PRACTICAL BIOLOGY

FOR

ADVANCED LEVEL, MEDICAL
AND INTERMEDIATE
STUDENTS

BY

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A

LABORATORY MANUAL

Covering the Syllabuses in *Biology* of the General Certificate
of Education (Advanced Level), First Examinations for Medical
and other Examinations of similar standard

FIFTH EDITION

LONDON

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1966

By the same author
Practical Botany
Practical Zoology

FOREWORD TO FIFTH EDITION

BIOLOGY is the science of life and therefore involves the study of living organisms, first the *variety of living organisms*, their form and structure (*morphology* and *anatomy*) and the microscopical structure of their tissues and organs (*histology*) and then the all important aspect of their mode of functioning (*physiology*). The chemistry of the substances which enter into the composition of the organism and its physiological processes (*biochemistry*), the development of new organisms (*embryology*) and the inheritance of characters from one generation to subsequent generations (*genetics*) all form part of this fascinating study. It must be understood that biology is not merely a combined study of botany and zoology; it is the science of life as such. Nevertheless, even in the early stages, it becomes apparent that living organisms can be divided into two distinct kingdoms and it should be appreciated that the study of these two kingdoms is plant biology and animal biology, branches of the same subject.

These various branches of biology cannot be properly understood without suitable practical work involving the examination of organisms, dissection, microscopical examination and the performance of experiments in biochemistry, and physiology and, if possible, in genetics. This book is an attempt to supply this need and it has been divided into appropriate parts as follows:—

Part   I. Microscopical Technique   Part   V. Physiology
Part II. The Variety of Living   Part   VI. Embryology
Organisms — Morph-   Part VII. Other Forms and
ology and Anatomy   Modes of Life
Part III. Cytology and Histology   Part VIII. Genetics
Part IV. Biochemistry

As stated in the Preface, it is *essentially a laboratory manual* and must be used in conjunction with text-books of biology. In choosing the material for the book I have endeavoured to cover the latest syllabuses in Biology of the various Examining Boards for the General Certificate of Education and of First Medical Examinations. At the same time I have not confined the text to these particular requirements. Some of the more recent of these syllabuses do not, in fact, specify particular types while others allow a wide latitude of choice and those included in the book are intended to give an overall picture. In a number of instances only external structure is given because this is all that is required by the syllabuses. Forms and modes of life which differ from those of typical plants and animals are dealt with in a separate part of the book. I have tried to give

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adequate instructions for carrying out the practical work *without long and tedious reading* of facts which can be learned from a text book. Illustrations have been included where it has been considered desirable in order to assist the student in his work and some of these are intentionally diagrammatic or semi-diagrammatic as it is *essential that he draws exactly what he sees in his own preparations.*

In the Introduction I have given general instructions for practical work, the keeping of practical notebooks and a list of apparatus and instruments required, while 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 classification. The appendixes contain information which it is hoped may be of some use to those in charge of biological laboratories while some of it may be helpful to students themselves.

My grateful thanks are due to my publishers, and to Mr. Owen R. Evans in particular, for their very considerable help and co-operation in the production of the book, to Mr. Frank Price for his assistance in preparing my drawings for the block-maker and to my wife for reading the proofs, thus making the task of checking and correction so much less arduous.

<div align="right">C.J.W.</div>

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* for Advanced Level and 1st M.B. examinations. It was therefore decided to revise the original book, (in effect to publish a new book) which would provide for their needs and it is hoped that the present volume will fill this gap. I have naturally drawn on the text and illustrations from my *Practical Botany* and *Practical Zoology* where these have proved suitable, but I have adapted them as need be to the purpose in hand and have included a great deal of extra material and a large number of new illustrations to satisfy the requirements of the subject *Biology*.

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.

As in my other 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

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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.
April, 1966

ACKNOWLEDGEMENTS

INTRODUCTION

I. GENERAL DIRECTIONS FOR PRACTICAL WORK

(1) Cleanliness, tidiness and accuracy are of the utmost importance. See that all your dissecting 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.*
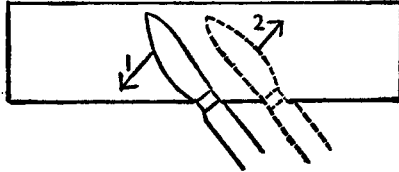

FIG. 1. **Sharpening a scalpel.**

For sharpening of section cutting razors see pp. 17, 18. Scissors must be sharpened by an expert. *Never use scalpels, scissors or razors for any purpose other than that for which they were 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 (anatomy) and functions (physiology) of the various organs and systems.

II. GENERAL DIRECTIONS FOR THE KEEPING OF PRACTICAL NOTEBOOKS

DRAWINGS

(i) Print the name of the animal or plant and the system, structure, organs or tissue on top; in the case of dissections state whether it is a dorsal or ventral dissection or an entire specimen, and in the latter case 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.*, removal or deflection of organs, method of staining.

(iii) Then make a **drawing** or **diagram** in pencil, 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.)

(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 or of part of the structure under the *high power*, all suitably named.

FIG. 2. LOW POWER DIAGRAM.
(Plant Tissues)
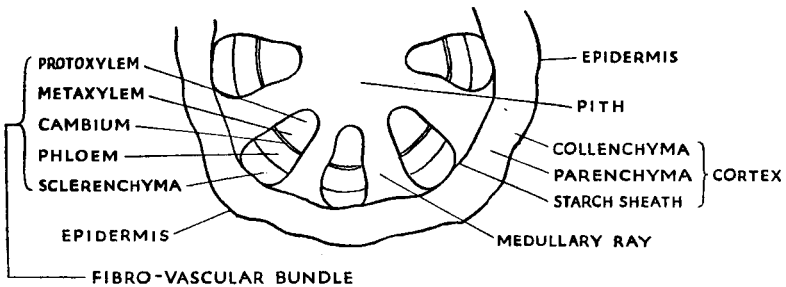
(vi) All drawings and diagrams should be drawn in pencil and should be *large*. When drawing a dissection the outline of the animal (or part of it) should be shown where possible in order to show the position and relationship of parts. The use of shading and colours

should be kept down to a minimum, *e.g.*, red for arteries, and blue for veins.

The names of all parts printed in **thick type** in the text which follows should be included in the drawings *provided that they can be seen in the specimen,* but in some cases *all* the structures mentioned in the text may not be seen or identified.
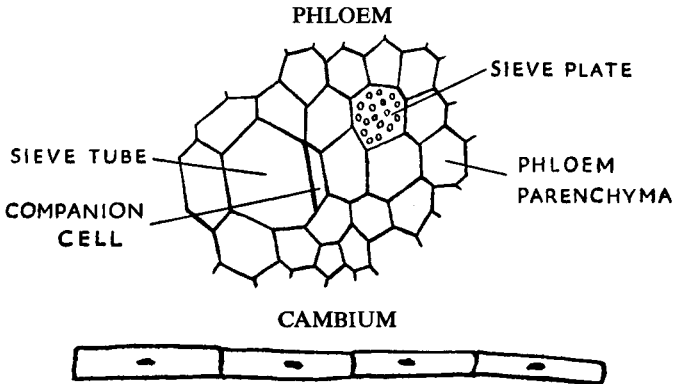
Diagrams (or plans) of systems may occasionally be desirable in addition to drawings of dissections (*e.g.*, blood systems). Here again *the general outline of the animal and, where desirable, of other*

FIG. 3. HIGH POWER DRAWINGS OF TISSUES INDICATED
IN LOW POWER DIAGRAM.
Walls thickened with lignin.

SCLERENCHYMA—

CUTICLE
EPIDERMIS
COLLENCHYMA
PARENCHYMA
INTERCELLULAR SPACE
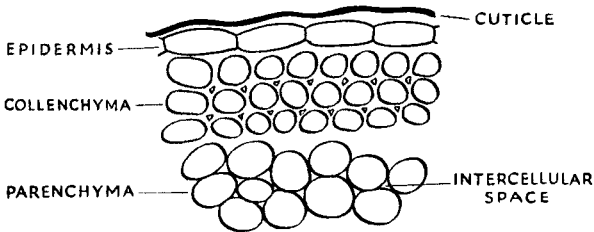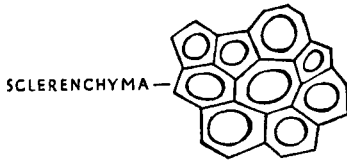
Walls thickened with cellulose, particularly at the corners.
Thin cellulose walls with many intercellular spaces.

PHLOEM

SIEVE PLATE
SIEVE TUBE
COMPANION CELL
PHLOEM PARENCHYMA

CAMBIUM

XYLEM

METAXYLEM
(VESSEL)

XYLEM
PARENCHYMA

PROTOXYLEM
(VESSEL)
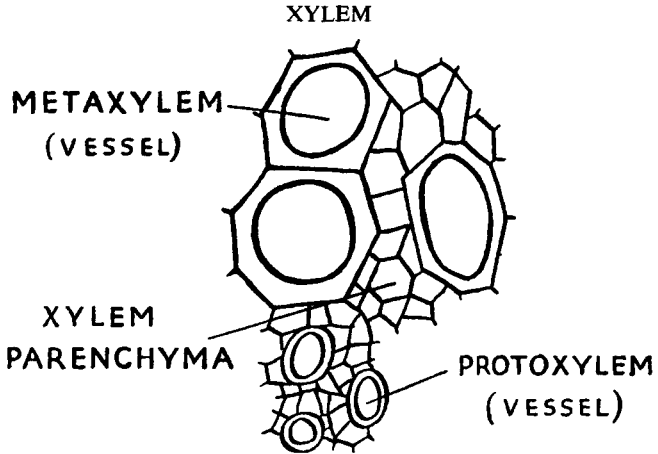
Walls thickened and lignified. No protoplasmic contents.

FIG. 4. LOW POWER DIAGRAM.
(Animal Tissues)

----Mucous Membrane

----Connective Tissue Layer

Muscular Layer
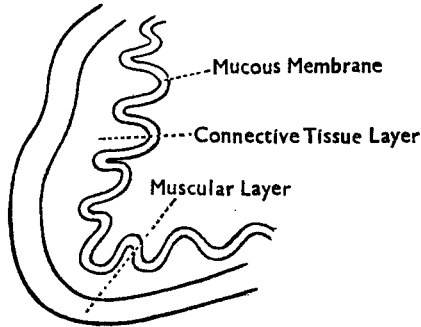
FIG. 5. HIGH POWER DRAWING OF TISSUES INDICATED IN LOW
POWER DIAGRAM.

Columnar
Epithelium

Areolar
Tissue

Unstriated
Muscle

Mucous
Membrane

Connective
Tissue
Layer

Muscular
Layer
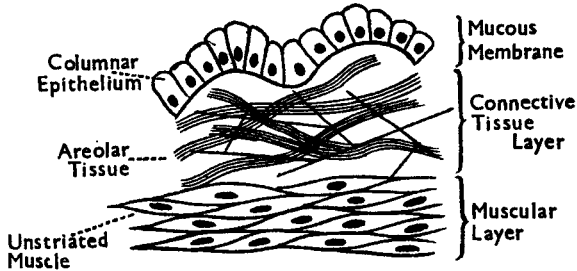
organs, should be indicated by a single line to show the relationship of the parts.

(vii) Never copy drawings or diagrams from text-books. 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 designed solely to help the student to find and identify the various structures and tissues. Many of the figures are diagrammatic or semi-diagrammatic, though this is not so in all cases. This is intentional.*

III. INSTRUMENTS AND APPARATUS REQUIRED

By each Student

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

    1 large all-steel *scalpel* (1¾ in. blade).
    1 medium all-steel *scalpel* (1½ in. blade).
    1 small all-steel *scalpel* (1 in. blade, or less).
    Alternatively, scalpel handles with detachable blades of various shapes and sizes can be purchased.
    1 pair of large *scissors* (5 or 5½ in. overall length).
    1 pair of small *scissors* with fine points (4 or 4½ in. overall length).
    1 pair of large *forceps*, blunt (5 in.).
    1 pair of small *forceps* with fine points (4½ in.).
    3 or 4 mounted *needles*.
    1 *seeker*, 1 *camel-hair brush*, 1 *section-lifter*.
    1 section-cutting razor.

    Other instruments, *e.g.*, *bone forceps*, may be added as desired.
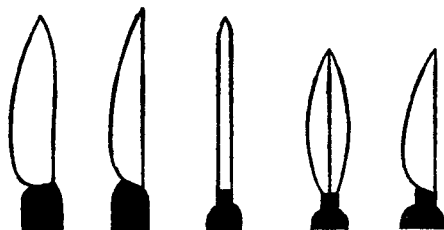

FIG. 6. Scalpel Shapes.

(2) Large-page practical *note-books* or *files*, with plain pages. The Elementary Biochemistry and the 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 (7) below.

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

(6) At least one *white coat* is advisable 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) *Dissecting dishes.* Rectangular enamel trays (about 8 × 6 in.) with black wax composition in the bottom are better than those which contain weighted cork.

(2) *Dissecting boards* with a rim round the edge (about 24 × 18 in.) (which may be fitted with hooks or rings at the corners) for larger animals.

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

(4) *Pins*, large and small; and *awls* for large animals.

(5) *Thread* for ligatures.

(6) *Preserving tank* for animal material containing 4 per cent. formaldehyde.

(7) *Microscopes* (unless provided by the students) with $\frac{2}{3}$ and $\frac{1}{6}$ in. objectives, on a triple nose-piece, and No. 2 (× 6) and No. 4 (× 10) eyepieces. A few better instruments fitted with sub-stage condensers, Nos. 2 (× 6), 4 (× 10) and No. 6 (× 15) eye-pieces, and a $\frac{1}{12}$ in. O.I. objective are also desirable. A blue filter to fit below the condenser giving a daylight effect is an advantage when using artificial light unless lamps with daylight bulbs are used. *All must be kept covered when not in use.*

(8) *Microscope lamps*, preferably fitted with daylight bulbs.
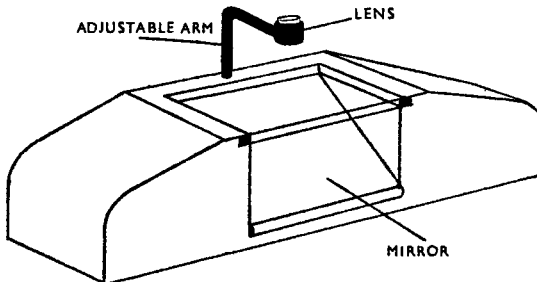
(9) A *dissecting microscope*.

FIG. 7.   A Simple Dissecting Microscope.

(10) *Stains* and *Reagents*. See Appendix I.

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

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

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

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 entire 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.

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 Plants and Animals** may be summarised as follows:—

Plants	**Animals**
1. Nutrition is *holophytic.* Inorganic food is synthesised into organic compounds to build up protoplasm and to provide energy.	1. Nutrition is *holozoic.* Food is organic and consists of plants or other animals or both.
2. *Chlorophyll* is necessary for this method of nutrition and occurs in plant cells.	2. Chlorophyll is not found in animal cells.
3. *Cellulose* 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 known as *Cryptogamia.* Flowering

Plants constitute the Division *Spermatophyta* (formerly known as *Phanerogams*).

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.

CLASSIFICATION OF ANIMALS

The **Animal (Animalia) Kingdom** is divided into two **Sub-Kingdoms, Protozoa** (non-cellular animals) and **Metazoa** (multicellular animals) The main divisions of these Sub-Kingdoms are the **Phyla,** of which the following are represented in this book, *e.g., Protozoa, Porifera, Cælenterata, Platyhelminthes, Nematoda, Annelida, Echinodermata, Arthropoda, Mollusca, Chordata,* etc.

Each Phylum is divided into **Classes,** the Classes into **Orders** and these into **Families.** Animals in the same Family are arranged in **Genera** and the varieties of animals in the same genus are classified into **Species.** In some cases, there are also Sub-Phyla, Sub-Classes, etc.

BINOMIAL NOMENCLATURE

Every plant and animal is given two names, its generic name and a specific name. Thus the creeping buttercup is *Ranunculus repens* and the bulbous buttercup *R. Bulbosus.* The common frog is *Rana Temporaria* and the continental edible frog *R. Esculenta.* This is known as **binomial nomenclature.**

The Divisions or Phyla, Classes and Orders to which each of the plants and animals treated in this book belongs are given, together with the bare outlines of the characteristics of these groups, at the beginning of the text in each case. Further details of the characteristics of the various groups will be found in the appropriate text-books.

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. 8):—

(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**
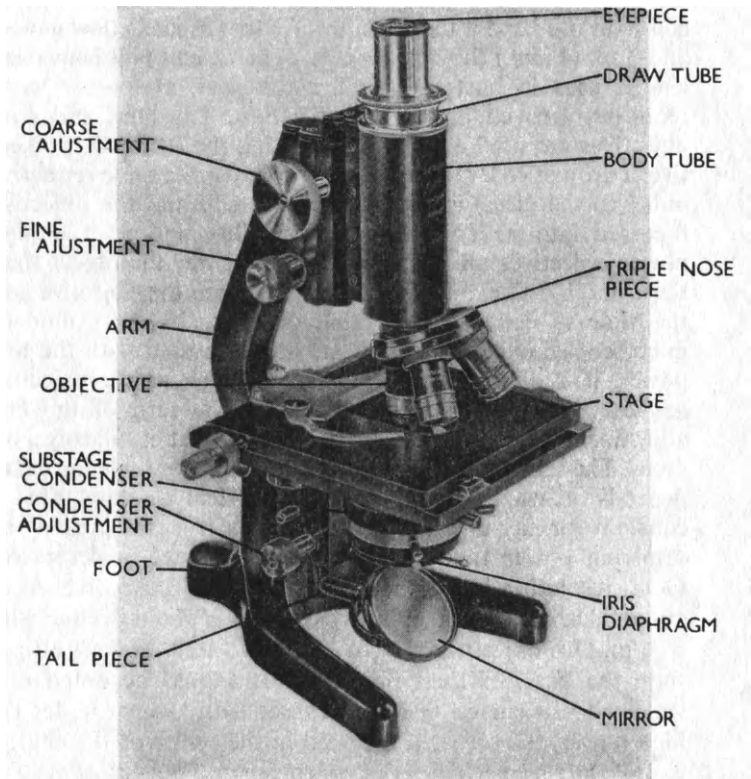

FIG. 8. The Microscope.

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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 eye-pieces, 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 $\frac{1}{12}$ 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 $\frac{1}{12}$ 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 $\frac{1}{12}$ 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), or 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
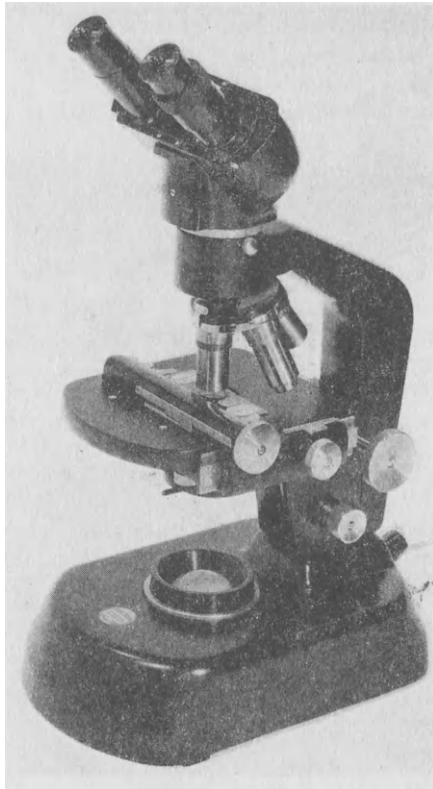

FIG. 9. **Binocular microscope with built in lamp.**

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—

$\frac{2}{3}$ in. objective magnifies 10 times,

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

and the $\frac{1}{12}$ 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.

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

(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, physiological saline, 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 **sagittal** (radial longitudinal), depending on the symmetry of the object from which they are cut.

---

*Several permanent mounts of tissues should be made. Suitable reagents and stains are given in the text. Methods of preparation of reagents and stains are given in Appendix 1.

†This information is included but in some practical examinations ready cut sections are provided.

For hand section-cutting a *sharp* **section-cutting razor,** hollow-ground on one side and flat-ground on the other, is necessary but this will normally be required only for work with plant material.
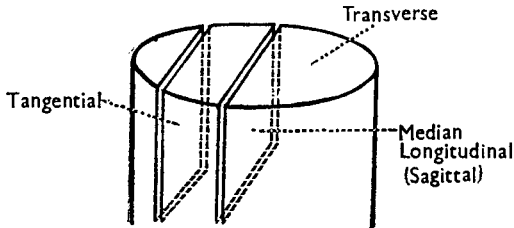

FIG. 10. Kinds of sections.

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, *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.
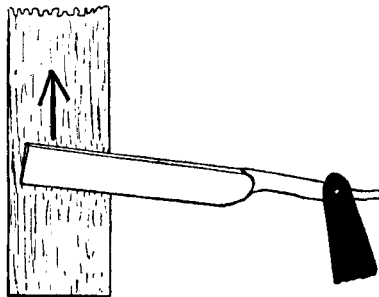

FIG. 11. Stropping a Razor.

(2) Honing

Periodically, stropping is insufficient and a razor requires sharpening on a stone. Put a small quantity of oil on an oil-stone and draw the razor, *edge foremost*, obliquely across the stone, keeping the
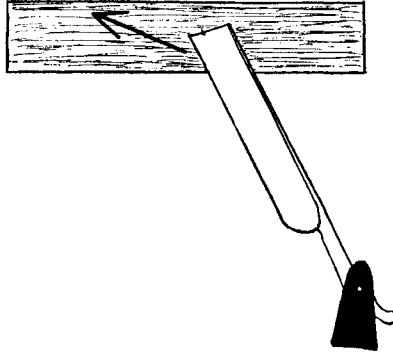
FIG. 12. **Honing a Razor.**

blade flat on the stone. Then turn the other edge to the stone and repeat the movement. This should be done several times.

Cutting Sections

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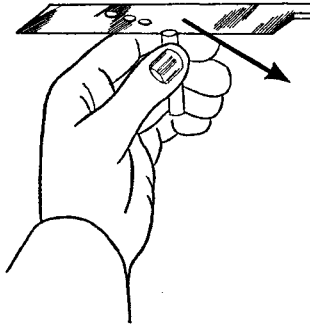

FIG. 13. **Hand Section-cutting.**

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. 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.) 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. 13.)

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 section lifter 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 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 molted 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* 10μ.

(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.*

*See Appendix 1 (8).

(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 stains and (iii) **hardens** it for section cutting. The tissues 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. 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	Use	Washing Medium
Alcohol (ethyl), 70%	Plant and Animal tissues	70% alcohol.
Acetic alcohol – –	Animal tissues.	70% alcohol.
Bouin's fluid – –	Plant and Animal tissues.	50 and 70% alcohol.
Mercuric chloride –	Plant and Animal tissues.	Iodine-alcohol. Decolorise with sodium thiosulphate.
Picric acid – –	Plant and Animal tissues.	50 and 70% alcohol.
Acetic acid – –	Animal nuclei.	50% alcohol.
Chromo-acetic – –	Plant tissues.	Water then 70% alcohol.
Corrosive acetic –	Animal tissues.	Iodine-alcohol. Decolorises with sodium thiosulphate.
Flemming's solution –	Plant tissues.	Water.
Formaldehyde –	Animal tissues.	70% alcohol.
Formalin-alcohol –	Plant tissues.	70% alcohol.
Müller's fluid – –	Animal tissues.	Water. Decolorise in 1% chloral hydrate.
Osmium tetroxide – "Osmic acid") –	Protozoa.	Water.
Potassium dichromate	Animal tissues.	Water. Decolorise in 1% chloral hydrate.

*Material is generally supplied to students already fixed by the laboratory.
†See Appendix I (1) for their preparation.

(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.

(ii) **Regressive staining** in which the tissue is intentionally overstained 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

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

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.

Stains for Animal Tissues

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

Stain	Solvent	Use
Borax-carmine – –	**Alcohol.**	**Nucleus-pink.**
Eosin Y – – –	**Water or alcohol.**	**Cytoplasm—pink.**
Haemalum – – –	**Water.**	**Nucleus—blue.**
Haematoxylin (Delafield) –	**Alcohol.**	**Nucleus—blue.**
Haematoxylin (Ehrlich) –	Alcohol.	Nucleus—blue.
Leishman's stain – –	Alcohol (methyl).	Blood corpuscles—red-pink. White (nuclei)—blue.
Methyl violet – – –	Water or alcohol.	Nucleus—violet.
Methyl blue – – –	Usually alcohol.	Nucleus, blood—blue.
Picro-carmine – – –	**Water.**	**Nucleus—red.** Cytoplasm—yellow.
Van Geison – – –	Water.	Epithelium—yellow. Connective tissue—red. Muscle—yellow.

Double Staining

$\Big\{$ Haematoxylin (Delafield or Ehrlich) or Haemalum.
Eosin Y.

$\Big\{$ Borax-Carmine.
Eosin Y.

$\Big\{$ Haematoxylin or Haemalum.
Van Geison.

*See Appendix I (4).
†Double staining is not permitted in some examinations.

Stains for Plant Tissues

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.
Aniline sulphate (or *hydrochloride*) – –	*Water.*	*Lignin—yellow.*
Bismarck brown – –	Alcohol.	Cellulose and nucleus— brown. Bacteria.
Carbol-fuchsin – –	Alcohol.	Bacteria and Fungi—red.
Congo red – – –	Water.	Fungal hyphae—red.
Eosin Y. – – –	**Water or alcohol.**	**Cyptoplasm—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.
Phloroglucin and conc. hydrochloric acid	Alcohol.	*Lignin—red.*
Safranin – – –	**Alcohol.**	**Lignin—red. Suberin—red. Nucleus—red.**
Schultze's solution (*chlor-zinc-iodine*)	*Water.*	*Cellulose—blue (or violet). Starch—Blue. Proteins—yellow. Lignin—yellow.*
Sudan III – – –	*Alcohol.*	*Fats—red.*

Double Staining

$\left\{\begin{array}{l}\text{Safranin.}\\\text{Light Green.}\end{array}\right.$

$\left\{\begin{array}{l}\text{Safranin.}\\\text{Haematoxylin or Haemalum.}\end{array}\right.$

$\left\{\begin{array}{l}\text{Haematoxylin or Haemalum.}\\\text{Eosin.}\end{array}\right.$

(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

*See Appendix I (2)

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.*
> 50   ,,   ,,
> 70   ,,   ,,
> 90   ,,   ,,
> *Absolute alcohol.*

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* and *natural oil of cedar wood* for animal tissues though oil of cloves may be used for both plant and animal tissues. Xylene can be used but it has a tendency to cause shrinkage.

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

*See Appendix I (5)
†See Appendix I (6).

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 Mounts

ANIMAL TISSUES

   (i) Physiological saline (R.I. = about 1·34).

      For Invertebrate tissues and vertebrate

blood	0·6 per cent.
Amphibian tissues (except blood) . .	0·75 ,, ,,
Mammalian tissues (except blood) . .	0·9 ,, ,,

or (ii) Ringer's Solution (Invertebrate tissues).
Locke's Solution (Mammalian tissues).

PLANT TISSUES

Glycerine 50 per cent. Aq. (R.I. = about 1·39–1·34).

(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 section lifter. 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 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.

FIG 14. **Levering down the Coverslip.**

*See Appendix I (7).

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.

Larvae, small insects and chitinous structures can be mounted in *Berlese's Medium.** If this is used or if the specimen is thick, the coverslip must be raised from the slide by means of a **cell** of the required height which is affixed to the slide. For some objects, a **cavity slide** is preferable.

(7) RINGING

Place the slide on a turntable (Fig. 15) and fix it in position by the springs provided.

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.

(a) Seen from above

(b) Side View
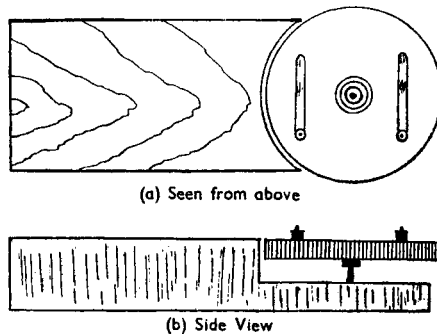
FIG. 15. Turntable for Ringing Slides.

(8) SMEARS

It is obviously impossible to cut sections of such tissues as blood and the contents of the seminal vesicle of the earthworm. The following procedure should therefore be used in these cases:—

*Make a thin film (called a **smear**) either on the slide or on the cover-slip. This may be done as follows: Place a drop of the fluid at one end of a slide and hold another slide in contact with the fluid at an angle of about 45°. Draw the second slide over the first to produce a film of even thickness. Allow this to dry in the air or by gentle heat well above*

*This is also a clearing agent for these objects.

the flame of a spirit lamp. Then treat the smear by placing the slide (or coverslip) in the stains and reagents in a similar manner to that used for sections.

(9) 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.
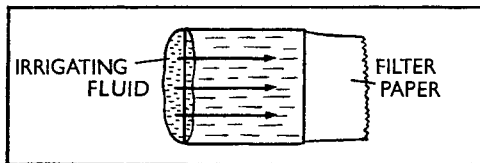
FIG. 16. **Irrigation.**

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 **Animal Tissues** generally.

(i) **Single Staining with Picro-Carmine**
  (1) Picro-Carmine (to stain). . . . 2-5 mins.
  (2) Distilled water (to remove excess stain) . 2 mins.
  (3) 30% Alcohol (to dehydrate) . . . 2 mins.
  (4) 50% Alcohol . . . . . 2 mins.
  (5) 70% Alcohol . . . . . 2 mins.
  (6) 90% Alcohol . . . . . 2 mins.
  (7) Absolute Alcohol . . . . . 2 mins.
  (8) Absolute Alcohol . . . . . 1 min.
  (9) Natural Oil of Cedar Wood (to clear) . 5 mins.
  (10) Mount in Canada Balsam.

(ii) Single Staining with Borax-Carmine

   (1) Borax-Carmine (to stain) . . . 2-5 mins.
   (2) 70% Alcohol (to remove excess stain) . 2 mins.
   (3) 90% Alcohol (to dehydrate) . . . 2 mins.
   (4) Absolute Alcohol . . . . . 2 mins.
   (5) Absolute Alcohol . . . . . 1 min.
   (6) Natural Oil of Cedar Wood (to clear) . 5 mins.
   (7) Mount in Canada Balsam.

(iii) *Single Staining with Ehrlich's Haematoxylin and †Double Staining with Ehrlich's Haematoxylin and Eosin Y

   (1) Ehrlich's Haematoxylin . . . 10-15 mins.
   (2) Acid Alcohol (to differentiate) . . 3 mins.
   (3) 70% Alcohol (to remove excess of stain) . 5 mins.
   (4) Tap water (to "blue" Haematoxylin) . 2 mins.
   (5) 70% Alcohol . . . . . 2 mins.
   (6) 90% Alcohol . . . . . 2 mins.
   (7) Eosin Y, alcoholic. . . . . 1 min.
   (8) 90% Alcohol (to remove excess stain and
          dehydrate). . . . . . 2 mins.
   (9) Absolute Alcohol . . . . . 2 mins.
  (10) Absolute Alcohol . . . . . 1 min.
  (11) Natural Oil of Cedar Wood (to clear) . 5 mins.
  (12) Mount in Canada Balsam.

These methods are suitable 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.
   (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.

*If Single Staining with Ehrlich's Haematoxylin is required, simply *omit processes* (7) *and* (8).
†It should be ascertained whether double staining is required or permitted in any particular examination syllabus.

(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% Alcohol (to remove excess Safranin)   2 mins.

    (4) Delafield's Haematoxylin (stains cellulose)   3 mins.

    (5) Distilled water (to remove excess Haema-
         toxylin)   .   .   .   .   .   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

THE VARIETY OF ORGANISMS

MORPHOLOGY AND ANATOMY

INTRODUCTORY NOTES

Reference should be made to the General Directions for Practical Work and the *Keeping of Practical Note-books* in the *Introduction* and to *Part I (Microscopical Technique) where necessary.*

(1) Personal examination of plants and animals is the only way to learn their physiology and anatomy. The student should avail himself of every opportunity to examine them *alive*, observing the development and seasonal changes of plants and how animals live by day and night, how they procure their food, breathe, move, grow, reproduce, avoid their enemies, etc. These observations may be made on plants grown in the garden and on animals kept in vivaria and aquaria, while visits to botanical and zoological gardens and observations made in the country, will be found most beneficial and instructive.

For an accurate knowledge of the anatomy and physiology of the living plant and animal, however, it is necessary to make a more detailed study. This often involves the examination of the dead organism at any rate in the case of animals. Small plants and animals are examined under a hand lens and microscope. Larger animals must be dissected and larger plants require a microscopical study of their cells and tissues.

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

(3) **Larger animals** are first examined externally, alive in the first place when possible. A series of dissections is than made on the killed animals to expose the various systems, the **anatomy** of which is studied in detail. The **histology** of the organs is also studied.†

Larger plants are also first examined externally, alive when possible, the **morphology** and modification of the different organs being studied separately. This may involve the dissection of some of them. Then a study of the **histology** of the organs must be made.

(4) Simple biochemical and physiological experiments should also be performed with both **plants** and **animals** in order to obtain an understanding of their **physiology**. The records of these experiments can be conveniently kept in a separate part of the note-book or file.

*See Part I for detailed instructions in microscopical technique.

The **embryology** of the flowering plant and of certain specified chordate types is also studied.

(5) The inheritance of characters will be better understood by the performance of some experimental **genetics.**

(6) Finally a study of **mycology** and **parasitology** will give an insight into the lives of organisms which differ in structure and live differently from typical plants and animals and, in the case of parasites, which may cause disease in other organisms.

(7) It may be desirable or necessary to study the organisms in a different order from that given in the book according to the availability of material.

(8) DISSECTION TECHNIQUE

Dissection is an art which can only be acquired by practice. Do not attempt any practical work until you have thoroughly studied the appropriate subject in your text-book.

   (i) Small animals, such as the earthworm and frog, are dissected under water in a dish. This gives support to the organs, keeps them apart, and renders them more easily visible. Always *keep the water clean*: replace it by fresh as soon as it becomes cloudy or dirty.

  (ii) Using pins of suitable size, fix the animal to the bottom of the dissecting dish or, in the case of large animals such as the rabbit and rat, tie the legs to the hooks or rings at the corners of the dissecting board or pin them in position by means of awls. *Keep the parts tightly stretched.*

 (iii) Always fix in the pins or awls *obliquely*, so that they do not get in your way.

 (iv) See that your scalpels and scissors are sharp and that all your instruments are clean.*

  (v) Read the directions carefully *before* beginning your dissection.

 (vi) Never cut anything until you are sure what it is.

(vii) Choose a scalpel of suitable size and shape and hold it as you would a pen.

(viii) Be gentle. If you are rough with your instruments you may damage the organs or cut blood vessels and your dissection will be a failure.

 (ix) Cut through the skin and muscular body wall and pin them down out of your way.

  (x) Mop up blood and body fluids with cotton wool.

*For sharpening of instruments see Introduction page 1.

(xi) You may displace or deflect organs if necessary to expose others to view. Black paper may be placed underneath obscure structures in order to make them more easily visible but it must not be used if it will hide important related structures or organs.

(xii) Always dissect *along* and not across blood vessels and nerves.

(xiii) When the required organs are exposed to view, examine them carefully and make a clear outline *drawing of exactly what you see in your specimen* and as near as possible to scale, making the organs the right size (in proportion) and the right shape. Make *large* drawings, be sparing in the use of colours —red for arteries and blue for veins—and keep shading down to a minimum.*

(xiv) Preserve your specimens, if required for future use, in 10 per cent. formalin or 70 per cent. alcohol.

(xv) The major dissections—those most frequently required in examinations—should be repeated until as near perfection as possible is attained.

(xvi) Thoroughly clean and dry your instruments immediately after use. A light coating of vaseline will prevent their rusting.

(9) Write notes on the method of your dissections and of observations made (*e.g.*, any abnormalities) where desirable. Do not let your note-book be simply a collection of drawings and diagrams.

*As already stated in the Introduction, a number of the illustrations in this book are intentionally diagrammatic or semi-diagrammatic though this does not apply in all instances. They are solely to *guide* the student in his practical work.

I. NON-CELLULAR ORGANISMS

AMOEBA PROTEUS

Amoeba is an animal belonging to the SUB-KINGDOM *Protozoa* and the PHYLUM *Protozoa* (Non-cellular animals, in most of which nutrition is holozoic) and the CLASS *Rhizopoda* (Lack a pellicle and have no definite body form. Reproduction asexual). It lives in the mud of ponds.

(1) *Examine a drop of water containing living* **Amoebae.** *Find an Amoeba under low power. Draw the outline at intervals of half a minute or so. Then examine under high power.*

Note the movement and the changes of shape due to the flowing out of promontories of protoplasm called **pseudopodia.** By careful focussing you will be able to distinguish a clear narrow outer layer of protoplasm, the **ectoplasm** (or **plasmagel**), enclosing the granular **endoplasm** (or **plasmasol**). Note that the endoplasm shows a flowing movement. In it may be seen small spaces, or vacuoles, some of which,
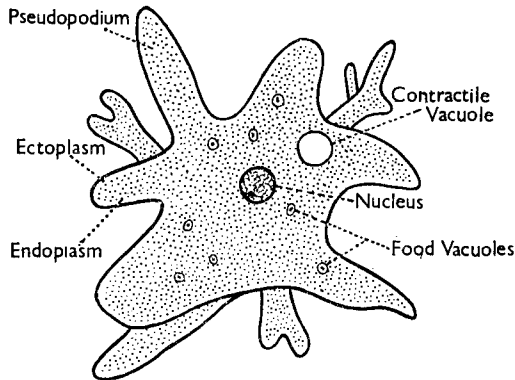
FIG. 17. Amoeba proteus.

food vacuoles, may contain particles of food such as algae. Look for the **contractile vacuole,** which disappears and reappears rhythmically at the same spot: its function is osmo-regulation and possibly to some extent excretory. Note also the **nucleus.** *If possible, make drawings at timed intervals of an organism* **undergoing binary fission.** During the winter months, **encysted amoebae** are sometimes found. These are spherical in shape, devoid of pseudopodia and are enclosed in a protective **cyst.** Placing the tube in warm water will often restore activity.

(2) *Irrigate with 1 per cent. acetic acid or ¼ per cent. "osmic acid" to*

*kill and fix the organisms; then stain with picro-carmine by irrigation.
Also examine a prepared slide.*

The structures in (1) will be more easily seen, but, of course, the
contractile vacuole will be at rest.

PARAMOECIUM

Paramoecium is also a non-c llular animal in the PHYLUM *Protozoa* and in
the CLASS *Ciliophora* (Movement by cilia. Two nuclei present. Reproduction
asexual but a sexual process may occur). It lives in pond water.

(1) *Examine a drop of water containing* **Paramoecia.** *The organisms
move rather quickly. Put a drop of weak gelatin sol or a few fibres
of cotton wool on a slide and add a drop of the Paramoecium culture.
Cover. The organisms cannot move so quickly and their structure can be
more easily seen.*

Note the rounded **anterior end** and the pointed **posterior end.**
Note also that the animal has a constant shape. This is due to the
pellicle which surrounds it. Movement is effected by the **cilia,** short
hair-like processes which cover the surface.

On one side is a shallow **oral groove,** leading to the **cytopharynx**
which runs obliquely backwards to the **cytostome** (or mouth). This
is called the **oral side,** as opposed to the other, which is the **aboral
side.** In the cytopharynx the cilia are fused, forming the so-called
undulating membrane.

The **ectoplasm** is a clear narrow outer layer, as in Amoeba, but it
contains a number of minute capsules, each containing a thread,
the **trichocysts.** The **endoplasm** is granular and contains a number
of **food vacuoles** and two contractile vacuoles, the **anterior** and
posterior contractile vacuoles, into which the water is poured from
ducts which radiate around them, the **formative vacuoles.** There are
two nuclei, a large oval **meganucleus** in which there is a niche on the
aboral side containing the smaller spherical **micronucleus.**
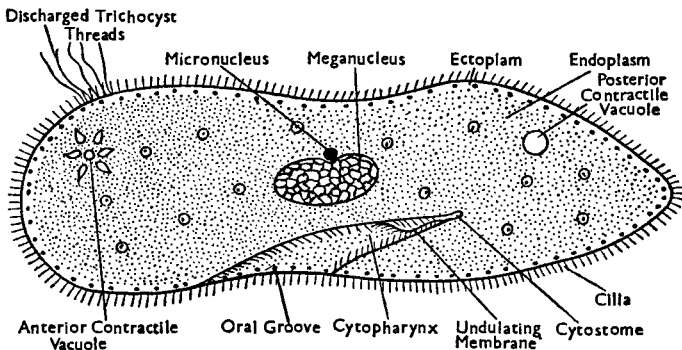
FIG. 18. Paramoecium.

Look for stages in **asexual reproduction** *and* **conjugation.**

(2) *Irrigate with iodine or feed with iodine ink.*

Note the discharged **trichocysts.**

(3) *Irrigate a fresh drop of the culture with* ¼ *per cent. "osmic acid" to kill and fix the organisms. Stain with picro-carmine by irrigation. Also examine a prepared slide.*

The structures in (1) will be more easily seen.

VORTICELLA

This "bell animalcule" is classified in the same **Phylum** and **Class** as Para-moecium. It is a fresh-water organism, living in ponds and is attached to weeds: It is sessile.

Examine a slide of **Vorticella.**

The animal is shaped like a **bell** on a long **stalk.** The free end of the bell has a thick **rim,** inside which is a groove called the **peristome** encircled by a retractable **disc.** The inside of the bell is filled with protoplasm, differentiated into **ectoplasm** and **endoplasm** and containing a **micronucleus** and a **meganucleus,** the whole being enclosed
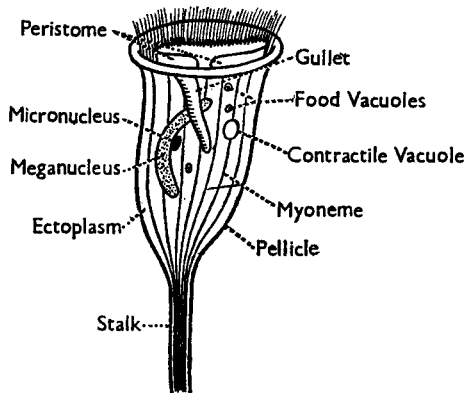
FIG. 19. **Vorticella.**

in a **pellicle.** Leading down from the peristome is a tubular **gullet** and round the edge of the disc and the upper part of the gullet are two rows of **cilia. Myoneme fibres** are present running longitudinally down the bell and these join to form a central **myoneme** which runs down the **stalk** where it is enclosed in **ectoplasm** and the **pellicle.** Near the upper end of the bell, lying in the endoplasm is a **contractile vacuole.**

PLEUROCOCCUS (PROTOCOCCUS) VIRIDIS

Pleurococcus is a non-cellular plant in the DIVISION *Thallophyta* (Plants without differentiation into root, stem and leaves: plant body a thallus) and the SUB-DIVISION *Algae* (Thallophytes living in damp or aquatic situations). The CLASS to which it belongs is *Chlorophyceae* (Green Algae) and the ORDER *Chaetophorales*. It grows on the damp side of tree trunks, fences, etc.

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

Under *low power* note that though the spherical units occur singly, they are 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

Chlorella has the same Classification as Pleuroccoccus, except that it is in the ORDER *Chlorococcales*. This organism lives in stagnant water. *Zoochlorella* lives in the endodermal cells of the animal *Hydra viridis*.

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
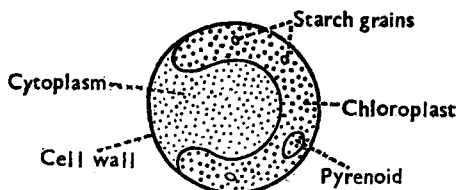
FIG. 20. Chlorella.

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

CHLAMYDOMONAS

Chlamydomonas has the same classification as *Pleurococcus* except that it is in the ORDER *Volvocales*. It lives in stagnant ponds and ditches.

Mount a drop of the water containing living **Chlamydomonas.** *Cover. Also examine a stained preparation.*

Under *high power* and in a prepared slide, preferably under the $\frac{1}{12}''$

O.I. objective, note the ovoid **wall** and the green, cup-shaped **chloroplast**, containing a large glistening **pyrenoid** (containing protein and probably also a centre of starch formation), at the rounded posterior end. In the centre and at the anterior end, *i.e.*, in the cavity enclosed by the chloroplast is colourless **cytoplasm** in contact with the wall. There are two **contractile vacuoles** anteriorly (difficult to see), a
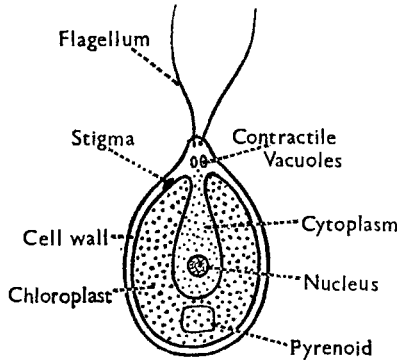
FIG. 21. Chlamydomonas.

nucleus (visible only in stained specimens) and a laterally placed red **pigment spot (stigma)**. The small projection at the anterior end is known as the **beak.** Look for two delicate 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.

DIATOMS

Diatoms are Thallophytes in the SUB-DIVISION *Algae* and the CLASS *Bacillariophyceae* (Non-cellular plants with a cell-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 elipsoid, though the sides are somewhat flattened. The delicate ornamentation of the wall will be clearly seen in this view. The **nucleus** is centrally situated

Pinnularia
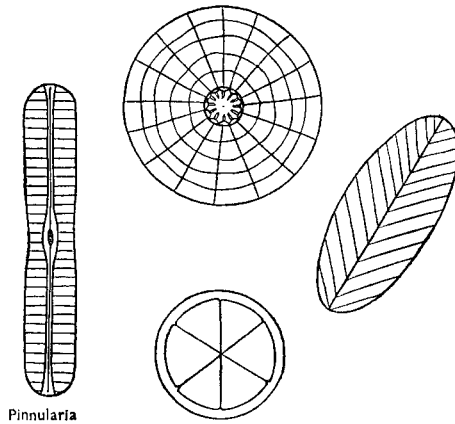
FIG. 22. **Diatoms.**

and is suspended by **cytoplasmic strands** which reach to the cyto-
plasmic lining of the wall. Two large chloroplasts, or rather **chroma-
tophores** 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.

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

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.

This green organism can be considered as an Alga, or as a proto-
zoon. It is, in fact, a "plant-animal". Saprozoic nutrition does occur
in Euglena when holophytic nutrition is impossible.

Peranema, a closely allied form, is similar to Euglena but is
colourless and its nutrition is holozoic.

(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
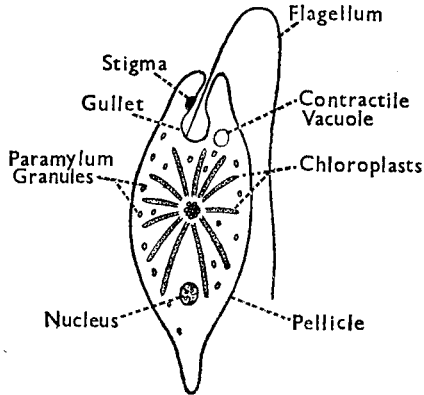
FIG. 23. **Euglena.**

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**
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.

II. SIMPLE MULTICELLULAR ORGANISMS

CARCHESIUM

Colonial types are common amongst the Protozoa except in the Sporozoa. An example is *Carchesium*.

Examine a slide of **Carchesium**

This is a colony of Vorticella-like organisms which lives in ponds. The individuals, known as **zooids**, are interconnected by **protoplasmic branches** enclosed in a **pellicle.** Each zooid has its own independent **myoneme** enabling it to contract quite independently of other zooids. Reproduction takes place asexually by the separation of buds which swim away to form a new colony.
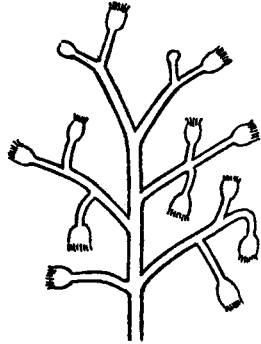
FIG. 24. Carchesium.

PANDORINA

Pandorina is a *colony* of Green *Algae* and is classified as for Chlamydomonas. This organism lives in pond and ditch water.

Examine a drop of water containing living **Pandorina** *or a prepared slide.* Note the spherical colony **coenobium** consisting of sixteen **cells** of structure similar to that of Chlamydomonas but rather pear-shaped. 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. A somewhat similar *Colony Eudorina*, contains thirty-two cells and shows slight differentiation into soma and germ-cells.
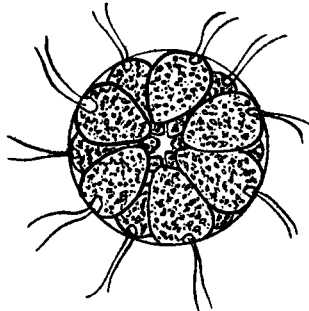
FIG. 25. Pandorina.

VOLVOX

Volvox is classified as for Pandorina. The colonies are visible to the naked eye and live in pond and ditch waters.

(1) *Mount a drop of the 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, up to 20,000 in *V. globator*, and up to 4,000 in *V. aureus*, but some species may contain less than 1,000. The cells are connected together by protoplasmic strands which may or may not be visible. These are more slender in *V. aureus*. Each rounded **somatic cell** contains cytoplasm, nucleus and chloroplast and is enclosed in the space bridged by
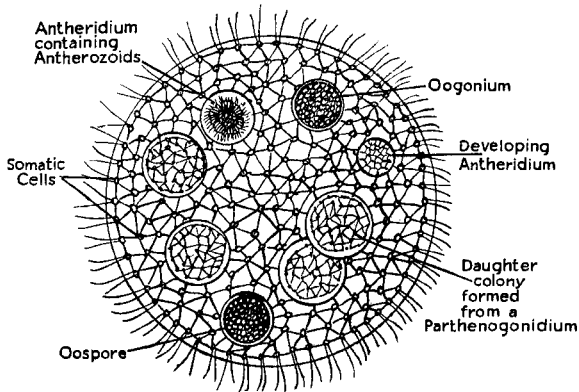
FIG. 26. **Volvox.**

protoplasmic strands. The flagella project outside the colony. Look for **gonidia** (which produce asexually) large rounded cells with large nuclei, and the **germ cells,** which are of two kinds: (*a*) tiny biciliate cigar-shaped male cells, **antherozoids (or spermatozoids)** devoid of chlorophyll and developing in **antheridia,** in plates or spheres, and (*b*) large spherical female cells, **oospheres,** without cilia, containing **chlorophyll** and developing in spherical **oogonia.** *Look for* **oospores** with thick walls, formed by fusion of gametes. In *V. globator* both male and female gametes are found in the same colony (*Monoecious*) whereas in *V. aureus* they are in different ones (*Dioecious*). There is a distinctly advanced division of labour in this type with differentiation into soma and germ cells.

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

SPIROGYRA

Spirogyra is a multi-cellular Green *Alga* and is classified as for Pleurococcus, except that it is in the ORDER *Conjugales*. It lives in ponds near the surface.

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

Under *low power* note that the **filament** is an unbranched thallus of identical cylindrical **cells.**

Under *high power* note the **cell wall** with **protoplasmic lining,** the **nucleus** containing a **nucleolus** and suspended by **protoplasmic strands** (or **bridles**). The spirally wound **chloroplast** has wavy 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.

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

Note **conjugation tubes** in various stages of formation joining two adjacent cells, usually in separate filaments (scalariform conjugation), the so-called **male gametes** (contracted cell contents) in various positions and the so-called **female gametes** (they are isogamous) and the empty cells which have lost their (male) gametes. Large brown ovoid **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 another filament in warm alcohol. Stain with safranin or haematoxylin. Mount in dilute glycerine.*

The nucleus and bridles will be more easily seen.

FUCUS

THE BROWN SEAWEED

Fucus is a Brown *Alga* and is therefore in the CLASS *Phaeophyceae* and the ORDER *Fucales.* It grows in the sea between low and high tide level.

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

The former has serrated edges and the latter bears small bladders to give it buoyancy. Note the colour of the body or **thallus** which 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 in the

outer parts of some of the lobes of the frond, showing small projec-
tions or papillae. These 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 elongated cells, the **meristoderm,** containing
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 larger vacuoles, fewer
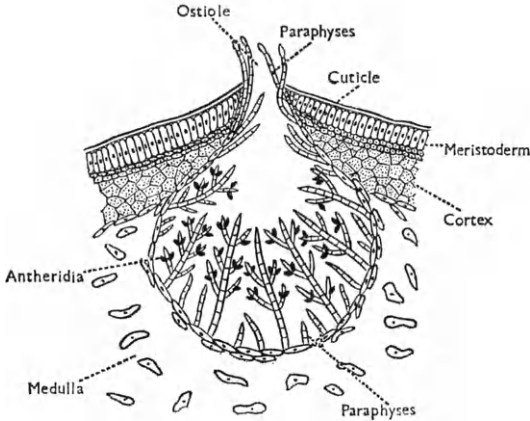
FIG. 27. **Fucus. T.S. Conceptacle—Male.**
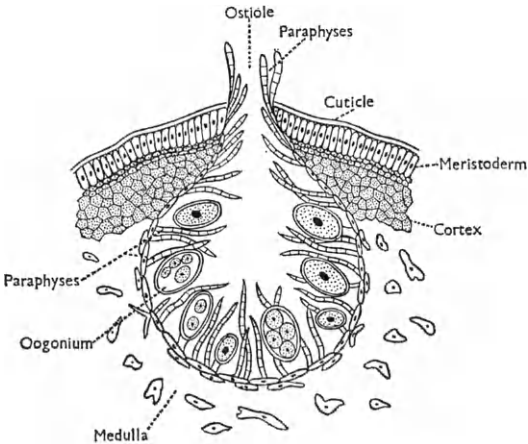
FIG. 28. **Fucus. T.S. Conceptacle—Female.**

phaeoplasts and beneath this layer is the central **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 conceptacles** under the *low* and *high powers.*

In *F. spiralis* the male and female gametes are developed in the same conceptacle, in *F. serratus* and *F. vesiculosus* male and female conceptacles are borne on separate plants.

