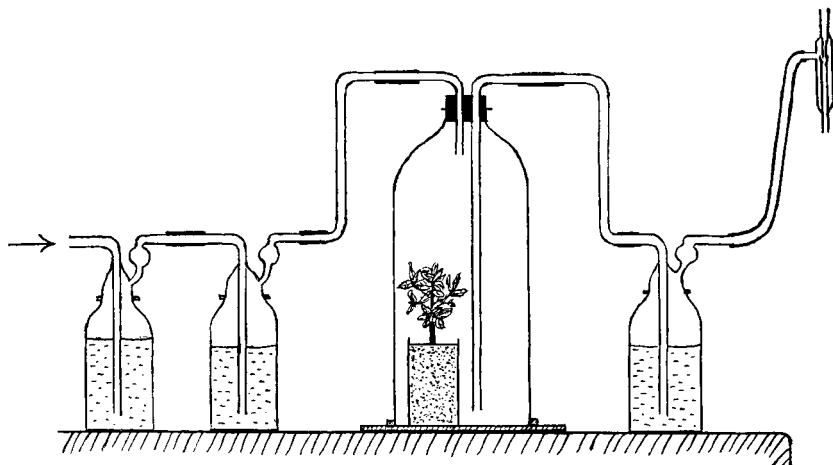


FIG. 96. Apparatus to demonstrate the Aërobic Respiration of a Green Plant.

remain clear so long as the plate is exposed to light (owing to the activity of photo-synthesis). *Cover the bell jar with black (light-proof) paper and observe the result.*

To show that Oxygen is necessary for Respiration

EXPERIMENT 3. *Take a small retort and put some soaked seeds on wet blotting-paper in the bulb. Clamp the apparatus with the tube standing vertically in a vessel of potassium pyrogallate (prepared by adding caustic potash to pyrogallic acid in the vessel after the apparatus is set up). Alternatively use an inverted U-tube with the soaked seeds with some wet cotton-wool in one limb closed with a cork, while the other stands in the solution. (Instruments used in this manner are referred to as respirosopes.) Observe whether the seeds germinate and draw your conclusion.*

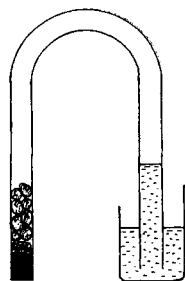


FIG. 97. Apparatus to show that Oxygen is necessary for Respiration.

To demonstrate the Respiration of Roots

EXPERIMENT 4. *Place a plant with its roots in Congo red solution in a bottle. Observe the colour change. As a control place some Congo red in another bottle without a plant. Confirm the result by passing a little carbon dioxide into some of the indicator.*

To demonstrate Anaërobic Respiration

EXPERIMENT 5. *Fill a small test-tube or specimen tube with mercury*

and invert it in a dish of mercury. Now insert three or four germinating pea seeds from which the testas have been previously removed to avoid introducing the contained air. The seeds will rise to the top of the tube. Clamp the tube in a small retort stand and set up a similar

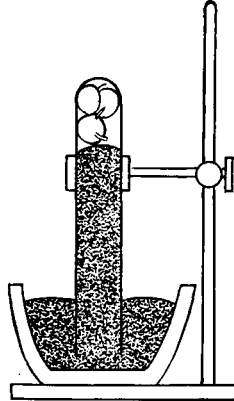


FIG. 98. Apparatus to Demonstrate Anaerobic Respiration.

experiment, using killed seeds as a "control". Examine the tubes a day or two later. The mercury level will have fallen in the tube containing the living seeds. Insert a small piece of caustic potash into the tube or a rod with a drop of lime-water on the end into the gas and observe what happens.

EXPERIMENT 6.* Fermentation: Fit a large flask with a rubber stopper and double right-angle tube as shown in Fig. 99. Pour some glucose solution into the flask and add some baker's yeast (*Saccharomyces cerevisiae*). Connect the right-angle tube to a Dreschel bottle containing lime-water and leave the apparatus for a day or two at the temperature of the room.

A froth consisting of bubbles of gas forms on the surface. Observe the effect on the lime-water. When the frothing has ceased, decant, filter and distil the liquid.† Then perform one or more of the following tests for ethyl alcohol with (a) alcohol from the reagent bottle and (b) the distillate.

(i) **Iodoform Test.** Add caustic soda solution and then, drop by drop, iodine solution until the liquid is yellow. Heat but do not boil.

* This experiment may be performed when studying yeast.

† Unless the liquid is distilled it may be found difficult to identify the smells produced in the tests owing to the strong "yeasty" smell of the original solution.

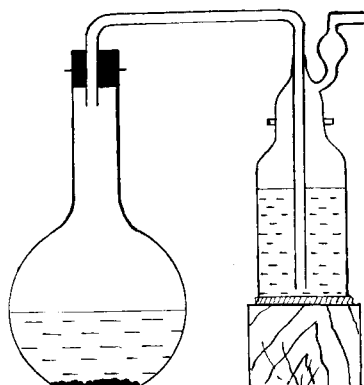


FIG. 99. Apparatus to demonstrate Fermentation.

A yellow precipitate of iodoform with characteristic smell is obtained.
 $C_2H_5OH + 6NaOH + 4I_2 = CHI_3 + HCOONa + 5NaI + 5H_2O$.

(ii) **Ethyl Acetate Test.** Add a few crystals of sodium acetate and a few millilitres of conc. sulphuric acid. Boil. A characteristic fruity smell of ethyl acetate is obtained.

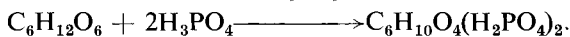


(iii) **Aldehyde Test.** Add a few crystals of potassium dichromate and a few millilitres of dilute sulphuric acid. Heat. The liquid turns green and a characteristic smell of acetaldehyde is produced.

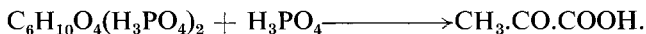


The fermentation of sugar is brought about by the enzymes complex **zymase**. The action is rather complicated but, briefly, it may be summarised as follows:—

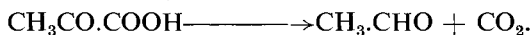
(i) Glucose + phosphoric acid $\xrightarrow{\text{phosphatase}}$ a hexosephosphate



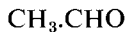
(ii) Hexosephosphate $\xrightarrow{\text{phosphatase}}$ pyruvic acid



(iii) Pyruvic acid $\xrightarrow{\text{carboxylase}}$ acetaldehyde + carbon dioxide



(iv) Acetaldehyde $\xrightarrow{\text{hydrogen}}$ ethyl alcohol



To show that Heat is liberated in Respiration

EXPERIMENT 6. Take two small thermos flasks and fit them with one-holed corks and thermometers graduated in $\frac{1}{2}$ (or better $\frac{1}{5}$) degrees. Fill one flask with germinating pea seeds and the other with killed seeds as a "control". Label the flasks. Moisten the living seeds with water and the killed seeds with weak formalin to prevent fermentation, and securely cork the flasks. Keep a record of the temperatures every few hours.

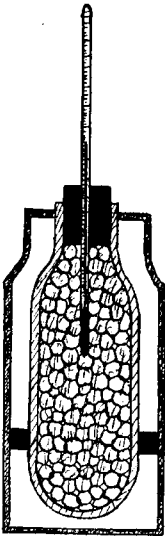


FIG. 100. Apparatus to show that Heat is liberated in Respiration.

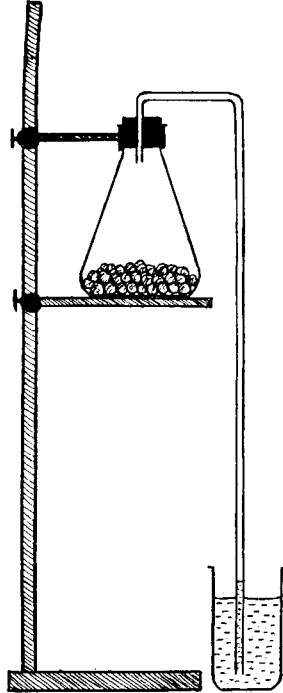


FIG. 101. Apparatus to Measure the Respiratory Quotient.