Note the male and female conceptacles (if separate) containing numerous unbranched hairs called **paraphyses.** In the **male conceptacle** are ovoid antherozoid-producing **antheridia** on branched hairs. Each antherozoid contains one or 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.

HYDRA

Hydra is a Multi-cellular animal and is therefore in the SUB-KINGDOM *Metozoa.* It belongs to the PHYLUM *Coelenterata (Diploblastic Metazoa,* primarily radially symmetrical and with the body wall composed of two layers of cells enclosing a single cavity). The CLASS is *Hydrozoa* (Coelenterates in which the mouth leads into a simple enteron). Jelly-fish, sea anemones and corals belong to different Classes in this Phylum.

There are two common forms—**Hydra viridis,** which is green, and **H. fusca,** which is brown. A third form **H. vulgaris** is almost colourless. All live in fresh-water ponds and are visible to the naked eye.

(1) *Examine the living animal under a hand lens in water in a watch-glass.*

Note the cylindrical **body** bearing a number of **tentacles** on its upper end. The lower end is called the **foot** (or **basal disc**). At the upper end of the body, inside the tentacles, is the conical **hypostome.** A **bud** (young hydra) may be developing from the side of the body.

Stimulate an animal by touching with a mounted needle. Observe the effect.

(2) *Mount in water and examine under the low power, supporting the coverslip on thin pieces of paper to avoid crushing the animal, or, better, use a cavity slide.*

The above structures will be more clearly visible. One or more bulges may be visible high up on the body: these are the **testes.** A

single similar swelling lower down is the **ovary**; thus the animal is hermaphrodite. You may be able to see the **mouth** on the hypostome.

(3) *Examine* **part of a tentacle** *under the high power.*

Note amongst the musculo-epithelial cells (which form the bulk of the cells) the **batteries** of **nematoblasts** (or **cnidoblasts**), pear-shaped cells from each of which projects a short bristle, or **cnidocil.**

(4) *Irrigate with dilute acetic acid or dilute iodine.*

The **nematocyst threads** will be ejected from oval sacs in the nematoblasts called **nematocysts.**

(5) *Examine a prepared slide of a* **T.S. of Hydra** *under the low power.*

Note the outer layer of cells, the **ectoderm** and the inner layer, the **endoderm,** separated by the structureless **mesogloea.** The central cavity is called the **enteron** (or **coelenteron**).

(6) *Examine a* **L.S. of Hydra.**

Note the parts as in (5). *Draw a diagram under low power.*

(7) *Examine the* **ectoderm** *under the high power.*
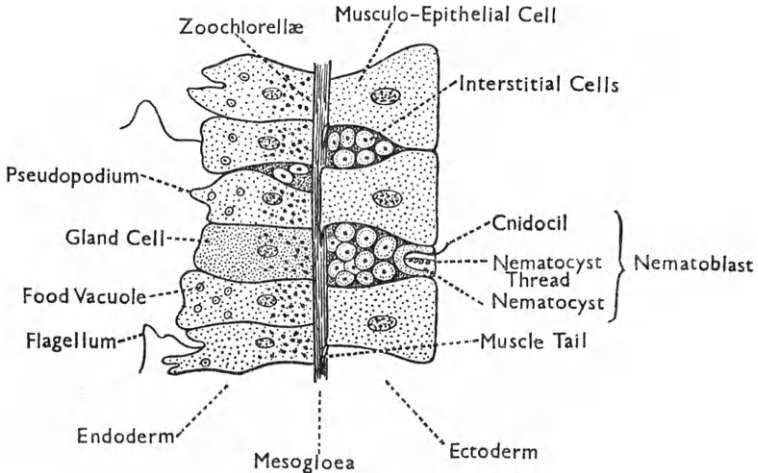
FIG. 29. **Hydra. Cells of Body Wall. High Power.**

Note the roughly conical **musculo-epithelial cells** with their narrow ends inwards, from which long **contractile processes (muscle tails)** project both upwards and downwards. The small rounded cells packed between the musculo-epithelial cells are the **interstitial cells,** and the small, less regularly shaped cells with branched processes, lying near the mesoglea, are the **nerve cells.** *Examine a* **nematoblast.**

These cells are found amongst the musculo-epithelial cells and occur in batteries on the tentacles as already seen. Note the oval sac, the **nematocyst,** inside which is the coiled **nematocyst thread.** The **cnidocil** is attached to the outside of the **nematoblast.** Here and there between the musculo-epithelial cells small **sense cells** will be found.

(8) *Examine the* **endoderm** *under the high power.*

Note the tall, columnar **endodermal cells,** which are widened towards the enteron. These edges are irregular, and are prolonged into **pseudopodia** in some cells, while others possess **flagella.** The cells contain **vacuoles** and **food particles** and, in *H. virids,* minute green plants (Algae) called **zoochlorellae,** which live symbiotically. In *H. fusca* the plants are **zooxanthellae. Interstitial cells** may be found here and there between these cells. Amongst these cells, too, are the **secretory** or **gland cells.**

(9) *Examine a* **T.S.** *or* **L.S. of Hydra through a testis.**

Note the spherical **testis** containing **developing spermatozoa.**

(10) *Examine a* **T.S.** *or* **L.S. of Hydra through an ovary.**

Note the spherical **ovary** containing **stages in development of the ovum** (or an **ovum** with a prominent nucleus and many yolk spherules).

OBELIA

Obelia is a colonial type of *Coelenterate* and is therefore classified as for Hydra, but it exists in three different forms, one of which is a *colony* of Hydra-like animals. The three forms are (i) a branched colony of Hydra-like organisms, the **hydranths** or **polyps,** which also bears (ii) **blastostyles** from which develop (iii) free swimming **medusae,** minute jelly-fish. The individual forms are termed **zooids.**

This is sometimes considered as *alternation of generations* or *metagenesis* but it is really a case of *trimorphism,* since there are three forms—the hydranth, the blastostyle and the medusa. The existence of many different forms of the same organism is called *polymorphism.*

There is no real alternation between the asexual and sexual forms and, furthermore, apart from the gametes, the cells in all forms have diploid nuclei.

The **Common Jellyfish** (*Aurelia aurita*) is in a separate Class (*Scyphomedusae*) in which the predominant stage is the medusa while the **Sea Anemones** such as *Actinia equina* is in a Class known as *Actinozoa* which lack the medusoid stage altogether. **Corals** are colonial Actinozoa some of which have an external skeleton of calcareous material and coral reefs have been formed from the skeletons of past generations of these.

(1) (*a*) *Examine a prepared slide of the* **hydranth** *colony.*

Note the **nutritive hydranths,** Hydra-like individuals growing alternatively on a stalk, the **hydrocaulus** which is attached to seaweeds by a branched **hydrorhiza** at the base (this may not be present). Note the **perisarc,** a non-cellular outer coat covering the entire colony and enclosing a common stalk, the **coenosarc,** consisting of an outer **ectoderm** and an inner **endoderm** separated by a thin **mesoglea.** In the centre of the coenosarc is the cavity, the **enteron** with which the

enteron of the hydranths is continuous. Each hydranth fits into a cup-shaped **hydrotheca** (a continuation of the perisarc) and bears at its free end a number of **tentacles** which surround the **hypostome,** on which is situated the **mouth.**

In the axils of the lower hydranths (which are the older ones) are the **blastostyles,** or asexual reproductive zooids. Each is enclosed in a club-shaped continuation of the perisarc, the **gonotheca.** It has no mouth or tentacles, but gives rise to a number of small **medusoid buds,** which develop into medusae.

(*b*) *Make a stained permanent preparation of a* **hydroid colony.** *Stain with borax carmine.*

(2) *Examine a prepared slide of a* **medusa.**
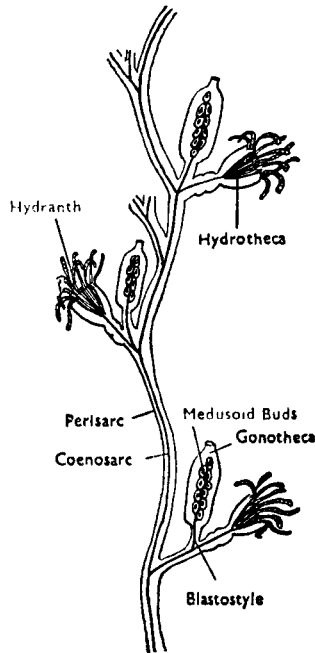
FIG. 30. Obelia colony.

It is umbrella-shaped and bears round the rim a large number of short **tentacles** with swollen bases. The convex upper side is the **ex-umbrellar surface,** and the concave side is the **sub-umbrellar surface,** from the centre of which is a short stalk, the **manubrium,** bearing on its tip the **mouth** (specimens are usually mounted to show the sub-umbrellar surface). A canal leads from this to the spherical **enteron,**

from which four **radial canals,** at right angles to one another, lead to the **circular canal** which runs round the edge of the umbrella. In the freshly formed medusa there are sixteen tentacles, four **per-radial tentacles,** one opposite each radial canal, four **inter-radial tentacles,** one between each pair of per-radials, and eight **adradial tentacles,** one between each per-radial and inter-radial (but others are formed as it becomes older). Look for the **statocyst,** a small sac containing a small calcareous object, the **statolith,** at the base of each adradial tentacle, and the **ocellus,** a small pigmented spot at the base of the per-radial, inter-radial and adradial tentacles. The **gonads,** or sexual reproductive organs, are rounded sacs, one below each radial canal. The sexes are separate, *i.e.*, the medusae are *diaecious.*

A TURBELLARIAN FLATWORM

PLANARIA

This free-living organism is a flatworm and lives in the mud of freshwater ponds. It belongs to the PHYLUM *Platyhelminthes* (Flatworms. Triploblastic Metazoa, bilaterally symmetrical. Complex reproductive system. Usually hermaphrodite) and the CLASS *Turbellaria* (Free living. Possess an enteron. Ectoderm ciliated).

Examine a living specimen or prepared slides of **Planaria.**

GENERAL STRUCTURE

The animal, which is a few mm. in length, is thin and flat. It is bilaterally symmetrical and has dorsal and ventral sides. The broader end is the anterior end and the somewhat pointed end posterior. The colour varies in different species. It may be white, grey or black. On the *dorsal side* near the anterior end are two **eyes** and on the *ventral side* in the posterior half of the body is the **mouth** which leads into a **protrusible pharynx.** When retracted this lies in a sheath. Near the posterior end on the ventral side is the **genital aperture.**

The body is covered by ectoderm composed of ciliated cells which enable the animal to move by a gliding movement. The body wall is muscular and this, too, can effect movement by a gliding motion.

III. SIMPLE TERRESTIAL PLANTS

PELLIA

THE LIVERWORT

Pellia is a *Liverwort* and belongs to the DIVISION *Bryophyta* (Moisture and shade-loving terrestial non-seed-bearing plants (*Cryptogamia*) bearing hair-like rhizoids for anchorage and for transporting water from the soil.) The plant has two generations, a sexual generation known as the **gametophyte** and the asexual generation called the **sporophyte.** This is referred to as **Alternation of Generations.** The CLASS to which Liverworts belong is *Hepaticae* (plants body is still a thallus) and the ORDER *Jungermanniales.* It lives on damp ground in woods and under hedgerows.

(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 **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
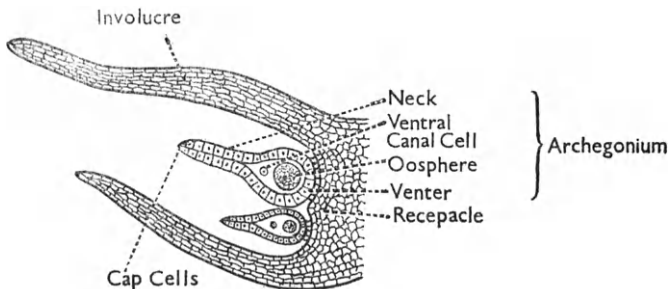
FIG. 31.   Pellia. L.S. Thallus showing Archegonia.

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
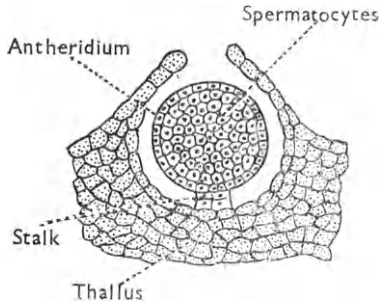
FIG. 32. Pellia. L.S. Antheridium.
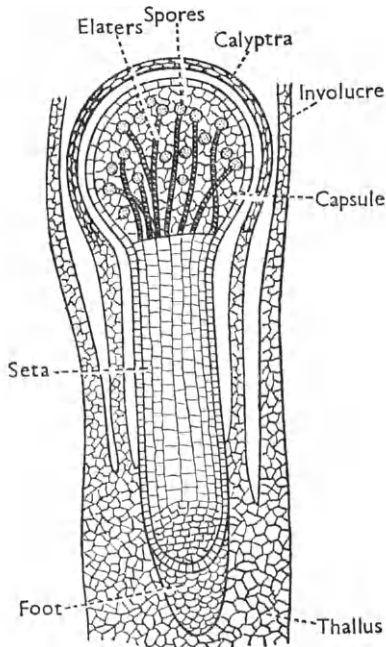
FIG. 33. Pellia. L.S. Sporognium.

protected by a wall formed 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.

FUNARIA

THE MOSS

Mosses are also in the DIVISION *Bryophyta* but belong to the CLASS *Musci* (Bryophytes which show a simple stem and leaf structure. Rhizoids are still present.) The ORDER is *Bryales.* They live in damp soil amongst other plants.

(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.**

Under *low power* note the club-shaped **antheridia** borne on multi-cellular stalks and containing numerous **spermatocytes.** These
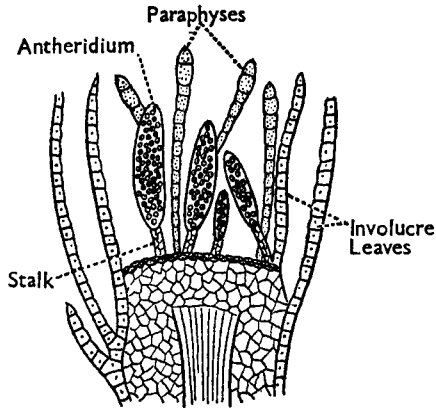
FIG. 34. Funaria. Antheridia.

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.**
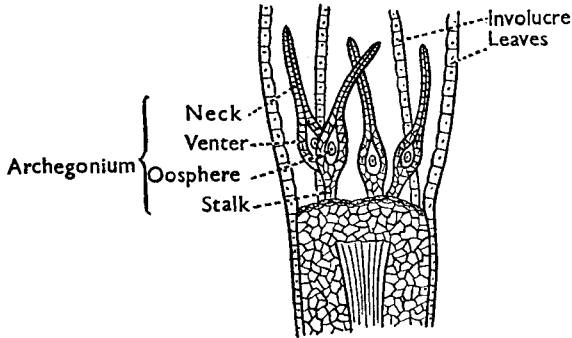
FIG. 35. Funaria. Archegonia.

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.
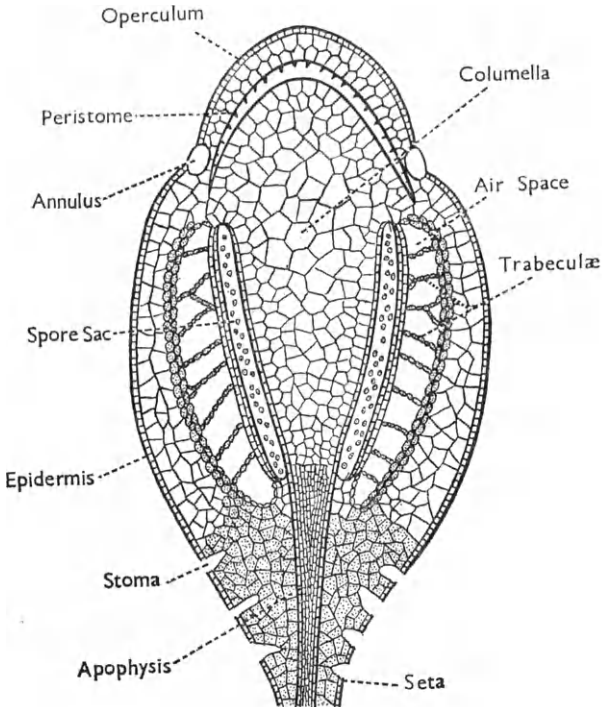
FIG. 36. Funaria. L.S. Sporogonium.

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.

(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.

DRYOPTERIS FELIX-MAS

THE FERN

Ferns, together with Horesetails and Club-Mosses, are in the DIVISION *Pteridophyta* (Terrestial plants showing a distinct stem leaf and root structure and further adaptation to a terrestial life. There is a definite Alternation of Generations,* the sporophyte* being well developed and the gametophyte* being very much reduced and on a separate plant. Vascular tissue is also well developed. Such plants are therefore called *Vascular Cryptogams*)† (vascular non-seed-bearing plants). The Ferns form the largest CLASS of the *Bryophyta* which is called *Filicales*. It grows in woods and hedgerows.

(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 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,** cells 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 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 *homospores.* **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.

*See classification of *Pellia*
†*Thallophyta*, *Bryophyta* and *Pteridophyta* constitute the **Cryptogamia.**
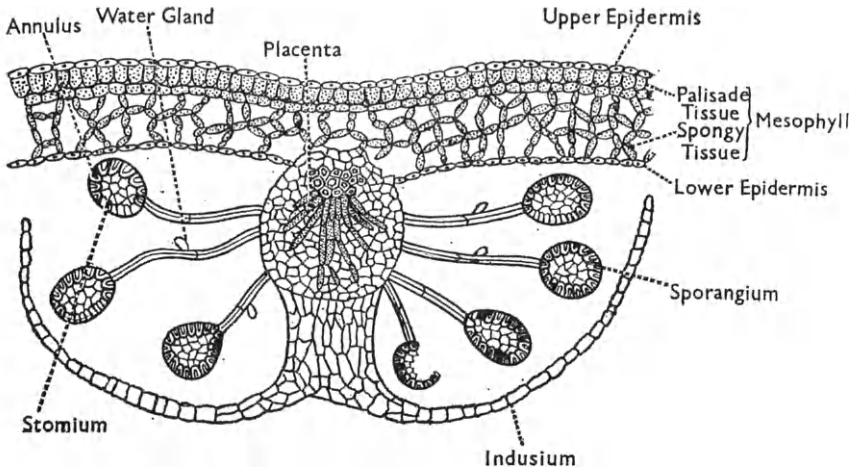
FIG. 37. **Dryopteris. T.S. Pinnule through a Sorus.**

(4) *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.

(5) *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).
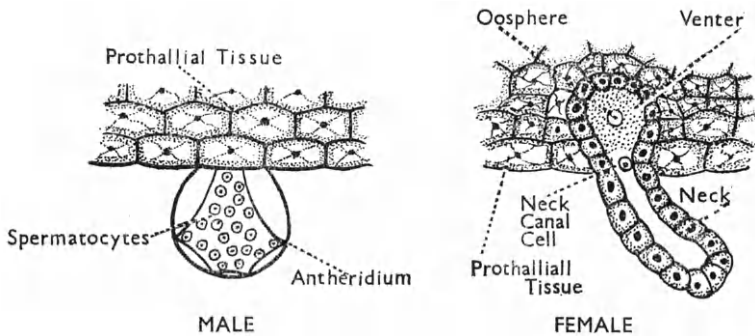
FIG. 38. **Dryopteris. Sexual Organs.**

(6) *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.

LYCOPODIUM

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

The creeping **stem** branches dichotomously and bears simple linear **leaves** spirally arranged and completely enveloping the stem. **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 **cones** (or **strobili**) of closely packed **sporophylls.**

(2) *Examine a* **L.S. through the centre of the** *strobilus.*

On the upper surface of the **sporophylls** are large **sporangia** containing numerous **spores** which are all of equal size *i.e.,* it is *homosporous.*

It is interesting to note that the monoecious gametophyte (prothallus) which develops from the spores and grows below soil level is devoid of chlorophyll and feeds *saprophytically* by means of a *mycorrhiza* (see Part VII p. 395 *seq.*).

SELAGINELLA

Selaginella is a *Club-Moss* and with another plant, Lycopodium, forms the CLASS *Lycopodinea* of the DIVISION *Bryophyta.* The ORDER is *Selaginellales.* It is mostly tropical in habitat.

(1) *Examine the* club-moss **Selaginella Kraussiana** entire.

The plant is the **Sporophyte.** It has a creeping **stem** which branches dichotomously and bears small simple **leaves** placed in four rows. The leaves in the two 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 structures called **rhizophores** from which **adventitious roots** arise. Erect **reproductive shoots** arise from the procumbant stem. These bear sporangia and the leaves are known as sporophylls. These are aggregated together to form cones or **strobili.**

(2) *Examine a median* **L.S. 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 the slightly larger **megasporangia** which contain four large **megaspores,** the microsporangia being 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 said to be *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.

Microsporophyll

Megasporophyll

Microsporangium

Microspores

Megasporangium

Megaspores
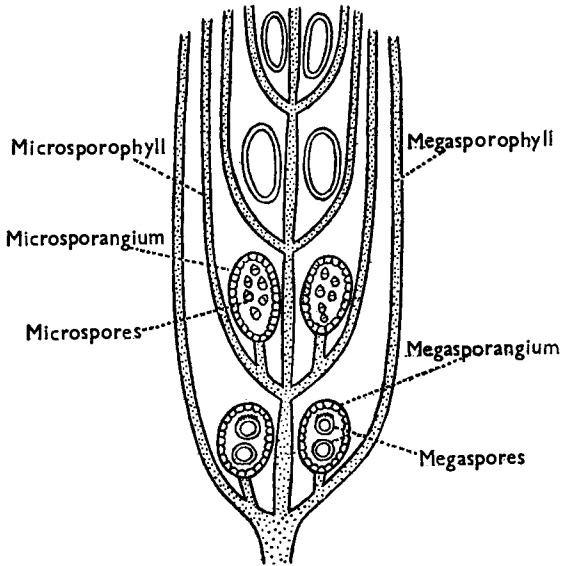
FIG. 39. **Selaginella. L.S. strobilus.**

IV. THE FLOWERING PLANT (SPERMATOPHYTA)

All flowering plants are seed-bearing plants and occupy the DIVISION *Spermatophyta* (or *Spermaphyta*). There are two separate and very much reduced gametophytes which are borne on the sporophyte. The sporogenous tissue is also very 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 still a greater adaptation to terrestial life and a more highly developed vascular system. Instead of ciliated antherozoids, a pollen tube germinates from the pollen grain (microspore) to carry the male gamete to the female gamete, thus eliminating the necessity of water for fertilisation. (There are some primitive seed-bearing plants which have ciliated sperms, *e.g.*, *Cycas* but this is not found in this country or in Europe).

There are two CLASSES of Spermatophyta, the *Gymnospermae* in which the seeds are exposed and the *Angiospermae* in which they are enclosed. The Gymnosperms are mostly evergreens and the Angiosperms include all the common Flowering plants.

A GYMNOSPERM LIFE-HISTORY

THE SCOTS PINE

Pinus sylvestris is the Scots Pine. This is a flowering plant in which the seeds are exposed and is therefore in the CLASS *Gymnospermae*. The sporophylls are aggregated together to form male and female cones. It is therefore classified in the ORDER *Coniferales*. The tree, which is an evergreen and can live in quite poor soil, is the **sporophyte**. It is a **conifer**.

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

Note the upright **stem** (trunk) with a reddish-brown, scaly bark, bearing lateral branches and flattened at the top. The ordinary branches are called **long shoots** and bear only **scale leaves.** On these long shoots, arising in the axils of the scale leaves, are **dwarf shoots** bearing **scale leaves** and needle-shaped **foliage leaves** amongst which **cones** may be seen. Dwarf shoots with their green leaves are known as **spurs. Terminal buds** and **axillary buds** occur on the long shoots. The tree may attain a height of a hundred feet.

The Cone

The sporophylls are borne in **strobili** or **cones** in which the sexes are separate.

(2) *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.

(3) *Examine a* **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.
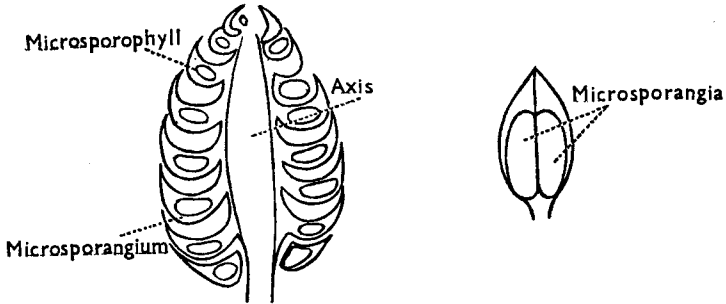
FIG. 40. Pinus. Male Cone.

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

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

(5) *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) larger 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.

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

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

Remove a **megasporophyll** *and examine with a hand lens.* Each is composed of a lower **bract scale** with a thicker **ovuliferous scale** above on which is a pair of **ovules** on its upper surface close to the axis.

(7) *Examine a* **L.S. of a First-year Female Cone.**
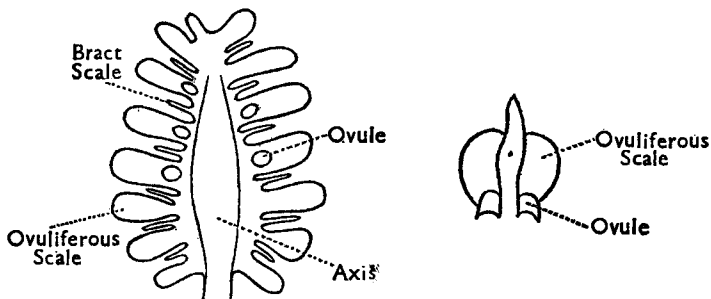
FIG. 41. Pinus. Female Cone.

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.

(8) *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.

(9) *Examine a* **L.S. of a Second-year Female Cone.**

The embryo-sac is now filled with a compact tissue, the **endosperm (female prothallus)** 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.**

(10) *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 side 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.
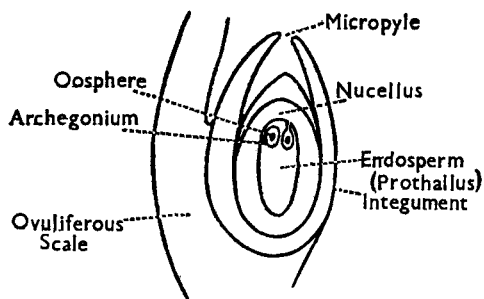
FIG. 42. **Pinus. L.S. Mature ovule.**

The Seed

(11) *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.**

ANGIOSPERMS

The Angiosperms are subdivided into two SUB-CLASSES *Monocotyledons* and *Dicotyledons*. The characteristics of these classes are as follows:—

MONOCOTYLEDONS	DICOTYLEDONS
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.	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.

I. THE ROOT

(1) ROOT SYSTEMS

(1) Tap Roots

These are elongated radicles.

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

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.)

(2) Fibrous Roots

(1) *Examine the* **roots** *of* **grass.**

The primary root ceases to grow as such, but branches considerably. There is thus no main root bearing lateral roots but a large number of roots growing from the base of the stem. (This is the Fibrous Root Sytem and is characteristic of monocotyledonous plants, though it is also found in a number of dicotyledons.) Note also that the roots are **fibrous**.

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

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

Test for inulin in Dahlia *and for starch in* Lesser Celandine. See Part IV (Biochemistry, p. 308).

(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** 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.

II. 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, the **internodes.** The angles between the upper part of the stem and the leaves are known as 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 stem 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 also* **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 **houseleek** (*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 but at the node.

(iv) Thorns (or Stem Spines)

Examine the **stem** *of* **hawthorn** (*Cratœgus oxyacantha*) *or* **gorse** (*Ulex europœus*).

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 the thorn. They are modified complete stems.

(v) Prickles (or Hooks)

Examine the **stem** *of a* **raspberry** (*Rubus idoeus*) or **blackberry** (*Rubus fruticosus*). Prickles or hooks are found at various places on the stem. These are modified parts of the stem, the outer tissues.

(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.** *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 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.** *of the* **corm.**

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*), also an **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.

III. THE FOLIAGE LEAF

(1) FORMS OF LEAF

(i) Simple Leaves

(1) *Examine the* **leaf** *of the* **garden pea** (*Pisum sativum*) or **broad bean** (*Vicia faba*) *attached to the stem.*

Note the leaf blade or **lamina,** the margin of which is **entire,** *i.e.,* not divided into leaflets, 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 annus*).

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** (*Æsculus 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

Characteristic of Dicotyledons

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

Note the **midrib** with an irregular network of veins on each side. This is *reticulate venation.*

(ii) **Parallel Venation**

Characteristic of Monocotyledons

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

The veins are parallel and there is no midrib. Here and there cross-connections occur. This is *parallel venation.*

(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 and so on 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 europæus*).

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. THE INFLORESCENCE

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

(i) 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.

(ii) 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.

V. 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) THE BUTTERCUP

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

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 therefore described as follows:—

The flower is symmetrical about any plane and is therefore said to be *radially symmetrical, actinomorphic* or *regular, 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*).

Write a **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:—

$\male$ = staminate (male) flower ⎫ Usually unnecessary as sub-
$\female$ = pistillate* (female) flower ⎬ sequent parts of the
$\hermaphrodite$ = hermaphrodite flower. ⎭ formula indicate this.

† or ↑ = zygomorphic (bilaterally symmetrical).

$\oplus$ = 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.

G͟ = Gynaecium, with a superior ovary (*hypogynous*).

G̿ = 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.

*The gynaecium was formerly known as the pistil.

( ) 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*).

$$\uparrow \; K(5) \; C\overset{\frown}{(5)} \; A \; 4 \; G \; (2)$$

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

(2) Wallflower (*Cheiranthus*).

$$\oplus \; K2 + 2 \; C4 \; A2 + 4 \; G \; (2).$$

= 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*).

(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.
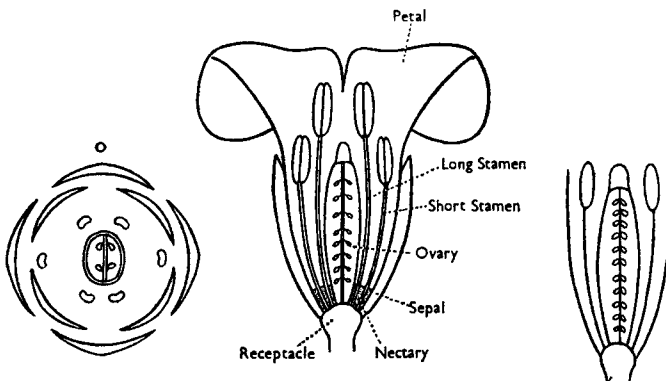
FIG. 43.   Floral Diagram, Longitudinal Half-flower and L.S. Flower.
of Wallflower (*Cheiranthus*).

(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) 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.**

(ii) THE PEA OR BEAN

(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,** note the number of petals and 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, which is inside the staminal tube, consists of one carpel (**monocarpellary**) and that the **ovary** is **superior.**
Write a **description of the flower.**
(2) *Cut and draw* (*a*) *a* **longitudinal half** *and* (*b*) *draw a* **longitudinal section** *in the antero-posterior plane.*
(3) *Draw the* **floral diagram.**
(4) *Write the* **floral formula.**

(iii) THE BLUEBELL OR LILY

(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 note the number of **stamens** and 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*) *draw a* **longitudinal section.**

(3) *Draw the* **floral diagram.**

(4) *Write the* **floral formula.**

(iv) THE SUNFLOWER

(1) *Examine the* **capitulum of the Sunflower** (*Helianthus*) *or* **Ox-Eye Daisy** (*Chrysanthemum leucanthemum*).

This is characteristic of the FAMILY *Compositae*. The inflorescence consists of a large number of miniature flowers called florets on the flattened surface of the head of the **peduncle.** It is sometimes referred to 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,** yellow in the sunflower, white in the daisy, externally; these are female and one of their important functions is to attract insects. (ii) **Disc florets,** yellow in colour, internally; these are hermaphrodite. Each floret arises in the axil of a bract and the oldest are on the outside.

(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
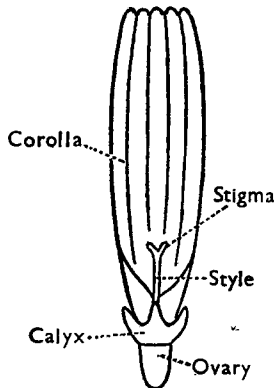
FIG. 44. Ray Floret.

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 called the **epigynous** arrangement.

(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
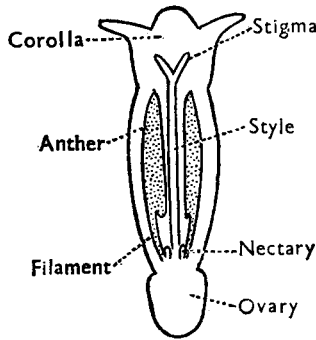
Fig. 45. Disc Floret.

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* **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) THE MEADOW GRASS

(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.

(2) *Remove a floret and examine with a hand lens.*
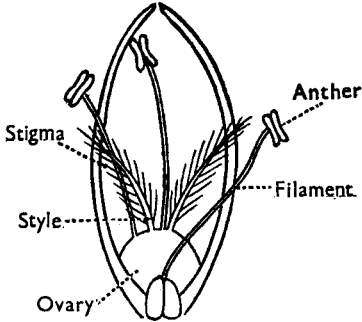
FIG. 46. **Meadow Grass. Flower.**

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.**

VI. THE FRUIT

I. SIMPLE FRUITS

(Developed from a single flower with a monocarpellary or synocarpous 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. (Characteristic of *Compositae*.)

(i) *Examine the* **fruit** *of the* **dandelion** (*Taraxacum officinale*) *or* **Thistle** (*Circium*).

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 or leathery.

N.B. The *Walnut* is not a nut: it is a Drupe.

(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.

(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**—simple follicles are very rare.) It is formed from a monocarpellary gynaecium, and dehisces along *one* suture (the **ventral suture**) only.

*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.

(4) Siliqua

Examine the **fruit** *of the* **wallflower** (*Cheiranthus*).

It is developed from a bicarpellary gynæcium. 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.** (Characteristic of *Cruciferae.*)

(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

These are legumes or siliquas which split transversely.

Examine the **fruit** *of the* **radish** (*Raphanus*).

It splits transversely into one-seeded portions.

(2) Double Samara

Examine the **fruit** *of the* **sycamore** *or* **maple.**

The two samaras are fused, but split apart when dispersed.

(3) Regma

Examine the **fruit** *of the* **geranium** (*Pelargonium*).

The one-seeded units are dehiscent and are called **cocci.**

(4) Cremocarp

Examine the **fruit** *of* **carrot** (*Daucus carota*) *or* **parsley** (*Pastinaca sativa*).

It splits longitudinally into two hanging indehiscent 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. The walnut is not a nut but a drupe (as already stated), the so-called nut being the endocarp.

(2) Berry

Cut a **longitudinal** *and a* **transverse section** *of a* **gooseberry** (*Ribes grossularia*), **grape** *or* **tomato** (*Solanum lycoperdicum*).

Note that the pericarp consists of a skin, the **epicarp,** and that both the **mesocarp** and **endocarp** are fleshy. The **seeds** are embedded in the endocarp.

(3) Pome (a 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. It is surrounded by the **swollen fleshy receptacle,** the skin of which is the **epidermis.** For this reason the pome is a pseudocarp.

II. 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 idoeus*). Each fruitlet is a tiny **drupe** or **drupel** (*not* a berry) and is therefore succulent.

(3) Etaerio of Follicles

This has already been seen in studying simple fruits.

III. COMPOSITE FRUITS
(Developed from an inflorescence)

There are few of these. *Fig, hop, pineapple* and *mulberry* are common examples.

VII. THE SEED AND SEEDLING
I. DICOTYLEDONOUS SEEDS
(i) 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 structures constitute the **embryo.**

Scarlet Runner (*Phaseolus multiflorus*)

Examine and compare with the Broad Bean seed.
Note the conspicuous micropyle.

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** and, at the pointed end of the seed, the **radicle** and small **plumule** with a **hypocotyl** between.

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.

(ii) **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 a longitudinal section.*

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.

(3) *Remove the testa and cut a transverse section.*

Note the **endosperm** and **cotyledons.**

II. MONOCOTYLEDONOUS SEEDS

Maize (*Zea mais*)

This is really a one-seeded *fruit* (caryopsis).

(1) *Examine a* **maize grain** *externally.*

Note the **pericarp** (fruit wall) 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.

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.

(iii) SEEDLINGS

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 germina‑ nation 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.

V. FURTHER MULTICELLULAR ANIMALS

THE RAG WORM

NEREIS

This is one of the **Bristle worms**. It lives under stones and burrows in the mud between tide-levels in the sea. It is in the PHYLUM *Annelida* (Segmented Worms. Triploblastic metamerically segmented Metazoa containing a coelom. Body-wall muscular. Ventral nerve cord. Bilaterally symmetrical) and the CLASS *Polychaeta* (show distinct cephalisation. Chaetae arise from protuberances on body-wall. Dioecious. Marine.)

EXTERNAL ANATOMY

(1) *Examine a specimen of* **Nereis,** *using a hand-lens when necessary.*

The animal is composed of a **head** and about eighty identical **segments,** each bearing bristles called **chaetae.** The dorsal surface is convex and the ventral surface more or less flat and the colour varies.

(2) *Examine the head.* It will be seen to be composed of a **peristomium** surrounding the **mouth** and a bluntly triangular **prostomium** on the dorsal side. At the anterior end of the prostomium two short **tentacles** will be seen on the dorsal side and pair of short **palps,** each consisting of two joints, on the ventral side. Also on the dorsal side four simple **eyes** will be found and, behind the palps, somewhat latero-dorsally, two pairs of fairly long **cirri.**

(a) SIDE VIEW   (b) DORSAL VIEW
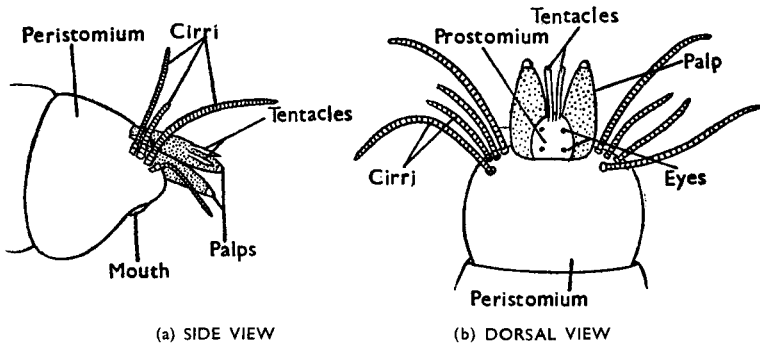
FIG. 47. Nereis. Head.

(3) *Examine a* **segment** *and a slide of a* **parapodium.**

The segment will be seen to bear a pair of lateral outgrowths known as **parapodia.** Each parapodium is composed of a dorsal bilobed **notopodium** and a ventral bilobed **neuropodium,** each bearing a bunch of **chaetae,** a slender **cirrus** and a short stiff bristle, the **aciculum.**
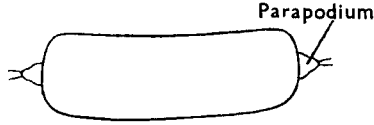
FIG. 48.   Nereis.   Segment.
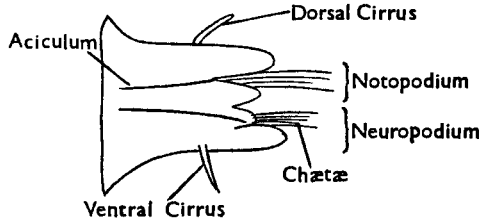
FIG. 49.   Nereis.   Parapodium

(4) *Finally examine the last segment or* **pygidium.**

This is devoid of parapodia and bears the **anus** and a pair of long, slender **anal cirri.**

THE EARTHWORM

LUMBRICUS

EXTERNAL ANATOMY

The earthworm is another representative of the PHYLUM *Annelida.* Its CLASS is *Oligochaeta* (Annelids which show little cephalisation and which have chaetae arising from sacs in the body wall. They are hermaphrodite.)

(1) *Examine a* **mature earthworm** *externally.*

Note the shape and colour of the animal and that the body consists of about 150 ring-like segments or **annuli.** At the anterior end is the **mouth** surrounded by the **peristomium** and protected by a fleshy upper-lip or **prostomium.** The **anus,** or intestinal aperture, is at the posterior end on the **pygidium** which is not a true segment. Note the **clitellum,** a thickened band stretching from segments 32 to 37 inclusive.

The **dorsal pores,** in the grooves between all segments after the 9th, except the last, are extremely difficult to see.

Rub the earthworm from back to front between the thumb and first finger.

The roughness on the lower, lighter (ventral) side is due to short bristles or chaetae, which are organs of locomotion.

(2) *Examine the* **first 20 segments on the ventral side,** *using a lens.*

Note the **mouth** and the four pairs of chaetae on each segment except the first and the last, two pairs of **ventral chaetae** and two pairs of **lateral chaetae.** Look in the grooves between segments 9 and 10 and 10 and 11 for the **spermathecal pores,** on segment 14 for the female **oviducal pores** (difficult to see) and on segment 15 for the male **spermaducal pores,** conspicuous on account of their prominent lips. Paired **nephridiopores** occur anterior to the ventral chaetae on all segments except the first three and the last.

(3) *Remove a chaeta with a pair of forceps, mount in dilute glycerin and examine under the low power.*

Draw the **chaeta.**

INTERNAL ANATOMY

1. THE ALIMENTARY SYSTEM

Kill a large worm by chloroform but do not allow the liquid to come in contact with the animal as it will make it brittle. (See Appendix II— Killing of Animals.)

Pin the animal, dorsal side uppermost, in a dissecting dish and cover with water. Put two small pins obliquely through segment 4 or 5 and two through the clitellum, stretching the body as much as possible. Insert the fine scissors in segment 30 or thereabout and cut in the mid-dorsal line up to segment 2. Pin back the body wall on each side, cutting through the septa, and examine the viscera with a hand lens.

Note that the adjacent segments are separated from each other by a transverse **septum,** and that there is a body cavity or **coelom** apart from the digestive cavity. The mouth leads into the **buccal cavity** which reaches to segment 3. In segments 4, 5 and 6 is the muscular **pharynx** with muscular strands radiating from it. The **oesophagus** is a thin-walled tube extending from segment 6 to segment 12 to 14. In segment 10 is a pair of **oesophageal pouches** and in each of segments 11 and 12 a pair of white **oesophageal glands** (which lead into the pouches), lateral protuberances on the sides of the oesophagus not readily visible until the alimentary canal is removed owing to their ventral position. The **crop** is a dilated sac reaching from the oesophagus to segment 16, and the **gizzard** is a thick muscular part of the alimentary canal extending from segment 16 to segment 18 or 19. The **intestine** stretches from segment 20 to the anus and bears a longitudinal infolding on the dorsal side (this is the typhlosole and will be seen later in the transverse section). The yellow cells lying on the intestine are the **chloragogen cells** which are concerned with nitrogenous excretion.
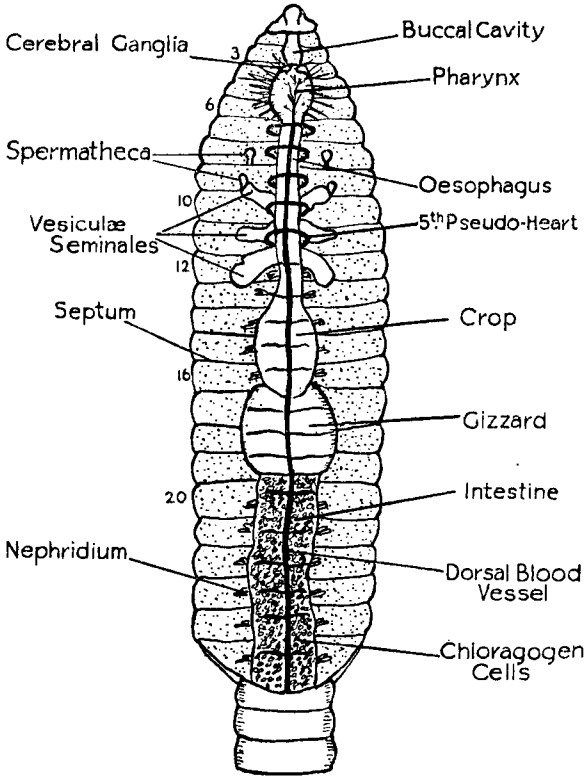
FIG. 50. **Lumbricus. General Dissection.**

Note also the paired coiled tubes or **nephridia** (excretory organs) on the ventral side, beginning in segment 4. Some of the **reproductive organs** (spermathecae and vesiculae seminales) will also be visible in segments 9 to 12.

2. THE VASCULAR SYSTEM

Use the worm already dissected.

The **dorsal blood vessel** (in which the blood flows forwards) is the largest and runs along the dorsal side of the alimentary canal. *By means of a camel hair brush, wash away any chloragogen cells hiding it.* On each side of the oesophagus is the **lateral** or **extra oesophageal blood vessel** which branches over the pharynx; it enters the **dorso-subneural** vessel (see p. 85) in segment 12. *By careful examination with a lens these vessels may be seen on the posterior sides of the cut*

septa where they run. The **contractile commissural vessels** or **pseudo-hearts** are paired loops in segments 7 to 11, which connect the dorsal vessel with the **ventral** or **sub-intestinal blood vessel** (in which the blood flows backwards) running on the ventral side of the alimentary canal. *To expose this vessel carefully release a part of the intestine by pulling it aside with forceps and cutting through the septa which hold it in place. Part of the ventral vessel will be seen on the underside of the intestine.*

Most of the following vessels will be seen later in a transverse section. **Ventro-intestinal vessels** in each segment take blood to the wall of the alimentary canal and **ventro-parietal vessels** to the body wall while **dorso-intestinal vessels** may be seen on the surface of the intestine. **Afferent nephridial vessels** run from these ventro-parietals to the nephridia while **efferent nephridial vessels** return the blood to the dorso-subneurals. Vessels from the ventro-parietals also take blood to the reproductive organs in segments 9 to 15. The **subneural vessel** lies ventral to the nerve cord. **Dorso-subneural vessels** connect this to the dorsal blood vessel from segment 12 backwards, these lie in the septa. On the sides of the nerve cord are two **lateral neural vessels.** The other blood vessels are difficult to see.

3. THE EXCRETORY SYSTEM

(1) *Dissect a worm dry and carefully remove the alimentary canal but nothing else. Holding the intestine in the forceps, cut across it transversely and then, still holding the canal, cut through the septa and connective tissue beneath it. Work forwards, carefully lifting it. Leave the buccal cavity and pharynx in situ (to preserve the cerebral ganglia) and before going any further, look for the oesophageal pouches (segment 10) and oesophageal glands (segments 11 and 12) if you were unable to see them earlier.*

Now note the **nephridia,** a pair of coiled tubes in each segment except Nos. 1, 2, 3 and the last. Each nephridium opens into the coelom in one segment, the **pre-septal part,** pierces the septum behind and the **post-septal part** opens to the exterior in the next segment.

(2) *Remove a **nephridium** from the animal dissected dry. To remove a nephridium complete with nephrostome is not an easy operation. The largest are in segments 13 to 20. Working under a lens and using very fine forceps and scissors, remove the nephridium and the septum, cutting transversely under the nephrostome which is anterior to the septum near the nerve cord. Mount in 0·6 per cent. physiological saline. Rearrange the specimen with mounted needles if necessary before putting on the coverslip and examine under the low power. Also make a permanent preparation stain with borax-carmine (see Part I, p. 28).*
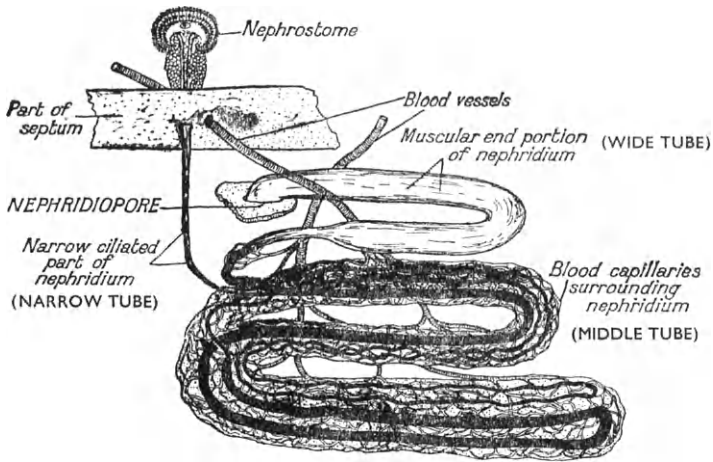
FIG. 51. **Lumbricus. Nephridium.**
(From Dakin's "Elements of Zoology". Clarendon Press.)

From the funnel-shaped **nephrostome** (if present) which is the coelomic opening (and in which you may be able to observe cilia still working), arises the ciliated **narrow tube** which passes through the septum into the wider looped **middle tube** surrounded by **connective tissue** and well supplied with **blood vessels.** The **wide tube** which follows opens to the exterior by the **nephridiopore.**

4. THE REPRODUCTIVE SYSTEM

This animal is hermaphrodite and the genital organs of both sexes are found in segments 9 to 15.

(i) Male Organs

(1) *Cover the worm with water and examine with a lens.*

The **anterior vesicula seminalis** is a white lobed body in segment 10, the lobes of which push the septum between segments 9 and 10 forwards. The **mid vesicula seminalis** is similar and lies in segment 11.

The **posterior vesicula seminalis** is in segment 12 and its posterior lobes protrude into segment 13, pushing this septum backwards. The central portions in segments 10 and 11 from which these arise are the **testis sacs.***

Cut open the testis sacs, wash out the contents with a pipette and examine with a lens.

*The designation of the vesiculae seminales varies with different authors.

Note the two pairs of digitate **testes** if visible, one pair in segment 10, hanging from the septum between 9 and 10, the other pair in segment 11, hanging from the septum between 10 and 11. They lie towards the mid-line but are seldom visible. Just behind each testis is a **seminal funnel,** the edges of which are convoluted (they are consequently often called **ciliated rosettes).** Each seminal funnel leads to a **vas efferens,** ventral to the posterior vesiculae seminales and often partly hidden by a nephridium, and each vas deferens runs backwards and outwards on the ventral body wall and unites with its fellow on that side in segment 12 to form the **vas deferens.** This runs straight back to open to the exterior by one of the spermaducal pores on segment 15. The vasa efferentia and the vas deferens are often difficult to see.
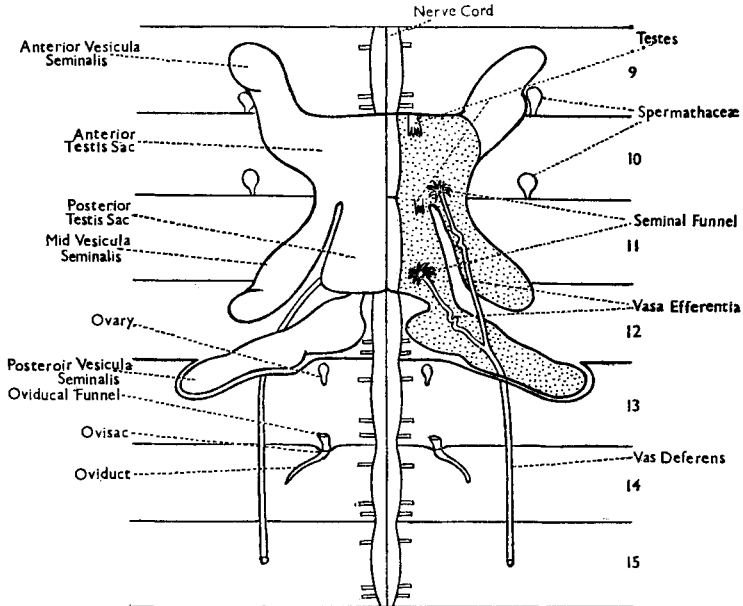
FIG. 52. **Lumbricus. Reproductive System.**

(2) *Dissect a worm dry. Remove a seminal vesicle and put it into a watch glass. Tease with mounted needles. Then make a smear of the milky contents on a coverslip.* * *Fix with 70 per cent. alcohol (5-10 minutes), stain with borax-carmine, picro-carmine or hæmatoxylin. Mount in dilute glycerine or make a permanent mount.*

*See p. 26 *seq.*

Look for **stages in spermatogenesis,** the development of sperma-tozoa. *Search also for the parasite* **Monocystis** which often infects the worm. The trophozoite stage is that most frequently seen (see p. 408).

Note the ripe **spermatozoa.**

(ii) Female Organs

(1) *Examine the same worm.*

A pair of **ovaries,** small pear-shaped bodies, are found in segment 13, suspended from the septum between 12 and 13 towards the mid-line on each side of the nerve cord. Behind them in the same segment are the **oviducal funnels,** continuous with which are the short **oviducts,** which open to the exterior by the oviducal pores on segment 14. Each oviduct swells into a sac, the **ovisac** or **receptaculum ovorum** in segment 14.

In each of segments 9 and 10 is a pair of **spermathecae,** small spherical white sacs which open to the exterior by the spermathecal pores in the grooves between segments 9 and 10, and 10 and 11. They store spermatozoa received from another worm in copulation.

(2) *Remove an ovary with a portion of the septum to which it is attached. Mount in water and examine under the lower power.*

Note the small pear-shaped **ovary** containing developing **ova,** the more mature ones being in the narrow posterior end.