To find the Respiratory Quotient

EXPERIMENT 7. Put some germinating pea seeds in a 250 ml. conical flask and fit the flask with a tube bent twice at right angles, one limb being about 50 cm. long. Clamp the flask in a retort-stand and place a beaker of coloured water under the long tube. Set up a similar apparatus without seeds as a "control". Observe the movement of water up the tube, if any. Now repeat the experiment using oily seeds, e.g., Linseed. Draw your conclusions in each case as to the ratio $\frac{\text{output of CO}_2}{\text{intake of O}_2}$ which is known as the **Respiratory Quotient**.

VII. GROWTH

GERMINATION OF SEEDS

EXPERIMENT 1. *Prepare several germination jars as follows: Insert a roll of white blotting-paper into a cylindrical or rectangular glass jar. Fill up the central cavity with bulb-fibre or sphagnum moss.*

Then place soaked seeds of Broad Bean (or Scarlet Runner), Maize (or Wheat), Sunflower and Onion between the blotting-paper and the glass in separate germination jars. Add water to the fibre and keep watered but do not saturate.

Examine the germination of the seeds and draw the various stages from time to time, noting the form, order of appearance and position of the following in so far as they apply, also noting whether germination is hypogeal or epigeal:—

Primary root (elongated radicle), **secondary** (lateral) **roots**, **root hairs** growing on the surface of the primary and secondary roots, **hypocotyl**, which by its elongation in the epigeal types raises the cotyledons (which turn green), **stem (epicotyl)** and **foliage leaves**. In the maize, note the **coleoptile**, a sheath which encloses the plumule in the early stages, and the **coleorhiza** or radicle sheath.

CONDITIONS NECESSARY FOR THE GERMINATION OF SEEDS

To show the Presence of Water in Unsoaked Seeds

EXPERIMENT 2. *Gently heat some “dry” unsoaked pea seeds in a test tube.*

Note the water which condenses on the cool sides of the tube. Test it with anhydrous copper sulphate.

To find the Influence of Light on Germination and Growth

EXPERIMENT 3. *Put some soaked broad bean seeds in a germination jar and leave it in the dark. Put some similar seeds in a similar jar kept in the light. Compare the growth of the two sets of seedlings from day to day for two or three weeks.* The seedlings which germinate in the dark grow more rapidly than those in the light, but the plants kept in darkness have weak, white stems with long internodes and small undeveloped leaves, which are yellow owing to the absence of chlorophyll and the presence of *etiolin*. These plants are said to be “etiolated”. They grow very tall but are unhealthy and soon die.

To find the Effect of Extremes of Temperature on Seeds

EXPERIMENT 4. (a) **Heat.** *Immerse two boiling-tubes containing a few soaked and unsoaked pea seeds respectively in the thermostat and keep them at 60° C. for a couple of hours or so. Then soak the dry*

seeds for twenty-four hours and put both groups in separate germination jars. Water the jars and examine a few days later. Compare the results.

(b) **Cold.** Repeat the above experiment but immerse the tubes in ice or a freezing mixture instead of the thermostat.

As a "control" to both experiments put some soaked pea seeds in a germination jar and keep them at room temperature.

To see whether Air is necessary for Germination

EXPERIMENT 5. Take two wide-necked bottles. Fill one completely with cold boiled water, drop in a few pea seeds and cork securely. As a "control", put some soaked pea seeds into the other bottle with a little water to keep them moist. Examine a few days later.

(Germination of the seeds deprived of air may just begin, owing to air already in the seed and to anaërobic respiration, but it will soon cease.)

To see whether Water is necessary for Germination

EXPERIMENT 6. Put some dry seeds into a dry germination jar and do not water. As a "control", put some soaked seeds in another jar, which is kept watered. Examine after several days.

GROWTH OF THE ROOT AND SHOOT

To find the Growing Region of the Root

EXPERIMENT 1. Dry the root of a bean seedling when it has grown to about 5 cm. length. Mark it transversely with indian ink lines 2 mm. apart from the tip to the base. Place the seedling in a tall germination jar or put the seed with wet bulb fibre in the head of a thistle funnel with the root in the stem of the funnel. Examine from day to day and observe where the marks become wider apart.

To measure the Rate of Growth in the Root

EXPERIMENT 2. Place a broad bean or pea seedling in a tall germination jar, or put the seed in damp bulb-fibre in the head of a vertically fixed thistle-funnel, with the root in the stem of the funnel. Clamp a half-metre scale vertically alongside. Note the position of the tip of the root from day to day and keep a record of the amount of daily growth. Compare the rate of growth during the day with that at night. Plot a graph of growth against time.

To find the Growing Region of the Stem

EXPERIMENT 3. Mark the stem of a broad bean seedling which is about 5 to 10 cm. high with transverse indian ink marks 2 mm. apart as in Experiment 1. Examine daily and compare with the result of Experiment 1.

To measure the Rate of Growth of the Shoot

EXPERIMENT 4. Join the apex of the stem of a potted plant to the short arm of the recording lever of an auxanometer or arc indicator by means of thread. These are instruments for measuring the growth of plants and for recording this growth magnified. The magnification is given with the instrument. The recording lever is a light

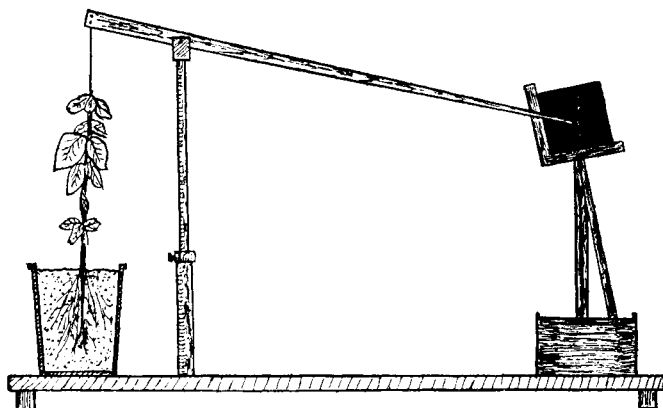


FIG. 102. Auxanometer.

wooden beam pivoted near one end, to which the plant is attached, and bearing a quill or pointer at the other. A sliding weight which can be fixed in position to adjust the balance may be provided on the short arm. The pointer is in light contact with a graduated arc, which shows the magnified growth (arc indicator), or with a smoked glass, which is moved automatically every hour by an arm in contact with the minute hand of an upturned clock, and on which the magnified growth is scratched (auxanometer). In this case, the intervals between the series of horizontal marks show the hourly growth.

Alternatively, the growth-lever can be in light contact with a vertically rotating drum, covered with smoked paper and driven by clockwork. *The graph can be varnished (see Appendix II) and a permanent record kept. Measurement of the growth should be made.*

Make a record of the hourly growth of the plant over several days and compare the rate of growth during the day and night. Plot a graph of growth against time.

HORMONES

Hormones are chemical substances produced in an organism which are responsible for the co-ordination and control of the behaviour of cells in the organism which may be distant from those in which the hormones is produced,

or of the organism as a whole. As most of these substances are stimulants they are known as **hormones** but a few are inhibitory in their actions and these are known as **chalones**. The term "**autacoid**" is more applicable to these substances in general though hormone is still more commonly used for all of them.

GROWTH HORMONES

Plant hormones are known as **auxins*** and are produced by the cells in the growing points of stems and roots. They are passed to the growing region immediately behind, where they control the elongation and direction of growth by their action on the meristematic tissue. The elongation is brought about by the enlargement of the existing cells in the growing region.

EXPERIMENT 1. *Cut off the tips of a few oat coleoptiles about 1 mm. from the apex. Carefully place the cut surfaces of the tips on small blocks of agar and leave them for a few hours.*

The decapitated coleoptiles cease to grow.

EXPERIMENT 2. *Replace the tips neatly on some of the decapitated coleoptiles and carefully place the agar blocks on which they have been standing on others. Examine later.*

In both cases growth is resumed.

EXPERIMENT 3. *Place some of the tips and some of the agar blocks slightly to one side on other decapitated coleoptiles. Examine later.*

Growth continues but it is curved. Growth is more rapid on the sides receiving the auxin. Light affects auxin distribution and it has been found that it accumulates on the side receiving less illumination, thereby accelerating growth on one side and causing phototropic curvature.

Similar experiments with roots have shown that the auxins retard growth and are responsible for curvature when the root is not in a vertical position thus enabling it to grow downwards, *i.e.*, the auxins are responsible for geotropic curvature.

VIII. IRRITABILITY AND MOVEMENT

(1) CYCLOSIS

Cyclosis is the circulation of cytoplasm in cells.

EXPERIMENT 1. *Very gently remove a leaf of Canadian Pondweed (*Elodea canadensis*) from near the apex of a shoot, taking care not to squeeze the leaf. Mount in a drop of its pond water and examine under the microscope.*

After a few minutes the chloroplasts will be seen circulating round the cell in the cytoplasm which is itself circulating and carrying the chloroplasts with it.

* *Auxin* is indole-3-acetic acid or β -indolyl acetic acid.

(2) TROPIC MOVEMENTS

A **tropism** is a growth movement on the part of a plant organ towards or away from a stimulus. The direction of the response is determined by the stimulus.

Phototropism

To Demonstrate the Positive Phototropism of the Stem

EXPERIMENT 2. Germinate mustard seedlings in a pot in a dark chamber in which light is allowed to enter through a narrow opening on

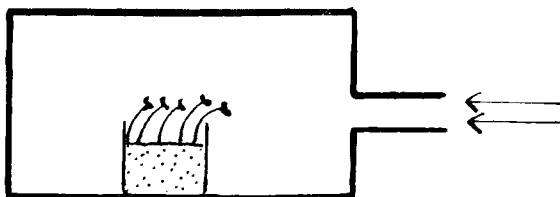


FIG. 103. Phototropic Chamber.

one side only. Germinate another set in a completely dark chamber as a "control". Examine a few days later when the plants have germinated.

Note the direction taken by the leaves: they are **dia-phototropic**.

To find the Region of Phototropism Curvature

EXPERIMENT 3. Grow a broad bean seedling in darkness and when the stem is about 5 cm. long mark it with transverse indian ink marks 2 mm. apart. Now put the plant in the phototropic chamber used in Experiment 1 so that it is exposed to one-sided light. Examine after a few days and observe where the curvature has taken place.

To demonstrate the Negative Phototropism of the Root

EXPERIMENT 4. Germinate a broad bean seedling, and when the radicle is about 3 cm. long, cover the germination jar with black paper except for a narrow vertical slit to expose the root to the light. As a "control" set up a similar experiment in which the jar is completely surrounded by black paper. Examine after a few days.

Geotropism

To demonstrate the Positive Geotropism of the Root and the Negative Geotropism of the Stem

EXPERIMENT 5. Place a bean seedling, germinated in darkness, with a shoot about 3 or 4 cm. in length, in a flat-sided germination jar with the root and shoot horizontal. Examine a few days later. Note the direction of the lateral roots: they are **dia-geotropic**.

To find the Region of Geotropism Curvature

EXPERIMENT 6. Germinate a broad bean seedling in darkness and when the root is about 5 cm. long mark it with transverse indian ink

marks 2 mm. apart. Put the seedling in a flat-sided germination jar with the root horizontal. Examine after geotropic growth has taken place. Note where the curvature has taken place.

To find the Effect of Gravity

EXPERIMENT 7. *The effect of gravity can be observed by means of an instrument called a clinostat. This consists of a vertical cork disc*

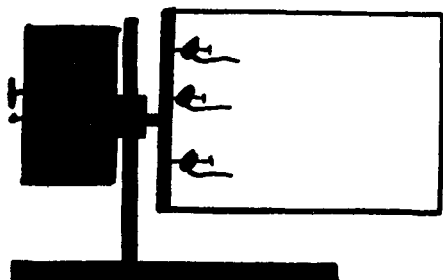


FIG. 104. Clinostat.

rotated once an hour by clockwork or by an electric motor. A cylindrical plastic cover fits on to the cork disc. (The instrument can also be arranged with the disc in a horizontal position for the demonstration of phototropism.)

Pin three or four pea or bean seedlings on the cork disc, each with two small

pins, with the roots and shoots horizontal. Pin some wet cotton-wool between the seedlings and fix the plastic cover on the disc. Start the clock or motor and leave the apparatus in the dark. Examine after two or three days, winding the clock, if necessary, in between times. Observe the direction of growth of the roots and shoots.

Hydrotropism

To demonstrate the Positive Hydrotropism of the Root

EXPERIMENT 8. *Put some pea or mustard seeds near the bottom of some bulb fibre in a sieve. Suspend the sieve obliquely from the under side of a shelf or prop it up in this position on the bench and keep the fibre well watered.*

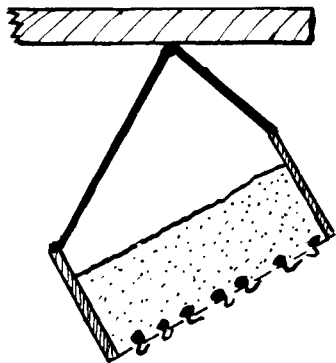


FIG. 105. Apparatus to demonstrate the hydrotropism of the root.

As a "control" set up a similar experiment with the sieve horizontally supported.

After a few days the roots will have grown down in response to gravity and, growing through the meshes of the sieve, will reach the air. A little later compare the further direction of growth by the roots in the two experiments.

Haptotropism (Thigmotropism)

To demonstrate the Response of Tendrils to Touch

EXPERIMENT 9. *Stroke several times the inside of the slightly curved apical part of a tendril of a pea with a mounted needle. Note that the curve becomes much greater, forming a complete circle in a very short time.*

Chemotropism

To demonstrate Positive Chemotropism

EXPERIMENT 10. *Mount some pollen grains from a flower of the pea or nasturtium in 15 per cent. sucrose solution or of bluebell in a 10 per cent. solution, on a microscopical slide (a cavity slide is best). Cut off the stigma or part of a stigma from one of the carpels of the same flower and arrange it in the centre or to one side of the sugar solution. Put on a coverslip and place the slide in a Petri dish with a small piece of cotton-wool or filter paper moistened with water to keep the atmosphere moist. Then cover the dish and put it in a dark cupboard. A few hours later, remove the slide and examine under the microscope.*

Note the pollen tubes which have grown out of the pollen grains by bursting of their inner coats (intine) through the outer ones (exine) and observe that they are growing towards the stigma. The attraction is a chemical one.

(3) TACTIC MOVEMENT

A **tactic movement** is the response of an entire organism to a stimulus and the direction of the stimulus determines the direction of the response.

To demonstrate Phototaxis

EXPERIMENT 11. *Chlamydomonas or Volvox will swim away from darkness or dull light to a diffuse light. This can be demonstrated in a long narrow vessel or tube one half of which is covered by a black light-proof paper while the other is well illuminated. If water containing a sufficient number of Volvox globator is put into the vessel, the coenobia will be seen as tiny specks congregated in the illuminated end.*

(4) NASTIC MOVEMENT

A **nastic movement** is a response to a diffuse stimulus and the direction of the movement is not determined by the stimulus.

To demonstrate Photonasty

EXPERIMENT 12. *Examine a Mimosa plant which has been kept in the light.*

Note the upwardly directed stem bearing alternate bipinnate leaves. *Place the plant in the dark.* Later note that the petioles now bend downwards, and that the leaflets have closed together.*

EXPERIMENT 13. *Place a plant of Wood Sorrel (Oxalis acetosella) in bright sunlight and note that the leaflets are open. Now put the plant in a dark cupboard.* It will be seen that the leaflets collapse.

A similar response will be observed with the leaves of Clover (*Trifolium pratense*) and with the inflorescence of the Daisy. *If the flowers of Evening Primrose (Oenothera lamarkiana) are examined by day and by night, it will be observed that the flowers are closed by day but open at night.*

IX. EXCRETION

Green plants, owing to the fact that they are much less active than animals, produce little in the way of excretory substances as compared with animals. The process is therefore somewhat limited in plants and they lack any specialised organs of excretion. *Carbon dioxide* produced in respiration is, of course, a waste product of metabolism and therefore an excretory product when liberated at night, though in the daytime it is used for photosynthesis. *Tannins* which are found in the bark of trees, *resins*, *volatile oils* responsible for the odour of flowers and *alkaloids* are examples of what may be excretory products though it may be that these substances also benefit the plant. One of the commonest excretory substances, however, is *calcium oxalate* which is deposited in crystalline form in the cells of many plants. It collects in the leaves of deciduous trees immediately prior to leaf-fall and the substance is thus eliminated from the tree at leaf-fall.