(3) *Mount a drop of the contents of a spermatheca adopting the method used for the seminal vesicle contents (see p. 87).*

5. THE NERVOUS SYSTEM

Use the worm left from the last dissection, i.e., with the alimentary canal removed.

In segment 3 are two white rounded bodies, the **cerebral ganglia.** These lie at the junction of the buccal cavity and the pharynx on the dorsal side. From the side of each arises a loop called the **circum-pharyngeal connective** or **commissure.** The two join and form the **nerve-collar.** In segment 4 where the commissures meet below the oesophagus is a **sub-oesophageal ganglion.** The **ventral nerve cord** is continuous with this and lies in the mid-ventral line, swelling slightly in each segment where three pairs of **segmental nerves** are given off. A pair of **prostomial nerves** arise from the cerebral ganglia and run forwards.

6. TRANSVERSE SECTION

(1) *Examine a prepared slide of the* **T.S. of the earthworm in the region of the intestine** *under the low power.*

The animal is *triploblastic.*

Note. (i) The **Body-Wall** composed of an outer protective **cuticle,** then the **epidermis,** the **circular muscular layer,** the **longitudinal muscular layer,** the **coelomic epithelium** and the **ventral and lateral chaetae** (if present) in **chaetigerous sacs** and provided with **retractor muscles.**

(ii) The **Coelom** in which may be seen the **dorsal blood vessel,** the **ventral blood vessel** connected to the alimentary canal blood vessels by the **ventro-intestinal blood vessel,** the ventral **nerve cord** in which three **giant fibres** may be visible, the **sub-neural blood vessel,** and portions of **nephridia.**

(iii) The **Alimentary Canal** surrounded by **chloragogen cells** and made up of the **circular muscular layer,** the **longitudinal muscular layer,** the **intestinal epithelium** and the **gut cavity.** The dorsal side is folded in to form the **typhlosole.**

(2) *You should now examine a* **T.S. in the posterior region of the oesophagus.**

From your knowledge of the anatomy of the animal, you should be able to identify the structures which are visible.
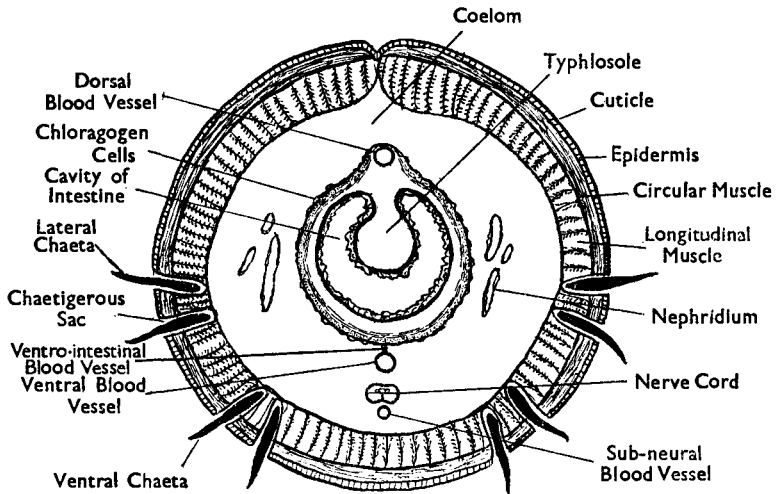
FIG. 53.   Lumbricus.   T.S. Intestinal region.

THE MEDICINAL LEECH

HIRUDO MEDICINALIS

Leeches are members of the PHYLUM *Annelida* and in the CLASS *Hirudinea* (Annelids which are ectoparasites and in which the number of segments is definitely limited. Have a sucker at each end of the body. Hermaphrodite).

Examine a specimen of the **Medicinal Leech** *(Hirudo medicinalis) using a hand lens.*

This leech lives in ponds, swamps and slow-flowing streams and attaches itself to fish and frogs from which it sucks blood. The adults will also suck the blood of warm-blooded animals including man.

The body is elongated and is about 6–10 cm. in length though it can expand to as much as 15 cm. There are 32 **segments** though there appear to be more because most of them, except at the extremities, consist of five **annuli.** There are 95 in all. *Examine the anterior end on the* **ventral side.** Here will be seen the **anterior sucker** in which lies the **mouth** which has three jaws. *Examine the posterior end.* The **posterior sucker** will be found at the other extremity and, just anterior to it, the **anus.** On the second annulus of segment 10 is the **male genital aperture** and on the second annulus of segment 11 the **female genital aperture,** both situated in the mid-line. Finally on the last annulus in each of the segments from the 6th to the 22nd is a pair of **nephridiopores.** Note the absence of chaetae.

Now turn to the **dorsal side** of the animal. On the first annulus of each segment is a row of **sensory papillae** and on the first segment, which is the **head,** is a pair of pigmented **eyes.**

The whole body is encased in a thin cuticle which is periodically shed and replaced. Note that the animal is rather flattened dorso-ventrally and that the colour varies from black to green and yellow and is darker above than on the ventral side.

THE STARFISH

ASTERIAS RUBENS

This is a member of the PHYLUM *Echinodermata* (Triploblastic marine coelomates. Adults radially symmetrical. Have a calcareous skeleton. *Sea Urchins* and *Sea Cucumbers* also belong to this Phylum). The CLASS is *Stelleroidea* (flattened five-rayed symmetry) *Brittle-Stars, Sea Urchins* and *Sea Cucumbers* are in different Classes.

Examine a specimen of a **starfish.**

This animal (which is not, of course, a fish) is shaped like a star with five arms radiating out from a central **disc.** The upper or **aboral side** is darker than the lower or **oral side** and varies from orange to a purplish colour. In the centre of the disc on the **oral side** is the **mouth** bearing a membranous lip called the **peristome.** Correspondingly on the **aboral side** is the **anus.** Each arm is referred to as a **radius** and between each pair is the **interradius.**

Tube Feet

Part of Arm
Oral Surface

Part of Arm
Aboral Surface

Oral Surface
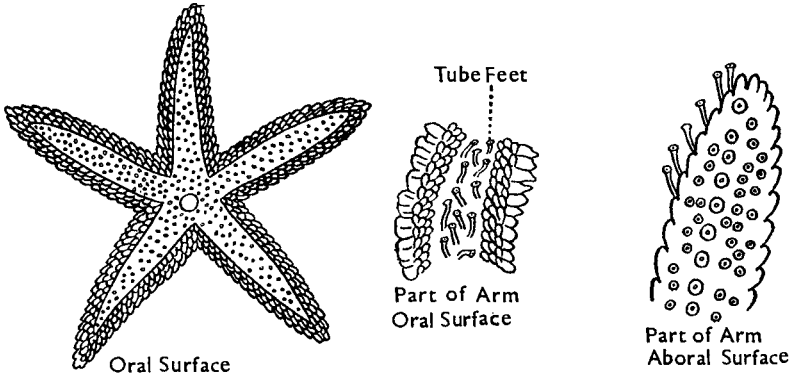
FIG. 54. Asterias rubens. Starfish.

Examine one of the **arms.**

Note that on the oral side is a deep **groove** which can be opened and closed by the animal. The grooves of all the arms meet around the mouth. These grooves contain what are known as **tube-feet** which end in **suckers** while at the end is a **sensory tentacle.** The body wall contains a large number of rod-shaped **ossicles** from which blunt **spines** arise and between the ossicles are **dermal gills.** Each spine is surrounded by a cushion and on and between these cushions are minute **pincers** (or **pedicellariae**) which are organs of defence and are of two sizes and forms. On the other, aboral, side are flattened rounded ossicles which bear grooves and in these grooves are **pores** through which water is drawn into the animal.

THE FRESHWATER CRAYFISH

ASTACUS

The Crayfish belongs to the PHYLUM *Arthropoda* (Bilaterally symmetrical, metamerically segmented animals possessing a well developed exoskeleton with paired jointed limbs. Body cavity a haemocoele), the CLASS *Crustacea* (Aquatic. Respire by gills. Thick exoskeleton. *Crayfish, crabs, shrimps*) and the *Order Decapoda*. It lives in rivers in chalky districts, remaining most of the time in holes in the bank. The common European freshwater crayfish is *Astacus fluviatilis*.

1. OBSERVATIONS ON THE LIVING ANIMALS

(1) *Observe the animal in water* and note its method of walking, swimming and feeding. Take note also of the way in which it uses its large pincers.

(2) *Place the animal on its back* and observe its movements, noting particularly the mouth parts.

II. EXTERNAL ANATOMY

(1) Cephalothorax and Abdomen

(i) Dorsal View

Note that the body is protected by a hard exoskeleton and that it is divided into a **head, thorax** and **abdomen**: each is segmented and
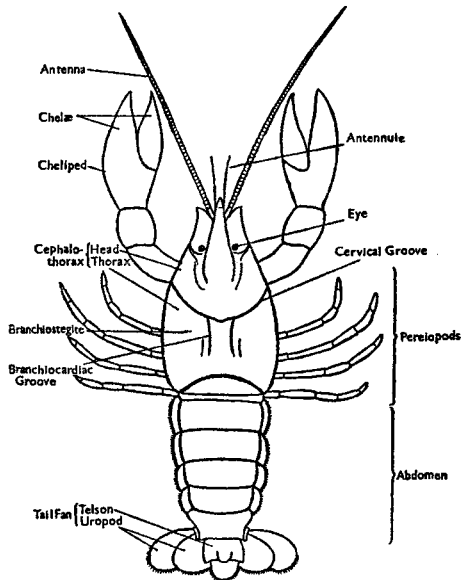
FIG. 55. Astacus. Dorsal view.

there are nineteen segments in all.* The head and thorax are almost completely fused to form what is known as the **cephalothorax,** the division between them being shown by the **cervical groove** running across the body, but here the segmentation is not visible externally.

It is clearly seen in the abdomen where each segment is composed of hardened plates called **sclerites,** that on the dorsal side being called the **tergum** and that on the ventral side the **sternum,** while at the sides are the < -shaped **pleura.** There is fusion between the tergum and the pleuron on each side and, to a certain extent, between the sternum and the pleura, but the appendages are inserted here and there is a small sclerite between the appendage and the pleuron on each side called the **epimeron,** seldom evident however.

In the cephalothorax the sclerites are fused to form the **carapace.**

(a) The Cephalothorax

The **head** consists of five segments (excluding the pre-antennal segment).

Note the pointed **rostrum** projecting anteriorly from the carapace, the short stalked **eyes** lateral to it, the two short **antennules** and the pair of very long **antennae.**

The **thorax** is composed of eight segments.

Running backwards from the cervical groove are two grooves on the thorax, the **branchiocardiac grooves.** From these the carapace bends down to form the sides of the thoracic cavity and these sides are known as the **branchiostegites.** The cavity beneath the two outer portions of the carapace thus formed encloses the gills and is called the branchial chamber while the central portion covers the heart. Note the four pairs of **walking legs** (or **pereiopods**) and the large **chelipeds** with large pincers or **chelae.**

(b) The Abdomen

This is composed of six movable segments and a structure called the **telson** at the posterior end, on each side of which is a wide **uropod,** the whole structure being called the **tail fan.**

(ii) Ventral View

Again note the division into **cephalothorax** and **abdomen.** Note also the **antennules, antennae, chelipeds,** four pairs of **pereiopods** and the **telson.** Between the bases of the appendages in the centre

*There is evidence that there are really twenty segments, a pre-antennal segment, bearing no appendages, preceding that which bears the antennules.

of the thorax will be seen small **sternal plates.** Find the **anus** on the telson and note the wide **uropods** on each side, forming the **tail-fan.**

(2) The Appendages

All the body segments except one on the head bear paired append-ages. There are nineteen pairs in all and they are all built on a common plan, though this is much modified in the various append-ages in adaptation to their functions. This common plan must be understood before any attempt to examine the appendages is made. Arising from a basal portion called the **protopodite**, itself composed of a proximal **coxopodite** and a distal **basipodite**, and bearing a small **epipodite**, are two branches (it is therefore said be *biramous*), an inner **endopodite** and an outer **exopodite**, each composed of several **podomeres.*

The most obvious appendages are the long **antennae** and short **antennules** on the head, the large **pincers** (or **chelipeds**), the four pairs of **walking legs** (or **pereiopods**) on the thorax and the **uropods** at the posterior end of the abdomen which, with the **telson**, form the **tail fan.** A complete list of the appendages is given in the table below.

Region	Seg-ment*	Appendage	Function
Head	1	Antennules.	Sensory (Olfactory).
	2	Antennae.	Sensory (Tactile).
	3	Mandibles.	⎫
	4	Maxillules.	⎪
	5	Maxillae.	⎬ Mouth parts.
Thorax	6	1st Maxillipeds.	⎪
	7	2nd Maxillipeds.	⎪
	8	3rd Maxillipeds.	⎭
	9	Chelipeds.	Pincers—protection.
	10	1st Pereiopods.	⎫ Walking legs.—Movement.
	11	2nd Pereiopods.	⎩ Bear genital pore in female.
	12	3rd Pereiopods.	⎫
	13	4th Pereiopods.	⎭ Bear genital pore in male.
Abdomen	14	1st Pleopods.	Vestigial in female. Modified in male for transference of spermatozoa.
	15	2nd Pleopods.	Swimmerets in female. Modified in male as in 14.
	16	3rd Pleopods.	⎫
	17	4th Pleopods.	⎬ Swimmerets.
	18	5th Pleopods.	⎭
	19	Uropods.	Form tail fan with telson.

*There is evidence that there are really twenty segments, a pre-antennal segment, bearing no appendages, proceeding that which bears the antennules.

THE WATER FLEA

DAPHNIA

This organism, which serves as food for Hydra, is found abundantly in ponds. It is a *Crustacean* but is in a different ORDER, *Branchiopoda*, from that to which the Crayfish belongs.

Examine **Daphnia** *in pond water in a small aquarium.*

Note the general appearance of the animals and the curious jerky way in which they row themselves through the water.
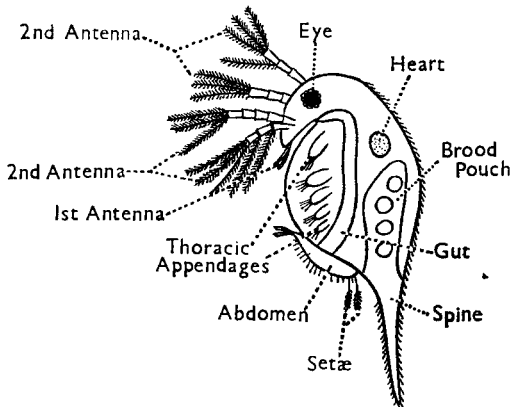
Fig. 56. Daphnia.

Examine a slide of **Daphnia**

Note that the whole of the body exclusive of the head is enclosed in a **carapace**, though on the ventral side this is incomplete.

On the **head**, which is unusual in shape, are the two **fused compound eyes**, large **antennae** by means of which the animal rows itself through the water and mouth parts which include **mandibles**. The **thorax** is large and bears **comb-like bristles** which are used for respiration and for collecting food. The **abdomen** is rudimentary. **Eggs** may be visible in what is known as the **brood pouch** towards the posterior end of the body. It is interesting to find that these develop parthenogenetically.

THE CENTIPEDE

SCOLOPENDRA

Centipedes are in the PHYLUM *Arthropoda* and the SUB-PHYLUM *Myriapoda* (body composed of a head and trunk, all the segments of the latter bearing legs. Respiration is by tracheae). They are in the CLASS *Chilopoda* (Each trunk segment bears one pair of legs and the first segment bears poisonous jaws).

The genital aperture is posterior. *Millipedes* also belong to the Myriapoda but are in the CLASS *Diplopoda* (each body segment really composed of two fused segments and bears two pairs of legs. Genital aperture anterior).

Examine the external structure of a **Centipede.**

There is a distinct **head** followed by a large number of segments which constitute the trunk. The **head** has a pair of **antennae** and two groups of **ocelli.** There are mandibles and two pairs of maxillae, the second pair being fused to form a labium. The **trunk** is composed of some twenty segments, the first of which bears a pair of appendages ending in a claw. A duct from a poison gland leads to this and these maxillipeds act as **poison jaws.** The segments of the trunk are flattened dorso-ventrally and in the soft tissue between the hard terga the spiracles open. Each segment bears a pair of **jointed legs.**

INSECTS

THE COCKROACH

PERIPLANETA

The Cockroach is in the PHYLUM *Arthropoda*, and belongs to the CLASS *Insecta* (Arthropods in which the body is divided into head, thorax and abdomen. They have three pairs of legs and, usually, two pairs of wings. Breathing is by means of tracheae and they generally undergo metamorphosis).

Periplaneta belongs to the ORDER *Orthoptera* (straight winged) which have mouthparts adapted for biting, anterior wings somewhat hardened with chitin, the posterior pair being membranous. This order also includes *grass-hoppers, locusts* and the *stick* and *leaf insects.*

There are two important species, **P. americana** and **Blatta orientalis** which is smaller, darker in colour and the female of which is deficient of wings. The young (known as nymphs) are similar to the adults except that they are small and wingless. They grow and undergo a series of moults or ecdyses, ultimately developing wings. Metamorphosis is thus incomplete and the insect is said to be *hemimetabolous.*

The following description refers to *P. americana.*

1. OBSERVATIONS ON LIVING ANIMALS

Observe the living insects.

Note the movements of the antennae and of the legs in walking.

Place the animal on its back and observe the motions of the mouth parts.

2. EXTERNAL ANATOMY

(1) Dorsal View

Examine the cockroach externally from the dorsal aspect. Use a lens as necessary.

The body is divided into head, thorax and abdomen and is protected by a chitinous exoskeleton which consists in each segment of **sclerites,** each composed of a **tergum** (dorsal), a **sternum** (ventral) and **pleura** (lateral).

Note the pear-shaped **head,** usually at right angles to the body and bearing tapering, many-jointed **antennae** and large **compound eyes.** The **thorax,** separated from the head by a short neck or **cervicum,** is subdivided into the **prothorax** (the **pronotum** or tergum of which hides the neck), the **mesothorax** covered by the **mesonotum** and bearing a pair of **elytra** (the anterior wings, somewhat hardened and covering the posterior wings when at rest) and the **metathorax** covered by the **metanotum** and bearing a pair of **membranous wings** strengthened by a framework of **nervures.** *Deflect the wings to the sides and pin them in position.* The segmented **abdomen** is covered by ten **terga** (the 8th and 9th being hidden under the 7th), and the membranous **podical plates,** *seen by lifting the* 10*th tergum.* The two jointed appendages under the lateral edges of the 10th tergum are the **cerci anales.** Internal to these in the male is a pair of short, many-jointed **styles** on the 9th segment.

(2) Ventral View

Examine the male and female animals from the ventral aspect. Again use a lens.

Note the **mouth** on the **head** and the three pairs of jointed **legs,** one pair attached to each segment of the **thorax.** The **abdomen** is covered by **sterna** similar to the terga. Nine are visible in the male and the 9th bears the **styles.** In the **female** only seven will be seen and the 7th is a large boat-shaped process serving as the floor of the **genital pouch.** In both sexes the **anus** is underneath the 10th abdominal tergum and the **genital pore,** surrounded by complicated structures, the **gonapophyses,** is below the anus. Look for the ten pairs of **spiracles,** the respiratory openings situated in the pleura near the anterior edges of the terga in the first and third thoracic segments and the first eight abdominal segments.

(3) Leg

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

Note the long proximal **coxa,** then the very small **trochanter,** followed by the longer **femur** and the long thin **tibia,** each of which

bear **bristles.** The distal joint is the **tarsus,** which is made up of five segments or **podomeres,** the last of which is often called the **pretarsus** sclerites and bears as its tip two **claws** with a pad or **arolium** between them.

(4) Head

This is actually composed of six segments but owing to fusion they are merely visible externally by sutures.

Examine the **head from the front,** *under a lens.*

Note the long, many-jointed **antennae** and the white oval areas near their bases known as **fenestrae.** Above the bases of the antennae are the curved black **compound eyes. Epicranial plates** cover the top and back of the head, the **frons** and **clypeus** covering the front and the **genae** the sides. Attached to the lower edge of the clypeus is the upper lip or **labrum** and three pairs of mouth parts: (i) the long-jointed **maxillary palps;** (ii) the shorter-jointed **labial palps** internal to them and (iii) the toothed **mandibles** posterior and dorsal to the labial palps.
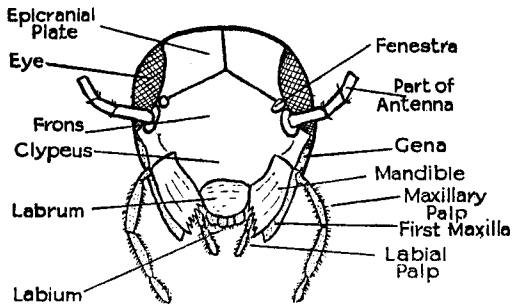
FIG. 57.   **Periplaneta.   Head Anterior View.**

(5) Mouth Parts

This should be left until last if the same insect is to be used for dissection.

Cut off the head, boil it gently in 2 per cent. caustic soda for a few minutes to remove the attached muscles. Pour off the caustic soda and wash thoroughly in water then transfer to a watch glass. Separate the parts with a small scalpel, starting with the labium, working forwards and finishing with the mandibles. Keeping them in their respective positions, examine under a hand lens. Then make a permanent preparation transferring the parts to a slide with small forceps. Dehydrate in 30, 50, 70, 90 per cent. and absolute alcohol. Clear in natural oil of cedar wood and mount, unstained, in balsam. The mandibles, being

thick, will cause rocking of the coverslip. To avoid this, a cell should be made to raise it. Pieces of cardboard of suitable thickness, placed where the edges of the coverslip will rest, will serve this purpose.
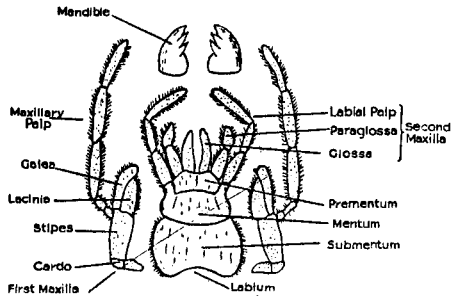
FIG. 58. **Periplaneta. Mouth Parts.**

Note the **Mandibles** or jaws with their inner margins toothed. The **Maxillae** (or **First Maxillae**) arise behind the mandibles and each consists of a **protopodite** made up of two sclerites, a proximal **cardo** and a distal **stipes** at the base of which is a small sclerite, the **palpifer,** the distal end bearing an inner, broad, pointed **lacinia** and an outer, softer, tapering **galea.** External to these is a **maxillary palp** consisting of five podomeres attached to the outside of the stipe.

The **Labium** (or **Second Maxillae**) shows a structure similar to that of the first maxillae but is smaller and partly composed of a large proximal podomere, the **submentum,** a smaller median one, the **mentum,** and a distal **prementum,** all being central and really composed of fused paired appendages. An outer **paraglossa** and inner **glossa** (or **lacinia**), constitute the **labial palp** (or **ligula**).

3. INTERNAL ANATOMY

It is essential that a freshly killed insect be used.

Melt a little of the wax in the centre of a dissecting dish, place the insect, dorsal side upwards, in the melted wax, keeping the edges of the terga above the wax. Allow the wax to cool, when the insect will be fixed. Cover with water. Remove the elytra and wings. Then carefully remove first the abdominal and then the thoracic terga one at a time, working forwards by lifting with forceps and cutting round the edge with small scissors. Examine under a lens.

1. THE HEART

The **heart** should be visible, enclosed in the pericardium as a long thirteen-chambered tube in the mid-dorsal line of the thorax and

abdomen, the chambers corresponding with the segments, and each chamber communicating with the pericardial cavity by a pair of openings, or **ostia,** on its sides. The pericardial cavity is in communication with the haemocoels around the viscera.

2. THE ALIMENTARY SYSTEM

Remove the heart and muscles carefully so as to avoid damaging the organs beneath.

Unravel the alimentary canal thus exposed from the white fluffy **fat body** *in which it is enveloped and pin it to one side.*

(1) The **oesophagus** runs into the thorax and joins the mouth to the **crop,** a dilated sac extending into the abdomen. The forked **visceral nerves** will be found on its surface. Note the two **salivary glands,** one on each side of the crop and lying in the thorax. *Take care not to damage them.* The ducts from the glands form a median **salivary duct** which opens into the **mouth.** The **proventriculus** or **gizzard** is a thick-walled muscular sac continuous with the crop, and this is followed by the mid-gut, or **mesenteron,** a narrow tube bearing at its anterior end seven or eight blindly ending tubes, the **hepatic caeca.**
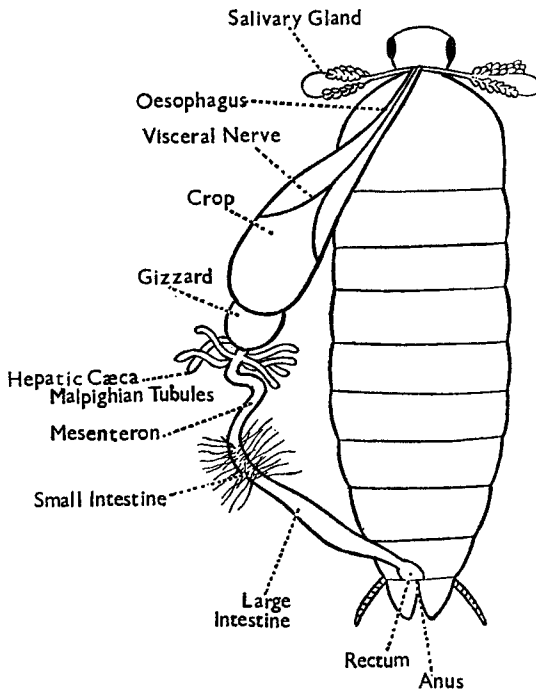
FIG. 59. Periplaneta. Alimentary System.

The coiled hind-gut is made up of the **small intestine** (sometimes called the ileum), a short tube, followed by the longer and wider **large intestine** (sometimes called the colon) which leads into the wide **rectum.** This opens to the exterior by the **anus.** At the beginning of the small intestine is a number of fine **Malpighian tubules.** They are not part of the alimentary system and will be considered later.

(2) *Cut open the* **proventriculus.**

Note the **cuticular teeth.**

(3) *Carefully remove one of the* **salivary glands** *with its ducts as follows: Remove the alimentary canal by cutting through the oesophagus and rectum. Then cut away the dorsal covering of the neck and head. Deflect the remains of the oesophagus forwards and cut through the salivary duct. Then carefully transfer the freed salivary gland with the duct to a watch glass of* 70 *per cent. alcohol to fix it. Stain with picro-carmine or Delafield's Haematoxylin, dehydrate, clear and mount in balsam. Examine under the lower power.*
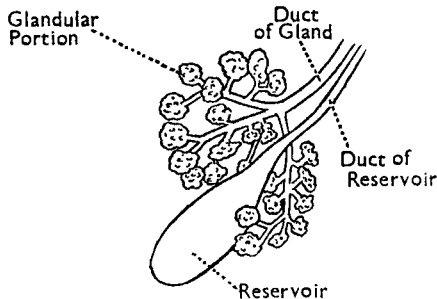
FIG. 60. **Periplaneta. Salivary Gland.**

Note that each gland consists of two diffuse **glandular portions** and a median sac, the **reservoir.** The duct from each glandular portion and that from each reservoir joins its fellow from the opposite side and the common ducts then unite to form the median **salivary duct.**

3. THE EXCRETORY SYSTEM

A large number of fine tubules at the beginning of the small intestine were seen when examining the alimentary canal. These are the **Malpighian tubules** and are the excretory organs. The excretions are passed into the intestine and are expelled with the faeces.

4. THE RESPIRATORY SYSTEM

This is composed of a number of tubular **tracheae** which open to the exterior by the **spiracles** (already seen) and which terminate internally in minute **tracheoles** in the tissues.

Remove one of the larger silvery-looking tracheae which ramify through the tissues of the body. Stain with picro-carmine and mount in dilute glycerine. Examine.
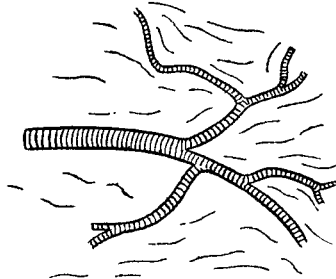
FIG. 61. **Periplaneta. Tracheae.**

Under *low power* note the **trachea** with spiral **chitinous lining** and the **cells** which secrete this lining.

Under *high power* note the **cells** as before and their **nuclei.**

5. THE REPRODUCTIVE SYSTEM

If a fresh insect is used very carefully remove the alimentary canal and the fat body.

(i) Male

The two **testes** and their **vasa deferentia** (which join) are embedded in the fat body with which they may have been removed. Note the two **vesiculae seminales** constituting the **mushroom-shaped gland** and composed of finger-like processes. The vasa deferentia after union
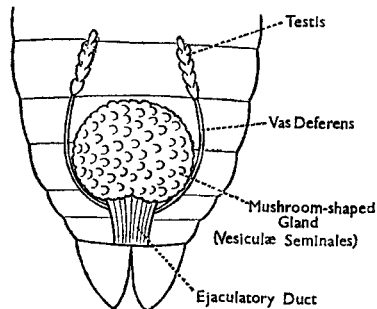
FIG. 62. **Periplaneta.**
Male Reproductive Organs.

lead into this and form the **ejaculatory duct,** a short muscular tube leading to the exterior by the **genital pore,** below the anus. Ventral to the ejaculatory duct is the elongated **conglobate gland.**

(ii) Female

There are two **ovaries** and each consists of eight **ovarian tubules,** showing swellings due to contained ova. The two **oviducts** are short and wide and join to form the **vagina** which opens to the exterior by a vertical pore in the genital pouch on the 8th abdominal sternum.
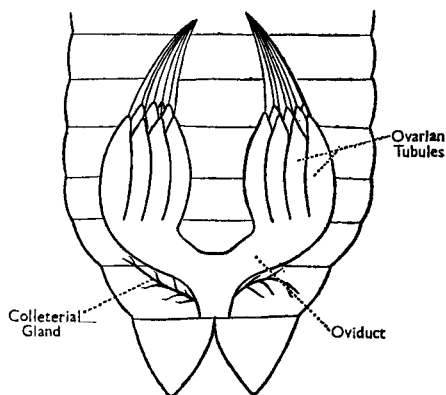
FIG. 63. Periplaneta.
Female Reproductive Organs.

On the 9th abdominal sternum is a pair of branched tubes, the **colleterial glands** which also open into the **genital pouch.** A pair of **spermatheceae** of unequal size will also be seen leading into the genital pouch.

Examine an **ootheca** *externally and then cut a* **longitudinal section** *and examine with a lens.*

This is a brown egg-case, shaped like a purse, which is formed by the colleterial glands. Sixteen fertilised ova are enclosed in two longitudinal rows of eight.

6. THE NERVOUS SYSTEM

Carefully remove the alimentary canal and fat body if a fresh insect is used and the dorsal coverings of the neck and head.

Note the white **cerebral ganglia** and the short, wide **circum-oesophageal connectives** which lead from them and join to form the
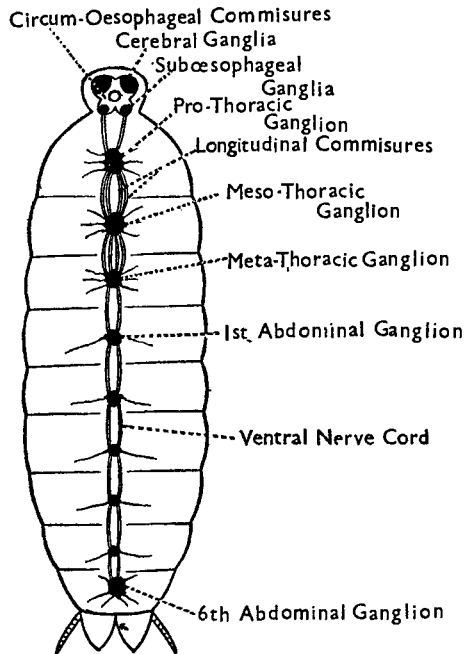
FIG. 64. Periplaneta. Nervous System.

suboesophageal ganglia. From this the double **longitudinal commissures** run to the **prothoracic ganglion**. The **meso-** and **metathoracic ganglia** follow and then six **abdominal ganglia**, all being joined by the **ventral nerve cord**. The three thoracic and the last abdominal ganglia are larger than the others. Nerves arise from all the ganglia.

INSECT LIFE HISTORIES

Note. The life-histories of the following insects, which are parasites or vectors, will be found in Part VII (Other Forms and Modes of Life):—*House-Fly, Mosquito, Aphis, Bug, Flea* and *Louse*.

THE CABBAGE WHITE BUTTERFLY

PIERIS BRASSICAE

Butterflies and Moths belong to the ORDER *Lepidoptera* (scaly-winged). The mouth-parts are adapted for sucking and the insect is *holometablolous* (complete metamorphosis).

Examine an **egg** *with a hand lens.*

It is bluntly conical in shape, yellow in colour and bears both vertical and horizontal ridges.

Examine the **larva,** *using a hand lens where necessary.*

FIG. 65. Pieris brassicae. Egg.

The *caterpillar* consists of a head and thirteen segments and is of a greyish-green colour with a yellow stripe dorsally and a wider one on each side. A number of short bristles protrude from black protuberances on its body.

The spherical **head** has a pair of toothed **mandibles** (which work sideways) posterior to the **labrum** with **maxillae** underneath. In the centre is the **spinneret** and externally on each side a pair of short **antennae.** On each side of the head, towards the ventral surface, is a group of six small black **ocelli** (simple eyes).

FIG. 66. Pieris brassicae. Larva.

The next three segments are the **thoracic segments.** Each bears a pair of five-jointed **true legs,** each of which terminates in a claw.

The remaining ten segments are the **abdominal segments.** No appendages are borne on the first two but each of the next four bears soft unjointed pro-legs, each ending in a pad and a semi-circle of hooks. The segments which follow have no appendages except the last which bears a pair of **claspers** similar to the pro-legs.

Careful examination with a lens will reveal the **spiracles** on the sides of the first thoracic and the first eight abdominal segments.

Examine the **pupa.**

The *chrysalis* is shorter than the caterpillar and is of a greenish colour with black and yellow spots.
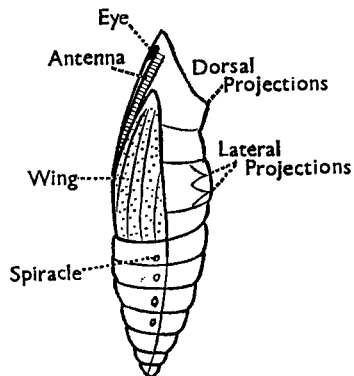
FIG. 67. Pieris brassicae. Pupa.

Dorsal and **lateral projections** (which hold the silken girdle in place when pupation takes place), spiracles, a segmented abdomen and the **outlines of developing** structures of the future imago such as **wings, legs** and **antennae** will also be seen.

Examine specimens of both **male** *and* **female imagines.**

The body is composed of **head, thorax** and **abdomen,** the thorax and abdomen being covered with hairs which makes it difficult to distinguish the three segments in the former and ten in the latter. The thorax bears three pairs of jointed **legs,** each composed of the usual five podomeres—coxa, trochanter, femur, tibia and tarsus—and two pairs of **wings.**

On the head note the two long, club-shaped and many-jointed **antennae** and the large **compound eyes** laterally placed.

Examine a slide of the **mouth-parts.**

There is a long coiled **proboscis** and a pair of **labial palps.**

Examine the **wings of both sexes** *from the dorsal and ventral aspects.* On the dorsal side these are creamy white in colour and the anterior
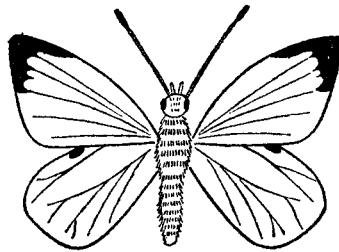
Male
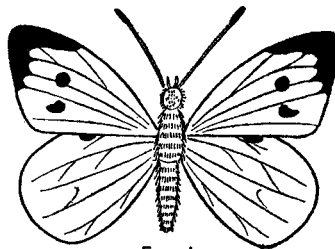
Female

FIG. 68. Pieris brassicae. Imagines. Dorsal View.

wings have black markings on the tips in both sexes. The female also has two black spots on each wing. The posterior wings also have black markings on their anterior edges in both sexes. The

ventral surfaces of the wings are pale greenish-yellow with black markings, though less conspicuous than those on the dorsal side.

Examine a slide of **scales from a butterfly's wing.**

These vary considerably in shape, size and colour. When in place on the wing they overlap like tiles on a roof.

THE HIVE BEE

APIS MELLIFICA

The Bee belongs to the ORDER *Hymenoptera* (membranous-winged) which have mouth-parts adapted for sucking. The larvae are limbless grubs and the insect is *holometabolous* (metamorphosis complete). This order includes ants and wasps.

Examine the **larva** *and* **pupa of the hive bee.**

Larva

This is white in colour, segmented and devoid of limbs.
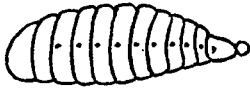
FIG. 69. **Apis mellifica. Larva.**

FIG. 70. **Apis mellifica. Pupa.**

Pupa

Segmentation can also be seen and the development of structures of the future imago will be clearly visible.

Imago

In the Summer there are three kinds of individuals in the hive— one **queen,** a fertile female whose sole duty is to produce ova, a few hundred males, **drones,** which are responsible for fertilising the queen and several thousand **workers,** sterile females in which the gonads do not develop and who build the hive and keep it clean, collect pollen and nectar from flowers, make honey, secrete wax and feed the larvae.

In consequence of their different functions, there are differences in structure in the three kinds of individual.

Examine and compare the structure of a **queen bee,** *a* **drone** *and a* **worker.**

The queen has a long abdomen and short wings, the drone has a much broader abdomen and large wings while the worker is the smallest of the three and has well developed wings.

The **head** of the worker bears complicated mouth-parts adapted to the obtaining of nectar from flowers, two large **compound eyes** (larger in the drone), three **ocelli** (simple eyes) and a pair of **antennae.**

Queen　　　　　　　Worker　　　　　　　Drone

Fig. 71. Apis mellifica. Imagines.

The **thorax** consists of three segments and each bears a pair of **legs** composed of the usual podomeres—**coxa, trochanter, femur, tibia and tarsus** but these are not identical, being adapted to the performance of different functions. The second and third thoracic segments bear a pair of **wings** which are all membranous, the two on each side being linked together by small hooks to enable them to function as a single wing in flight.

The **abdomen** is segmented and at its posterior end in the queen and the worker is a protrusible **sting** which contains a duct from a poison gland. In the worker it is barbed and can therefore be used only once. In the queen this is known as the **ovipostor** since it is used in the deposition of eggs. Note that the abdomen of the queen is long and narrow and extends beyond the wings when they are folded back.
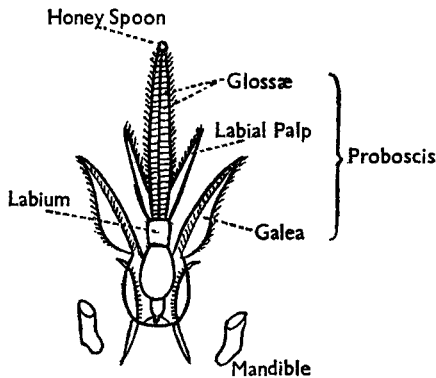
Fig. 72. Apis mellifica. Worker. Mouth-parts.

Examine a slide of the mouth-parts of the worker bee. (Refer to the diagram of the mouth-parts of the cockroach on p. 99.)

The **mandibles** are devoid of toothed edges and are somewhat spoon-shaped. They are used for kneading wax. In the first maxilla

there is an elongated **blade-like galea** but no lacinia while the maxillary palp is vestigial. In the second maxillae (labium) the two glossae are much elongated and fused together to form at the tip a small expansion known as the **honey spoon.** External to the glossae are the elongated **labial palps.** These very long maxillae form a protective sheath for the sucking tube or **proboscis** formed by the galeae of the first maxillae and the glossae and labial palps of the second maxillae or labium.

Examine a slide of the **first leg of the worker.**
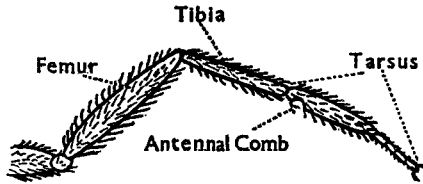
FIG. 73. Apis mellifica. Worker. First Leg.

The joint between the tibia and the tarsus is open and the anterior end of the tarsus is provided with a comb-like structure of short setae, used for removing pollen from the antennae which can be passed into it. This is known as the **antennal comb.**

Examine a slide of the **second leg of the worker.**
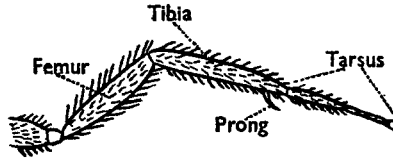
FIG. 74. Apis mellifica. Worker. Second Leg.

The tibia bears a short stiff seta known as the **prong;** it serves to remove pollen from the pollen basket on the third leg.

Examine a slide of the **hind leg of the worker.**
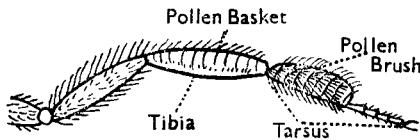
FIG. 75. Apis mellifica. Worker. Hind Leg.

The tibia bears a deep groove lined by bristles; this is the **pollen basket.** The first podomere of the tarsus bears stiff setae forming the **brush** (or **pollen comb**), used for placing pollen in the pollen basket.

THE DRAGONFLY

AESHNA CYANEA

Dragonflies belong to the ORDER *Odonata* (having strong, biting mouth-parts) and are hemimetabolous, there being no pupal stage. The larvae are aquatic *nymphs*.
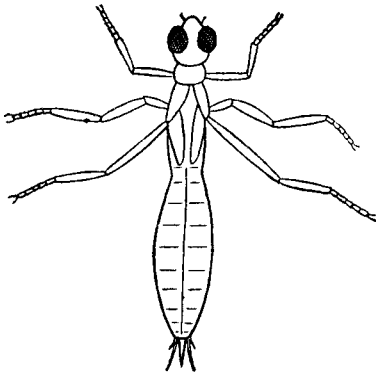
FIG. 76. Aeshna cyanea. Larva.

Examine the larva and the imago.
Larva
    This is an aquatic **nymph** with a broad **head** and two large protruding **compound eyes**. The labium is enlarged and modified into a tubular structure which is known as the **mask**. It is situated below the mouth and covers the front of the head. Composed of two sclerites, it terminates in slightly curved pincers.

The **thorax** bears three pairs of **legs** and, except in young larvae, two pairs of **rudimentary wings**.

FIG. 77. Aeshna cyanea. Mask of Larva.

The **abdomen** is composed of ten segments and bears two short and three long pointed processes at its posterior end.

Imago
    The broad **head** has two large, prominent blue **compound eyes** and three small **ocelli.** The **antennae** are very short and the mouth-parts are adapted for biting with powerful **mandibles.**
    The **wings** are large, equal in size and similar in shape and in some species they are coloured. A fine network of nervures gives each wing support.
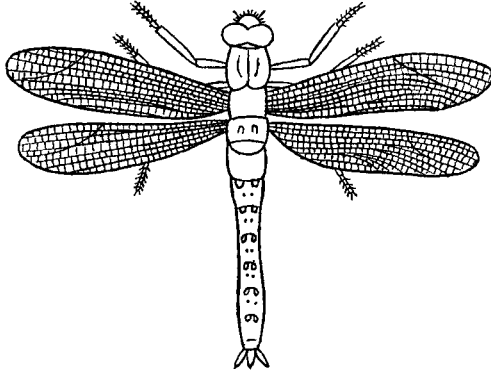
FIG. 78. Aeshna cyanea. Imago.

The **abdomen** is long and tapering and is composed of ten segments. At its posterior end are **claspers,** two in the male and three in the female. The body has a brilliant metallic colour which varies in different species.

BEETLES

Beetles are in the ORDER *Coleoptera* (sheath-winged), the anterior pair of wings serving as wing cases. The mouth parts are adapted for biting and the insects are *holometabolous*.

The Seven Spot Ladybird is a small but very common example of this Order and the Great Water Beetle a large aquatic type. Both will be examined.

THE SEVEN SPOT LADYBIRD
COCCINELLA SEPTEMPUNCTATA

Egg

Examine the **eggs** *and* **larvae,** *if available, with a hand lens* (they may be found on the leaves of plants infected with *aphis* on which the imagines feed).

The eggs are minute, ovoid in shape and yellow in colour.

Larva

These, too, are minute. They are grey in colour with black and yellow spots and the **abdomen** tapers posteriorly. The mouth-parts contain **mandibles** and the three **thoracic segments** a pair of jointed **legs** apiece. There are eight **abdominal segments** which decrease in width as they pass backwards. On the terga are small **tubercles** bearing **setae.**
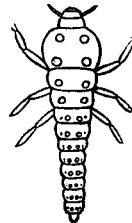
FIG. 79.
Coccinella. Larva
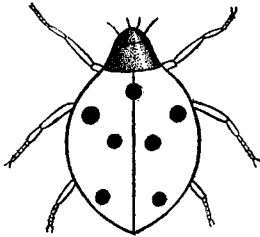
FIG. 80. Coccinella
septempunctata. Imago.

Examine the **Imago.**

Note the bright red colour of the ovoid body on the dorsal side of which are seven black spots, three on each elytrum and one, the anterior spot, shared between the two. (Other common species have five and two spots respectively and there is one with twenty-two. *C. ocellata* is larger than the others, has orange elytra, with numerous black spots each with a yellow margin.)

The **head** bears sickle-shaped **mandibles, compound eyes** and short **antennae** and is almost hidden by the **pronotum** which is black. The **anterior pair of wings** are large (as already seen), strongly thickened with chitin, red with seven black spots (already observed) and serve as wing cases when at rest. These are known as **elytra.** They almost completely cover the body. Beneath the elytra (at rest) is a pair of **membranous posterior wings.**

THE GREAT WATER BEETLE
DYTISCUS MARGINALIS

All stages of this insect's life-history are aquatic though atmospheric oxygen is used in breathing.

Examine the **Larva.**

The larva is about two inches in length and brownish in colour. The **head** is rounded and somewhat flattened and bears a pair of **antennae** and six **ocelli** on each side. The mouth-parts are adapted for biting by means of long sickle-shaped **mandibles** which are grooved on the inside. Each of the three segments of the **thorax** bears a pair of jointed **legs.** The **abdomen** tapers posteriorly and consists of eight segments, the last two having a chitinous fringe. A pair of pointed appendages, also fringed, project from the posterior end. The abdomen is arched in an upward direction and **spiracles** are situated at the posterior end.
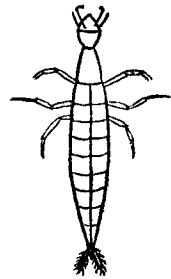
FIG. 81.
Dytiscus. Larva.

Examine the **Imago.**

This large carnivorous insect is oval in shape, the dorsal surface being slightly convex, the ventral side slightly keeled, the entire surface being extremely smooth and the colour brownish-black with a yellow edge.

The **head** bears a pair of **compound eyes,** long **antennae** and strong toothed **mandibles.** The **thorax** bears a pair of highly polished **anterior wings,** well thickened with chitin which serve as wing cases and which are known as **elytra.** In the male these are smooth but those of the female have deep furrows along the greater part of their length. The **posterior wings,** completely hidden by the elytra when the animal is in the water, are membranous. The three pairs of jointed **legs** are used for swimming and the **tibia** and **tarsus** are flattened and bear a fringe of stiff **setae.** The flat first three podomeres
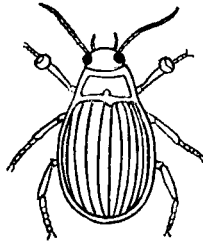
FIG. 82. **Dytiscus. Imago.**

of the tarsus of the front legs of the male are enlarged to form a kind of disc and on the ventral surface of them are several **suckers** (used for gripping the female). Eight pairs of **spiracles** are found dorsally towards the sides. All the thoracic segments are firmly interlocked as is the thorax to the abdomen and the meso- and meta-thorax and the first three abdominal segments are joined together. This gives the body considerable strength.

THE SNAIL

HELIX POMATIA

The Snail belongs to the PHYLUM *Mollusca* (Unsegmented coelomates with an exoskeleton, in most cases a shell. Body composed of head, foot and visceral mass enveloped in a respiratory mantle) and the CLASS *Gastropoda* (Head bears tentacles. Foot flattened. Enclosed in shell. *Snails, limpets, whelks* = Univalves.)

1. OBSERVATIONS ON THE LIVING ANIMAL

Note the part of the body which protrudes from the shell and which can be retracted into it. There are two pairs of **tentacles.** *Touch them* and note the rapid withdrawal.

Place the animal on a piece of glass and examine from underneath.

Note the forwardly moving waves of muscular contraction of the foot and the movements of the mouth. Observe the trail of slime left behind.

2. EXTERNAL STRUCTURE

(1) Before Removal of Shell

Examine a snail which has been freshly killed with the body extended. (See Appendix II.).

The Shell. Note the conical right-handed helix composed of about four and a half turns when completely formed. Each turn almost completely hides the previous one. The oldest part of the shell is the apex and this is known as the **nucleus of the shell.** Observe the coloration of the shell. Find the **shell mouth** and the **umbilicus,** an opening on the under surface which leads into the hollow columella, which is the axis around which the shell is coiled.

Cut away one side of the shell and find the **columella.** *Examine the cut edge.*

It will be seen that the **shell** consists of three layers: (i) the **periostracum,** the thin, horny, external layer made of conchiolin; (ii) the **prismatic** (or **middle) layer,** thick and densely calcified; and (iii) the smooth, glistening, pearly **nacreous layer** on the inside.

The Head. This is rounded though not distinctly separated from the foot and bears the slit-like mouth, which has distinct lips, on the ventral side and two pairs of tentacles. One pair, the **posterior tentacles,** are long and each bears at its free end a complex **eye,** visible only when the tentacle is fully extended. The **anterior tentacles** are smaller.

The Foot. This is large, oval, flattened and muscular and forms the ventral surface of the body. It bears a **pedal (mucous) gland** on its ventral side just ventral to the mouth.

The Collar is a fleshly structure surrounding the mouth of the shell, somewhat thicker at the sides. It is the free edge of the mantle.

The Genital Pore is situated on the right side, ventral to the optic tentacle. From it the **genital groove** runs backwards. The **Pulmonary Aperture** (or **pneumostome**) is on the right side of the collar. *Wash away any mucus and pass a seeker into this aperture.* It leads into the pulmonary or mantle cavity. The **Excretory Pore** is just inside the pneumostome and immediately behind it is the **anus.**

(2) After Removal of Shell

Carefully remove the shell with strong scissors, taking care not to damage the internal organs with the points. Cut upwards at first from the shell mouth and then round the sides of the coils, removing a portion of the shell at a time. Cut through the muscular attachment to the columella and remove the latter.

The Visceral Hump is that part of the snail which remains permanently in the shell. Note that it is coiled, the turns corresponding with those of the shell.   It is covered by a thin, transparent tissue through which the internal organs or viscera are visible.

FIG. 83. **Helix. After Removal of Shell. Dorsal View.**

Note the **mantle** (which secretes the shell), a thin layer of tissue forming the roof of the pulmonary cavity, the **kidney**, yellowish-white in colour, about half-way round the basal turn of the visceral hump and the **pericardium**, enclosing the **heart**, lying alongside the anterior side of the kidney. The **digestive gland**, dark reddish brown, reaches from the end of the kidney, beyond the pericardium to the apex of the hump and the **rectum** will be seen passing along the right edge of the basal turn of the hump.

THE FRESHWATER MUSSEL
ANODONTA CYGNEA

Anodonta cygnea, the Swan Mussel, is found partly buried in the mud at the bottom of rivers, lakes and ponds. It is also in the PHYLUM *Mollusca*. The CLASS is *Lamellibranchiata* (Head small. Foot wedge-shaped. Shell composed of two parts. Bilaterally symmetrical. *Mussels, oysters.*) These are known as *Bi-valves*.

The Octopus, squids and cuttlefish belong to a separate CLASS, *Cephalopoda* (Head well developed and bears large eyes. Foot modified into prehensile tentacles. Siphon for expelling water from mantle cavity. Shell sometimes present. Bilaterally symmetrical.)

1. OBSERVATIONS OF THE LIVING ANIMAL

Examine a fresh-water mussel in an aquarium.

Note that it lies, usually obliquely and with valves slightly opened, partly buried in the sand with one end, the posterior end, projecting upwards into the water. It moves slowly by a muscular foot. A stream of water enters the open end on one side, carrying food and oxygen.

Place a few grains of carmine near the partially opened valves.

Note the direction of the water current, It enters by what is known as the inhalent or ventral siphon on the ventral side and leaves by the exhalent or dorsal siphon.

P.B.—5

2. EXTERNAL STRUCTURE
The Shell

Note that the **anterior end** is rounded and the **posterior end** more pointed and that the animal is bilaterally symmetrical, the two halves or **valves** being joined along a straight **hinge line** by a **ligament.** This is dorsal. It will be easy, therefore, to determine which are the right and left sides. Each valve shows a series of lines which start from a small elevation near the anterior edge of the hinge line. This is the oldest part of the shell and is called the **umbo**: the lines are **lines of growth.**

Wedge open the shell with the handle of a scalpel.

Note the **mantle lobes** lining the valves and the **adductor muscles** which are for closing the shell.

Place the animal in a dish with either valve uppermost and carefully cut through the mantle lobes and muscles with a small scalpel, cutting through the muscles close to the shell. Remove the valve and examine the inside of it.

On the inside of the valve note the following **muscle impressions:—**

The **anterior adductor,** large and oval in shape, near the anterior end of the shell, towards the dorsal side.