* Photonastic movements are often affected by other influences, and plants such as *Mimosa* assume this position at night. It is therefore referred to as "sleep movement" or nyctinasty.

PART V

GENETICS

INTRODUCTORY NOTES

Genetics is the study of the variation and inheritance of characters.

Adequate time must be available for the performance of experiments on heredity in plants. Considerable care must be exercised in the performance of these experiments and in the ascertaining of the results. Satisfactory numerical ratios will be obtained only if *large* numbers are taken and the mean result of a number of such experiments performed by different students is found. The larger the number the better the result will always be. The Maize cob (*Zea mais*) serves as suitable material for these experiments, though, if time, labour and space permit, the Garden Pea (*Pisum sativum*) will give good results. Both provide suitable alternative allelomorphs. Experiments which involve plant breeding will necessarily take a long time and will require adequate ground space and suitable external conditions quite apart from the length of time which must ensue before results can be obtained. If experiments with the garden pea, which include the study of height of plant, colour of flowers or colour or form of seeds, are to be performed, it will be necessary, of course, to germinate the seeds which result from the fertilisation which follows cross-pollination and this will take several months. These experiments are clearly outside the possibility of the normal first year or Sixth-Form course.

Suitable material (*e.g.*, maize cobs and seeds) can be purchased from biological supply firms (see Appendix V).

Mendelian Segregation

EXPERIMENT 1. *Examine a Cob of maize (Zea mais).*

The cob will be seen to be composed of a large number of fruits (caryopses) but these fruits may not all be of the same colour and this will depend on the colour of the fruits from which the parent plants germinated. The colours may be red and white or purple and white.

Count the number of fruits of each colour in the cob and correlate your results with those obtained from the cobs examined by other students. The larger the number studied, the more accurate will be the result. Then calculate the mean ratio of one colour to the other.

The result should work out as follows:—

Red: white 3 : 1

Purple: white 1 : 3

This shows that in the F1 generation red is dominant in the first pair of allelomorphs and white in the other and clearly illustrates Mendelian Segregation.

Monohybrid Inheritance

1. Preparation for the Experiments

From the instructions given below it will become obvious that these experiments will take up a great deal of time, not only in their preparation but also in their performance and that it will take a long time for them to be completed. Furthermore, adequate and suitable ground and many willing gardeners must be available. *It will not, therefore, be practicable in the ordinary course to carry them out.* Nevertheless they are very instructive and the method should be known.

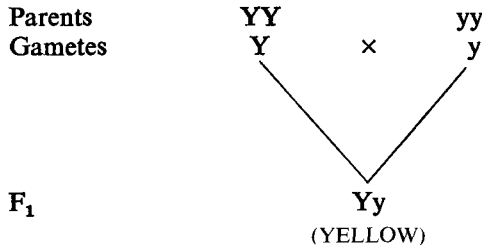
They are really repetitions of Mendel's original experiments, when he chose such contrasted characters as height of plants (tall or dwarf), colour of flower (purple or white), colour of seed (green or yellow) and surface of seed (smooth or wrinkled). Any of these characters may be chosen for the experiments and as an example, seed colour is illustrated below.

Separate beds must be prepared for the sowing and germinating of the seeds and they will need constant attention during the period of growth. Large numbers of seeds must be sown.

2. The Experiments

EXPERIMENT 1. *Germinate the plants from green and yellow seeds in separate beds. When the plants have flowered, remove the stamens from all the flowers in one bed to prevent self-pollination from taking place. Leave the flowers to develop to maturity. Allow the flowers in the other bed to open and mature and, using a camel-hair brush, transfer the pollen from the anthers of these flowers to the stigmas of the de-staminated flowers in the other bed. It does not matter whether flowers from green seeds are pollinated by those from yellow seeds or vice-versa.*

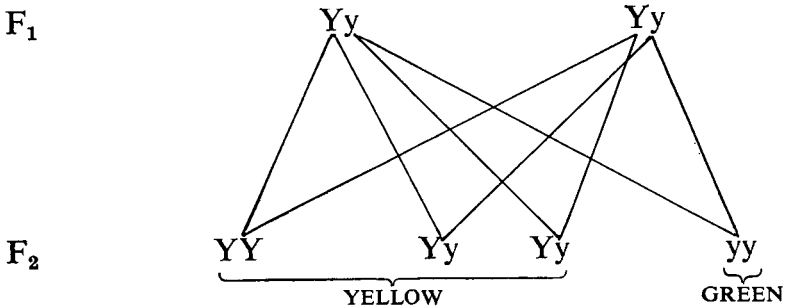
After cross-pollination has been effected cover the flowers with close-



mesh muslin secured round the pedicels with thin string to prevent insects from entering and interfering with the experiments. When the fruits have developed, allow them to grow to full size, then open the legumes and remove the seeds. Observe the colour of the seeds in this

F1 generation. They should all be yellow, showing that yellow is dominant to green.

EXPERIMENT 2. Plant these seeds and allow them to germinate. When flowers have been produced, cross-pollinate as before or allow them to self-pollinate. When seeds have been developed, observe the colour as before and count the number of each. The result should show both yellow and green seeds and they should be in the ratio of 3 yellow: 1 green.



It will have been seen from the text-book studies that one-third of the yellow seeds are homozygotes and two-thirds heterozygotes, and that the green seeds are, of course, homozygotes. To verify this, further experiments will be necessary in which only self-pollination is allowed to take place but this will lengthen the time even more.

Back-crosses between the F_1 generation and the parental dominant and recessive types could also be carried out.

Note

Experiments on **Dihybrid Inheritance** can also be performed, using methods similar to those above, but much more time and labour will, of course, be involved.

APPENDIX I

THE PREPARATION AND USES OF REAGENTS

MICROSCOPICAL REAGENTS

(1) HARDENING AND FIXING AGENTS

Alcohol (Ethyl), Absolute or 70%

Acetic Acid (1 per cent.)

Acetic acid (glacial)	1 ml.
Distilled water	99 ml.

Bouin's Fluid (Picro-Formol)

Picric acid (sat. sol. aq.)	75 ml.
Formaldehyde	25 ml.
Acetic acid (glacial)	5 ml.

Chromo-acetic Acid

Chromic acid	0.3 gm.
Distilled water	100 ml.
Acetic acid	1 ml.

Flemming's Solution

Chromic acid, 1% aq.	90 ml.
Osmic acid (osmium tetroxide), 2% aq.	24 ml.
Acetic acid (glacial)	6 ml.

Formalin Alcohol

Alcohol, 70%	100 ml.
Formaldehyde	6 ml.

(2) MACERATING FLUIDS

Chromic acid (0.2%)

Chromium trioxide	0.2 gm.
Distilled water	100 ml.

Schultze's Macerating Fluid

Alcohol, 50%	100 ml.
Formaldehyde	6.5 ml.
Acetic acid, glacial	2.5 ml.

(3) MICROSCOPICAL STAINS

Many of these are best bought ready prepared, particularly in small laboratories. They can be purchased in solid form and in solution.

Acid Fuchsin

Acid fuchsin 0.5 gm.

Distilled water 100 ml.

Use: *Leucoplasts.*

Alkannin

Alkanna roots sat. sol. in 96% alcohol.

Use: *Fats, cork.*

Aniline Blue (Alcoholic)

Aniline blue 0.2 gm.

Alcohol (70%) 100 ml.

Use: *Sieve plates, Algae.*

Aniline Blue (in Lacto-phenol)

Aniline blue 0.4 gm.

Lacto-phenol 100 ml.

Use: *Algae, Fungi.*

Aniline Sulphate (or Hydrochloride)

Aniline sulphate (or hydrochloride) . Sat. sol. in distilled water.

Add Sulphuric (or hydrochloric) acid (conc.) A few drops until its reaction is acid.

Use: *Lignin (temporary stain).*

Bismarck Brown

Bismarck brown, sat. sol. aq.* 90 ml.

Alcohol, 90% 30 ml.

Best purchased.

Use: *Bacteria, cellulose, nuclei.*

Carbol Fuchsin

Fuchsin 1 gm.

Phenol 5 gm.

Alcohol 10 ml.

Distilled water 100 ml.

Use: *Fungi, Bacteria.*

Congo Red

Congo red 0.5 gm.