The **posterior adductor,** larger than the anterior, near the posterior end of the shell, and near the dorsal edge.

The **protractor of the foot,** small, behind the anterior adductor.

The **anterior retractor of the foot,** also small, beside the anterior adductor but nearer the hinge line.

The **posterior retractor of the foot,** again small, next to the posterior adductor.

Note also the streak between the anterior and posterior adductors, which marks the insertion of the mantle and which is known as the **pallial line.**
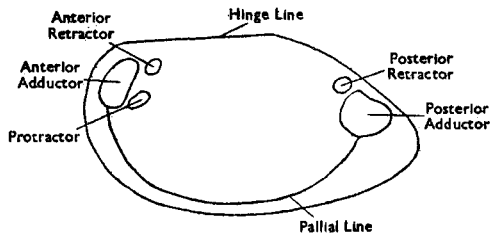
FIG. 84. Anodonta. Interior of Right Valve. Muscle Impressions.

Now break the shell near its rounded edge away from the muscle impressions, and examine the broken edge with a lens.

The shell will be seen to consist of three layers:—

(i) a thin, horny outer layer called the **periostracum**. It is made of a substance called conchiolin.

(ii) a **prismatic layer** (or **middle layer**), also made of conchiolin, but partly calcified.

(iii) an inner layer which lines the inner surface except at the edge, also partly calcified. This is the **nacreous layer.**

The Mantle Cavity

Examine a freshly killed animal if possible, but preserved material may be used.

Find the **anterior** and **posterior adductor muscles** and the **anterior** and **posterior retractors of the foot.** *They can be identified from the corresponding muscle impressions on the inside of the valve.*

The two **mantle lobes** cover the side of the body and were joined to the valves along the pallial line. At the posterior end below the posterior adductor muscle, the mantle is thickened and pigmented at the edge. Here will be found a ventral slit, the **inhalent** or **ventral siphon** bounded by small **tentacles.** Immediately dorsal to it and much smaller, is the **exhalent** or **dorsal siphon,** also known as the **cloacal aperture.** This is not surrounded by tentacles. Between the two mantle lobes is the **mantle cavity.** This is divided into a large **branchial chamber** on the ventral side and smaller **supra-branchial**
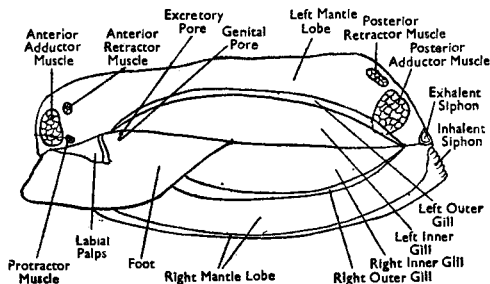
FIG. 85. **Anodonta. After Removal of Left Valve. Left Gills deflected upwards (Semi-Diagrammatic).**

passages on the dorsal side. The two join posteriorly to form the **cloacal chamber**. *Deflect back the right mantle lobe.* In the branchial chamber lie the muscular **foot,** extending downwards, the **visceral mass** which form the upper and larger portion, and the gills.

At the sides of the visceral mass and reaching from the posterior adductor muscle to the posterior edge of the mantle cavity are

two pairs of flaps known as the **inner gills** and **outer gills** though they do not properly function as such. The two pairs of folds at the sides of the anterior part of the foot between the anterior adductor muscle and the gills are two pairs of flaps called the **labial palps.** Between the anterior adductor muscle and the anterior edge of the foot is the **mouth.**

VI. CHORDATE ANIMALS

These are in the PHYLUM *Chordata* (bilaterally symmetrical animals possessing a dorsal **notochord** in the embryo which persists only in the simpler types, a tubular dorsal nerve cord, a ventral heart, branchial clefts penetrating the wall of the pharynx in the embryo but which persist throughout life in fish, post anal tail usually present).

This Phylum is divided into two SUB-PHYLA—*Acrania* (simple types devoid of a skull or a true brain. The notochord persists and there is no endoskeleton or heart, *e.g., Amphioxus*) and *Craniata* (in which there is definite cephalisation, skull and brain. The notochord is replaced by a vertebral column [in some types not entirely]. An endoskeleton and a heart are both present). There are five CLASSES. in this SUB-PHYLUM—*Pisces, Amphibia, Reptilia, Aves* and *Mammalia.*

THE DOGFISH
SCYLIORHINUS

Dogfish are in the PHYLUM *Chordata*, SUB-PHYLUM *Craniata* and CLASS *Pisces*. There are two chief SUB-CLASSES—*Elasmobranchii* (fish with cartilaginous endoskeleton and exoskeleton of dermal denticles. Tail heterocercal. *Dogfish, sharks, skates, rays*) and *Teleostei* (fish with skeleton mainly of bone and exoskeleton of bony scales. Have opercula. Tail homocercal. Have air-bladder. *Bony fish*. The dogfish is in the SUB-CLASS. *Elasmobranchii* and the ORDER *Selachii* (hyostylic jaws and no operculum).

The commonest SPECIES of dogfish which frequents the British coast is **Scyliorhinus canicula**, the lesser spotted dogfish or Rough Hound. The greater spotted dogfish or Nurse Hound is S. **stellaris** and the spiny dogfish is **Squalus acanthias**. There are certain minor anatomical differences between Scyliorhinus and Squalus, and while the former is **oviparous** (*i.e.*, lays eggs), the latter is **ovo-viviparous** (*i.e.*, the young are hatched before leaving the body of the female).

The following description applies to **Scyliorhinus.**

I. OBSERVATIONS ON LIVING FISH

If possible, observe living fish in an aquarium.

Note how they swim, and pay particular attention to the way in which the fins and tail are used. Examine the method of breathing, how they eat their food and how they rest.

II. EXTERNAL ANATOMY

(1) *Place a complete dogfish on a dissecting board and examine its external structure.**

Note the shape and colour of the body, which is divided into **head, trunk** and **tail.** Note the **lateral line,** marking the position of a sense organ running along each side of the head and body.

(i) The Head

The head bears on the ventral side a large crescentic **mouth,** in front of which are the two **nostrils,** circular apertures connected to

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*Specimens preserved for dissection generally have the greater part of the tail removed and the ventral body wall is sometimes cut open. Fresh specimens are better and are sometimes obtainable.

the mouth by the **oro-nasal grooves.** The **eyes,** with immovable upper and movable lower lids, are at the sides of the head, and immediately behind each is a small round aperture, the **spiracle,** which is a modified gill-cleft. The five slits on each side behind the eyes are the **gill-slits.** The last of these marks the posterior end of the head. *Pass a seeker through the spiracle and gill-slits into the pharynx.*

On the surface of the head are rows of minute apertures of the **sensory canals** or **ampullary canals,** the latter being abundant on the snout. These apertures contain a gelatinous substance. *Squeeze the head to see this substance exude.*

(ii) The Trunk

The trunk bears paired and unpaired fins. The anterior pair of paired fins are the **pectoral fins.** They are roughly triangular in shape and project horizontally from the ventral side. The posterior pair of paired fins are the **pelvic fins,** somewhat similar to the pectoral fins, though smaller. In the **male,** a pair of so-called **claspers,** muscular rods strengthened by cartilage internally, will be seen between the pelvic fins. Their function is that of an intromittent organ in copulation. The pelvic fins mark the posterior end of the trunk. Between their bases is the **cloacal aperture,** just posterior to which on either side are the **cloacal pouches** bearing **abdominal pores.**

(iii) The Tail

The unpaired fins are the **anterior** and **posterior dorsal fins** which project vertically from the animal's back, the **ventral fin** projecting vertically from the ventral side, and the **caudal fin** which surrounds the end of the **heterocercal tail** (a tail with its axis directed upwards and having a large part of the caudal fin on its ventral side).

Make drawings of (i) **the lateral view** of the entire fish, (ii) the **ventral view of the head,** (iii) the **ventral view of the male and female pelvic fins,** noting the structures above.

(2) *Examine the surface of the body with a lens and rub the finger along the skin both backwards and forwards.*

Embedded in the skin are small tooth-like structures, **dermal denticles** (or **placoid scales**).

Open the mouth as far as possible and note the three or four rows of teeth on the jaws, similar in structure to the dermal denticles.

(3) *Examine a prepared slide of a* **vertical section of a dermal denticle** *under the low power.*

It is composed of a **basal plate** which is embedded in the skin and which bears a backwardly projecting **spine.** The basal plate is made

of **cement** and the spine of **dentine** which is covered externally by **enamel.** The **pulp cavity** in the basal plate passes into the spine where it branches considerably in the **dentine.**

III. THE SKELETON

The skeleton is entirely cartilaginous and consists of (i) an **axial skeleton** (the skull and verterbral column) to which should be added a **visceral skeleton** (the skeletal parts of the jaws, the hyoidean arch and the branchial arches), and (ii) **an appendicular skeleton** (the girdles and the skeletal part of the fins).

Examine a prepared disarticulated skeleton.

The Axial Skeleton

The Skull

This consists of the cranium, to which is attached the visceral skeleton which supports the jaws and pharynx, and the sense capsules.

Examine a skull from which the visceral arch skeleton has been removed.

(1) Dorsal View

The somewhat oblong **cranium** or **chondrocranium** (because it is cartilaginous) is bounded in front by the two large oval **olfactory capsules** with a large dorsal hole, the **anterior fontanelle,** in the centre between and behind them. The olfactory capsules bear three **rostral cartilages** projecting from the anterior end and constituting the **rostrum.** The sides of the cranium project as the **supra-orbital ridges** and the **auditory capsules** are fused laterally to the posterior ends. Note the ridges which mark the positions of the anterior and posterior semi-circular canals of the "ear". The large hole in the hinder end of the cranium is the **foramen magnum** through which the spinal cord passes, and on either side of the foramen magnum are the rounded **occipital condyles** which articulate with the first vertebra.
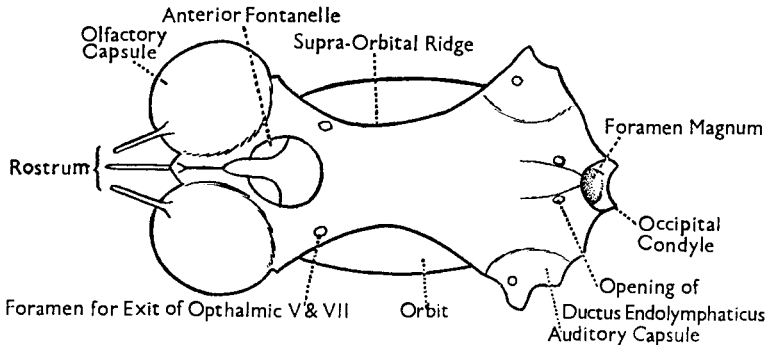
FIG. 86. **Scyliorhinus. Chondrocranium. Dorsal View.**

The two **foramina for the exit of the ophthalmic** branches of the **Vth and VIIth nerves** will be seen just posterior and slightly lateral to the anterior fontanelle and the **opening of the ductus endolymphaticus** will be seen on each side where the auditory capsule fuses with the cranium at its inner end.

(2) Ventral View

The base of the skull is a wide flat cartilage. Running across the posterior end will be seen a pair of **grooves for the carotid arteries.** These end in two **foramina for the carotid arteries.** Note also the **olfactory capsules** and the **olfactory openings,** considerably covered by the **nasal cartilages** and the rostrum. The **upper jaw** and **lower jaw** and the **ligaments** connecting the two halves of each will be seen. These will be described below.

(3) Lateral View

Note the ventral rostral cartilage, which with the two dorsal rostral cartilages constitute the **rostrum,** the **olfactory capsule,** the **auditory capsule,** the **occipital condyles** and the **orbit,** bounded above by the **supra-orbital ridge** and below by the **sub-orbital ridge.** In the orbit will be seen the following **foramina:**

The most posterior dorsal foramen is the **foramen for the entry of the ophthalmic branch of the VIIth nerve.** Immediately below and slightly anterior to this is the **foramen for the entry of the ophthalmic branch of the Vth.** From both these foramina, the **ophthalmic grooves** run forwards to a foramen at the anterior dorsal end of the orbit which is the common **foramen for the exit of the ophthalmic branches of the Vth and VIIth nerves** (already seen in the dorsal view). Ventral and slightly anterior to the foramen by which the ophthalmic branch of the Vth enters is the **foramen for the IIIrd nerve,** and dorsal and
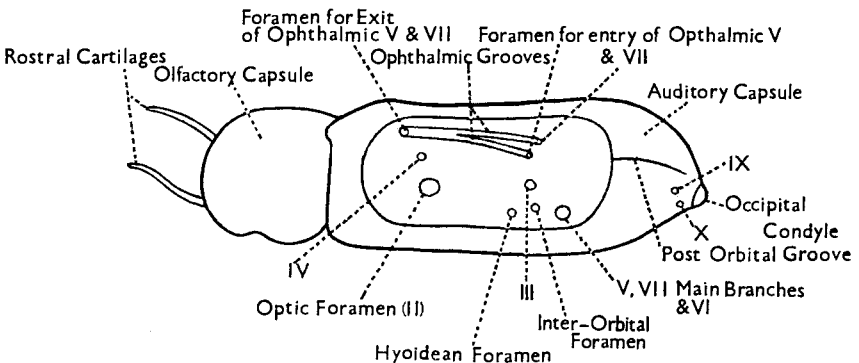
Fig. 87. **Scyliorhinus. Chondrocranium. Lateral View.**

anterior to this, under the ophthalmic groove, is the **foramen for the IVth.** Beneath this is a large foramen for the **IInd nerve,** the **optic foramen,** ventral and posterior to which is a **hyoidean foramen** for the "hyoidean" artery. Posterior to this is the **inter-orbital foramen,** the aperture of the inter-orbital canal, behind which is a large **foramen for the main trunk of the Vth and VIIth nerves** and the **VIth.** Note the **post-orbital groove** which runs back from the orbit, at the posterior end of which is the **foramen for the IXth nerve.** The **foramen for the Xth nerve** is just at the side of the foramen magnum and may not be seen in this view.

The Visceral Arch Skeleton

There are seven hoops of cartilage. The first or **Mandibular Arch** is modified to support the upper and lower jaws. The **upper jaw** is made up of the **palato-quadrate cartilage** which meets its fellow of the opposite side anteriorly. These cartilages are attached to the skull by the **ethmo-palatine ligaments** in front of the orbit and by the **pre-spiracular ligaments** in the region of the auditory capsule. The **lower jaw** is made up of a pair of **Meckel's cartilages.** Both jaws bear **labial cartilages.**

The second or **Hyoid Arch** is partly concealed by the jaws and consists on each side of an upper cartilage, the **hyomandibular cartilage,** and a lower one, the cerato-hyal cartilage, which articulates with a ventral plate of cartilage supporting the tongue, the **basi-hyal.**

The five remaining arches are the **Branchial Arches** which support the gills and are similar to each other except that the first and fifth have certain modifications.

FIG. 88. **Scyliorhinus. Structure of Branchial Arch Skeleton.** (Diagrammatic.)

Each arch contains a rod-like **pharyngo-branchial cartilage,** the most dorsal, an **epi-branchial cartilage,** short and plate-like, a rod-like **cerato-branchial cartilage** running inwards and forwards and a **hypo-branchial cartilage,** a short rod, running backwards and inwards. The **basi-branchial cartilage,** a long flat plate, rather pointed posteriorly, runs along the ventral side and is common to all.

In the 1st branchial arch, the hypo-branchial is directed forwards and it is not joined to the basi-branchial but to the basi-hyal. The 5th branchial arch has no hypo-branchial; consequently its cerato-branchial is much wider than in the other four and directly articulates with the basi-branchial. The 4th and 5th pharyngo-branchials

are fused. On the 2nd, 3rd and 4th branchial arches are the **extra-branchials,** ventral and external to the arches, often missing from prepared skeletons. The 5th cerato-branchials bear notches for the Cuverian ducts.
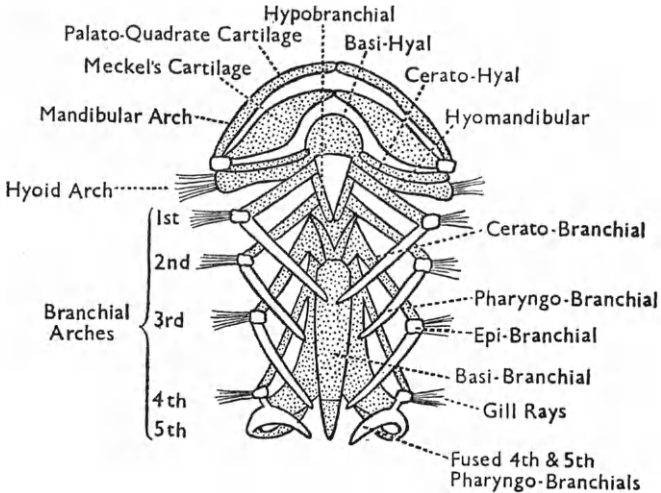
FIG. 89. **Scyliorhinus. Visceral Arches. Dorsal View.**

The **gill rays (branchial rays)** are borne on the posterior edges of the epi-branchials and cerato-branchials of the first four branchial arches and on the hyomandibular and cerato-hyals of the hyoid arch. *Draw* a dorsal view of the Visceral Arch skeleton.
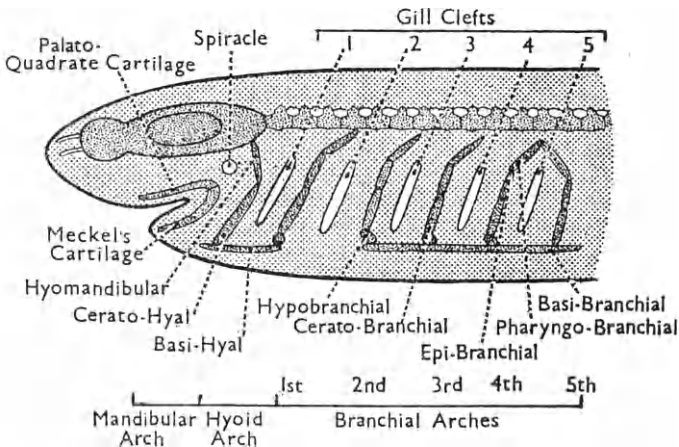
FIG. 90. **Scyliorhinus. Relation of Visceral Arches to Gill Clefts. Diagrammatic.**

The Vertebral Column

This consists of about 130 similar vertebrae.

(1) *Examine a few vertebrae from the trunk region in lateral view.*

Note the body or **centrum**, bearing the **vertebral neural plates** on the dorsal side with the **intervertebral neural plates** between them. Between the vertebral neural plates and the intervertebral neural plates are the rounded **neural spines.** The notches projecting from the sides of the centra in this region are called **transverse processes*:** they have slender **ribs*** attached to them.
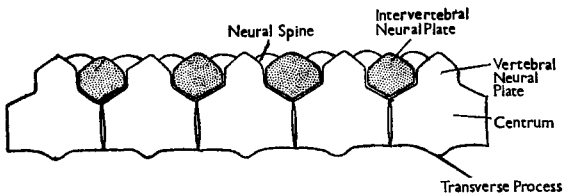
(a) Five vertebrae from trunk region. Lateral view.
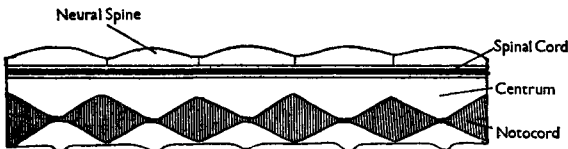
(b) L.S. of part of vertebral column.
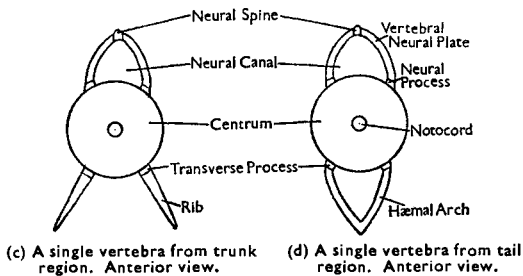
(c) A single vertebra from trunk region. Anterior view.

(d) A single vertebra from tail region. Anterior view.

FIG. 91. Scyliorhinus. Vertebral Column.

(2) *Examine the anterior end of a vertebra from the trunk region.*

Note that the **centrum** is concave at both ends and therefore said to be amphicoelous and that it contains **notochordal tissue** in the centre. The centrum is surmounted on the dorsal side by the **neural arch** composed of the two **neural processes** in contact with the centrum, above which are the **vertebral neural plates** with the **neural spine** between them on the dorsal side. The two **transverse processes**

*These are not homologous with those of higher vertebrates.

are produced laterally from the lower region of the centrum and bear very short **ribs.**

(3) *Examine the anterior end of a vertebra from the tail region.*

Note the structures in (2) and that the transverse processes, or rather **haemal processes,** join to form the **haemal arch,** in which the caudal artery and vein are situated.

(4) *Cut a portion of the vertebral column vertically in the mid-longitudinal (sagittal) plane.*

Note the structures in (2) above except the lateral processes and ribs, observing the widening of the notochordal tissue between each pair of centra, and noting, inside the neural arch, the **spinal cord.**

The Appendicular Skeleton
(1) The Pectoral Girdle

This is an incomplete hoop of cartilage, complete on the ventral side only, which is known as the **coracoid portion;** it has a concave **pericardial depression** on the dorsal surface for the ventricle of the heart. The curved pointed processes which arch round to the dorsal side constitute the **scapular portion.** Where the two portions join are three smooth **glenoid facets** for the articulation of the pectoral fin.
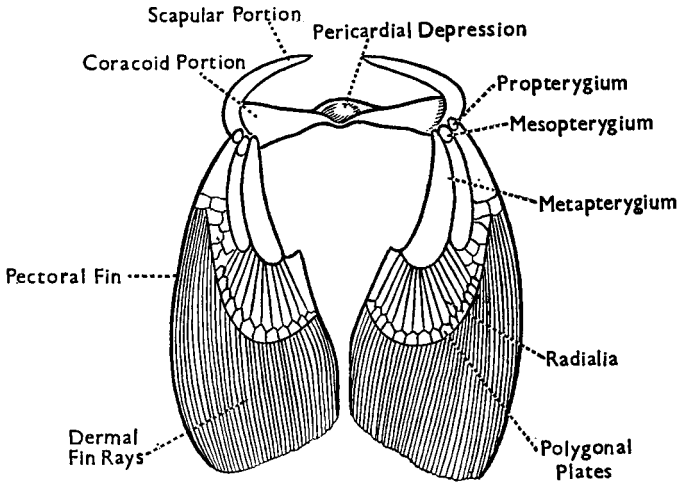
FIG. 92. Scyliorhinus. Pectoral Girdle and Fin.

(2) The Pectoral Fins

Each articulates with the glenoid facets at the bend of the hoop, and is composed of three basal cartilages, the small anterior **pro-pterygium,** the longer middle **mesopterygium,** and the still longer

posterior **metapterygium.** Radiating out from these three cartilages, are the **radialia** (or **cartilaginous fin rays**) which bear at their distal ends a few rows of very small cartilages, **polygonal plates.** The rest of the fin is supported by the elastic **dermal fin rays (dermotrichia).**

(3) The Pelvic Girdle

This consists of a more or less straight bar of cartilage, the middle part of which is called the **ischio-pubic portion,** the outer parts being known as the **iliac portions.** The **acetabular facets** for the attachment of the pelvic fins are on the iliac portions.
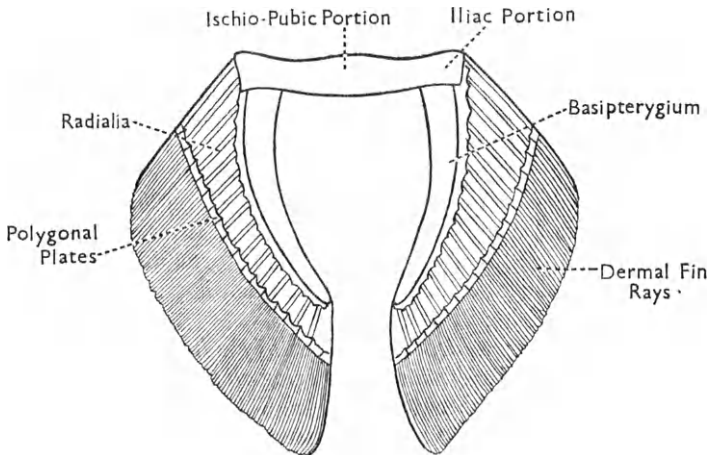
FIG. 93. Scyliorhinus. Pelvic Girdle and Fin. (Female.)

(4) The Pelvic Fins

These articulate with the acetabular facets near each end of the bar, and each is supported by a single long curved **basi-pterygium,** bearing on its outer edge a number of long **radialia** and then a few tiny **polygonal plates** as in the pectoral fin, the rest of the fin being supported by **dermal fin rays (dermotrichia).** In the **male,** each **clasper** is composed of a rod of cartilage, grooved on its inner border and attached to the posterior end of the basi-pterygium.

(5) The Dorsal, Ventral and Caudal Fins

Each consists of a row of rod-like **basal cartilages** and a row of **radialia** bearing **polygonal plates** at their distal ends. The cartilaginous parts are sometimes absent in the ventral and caudal fins.

(6) The Articulated Skeleton

Examine the articulated skeleton to see the relationship of the various parts.

IV. INTERNAL ANATOMY

(1) THE MUSCLES

Cut out a large rectangle of skin from the side of the fish in the tail region.

The muscles are arranged in zig-zag shaped segments, **myotomes,** separated by septa of connective tissue called **myocommata.** This shows metameric segmentation.

(2) THE ALIMENTARY SYSTEM

(1) *Place the dogfish, ventral side upwards, on a dissecting board and fix awls through the basal parts of the pectoral and pelvic fins. Make a median, but not too deep, incision from the level of the pectoral fins to the cloaca cutting through the skin and body-wall, and pin back the flaps on either side with awls. Transverse cuts may be made at both ends to facilitate the deflection of the body wall.* (Preserved specimens if not injected are partially opened to admit formalin.)

The **Abdominal** or **Peritoneal Cavity,** which is part of the **Coelom,** contains the following digestive viscera.

It will be necessary to deflect the lobes of the liver outwards and the stomach to the animal's left in order to bring all the structures into view.

The lower end of the **oesophagus** leads from the pharynx to the **stomach,** a large U-shaped sac lying between the **left** and **right lobes** of the **liver.** The left lobe is somewhat divided as the proximal end forming what is sometimes called the **median lobe.** The liver is attached to the anterior abdominal wall by a **suspensory** or **falciform ligament.** The large side of the stomach is the **cardiac portion,** the narrow side the **pyloric portion** and this leads by the **pylorus** into the **intestine.** Find the **gall-bladder,** embedded in the median lobe of the liver and the **bile duct** between the liver and the stomach, which leads from the gall-bladder to the dorsal side of the intestine (this part sometimes being called the duodenum). The **pancreas** is a whitish body lying between the pyloric portion of the stomach and the intestine, which it enters by the **pancreatic duct.** It will be seen that the intestine gradually gets wider. Continuous with it is the narrow **rectum** into which the small dorsally placed **rectal gland** opens. The rectum opens into the **cloaca.** The alimentary canal is suspended by **mesentery,** that supporting the stomach being called the **mesogaster.**

*The terms duodenum and ileum into which the intestine are sometimes differentiated are not aptly applicable in the dogfish as there is insufficient demarcation.

Note also the **spleen,** a large reddish body attached to the posterior end of the stomach with a branch running up alongside the pyloric portion, though it is not part of the alimentary system.

(2) *Cut lengthwise along the sides of the intestine, remove the ventral wall carefully and wash out the contents.*

Examine the so-called **spiral valve** in the intestine.

(3) *Now examine the* **blood vessels associated with the alimentary canal.**

Deflect the alimentary canal as far as possible outwards and to the animal's left and turn it over in order to expose the dorsal side without damaging any of the supporting tissues. Find the following arteries which take oxygenated blood to the organs.

The short **coeliac artery** arises from the **dorsal aorta** near the anterior end of the stomach and divides into (i) the **hepatic artery**
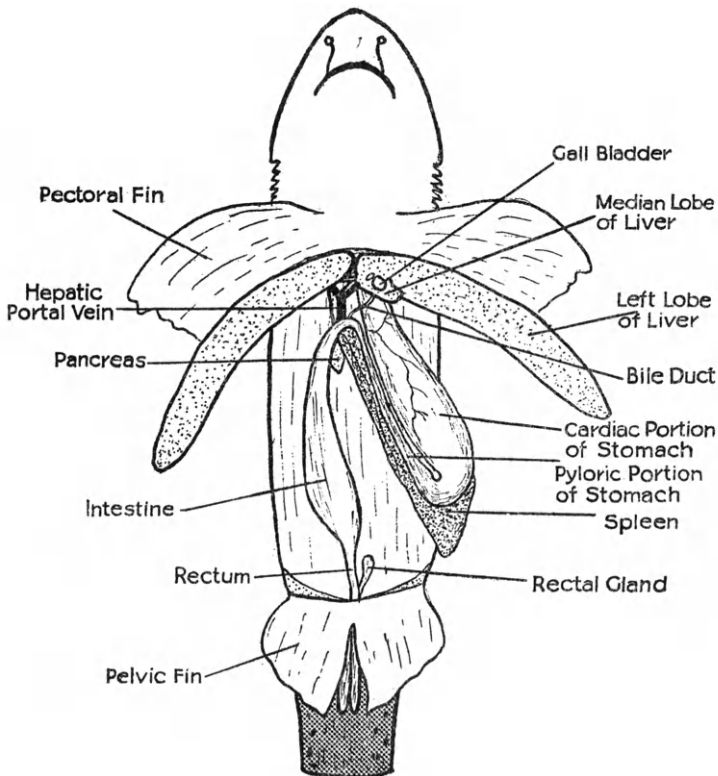
FIG. 94. Scyliorhinus. Alimentary Canal. Liver Displaced
Outwards and Stomach to Animal's Left.

going straight to the liver, (ii) the **gastric artery** to the stomach, and (iii) the **intestino-pyloric artery** to the anterior part of the intestine and the pancreas.

The **anterior mesenteric artery** arises about an inch or so behind the coeliac and goes to the rest of the intestine and the rectum. It will be seen on the posterior side of the intestine.

The **lieno-gastric artery** arises immediately behind the anterior mesenteric and the two arteries cross: it runs to the stomach and spleen.

The **posterior mesenteric artery** arises about one and a half inches behind the lieno-gastric and goes to the rectal gland.

Now find the following veins which carry deoxygenated blood from the organs.

Find the large **hepatic portal vein** which will be found to enter the liver slightly right of the middle line. Before doing so it divides into three branches, one to each lobe. *It is best to trace this vessel backwards.* It runs alongside the bile-duct and pancreas and is formed by the union of veins from the intestine, stomach, spleen and pancreas.

The **hepatic sinuses,** one from each lobe of the liver, will be seen at the anterior end of the liver. They will be examined again later when studying the venous system.

(3) THE VASCULAR SYSTEM

The circulation of the blood is a single circulation, *i.e.,* the blood is pumped by the heart to the gills and straight on to the rest of the body and so back to the heart.

It is necessary first to remove the alimentary canal and then to expose the heart.

Open the pericardial cavity, which is the anterior part of the coelom, by cutting through the pectoral girdle and removing the ventral body wall and the central part of the coracoid portion of the girdle.

The **pericardial cavity** is triangular in shape, the apex being directed forwards. It is almost completely filled by the heart. *Pass a seeker into the* **pericardio-peritoneal canal,** which lies dorsal to the sinus venosus (see below), and puts the pericardial and peritoneal cavities into communication.

By carefully opening the pericardium the heart will be exposed.

(1) The Heart—External Ventral View

This consists of four chambers, and is bent dorso-ventrally into an S-shape.

Note the **sinus venosus,** a roughly triangular sac at the posterior end with the apex directed forwards. *This will be rendered more easily visible if the ventral chamber is pressed slightly forwards.* The

apex of the sinus leads into the **auricle,** a large thin-walled triangular chamber on the dorsal side, part of which can be seen on each side of the ventral chamber. *To see this more fully, press the ventral chamber to one side.* The auricle leads into the prominent thick-walled ventral chamber, the **ventricle,** continuous with which is a straight tube, the **conus arteriosus,** leading in an anterior direction.

(2) The Venous System

It is seldom necessary to do this dissection but if this system is dissected a fresh specimen will be required for subsequent dissections.

The venous system is made up mostly of a number of dilated cavities called sinuses and if required, should be studied before dissecting the arterial systems. Apart from the hepatic sinuses, which enter the sinus venosus direct, all the main sinuses enter two Cuverian sinuses (or ducti Cuvieri) which lead transversely into the corners of the sinus venosus. *These should therefore be opened in order to find the entrances of these sinuses, this will be simplified if seekers are inserted into their openings. Wash the blood out of the sinus venosus and ducti Cuveri after opening them.*

Trace the sinuses away from the sinus venosus (but remember that the blood in them flows to the heart).

Each of the two short, narrow **Cuvierian sinuses,** or **ducti Cuvieri,** which enter the sinus venosus laterally from the dorsal side receives (i) at its dorsal end the **anterior cardinal sinus,** a large space between the dorsal body wall and the gill pouches, (ii) the **posterior cardinal sinus,** lying alongside its fellow on the dorsal body wall of the abdominal cavity almost in the middle line and formed posteriorly by a number of **efferent renal veins** from the kidneys, (iii) at about its middle, the small **inferior jugular sinus** from the floor of the pharynx, and at its anterior end (iv) the **subclavian vein** from the pectoral fin and neighbouring parts and (v) the **lateral abdominal vein** from the ventral body wall.

Expose and trace the **anterior cardinal sinus** *forwards on one side by making an incision from a point just dorsal to the spiracle, straight back dorsal to the gill clefts to a point just beyond the last one.*

Note that this sinus communicates with the large **orbital sinus** at the back of the eye by a narrow tube, the **post-orbital sinus.** *Insert a seeker into it.* The two orbital sinuses communicate with each other by a transversely running **inter-orbital sinus** (the foramen was seen in the lateral view of the skull). The **nasal sinus** on the posterior side of the olfactory organ also communicates with the orbital sinus. The **hyoidean sinus** from the floor of the mouth enters the anterior cardinal sinus just anterior to the first gill.

Nasal Sinus

Inter-orbital Sinus

Orbital Sinus

Post-orbital Sinus

Hyoidean Sinus

Anterior Cardinal Sinus

Sinus Venosus

Inferior Jugular Sinus

Ductus Cuvieri

Right
Hepatic Sinus

Subclavian Vein

Hepatic
Portal Vein

Posterior Cardinal
Sinus

Lateral
Abdominal Vein

Lateral
Cutaeous Vein

Afferent Renal
Veins

Renal Portal
Vein

Iliac Vein

Caudal Vein
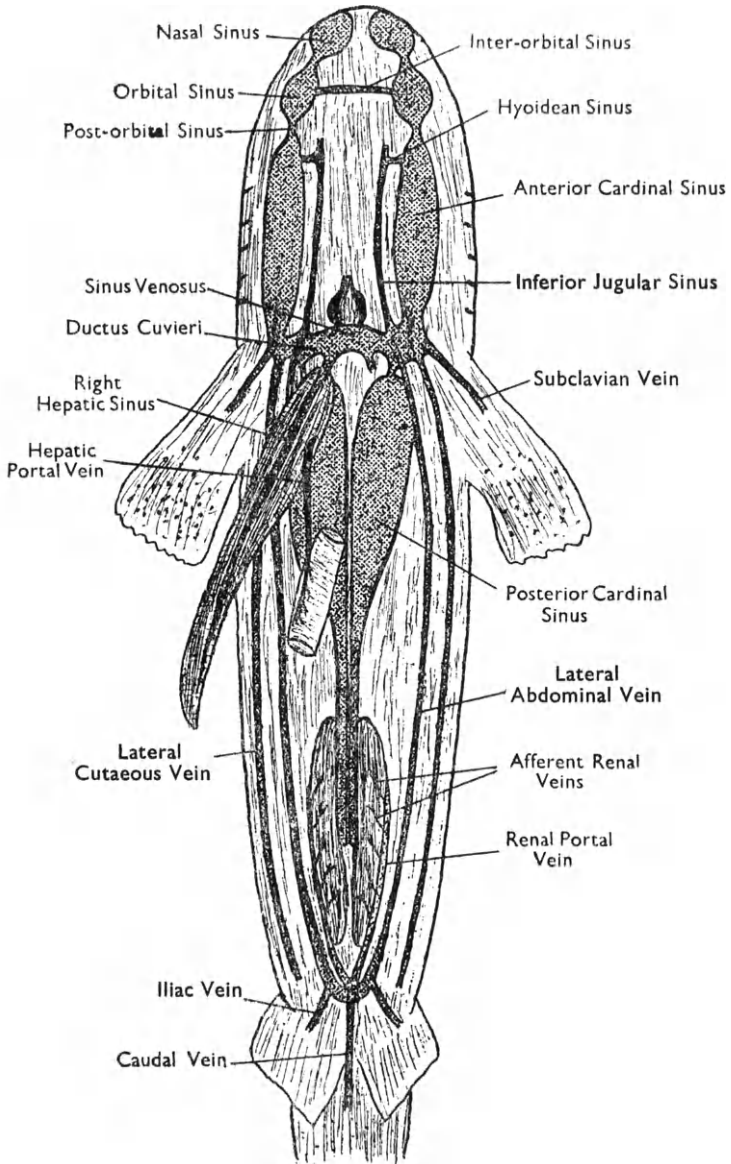
FIG. 95. Scyliorhinus. Venous System. Semi-diagrammatic.

Trace the posterior cardinal sinuses backwards: they are very wide sacs which narrow down as they pass backwards and pass between the kidneys, where they receive a number of **efferent renal veins.** *Cut open the posterior cardinal sinus.* A number of small holes will be seen where the efferent renal veins enter it from the kidney.

Completely cut open these sinuses ventrally and wash out the blood.

Note that they freely communicate with each other somewhat irregularly. The **lateral abdominal veins** are formed by the anastomosis of the two **iliac veins** from the pelvic fins. There are also two **lateral cutaneous veins** immediately beneath the lateral line and running superficially from the tail. *Examine the cut tranverse edges of the body wall.* The veins will be seen immediately beneath the lateral line. They enter the posterior cardinal sinuses near their anterior ends.

The two **Hepatic-Sinuses** open directly into the sinus venosus on its posterior side near the centre. Into them lead the **hepatic veins** running from the two lobes of the liver. The two sinuses are incompletely separated from each other.

The **Hepatic Portal System** has already been seen in the dissection of the alimentary system.

The **Renal Portal System** consists of the two **renal portal veins** which run along the outer edge of each kidney on the dorsal side, which they enter along the entire length by **afferent renal veins.** They are formed by the bifurcation of a median **caudal vein** from the tail at the posterior end of the kidneys. The caudal vein lies in the haemal arches of the vertebral column. This system will be examined later when dissecting the urino-genital organs.

(3) The Arterial System

The conus arteriosus continues forwards into the **ventral aorta,** from which arise the **afferent branchial arteries,** taking blood to the gills. The re-oxygenated blood from the gills is carried in the **efferent branchial arteries** which form the **dorsal aorta** from which arise arteries taking blood to the organs of the body.

These three parts of the system will be studied separately.

(i) The Afferent Branchial Arteries

First insert a test-tube or a wad of paper into the mouth to expand it and fix the animal on the dissecting board, ventral side up with the head towards you. Cut through the skin only in the mid-ventral line from the heart to the mouth. Holding the cut edges with forceps, carefully remove the skin only on both sides of the cut on both sides as far as the pectoral girdle. This exposes the superficial muscle layer. *Carefully remove this thin layer only by making a very shallow longitudinal*

Test Tube or wad of Paper
inserted in mouth

Superficial
Muscle

Coraco-
Mandibular
M.

1. After removal of Skin

2. After removal of Superficial Muscles

Cut end of Coraco-
Mandibular M.

Cut ends of Coraco-
hyoid Muscles

Thyroid Gland

Coraco-hyoid M.

1st Coraco-
branchial M.

Innominate
A.

3. After removal of Coraco-Mandibular
Muscle

4. After removal of Coraco-Hyoid
Muscle

Ventral Aorta

Cut ends of 1st & 2nd
Coraco-branchial Ms.

1st &
2nd. Afferent
Branchial A.

3rd. Coraco-
branchial Ms.

3rd. Afferent
Branchial A.

5. After removal of Thyroid Gland

6. After removal of 1st & 2nd Coraco-
Branchial Muscles

4th Afferent
Branchial
A.

Opening of Inferior
Jugular Sinus
Direction of cut in
Ventral wall in
Inf. Jugular Sinus

5th. Afferent
Branchial A.

4th. Coraco-
branchial Ms.

Cut ends of 4th.
Coraco-
branchial
Ms.

7. After removal of 3rd Coraco-Branchial
Muscles

8. After removal of 4th Coraco-Branchial
Muscles

FIG. 96. Scyliorhinus. Stages in Dissection of Afferent Branchial Arteries.

incision in the mid-line, following this up by gently peeling off the muscle. Observe the long median coraco-mandibular muscle now exposed. Remove this by dissection from the pectoral girdle to the lower jaw to which it is attached and paired median longitudinal muscles will be seen. These are the coraco-hyoids which are attached to the basi-hyal cartilage. *Carefully separate them from the pectoral girdle and remove them, severing them at their anterior ends.* The thyroid gland will now be visible just behind the severed ends of the coraco-hyoids, as a pear-shaped structure in the mid-ventral line. *Remove the thyroid gland carefully* and a pair of **innominate arteries** will be seen below it, forming a "T" with the anterior end of the **ventral aorta.** These divide into the **1st and 2nd afferent branchial arteries.** Behind the innominate arteries on each side of the ventral aorta is a coraco-branchial muscle running between the coracoid and hypo-branchial cartilages. *These muscles must be severed and removed as for the coraco-hyoids.* They are followed, posteriorly, by another pair *which must also be severed and removed.* The **3rd pair of afferent branchial arteries** will then be seen, followed by another pair of coraco-branchial muscles. *Sever and remove these as before.* The **4th pair of afferent branchial arteries** are posterior to these. *Remove the coraco-branchial muscles which follow* and the **5th** (and last) **pair of afferent branchial arteries** will be found arising close to the previous pair.

Now trace these afferent branchial arteries to the gills, which are enclosed in membranous gill pouches, by careful dissection as follows:—Insert a seeker into the opening of the inferior jugular sinus, which will be found where the 2nd afferent branchial artery disappears from view. Carefully cut along the upper (ventral) wall of this sinus with small scissors, keeping the inner blade as horizontal as possible, as far as its posterior end where it enters the ductus Cuvieri. The remaining afferent branchial arteries can then be seen on the dorsal wall of the sinus and can be carefully traced to and along the gills.

Auricle

Conus Arteriosus

Ventricle

9. Afferent Branchial Arteries traced along Gills. Dissection Completed.

FIG. 97. **Scyliorhinus. Final Stage in Dissection of Afferent Branchial Arteries.**

The innominates divide into (i) the 1st afferents running along the outer border of the hyoid arch to supply the hemibranch borne on that arch and (ii) the 2nd afferents supplying the gills of the first branchial arch.

The pairs which follow supply the holobranchs on the remaining arches. Each afferent branchial artery runs in the septum between two gill pouches. *Gently trim the gills as necessary to expose the arteries.*

Finally, carefully pare off the cartilage of the ventral side of the pectoral girdle and so expose and study the ventral view of the heart unless previously done. (See p. 130.)

Then remove the heart by cutting through the anterior end of the conus arteriosus and the posterior end of the sinus venosus. Turn it on its side and observe the S-shaped form.

The Heart—Internal Structure

To study its internal structure *cut along the mid ventral line of the conus and ventricle. Remove the ventral wall of the ventricle but pin down the walls of the conus. Wash out the blood from both.*
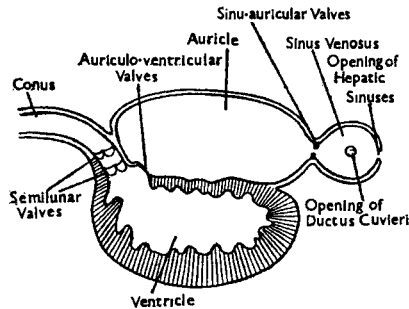
FIG. 98. Scyliorhinus. V.S. Heart.

Six **semi-lunar valves** in two rows of three will be seen at the entrance to the **conus arteriosus** and a large **auriculo-ventricular aperture** in the dorsal ventricular wall, slightly to the left of the midline. Note that the **walls of the ventricle** are thick and muscular while that of the **auricle** is thin. *Cut open the* **sinus venosus.** At the anterior end is the centrally placed **sinu-auricular aperture** which leads into the auricle.

(ii) The Efferent Branchial Arteries

Insert the scissors into the mouth and cut back horizontally along one side as far as the last gill arch, cutting through the visceral arches. Now cut straight across to the other side, through the sinus venosus, behind where the heart was situated. Turn the flap over to the other side and pin it down with an awl. Do not remove the lower jaw or you will cut through a branch of the Vth nerve which must be seen later. Clean the mouth and carefully remove the mucous membrane covering

Bifurcation   Cerato-Branchial
of Dorsal Aorta   Cartilage
1st Epibranchial A.   Dorsal Aorta
Mucous Membrane   Pharyngo-
Buccal Cavity Oro-Nasal Groove   Branchial
Pharynx   Nostril   Cartilage
Gill Slit   Spiracle

Connective Tissue

1. Floor of Mouth Deflected   2. Mucous Membrane removed

Left Subclavian Artery

Efferent
Pseudo-Branchial ("Hyoidean") A.
Orbital (Stapedial) A.

Internal Carotid As.

Root of
Carotid A.

1st Epibranch-
ial A.

Dorsal
Aorta

R. Subclavian
A.

3. Pharyngo-Branchial Cartilages   4. Efferent Branchial Arteries traced
   removed on Animal's left   Dissection Completed

Fig. 99. **Scyliorhinus. Stages in Dissection of Efferent Branchial Arteries.**

the roof of the mouth but avoid removing or cutting the arteries which are now exposed. If preferred the arteries may be dissected on one side (the cut side) only. This will avoid cutting nerves which will be required later.

Four pairs of **epibranchial arteries** run inwards and backwards from the inner (dorsal) ends of the first four gill arches along the edges of the pharyngo-branchial cartilages, parallel with each other on either side, and join the **dorsal aorta** in pairs in the mid-line.

Carefully remove the pharyngo-branchial cartilage between these arteries so as to expose them fully.

Four **efferent branchial arteries** arise from the outer ends of the epibranchial arteries and loop round the first four gill-clefts. There is a half-loop on the anterior border of the fifth, the loops being connected with each other about half way along by **connecting canals.** They lie in the septa between the gill pouches.

The following arteries should also be traced:—

(iii) **Arteries arising from the Dorsal Aorta**

The two **roots of the carotid arteries** arise from the inner ends of the 1st efferent loops where the 1st epibranchial artery originates. Each is joined by a branch from the anterior end of the dorsal aorta, more or less opposite the hinder edge of the orbit, and immediately divides into (i) an **orbital** (or **stapedial artery**) (formerly thought to be and wrongly called the external carotid), which runs across the floor of the orbit forwards and outwards to the anterior part of the head, and (ii) an **internal carotid artery** which loops inwards and enters the cavity of the cranium, where it crosses the corresponding artery from the other side and unites with the "hyoidean" artery of the opposite side. There is no true external carotid artery in the dogfish. These arteries are embedded in the cartilage of the roof of the mouth.

The so-called **"hyoidean"** (**efferent pseudo-branchial**) **artery** arises from the middle of the anterior side of the 1st loop on each side but almost immediately disappears from view. *It is necessary to remove the cerato-hyal cartilage to see this.* It goes to the anterior wall of the spiracle then crosses the floor of the orbit below the eye (where it will be seen in a later dissection) and enters the cavity of the cranium. *A little more of this artery can now be traced if the edge of the spiracle and the cartilage underneath which it passes is removed.*

Trace the further course of the so-called hyoidean artery from the side of the head. Place the fish on the dissecting board, dorsal side upwards. Remove the skin so as to expose the hinder part of the mandibular arch, the hyoid arch and part of the orbital cavity. The **" hyoidean"** (**efferent pseudo-branchial**) **artery** will again be seen

arising from the first efferent branchial artery. It then crosses the hyomandibular cartilage under the hyomandibular division of nerve VII, running forward to the orbit (outside the spiracle) and then turns inwards to cross the floor of the latter cavity. It will be seen again later when examining the orbit.

Now return the fish to its original position, ventral side up. Trace the dorsal aorta by careful dissection between the kidneys and find the following arteries which arise from it. Only the roots of these vessels will be seen. Most of them have already been seen in this dissection of the alimentary system.

The two **subclavian arteries** arise between the 3rd and 4th epibranchial arteries on the dorsal side of the aorta and run backwards and outwards to the pectoral fins.

The **coeliac artery** is a single vessel which arises from the aorta just behind the 4th pair of epibranchial arteries and runs in the mesentery dorsal to the stomach, where it divides into branches, to the liver (hepatic), stomach (gastric) and to the anterior end of the intestine and pancreas (intestinal-pyloric).

The **anterior mesenteric artery** arises about an inch or so behind the coeliac and goes to the intestine and rectum, with branches to the genital organs.

The **lieno-gastric artery** arises immediately behind the anterior mesenteric. It runs to the stomach and spleen. This and the anterior mesenteric cross one another as previously seen.

The **posterior mesenteric artery** arises about one and a half inches behind the lieno-gastric and runs backwards in the mesentery to the rectal gland.

The **renal arteries** and the **iliac arteries** will be seen when the urino-genital system is dissected. The continuation of the aorta as the **caudal artery** into the tail will also be seen.

(4) THE RESPIRATORY SYSTEM

(1) *Cut through the ventral wall of a gill pouch longitudinally.*

The **gills** are borne on the sides of the **gill arches** (or visceral arches) between which are the **branchial clefts** consisting of the **gill pouches,** cavities where the gills are situated on either side except in the fifth which lacks a gill on its posterior side. These decrease in size from first to last, each having an **internal pharyngeal opening** and an external **gill slit.***

The gill pouches are separated from each other by **inter-branchial septa.** The cartilaginous gill rays, already seen in the visceral arch skeleton, pass through the septa into the gills.

* Gill slits are often called gill clefts.

The first visceral arch is modified as already seen, to form the jaws, and is called the mandibular arch. The second, the **hyoid arch,** is between the first gill-cleft and the spiracle. It bears a gill on its posterior side, known as a **hemibranch.** There are then five **branchial arches,** the first four of which bear hemibranchs on each side (each pair of hemibranchs on a branchial arch being called a **holobranch),** the fifth bearing no gill as already stated. There are five gill slits on each side.

Remove and examine a **gill.**

Each consists of a comb-like series of vascular structures, called **gill filaments** or **lamellae.** The blood vessels in connection with the gills have already been seen.

(2) *Cut through and expose the spiracle.*

It contains a non-vascular, vestigial gill, or **pseudobranch,** on its anterior wall.

(5) THE URINO-GENITAL SYSTEM*

This dissection must be performed on an animal from which the alimentary canal and liver have been removed.

Before commencing the dissection of the male, remove the skin only on the ventral side of the pelvic region. This exposes a sac called the **siphon.**

Then cut through the centre of the pelvic girdle and through the muscle so as to expose the cloaca. Cut through this in the mid-ventral line. Fix awls through the pelvic fins.

(i) Male

Note the two large, soft **testes** suspended from the body wall by the **mesorchium** and united at the posterior ends by a membrane (unless this has been cut or torn). The testes open at their anterior ends by a number of tiny ducts, the **vasa efferentia,** which pass through the mesorchium into the long and very sinuous **mesonephric (or Wolffian) duct,** which serves as a **vas deferens.**

Deflect the testes to one side. Carefully remove the peritoneum covering the ventral side of the kidneys.

Note the functional **kidney** on each side (the posterior part of the mesonephros), on the ventral side of which is the mesonephric or Wolffian duct which, as already stated, is the vas deferens. Towards the posterior end of the mesonephros, the vas deferens swells out to form the **vesicula seminalis,** which opens into the **urino-genital sinus** in front of the **cloaca,** into which it opens with its fellow, by the

*The dissection of this system is not required in some examination syllabuses.
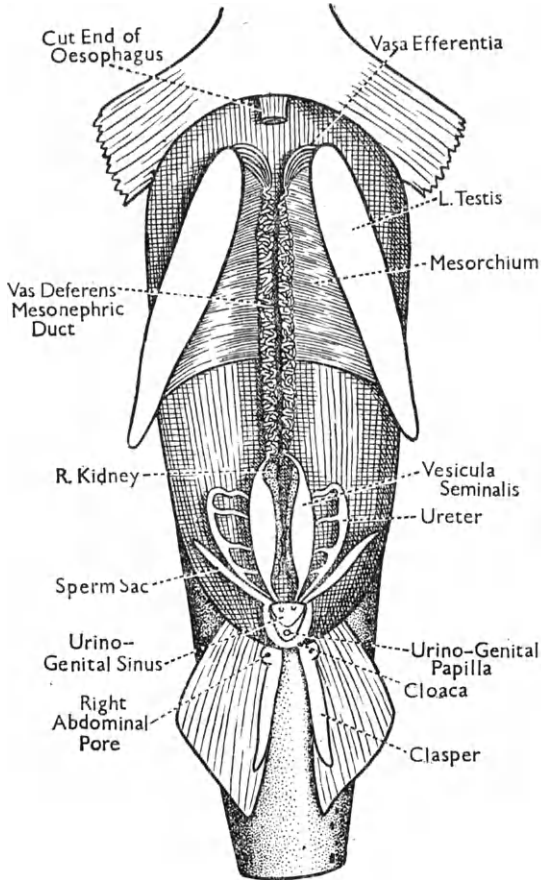
FIG. 100. Scyliorhinus. Urino-genital Organs. Male.

urino-genital papilla. *Carefully release the vesicula seminalis from the kidney.*

The two **sperm sacs** are blindly ending pouches originating from the urino-genital sinus. They are attached to the vesiculae seminales. *Separate them carefully from these structures.* The **ureters** are formed from about five thin tubules arising from the posterior (functional) part of the mesonephros and open into the urino-genital sinus. *It will be necessary to dissect from the vas deferens back to the cloaca to follow the course of the ureters.*

Gently dissect one kidney from the body wall at its outer edge and deflect it inwards.