Distilled water 100 ml.

Use: *Parasitic Fungi, Fungal hyphae.*

* Not readily soluble in water.

Cotton Blue (*see Aniline Blue*).

Crystal Violet (*see Gentian Violet*).

Eosin (Aqueous)

Eosin Y	1.0 gm.
Distilled water	100 ml.

Use: *Plant tissues in general.*

Eosin (Alcoholic)

Eosin Y	1.0 gm.
Alcohol (70%)	100 ml.

Use: *Plant tissues in general.*

Fuchsin (Aqueous)

Fuchsin	10 gm.
Distilled water	100 ml.

Use: *Counterstain with Gram's Iodine.*

Gentian Violet

Gentian violet	1.0 gm.
Distilled water	100 ml.

Use: *Bacteria, Fungi.*

For Mitosis, use

Gentian violet	sat. sol. aq.
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Gram's Iodine

Potassium iodide	0.66 gm.
Distilled water	100 ml.
Add Iodine	0.33 gm.

Counterstain with **Gentian Violet (Crystal Violet)** and **Fuchsin (Aqueous)** (*see above*).

Use: *Bacteria—Gram's Test.*

Haemalum (Mayer)

Haematoxylin	0.25 gm.
Distilled water	250 ml.

When dissolved add

Sodium iodate	0.05 gm.
Alum	12.5 gm.

When dissolved, add

Chloral hydrate	12.5 gm.
Citric acid	0.25 gm.

Use: *A good general stain for plant tissues.*

Haematoxylin (Delafield)*Best purchased.*

Haematoxylin	4 gm.
Absolute alcohol	25 ml.
Add Ammonium alum, sat. sol. aq.	400 ml.
<i>Leave exposed to light for three or four days. Filter.</i>	
Add Glycerine	100 ml.
Methyl alcohol	100 ml.

*Allow to stand for about 8 weeks. Filter.**Use: A good general stain for plant tissues.***Hoffman's Blue**

Aniline blue	1.0 gm.
Alcohol (50%)	99 ml.
Acetic acid (glacial)	1.0 ml.

*Use: Sieve plates.***Iodine**

Potassium iodide (sat. sol. aq.)	50 ml.
Add Iodine	until no more dissolves
Add Distilled water	until pale in colour

*Use: Starch, glycogen, cellulose (temporary stain).***Iodine Green**

Iodine green	1 gm.
Distilled water	100 ml.
Acetic acid (glacial)	1 ml.

*Use: Lignin.***Light Green (Alcoholic)**

Light Green	sat. sol. in 90% alcohol
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*Use: Cellulose.***Light Green (in Clove Oil)**

Light Green	0.2 gm.
Alcohol (absolute)	50 ml.
Clove oil	50 ml.

*Use: Cellulose.***Methylene Blue**

Methylene blue, sat. sol. in absolute alcohol	30 ml.
Add Potassium hydroxide	0.01 gm.
Distilled water	100 ml.

Use: Bacteria, Saccharomyces.

Methyl Blue

Methyl blue	1.0 gm.
Distilled water	100 ml.

Use: *Cellulose.*

Methyl Green

Methyl green	1.0 gm.
Alcohol	100 ml.
Add Acetic acid (glacial)	1.0 ml.

Use: *Lignin.*

Methyl Violet

Methyl violet	1.0 gm.
Alcohol (70%)	100 ml.

Use: *Bacteria.*

Orange G

Orange G	0.5 gm.
Distilled water	100 ml.

Use: *Cellulose.*

Orcein

Orcein	1.0 gm.
Alcohol (absolute)	100 ml.
Hydrochloric acid (conc.)	1.0 ml.

Use: *Inulin.*

Osmium Tetroxide ("Osmic Acid")

Purchase 2% solution.

Use: *Fats. (Temporary stain.)*

Phloroglucin

Phloroglucin	10 gm.
Alcohol (absolute)	100 ml.

Use: (With conc. HCl.) *lignin. (Temporary stain.)*

Safranin

Safranin	1.0 gm.
Distilled water	100 ml.

Use: *Lignin (red), cutin (pink), suberin (red), nuclei (red).*

Schultze's Solution (Chlor-zinc-Iodine)

Zinc chloride	30 gm.
Potassium iodide	5 gm.
Iodine	1 gm.
Water	15 ml.

Use: *Cellulose* (blue), *starch* (blue), *proteins* (yellow), *lignin* (yellow) (*temporary stain*).

Sudan III

Sudan III	sat. sol. in 70% alcohol
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Use: *Fats* (red).

Sudan IV (Scharlach Red)

Sudan IV	5.0 gm.
Alcohol (70%)	100 ml.

Use: *Fats* (red).

(4) DIFFERENTIATING FLUID**Acid Alcohol**

Alcohol, 70%	100 ml.
Hydrochloric acid conc.	2.5 ml.

(5) DEHYDRATING AGENTS**Ethyl Alcohol**

30% Alcohol.	<i>To prepare dilute solutions from Industrial Methylated Spirits (=95% ethyl alcohol), stand the spirit over anhydrous copper sulphate for three or four days, replacing the anhydrous copper sulphate as necessary. Then prepare as follows:—</i>
50% Alcohol.	
70% Alcohol.	
90% Alcohol.	
Absolute Alcohol.	

30% Alcohol

Alcohol, 95%	35 ml.
Distilled water	65 ml.

50% Alcohol

Alcohol, 95%	55 ml.
Distilled water	45 ml.

70% Alcohol

Alcohol, 95%	75 ml.
Distilled water	25 ml.

90% Alcohol

Alcohol, 95%	95 ml.
Distilled water	5 ml.

Cellosolve

= Ethylene glycol monoethyl ether. Purchase ready prepared.

(6) CLEARING AGENTS**Chloral Hydrate**

Chloral hydrate 250 gm.
Distilled water 100 ml.

Use: *Plant tissues*, especially for embryos in ovules and archegonia.

Clove Oil

Purchase as such.

Use: *Best clearing agent for plant tissues.*

Eau de Javelle

Bleaching powder 10 gm.
Distilled water 50 ml.

Allow to stand for 24 hours, then add the following solution:—

Potassium oxalate 7.5 gm.
Distilled water 50 ml.

until no further ppt. is formed. Filter. If a film forms on the surface on exposure to air, add more potassium oxalate solution. Keep well stoppered in an amber bottle.

Use: *Plant tissues, especially growing points and temporary mounts.*

Lacto-phenol

Lactic acid 20 gm.
Phenol 20 gm.
Glycerine. 40 gm.
Distilled water 20 ml.

Use: *Best clearing agent for Algae and Fungi.*

Xylene

Use: *Plant tissues generally* but it is *not* recommended because it tends to cause shrinkage.

(7) MOUNTING MEDIA**(a) Permanent Mounts****Canada Balsam**

Purchase ready prepared.

The best solvent is xylene.

Euparal and Euparal Vert

Purchase ready prepared.

Glycerine Jelly*Best bought ready prepared.*

Gelatin	25 gm.
Distilled water	150 ml.

Soak the gelatine in the water for a few hours. Then pour off the water, melt the gelatine and add:—

Glycerine	175 gm.
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Add:

Phenol (5% aq.)	a few drops
or, better, Thymol	a few crystals

*Stir well while hot then heat gently and filter through glass wool in a hot-water funnel.***(b) Temporary Mounts****Dilute Glycerine**

Glycerine	50 ml.
Distilled water	50 ml.

*Add a few crystals of thymol.***(8) EMBEDDING WAX****Paraffin Wax**

Mix Paraffin wax, melting point 50°C.	2 parts
Paraffin Wax, melting point 36°C.	1 part

Melt and stir.

M.P. of Mixture = 48°C.

(9) RINGING CEMENT*Purchase Ringing Cement or Gold Size, Black Varnish or Black Enamel.***BIOCHEMICAL AND GENERAL REAGENTS, ETC.****Acetic Acid, Dilute**

Glacial acetic acid	23 ml.
Water	77 ml.

Albumen*Purchase dried albumen.***Ammonium Hydroxide**

Ammonium hydroxide S.G. 0.88.	25 ml.
Distilled water	75 ml.

Ammonium Molybdate

<i>Dissolve</i> Ammonium molybdate	15 gm.
<i>in ammonium hydroxide prepared as follows:—</i>	
Ammonium hydroxide, S.G. 0·88	10 ml.
Distilled water	5 ml.
<i>Add</i> Distilled water	120 ml.
<i>Shake and add this to dilute nitric acid prepared as follows:—</i>	
Nitric acid (conc.)	18 ml.
Distilled water	95 ml.

Ammonium Sulphate

Ammonium sulphate	Sat. sol. in distilled water
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Antimony Chloride (for Vitamin A Test)

Antimony trichloride	Sat. sol. in chloroform
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Antiseptics**Carbolic Acid**

Phenol (cryst.)	5·0 gm.
Distilled water	95 ml.

Corrosive Sublimate

Mercuric chloride	1·0 gm.
Distilled water	99 ml.

Dettol

Dettol	15 ml.
Water	135 ml.

Formaldehyde (4%)

Formalin (40% formaldehyde)	10 ml.
Water	90 ml.
(= 10% Formalin)	

Barium Chloride

Barium chloride	3·0 gm.
Distilled water	97 ml.

Barfoed's Reagent

Copper acetate	4·5 gm.
Distilled water	100 ml.
Acetic acid (50%)	1·0 ml.

Calcium Chloride

Calcium chloride	2·0 gm.
Distilled water	98 ml.

Chloral Hydrate

Chloral hydrate	15 gm.
Distilled water	85 ml.

Chlorine Water

Prepare chlorine by heating manganese dioxide with concentrated hydrochloric acid or by treating potassium permanganate with concentrated hydrochloric acid. Pass the chlorine into water until it is saturated. Store in an amber bottle.

Chromic Acid

Chromium trioxide	10.0 gm.
Distilled water	90 ml.

or

Potassium dichromate	10.0 gm.
Distilled water	100 ml.
Sulphuric acid (conc.)	10 ml.

Use: Cleaning glass apparatus.

Cobalt Chloride Paper

Cobalt chloride	5.0 gm.
Distilled water	95 ml.

Soak filter paper in this solution and dry in an oven. Store in air-tight bottle.

Copper Sulphate

Copper sulphate (cryst.)	10.0 gm.
Distilled water	90 ml.

Copper Sulphate 1% (for Biuret Test)

Copper sulphate	1.0 gm.
Distilled water	99 ml.

Culture Solutions**(1) Knop's Solution (for Plant Water Culture Experiments)****(a) Stock Solution—Complete**

Calcium nitrate	2.0 gm.
Potassium nitrate	0.5 gm.
Magnesium sulphate	0.5 gm.
Potassium phosphate.	0.5 gm.
Ferric chloride, sol. aq.	a few drops
Distilled water	1 litre

Keep in an amber bottle for storage.

(b) Solution for use—complete

Dilute stock solution with distilled water 1 : 5.

(c) Deficient Solutions

- Minus **Calcium**—Substitute potassium nitrate for calcium nitrate.
- „ **Iron**—Omit the ferric chloride.
- „ **Magnesium**—Omit the magnesium sulphate or substitute potassium sulphate.
- „ **Nitrogen**—Substitute chlorides for nitrates.
- „ **Phosphorus**.—Omit the potassium phosphate or substitute potassium sulphate.
- „ **Potassium**—Substitute calcium nitrate and phosphate for potassium nitrate and phosphate.
- „ **Sulphur**—Substitute magnesium chloride for magnesium sulphate.

(2) Pasteur's Solution (for Yeast Cultures)**(a) Stock Mixture**

Potassium phosphate	10.0 gm.
Calcium phosphate	1.0 gm.
Magnesium sulphate	1.0 gm.
Ammonium tartrate	50.0 gm.

(b) Solution for use

Stock mixture	4.0 gm.
Distilled water	200 ml.
Add Sucrose	30.0 gm.

Cuprammonia (Schweitzer's Reagent)

Add sufficient ammonium hydroxide solution to copper sulphate solution to precipitate cupric hydroxide and then excess to dissolve it. The solution does not keep and should be prepared when required.

Diastase

Purchase commercial diastase.

2 : 6 Dichlorophenol-indophenol (for Vitamin C test)

Dissolve 0.2 gm. in 100 c.c. of distilled water, allow to stand 24 hours. Filter.

Or purchase tablets from B.D.H.

Dissolve one tablet in

Distilled water	10 ml.
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This can be used immediately.

Diphenylamine

Diphenylamine	20 gm.
Alcohol (95%)	80 ml.

Fehling's Solution**Solution A**

Copper sulphate	36.64 gm.
Water	500 ml.

Solution B

Sodium potassium tartrate	176.0 gm.
Potassium hydroxide	77.0 gm.
Water	500 ml.

Keep in separate bottles.

For use mix equal quantities of A and B.

Ferric Chloride

Ferric chloride	10.0 gm.
Distilled water	90 ml.

Formaldehyde

Formalin = 40% aqueous solution of formaldehyde.

To prepare a 10% solution of formalin *see Antiseptics* above.

Hydrochloric Acid, Dilute

Hydrochloric acid (conc.)	26 ml.
Distilled water	73 ml.

Indigo Sulphate (for demonstrating the evolution of oxygen in photosynthesis)

Indigo sulphate.	0.01 gm.
Distilled water	1.0 litre

This solution is blue but the addition of the following solution *reduces* it and discharges the colour.

Do not mix until required and then take care not to shake.

Sodium hydrosulphite ($\text{Na}_2\text{S}_2\text{O}_4$).	10 gm.
Distilled water	100 ml.

Iodine

Potassium iodide	6.0 gm.
Water	100 ml.

Add Iodine 2.0 gm.

Lead Acetate

Lead acetate	10.0 gm.
Distilled water	90 ml.

Lime Water

Make a saturated solution in water. Shake well. Filter.

Millon's Reagent (for Protein Test)*Best purchased.**Dissolve*

Mercury	50.0 gm.
in Nitric acid (conc.)	35 ml.
Add Distilled water	35 ml.

 α -Naphthol (for Carbohydrate Test)

α -Naphthol	2.0 gm.
Alcohol (95%)	100 ml.

Osmium Tetroxide ("Osmic Acid")

1% or 2% solution. Buy ready prepared.

Osmosis Solutions**Sodium chloride**

Sodium chloride	5.0 gm.
Distilled water	95 ml.

Sucrose

Sucrose	15.0 gm.
Distilled water	100 ml.

Peptone*Purchase commercial peptone.***Plasmolysis Solutions****(a) Molar Solutions****(1) Potassium Nitrate**

Potassium nitrate	10.1 gm.
Distilled water	100 ml.

(2) Sucrose

Sucrose	34.2 gm.
Distilled water	100 ml.

(b) Solutions for Use

Dilute solutions with distilled water as instructed in Part IV (Physiology).

Potassium Dichromate

Potassium dichromate	5.0 gm.
Distilled water	95 ml.

Potassium Ferrocyanide

Potassium ferrocyanide	10.0 gm.
Distilled water	90 ml.

Potassium Hydroxide

*Potassium hydroxide	10.0 gm.
Distilled water	90 ml.

Potassium Pyrogallate

To Pyrogallol	5.0 gm.
Distilled water	95 ml.
Add Potassium hydroxide	25.0 gm.
Distilled water	15 ml.

Use immediately.

Preserving Media. See Appendix II.

Schweitzer's Reagent

See Cuprammonia.

Silver Nitrate

Silver nitrate	1.0 gm.
Distilled water	99 ml.

Keep in an amber bottle.

Sodium Hydroxide

*Sodium hydroxide	8.0 gm.
Distilled water	92 ml.

Sodium Nitroprusside

Sodium nitroprusside	about 2.0 gm.
Distilled water	100 ml.

The solution must be freshly prepared for use.

Starch Sol

Mix Starch	1.0 gm.
<i>with a little cold water to a paste. Add this gradually to</i>	
Salicylic acid	0.5 gm.
Boiling water	100 ml.

stirring well all the time.

The salicylic acid is a preservative and the "solution" will keep for a long time.

Sugar Solution for Germination of Pollen Grains

Sucrose 5 to 20% aqueous solution.

Different concentrations prove more effective with different plants, e.g., Bluebell 10%; Pea 15%.

* The pellets will be found easier to weigh than the sticks.