The **renal arteries** from the dorsal aorta go direct to the kidneys and the efferent renal veins from the kidney enter the **posterior cardinal sinus** between the kidneys. *Open up the sinus* and observe the holes which are the **openings of the efferent renal veins.** Note the **spermatic artery** and the **spermatic vein** in the mesorchium and the **iliac arteries** which arise from the dorsal aorta and go to the pelvic fins. Note also the so-called **claspers,** in the longitudinal groove of which the sperm passes from the cloaca. Two channels from the siphon lead to the grooves on the claspers.

Find the **renal portal veins** on the inside of the dorsal edges of the kidneys and formed by the bifurcation of the caudal vein in the haemal arch. *To see this it is necessary to remove the muscle here and then carefully pare off the cartilage of the ends of the haemal arches.*

FIG. 101. **Scyliorhinus. Urino-genital Organs. Female.**
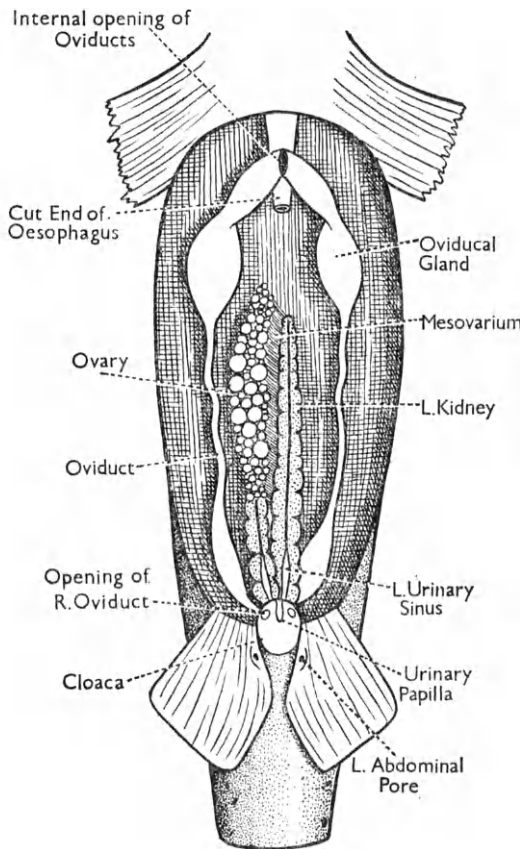
(ii) **Female**

There is one **ovary** (the right), a more or less median organ containing **ova** and attached to the body wall by the **mesovarium.** *Deflect it to the side.* The two thick-walled **oviducts** (modified Müllerian ducts) join at their anterior ends immediately in front of the liver, where the oesophagus joins the stomach, and open by a single aperture, the **internal opening of the oviduct.** They pass down the cloaca, and near the anterior end each swells to form the **oviducal (or shell) gland.**

The posterior ends of the oviducts open into the cloaca by a single **cloacal opening** The anterior end of

each **kidney** (or **mesonephros**) consists solely of a few pieces of tissue, the posterior ends only being well developed and functional. From them arise the **mesonephric** (or **Wolffian**) **ducts,** which widen and then join to form the **urinary sinus.** This opens into the **cloaca** by a **urinary papilla.** The **ureters,** composed of a series of tubules from the posterior part of the mesonephros, also open into the urinary sinus. They are difficult to identify owing to the position of the oviducts. Observe the **ovarian artery** and the **ovarian vein** in the mesovarium and *find the* **renal portal vein** as in the male.

(6) THE NERVOUS SYSTEM

This consists of a **central nervous system** (the brain and spinal cord), a **peripheral nervous system** (the cranial and spinal nerves) and a **sympathetic** or **autonomic nervous system,** which, however, is not well marked in the dog-fish.

(1) The Cranial Nerves

I. The Olfactory Nerve

This arises from the anterior end of the olfactory bulb and supplies the olfactory organs as will be seen in examining those organs.

II. The Optic Nerve

This arises from the optic lobe, crosses its fellow on the ventral side as will be seen when examining the ventral side of the brain and, passing through a foramen on the opposite side of the cranium, enters the orbit and supplies the retina of the eye, as will be seen in the examination of the orbit.

III. The Oculo-Motor Nerve

This arises from below the optic lobes on the ventral side, leaves the cranial wall behind the IInd nerve, where it branches and goes to the rectus superior, inferior and internus (or anterior) and the obliquus inferior muscles, as will be seen in the orbit.

IV. The Pathetic or Trochlear Nerve

This arises between the optic lobe and the cerebellum on the dorsal side, and leaves the cranial wall by a foramen just dorsal and anterior to the IInd nerve. Its course to the obliquus superior muscle will be seen.

V. The Trigeminal Nerve

This arises from the side of the medulla and enters the orbit. Courses of its three branches will be seen in the orbit. These three branches will be traced to their destinations (together with the branches of VII), the **ophthalmic branch** to the snout, the **maxillary**

branch to the upper jaw and the **mandibular branch,** bending round the angle of the jaw, to the lower jaw.

VI. The Abducens Nerve

This slender nerve arises from the ventral side of the medulla just behind the root of the VIIth nerve, which is close to the root of the Vth. It leaves the skull with the Vth nerve and, as will be seen in the orbit, supplies the rectus externus (or posterior) muscle.

VII. The Facial Nerve

This arises very close to the root of the Vth nerve and has four branches, the **ophthalmic branch** entering the orbit separately just behind and above the ophthalmic branch of the Vth nerve. The main stem branch enters the orbit with the Vth nerve, after which it divides into the **buccal branch** to the mouth, the **palatine branch** to the roof of the mouth, and the **hyomandibular branch** to the spiracle, ampullary canals and hyoid arch. These, too, will be seen in the orbit and they will be traced to their destinations (along with the branches of V).

VIII. The Auditory Nerve

This arises very close to the Vth and VIIth nerves, enters the auditory capsule at its inner end and goes to the membranous labyrinth. No further dissection is necessary.

IX. The Glosso-pharyngeal Nerve

This arises from the medulla just behind and ventral to the VIIIth nerve. It enters the auditory capsule on its inner side, runs backwards and outwards on its floor, and then leaves it at its posterior end and above the 1st gill cleft, divides into two branches, the **pre-trematic branch** running along the anterior edge of the 1st gill cleft, and the **post-trematic branch** running along the posterior edge.

It will be seen that this nerve is inappropriately named in the dogfish. Dissection of this nerve will accompany that of the Xth.

X. The Vagus Nerve

This is a large nerve arising from the posterior end of the medulla by several roots.

It leaves the posterior end of the skull between the auditory capsule and the cranium and runs along the inner wall of the anterior cardinal sinus when it divides into:—

The **branchial branch** running to the 2nd, 3rd, 4th and 5th branchial arches, each nerve dividing into a **pre-trematic** and **post-trematic branch.**

The **visceral** branch dividing into several branches which supply the heart, stomach and other viscera.

The **lateral** branch supplying the sense organs in the lateral line canal.

This nerve will be traced together with the IXth.

Dissect and trace the cranial nerves on one side as directed below. The roots of these nerves will be seen when examining the ventral surface of the brain.

(1) The Eye in the Orbit

(1) *With the animal dorsal side up and the head towards you, cut away the skin surrounding one eye and dissect away the eyelids. This should be the one on the side of which the lower jaw has not been cut for the dissection of the efferent branchial arteries. The complete dissection of the nerves can then be done on this side. Do not cut away any cartilage. Pull the eye slightly outwards.*

Note the spherical **eyeball** somewhat flattened on its outer surface, situated in the **orbit** and supported by six muscles.

Note the four recti muscles which have their *origins* close together in the posterior end of the wall of the orbit:—

The **rectus superior** runs forwards and outwards and is *inserted* in the dorsal side of the eyeball. It raises the eye.

The **rectus inferior** runs forwards and outwards and is *inserted* in the ventral side of the eyeball. It lowers the eye.

The **rectus internus** (or **anterior**) runs forwards at the back of the eyeball and is *inserted* in the anterior part of the eye. It moves the eye forwards.
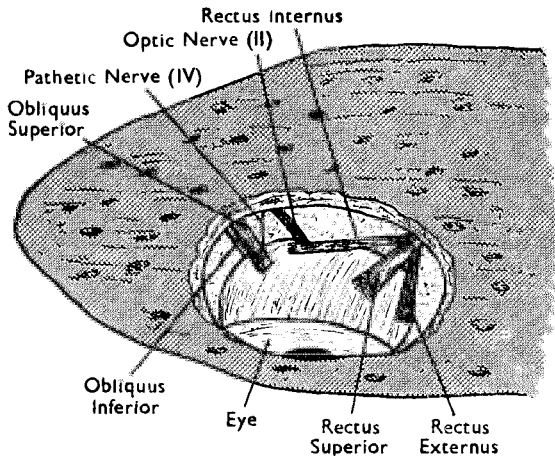
FIG. 102. **Scyliorhinus. Left Orbit dissected open.**

The **rectus externus** (or **posterior**) runs outwards and is *inserted* in the posterior part of the eye. It moves the eye backwards.

Note also the two oblique muscles which have their *origin* close together in the anterior end of the wall of the orbit:—

The **obliquus superior** runs backwards and outwards and is *inserted* in the dorsal side of the eye, just in front of the rectus superior.

The **obliquus inferior** runs backwards and outwards and is *inserted* in the ventral side of the eye, just below the rectus inferior.

They give a turning movement to the eye when used in conjunction with the recti muscles.

Note the stout **optic nerve** (cranial nerve **II**) which enters the eye at the back, and the more slender **pathetic or trochlear nerve** (cranial nerve **IV**) which enters the orbit dorsal to the optic nerve and runs to the obliquus superior muscle.

(2) The Orbit after removal of the Eye

(2) *Cut through the eye muscles and optic nerve close to the eyeball, and remove the eye. Wash out the blood from the orbit and examine it.*

Note the muscles and nerves as in (1):—

The **obliquus superior** is in the anterior dorsal corner of the orbit, with the **obliquus inferior** just below it. The four **recti** muscles are in the form of a cross in the posterior dorsal corner, the **rectus superior** above, the **rectus inferior** below, the **rectus internus** (or **anterior**) running forwards and the **rectus externus** (or **posterior**) backwards.

The **optic nerve** (**II**) enters at the back of the orbit towards the anterior end and the **pathetic nerve** (**IV**) in the anterior dorsal corner and runs down obliquely to the obliquus superior muscle.

Note also the following nerves:—

The **oculo-motor nerve** (**III**) enters behind the optic nerve, runs across the orbit, dividing into branches, one to the rectus internus, one to the rectus superior and one to the rectus inferior and obliquus inferior.

The **trigeminal nerve** (V) consists of three branches:—

(i) A broad band, the **maxillo-mandibular branch,** running across the floor of the orbit which divides near the outer edge of the orbit into (*a*) the **maxillary branch,** which passes forwards and downwards to the upper jaw, and (*b*) the **mandibular branch,** which passes downwards to the lower jaw.

(ii) A separate **ophthalmic branch** entering the orbit above the recti muscles and running forwards (with the corresponding branch of VII), to leave the orbit and its anterior end.*

*In Squalus this is known as the superficial ophthalmic as there is also a deep ophthalmic branch.

The **Abducens Nerve (VI)** is slender and runs along the inferior edge of the rectus externus, which it supplies.

The **Facial Nerve (VII)** consists of four branches:—

(i) The **ophthalmic branch** enters the orbit just posterior and dorsal to the ophthalmic branch of the Vth nerve, which it then accompanies.

(ii) The **buccal branch,** a small nerve, accompanies the maxillary branch of the Vth and may be seen between this branch and the mandibular branch.
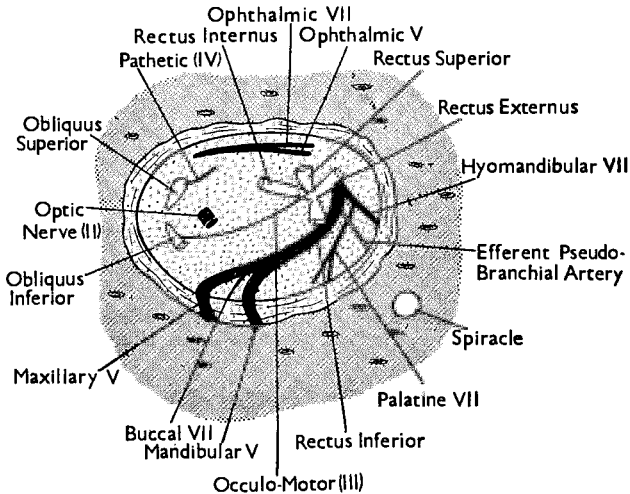
FIG. 103. **Scyliorhinus. Left Orbit after Removal of Eye.**

(iii) The **palatine branch** runs across the floor of the orbit behind the main branch of the Vth. It is crossed by the **efferent pseudobranchial ("hyoidean") artery,** which should be noted.

(iv) The **hyomandibular branch** is a large branch which runs outwards in the posterior wall of the orbit and goes to the hyoid arch. It will be traced out later. The bulge in the posterior region of the orbit marks the position of the spiracle.

Remove the skin from the lateral side of the head on one side as far as the pectoral fin. Care must be exercised in the region of the spiracle, gill clefts and round the eye.

Dissection of the Vth and VIIth Nerves

(i) Ophthalmic Branches of V and VII

Remove the cartilage of the upper edge of the orbit by cutting parallel with it very carefully until the forward continuance of

the **ophthalmic branches** *of* V *and* VII *are exposed. Now trace these nerves to the ampullary canals of the snout by removing the tissue immediately dorsal and ventral to them, exercising great care to avoid cutting the nerves.*
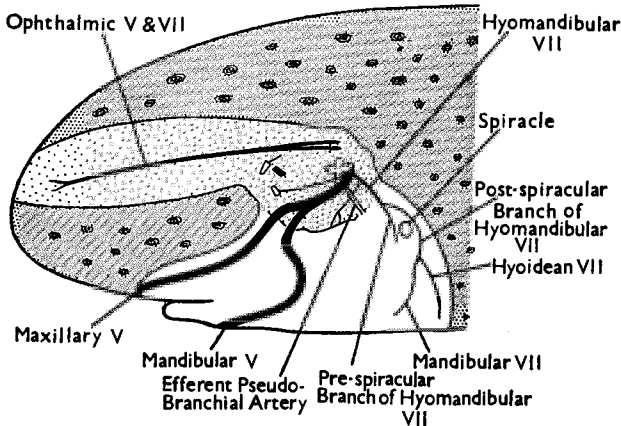
FIG. 104. Scyliorhinus. Dissection of Ophthalmic, Maxillary and Mandibular Branches of Vth and VIIth Nerves and Hyomandibular Branch of VIIth Nerve.

(ii) Maxillary and Mandibular Branches of V

These branches have already been traced to the lower edge of the orbit. By careful dissection, trace each to its destination as follows:—

The **Maxillary Branch** *along the cartilage of the upper jaw.*

The **Mandibular Branch** *running backwards behind the angle of the jaw and then forwards along the cartilage of the lower jaw.*

(iii) Hyomandibular Branch of VII

This was seen in the orbit as a large nerve running posteriorly in the direction of the bulge marking the position of the spiracle. It divides into a slender **pre-spiracular** branch which runs anterior to the spiracle and a large **post-spiracular branch** which passes posterior to the spiracle. *Trace the continuation of the latter branch backwards to the main branch by very carefully paring away the surface only of the muscle below and behind the spiracle. It is easier than tracing it forwards. When the nerve has been identified, follow it back to the main branch in the orbit by careful dissection, cutting upwards and carefully following the nerve.* Below the spiracle the post-spiracular

branch will soon be seen to divide into an **external mandibular
branch,** which ultimately supplies the ampullary canals on the
lower jaw and a **hyoidean branch** which runs down the hyoid
arch. *Find these branches, exercising the same care as before.
Then find the pre-spiracular branch which arises from the same
point on the main branch as the post-spiracular branch.*

(iv) **Buccal and Palatine Branches of VII**

No further dissection of these branches, already seen in the
orbit, is necessary.

Dissection of the IXth and Xth Nerves

*The dissection should also be performed on the side opposite to that
used for the examination of the venous and efferent branchial systems
if the same specimen is used. Open up the anterior cardinal sinus in
which part of the course of the nerves lie. It lies dorsal and parallel to
the gill slits between the body wall and the muscles of the back. It can
be felt where there is less resistance to the pressure of the fingers. Make*
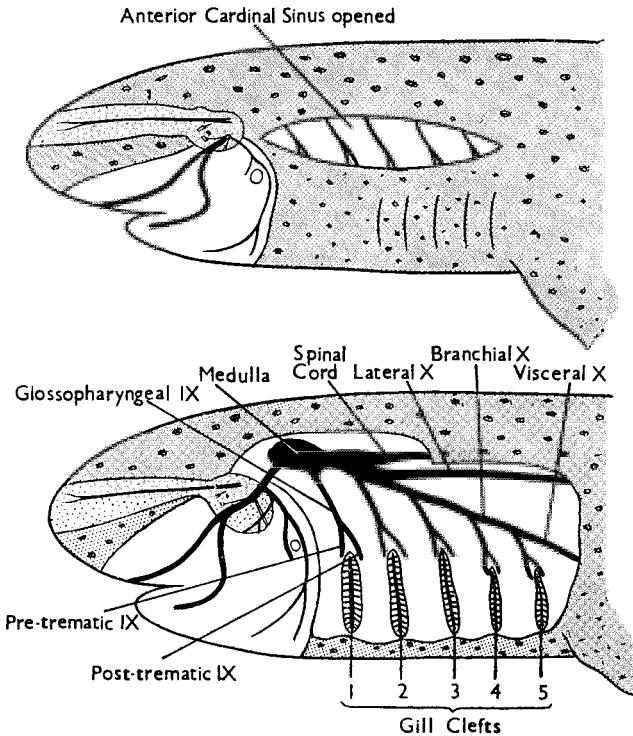
FIG. 105. **Scyliorhinus. Dissection of IXth and Xth Cranial Nerves.**

the incision just dorsal to the spiracle and cut straight back, dorsal to the gill clefts, until the posterior end of the sinus is reached. Wash out any blood in the sinus and remove the roof of the sinus, taking care not to cut away any nerves.

(i) IXth Nerve

In the anterior corner of the sinus will be found a comparatively slender nerve entering from the skull. This is the IXth nerve. *Trace this by careful dissection of the 1st gill cleft.* It will be found to divide into a slender **pre-trematic branch** which runs along the anterior side of the cleft and a less slender **post-trematic branch** running along the posterior edge. *These two branches can be traced by very careful dissection and removal of such parts of the gills as is necessary, though the thinner pre-trematic is more difficult to follow for any great distance.*

(ii) Branchial Branches of X

The Xth nerve leaves the cranium and enters the sinus as a stout structure, the main stem, posterior to the IXth nerve. The **branchial branch** has four branches supplying the 2nd to 5th gill clefts and lies on the floor of the sinus. Each has a **pre-trematic** *and* **post-trematic branch.** *Trace them to the gills, following the same procedure as for those of the IXth.*

(iii) Visceral Branch of X

This arises from the main stem and closely accompanies the branchial branch to a point just before it passes to the last gill cleft; it is difficult to distinguish the two nerves anterior to this. Then it passes out of the sinus in a posterior direction to give branches to the heart, stomach and other viscera.

Trace this nerve back for a short distance by careful dissection. It is not necessary (or possible, if the alimentary system and other viscera have been removed) to trace its branches.

(iv) Lateral Branch of X

This branch is dorsal to the branches already seen and is more deeply seated at first, being embedded in the muscle of the body wall.

Trace it from the main stem. It soon runs inwards into the muscle. *Carefully dissect away sufficient of the muscle to expose it for a short distance. Its path to the sense organs of the lateral line need not be followed.*

Now expose the brain as directed below in order to trace the nerves back to it.

Very carefully pare off the cartilage from the roof of the cranium, starting at the posterior end, and gradually work forwards until the whole of the dorsal side of the brain is exposed. Take care not to damage the brain with your scalpel while doing this. Still being very careful, continue to remove all such cartilage as is necessary to expose the continuation of the IXth and Xth nerves to the brain. Now remove the muscle posterior to the cranium for a short distance until you come down to the vertebrae. Remove the top of the exposed vertebral column to expose the spinal cord. Continue to remove the cartilage, exercising great care, so as to expose the continuation of the IXth and Xth nerves to the brain.
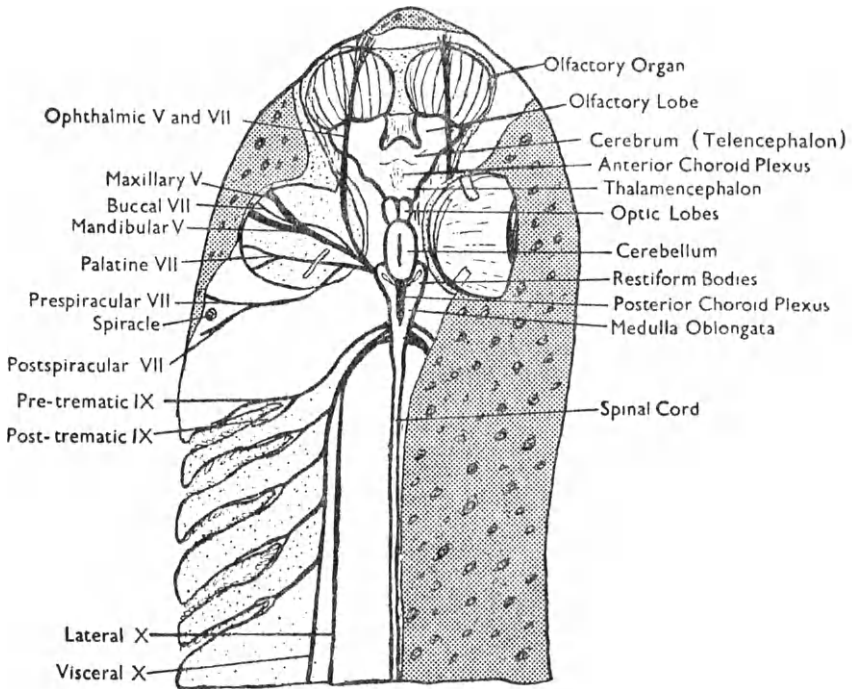
FIG. 106. Scyliorhinus. Brain and Cranial Nerves in situ. Dorsal View.

(2) The Brain and Spinal Cord

(a) Dorsal View

Expose the rest of the brain by carefully removing the cartilage at the anterior end of the cranium, both dorsally and laterally. To avoid damage to the ophthalmic branches of the Vth and VIIth nerves while

removing the cartilage beneath them, sever them at their anterior ends and deflect them while paring off this cartilage.

In the **Fore-Brain** note the **olfactory lobes** (or **bulbs**), the anterior surfaces of which are in close proximity to the large olfactory organs. These lobes arise from a rounded part of the fore-brain or **telencephalon** (sometimes called the cerebrum), behind which is the narrow **thalamencephalon,** from the hinder part of the thin-walled vascular roof (or **anterior choroid plexus**) of which arises the **pineal stalk** bearing the **pineal body.** This will almost certainly have been removed with the roof of the cranium. The **Mid-Brain** consists of the rounded **optic lobes** which are partly hidden by the long flattened **cerebellum** belonging to the **Hind-Brain,** continuous with which is the thin-roofed **medulla oblongata** covered by a triangular **posterior choroid plexus,** and which bears at its anterior end the wing-like laterally placed **restiform bodies.**

Press the brain carefully to one side and *look for the inner ends of the ten cranial nerves.* III and VI will not be seen in this view.

Note that the **spinal cord** is continuous with the medulla oblongata and bears a groove, the **dorsal fissure,** on the dorsal side.

(b) Ventral View

Cut through the olfactory nerves which run from the olfactory lobes to the olfactory organs in front of the brain, and through the posterior end of the medulla where it joins the spinal cord or, better, a little further back. Then, carefully lifting the brain with forceps and working
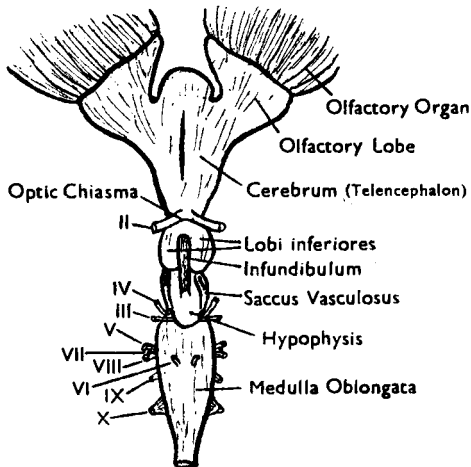
FIG. 107. Scyliorhinus. Brain. Ventral View.

forwards, cut through the roots of the cranial nerves in succession. Finally gently remove the brain from the cranial cavity and examine its ventral surface.

Note the **olfactory lobes** (or **bulbs**), **telencephalon, thalamencephalon** and **optic lobes,** already seen in the dorsal view. At the posterior end of the telencephalon the optic nerves (**II**) cross over one another forming the **optic chiasma,** behind which is the **infundibulum,** the sides of which consist of two thick lobes, the **lobi inferiores,** and between which is the **hypophysis** or **pituitary body,** the end of which will probably have been left in the cranium. The unpaired vascular outgrowth beside and behind the infundibulum is the **saccus vasculosus.** Behind this is the **medulla oblongata.**

Look for the roots of the cranial nerves. Their positions have been given on pp. 143-145. IV will probably not be seen. To see III, *lift the infundibulum.*

There is also a series of three slender nerves arising from the lower side of the medulla below the roots of the Xth nerve which unite immediately outside the skull, forming a thin trunk which joins the first few spinal nerves. These slender nerves are called the **occipital nerves.** The fused nerve formed by the occipital nerves is probably more or less homologous with the hypoglossal nerve of terrestrial vertebrates, and can be seen by a dissection from the side. It runs with the vagus for a short distance then leaves it to pass downwards and forwards round the Cuverian duct to supply the hypo-branchial muscles.

(*c*) **Longitudinal Section**

Cut a sagittal (median longitudinal) section of the brain after hardening in alcohol for a few days.
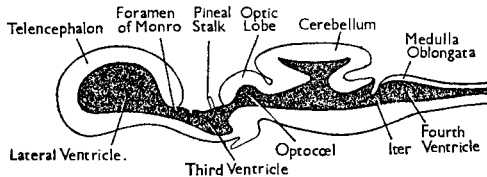
FIG. 108. **Scyliorhinus. Brain. Sagittal section.**

Note one of the **lateral ventricles** in the **telencephalon** (these two ventricles are separated by a septum), each leads into the **third ventricle** in the **thalamencephalon** by a **foramen of Monro.** The third ventricle leads by the narrow **iter** (or **aqueduct of Sylvius**) into the **fourth ventricle** in the **medulla.** Each **optic lobe** contains an **optocoel cerebellum.**

(5) **The Spinal Nerves**

Carefully press the spinal cord to the side to show the roots of the spinal nerves.

Note that a pair of **spinal nerves** arises between each pair of vertebrae on the sides. Each nerve arises by a **dorsal (afferent or sensory) root** bearing a **ganglion** and a **ventral (efferent or motor) root** which join outside the vertebral column to form the (mixed) spinal nerve.

(7) THE SENSE ORGANS

The Eye

Remove the remains of the muscles from the eyeball and cut a longitudinal section of the eye by cutting round it, thus dividing it into inner and outer parts.

Note that the eyeball is divided into an **anterior chamber** containing a watery fluid, the **aqueous humour,** and a **posterior chamber (vitreous body)** containing a gelatinous fluid, the **vitreous humour,** by a solid spherical **lens,** which is kept in position by a circular band containing muscle fibres and known as the **ciliary body** to which it is joined by the **suspensory ligament.**

The wall of the eye consists of three coats:—

  (i) The opaque white outer coat is the **sclerotic** which encloses the eye except where the optic nerve enters. In front it is transparent, where it is known as the **cornea.**

 (ii) The black middle coat, or **choroid,** is pigmented and vascular and is lined by a silvery-looking membrane, the **tapetum** (peculiar to the dogfish). It lines the sclerotic but not the cornea. In front is the contractile pigmented **iris,** which is perforated in the centre by an aperture, the **pupil.**

(iii) Lining the choroid is the **retina,** a membrane containing cells sensitive to light, which are continuous with the fibres of the optic nerve.

The Membranous Labyrinth

This dissection is not required in most examination syllabuses.

This is a very delicate and time-consuming dissection. If required the following procedure should be adopted.

Cut away the skin from the posterior part of the skull on one side and scrape the latter clean. The side used for tracing the Vth and VIIth nerves to the brain will have been damaged. Therefore where this dissection has to be done (in the auditory capsule) use the other side. Find the ridge running inwards and backwards from the posterior edge of the orbit and the ridge running outwards and backwards to the back of the skull. These mark the positions of the two vertical semicircular canals (see below). This is the dorsal side of the auditory capsule.
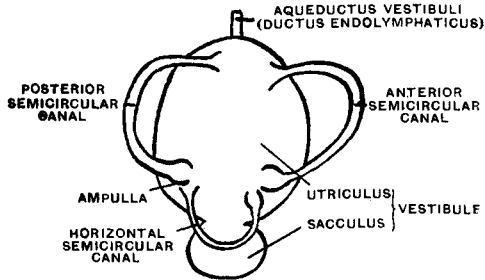
FIG. 109. Scyliorhinus. Membranous Labyrinth dissected out of Auditory Capsule.

Note the **external opening of the ductus endolymphaticus** (or **aqueductus vestibuli**), a small hole where the auditory capsule fuses with the cranium on its inner dorsal surface just behind the level of the eyes and already seen in the dorsal view of the skull.

Cut away the cartilage very carefully so as to expose the membranous labyrinth. If care is not exercised it will be damaged. The semicircular canals are similar in appearance to the surrounding cartilage and are very easily cut or broken.

Note the **ductus endolymphaticus** (or **aqueductus vestibuli**) which leads from the exterior to the **vestibule**. The latter is composed of:

(i) A dorsal swelling, the **utriculus,** from which arise three **semicircular canals,** each widened at its anterior end into an **ampulla,** the **anterior canal** being vertical and running from front to back, the **posterior canal** also being vertical but behind the anterior canal, and the **horizontal canal** running laterally.

(ii) A ventral swelling, the **sacculus.**

The **Auditory Nerve** (Cranial Nerve **VIII**) enters the auditory capsule at the anterior end of its inner wall and gives off branches to the various parts of the membranous labyrinth.

The entire membranous labyrinth can be removed from the auditory capsule but it is very difficult to avoid damaging it and it takes a long time.

The Olfactory Organs

Cut away the skin from the dorsal surface of the snout and cut away the cartilage of the olfactory capsules if not already done.

Note that the **olfactory organs** are large spherical sacs, the lining of which is thrown into folds. *Cut one open to see this.* It is covered by a sensory epithelium which is supplied with twigs from the **olfactory nerve** (Cranial nerve **I**), which enters each organ from the posterior side.

(8) TRANSVERSE SECTIONS

Now that the whole animal has been dissected, *prepared transverse sections cut through the branchial and visceral regions of the trunk and through the tail should be examined and drawn.* The structures visible will depend, of course, on the exact regions in which the sections have been made.

(1) Branchial Region

Note (i) the **vertebra** with its **centrum** containing **notochordal tissue,** and **neural arch** enclosing the **spinal cord** (ii) the **dorsal aorta** ventral to the vertebra, (iii) the segmented **dorsal muscles (myotomes)**
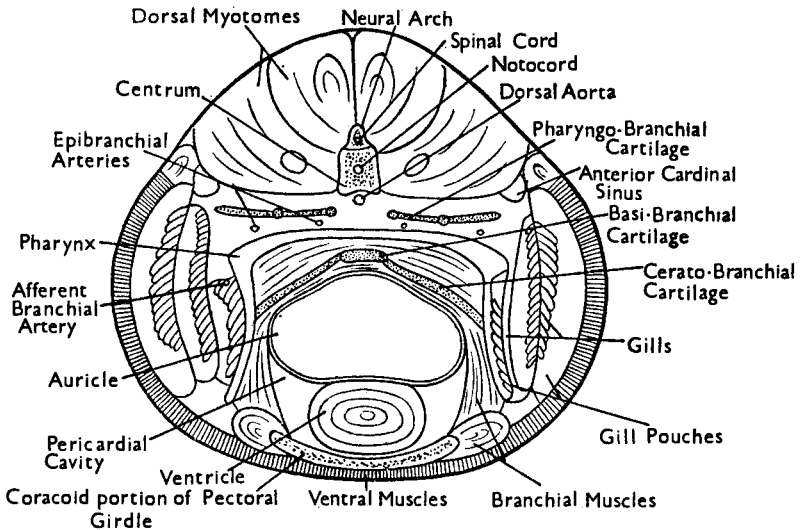
FIG. 110. Scyliorhinus. T.S. Branchial Region.

of the body wall, (iv) the **auricle** and **ventricle** of the **heart** enclosed in the **pericardial cavity** on the ventral side, (v) the **pharynx** across the body dorsal to the heart, (vi) the **gill pouches** laterally, (vii) the **gills,** (viii) the **coracoid portion of the pectoral girdle** ventrally, (ix) the **ventral muscles** of the body wall, (x) the **pharyngo-branchial cartilages,** (xi) the **basi-branchial** and **cerato-branchial cartilages.** (xii) Look for **afferent, efferent** and **epibranchial** arteries and (xiii) the **anterior cardinal sinus.**

(2) Visceral Region

Note (i) the **vertebra,** (ii) the **dorsal muscles** and (iii) **dorsal aorta** as before. Also (iv) the **posterior cardinal sinus,** (v) the **perivisceral**

cavity and parts of such of the following organs as are present; (vi) the **liver,** (vii) the **cardiac** and **pyloric portions** of the **stomach,** (viii) the **intestine** (look for the **spiral valve**); (ix) **testis** with **mesorchium** or **ovary** with **mesovarium** and **oviduct;** (x) **kidney,** (xi) **lateral** and **ventral muscles,** (xii) **lateral line canal,** (xiii) **lateral cutaneous vein,** (xiv) **lateral abdominal vein.**
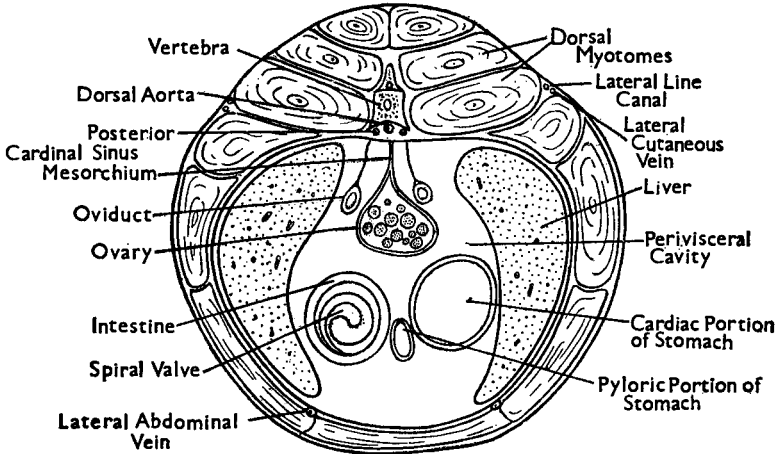
FIG. 111. Scyliorhinus. T.S. Visceral Region.

(3) Caudal Region

Note the (i) **vertebra** with the **neural arch** on the dorsal side and the **haemal arch** on the ventral side, the latter containing the **caudal artery** above and the **caudal vein** below; (ii) the **myotomes** and **myocommata** of the muscles completely surrounding the vertebra.
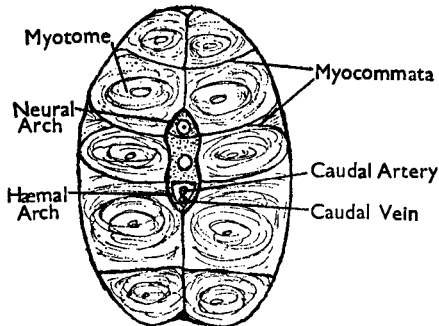
FIG. 112. Scyliorhinus. T.S. Caudal Region.

A BONY FISH

The typical and common bony fish belong to the CLASS *Osteichytes* and the ORDER *Teleostei* (Body covered with bony scales. No spiracle. Four gill-slits covered by an operculum. Urinary and genital ducts open separately. Possess a swim-bladder).

Examine a **typical bony fish** *such as a* **minnow** (*Phoxinus*) *or* **herring** (*Clupea*).

The body is streamlined, somewhat flattened laterally and in some fish quite wide dorso-ventrally. The body is covered with **bony scales,** overlapping like tiles on a roof and is darker above than it is beneath. A **lateral line** is seen running along the sides of the body, which is divided into **head, trunk** and **tail.** The **mouth** is at the anterior end and dorsal to it are the **external nostrils.** The **eyes** are laterally placed and the gill-slits are hidden beneath an **operculum** which is free along its posterior edge. One, or as many as three, vertically placed **dorsal fins** and one or more **ventral fins** are present. The paired **pectoral fins** and **pelvic fins** are often closer together than is the case in the dogfish. The **caudal fin** is bilobed and symmetrical and the tail is said to be *homocercal.*

The colour varies considerably in different fish and in the minnow may be grey or yellow and is brightest in the male during the breeding season. (Internally these fish differ from the *Elasmobranchii* in the presence of an air-bladder, known also as the swim-bladder, which lies ventral to the vertebral column and which leads in most cases into the oesophagus. This helps to maintain balance and may also serve a respiratory function.)

THE COMMON FROG

RANA TEMPORARIA

The Frog belongs to the PHYLUM *Chordata*, SUB-PHYLUM *Craniata*, CLASS *Amphibia* (craniates which breed in water and the adults of which either live in water or inhabit damp places) and the ORDER *Anura* (Amphibia which become tailless at metamorphosis). Toads also belong to this order while tailed adults such as newts and salamanders are in the ORDER *Urodela*.

I. OBSERVATIONS ON LIVING ANIMALS

Observe living frogs in a vivarium and out of doors.

Note how they sit, jump and swim, and examine the movement of the floor of the mouth in respiration.

II. EXTERNAL ANATOMY

Note that the body is composed of **head** and **trunk,** and that there is no neck or tail. The skin is green or yellow with black pigment spots, **melanophores,** and is slimy and loose. The ventral side is lighter in colour and the "throat" of the male is white.

Put two frogs of similar colour on light and dark backgrounds respectively and examine their colours a few hours later.

(1) Head

On the bluntly triangular head note the two dorso-laterally placed prominent **eyes,** which have slightly movable **upper lids** and translucent **nictitating membranes** below, which can be lifted up over the eye. There are no lower lids. Behind and slightly lower than the eye on each side is the **tympanic membrane** or ear drum, well camouflaged in a pigmented area of skin, and well forward, anterior to the eyes are two small openings, the **external nares** or nostrils. The **mouth** is crescentic and large and is at the anterior end of the head.

(2) Trunk and Limbs

On the trunk are two pairs of limbs. The **fore-limb** is short, and is composed of four parts, the **brachium** (upper arm), **ante-brachium** (forearm), the **carpus** (wrist) and the **manus** (hand). The **hind limb** is much longer and also consists of four parts, the **femur** (thigh), the **crus** (shank), the unusually long **tarsus** (ankle) and the **pes** (foot). On the **dorsal side** of the trunk towards the posterior end, note the prominent ridge; this is the **sacral prominence.** Between the hind limbs on the posterior end of the trunk is the **cloacal aperture,** the common opening of the intestine and the urino-genital duct.

Draw a **lateral view** *of the animal, showing the structures mentioned on the previous page.*

(3) Manus

Examine the **palmar surface** *(lower surface) of the* **manus** *of a* **male** *and of a* **female frog.**

There are four short **digits,** numbered 2, 3, 4, 5 (No. 4 is the longest), the pollex, or thumb, being absent. The digits are not webbed.

In the **male,** the second digit bears a **nuptial pad,** prominent and brown in the breeding season. This is absent in the **female.**

(4) Pes

Examine the **plantar surface** *(lower surface) of the* **pes.** There are five long **digits** (No. 4 is the longest), which are **webbed.**

III. THE SKELETON

The skeleton is composed of the **axial skeleton,** the **visceral skeleton** and the **appendicular skeleton** as in the dogfish.

Cartilage in the skeleton of the tadpole is partly ossified when the larva develops into a frog and bones thus formed are called **cartilage bones.** Some bones, however, are formed by ossification of the enveloping membranes of the cartilage and these are known as **membrane bones.**

Examine a prepared disarticulated skeleton.

The Axial Skeleton
The Skull

This consists of the chondrocranium, the sense capsules and the jaws. The skull is partly ossified and most of the bones are membrane bones though a few are cartilage bones as will be seen below.

(1) Dorsal View

The **chondrocranium** is in the centre with the **orbit** on either side. The two **fronto-parietals** (membrane-bones) cover the chondro-cranium dorsally. They partially cover the **sphenethmoid** (a cartilage-bone), visible beyond their anterior ends, and their posterior ends widen outwards to the auditory capsules. The sphenethmoid actually forms the anterior end of the cranium. Behind the fronto-parietals are the two **ex-occipitals** (cartilage-bones) forming a ring, the cavity of which is the **foramen magnum;** it is through this that the spinal cord passes.

The **Auditory Capsules** are mainly cartilaginous and are fused with the sides of the posterior ends of the cranium. Part is ossified as the **pro-otics** (cartilage-bones), which constitute part of the roof, anterior end and part of the floor.

The **Nasal or Olfactory Capsules** are at the anterior end of the skull. They are mostly cartilaginous, though in the dorsal view will be seen the two **nasal bones** (membrane-bones), roughly triangular in shape with the apices directed laterally. The **external nares** will be seen anterior to these in the cartilage.

The **upper jaw** is composed of cartilage covered by the **maxillae,** which form the greater part of the upper jaw, the **premaxillae,** small bones continuous with the maxillae and meeting one another in the middle line in front of the skull, and the **pterygoids,** λ-shaped bones which stretch from the auditory capsules to the maxilla on each side and form the outer border of the orbits. The jaw is supported by
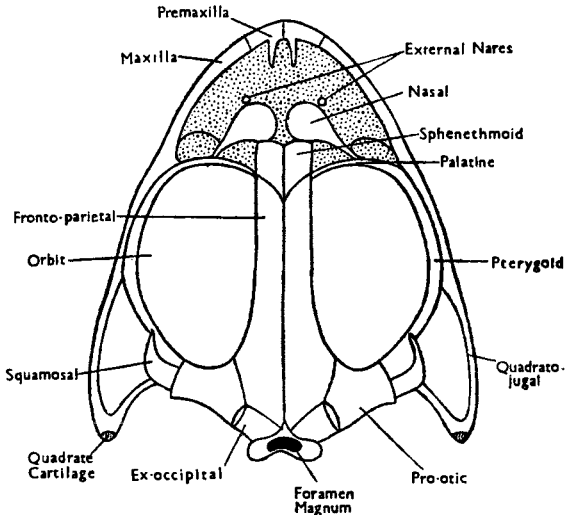
FIG. 113. Rana. Skull. Dorsal View.

the **palatine,** a transversely placed bone joining the sphenethmoid to the pterygoid, the **quadrato-jugal,** joined to the hinder end of the maxilla on each side, and which runs backwards and joins one of the cross pieces of the **squamosal bone,** which is shaped somewhat like an inverted T. Joining the quadrato-jugal and the pterygoid posteriorly is the **quadrate cartilage** but only the tip could be visible in this view.

(2) Ventral View

The floor of the chondrocranium is formed by the **sphenethmoid** anteriorly and supported by the **parasphenoid,** shaped rather like a dagger with the blade pointing forwards, the cross pieces of the

handle reaching to the auditory capsules. The two **ex-occipitals** on this surface enclose the **foramen magnum** at the posterior end of the skull and bear convex processes called **occipital condyles** for articulation with the first vertebra. The bases of the **pro-otics** in the auditory capsules can be seen. The base of the nasal capsules is composed of cartilage strengthened by the two **vomer** bones which are roughly triangular or rather triradiate and which bear the **vomerine teeth** on their posterior edges. The **internal nares** are situated on the outer edge of the vomers.

Note that the **pre-maxilla** and **maxilla** on each side bear **maxillary teeth.** The under surfaces of the **palatines, pterygoids** and **quadrato-jugals** will also be seen. The **quadrate cartilage,** will be seen where the pterygoid and quadrato-jugal join posteriorly on each side.
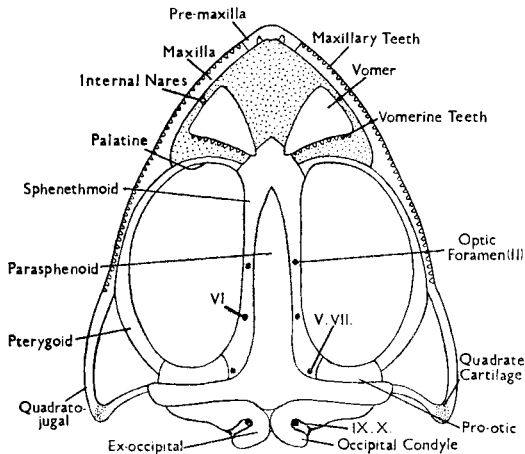
FIG. 114. Rana. Skull. Ventral View.

Note the following **foramina** for the cranial nerves:—

II (optic) in the cartilage on either side of the cranium.

VI (abducens) behind the foramen for II near the pro-otic.

V (trigeminal) and VII (facial) behind the foramina for VI, almost in the angle between the "blade" and the "handle" of the parasphenoid, close to the pro-otic.

IX (glossopharyngeal) and X (vagus) in the pit surrounded by the ex-occipitals.

The **Lower Jaw** or **Mandible** is an arch composed of two rods of cartilage, Meckel's Cartilage, fused in the centre in front and partially enclosed by membrane-bone and cartilage-bone. It is called the **dentary** although it bears no teeth. Articulation with the upper jaw is

effected by the posterior part of the **angulo-splenial** bone which stretches along the inside and underside of the mandible.

(3) The Hyoid Apparatus

This is a cartilage in the floor of the mouth and is roughly rectangular in shape, the **body** bearing at its corners processes called the **anterior cornua** and the **posterior cornua,** partly ossified.

The Vertebral Column

This consists of nine vertebrae, bony rings forming a canal for the protection of the spinal cord and, continuous with the last vertebra, the tapering urostyle.
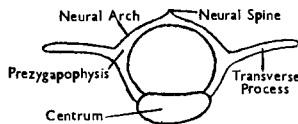
FIG. 115. **Rana. A Typical Vertebra. Anterior View.**

Each **vertebra** is a bony ring, the lateral and dorsal sides of which form the **neural arch,** while the thicker base is the **centrum,** concave anteriorly and convex posteriorly, and therefore said to be procoelous. The enclosed cavity is the **neural canal.** Projecting from the top of the neural arch is the **neural spine** and, except in the first vertebra, the **transverse processes** arise from the sides. These are directed forwards in the second vertebra, outwards in the third and backwards in the rest. The vertebrae articulate with one another by processes called zygapophyses on the anterior and posterior edges of the neural arches. These are called the **prezygapophyses** and **postzygapophyses** respectively. The articular surfaces on the prezygapophyses face upwards while those on the postzygapophyses face downwards.

The first vertebra or **atlas,** which has a very much reduced centrum and no transverse processes, articulates anteriorly by its concave occipital facets with the occipital condyles of the skull. It lacks prezygapophyses.
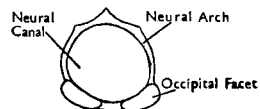
FIG. 116. **Rana. Atlas. Anterior View.**

The **eighth vertebra** has concave surfaces at both ends of its centrum and is therefore said to be amphicoelous.

The **ninth (or sacral) vertebra,** or **sacrum,** has large backwardly directed transverse processes, the ends of which, the **ilial facets,** articulate with the pelvic girdle. The centrum, the anterior surface of which is convex, has no postzygapophyses,
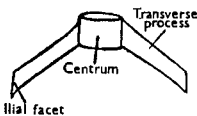
FIG. 117. **Rana. IXth Vertebra.**
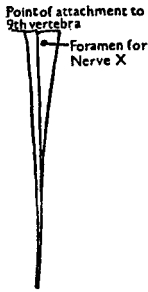
of course, but it has two convex surfaces for articulation with the urostyle.

The **urostyle,** though really composed of fused vertebrae, is unsegmented and tapers towards the hinder end. Anteriorly are two concave **surfaces for articulation with the ninth vertebra.** A ridge runs along its dorsal surface. There are two **foramina,** through which the **Xth** spinal nerves pass, on the sides.

The Visceral Skeleton

This is composed of the cartilaginous parts of the jaws (mandibular arch skeleton), the hyoid and the columella auris of the ear (hyoid arch skeleton).

FIG. 118.
Rana. Urostyle.

Point of attachment to 9th vertebra

Foramen for Nerve X

The Appendicular Skeleton

(1) The Pectoral Girdle

This consists of two half-hoops of cartilage and bone, joined ventrally but free dorsally, and serves for the articulation of the forelimbs. On the dorsal side above the shoulder-joint is a blade-like bone, the **scapula,** and dorsal to this a partially ossified cartilage, the

Episternum
Clavicle   Omosternum
Coracoid Fontanelle   Scapula
Suprascapula
Coracoid
Epicoracoid
Xiphisternum   Xiphoid Cartilage
(Mesosternum)   (Xiphisternum)
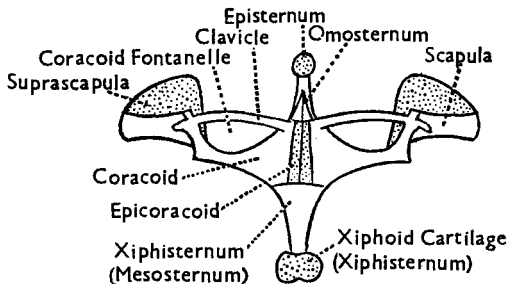
Fig. 119. Rana Pectoral Girdle. Ventral View.

suprascapula. On the ventral side below the shoulder joint is the **coracoid,** which widens out towards the inner end where the **epicoracoid,** a narrow strip of calcified cartilage, joins its fellow. In front of the epicoracoid is another strip of cartilage, the **precoracoid.** The epicoracoid and precoracoid may not be easy to identify except in a fresh specimen. The oval space between the precoracoid and the coracoid is the **coracoid fontanelle** and the slender bones overlying the precoracoids are the **clavicles,** the only membrane-bones in the pectoral girdle. The **glenoid cavity,** into which the head of the forelimb fits, is formed by the scapula and coracoid.

The central part of the girdle is composed of (*a*) the **omosternum** directed forwards and bearing at its tip a small circular plate of cartilage, the **episternum,** and (*b*) the **xiphisternum** (or **mesosternum**) directed backwards and bearing a larger plate of cartilage at its tip, **xiphoid cartilage** (or **xiphisternum**).

(2) The Pelvic Girdle

This is also composed of two half-loops, but they are joined at the posterior end.

The long slender bone is the **ilium,** which articulates with the transverse process of the ninth vertebra in front and which widens out behind. Posterior to this is the **ischium,** and on the ventral side, a calcified cartilage, the **pubis.** The cavity formed by these three bones is the **acetabulum,** into which the head of the hind-limb fits.
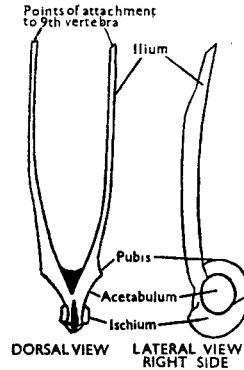
FIG. 120. **Rana. Pelvic Girdle.**

(3) The Fore-Limb

The skeleton of the upper arm consists of a single bone, the **humerus.** It bears a rounded swelling at its upper end, the **head** (which fits into the glenoid cavity) and a more irregular process, the **trochlea,** at its lower end. Note the **deltoid ridge** along the inner side for the insertion of the deltoid muscle.
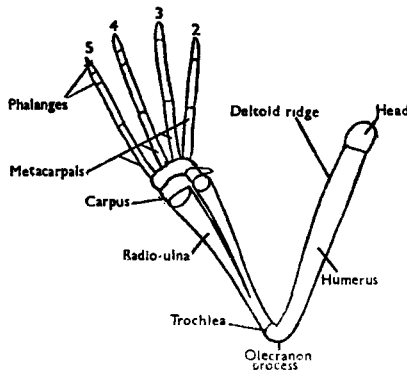
FIG. 121. **Rana. Fore Limb Skeleton.**

The skeleton of the forearm is composed of the **radio-ulna,** which corresponds to the radius and ulna of higher vertebrate animals, and which shows a groove running longitudinally almost to the proximal

end. This bone articulates with the trochlea of the humerus by
hollows in its proximal end where the ulnar portion projects back-
wards forming the **olecranon process.**

The skeleton of the wrist is made up of six **carpals,** in two rows of
three. These may be difficult to identify individually. The proximal
row consists of the **radiale, intermedium** and **ulnare,** and those in the
distal row are simply called the **distal carpals.**

The palm of the manus is composed of five short **metacarpals,**
though only the 2nd, 3rd, 4th and 5th bear **phalanges,** which form
the digits. The 2nd and 3rd have two and the 4th and 5th three.

(4) The Hind Limb

The bones of the hind-limb correspond with those of the fore-limb,
but are much longer.

The thigh-bone, or **femur,** bears a rounded **head** (which fits into
the acetabulum) at its proximal end, and a **condyle** at its distal end.
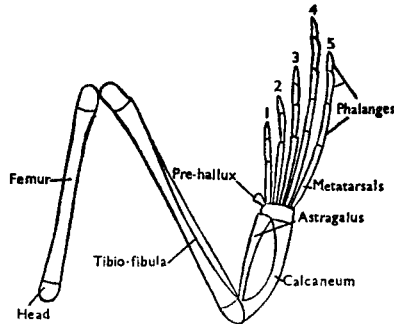
FIG. 122. **Rana. Hind. Limb Skeleton.**

The **tibio-fibula** corresponds to the tibia and fibula of higher
vertebrate animals. The grooves are less conspicuous than those in
the radio-ulna. Its proximal end articulates with the condyle of the
femur.

The ankle, like the wrist, is composed of two rows of small bones,
tarsals, but there are only two bones in each row, (*a*) the long
astragalus (tibiale) and **calcaneum** (fibulare), in the proximal row,
separate from each other except at the ends, and (*b*) the two small
distal tarsals in the distal row.

Six metatarsals are found in the sole of the pes, one being very
small and corresponding to an extra toe, the **prehallux,** or **calcar,**
which is not visible externally. The other five metatarsals bear
phalanges, forming the digits. The 1st, the big toe or **hallux,** has

two; so has the second; the 3rd has three; the 4th four and the 5th three.

(5) The Articulated Skeleton

Examine an articulated skeleton in order to see the relationship of the various parts.

IV. INTERNAL ANATOMY

It is an obvious advantage to carry out dissections on the large edible frog, *Rana esculenta,* if available.

(1) THE MUSCULAR BODY WALL

Fix the frog, ventral side uppermost, to the bottom of a dissecting dish by placing pins obliquely through each manus and pes, stretching the limbs well. Hold up the skin with fine forceps in the mid-ventral line and cut through it from cloaca to mouth. Then, holding the skin with the forceps, cut through the connective tissue which joins it to the muscular body wall beneath, taking care to avoid cutting the musculo-cutaneous veins at the armpits.

The spaces between the **connective tissue** are the **subcutaneous lymph sacs.**

Make transverse cuts through the skin level with the fore-limbs and pin it back on either side.

Note the **musculo-cutaneous** veins on the inside of the skin, the **rectus abdominis muscle** running along the lower part of the trunk, divided longitudinally in the centre by the **linea alba,** a white line of connective tissue, and transversely by **tendinous intersections.** The large fan-shaped muscle radiating from the forearm to the centre on each side is the **pectoralis muscle** and the small triangular piece of cartilage in the centre above the rectus abdominis muscle is the **xiphoid cartilage** (or **xiphisternum).** Note the **mylo-hyoid muscle,** running from the mid-line to the edges of the lower jaw. The bluish **anterior abdominal vein** will be seen through the lower part of the body wall in the mid-ventral line.

Make a median incision in the mylo-hyoid muscle and deflect the flaps to each side. The **hypoglossal nerves** (1st spinal) will be seen running up (from the spinal cord) to the mouth.

Cut longitudinally through the skin of the thigh and shank and pin it back or cut it away. Note the large spindle-shaped **gastrocnemius muscle** of the calf. It has its origin in the lower end of the femur bone and its insertion in the sole of the foot, the insertion being effected by the long **tendo achillis.**

(This muscle is used in experiments in muscular contraction by electrical stimuli in the study of physiology.)

(2) THE ALIMENTARY SYSTEM
Buccal Cavity and Pharynx

Open the mouth wide.

Note the ridge of **maxillary teeth** on the **upper jaw** and the group of teeth, **vomerine teeth,** on the roof of the mouth. There are no teeth on the **lower jaw.** Pull out the **tongue.** Note that it is fixed in front and that the tip is forked. The **internal nares** can be seen on the front of the roof of the mouth. At the back of the **buccal cavity,** *i.e.,* in the **pharynx,** is the entrance to the **oesophagus,** which leads to the stomach, and below it the slit-like **glottis** leading to the lungs. The downward projections of the large prominent eyes can be seen and, just behind them on each side, is the entrance to the **Eustachian tube** which leads to the tympanic membrane.

Pierce one of the tympanic membranes with a mounted needle and pass a bristle through one of the Eustachian tubes into the buccal cavity in order to see where it enters it. Pass another bristle through one of the external nares into the buccal cavity.

GENERAL DISSECTION

Ligature the anterior abdominal vein (to prevent loss of blood) in two places, as follows: with a small scalpel, or the small scissors, make an
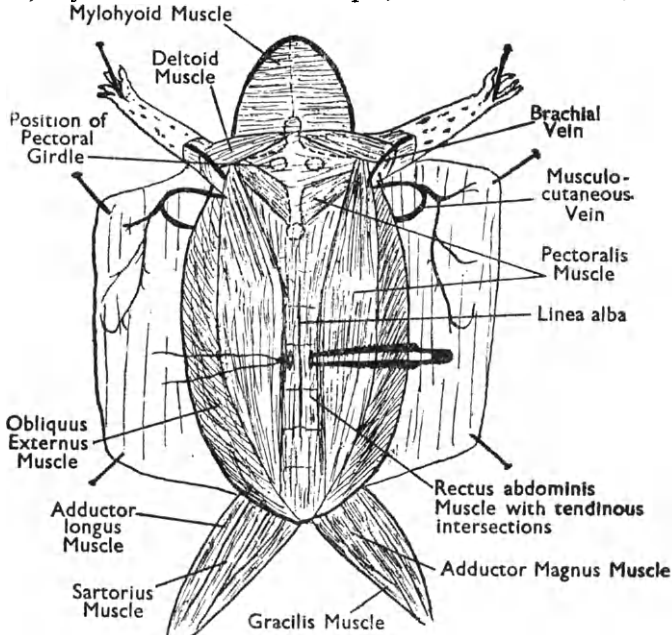
FIG. 123. **Rana. Muscular Body Wall and Method of Ligaturing the Anterior Abdominal Vein.**

incision on either side of the vein. Pull a loop of thread underneath it with the small forceps and then cut the loop at the bend (see Fig. 123). Tie each of the pieces of thread thus separated in a double knot round the vein. Cut across the vein between the ligatures, free it and deflect it out of the way.

Now expose the internal organs or **viscera** *by continuing your incisions as far back as possible and as far forward as the pectoral girdle. Cut through the coracoid and clavicle of the pectoral girdle close to the humerus on each side with the large scissors, taking care not to cut the blood vessels underneath or those going to the forelimb.*

Anterior abdominal vein ligatured and cut
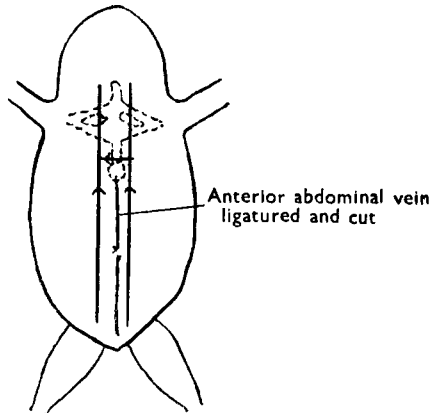
Fɪɢ. 124. **Rana. Method of opening the Body Cavity.**

Remove the freed central part of the girdle and gently pull the forelimbs farther out. Pin back the body wall and then cover the animal with water. In the **coelom** *or body cavity note the following, deflecting organs as necessary:—*

The large reddish-brown **liver** consists of two large lobes, the left one being subdivided into two. A small dark green sac, the **gall-bladder,** lies between them. *Deflect the liver forwards.* The **stomach** is a muscular sac on the left side of the animal into which the **oesophagus** leads from the pharynx. *Deflect the organs to the animal's right to see the latter.* Continuous with the stomach is the first part of the intestine (sometimes called the **duodenum**), and between them is a slight constriction, the **pylorus.** In the mesentery between the stomach and the duodenum lies the **pancreas,** a whitish gland. *Squeeze the gall-bladder with forceps and thus inject bile into the* **bile duct** which comes from the gall-bladder and traverses the pancreas. The **hepato-pancreatic duct** leads from the pancreas into the duodenum. The intestine continues as a coiled tube, the **small intestine** (sometimes

called the **ileum**) between the coils of which is the vascular membrane, the **mesentery.** The small intestine leads into a short wider tube, the **rectum,** which opens into the **cloaca.**

Note also the following viscera:

The **heart** in its **pericardium** is a conical reddish organ lying more or less in the centre at about the level of the fore-limbs (it may still be beating). The **lungs** lie one on each side of the heart, and the **urinary bladder** is a thin-walled bilobed sac in the posterior end of the coelom, which opens ventrally into the cloaca.
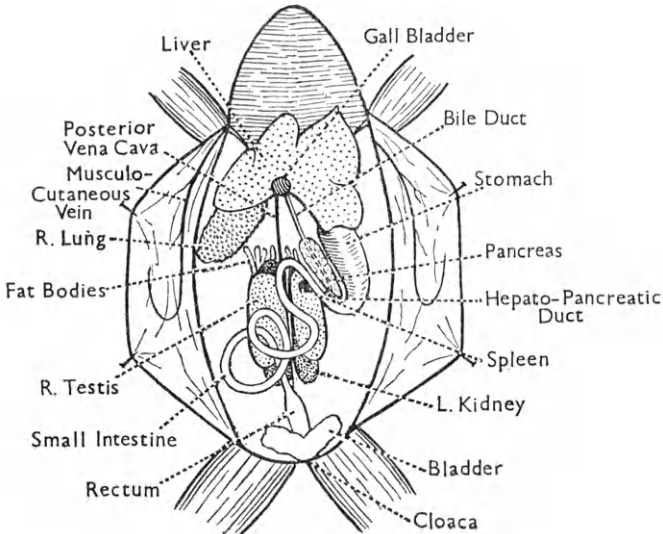
FIG. 125. Rana. Alimentary Canal. Ventral Dissection. Male. Liver Deflected Forwards.

Deflect the viscera slightly to the left of the animal.

In the *male* note the two yellowish oval bodies, the **testes,** and dorsal to them the reddish **kidneys** will be seen. In the *female*, the **ovaries** (containing a mass of **ova,** in the breeding season) and the coiled **oviducts** which may be distended with ova in the breeding season, will be seen. The ovaries and oviducts may considerably obscure the other viscera if they are filled with eggs. In both sexes the **corpora adiposa,** or fat bodies, yellow finger-like processes are situated at the anterior ends of the kidneys and the **posterior vena cava,** a large vein, runs between the kidneys up to the heart. Note also the **anterior abdominal vein,** already ligatured and cut, and the **spleen,** a small red spherical body in the mesentery near the beginning of the rectum.

Look for branches of the **mesenteric artery** and **vein** in the mesentery.

(3) THE VASCULAR SYSTEM

A mounted lens on a stand will be found useful in the following dissections which are best performed on a freshly killed male animal.

The circulation of the blood in the frog is a double circulation, *i.e.*, the blood is pumped by the heart to lungs back to the heart and then on to the rest of the body and so back to the heart again.

(1) The Heart

In a freshly killed frog, the heart may still continue to beat for some time. If this is so, note how it contracts. This will be more visible after removal of the pericardium.

Carefully free the heart from the pericardium by cutting through the latter with the fine scissors and removing it.

Note that the heart is roughly conical with the apex directed backwards and that it is composed of a single thick-walled **ventricle** at the free end with two **auricles** anterior to it. The **truncus arteriosus** arises from the right upper side of the ventricle on the ventral side and passes between the auricles, at the upper edge of which it divides into three **aortic arches.**

Turn the heart upwards and examine the dorsal view.

Note the thin-walled **sinus venosus,** roughly triangular in shape, at the apices of which the two **anterior venae cavae** and the **posterior vena cava** enter. The sinus leads into the right auricle, and the **pulmonary vein,** formed by a branch from each lung, leads into the left auricle.

Deflect the lungs to the sides of the animal when the pulmonary veins will be seen on their inner surfaces.

(2) The Venous System

It is easier to trace the veins from *the heart,* but remember that the blood flows in them to the heart.

Deflect the heart forwards or, better, to one side. A small pin through the tip of the ventricle will keep it in place.

(a) ANTERIOR TO THE HEART

Find the **Anterior Venae Cavae** which, as already seen, lead into the **sinus venosus.**

Just after leaving the auricle, the **anterior vena cava** receives:—

(i) The **External Jugular Vein,** which is the most anterior of three veins entering at this point. It runs underneath the mylo-hyoid

muscle, alongside the hypoglossal nerve (already seen), and is formed by the union of the (*a*) **mandibular vein,** a small vessel from the lower jaw and (*b*) the slender **lingual vein** from the tongue and continuous with the external jugular.
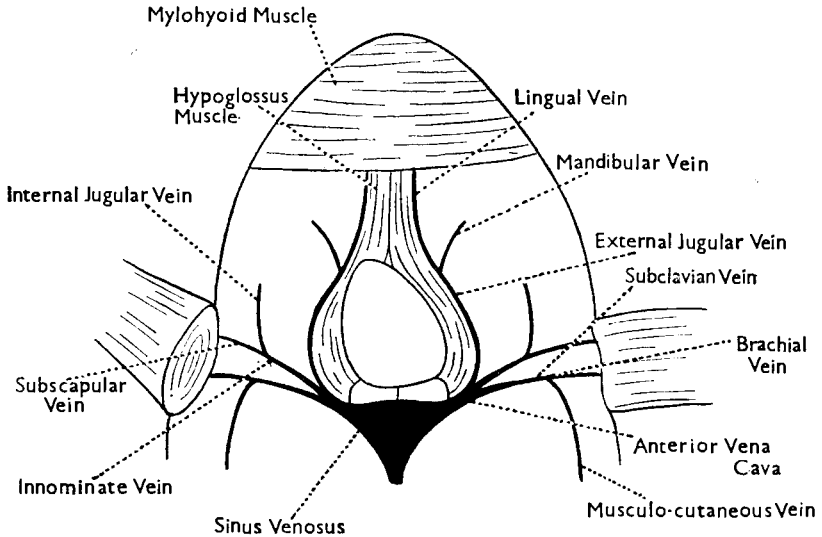
FIG. 126. **Rana. Veins anterior to the heart.**

(ii) The **Innominate Vein** is the middle one of the three veins and leads to the angle between the shoulder and the jaw. It receives:—

(*a*) the **internal jugular vein** from the angle of the jaw;

(*b*) the **subscapular vein** which comes from the shoulder.

(iii) The **Subclavian Vein** is the posterior and largest of the three veins entering the anterior vena cava. It comes from the fore-limb, where it is formed by the union of the **brachial vein** from the arm and the **musculo-cutaneous vein** from the skin and body wall. *Gently dissect apart the muscle fibres in the limb in order to see these two veins.*

The musculo-cutaneous vein will be seen curved round on the inside of the skin which has been pinned back on each side.

(*b*) POSTERIOR TO THE HEART

Find the **Posterior Vena Cava** which, as already seen, also leads into the **sinus venosus.**

At this point it receives the two **hepatic veins** from the liver (one from each lobe).

Deflect the alimentary canal to the animal's right and trace the posterior vena cava backwards.
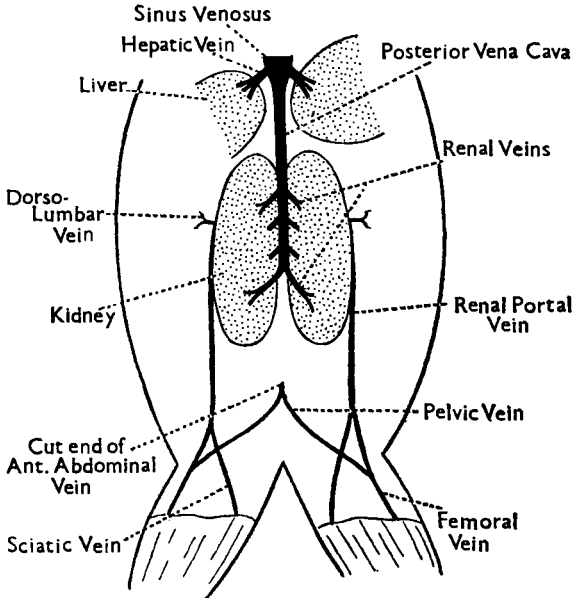
FIG. 127. **Rana. Veins posterior to the heart.**

It passes between the kidneys, where it has its origin in the **renal veins,** four or five pairs of small vessels coming from the kidneys.

The **Genital Veins (Spermatic** or **Ovarian)** usually enter one of the renal veins, though they sometimes lead directly into the posterior vena cava.

The **Renal-Portal Vein** runs along the outer edge of each kidney, in which it terminates in capillaries.

Trace one of the renal-portal veins backwards on one side.

Just before entering the kidney it receives the small **dorso-lumbar vein** from the muscles of the dorsal body wall. Further back it is formed by the union of the **sciatic vein** from the inside of the thigh, and the **femoral vein** from the other side of the hind limb.

The **femoral vein** divides anteriorly into the renal portal vein (already seen) and the **pelvic vein** which runs inwards and ventralwards to join its fellow.

Find the femoral vein in the outer part of the thigh muscles immediately below the surface. Trace it backwards by carefully separating the muscles of the thigh.

Now trace the **anterior abdominal vein** *backwards.*

It is formed by the union of the two **pelvic veins.** This will usually be seen on the back of the part of the body wall where the anterior abdominal vein was ligatured.

Trace the anterior end of the severed anterior abdominal vein forwards.

It divides into two branches, one to each lobe of the liver.

The **Hepatic Portal Vein** will be found as follows:—

At the bifurcation of the anterior abdominal vein the **hepatic portal vein** will be seen entering it. It is formed by union of **gastric, intestinal** and **splenic veins** from the stomach, intestine and spleen.
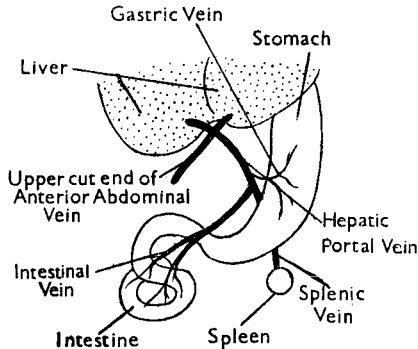
FIG. 128. **Rana. The hepatic portal vein.**

Examine the mesentery again. In it will be seen the intestinal vein. The gastric will be found on the stomach surface and the splenic coming from the spleen.

(3) The Arterial System

Ligature the anterior vena cava on one side in two places and cut between the ligatures, then carefully remove the three veins which form the anterior vena cava on that side, i.e., the external jugular, the inno-minate and the subclavian. (If the frog has been preserved since your last dissection, there should be little, if any, bleeding, but if it is a fresh specimen, a considerable amount of blood will be lost unless the ligaturing is done as directed above.) Make a small roll of paper, insert it into the mouth and push it down into the oesophagus to distend it. This will make the arteries more easily seen.

The **Aortic Arch** which as already seen arises from the **truncus arteriosus** on each side, gives rise to three arches:—

(i) **The carotid arch** is the most anterior. It runs upwards and soon gives off a small **external carotid** (also called the **lingual artery**) running under the hypoglossal nerve to the tongue. Immediately after giving off the lingual artery, the carotid arch forms a labyrinth of minute vessels appearing as a swelling, called the **carotid labyrinth.**

From this point the artery continues as the **internal carotid** to the orbit, though it cannot be traced without further dissection, where it continues to the brain, giving a branch, the **pharyngeal,** to the pharynx, palate, and orbit.
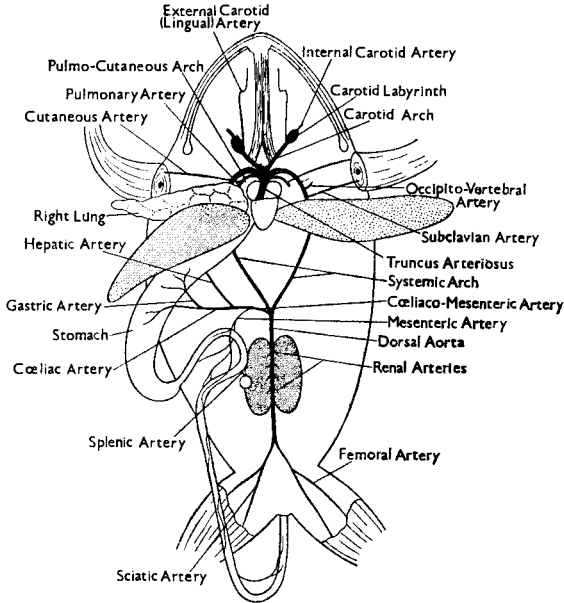
FIG. 129. **Rana. Arterial System.**

(ii) **The pulmo-cutaneous arch** is the hindermost arch.

Deflect one lung to the opposite side, pin it in place, and find the arch on the exposed side.

It is a short arch which soon divides into a **pulmonary artery** running dorsally to the lung and a **cutaneous artery** which runs out towards the shoulder region on the dorsal side and then continues to supply blood to the skin of the back, along the entire length of the body.

(iii) **The systematic arch** is the middle arch.

Deflect the alimentary canal to the animal's right and pin it in this position. Trace the arch on the exposed side.

On leaving the aorta the systemic arch on each side loops round the oesophagus to the dorsal side and runs backwards, joining its fellow just above the anterior edge of the kidneys to form the **dorsal aorta.** Find the **subclavian artery,** a large vessel which arises just after the loop of the systemic arch begins to turn downwards and which goes to the shoulder and fore-limb.

Just anterior to the origin of this artery is the **occipito-vertebral artery,** a short vessel dividing into the **occipital artery** to the head and the **vertebral artery** to the spinal cord.

The Coeliaco-mesenteric Artery arises at the junction of the systemic arches. It divides into:—

(i) the **coeliac artery,** which branches into the **gastric artery** to the stomach and the **hepatic artery** to the liver:

(ii) the **mesenteric artery,** which divides into:—

(*a*) the **anterior mesenteric artery** to the first part of the small intestine;

(*b*) the **posterior mesenteric artery** to the hinder part of the small intestine and, in some specimens, to the rectum.

(*c*) the **splenic artery** to the spleen.

The Dorsal Aorta runs in the mid-dorsal line between and dorsal to the kidneys and gives rise to:—

(i) the **renal arteries,** which enter the kidneys;

(ii) the **genital arteries (spermatic** or **ovarian),** which go to the gonads (testes or ovaries). Sometimes these arteries arise from the renal arteries;

(iii) the **rectal** (or **haemorrhoidal) artery,** which goes to the hinder part of the intestine or rectum, present only in specimens in which this is not supplied by the posterior mesenteric artery.

Just posterior to the kidneys the aorta bifurcates. The two arteries thus formed are the **iliac arteries** which go to the legs.

Cut through the bony pelvic girdle with the large scissors in the mid-central line and artery on one side, trace the iliac downwards into the leg.

It divides into the **sciatic** and **femoral arteries** on the inside and outside of the leg respectively.

(4) THE URINO-GENITAL SYSTEM

Remove the alimentary canal by cutting through the oesophagus, the mesentery, holding the alimentary canal with forceps meanwhile, and the rectum.

(i) Male

Note the two red, flat, oval **kidneys.** They are partly hidden by the testes. Trace the kidney duct, which also serves as a genital duct and is known as the **urinogenital duct** (Wolffian duct), from the kidney to the **cloaca** which it enters by a **urino-genital papilla.** After leaving the kidney, it bears a sac, the **vesicula seminalis.** Note also the bilobed

bladder. On the ventral side of each kidney is the **testis.** Ducts from this organ, the **vasa efferentia,** lie in a supporting tissue, the **mesorchium,** and lead into the kidney.

Slit open the cloaca which will be found beneath the centre of the pelvic girdle, already severed when dissecting the arterial system. On its dorsal side the two **urino-genital papillae** will be seen side by side
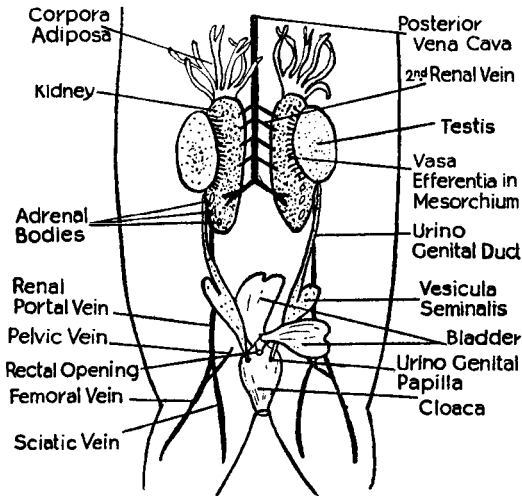
Fig. 130.   **Rana.   Urino-Genital Organs.   Male.   Bladder Deflected.**
Anterior Abdominal Vein not shown.

in the mid-line. *Then by means of a seeker or bristle find* the **opening of the bladder,** the **rectal aperture** and the **external cloacal aperture.**

Note also the yellow digitate **corpora adiposa** at the anterior end of the kidneys and the **inferior vena cava** between the kidneys, into which the **renal veins** lead. Find the **femoral vein** seen already in the muscles of the outer side of each leg, the **sciatic vein** from the inner side of the leg, the **renal-portal vein,** formed by the union of the sciatic and femoral veins and running along the outer edge of each kidney and the **pelvic vein** joining its fellow from the opposite side to form the **anterior abdominal vein** (as seen in the dissection of the venous system).

Deflect one of the testes to one side so as to expose the ventral surface of the kidney. Observe the light patches on the kidney: these are the **adrenal bodies.**

(ii) Female

Note the **kidneys,** largely hidden by the ovaries, and their ducts, the **ureters** (Wolffian ducts), each of which enters the **cloaca** by a

urinary papilla, the **adrenal bodies,** and the **bladder.** The lobed **ovaries** are supported by a tissue, the **mesovarium,** and may contain a large number of **ova.** The **oviducts** (Müllerian ducts) are two coiled tubes which lead to the cloaca and near the posterior ends bear swellings, the **ovisacs.** During the breeding season these may be distended with eggs. *In this case remove one of the oviducts. Trace one oviduct forwards and find* the funnel-shaped **internal opening of the oviduct** at the side of the oesophagus on a level with the base of
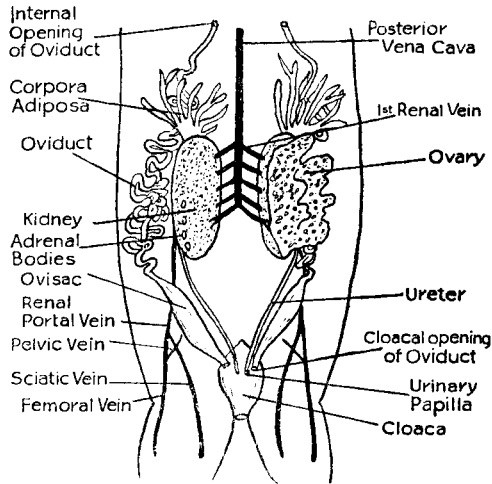
FIG. 131. **Rana. Urino-Genital Organs. Female. Right Ovary and Bladder Removed. Anterior Abdominal Vein not shown.**

the lung. *Open up the cloaca as explained for the male (p. 177). By means of a seeker or bristle find the* **oviducal apertures** *and the* **ureter openings.** *They will be found on the sides and ends of two small papillae.* *Find also the* **opening of the bladder,** the **rectal aperture** and the **external cloacal aperture.** Note also the **inferior vena cava, renal femoral, renal-portal, sciatic, pelvic** and **anterior abdominal veins** and the **corpora adiposa** as in the male.

(5) THE NERVOUS SYSTEM

This consists of the **central nervous system,** the **peripheral nervous system** and the so-called **sympathetic nervous system,** * which is more developed than in the dogfish.

*The term **autonomic nervous system** is more appropriate than sympathetic nervous system.

(1) The Spinal Nerves

Remove the heart, lungs, liver, kidneys and testes (or ovaries), exercising great care. If a fresh animal is being used, first remove the alimentary canal, of course. Leave the systemic arch and dorsal aorta in position.

Note the bony **vertebral column** and the ten pair of **spinal nerves,** white cords which pass out between the vertebrae, being surrounded by white **calcareous concretions** as they emerge.

Trace out the following nerves:—

I. The **Hypoglossal** nerve leaves the spinal column between the 1st and 2nd vertebrae and runs forwards underneath the mylo-hyoid muscle to the tongue. *The mylo-hyoid muscle must be cut in the midline and the flaps deflected and pinned out if this has not already been done, to see the nerve, but it should already have been seen in earlier dissections.*

II, III. The 2nd nerve leaves the spinal column between the 2nd and 3rd vertebrae and is joined by the 3rd nerve, which emerges between
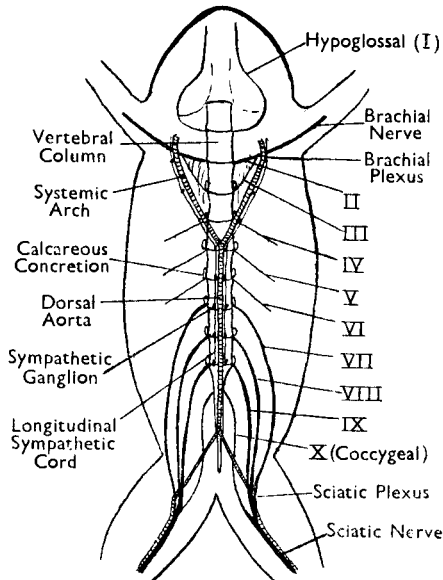
FIG. 132. **Rana. Spinal Nerves.**

the 3rd and 4th vertebrae. A branch from the hypoglossal joins them, thus forming the **brachial plexus.** The main trunk then continues into the arm as the **brachial nerve.**

IV, V, VI leave the spinal column between the 4th and 5th, 5th and 6th, and 6th and 7th vertebrae respectively. They are all small and run outwards and backwards to the skin and muscles of the body wall.

VII, VIII, IX emerge from the nest three intervertebral spaces and unite to form the **sciatic plexus.** Several branches arise from this, the large **sciatic nerve** to the leg being the most easily seen and being formed by the union of VIII and IX.

X. The **Coccygeal nerve** leaves the vertebral canal by a foramen in the urostyle. Branches go to the bladder, etc., and to the sciatic nerve.

(2) The Sympathetic Nervous System*

Note the chain of pigmented **sympathetic ganglia** outside the vertebral column on either side of the dorsal aorta, joined by the **longitudinal cords** of the sympathetic chain. Branches, the **rami communicantes,** connect the spinal and sympathetic nervous systems.

(3) The Brain and Spinal Cord

Examine again the skull and vertebral column of an articulated skeleton before you begin this dissection.

Pin the frog, dorsal side uppermost, in the dissecting dish. Remove the skin from the dorsal side; then, carefully inserting the scissors from behind, cut through the sides of the cranium on both sides, keeping the lower blade of the scissors flat. Then cut through the occipito-atlantal membrane covering the space between the skull and the first vertebra and through any remaining tissues which prevent the lifting of the roof of the cranium. Deflect the roof of the cranium forwards and then remove as much as possible of the sides.

Now turn the dish round so that the head of the frog points towards you, insert the scissors into the neural canal of the first vertebra taking care not to injure the spinal cord within and carefully remove the roof (neural arch) of the vertebra. Remove the tops of the other vertebrae in succession and so expose the spinal cord.

(a) Dorsal View

Examine in situ from front to back, removing the pigmented membrane, the **pia mater,** *as you proceed.*

In the **Fore-Brain** note the anteriorly placed **olfactory lobes** (or, better, **bulbs**), joined in the middle line so that they appear undivided. Behind them are the large **cerebral hemispheres** separated by a median fissure. These are followed by a small portion, the **thalamencephalon,**

*Better called the **autonomic nervous system.**

covered by a vascular membrane, the **anterior choroid plexus.** This part of the brain bears the **pineal body** on a short stalk. (The pineal body will probably have been removed with the roof of the skull so that only the stalk remains.) The side walls of the thalamencephalon are thick and are known as the **optic thalami.** The **Mid-Brain** consists of two spherical **optic lobes** immediately behind the thalamencephalon. The **Hind-Brain** follows first as a narrow transverse band, the **cerebellum,** and then by the tapering **medulla oblongata,** covered by the vascular **posterior choroid plexus.**
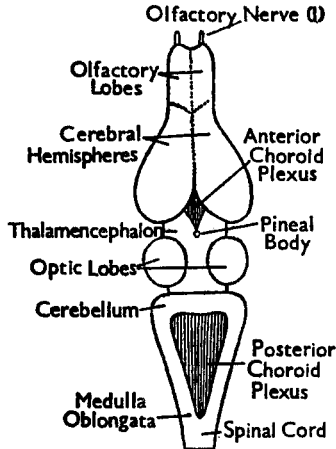
FIG. 133. **Rana. Brain. Dorsal View.**

The **spinal cord** is continuous with the medulla oblongata. It tapers towards the posterior end, terminating in the thin **filum terminale** in the urostyle. Note that it is wider where the nerves for the fore-limb originate, the **brachial enlargement,** and again where the nerves for the hind-limb arise, the **lumbar enlargement.** Note also that the cord is somewhat flattened and that it has a fairly deep groove, the **dorsal fissure,** along its length. The hindmost nerves run backwards in the vertebral canal for some distance before they pass out of it. This group of nerves, together with the filum terminale, is called the **cauda equina.**

(b) Ventral View

It is best to hold the frog in the hand, resting the head along the fingers when removing the brain.

Cut through the olfactory nerves which hold the brain in position in front, then cut through the remaining nerves at the sides and base, keeping as far away from the brain as possible.

Cut along each side of the spinal cord with small scissors or scalpel; this will sever the spinal nerves. The spinal cord can then be removed from the vertebral column, the filum terminale being gently pulled out of the urostyle with forceps.
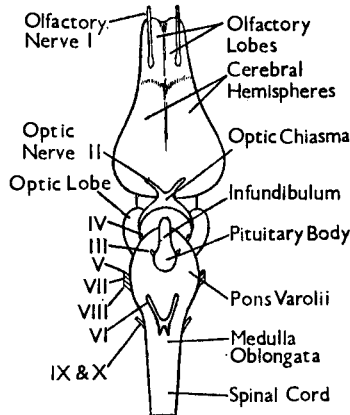
FIG. 134. **Rana. Brain.**
Ventral View.

Gently turn the brain and spinal cord so as to expose the ventral surface. You may find it easier to hold the frog vertically in your hand to remove the brain and spinal cord.

Note the **olfactory lobes** (or **bulbs**) and **olfactory nerves (I), cerebral hemispheres** and the sides of the **optic lobes.** The **optic nerves (II)** cross over one another forming the **optic chiasma.** Immediately behind this is the **infundibulum,** a grooved median swelling on the floor of the thalemencephalon, attached to which is the **pituitary body** or **hypophysis.** (This may have been broken off when the brain was removed.) The **crura cerebri** join the thalamencephalon to the medulla but are hidden by the posterior end of the pituitary body.

The Spinal Cord shows a groove, the **ventral fissure,** along its length which is deeper than the dorsal fissure.

(c) Horizontal Section of the Brain

The brain must be hardened in 90 per cent. alcohol before a section is made. This takes three or four days.

With the brain preferably on its side, carefully cut off the roof of the brain with a small scalpel, inserting it into the side of the brain.

Note that the brain contains cavities or ventricles as follows:—

The **lateral Ventricles** are in the **cerebral hemispheres** and

communicate in the centre with the **third ventricle** in the **thalamen-cephalon** by the **foramen of Monro.** The third ventricle communicates by the **iter** (or **aqueduct of Sylvius**) with the **fourth ventricle,** which is in the **medulla.** The **optocoels** in the optic lobes open into the iter.
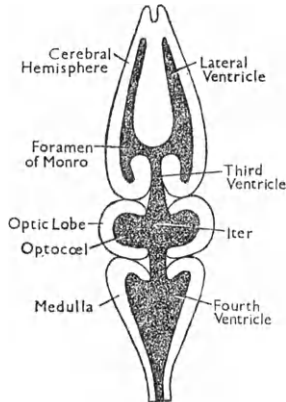
FIG. 135. **Rana. H.S. Brain.**

(4) The Cranial Nerves

Using a fresh frog (preferably hardened in alcohol), expose the dorsal surface of the brain as before, pour in 90 per cent. alcohol and remove the skin round the eye. Trace out the cranial nerves on one side as follows:—

I. The **Olfactory Nerve** arises from the olfactory lobe and goes to the epithelium lining the nasal cavity. It is not worth while dissecting this out.

II. The **Optic Nerve** which arises from the optic lobe on the opposite side, thus forming the **optic chiasma** goes to the eye-ball. *Separate the recti muscles at the posterior end of the orbit. The optic nerve will be seen in the* **retractor bulbi muscle** *in the midst of them.*

III. The **Oculomotor Nerve** is a very small nerve arising from the ventral side of the brain between the crura cerebri and supplies four of the eye muscles, namely, the rectus superior, inferior and internus and the obliquus inferior. (These muscles have been studied in the dog-fish.) This nerve is difficult to trace.

IV. The **Pathetic or Trochlear Nerve** is another slender nerve, arising between the optic lobes and the cerebellum on the

dorsal side and is difficult to trace. It supplies the obliquus superior muscle, and is too small to be worth tracing out.

VI. The **Abducens Nerve** is also very slender and difficult to trace. It arises from the ventral surface of the medulla and supplies the rectus externus muscle.

VIII. The **Auditory Nerve** arises from the side of the medulla, immediately behind the VIIth nerve, enters the auditory capsule and supplies the internal ear.

Dissection of the IXth and Xth nerves

It is best to dissect these nerves before the Vth and VIIth as the lower jaw must be removed to see the latter.

IX. The **Glossopharyngeal Nerve** arises behind the VIIIth nerve in the side of the medulla by a root common with the Xth nerve. It passes through the skull immediately behind the auditory capsule and then divides into:—

    (*a*) An **anterior branch,** running forwards and downwards, which joins the VIIth (facial) nerve.

    (*b*) A **posterior branch,** also running forwards and downwards, which goes to the base of the tongue.

X. The **Vagus Nerve.** This arises with the IXth nerve as already
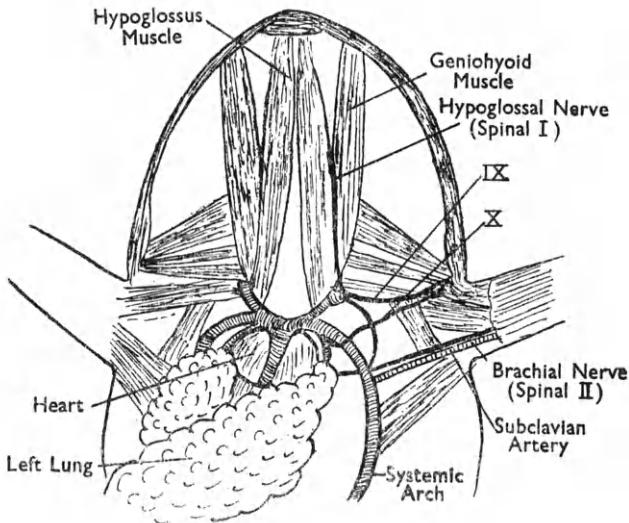
FIG. 136. Rana. **Dissection of IXth and Xth Cranial Nerves.**

seen. It also leaves the skull with the IXth nerve and then, after a ganglionic swelling, gives rise to four branches as follows:—

(*a*) The **laryngeal branch** which runs under the hypoglossal nerve anteriorly to the larynx.

(*b*) The **cardiac branch** which runs to the heart.

(*c*) The **pulmonary branch** which runs alongside the pulmonary artery to the lung.

(*d*) The **gastric branches** (usually two) which go to the stomach.

> *Open up the ventral side of the body to expose the viscera in the usual way.*

> *Carefully remove the mylohyoid muscle. Find the hypoglossal nerve (Spinal I) and the geniohyoid muscle* running longitudinally from the anterior part of the lower jaw and dividing posteriorly. Note also the hypoglossus muscles in the mid-line running from the anterior end of the lower jaw and dividing posteriorly. *Now find the external jugular vein.* The **IXth nerve** runs from the angle of the jaw, crosses the hypoglossal nerve alongside the external jugular vein and runs under the geniohyoid muscle to the floor of the mouth. The **Xth nerve** runs more or less parallel with the IXth with some muscle between before it divides into the four branches mentioned above. *Find the* **cardiac, pulmonary** *and* **gastric branches.**

> *Cut through the external jugular, innominate and sub-clavian veins on one side where they join the anterior vena cava and remove these veins: otherwise the nerves will be hidden. Deflect the lung of this side to the opposite side of the body.* The IXth and Xth nerves will then be seen.

Dissection of the Vth and VIIth Nerves

This dissection may not be required by some examination syllabuses.

V. The **Trigeminal Nerve** is a large nerve—the largest cranial nerve—and arises from the side of the anterior end of the medulla. It bears a swelling or ganglion, the **Gasserian ganglion,** just before it enters the bone of the skull. After leaving the skull, just anterior to the auditory capsule, it divides into:—

(*a*) the **Ophthalmic Branch** which passes through the orbit between the bone of the skull and the eye, and out at

the anterior end where it divides into two branches supplying the skin of the anterior part of the head. *Move the eyeball away from the inner wall of the orbit to see this branch.* It will be seen again when studying the VIIth nerve.

(*b*) the **Maxillary Branch.** This branch, which will also be seen later, runs at the back of the orbit to the upper jaw or maxilla and arises from a second branch of the Vth nerve, the maxillo-mandibular branch.

Press the eyeball sideways to see this branch.

(*c*) the **Mandibular Branch** arises from the maxillo-mandibular branch and runs behind and at first parallel to the maxillary branch. The mandibular branch leaves the maxillary branch at the outer edge of the eye and runs to the lower jaw or mandible, in close association with the mandibular branch of the VIIth nerve but external to it. It, too, will be seen later in the dissection.
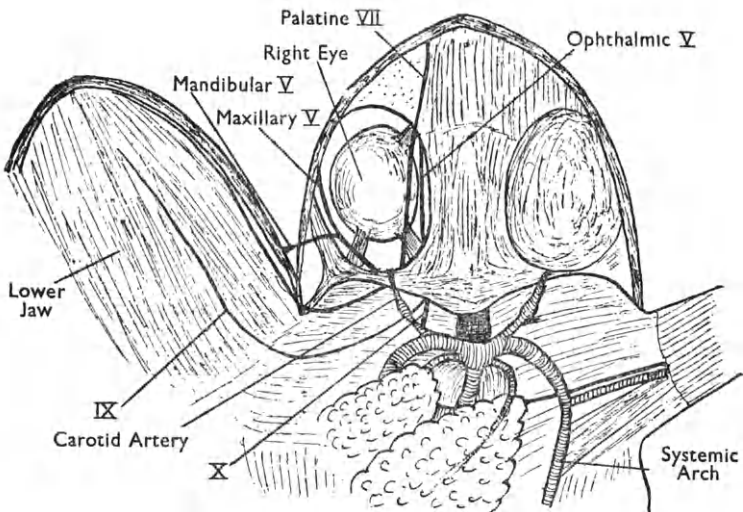
Fig. 137.   **Rana.   Dissection of the Vth and VIIth Cranial Nerves.**

VII. The **Facial Nerve** arises from the side of the medulla immediately behind the Vth nerve, leaving the skull behind it and then divides into a **palatine branch** and a **hyomandibular branch.**

(*a*) The **Palatine Branch** runs straight forwards in the floor of

the orbit from the inner posterior corner to the nasal region on the roof of the mouth. *Trace this branch as follows:*—

With the frog ventral side up, cut transversely from the angle of the jaw on one side and deflect the lower jaw to the opposite side. If the mucous membrane covering the roof of the mouth is now carefully cut away, this branch will be seen on the ventral surface of the eye.

Move the eyeball outwards.

The **ophthalmic branch of the Vth nerve** will now be seen running forwards along the inner edge of the orbit.

Now find the **maxillo-mandibular branch of the Vth nerve** *at the posterior end of the orbit and trace it forwards to the point where it divides. Follow the* **maxillary branch** *in the floor of the orbit to the upper jaw. Then trace the* **mandibular branch** *across to the now deflected lower jaw.* It runs along the outer edge of the jaw and will be seen *if the muscles there are carefully separated.*

(*b*) The **Hyomandibular Branch of the VIIth nerve** is not easy to follow.

It runs round the anterior end of the auditory capsule where it divides into a posterior **hyoidean branch** to the muscles of the hyoid in the floor of the mouth, and a **mandibular branch** running along the inner edge of the lower jaw, in close association with the mandibular branch of the Vth.

Again examine the edge of the **lower jaw.** *Carefully separate the muscles there and find the* **mandibular** branch running along the inner edge of the jaw. After a short distance it gives off a branch which anastomoses with the mandibular branch of the Vth nerve.
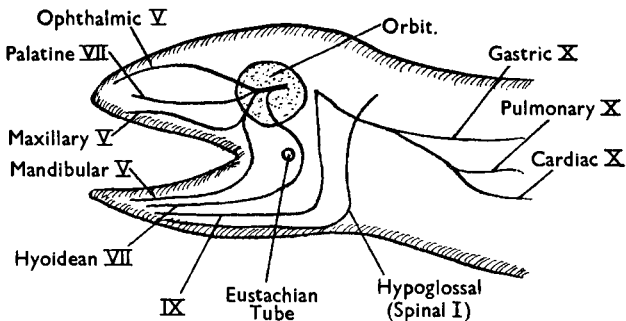
FIG. 138. Rana. Cranial Nerves. Lateral View. Diagrammatic.

THE NEWT

Newts belong to the CLASS *Urodela* (tailed Amphibia). The body is similar to that of the frog, except that it is longer and thinner and that in addition to the head and trunk it has a tail. The common newt is *Trituris vulgaris* and the Warty newt *Molge cristata*. In the breeding season the male develops a crest on the dorsal side.

THE LIZARD

LACERTA

Lizards belong to the CLASS *Reptilia* (animals completely adapted to a terrestial life, though some live in rivers and lakes. The skin is covered with horny scales. Respiration is by lungs. There is no external ear. Eggs are large and contain large yolks. This CLASS contains alligators, crocodiles, turtles and snakes as well as lizards). The lizard is in the ORDER *Squamata* and the SUB-ORDER *Lacertilia*.

Examine the **Common Lizard** (*Lacerta vivipara*).

The body is divided into head, neck, trunk and tail and is completely covered with **horny scales.** Dorsally the animal is green but on the ventral side it is brown or yellow.

The **head** is comparatively small, somewhat pyramidal in shape and slightly flattened dorso-ventrally. The **mouth** is wide and anteriorly placed, the **external nostrils** being dorsal to it. The **eyes** are dorso-lateral and have upper and lower lids and a nictitating membrane. Behind each eye is a brown circular patch which is the **tympanic membrane,** there being no external ear.

The long **trunk** is rather flattened laterally and at its posterior end is the **cloaca.** The limbs are short, the **fore-limbs** being situated at the anterior end of the trunk and the **hind limbs** at the other end. Both manus and pes have five digits which terminate in claws.

The tail is extremely long, almost twice the length of the rest of the body, and is cylindrical and tapers towards its extremity.

THE GRASS SNAKE

NATRIX NATRIX

Snakes are also in the CLASS *Reptilia*, of course, and though they are in the same ORDER, *Squamata*, as the lizards, their SUB-ORDER is *Ophidia*.

Examine the **external structure** *of a* **Grass Snake.**

Snakes are, of course, limbless reptiles and the body is divided into **head** and **trunk.** The shape of this body is cylindrical and it may attain a length of 1·2 to 1·8 m. (4–6 ft.). The skin is covered with **scales** which are shed periodically as a continuous slough. The colour varies in different species of snakes and the grass snake is greenish with black and yellow markings on its head. The **eyes** are dorsally situated. The **mouth** is expansible and in the buccal cavity is a long and **protrusible tongue.** The **teeth** are fused to the bone of the upper and lower jaws. The **cloaca** is at the posterior end of the trunk. (Venomous snakes have pointed poison fangs in the mouth and these have grooves which serve as ducts for the poison which is secreted by the poison gland.)

THE PIGEON

COLUMBA

The CLASS *Aves* includes all birds (body covered with feathers, fore-limbs modified into wings. Large sternum with keel for attachment of flight muscles. Feet covered with horny scales. Jaws devoid of teeth and enclosed in horny beak. Lay large yolked eggs in nests and show parental care of offspring).

Pigeons are varieties of the wild Rock Dove (*Columba livia*). The well-known Wood Pigeon is *C. palumbus*.

Examine a **pigeon** *externally. A plucked bird will also be required.*

The body, which is divided into head, neck, trunk and tail is completely covered with feathers except on the beak and feet. The colour is bluish-grey with a greenish blue sheen on the neck.

The **head** is spherical and bears a pointed **beak** composed of upper and lower jaws encased in horny skin, and devoid of teeth. At the base of the beak are the **external nostrils,** partly hidden by the **cere,** a swollen, sensory patch of skin of waxy appearance. The **eyes** are laterally placed, each has its own field of vision and each having upper and lower lids and a nictitating membrane. Posterior to the eyes, hidden in feathers known as the **ear coverts,** are the **auditory apertures.** The long **neck** can be turned through 180°. *Examine the plucked bird* and note that the **trunk** is boat-shaped. It has a distinct **keel** on the ventral side and at the posterior end is the **cloacal** aperture. The fore-limbs are modified to serve as **wings.** The skeleton of this limb is of typical vertebrate form but is devoid of digits. The wing is folded flat against the body when at rest and it cannot be straightened out completely. This is due to two folds of skin, one across the elbow, the **propatagium,** and the other across the arm-pit, the **postpatagium.** *Examine the plucked bird to see these.* The **hind-limbs** are vertically placed, the thigh being hidden beneath the skin, and the **pes** is covered with **scales** similar to those found in reptiles. There are four digits, three directed forwards and one, the **big-toe,** backwards. This enables the bird to perch. The toes end in claws. *Again examine the plucked bird.* The **tail** is short and stumpy and at its apex on the dorsal side is the **oil gland aperture.**

Feathers

There are three kinds of feathers:—

Contour feathers which include the **covert** feathers which cover the whole body (except the beak and feet) and the **down feathers** between them. **Quill feathers** found on the wings and tail and **filoplumes,** minute thread-like feathers all over the body between the coverts. (These are left when a bird is plucked and their removal entails singeing).

Examine a **quill feather from the wing** *or* **tail.** *Use a lens for detail.*

The feather is composed of a **shaft** or **rachis,** convex on its upper surface and with a groove on the lower, on each side of which is the flattened and expanded **vane** or **vexillum.** This is made up of **barbs** on either side of the rachis arranged like teeth on a comb, though they are obliquely placed. *Examine with a lens.* It will be seen that each barb bears branches called **barbules** which are interlocked by means of tiny hooks known as **barbicels.** This cohesion gives the feather a continuous surface during flight. At the lower end of the vane is a small tuft of small feathers devoid of barbicels and known as the **aftershaft.** The continuation of the rachis beyond the vane is known as the **quill** or **calamus** and at its upper end is a minute pit called the **superior umbilicus** while at its lower extremity is a hole called the **inferior umbilicus.** The quill feathers on the wing are known as **remiges** and those on the tail as **rectrices.** They differ slightly in that in the former the vane is asymmetrical, one side being wider than the other and somewhat concave on the upper surface while the rectrices are flat and have a symmetrical vane. Feathers are made of keratin.

Vane

Barbs

Rachis (Shaft)

Superior Umbilicus

Calamus (Quill)

Inferior Umbilicus
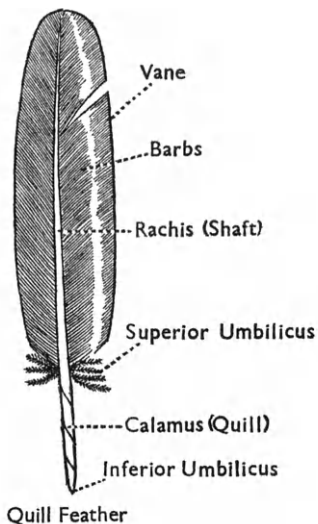
Quill Feather

FIG. 139. **Columba.**

Examine a **covert feather.**

This is similar to a quill feather but the rachis is pliable and the vane much softer.

Examine a **down feather.**

This, too, is very soft. The rachis is short and very soft barbs stretch out from it.

Down Feather

FIG. 140. **Columba.**

Filoplume

FIG. 141. **Columba.**

Examine a **filoplume.**

This is composed of a thin thread bearing a small tuft at its tip.

Examine a **complete wing.**

The arrangement of the coverts and quill feathers will be seen in the illustration. The **primary quills** are at the tip. These are followed
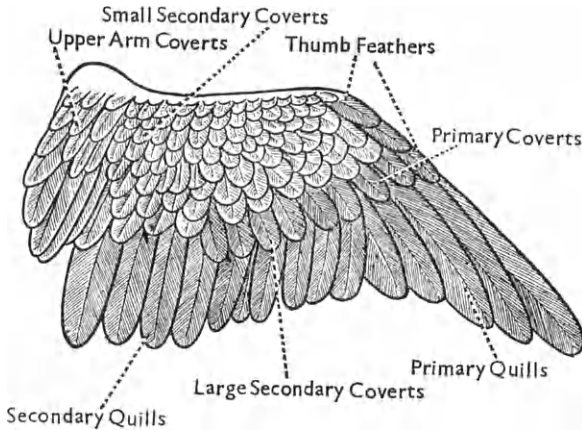
FIG. 142. **Columba. Wing.**

by the **secondary quills** and then the **primary coverts** followed by the smaller **secondary coverts.** A small group of feathers, known as the **thumb feathers** and also called the **bastard wing,** will be found on the upper surface.

Examine the plucked bird again.

The feathers are arranged in definite tracts known as **pterylae,** the tracts devoid of feathers being called **apterylae.** This may not be clearly visible.

MAMMALS

General Characteristics of Mammals

The following are the *chief general* characteristics of mammals, though certain types are exceptional in some respects:—

(1) Mammals are vertebrate animals in which the body is divided into a head and trunk, separated by a distinct neck, the trunk bearing **four limbs.**

(2) The body is covered with **hair,** or some modification of it.