Sulphuric Acid, Dilute

*Add Sulphuric acid (conc.) 5 ml.
gradually to*

*Distilled water 95 ml.
in a vessel standing in cold water.*

Thymol (for Carbohydrate Test)

Thymol 3.0 gm.

Alcohol (90%) 97 ml.

APPENDIX II

BIOLOGICAL METHODS

CLEANING OF GLASS APPARATUS

Wash with commercial hydrochloric acid or aqua regia (HCl, 3 parts : HNO₃, 1 part, by volume).

Very dirty apparatus may be cleansed with chromic acid which renders it chemically clean. This is prepared as follows:—

Dissolve

Potassium dichromate	10 gm.
<i>in</i> Water	100 ml.
Sulphuric acid	10 ml.

Grease is best removed by caustic soda solution.

In all cases wash thoroughly with water after cleansing as above. For cleaning of microscopical slides see below under "M".

CULTURE METHODS

Algae

Knop's solution. *See* Culture Solutions, Appendix I, p. 175.

Bacteria

Nutrient agar or nutrient gelatine. The former is better for general use as some bacteria liquefy gelatin.

All apparatus must be sterilised before use. (*See* Sterilisation Methods under "S" below.)

Cultures may be made in bacteriological test tubes or in Petri dishes. Sterile nutrient media may be purchased in bottles and may be melted and poured into sterilised tubes and plugged with sterile cotton-wool in the laboratory or the tubes may be bought ready prepared.

Further details are given under the heading, "Elementary Bacteriology" in Part III (Morphology, Cytology and Histology), pp. 54, *seq.*

Eurotium

Dry bread, Cover with bell-jar to exclude dust.

Moulds (in general)

Inoculate the following medium with the mould to be cultured:—

Maltose	4.0 gm.
Peptone	1.0 gm.
Agar	1.8 gm.
Distilled water	100 ml.

Mucor

Expose a piece of damp bread to the air for about half an hour in dry weather. Cover with a bell-jar to exclude dust.

Penicillium

Damp bread as for Mucor which develops first, followed by Eurotium and Penicillium.

Pythium

Sow cress seeds closely in damp soil. Keep well watered.

Yeast

Pasteur's solution. *See Culture Solutions, Appendix I, p. 176.*

GRAPHIC RECORDS—TO PRESERVE

Graphic records made on smoked glass or paper may be preserved for permanent storage by immersion in the Varnish given below, afterwards allowing them to dry in the air.

Methylated spirit	75%
White hard varnish	25%

Stir well.

JOINTS—TO MAKE AIRTIGHT

Apply the wax mixture given below. This is more effective and cleaner to use than vaseline.

Beeswax	30 gm.
Vaseline	40 gm.

Melt and add—

Resin, powdered	15 gm.
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Stir.

LABELS

(1) All bottles should be clearly and neatly labelled in a uniform style. Books of printed labels can be purchased, but they are seldom sufficient or complete. If they are used, supplementary labels should be written in similar style. When all are done by hand the style known as "bold old face" is perhaps the most suitable, *e.g.*:

<p style="text-align: center;">ALCOHOL 50%</p>
--

All labels should be written in indian ink and should be varnished when on the bottle. A suitable varnish may be prepared as follows:—

Celluloid	6.5 gm. (approx.)
Acetone	100 ml.

The celluloid may be obtained from old photographic films which should first be soaked in hot water until the negative has been removed.

(2) Specimens being temporarily stored in bottles may be labelled internally by writing the name in pencil on a small strip of paper, which should be put into the preserving fluid in the bottle. This method is not really suitable for specimens in permanent storage as the labels are not readily visible. *See also Microscopical Slide Labels.*

Gummed labels will not adhere to plastic vessels. The Perspex Cement used to seal lids on plastic museum jars is most suitable for this purpose.

MACERATION

The purpose of maceration is to soften and isolate parts of a tissue. One method is to put the structure (stem or root) in a test-tube and add a few crystals of potassium chlorate. Cover this with conc. nitric acid and then heat gently. When fumes cease to be given off add water. Wash the tissue and mount in glycerine ready for teasing out (Schultze's method). Alternatively the structures may be immersed in macerating fluid (see Appendix I, p. 166) and then washed and mounted as above.

MICROSCOPICAL SLIDE LABELS

Gummed labels may be purchased but the self-adhesive "Micro-tabs" obtainable from Messrs. T. Gerrard & Co. are most convenient to use.

MICROSCOPICAL SLIDES—TO CLEAN

It is best to keep a vessel of methylated spirit into which permanent preparations no longer required can be placed. Leave the slides to soak and keep the jar covered.

Periodically remove, boil in water, preferably with soap powder or detergent, rinse well and dry. A number of coverslips will also be retrievable.

MUSEUM SPECIMENS

(1) Preserved Specimens

Flat-sided museum jars are better than the cylindrical ones for

most purposes as the latter sometimes cause distortion. Either glass or plastic jars can be used.

Specimens can be fixed to a glass or plastic background by thread or fine catgut through holes drilled in it. This makes it possible for both sides of the specimen to be examined. Ebonite, giving a black background, is sometimes an advantage and is unaffected by formaldehyde. Specimens such as brains can be fixed to the background by Durofix, but the specimen must be dry at the time of fixation. It can afterwards be preserved in formaldehyde without ill effect on the Durofix.

For preserving fluids, see "Preservation and Storage of Biological Material", see below.

The lids of *glass jars* should be securely sealed as follows:—

(i) **When the specimen is preserved in alcohol**

Best glue 8 parts

"Dissolve" in water.

Add Glycerine 1 part

Apply hot to the edge of the cover. Press.

(ii) **When the specimen is preserved in formalin**

Shellac varnish. Dissolve in alcohol.

Apply to the edge of the cover and the jar. Press.

The tops may be covered with molten pitch, a smooth surface being obtained by means of a small heated trowel or knife.

The lids of *plastic jars* are sealed with a special cement such as "Perspex Cement" which can be purchased.

PRESERVATION AND STORAGE OF BIOLOGICAL MATERIAL

Material should be stored in wide-neck stoppered vessels for preference and the bottles should be clearly labelled with the name of the specimen and preserving fluid.

(a) **Plant Material—General**

70% alcohol *or* 4% formaldehyde (= 10% formalin) *or* in the following solution:—

Alcohol 300 ml.

Distilled water 300 ml.

Glycerine 200 ml.

(b) Algae (Green)

50% Glycerine (alcoholic solution).

To preserve green colour place in the following solution:—

Copper acetate	0.3 gm.
Camphor water	75 ml.
Glacial acetic acid	1 ml.
Copper chloride	0.3 gm.
Distilled water	75 ml.

(c) Bacterial Cultures

Tube Cultures. Remove the cotton-wool plug. Moisten its under surface with formalin and replace it in the tube. Cover with a rubber cap.

Plate Cultures. Remove the cover and place 2 drops of formalin on the inside of the cover. Replace the cover and allow to stand for 24 hours. Take care that no formalin drops on to the culture. Remove any unevaporated formalin and again replace the cover. Fix a broad band round the edge of the petri dish.

(d) Plant Material—Dry, Herbaria

Collect in a *vasculum* and as soon as possible place the specimen (preferably the entire plant) between layers of absorbent paper (special *Herbarium Drying Paper* can be purchased). Do not use blotting paper. Place between boards and clamp together or bind together with straps (special *botanical presses* can be purchased). Change the paper frequently and leave the press in a warm place. When the specimens are quite dry, mount them on herbarium paper or good cartridge paper using *herbarium paste* (which can be purchased).

STERILISATION

Glass: Dry heat: 150° C. for 1 hour. Use a hot air oven.

Pipettes and small glass apparatus: Rinse in mercuric chloride (0.1% aq.) or boil in water for $\frac{1}{4}$ hour.

Used culture-tubes and petri dishes: Boil in water for 1 hour.

Rubber: Steam for $\frac{1}{2}$ hour.

Cotton-wool: Dry heat: not above 180° C.

Instruments: Boil in water containing soda (to prevent rusting) for $\frac{1}{4}$ hour.

Minor instruments (e.g., platinum needles): Flame: heat to redness.

Nutrient media: Sterilise on three consecutive days by steam for $\frac{1}{4}$ to $\frac{1}{2}$ hour.

THERMO-REGULATOR

To set Reichart's Thermo-Regulator.

Connect the top tube, A, to the gas-supply and the side-tube, B, to the burner as shown.