(3) The skin possesses **sebaceous glands** for lubrication of the hair, and **sudorific** or **sweat glands** for excretion and osmo-regulation.

(4) The trunk is divided into an anterior **thorax** and a posterior **abdomen,** separated internally by a muscular partition, the **diaphragm.**

(5) The bones of the skull are immovably articulated by dovetail joints called **sutures.** The long bones and vertebrae bear epiphyses and between the vertebrae are inter-vertebral discs.

(6) The **cerebrum** is large and the surface is thrown into folds or **convolutions,** as also is the **cerebellum,** in the higher types.

(7) The heart is **four-chambered,** having two auricles and two ventricles, and there is a double circulation (though not in the same sense as in Amphibia). The heart is situated between the lungs which are enclosed in sacs, the **pleura,** in the thorax.

(8) There is **no** renal-portal system.

(9) The temperature of the blood (25°—40°C.) does not vary with the surrounding atmosphere. Mammals are **"warm-blooded" (homoiothermic).** The red corpuscles are **not** nucleated.

(10) The intestinal and urino-genital openings are **separate.**

(11) Except in the Monotremata which lay eggs, the offspring develop in a special organ in the abdomen, the **uterus,** to the wall of which they are vitally joined by an **allantoic placenta.** The ova are small and with little yolk **(micro-lecithal).**

(12) The newly born young are fed for a limited period on milk secreted by special glands, **mammary glands,** in the skin of the mother.

The CLASS *Mammalia* is divided into three SUB-CLASSES, *Monotremata* (primitive mammals showing clear evidence of their reptilian ancestry. Lay large eggs with large yolks. Young fed by milk secreted by modified sudorific glands in skin of female: no teats are present. Have a cloaca. *E.g., Duckbilled Platypus, Spiny anteater*), *Metatheria* (*Marsupials.* Primitive mammals in which young are developed in body of female but are immature at birth. Development continues in a pouch or marsupium where the young are fed on milk secreted by modified sebaceous glands. Separate urino-genital and anal apertures. *E.g., Kangaroo, Wallaby*), and *Eutheria* (most highly developed mammals. Young "fully developed" in uterus of female to which they are united by an allantoic placenta and suckled by milk secreted by mammary glands. Separate urino-genital and anal apertures. Highly developed brain. *All the common mammals*).

Two examples of mammals follow, the *rabbit* and the *rat*.

THE RABBIT

ORYCTOLAGUS

The *rabbit* belongs to the ORDER *Lagomorpha* and is in a different genus from that to which the *hare*, **Lepus,** belongs.

I. EXTERNAL ANATOMY

Examine a killed specimen.

Note the colour of the body, which is covered with fur and is divided into head, neck, trunk, and tail.

Head

The head tapers towards the anterior end, or snout, where the **external nares,** or **nostrils,** will be seen as two slits on skin devoid of hair. The **mouth** below the nostrils, is bounded above by an *upper lip*, cleft in the middle, showing the *incisor teeth*, and by a **lower lip.** At the sides of the nostrils are stiff whiskers, or **vibrissae,** which are tactile organs. The eyes are on the sides of the head: each has an **upper lid** and a **lower lid,** both bearing **eyelashes** and a hairless **nictitating membrane.** The **external ears,** or **pinnae,** are large and are situated on the sides of the head at the posterior end. The entrance to the ear is called the **external auditory meatus.**

Neck and Trunk

A distinct **neck** connects the head to the trunk. The **trunk** consists of the **thorax** above, enclosed by the ribs and sternum, and the **abdomen** below, with simply a muscular body wall on the ventral side. The posterior end of the trunk bears a short **tail,** white on its under surface. The **anus** is situated at the root of the tail between the hind limbs.

In the **male,** note the **penis,** an intromittent organ in its sheath, the **prepuce,** separated from the anus by a hairless tract, the **perinaeum.** On either side of the penis is a **scrotal sac,** which contains a testis.

In the **female,** note the **vulva,** the slit-like urino-genital opening separated from the anus by the **perinaeum,** with a small, rod-like **clitoris,** in its ventral wall. Note also the four or five pairs of **mammary glands,** bearing **teats,** on the ventral side of the abdomen.

Limbs

The **fore-limbs** of the rabbit are much shorter than the hind limbs, and the **manus** bears five **digits,** while the **hind-limb** bears a **pes** with only four **digits,** the big toe, or hallux, being absent.

II. THE SKELETON

The Axial Skeleton

This includes the ribs and sternum as well as the skull and vertebral column.

The skull of the rabbit is in many respects not typical of mammalia, particularly as regards the dentition. It is therefore usual to study the skull of the dog (*Canis*) in detail and then to compare with it the skull of the rabbit.

(1) The Skull of the Dog

Cranium

The **cranium** is large and consists of three rings:—

(i) A posterior **occipital ring** composed of a **supra-occipital** bone above, a **basi-occipital** below, and an **ex-occipital** on each side surrounding the **foramen magnum.** Note the **occipital condyles,** which articulate with the first vertebra (atlas) on the lower sides of the foramen magnum and the **paroccipital processes,** anterior to the occipital condyles and projecting downwards, both on the ex-occipitals.

(ii) A median **parietal ring** composed of the two **parietal bones** above, the **alisphenoids** at the sides, and the **basi-sphenoid** below. The small bone between the posterior ends of the parietals is the **interparietal,** forming a ridge, the **sagittal crest.**

Between the occipital and parietal rings is the **squamosal,** bearing a forwardly directed **zygomatic process** which with the jugal bone forms the **zygomatic arch** as will be seen below, and a **glenoid fossa** for the articulation of the lower jaw.

(iii) An anterior **frontal ring** composed of two **frontal bones** above, the **orbito-sphenoids** at the sides, and a **presphenoid** below. Note also the **post-orbital ridge** running from the frontals to form part of the posterior wall of the orbit.

Note the immovable joints or **sutures** between the bones of the cranium.

The anterior wall of the cranium consists of the **ethmoid** in which is the **cribriform plate.** This and the presphenoid, continue forwards as the **mesethmoid,** forming the posterior part of the **septum** of the nose, the anterior part being composed of cartilage.

Sense Capsules

The Auditory Capsule

This is formed by the **periotic bone,** divisible into (*a*) a **petrous** portion, placed internally, containing the auditory organ, and (*b*) a **mastoid portion,** situated partly on the surface between the ex-occipital and the **external auditory meatus.** This will be found on

another bone, the **tympanic bone,** ventral and partly external to the periotic between the squamosal and the basisphenoid, bearing the **opening of the Eustachian tube** and protruding as a swelling, the **tympanic bulla.**

Examine the isolated **auditory ossicles,** *or bones of the ear, as follows:—*

The **malleus,** or hammer bone, the **incus,** or anvil bone, and the **stapes,** or stirrup bone. They are situated in the tympanic cavity.

Nasal Capsule

This is composed of two narrow **nasal** bones above, connected behind with the frontals, the trough-like **vomer** supporting the
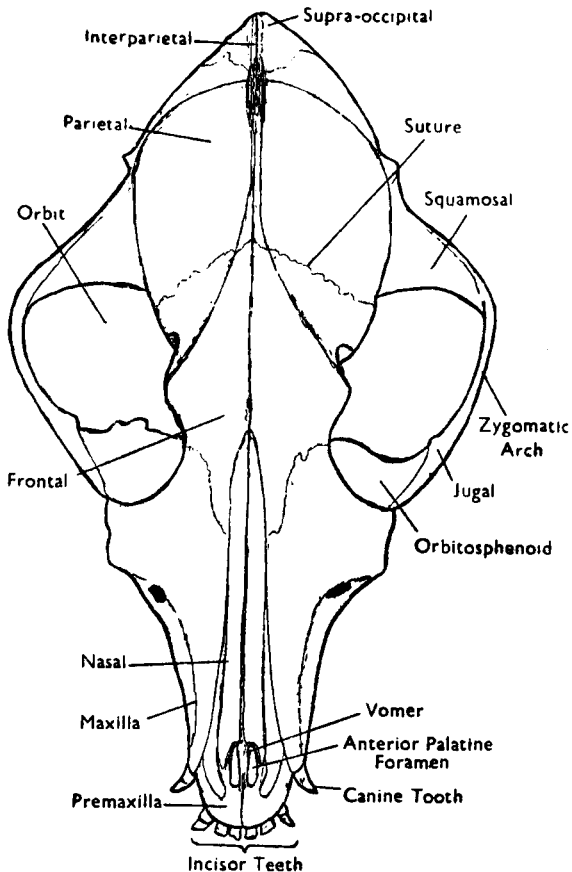
FIG. 143. Canis. Skull. Dorsal View.

cartilaginous nasal septum from below and the **facial portions of the maxillae** at the sides. The chamber is divided longitudinally by the **mesethmoid** posteriorly and by cartilage anteriorly which together form the **nasal septum,** and is bounded behind by the **cribriform plate,** as already seen. Note the **naso-turbinals,** the **maxillo-turbinals** and the **ethmo-turbinals,** three pairs of scroll-like bones on the inner sides of the chamber, and the **internal** and **external nares.**

Jaws and Orbit

The **Upper Jaws** are composed (i) posteriorly of the **maxillae,** with (*a*) the **facial** portions, each bearing **1 canine, 4 premolar** and **2 molar teeth,** and (*b*) the **palatal** portions: the last pre-molars are pointed and are known as **carnassial teeth,** (ii) anteriorly of the **pre-maxillae,** each of which bears the **3 incisor teeth.** The posterior part of the roof of the mouth is formed by the two **palatine** bones which surround the external nares and continue backwards with the vertically placed **pterygoids.** The zygomatic arch continues forwards as the **jugal** bone to the maxilla on each side.

The **Orbit** is bounded above by the frontal, and below by the jugal, anteriorly by the maxilla, and posteriorly by processes from the jugal and frontal. In the anterior wall is the **lacrimal,** in which the lacrimal canal leads to the nasal chamber. The orbito-sphenoid surrounds the optic foramen in the orbit.

The **Lower Jaw** (or **mandible**) consists of **2 dentaries** or **rami** fused anteriorly at the **mandibular symphysis,** each of which articulates posteriorly with the glenoid fossa of the squamosal by the somewhat rounded and elongated **condyle.** The **3 incisor, 1 canine, 4 premolar** and **3 molar teeth** are borne on each ramus. The first molars are **carnassial teeth** similar to those in the upper jaw. Between the condyle and the teeth is the flat upwardly directed **coronoid process,** and below the condyle is the **angle of the jaw** for muscle attachments.

From the above description and with the aid of the illustrations note the bones as follows:—

(1) **Dorsal View**

The **supra-occipital, inter-parietal, parietal, squamosal, jugal, zygomatic arch, orbit, orbito-sphenoid, frontal, nasal,** anterior end of the **vomer, maxilla, pre-maxilla, incisor** and **canine teeth** (the others may not be visible in this view) and the **sutures.**

(2) **Ventral View**

The **supra-occipital, ex-occipital, occipital condyles, foramen magnum, basi-occipital, condylar foramen** for the XIIth nerve anterior to the condyle; **tympanic bulla; foramen lacerum posterius,** a long foramen for the IXth, Xth and XIth nerves between the

ex-occipital and the tympanic bulla; **squamosal; stylo-mastoid foramen** for the VIIth nerve outside the tympanic bulla; **basi-sphenoid; alisphenoid; foramen rotundum** for the maxillary branch of the Vth nerve in the alisphenoid; **foramen ovale** for the mandibular branch of the Vth nerve posterior to the foramen rotundum; the **Eustachian foramen** for the Eustachian tube just inside and slightly posterior to the foramen ovale; **alisphenoidal canal** for the external carotid artery between the foramen ovale and the foramen rotundum; and the **foramen lacerum medium** for the internal carotid artery next
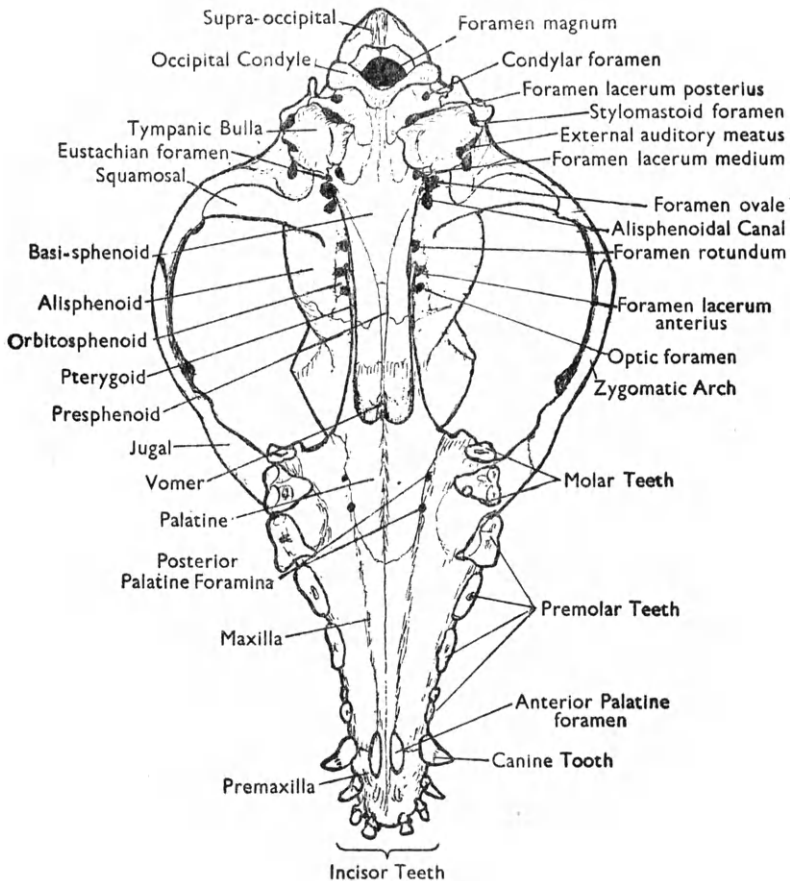
FIG. 144. Canis. Skull. Ventral View.*

*In this and the subsequent illustrations it will be noticed that an extra premolar tooth is present in the upper jaw. This abnormality was present in the specimen from which the drawing was made.

to the Eustachian foramen on the inside; **pterygoid; presphenoid; jugal; zygomatic arch; orbito-sphenoid; foramen lacerum anterius,** a large foramen between the alisphenoid and orbito-sphenoid for the IIIrd, IVth, VIth and ophthalmic branch of the Vth nerve; **optic foramen** for the IInd nerve anterior to the foramen lacerum anterius in the orbito-sphenoid; **palatine; pre-maxilla;** a large aperture at the anterior end of the maxilla on the ventral side the **anterior palatine foramen** which leads to the nasal chamber, and the **posterior palatine foramen** between the palatine and the maxilla; **incisor, canine, premolar** (including **carnassial**) and **molar teeth.**

(3) Lateral View

Pre-maxilla; maxilla; teeth as before, **infra-orbital foramen** for the maxillary branch of the Vth nerve on the side of the maxilla; **frontal; orbit; squamosal; zygomatic arch; glenoid fossa** on the squamosal for the articulation of the lower jaw; part of the **palatine; pterygoid; parietal; sagittal crest; supra-occipital; ex-occipital** and **occipital condyle; paroccipital process; tympanic bulla; external auditory meatus; foramen ovale; foramen rotundum; foramen lacerum anterius; optic foramen; lacrimal,** a small bone in the anterior corner of the orbit bearing the **lacrimal foramen** leading to the lacrimal canal which runs to the nasal cavity.

Mandible—incisor, canine, premolar and molar (including **carnassial**) teeth; coronoid process; angle; condyle.

Write the dental formula.
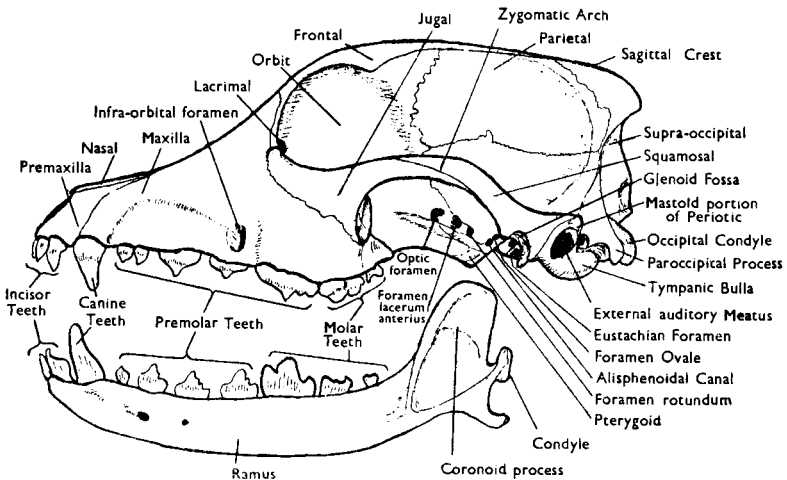
FIG. 145. **Canis. Skull. Lateral View.**

(4) Longitudinal Section of Skull

Pre-maxilla; nasal; mesethmoid; turbinals; vomer; frontal; parietal; inter-parietal; alisphenoid; squamosal; periotic; supra-occipital; ex-occipital; paroccipital process; tympanic bulla; basi-occipital; basi-sphenoid; presphenoid; orbito-sphenoid; pterygoid; palatine; maxilla; **ethmoid** and **cribriform plate** bearing **foramina for branches** of Ist **nerve; teeth** as in lateral view.
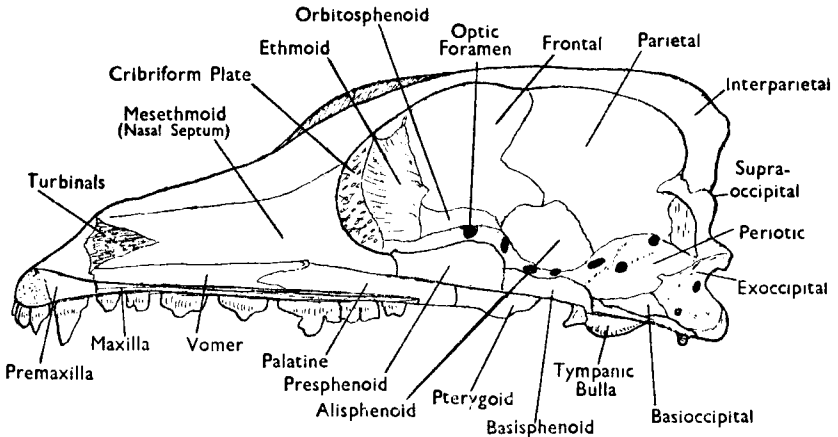
FIG. 146. Canis. Skull. Longitudinal Section.

The **hyoid bone** (part of the visceral skeleton) will be best examined later, in position, in the dissection of the neck.

(2) The Skull of the Rabbit

Examine the skull of the rabbit and compare it with that of the dog.

Note the difference in shape of the skull and the absence of canine teeth (the rabbit is herbivorous in its diet). There are two incisors, one *behind* the other, three premolars and three molars on each side in the upper jaw, but only one pair of incisors, two premolars and three molars, in the lower jaw. The space between the incisors and premolars is called the **diastema.** The mastoid portion of the periotic is more easily seen on the surface, and the paraoccipital process is closer to the tympanic bulla. The zygomatic arch is closer to the skull. The maxillae take part in the formation of the palate only across the posterior end. In front of this are the two long, narrow palatine foramina, separated by the palatine processes of the premaxillae. The

posterior part of each ramus of the lower jaw is deeper than it is in the dog, and the coronoid process is hardly noticeable.

The foramen rotundum and foramen lacerum anterius are absent and are replaced by an elongated aperture, the **sphenoidal fissure.**

Write the dental formula of the rabbit.

(3) The Vertebral Column of the Rabbit

The vertebral column is divided into five regions as follows:—

Cervical . .	7 vertebrae, **atlas, axis** and 5 others.
Thoracic . .	12 vertebrae (sometimes 13).
Lumbar . .	7 vertebrae (6, if 13 thoracic).
Sacral . .	4 vertebrae fused as the **sacrum.**
Caudal . .	15 vertebrae (approximately).

(*a*) **Cervical**

(i) **Atlas**

This has no body or centrum, and has wide **transverse processes.** On the anterior side there are two **occipital facets** for articulation with the occipital condyles of the skull and on the posterior side a central **odontoid facet** for articulation with the odontoid process of axis, and two lateral **facets for articulation with axis.** Above the

Dorsal View.   Anterior View.
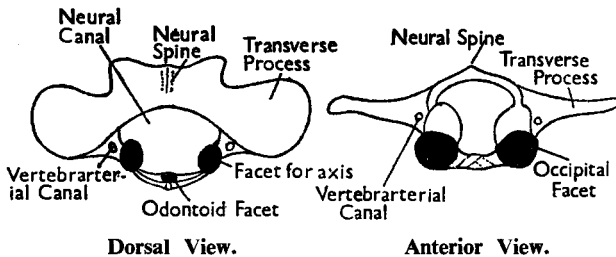
FIG. 147. Oryctolagus. Atlas.

ligament, traversing the central cavity (often missing from a prepared skeleton), is the **neural canal;** the odontoid process of axis fits in below. The tiny hole on either side of the vertebra is the **vertebrarterial canal** for the vertebral artery. These are found in the cervical vertebrae only. The **neural spine** is an inconspicuous ridge on the dorsal side.

Draw the dorsal and anterior views.

(ii) Axis
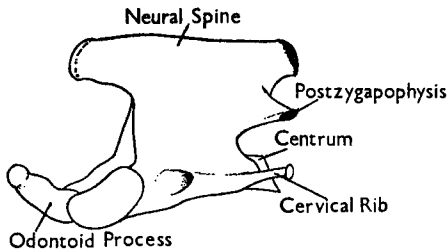
FIG. 148. **Oryctolagus. Axis.**
Lateral View.

This has a wide flat **centrum,** produced forwards as the **odontoid process** (probably originally the centrum of atlas), with an **articular facet** on each side, also for articulation with atlas, small backwardly directed transverse processes called **cervical ribs, vertebrarterial canals,** and a large vertical bladelike **neural spine.** Note the **post-zygapophysis,** a backwardly directed process on each side of the posterior end of the **neural arch,** dorsally, and the **neural canal.**

Draw a lateral view.

(iii) 3rd to 7th cervical vertebrae

Each has a short broad **centrum,** a small **neural spine,** transverse processes called **cervical ribs** through which pass the **vertebrarterial canals,** two **prezygapophyses** projecting anteriorly, and two **post-zygapophyses** projecting posteriorly. Note also the **neural canal.**

Draw the anterior view.
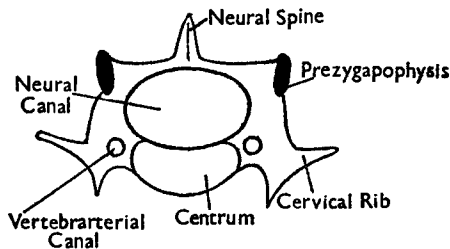
FIG. 149. **Oryctolagus. A typical Cervical Vertebra.**
Anterior View.

(b) Thoracic

Each **thoracic vertebra** has a short thick **centrum,** with a **capitular demi-facet** for the articulation of the capitulum of a rib (together with a corresponding demi-facet on the next vertebra) on the upper side at each end.

The short **transverse processes** bear on their lower surfaces a **tubercular demi-facet** for articulation with the tuberculum of a rib The **prezygapophyses** project upwards and outwards and the **post-zygapophyses** project downwards and inwards. The **neural spine** is

Anterior View.   Lateral View.
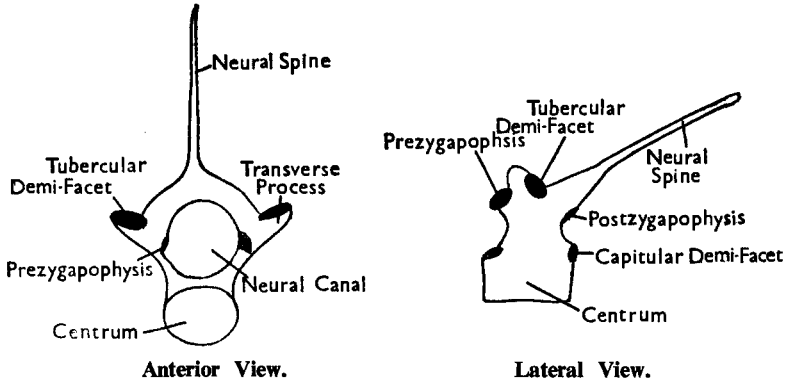
FIG. 150. Oryctolagus. Thoracic Vertebra.

long and backwardly directed except in the last three or four thoracic vertebrae which are somewhat similar to lumbar vertebrae, and have larger centra and shorter neural spines. They bear rib facets, though they differ from the others in that there is only one, the anterior one, the capitular demi-facet, because the ribs articulating with them have a capitulum only. Note also the **neural canal.**

Draw the anterior and lateral view.

(c) Lumbar

Each **lumbar vertebra** has a large **centrum,** two long **transverse processes,** projecting forwards and downwards, a large flat **neural spine,** on each side of which is a **metapophysis** anteriorly, a large forwardly directed process bearing a **prezygapophysis** on its inner

Anterior View.   Lateral View.
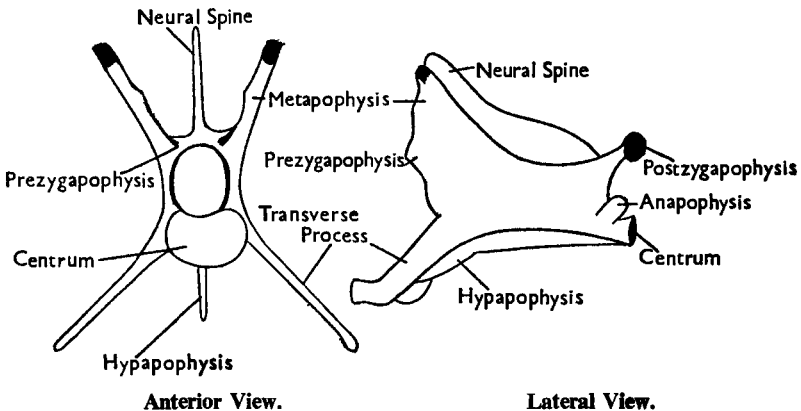
FIG. 151. Oryctolagus. Lumbar Vertebra.

side. The **postzygapophyses** are backwardly directed processes on the dorsal side of the posterior end of the neural arch, which, in some cases, also bear small backwardly directed **anapophyses.** The first two or three lumbar vertebrae also bear a **hypapophysis,** a downwardly projecting process on the middle of the ventral side of the centrum.

(*d*) Sacrum

The **sacral vertebrae** are fused together as the **sacrum.** The first is the largest and has broad **transverse processes** bearing **ilial facets** for attachment to the ilium of the pelvic girdle, **prezygapophyses,** small **metapophyses,** and a large **neural spine.** The other sacral vertebrae are smaller, decreasing in size from before backwards. The true sacral vertebrae are those which support the pelvic girdle. Foramina for the exit of spinal nerves occur between the fused vertebrae.

Draw the dorsal and lateral views.
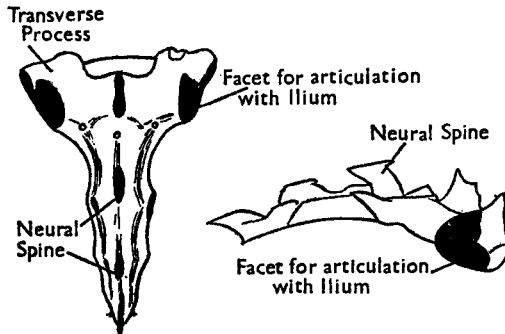
Dorsal View.   Lateral View.

FIG. 152. Oryctolagus. Sacrum.

(*e*) Caudal

The **caudal vertebrae** become smaller as they are traced backwards, gradually losing their processes and neural arches until the last few vertebrae consist merely of solid centra.

These need not be drawn.

(4) The Ribs and Sternum

(*a*) Ribs

There are twelve pairs of ribs articulated dorsally with the thoracic vertebrae, as already seen. The first seven are joined ventrally to a bone, the sternum, but the remaining five are not, the eighth and ninth being connected to the seventh and called **false ribs,** while the tenth, eleventh and twelfth are not connected with the sternum at all and are called **floating ribs.**

Each **rib** is a curved, flattened, bony rod bearing at its dorsal end a process, the **capitulum,** which articulates with the demi-facets on the centra of the vertebrae, and a smaller dorsal **tuberculum,** which articulates with the demi-facets on the transverse processes. The bony shaft of the rib is called the **vertebral portion;** to this is joined the imperfectly ossified **sternal portion,** which is joined to the sternum.

(d) Sternum

The **sternum** consists of a series of seven bony **sternebrae.** The anterior sternbra is the largest, and is called the **manubrium** The last, the **xiphisternum,** is long and bears a flat cartilaginous plate, the **xiphoid cartilage.**

The Appendicular Skeleton

(1) The Pectoral Girdle

This is very much reduced in the mammal and consists of two scapulae or shoulder blades kept in position by muscles.

Examine a scapula from its dorsal side, the side bearing a ridge.

The **Scapula** consists of a triangular **blade** bearing at its apex a cavity for the articulation of the fore-limb, the **glenoid cavity.** Projecting over this is the hook-like **coracoid process.** The ridge on the
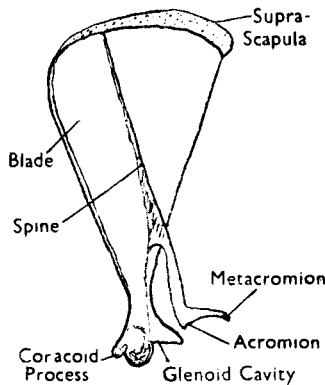
FIG. 153.   Oryctolagus.   Left Scapula.

blade is called the **spine:** this continues beyond the blade as the **acromion process,** at right angles to which is the **metacromion process.** In the living animal, a piece of cartilage, the **supra-scapula,** is situated on the base of the triangle and may or may not be present in the prepared skeleton.

The **clavicle** is a small membrane bone connected by ligaments to the coracoid process at one end and to the manubrium at the other.

Like the hyoid, it will probably have been lost in the preparation of the skeleton. It should be seen in the articulated skeleton.

(2) The Pelvic Girdle or Pelvis

This is strongly developed and is composed of two bones, known as the **ossa innominata,** which are joined ventrally by ligaments.

Each os innominatum is composed of (i) a large anterior wing-like bone, the **ilium,** bearing on its inner surface a horse-shoe shaped **sacral facet** for the articulation of the 1st sacral vertebra; (ii) the
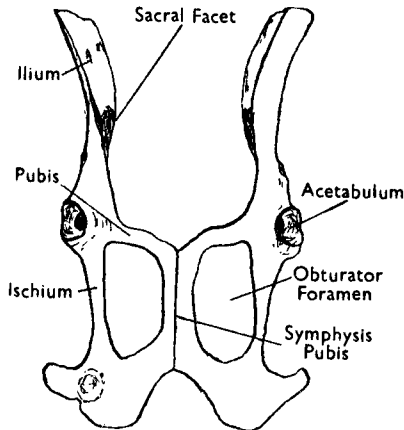
Fig. 154.   Oryctolagus.   Pelvis.

ischium, posterior to the ilium and bearing the **ischial tuberosity** posteriorly; (iii) the **pubis,** on the inner side, joining its fellow of the other os innominatum at the **symphysis pubis.** The ilium, ischium and pubis enclose (iv) a large hole, the **obturator foramen.** (v) The **acetabulum** is a deep cup for the articulation of the hind-limb, formed by the ilium, ischium and pubis where they join on the other side. On its ventral side is a small bone, the **cotyloid.**

(3) The Fore-Limb

The upper bone is the **humerus,** bearing a rounded **head** at its proximal end for articulation with the glenoid cavity of the scapula, and a groove, the **trochlea,** with ridges or **condyles** on each side, at its distal end. Just below the head, on the outer side, is a process, the **greater tuberosity,** and on the inner side the **lesser tuberosity,** both for the insertion of muscles. The groove between the tuberosities is the **bicipital groove.** The **deltoid ridge** on one edge of a lateral flattened part of the shaft below the head is for muscle attachment. At the

distal end above the trochlea are two depressions, the **supratrochlear fossae,** connected by the **supratrochlear foramen,** the upper or posterior fossa being known as the **olecranon fossa.**
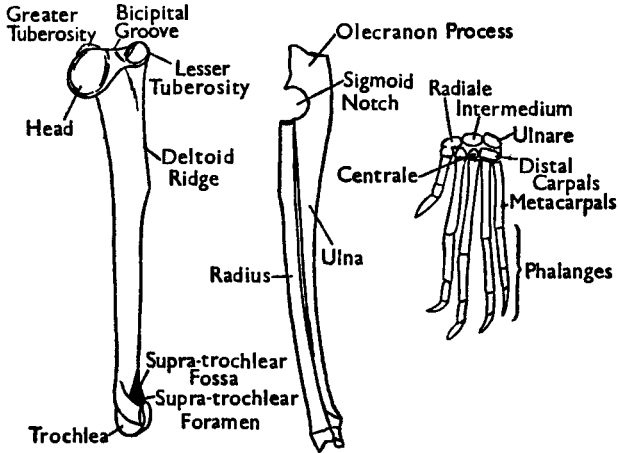
Fig. 155. Oryctolagus. Left Fore-Limb Skeleton.

The fore-arm consists of two long bones firmly attached but not fused, the **radius** and **ulna.** The ulna is longer and projects beyond a groove, the **sigmoid notch,** which fits into the trochlea of the humerus, the projection being known as the **olecranon process.** This fits into the olecranon fossa of the humerus. The distal ends articulate with the wrist bones.

The wrist or **carpus** consist of 9 **carpals** arranged in 3 rows as follows: (i) in the proximal row, **radiale** and **intermedium** articulating with the radius, and **ulnare** with the ulna. (ii) In the middle, **centrale** articulating with radiale and intermedium. (iii) In the distal row, five **distal carpals,** 4 and 5 being fused.

The **manus** or hand is composed of 5 **metacarpals,** the first or **pollex** being the shortest and bearing two **phalanges,** while the others bear three. The distal phalanges bear the pointed **claws.**

(4) The Hind-Limb

This is longer than the fore-limb. The upper bone is the **femur,** a long stout bone bearing a rounded **head** at its proximal end, which articulates with the acetabulum of the pelvis, and at its distal end two **condyles** with a deep depression between them on the ventral side, the **inter-condylar notch.**

At the proximal end, just below the head, are three protuberances, the **greater trochanter,** the outside process on top, the **lesser trochanter** inside below the head and the **third trochanter** below the greater trochanter, all for the insertion of muscles. Continuous with the inter-condylar notch at the distal end on the dorsal side, is the **patella groove,** in which the knee-cap or **patella** glides.

The shank is composed of the **tibia** and **fibula,** separate but partially fused bones, the tibia being the larger. The tibia bears at its proximal end two prominences for articulation with the condyles of the femur, and on its anterior side a ridge, the **cnemial crest.**
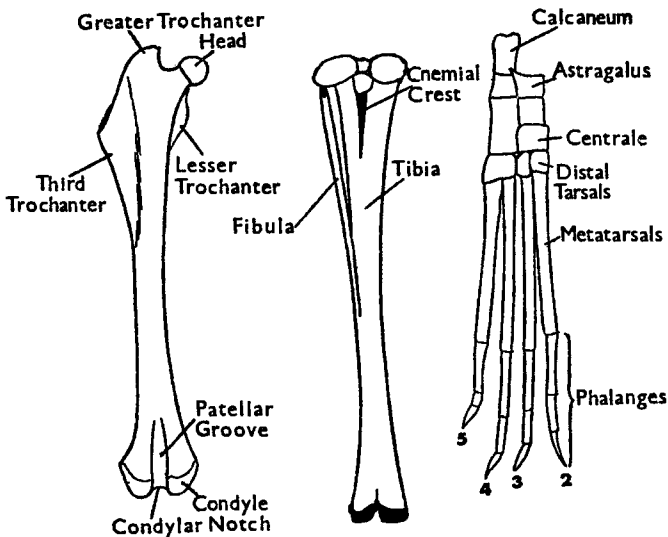
FIG. 156. **Oryctolagus. Right Hind-Limb Skeleton.**

The tibia fuses with the more slender fibula about half way down, and at the distal end of the fused bone will be seen the surfaces for articulation with the ankle bones.

The ankle, or **tarsus,** consists of six bones arranged in three rows as follows:—

(i) In the proximal row, a large **calcaneum** (or fibulare), bearing a backwardly projecting **heel** and a smaller **astragalus** (fused tibiale and intermedium), both articulating with the tibia. (ii) In the middle, the **centrale,** which is, however, to one side almost under the astragalus. (iii) In the distal row, three **distal tarsals,** one being composed of two fused tarsals.

The **pes,** or foot, is composed of four **metatarsals,** numbered 2, 3, 4, 5, No. 2 being under the centrale, and each bearing three **phalanges,** the distal ones bearing **claws.** No. 1, the hallux, is missing.

(5) The Articulated Skeleton

Examine an articulated skeleton to see the relationship of the skull, vertebrae, ribs, girdles and limbs and observe how to distinguish between bones on the left and right.

III. INTERNAL ANATOMY

Dissection is best performed on a freshly killed animal.

If the animal which is used for the general dissections is also to be used for the study of the brain, it will be necessary to take precautions at this stage to preserve the brain. The procedure is as follows:—

Remove the skin from the dorsal side of the head so as to expose the bones of the cranium. Insert the point of one blade of a pair of large scissors or small bone forceps into the cranium where the parietal and frontal bones join, and carefully remove a portion of one or both parietal bones, taking care not to push the point of the scissors into the brain. A small scalpel can be inserted in the interparietal suture and given a slight twist to facilitate the entry of the scissors.

If a trephine is available, insert this at the same point as above and remove a small circular piece of bone.

This exposure of the brain will enable the preservative fluid (usually 4 per cent. formaldehyde) in which the animal is kept between the various dissections, to penetrate into the cranial cavity.

THE MUSCULAR BODY WALL

It is advisable to wet the fur of the animal before beginning the dissection. Alternatively it can be skinned.

Place the animal, ventral side upwards, on a dissecting board. Tie the fore-limbs to the hooks in the top corners of the board or fix awls through the wrists, stretching the body as much as possible, tie the hind-limbs to the hooks in the bottom corners of the board or fix awls through the ankles.

Pinch the skin in the centre of the abdomen and cut with the scissors, making a median incision upwards to the top of the thorax and down to the tail. Holding the skin with forceps, cut away the connective tissue between the skin and the muscular body wall as far round as possible with a scalpel and pin back on either side with awls.

Note the strip of muscle down the centre of the **abdomen,** the **rectus muscle** with the **oblique muscles** on either side.

In the **thorax,** note the **ribs** which can be somewhat vaguely seen, the **pectoralis muscle,** a large fan-shaped muscle extending from the **sternum** (with the **xiphoid cartilage** at its lower end) to the upper-arm and the **latissismus dorsi muscle** at the side of the thorax.

Note also the **cutaneous blood-vessels** in the inner (exposed) side of the skin.

Regional Anatomy

In the mammal it is more convenient, from a practical point of view, to study the anatomy of the various regions of the body (*i.e.*, abdomen, thorax, neck and head) than to study one system at a time, though this is, of course, possible.

I. THE ABDOMEN

Carefully lift the wall of the abdomen with forceps and make a median incision stretching up to the xiphoid cartilage and down to the pubic symphysis. Take care not to pierce, cut or puncture any of the abdominal viscera. Now make transverse cuts in the abdominal wall just posterior to the last ribs and pin it back on either side.

FIG. 157. **Oryctolagus. Abdominal Viscera. In situ.**

(1) THE ABDOMINAL VISCERA IN SITU

Note the shining mucous membrane, the **peritoneum**, which lines the **peritoneal cavity** (part of the coelom) and covers the organs, and the muscular partition separating the abdomen from the thorax, the **diaphragm**. This will be seen if the liver is gently lowered a little. The large red organ under the diaphragm is the **liver**; it partially covers the large white **stomach** on the (animal's) left, stretching across the body, below which is part of the large brown **caecum**, another part of which will be seen lower down. The sacculated **colon** will also be seen going across the centre of the body and parts of the **ileum** may be visible. The **urinary bladder** may be seen, particularly if it contains a quantity of urine, at the posterior end of the body cavity.

(2) THE ABDOMINAL VISCERA AFTER DEFLECTION OF THE INTESTINES

Deflect the liver forwards, and the caecum and colon to the animal's right.
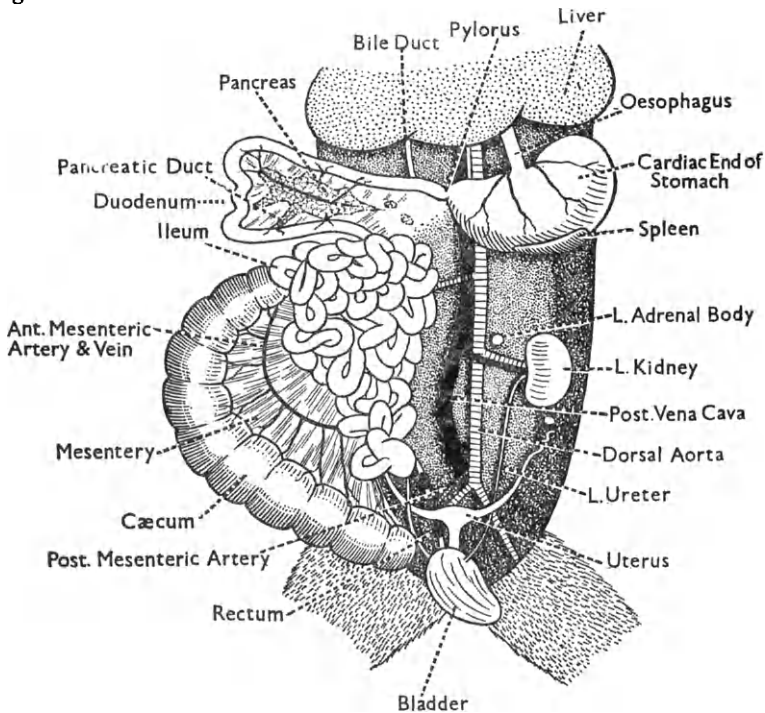
Fig. 158. Oryctolagus. Abdominal Viscera. Intestine deflected to Right.

Find the **duodenum,** the first part of the small intestine, continuous with the stomach, the distal loop of which is attached to a part of the rectum by **mesentery.** Examination of the mesentery may reveal a number of small vesicles in it. If so, these are cysticerci (bladder-worms) of Taenia. The **rectum** contains pellets of faeces.

Examine the **liver.** It is suspended from the diaphragm by the **falciform ligament** and is composed of five **lobes**—the **left lateral** (the larger and, in its deflected position, the lower of the two lobes on the left) **left central** (the other left lobe and, in its present position, the upper), **right central** (upper right in present position), **caudate** (partly covering the right kidney when in its normal position) and **spigelian** (in the centre and the smallest). The green **gall-bladder** is
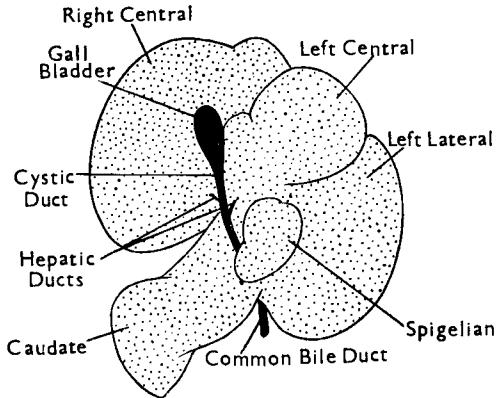
FIG. 159. **Oryctolagus. Liver. Deflected forwards.**

between the right and left central lobes and is partly embedded in the right central lobe. From it arises the **cystic** duct which is joined by the **hepatic ducts** from the lobes of the liver, thus forming the **common bile duct,** which passes down on the dorsal side of the proximal loop of the duodenum, which it enters at its proximal end. Note the wide **cardiac end** and narrow **pyloric end** of the **stomach,** and the constriction, the **pyloric sphincter** (or **pylorus**) at the outlet of the stomach where it joins the duodenum.

Pull the stomach down slightly in order to see the **oesophagus** *entering the cardiac end.*

Spread out the loops of the duodenum without cutting the mesentery.

The **pancreas** is a diffuse gland between these loops. The **pancreatic duct** leads into the distal end of the duodenum about $1\frac{1}{2}$ inches beyond the bend.

The **spleen** is a dark red body under the cardiac end of the stomach.

The left **kidney,** a dark red organ, will be seen on the dorsal body wall.

Find and trace the following blood-vessels associated mostly with the alimentary canal. The veins are dark purplish red, the arteries being lighter in colour and the larger ones thicker-walled:—

The **dorsal aorta** and the **posterior vena cava** both run in the mid-dorsal line on the dorsal body wall underneath the peritoneum, through which they can be seen if adequate deflection is made, the dorsal aorta being mostly dorsal to the vein.

The single **anterior mesenteric artery** arises from the dorsal aorta just above the left kidney and runs in the **mesentery** supporting the intestine, to the duodenum, pancreas, ileum, caecum and colon.

The single **coeliac artery** arises in front of the anterior mesenteric just posterior to the diaphragm and almost immediately divides into the **hepatic artery** which goes to the liver (first giving a branch to the
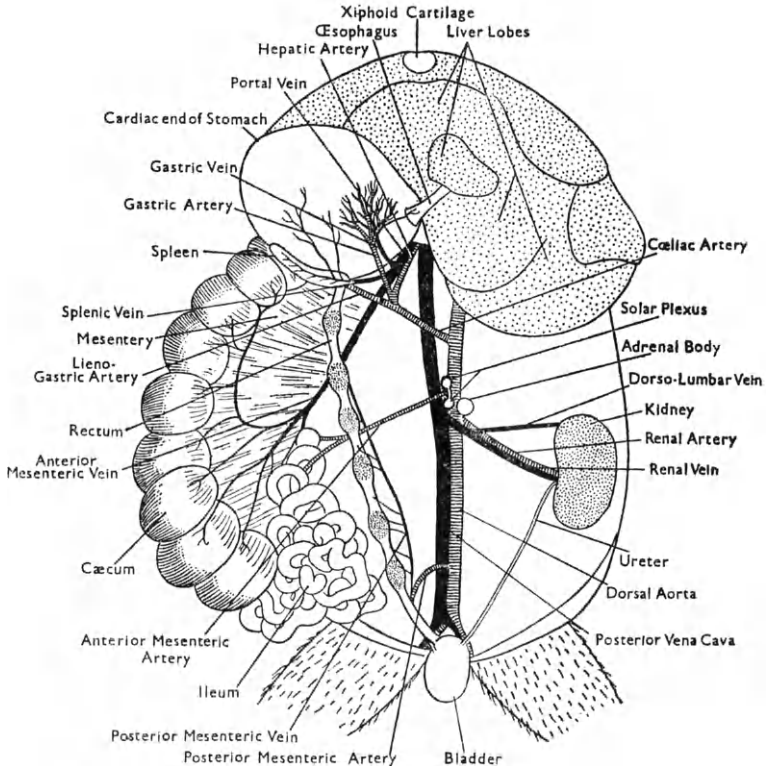
FIG. 160. Oryctolagus. **Blood vessels of alimentary canal: Solar Plexus.**

stomach) and the **lieno-gastric artery,** which supplies the stomach **(gastric artery)** and spleen **(splenic artery).**

The **posterior mesenteric artery** is another single vessel which arises just before the aorta bifurcates posteriorly and goes to the posterior part of the rectum. It will be seen underneath the bladder.

The **duodenal vein** will be seen running alongside the **duodenal artery** (a branch of the anterior mesenteric artery) in the pancreas, where it receives branches from the duodenum itself. The vein leads into the hepatic portal vein which enters the liver.

The branches of the **anterior mesenteric vein** will be seen in the mesentery. This vein also joins the portal vein at about the same point as the duodenal.

The **lieno-gastric vein** from the spleen and stomach accompanies the lieno-gastric artery but leads into the portal vein near its junction with the duodenal and anterior mesenteric veins.

The **posterior mesenteric vein** runs alongside the posterior mesenteric artery from the posterior end of the rectum on the right of the posterior vena cava to join the portal vein.

The **portal vein** is thus formed by the union of the anterior and posterior mesenteric, the lieno-gastric and the duodenal veins. It is the large vein entering the liver and can hardly be missed.

The **left renal artery** and **renal vein** will be seen running side by side to the left kidney from the dorsal aorta and posterior vena cava respectively.

Now find the left adrenal body and the solar plexus.

Just above the left kidney, towards the mid-line near where the renal vein joins the posterior vena cava, note the small round whitish **adrenal body,** a ductless or endocrine gland.

The **solar plexus** consists of two ganglia, one in front and one behind the point where the anterior mesenteric artery leaves the dorsal aorta, level with the left adrenal body. It may, however, be single and more central, lying on the posterior vena cava. It is white and star-shaped, is the end of the **splanchnic nerve,** which can be seen joining the ganglia, and is part of the sympathetic nervous system.

(3) REMOVAL OF THE ALIMENTARY CANAL

Remove the alimentary canal as follows: ligature the portal vein in two places and cut across it between the ligatures. It may be as well to ligature the anterior mesenteric and coeliac arteries also. Cut across the rectum near its lower end. Now carefully cut through the mesentery, close to the intestine, gradually unravelling the intestine. Take great care not to puncture the caecum, and when you reach the duodenum try

to keep the loop intact. Finally cut across the oesophagus. Now neatly lay out the alimentary canal on another dissecting board.

Note the **oesophagus, stomach (cardiac end** and **pyloric end), pyloric sphincter, duodenum, ileum** ending in a swelling, the **sacculus rotundus,** where it joins the **caecum.** This terminates in the blunt **vermiform appendix.** Note the **colon,** with its **longitudinal muscle** and **sacculations,** and the **rectum** containing pellets of **faeces.**

(4) THE ABDOMINAL VISCERA REMAINING AFTER THE REMOVAL OF THE ALIMENTARY CANAL, IN SITU

Note the two **kidneys,** the right being more anterior than the left: these are dorsal to the peritoneum. The position of the **left adrenal body** has already been seen. The **right adrenal body** is anterior to the right kidney though usually hidden by it.

Note the **ureters,** narrow white tubes leading from the kidneys to the **urinary bladder,** and the **renal artery** and **vein** on each side leading from the **dorsal aorta** and to the **posterior vena cava** respectively. *Gently pull back the bladder slightly in order to see the entry of the two ureters.* The origins of the **coeliac** and **anterior** and **posterior mesenteric arteries** will be seen, also the bifurcation of the aorta to form the two **common iliac arteries** to the legs. Each of these gives off an **ilio-lumbar artery** to the dorsal body wall almost at once and then a small **vesical artery** to the bladder. The iliac then divides on its dorsal side into an **internal iliac artery** to the back of the pelvic cavity (consequently only its origin will be visible) and an **external iliac** or **femoral artery** to the leg.

The **internal iliac veins** from the back of the legs unite to form the posterior vena cava. They can be seen on the posterior side of the thigh muscle. Find also the **external iliac veins,** which are continuations of the **femoral veins** on the inside of the thighs, and which open into the inferior vena cava anterior to the internal iliacs. The small **vesical veins** from the bladder run near the vesical arteries and lead into the external iliac veins. The **ilio-lumbar veins** run alongside the ilio-lumbar arteries and enter the posterior vena cava, though that on the left sometimes curves forwards and runs up alongside the posterior vena cava for a short distance before joining it. The reproductive organs in the posterior part of the abdomen will be studied separately.

The Diaphragm

Now turn to the anterior end of the abdomen and examine the **diaphragm.**

Note the **central tendon** and **marginal muscular portions** of the **diaphragm,** in which will be seen the **phrenic artery** and **vein.**

(5) THE REPRODUCTIVE SYSTEM
(i) Male

At the posterior end of the body note the two **scrotal sacs** which contain the ovoid or elongated **testes.** *Open up one of the scrotal sacs lengthways* and note a mass of coiled tubes, the **epididymis,** on its inner edge, the upper part of which is the **caput epididymis** and the lower part the **cauda epididymis.** The cauda epididymis is connected with the base of the scrotal sac by a cord, the **gubernaculum.** The **vas deferens** (modified mesonephric duct) runs from the cauda epididymis into the abdomen where, after curling round the ureter, it runs to the dorsal side of the bladder and opens into the **urethra.** The testis is suspended by the **spermatic cord** joined to the dorsal body wall and composed of the **spermatic artery, vein** and **nerve** and connective tissue. It passes through what is known as the **inguinal canal** from the scrotal sac into the cavity of the abdomen. There is no vesicula seminalis in the rabbit.
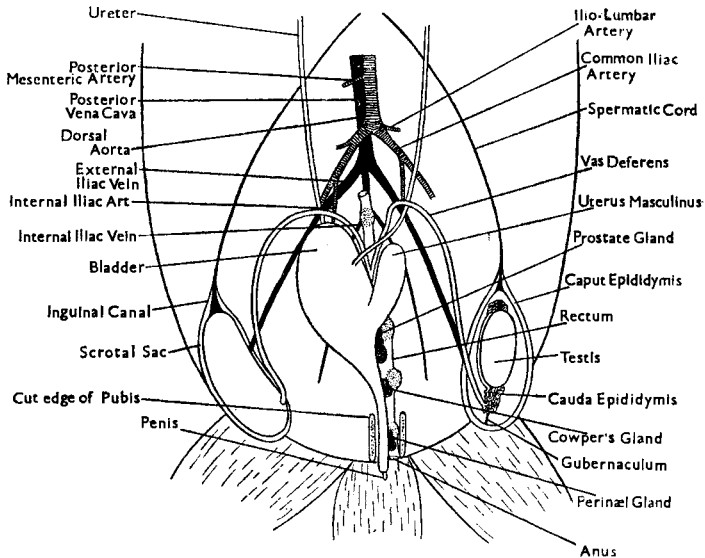
Fig. 161. **Oryctolagus. Male. Reproductive System.**

Carefully cut through the symphysis pubis with a scalpel and separate the two halves, or better, cut longitudinally through the centre of each os innominatum on each side of the symphysis pubis with strong scissors or small bone forceps and remove the central part of the pelvis. Deflect the bladder to the side and remove any obscuring connective tissue.