Turn the screw S full out. Light the gas at the burner. When the required temperature is reached, turn in the screw S sufficiently to close the jet J with mercury. Sufficient gas to maintain an even temperature will enter through the by-pass and the gas flame will be lowered. By contraction of the mercury when the temperature falls slightly (thus allowing more gas to enter) and by its expansion to the limit at which it was set (thus lowering the flame again) when the temperature rises, a constant temperature will be maintained for any length of time.

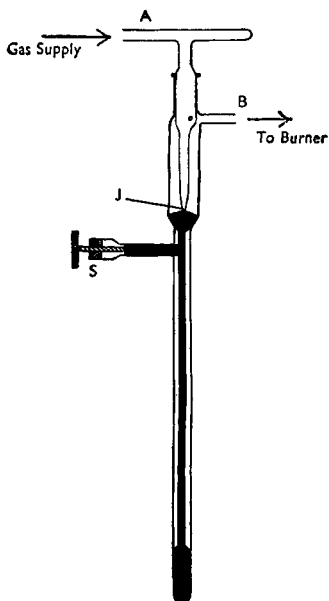


FIG. 104. Thermo-Regulator.

THERMOSTAT

A satisfactory thermostat can be made from an ordinary "steamer" as follows:—

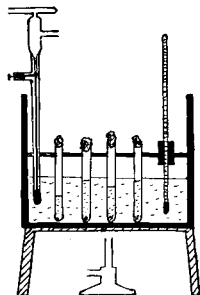


FIG. 105. Thermostat.

Enlarge the existing holes in the bottom of the upper vessel sufficiently to take test-tubes. Fit a thermometer and a heat regulator through corks in two of the holes. A hole may be bored in the side of the lower vessel and a cork fitted into this, through which passes a piece of bent glass tubing to act as a water gauge. Stand the thermostat on a low tripod and place a small burner underneath.

Alternately, a *hot water oven* fitted with a thermo-regulator and thermometer, may be used.

APPENDIX III

Equivalents

1 in. = 2.54 cm.	1 grain = 0.0648 gm.
1 cm. = 0.3937 in.	1 gm. = 0.03527 oz. (av.)
1 metre = 39.37 in.	= 0.0022 lb.
1 cu. in. = 16.39 ml.	= 15.43 grains
1 gallon = 4.546 litres	1 kg. = 2.205 lb. (av.)
1 litre = 1.76 pints	1 oz. (av.) = 28.35 gm.
$1 \mu = 0.00003937 \text{ in.} = \frac{1}{25,400} \text{ in., or, more approximately, } \frac{1}{25,000}.$	

Conversion Table

	<i>Multiply by</i>
Cm. to in.	0.3937
Ml. to cu. in.	0.06102
Ml. to cu. ft.	0.0003532
Cu. ft. to ml.	28320
Cu. in. to ml.	16.39
Gallons to litres	4.546
Grains to grams	0.0648
Grams to ounces	0.03527
Grams to grains	15.43
Ins. to cm.	2.54
Ins. to μ (microns)	25,400
Litres to gallons	0.22
Metres to yards	1.094
μ (microns) to in.	0.00003937
Ounces to grams	28.35
Sq. cm. to sq. in.	0.155
Sq. in. to sq. cm.	6.452
Yards to metres	0.9144
$^{\circ}\text{C}$ to $^{\circ}\text{F}$	$^{\circ}\text{C.} \times \frac{9}{5} + 32$
$^{\circ}\text{F}$ to $^{\circ}\text{C}$	$^{\circ}\text{F} - 32 \times \frac{5}{9}$

NOTE. The measure cubic centimetre (c.c.) is no longer used in scientific works or on graduated vessels. Millilitre (ml.) is used instead. The two measures are not quite identical but for practical purposes they may be regarded as the same.

APPENDIX IV

TREATMENT OF ACCIDENTS IN THE LABORATORY

Burns

- (i) **By dry heat:** Treat with gentian violet jelly or tannic acid jelly or 1% picric acid. Bandage to exclude air.
- (ii) **By acids:** Wash with plenty of water and then a saturated solution of sodium bicarbonate.
- (iii) **By alkalis:** Wash with much water and then 1% acetic acid.
- (iv) **Scalds:** Cover with gentian violet jelly; failing this, treat with sodium bicarbonate solution or boracic or zinc ointment. Bandage to exclude air.

Cuts

Wash thoroughly with T.C.P. or Dettol. Cover with elastoplast dressing. If large or deep cover with lint and bandage. Iodine should *not* be applied to an open wound.

Eye Accidents

- (i) **Acid in the Eye.** Using an eye-bath, wash with weak (1%) sodium bicarbonate solution.
- (ii) **Alkali in the Eye.** Wash with 1% boric acid.

Fainting (Syncope)

Lay the patient on his back; loosen the clothing. Administer sal volatile.

Poisons

- (i) **Acids or alkalis.** Wash the mouth and drink much water. Drink a tumbler of lime-water if due to acid or 2% acetic acid if due to alkali.
- (ii) **Mercuric chloride.** Take an emetic—a tablespoonful of common salt in a glass of water.

First-aid Cabinet

Every Laboratory should be fitted with a First-aid Cabinet, which should contain:—

Gentian violet jelly
or Tannic acid jelly
or Picric acid 1% aqueous solution
Acetic acid 1% aqueous solution

Acetic acid	2% aqueous solution	
Boric acid	1% aqueous solution	
Sodium bicarbonate	1% aqueous solution	
Sodium bicarbonate	Saturated aqueous solution	
Sal volatile	Sodium chloride	Zinc or boracic ointment
Lime-water	Vaseline	T.C.P. or Dettol
Adhesive tape	Lint	Eye-bath
Bandages, assorted	Oiled silk	Forceps
Cotton-wool	Elastoplast dressings	Safety pins
Gauze	(assorted sizes)	Scissors

APPENDIX V

FIRMS SUPPLYING BIOLOGICAL APPARATUS AND MATERIAL*

BIOLOGICAL APPARATUS, MICROSCOPES, MICROSCOPICAL SLIDES, STAINS AND REAGENTS, DISSECTING INSTRUMENTS, CHARTS, FILM STRIPS, LANTERN SLIDES, BOTANICAL MODELS, CHEMICAL APPARATUS AND REAGENTS, ETC.

Baird & Tatlock (London) Ltd., 14 St. Cross Street, E.C.1.

Flatters & Garnett Ltd., Mikrops House, Bradnor Road, Manchester, 22.

A. Gallenkamp & Co. Ltd., 6 Christopher Street, E.C.2.

T. Gerrard & Co. Ltd., Gerrard House, Worthing Road, East Preston, Nr. Littlehampton, Sussex.

Griffin & George Ltd., Ealing Road, Alperton, Wembley, Mddx.

MICROSCOPICAL STAINS AND REAGENTS ONLY

Edward Gurr Ltd., 47 Upper Richmond Road West, S.W.15.

George T. Gurr Ltd., 136 New Kings Road, S.W.6.

MICROSCOPES & ACCESSORIES

R. & J. Beck Ltd., Bushey Mill Lane, Watford, Herts.

E. Leitz (Instruments) Ltd., 20 Mortimer Street, W.1.

T. Gerrard & Co. Ltd., Address: see above.

†**Wallace Heaton Ltd.**, 127 New Bond Street, W.1.

W. Watson & Sons Ltd., Barnet, Herts.

BOTANICAL MATERIAL (Living or Preserved)

Flatters & Garnett Ltd., Address: see above.

Galloway's Biological Agency, Rhydyfelin Aberystwyth, Wales.

T. Gerrard & Co. Ltd., Address: see above.

National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London, N.W.9 (Living Cultures of Saprophytic Bacteria).

* This list is not exhaustive.

† This firm also sells second-hand instruments.

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- Biochemical substances and processes* are in their alphabetical positions.
Biological methods (Appendix II) are arranged alphabetically.
Microscopical processes and other matter relating to this technique are in their alphabetical places.
Physiological processes and experiments in connection with them will be found under the names of those processes.
Plants are arranged under their *generic* names and also under their English names where applicable.
Plant organs, tissues and other structures may be found under the generic names of the plants *except in Angiosperms* in which they are classified under the names of the organs. Plant tissues will also be found in their alphabetical places.
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