Note the **urethra** (which is a **urino-genital canal),** the continuation of the neck of the bladder and the **penis,** through which this passes.

The vascular ventral wall of the penis surrounding the urethra is the **corpus spongiosum,** (or **corpus cavernosum urethrae),** and the dorsal wall is composed of the paired **corpora cavernosa** (or **corpora cavernosa penis).** Both are erectile tissues. The distal end of the penis, the **glans penis,** bears the opening of the urethra, and is covered by a loose sheath, the **prepuce.** The large sac on the dorsal side of the urethra where the vas deferens joins it, is the **uterus masculinus** (vestige of Müllerian ducts).

Cut open the uterus masculinus.

Note that it opens into the urethra by a large aperture, in front of which are the openings of the vasa deferentia. Note the **prostate,** a gland round the dorsal and lateral surfaces of the uterus masculinus. *Find* **Cowper's glands** posterior to the prostate, and the **perineal glands,** one on either side of the penis.

The **spermatic artery** runs from the dorsal aorta to the testis, and the **spermatic vein** runs from the testis to the posterior vena cava.

The **rectal gland** will be seen on the dorsal side of the rectum.

(ii) **Female**

Carefully cut through the symphysis pubis and separate the two halves as in the male (p. 216).
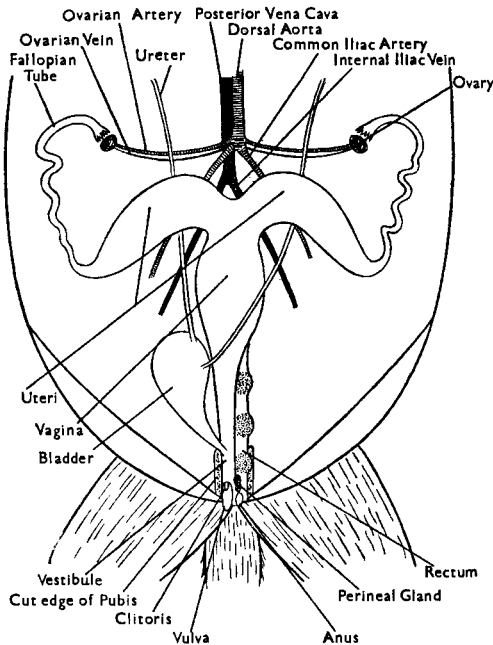
FIG. 162. **Oryctolagus. Female. Reproductive System.**

Note the two oval **ovaries** on the dorsal body wall, posterior to the kidneys and the convoluted oviducts (Müllerian ducts) called **Fallopian tubes,** with their funnel-shaped **internal openings** close to the ovaries and partially enveloping them dorsally. These tubes lead posteriorly into the two thick-walled and slightly convoluted **uteri,** in which the embryos develop and which lead by separate openings into the **vagina,** a wide median tube which, with the **urethra** which it joins, forms the **urino-genital canal** (or **vestibule**), opening to the external **vulva.** The **perineal glands** are situated one on each side of the vestibule. The **clitoris** (the counterpart of the penis in the male) is a small rod-like organ in the ventral wall of the vulva.

The **ovarian artery** runs from the aorta to the ovary and the **ovarian vein** from the ovary to the posterior vena cava, though it may open into the ilio-lumbar vein on the left.

Note the **rectal gland** on the dorsal side of the rectum as in the male.

(6) THE KIDNEY

Examine a kidney in situ.

Note that it is of typical "kidney", or bean shape, the concave edge, directed inwards, being called the **hilus.** It is here that the **renal artery** enters and the **renal vein** and **ureter** leave the kidney.
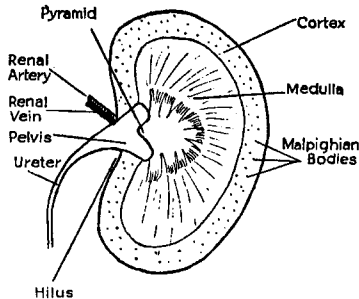
Fig. 163. **L.S. Kidney.**

Remove one of the kidneys and cut a longitudinal section. Examine with a lens.

Note that it consists of (i) an outer rind, or **cortex,** in which is a number of **Malpighian bodies,** appearing as specks, and (ii) an inner **medulla.** The ureter widens after entering the hilus as the **pelvis** of the kidney into which the conical **pyramid** opens. The tubules of the kidney open on to the pyramid.

II. THE THORAX

Carefully remove the ventral and as much as possible of the lateral walls of the thorax, leaving the diaphragm in situ as follows:

Cut through the ribs on each side from the 9th rib (inclusive) upwards to the 1st (exclusive), as low as possible. Then cut transversely in the intercostal space between the 9th and 10th ribs and continue to cut upwards through the ribs up to the 6th sternebra above the xiphisternum on both sides. Cut through this sternebra, and, lifting up the ventral wall of the thorax and cutting through the **pleura** *in the midventral line which divides the thorax into two* **pleural cavities,** *continue the lateral cut on either side through the manubrium (1st sternebra), between the 1st and 2nd ribs. Remove the ventral thoracic wall and retain it for the time being.*
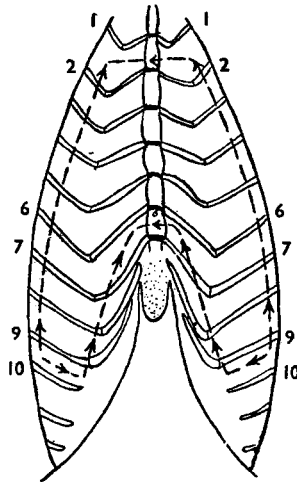
FIG. 164. Oryctolagus. Method of Removal of Ventral Thoracic Wall.

(1) THE THORACIC VISCERA IN SITU

Note **the cut surfaces of the ribs,** the **diaphragm** and the remains of the **pleura** lining the thorax on the sides. The **heart** is centrally placed with its apex directed to the left, and is enclosed in the thin **pericardium** between the two pleural cavities. On each side are the pinkish **lungs** enclosed in the **pleural cavities.** (The **pleura,** lining the pleural chambers, will have been removed with the central thoracic wall, as already seen except at the sides where it has already been observed.) The cavity between the pleural cavities and which encloses the roots of the trachea and the chief blood vessels leaving the heart and part of the oesophagus, is the **mediastinum.** It reaches from the diaphragm

to the heart, enclosing this organ in its pericardium. On its lateral edges will be seen the white **phrenic nerves** going to the diaphragm. The pink organ above the heart is the **thymus gland,** an endocrine gland; it is large in a young rabbit. The two **bronchi,** branches of the **trachea,** enter the lungs. The pericardial and pleural cavities and the mediastinum are all parts of the coelom.

(2) THE HEART AND GREAT VESSELS

Carefully dissect away the thymus gland and free the heart from the pericardium. Exercise great care in the removal of the pericardium from the base of the heart (anterior end) where the great vessels are situated. Deflect the heart as necessary so as to expose the blood vessels dorsal to it.

Note that the **heart** consists of thin-walled **right** and **left auricles** anteriorly and thick-walled **right** and **left ventricles** posteriorly.

The **pulmonary artery** is a large vessel leading from the right ventricle to the lungs. It bends towards the left and divides into two branches, one to each lung. It is crossed by the **left anterior vena cava,** which runs on the dorsal side to the right auricle. The **right anterior vena cava** will also be seen entering the right auricle. The **pulmonary veins** come from the lungs and join, the single vein thus formed entering the left auricle. The single **posterior vena cava** passes up from the abdomen, through the diaphragm, and, running dorsal to the heart, enters the right auricle. *Now find* the **aortic arch,** which leaves the left ventricle, bends over to the left and, running dorsal to the heart, passes through the diaphragm into the abdomen as the dorsal aorta. *Find the* **ductus arteriosus,** a ligament which runs across from the aortic arch to the pulmonary artery. It will be found where the left anterior vena cava crosses the latter. On the aortic arch, note the short **innominate artery** which gives off the **right common carotid artery** almost at once and then the **right subclavian artery.** The **left common carotid artery** arises from the aortic arch just beyond the origin of the innominate artery and the **left subclavian artery** arises direct from the left side of the aortic arch. Both subclavian arteries run dorsal to the anterior vena cava. (Sometimes both carotid arteries arise from the innominate: sometimes they arise directly from the aortic arch, the innominate artery being absent.)

The **right** and **left subclavian veins** will be seen alongside the corresponding arteries. Each enters the anterior vena cava on its own side.

The **vertebral arteries** to the head arise from the subclavian artery just beyond its origin and enter the vertebrarterial canals of the cervical vertebrae.

The **internal mammary artery** on each side arises from the subclavian artery just after it has given off the vertebral artery and runs along the ventral wall of the thorax.

The **internal mammary vein** enters the anterior vena cava level with the first rib.

These two vessels will have been removed with the ventral thoracic wall.

Examine the inner side of the ventral thoracic wall which has been removed and note the internal mammary artery and vein running along each side of the sternum.

The **azygos vein** enters the right anterior vena cava just before it enters the auricle. It is unpaired, there being none on the left side.

Look for the **oesophagus** on the dorsal side of the thoracic cavity and dorsal to the trachea with the **vagus nerve,** a white cord, on either side.

Move the aorta sideways. Observe the chain of **sympathetic ganglia** behind it, connected together by a slender nerve cord.

Finally you should find the origin of the **left recurrent laryngeal nerve** which arises from a large nerve, the left **vagus nerve** just behind the subclavian artery, loops round the ductus arteriosus and then passes forwards. *Turn to the other side and find the origin of the* **right recurrent laryngeal nerve** from the **right vagus nerve** in front of the subclavian artery, afterwards looping round it and passing forwards.

(3) THE HEART OF THE SHEEP

The sheep's heart is much larger than the rabbit's heart and is therefore more suitable for the study of the internal structure. It differs little from that of the rabbit.

(*a*) External Structure (Ventral View)

Examine a sheep's heart with the great vessels attached.

Note the two ventricles separated on each side by a groove containing **fat,** the **longitudinal sulcus.** *Feel the walls of the ventricles.* That with thicker walls is the **left ventricle,** the other the **right ventricle.** Above then are the thinner walled **left** and **right auricles** bearing small **auricular appendages.** Note the **coronary artery** and **vein** which branch over the tissue of the heart, also the **aortic arch, anterior vena cava** (single in the sheep, the right and left vessels joining before entering the heart), **posterior vena cava, pulmonary artery** and **pulmonary vein.**

(*b*) Internal Structure

(i) Right Auricle and Ventricle

Place the heart with the ventral surface uppermost and make a longitudinal incision through the right auricle and ventricle just to the

right of the longitudinal sulcus. You will thus expose the cavities of the
right side of the heart. Wash out any contained blood.

Note the comparatively thin but muscular **wall of the right auricle**
and the thicker **wall of the right ventricle.** The **inter-auricular septum**
separates the two auricles, and the **inter-ventricular septum** separates
the two ventricles. In the inter-auricular septum is a thin oval area,
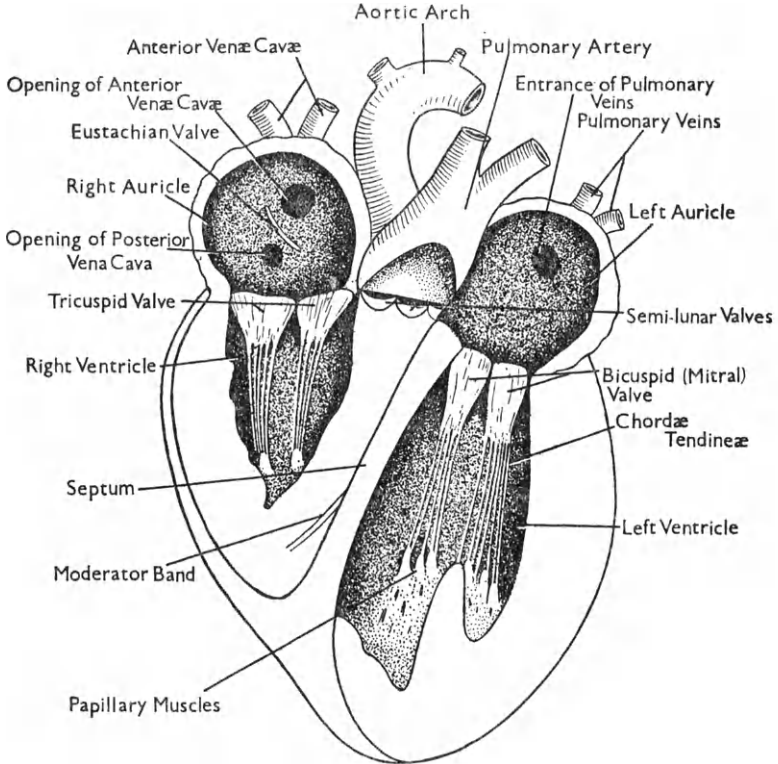
FIG. 165. Ovis. Heart. Ventral Dissection.

the **fossa ovalis** (this was open in the embryo). Note the openings of
the **anterior** and **posterior vena cava** into the right auricle and the
Eustachian valve, a fold of membrane between them (representing the
remains of a sinu-auricular valve). The valve between the right auricle
and ventricle is called the **tricuspid valve,** because it consists of three
flaps or cusps. To these cusps are attached the **chordae tendineae,**
tendinous cords which connect them to the **papillary muscles** in the
wall of the ventricle. Note the obliquely running muscular **moderator**

band in this ventricle (not present in the rabbit). The other muscular columns on the ventricle wall are called **columnae carnae.** The **pulmonary artery** leads out of the right ventricle.

Cut open the pulmonary artery.

Note the three **semilunar valves** just inside the entrance.

(ii) Left Auricle and Ventricle

Now make a longitudinal incision through the left auricle and ventricle and wash out any blood that may be present.

Examine the **wall of the left auricle** and the entrance into it of the **pulmonary vein.** The **wall of the left ventricle** is thicker than that of the right. On this side the valve between the auricle and ventricle consists of only two cusps and is called the **bicuspid (or mitral) valve.** Note the **chordae tendineae, papillary muscles** and **columnae carnae.** The **aorta** leads out of the left ventricle.

Cut open the aorta.

Note the three **semilunar valves** guarding its entrance. Just beyond this look for the openings of the **coronary artery.**

III. THE NECK

This is a dissection which needs patience and great care is necessary.

Extend the head and neck as much as possible and fix down the head. Cut through and dissect away the skin only of the neck from the thorax
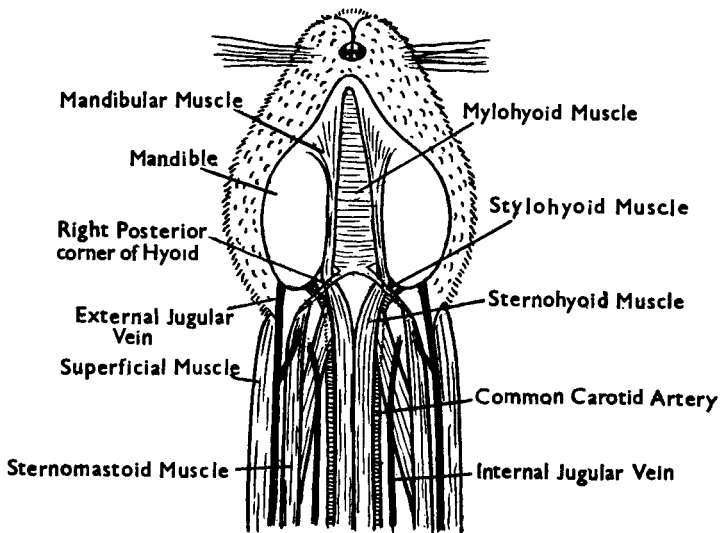
FIG. 166. Oryctolagus. Neck Muscles.

up to the lower lip, taking care not to injure the muscles. Pin back the skin on each side or remove it entirely.

Note the large **external jugular veins** on each side of the neck on the surface of the muscles and connected by a transverse **jugular anastomosis** towards their posterior ends. The **mylohyoid muscle** stretches across the **mandible** and covers the hyoid and the **mandibular muscle** runs along the inner side of each ramus.

Now very carefully cut through the surface muscles in the mid-ventral line and pin them back on either side.

The **hyoid bone** will now be exposed. It has two **posterior cornua,** each bearing a **sternohyoid muscle.** These meet in the mid-ventral line, continue backwards and cover the trachea, or windpipe, at the anterior end of which is the **larynx.** At their tips, the posterior cornua bear the smaller **stylohyoid muscles** which pass obliquely backwards to the dorsal side. Between the cornua is the floor of the pharynx. The **anterior cornua** of the hyoid are short.

Note that the **trachea** is a straight tube passing down the middle of the neck; in its wall are rings of cartilage incomplete on the dorsal side. Dorsal to it is the **oesophagus** and on either side of it, dorsal to the stylohyoid muscle, is the **common carotid artery.**

Now note the **sternomastoid muscles** which are attached to the mastoid process of the skull at one end and which were attached to the sternum at the other. The **internal jugular vein** runs along the inner side of each sternohyoid muscle and alongside the trachea, opening into the external jugular vein lower down in the neck.

The trilobed **thyroid gland,** another endocrine gland, lies at the anterior end of the trachea with one lobe on either side and one on its ventral surface.

Remove the stylohyoid muscle, displace the carotid artery inwards and the sternomastoid muscle outwards and stretch and cut the connective tissue joining it to the trachea. Pin the artery by a pin alongside it and the muscle in their new positions.

Note that the **common carotid artery,** near its division, divides, under the stylohyoid muscle, into an **internal carotid artery** to the brain and an **external carotid artery** to the face. The **hypoglossal nerve (XIIth** cranial) crosses it and then runs outside the posterior cornu of the hyoid to the tongue. This nerve gives off a branch, the **ramus descendens,** just before it meets the internal carotid artery and crosses the carotid artery on the ventral side, supplying the sternohyoid and sternothyroid muscles.

The **Spinal Accessory Nerve (XIth** cranial) runs behind the mandible to the sternomastoid and other neck muscles.
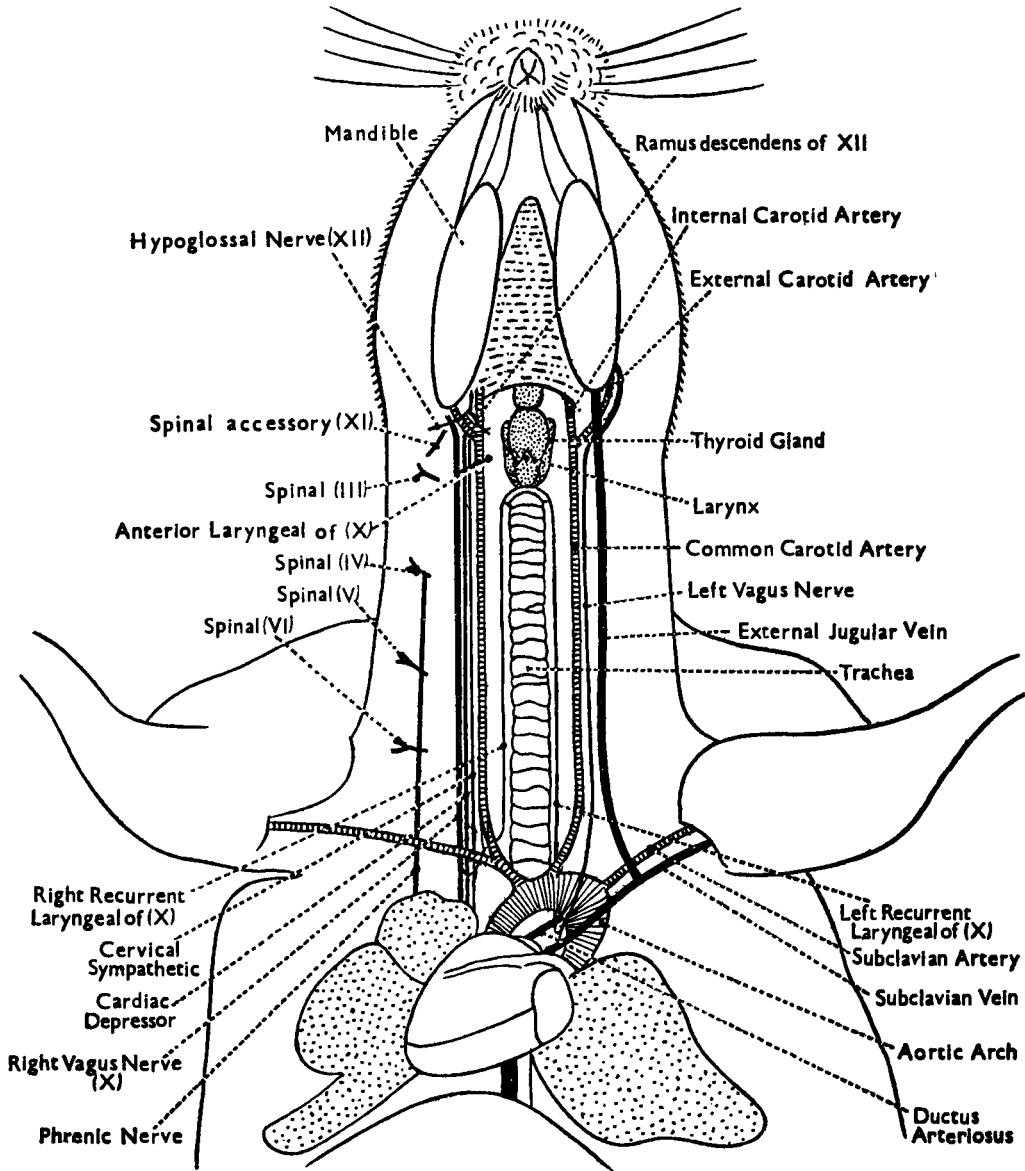
FIG. 167. Oryctolagus. Neck Dissection.

The left anterior vena cava has been deflected to the animal's left, the right external jugular vein has been omitted and the jugular anastomosis removed. (Semi-Diagrammatic.)

The **Vagus Nerve (Xth** cranial) is large, leaves the skull with the XIth and the runs along the outside of and somewhat dorsal to the carotid artery, between it and the external jugular vein. *Trace it into the thorax.* In the throat region by the larynx it forms a ganglion from which a branch known as the **anterior laryngeal nerve** arises. It supplies the larynx and is dorsal to the sternohyoid muscles and the carotid artery which it crosses. The **cardiac depressor nerve** arises with the anterior laryngeal but it is very slender and not easy to see. It runs along the inside of the main branch of the vagus and supplies the heart.

The vagus gives off a third branch, the **recurrent laryngeal** (or **posterior laryngeal) nerve,** which arises at the posterior end of the neck, runs alongside the trachea dorsal to the carotid artery and supplies the muscles of the larynx. On the *right*, it arises just *in front of* the subclavian artery, round which it loops and passes forward alongside the trachea. On the *left*, it arises *behind* the subclavian artery, looping round the ductus arteriosus and then passes alongside the trachea. The origins of these two nerves were seen when dissecting the thorax.

In the thorax, the vagus also gives other branches to the oesophagus, heart and lungs.

Now note the **cervical sympathetic ganglia,** which are ganglia on the **cervical sympathetic nerve** running alongside the trachea and near the vagus and depressor nerves, this nerve being the middle of the three parallel nerves. The ganglia are situated one at the anterior end under the internal carotid artery and one at the posterior end near the subclavian artery.

The **Phrenic Nerve,** which has already been seen on the diaphragm, is formed from twigs of the **4th, 5th** and **6th spinal nerves** which will be found on the neck.

IV. THE RESPIRATORY SYSTEM

Examine the respiratory organs in the neck and thorax.

At the anterior end of the **trachea** (already seen) is the sound box or **larynx,** the ventral and lateral sides of which are composed of the **thyroid cartilage.** At the base is the **cricoid cartilage** while the dorsal side is supported by the two **arytenoid cartilages.** The trachea, as already seen, is composed of a series of cartilaginous rings, incomplete on the dorsal side. In the thorax it divides into two **bronchi,** one to each lung. In the lungs these branch into bronchioli, which terminate in minute air-sacs or alveoli.
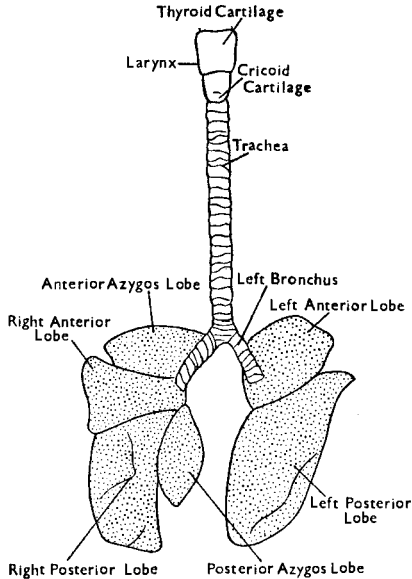
FIG. 168. **Oryctolagus.** **Respiratory system.**

The lungs are pink, spongy, vascular organs situated in the pleural cavities as already seen. The **left lung** is divided into an **anterior** and a **posterior lobe** and the **right lung** into an **anterior azygos lobe,** a **posterior lobe** and a **posterior azygos lobe.**

Make a drawing of the respiratory system.

V. THE VASCULAR SYSTEM

Now that all the blood-vessels have been seen *you should do separate dissections of the complete* **Arterial** *and* **Venous Systems.** *You should also draw connected diagrams of them.*

VI. THE HEAD

Remove entirely the skin from the head and cut off the pinnae close to their points of attachment to the head.

(1) LATERAL VIEW

Note the **eye** and, immediately below it, the **infraorbital gland,** one of the four salivary glands of the mouth. It will be rendered more easily visible by carefully removing the tissue on the surface. The large **masseter muscle** covers the mandible, and posterior to it, at the angle of the jaw, is a second salivary gland (the largest), the

parotid gland, immediately below the **external auditory meatus.** Branches of the **Facial Nerve (VIIth** Cranial) may be seen on the surface of the masseter muscle. The other salivary glands will be seen later.
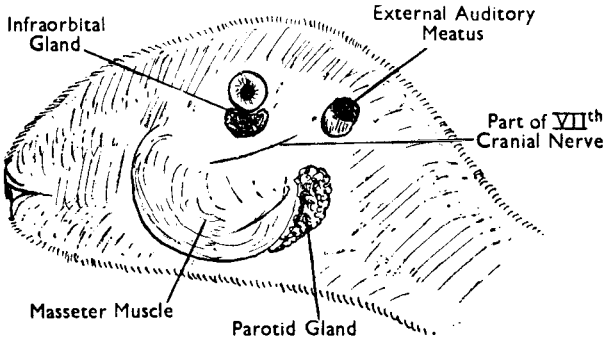
FIG. 169.   Oryctolagus.   Head.   Lateral View.   Skin removed.

The **lacrimal gland** in the posterior corner of the orbit will doubtless have been removed with the skin.

(2) THE BUCCAL CAVITY

Cut through the muscles at the sides of the mouth and open it wide.

In the **open mouth** again note the **incisor, premolar** and **molar teeth** in the upper and lower jaws. Also observe the ridges or **rugae of the hard palate,** the **soft palate** behind and the **pharynx** at the back. This leads (i) ventrally into the **glottis** or opening of the trachea, with a cartilaginous flap, the **epiglottis,** on its ventral side (*seen by pressing the tongue forwards*), and (ii) dorsally into the **oesophagus.** The **posterior narial aperture** is behind the soft palate and behind the **tonsils,** ridged depressions on either side; it leads into the **narial chambers** in front. Note also that the **tongue,** free at its front end, has **papillae** on is surface. These bear microscopical taste-buds.

Cut open the soft palate in the centre from front to back.

Note the **openings of the Eustachian tubes** at the sides of the narial chamber. *Insert a seeker into one of them.* It passes into the tympanic cavity.

Cut through the mylohyoid muscle which stretches across between the two mandibles in the middle line and deflect the flaps outwards (if not already done). Cut through the mandibular symphysis and separate the two half jaws slightly.

Note the **submaxillary** (or **submandibular**) **glands,** the third pair of salivary glands, at the angles of the jaw inside the mandible on the same level as the posterior cornua of the hyoid. The duct from each, **Wharton's duct,** *will be seen if the mandibular muscle is displaced to one side or removed.* Note also the fourth of these glands, the **sublingual gland,** which was under the tongue and will now be on the anterior inner side of the mylohyoid muscle on each side.

(3) THE BRAIN

The brain must be examined in a freshly killed rabbit or one in which it has been preserved by treatment in accordance with the instructions on page 209, as it decomposes rapidly.

Cut through the neck between a pair of the anterior cervical vertebrae and thus separate the head completely from the body. The skin of the head having been removed, cut away the muscle and immerse the entire head in 10 per cent. hydrochloric acid to decalcify the bone. Leave it in the immersion fluid for three or four days: the bone will then be much softer and easier to cut. At the end of this period wash the head in water to remove the acid and immerse it in 70 per cent. alcohol or 4 per cent. formaldehyde (= 10 per cent. formalin) for three or four days to harden it.

It should be noted that if a fresh animal is used, the brain should be hardened before the bone is decalcified by removal of part of the cranium by the method described on page 209, and immersion in 70 per cent. alcohol or 4 per cent. formaldehyde for three or four days.

Now remove sufficient of the cranium to enable you to remove the brain, taking care throughout the operation not to dig the scissors into the brain, by keeping the blades horizontal.

With the anterior end of the head facing you, insert the scissors into the hole cut in the cranium and cut through the bone back to the foramen magnum on one side. Repeat on the other side. Now turn the head right round so that it faces the opposite direction and again inserting the scissors into the hole, cut forwards round the orbit and along the outer edge of each nasal bone. Carefully remove the portions of bone thus set free. The brain will now be partially exposed. Finally remove the bone on either side of the cerebellum, the convoluted part of the hind brain, so as to expose the flocculi on each side of it. The brain should now be removed from the cranium as follows:—

Cut through the spinal cord as far back as is practicable: then, lifting the free end joined to the brain, cut through the spinal nerves on each side as far from the cord as possible, working forwards. Now turn the head upside down over a dish and, holding it as close to the dish as possible, cut through the cranial nerves as far from the brain as you

can, *working forwards and allowing the brain to release itself from the cranium into the dish by its own weight. Finally, sever the anterior attachments of the olfactory lobes. The brain will then lie in the dish, ventral side uppermost.*

It should be preserved in 70 *per cent. alcohol or in formaldehyde if required on a future occasion.*

(a) Dorsal View

Note the **pia mater,** a thin vascular inner membrane which closely invests the brain. This with the **dura mater** which lines the cavity of the cranium (with which it will have been removed) and the **arachnoid** between them constitute the **meninges.** Between these membranes is the cerebro-spinal fluid.

Remove the pia mater carefully.
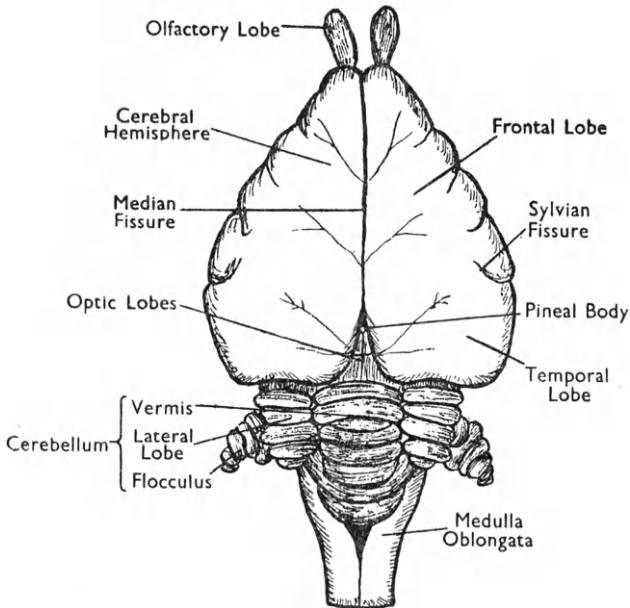
FIG. 170. Oryctolagus. Brain. Dorsal View.

In the **Fore-Brain** note the **cerebral hemispheres,** the two large anterior lobes with a **median fissure** between them. Their surfaces are smooth except for a few shallow grooves or **sulci,** the most prominent being the **Sylvian fissure** which begins about half-way along the outer edge of each hemisphere and runs obliquely

backwards dividing the hemisphere into an anterior **frontal lobe** and a posterior **temporal lobe.**

Gently part the two hemispheres slightly.

You will see the **corpus callosum,** a transverse band of fibres which connects the two hemispheres together.

Note the small **olfactory lobes** (or **bulbs**) at the anterior end of the hemispheres.

The **Mid-Brain** consists of the **optic lobes** (or **corpora quadrigemina,** so called because each lobe is sub-divided into two). They are almost completely hidden by the posterior ends of the cerebral hemispheres but *they will be visible if the cerebral hemispheres are gently separated by the fingers.*

The **pineal body** will most probably have been removed with the dura mater, but the **pineal stalk** may remain. It is situated between the hinder ends of the cerebral hemispheres on the thalamencephalon (the posterior part of the fore-brain) which is hidden by the hemispheres.

The **Hind-Brain** is composed of the transversely grooved **cerebellum,** consisting of a central **vermis,** on each side of which is a **lateral lobe** bearing a **flocculus** and behind it is the **medulla oblongata,** continuous with which is the spinal cord.

(b) Ventral View

Note the **olfactory lobes** (or **bulbs**) bearing anteriorly the roots of the **olfactory nerves (I)** and the **cerebral hemispheres** on which will be seen the **Sylvian fissure** separating the **frontal lobe** from the **temporal lobe.** The longitudinal **rhinal fissures** mark the outer edges of the **olfactory tracts,** which are continuous with the olfactory lobes. The inner posterior **hippocampal lobes** of the cerebral hemispheres should be noted. The **optic chiasma** formed by the junction of the **optic nerves (II),** is situated between the hippocampal lobes at their anterior ends, and immediately behind it is the **infundibulum,** a median rounded prominence on the floor of the **thalamencephalon** bearing an endocrine gland, the **pituitary body,** which will probably have been left in the floor of the skull. A small rounded projection, the **corpus albicans** (or **corpus mammillare**) at each side of which are the roots of the **oculo-motor nerves (III),** is situated just behind the infundibulum, and behind this are the **crura cerebri,** two prominent bands of fibres which form the floor of the mid-brain. Next you will see the **pons Varolii,** a transverse band of fibres passing up on each side into the cerebellum. Note the **flocculus of the cerebellum** on each side. The **medulla oblongata** is broad in front but tapers as it runs posteriorly and shows a median longitudinal **ventral fissure,** on each side of which is a narrow strip, the **ventral pyramid** (or **pyramidal**
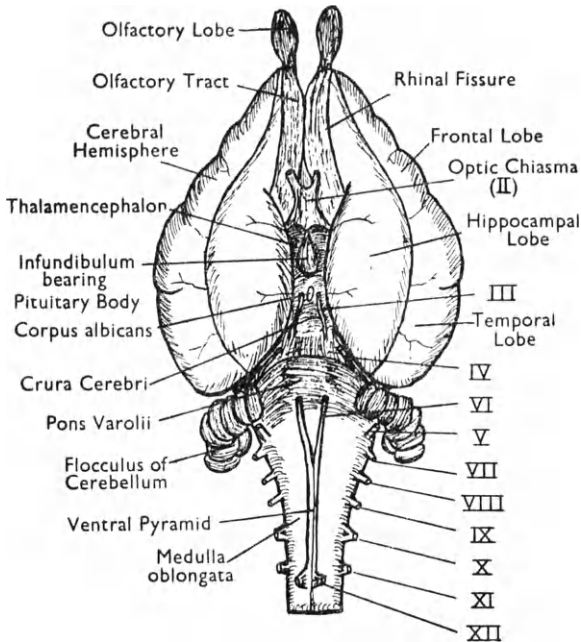
Fig. 171. Oryctolagus. Brain. Ventral View.

tract). On the ventro-lateral sides of the medulla will be seen the roots of the following nerves in order:—

Trigeminal Nerve (V).
Facial Nerve (VII).
Auditory Nerve (VIII).
Glossopharyngeal Nerve (IX).
Vagus Nerve (X), by several small roots.
Spinal Accessory Nerve (XI).

On the ventral surface of the medulla, more centrally placed, will be seen the roots of the following:—

Abducens Nerve (VI), just behind the pons.
Hypoglossal Nerve (XII) arising by several small roots posterior to the root of XI.

(c) Horizontal Section

Place the brain, hardened in spirit, dorsal side upwards and very carefully cut a horizontal section of one of the cerebral hemispheres by inserting the point of a scalpel not more than a quarter of an inch in the side of one of the hemispheres. Continue to cut carefully round the posterior and lateral edges of the hemisphere as far forwards as the

olfactory lobe and remove the roof of the hemisphere. Examine with a lens.

Note the cavity of the hemisphere, the **lateral ventricle,** towards its posterior end, a rounded thickening of the floor, the **hippocampus major,** and a further rounded thickening, the **corpus striatum,** alongside and in front of the hippocampus major. *Make a drawing at this stage.*

Now very carefully remove the floor of the lateral ventricle by cutting round the edge of the hippocampus and wall of the hemisphere. Gently separate the two hemispheres slightly with the fingers.

(a) Early dissection.   (b) Later stage.
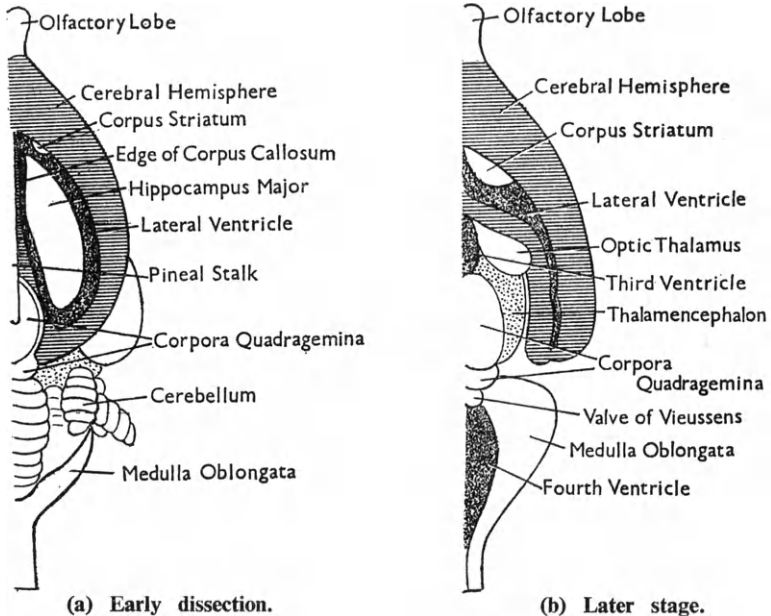
FIG. 172. **Oryctolagus. H.S. Brain.**

Note the floor of the **thalamencephalon** the posterior part of the fore-brain, behind which are the **corpora quadrigemina** with lateral thickenings, the **optic thalami,** and the **pineal stalk** anterior to them. The **anterior choroid plexus,** the vascular roof of the **third ventricle** in the thalamencephalon, will also be seen in front of the pineal stalk. Each lateral ventricle communicates with the third ventricle by a **foramen of Monro.** *Make another drawing.*

Gently remove the central vermis of the cerebellum.

Note the small cavity of the medulla, the **fourth ventricle,** which communicates with the third ventricle by the **iter.** Note also the

membranous **valve of Vieussens** between the corpora quadrigemina and the vermis, and the hinder vascular part of the roof of the fourth ventricle, the **posterior choroid plexus**. *Complete your drawing.*

(*d*) Longitudinal Vertical Section

Divide the brain into two halves longitudinally by a careful vertical cut. Examine the half which is complete with a lens.

Identify the **olfactory lobes,** the **cerebral hemispheres,** the edge of the **corpus callosum,** the **body of the fornix**—a thickening formed by the union in the middle line of two bands of fibres, one from the anterior border of each hippocampus. From the body of the fornix, the **anterior pillars of the fornix** run in a ventral direction to the **corpus albicans** and the **posterior pillars of the fornix** (or **fimbriae**) run in a dorsal direction and, in fact, not posterior but anterior to the anterior pillars. Note the **lateral ventricle,** the **foramen of Monro,** the **third ventricle,** the **anterior commissure,** an oval structure ventral to the fornix, the **middle commissure** (or **massa intermedia**), a large rounded band of fibres joining the optic thalami in the thalamencephalon, and the **posterior commissure,** a small transverse band of fibres in the roof of the third ventricle. The **anterior choroid plexus** (or its remains), the **pineal stalk,** arising from the posterior end of the roof of the thalamencephalon, the **infundibulum,** the **corpus albicans**
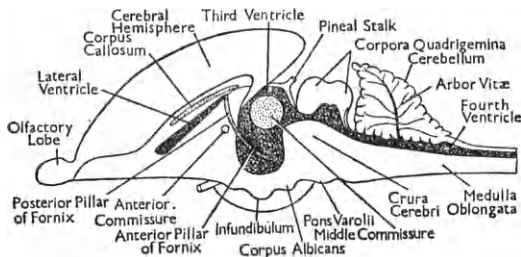
FIG. 173. **Oryctolagus. L.S. Brain.**

and the **anterior** and **posterior lobes** of the **corpora quadrigemina** with the thick **crura cerebri** forming their floor and the **pons varolii,** continuous with the crura cerebri, will also be seen. Look for the **iter** joining the third and fourth ventricles and the **fourth ventricle** in the **medulla oblongata** with the vascular **posterior choroid plexus** forming its roof. Examine the **cerebellum** and note the tree-like lines inside called the **arbor vitae.**

You may experience a little difficulty in finding all the **above** structures.

THE RAT

RATTUS

Like the rabbit, the rat is a member of the CLASS *Mammalia* and the ORDER *Rodentia* to which hares, mice, squirrels and guinea-pigs also belong. The black rat is *Rattus rattus* and the brown rat *R. norvegicus* but the colour varies considerably. The tame rats frequently used for dissection and experiments, whether black, white or pied, are all domesticated forms of *R. norvegicus*.

I. EXTERNAL ANATOMY

Note the colour and shape of the body and that it is covered with short hairs except on the ears and feet while a few are found on the tail. The body is divided into head, neck, trunk and tail.

Head

The head is pointed anteriorly where the **nostrils** will be seen as two slits. The black rat has a sharp snout whereas that of the brown rat is blunt. The **mouth** is below the nostrils and is bounded by two lips. At the sides of the snout are long whiskers or **vibrissae** which are tactile organs. The **eyes** are small and the external ears or **pinnae** are rounded and smaller in the brown rat than they are in the black. The entrance to the ear is called the **external auditory meatus.**

Neck and Trunk

The **neck** is short and the trunk consists of the **thorax** anteriorly and the **abdomen** posteriorly. The **tail** of the black rat is naked, slender and at least as long as the body whereas the tail of the brown rat is somewhat hairy and shorter than the body. At the root of the tail on the ventral side is the **anus.** In the *male* note the **penis,** an intromittent organ, in its sheath, the **prepuce,** and the two large **scrotal sacs** enclosed in a single fold of skin and which enclose the testes. In the female note the vulva, the slit-like genital aperture, with a small rod-like **clitoris** in its ventral wall. The genital and urinary openings are separate, unlike the rabbit, the latter opening anteriorly to the former. There are six pairs of **mammary glands** bearing teats, three on the thorax and three on the abdomen.

Limbs

The **fore-limbs** are shorter than the hind-limbs and the **manus** bears four distinct digits each terminating in claws while the **pes** has a short hallux or big toe and four other digits, all ending in claws.

II. THE SKELETON

The skeleton of the *rabbit* should be studied in preference to that of the rat, the skull of the *dog (Canis)* being substituted for that of the rabbit. Details for the examination of the rabbit's skeleton and the skull of the dog will be found on pp. 195 *seq.*

The chief differences between the skeletons of the rabbit and rat are as follows:—

Dental formula of Rat $\frac{1033}{1033}$. Thoracic vertebrae 13. Ribs 13 pairs.

Clavicle articulates with the manubrium and the acromion process of the scapula. Six lumbar vertebrae. Two sacral vertebrae are fused with two caudal to form the sacrum. Caudal vertebrae 27-30. The pes has 5 digits.

III. INTERNAL ANATOMY

Dissection is best performed on a freshly killed animal.

If the animal which is used for the general dissections is also to be used for the study of the brain, it will be necessary to take precautions at this stage to preserve the brain. The procedure is as follows:—

Remove the skin from the dorsal side of the head so as to expose the bones of the cranium. The brain will be visible through the bones of the cranium. Holding the head firmly but gently, insert a small scalpel horizontally into the inter-parietal bone from behind in order to force apart the parietal bones at the sutures. Then carefully remove all these bones. The brain will then be exposed. Cover the exposed part with 4 per cent. formaldehyde. When the animal is kept in formalin between dissections, the fluid will penetrate into the cranial cavity.

THE MUSCULAR BODY WALL

Place the animal, ventral side upwards, on a dissecting board. Fix awls through the wrists and, stretching the body as much as possible, fix awls through the ankles.

Pinch the skin in the centre of the abdomen and cut with the scissors, making a median incision upwards to the top of the thorax and down to the tail. Holding the skin with forceps, cut away the connective tissue between the skin and the muscular body wall as far round as possible with a scalpel and pin back the skin on either side with awls.

Note the strip of muscle down the centre of the **abdomen,** the **rectus muscle** with the **oblique muscles** on either side.

In the **thorax,** note the **ribs,** which can be somewhat vaguely seen, the **pectoralis muscle,** a large fan-shaped muscle extending from the **sternum** (with the **xiphoid cartilage** at its lower end) to the upper-arm and the **latissimus dorsi muscle** at the side of the thorax.

Note also the **cutaneous blood-vessels** in the inner (exposed) side of the skin.

Regional Anatomy

In the mammal it is more convenient, from a practical point of view, to study the anatomy of the various regions of the body (*i.e.*, abdomen, thorax, neck and head) than to study one system at a time, though this is, of course, possible.

I. THE ABDOMEN

Carefully lift the wall of the abdomen with forceps and make a median incision stretching up to the xiphoid cartilage and down to the pubic symphysis. Take care not to pierce, cut or puncture any of the abdominal viscera. Now make transverse cuts in the abdominal wall just posterior to the last ribs and pin it back on either side. A great deal of fat will be found which may hide some of the structures sought. This should be carefully removed with forceps. Bleeding from capillaries will readily occur in this animal. The blood should be absorbed with cotton wool.
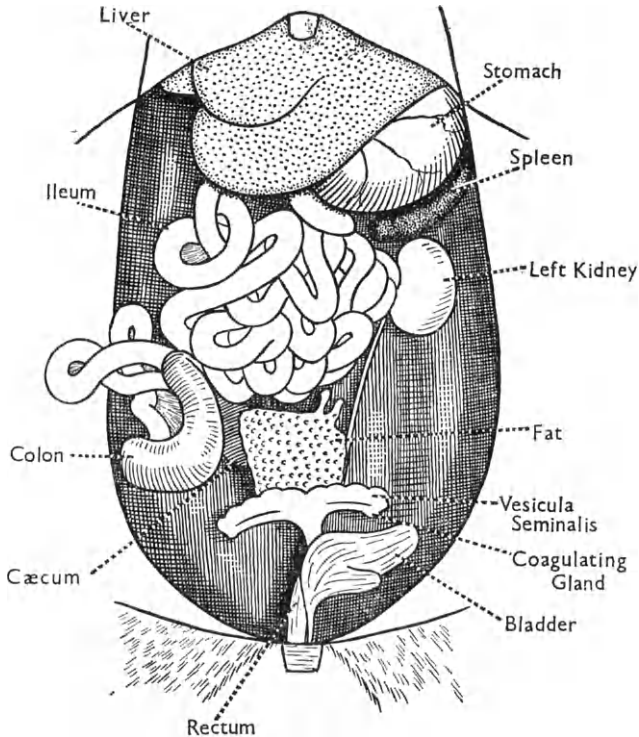
FIG. 174. **Rattus. Abdominal Viscera.**

(1) THE ABDOMINAL VISCERA IN SITU

Note the shining mucous membrane, the **peritoneum,** which lines the **peritoneal cavity** (part of the coelom) and covers the organs, and the muscular partition separating the abdomen from the thorax, the **diaphragm.** This will be seen if the liver is gently lowered a little. The large red organ under the diaphragm is the **liver;** it partially covers the large white **stomach** on the (animal's) left stretching across the body, below which is part of the short **caecum.** Part of the **colon** may also be seen and some coils of the **ileum** will be visible. The **urinary bladder** may be seen, particularly if it contains a quantity of urine, at the posterior end of the body cavity. The hook-like **vesiculae seminales** may also be visible in this region.

Two large **fat bodies** may obscure some of the organs at the posterior end of the abdomen.

(2) THE ABDOMINAL VISCERA AFTER DEFLECTION OF THE INTESTINES

Deflect the liver forwards and the viscera to the animal's left to ensure as complete an exposure of the alimentary canal as possible. Care must be exercised as the small and large intestines are coiled over one another. They should be released from each other by cutting through the **mesentery** *which supports them. Remember to remove any fat which obscures the organs.*

Find the **duodenum,** the first part of the small intestine, continuous with the stomach, continuous with which is the ileum. This leads into the small, short **caecum** from which arises a short **colon.** The last part of the large intestine is the **rectum.**

Examine the **liver.** It is suspended from the diaphragm by the **falciform ligament** and is composed of four **lobes**—the large subdivided **right lobe** (the posterior one on the animal's right), the large undivided **left lobe** (the anterior one on the left), the **median lobe** (in the centre
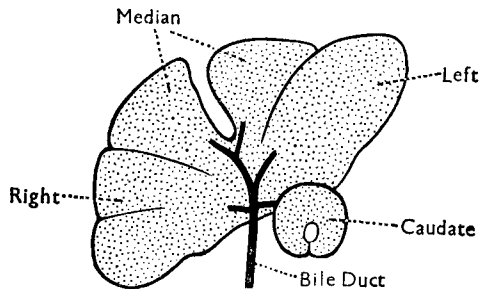
Fig. 175. **Rattus. Liver.**

anteriorly) and a curved **caudate lobe** (posteriorly on the right and looping round the oesophagus). There is no gall gladder. The **bile duct** formed from the hepatic duct from each lobe enters the proximal loop of the duodenum. Note the wide translucent **cardiac end** and narrow **pyloric end** of the **stomach,** and the constriction, the **pyloric sphincter** (or **pylorus**) at the outlet of the stomach where it joins the duodenum.

Pull the stomach down slightly in order to see the **oesophagus** *entering the cardiac end.*

Spread out the loops of the duodenum without cutting the mesentery.

The **pancreas** is a large diffuse gland between these loops. The **pancreatic ducts,** (there are several), open into the bile duct along its length as the latter traverses the pancreas.
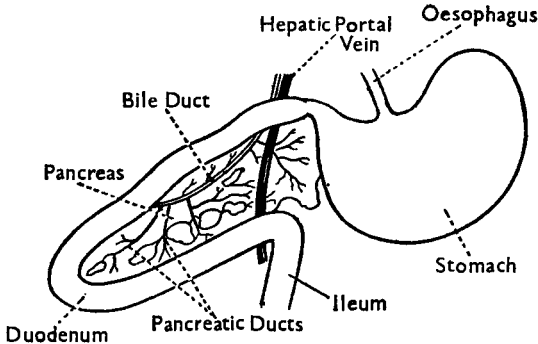
FIG. 176. **Rattus. Duodenum and Pancreas.**

The **spleen** is a dark red body lying alongside the cardiac end of the stomach.

Now find two of the blood vessels associated with the alimentary canal. The others will not be seen until it has been removed.

The **portal vein** to the liver will be found in the mesentery alongside the colon. *Turn the stomach over to the animal's right.* The **coeliac artery,** which arises from the dorsal aorta, will be revealed running across to the stomach.

The left **kidney,** a dark red organ, will also be seen on the dorsal body wall.

(3) REMOVAL OF THE ALIMENTARY CANAL

Remove the alimentary canal as follows: ligature the portal vein in two places and cut across it between the ligatures. It will be as well to ligature the anterior mesenteric and coeliac arteries also. Cut across

the rectum near its lower end. Now carefully cut through the mesentery, close to the intestine, gradually unravelling the intestine. Take great care not to puncture any part of the intestine, and when you reach the duodenum try to keep the loop intact. Finally cut across the oesophagus. Now neatly lay out the alimentary canal on another dissecting board.

Note the **oesophagus, stomach (cardiac end** and **pyloric end), pyloric sphincter, duodenum, ileum** and **caecum.** At its apex is **lymphoid tissue** which corresponds to the **appendix.** Continuous with the caecum is the short **colon** followed by the **rectum.**

(4) THE ABDOMINAL VISCERA REMAINING AFTER THE REMOVAL OF THE ALIMENTARY CANAL, IN SITU

Note the two **kidneys,** the right being only slightly anterior to the left: these are dorsal to the peritoneum. The **left adrenal body** is situated just above the left kidney. The **right adrenal body** is anterior to the right kidney though usually hidden by it.

Note the **ureters,** narrow white tubes leading from the kidneys to the **urinary bladder,** and the **renal artery** and **vein** on each side leading from the **dorsal aorta** and to the **posterior vena cava** respectively. The dorsal aorta and the vena cava run in the mid line, the latter being hidden by the aorta which lies ventral to it. *Gently pull back the bladder slightly in order to see the entry of the two ureters.* The reproductive organs in the posterior part of the abdomen will be studied separately.

The Diaphragm

Now turn to the anterior end of the abdomen and examine the **diaphragm,** which separates the abdomen from the thorax. *Cut through the falciform ligament.*

Note the **central tendon** and **marginal muscular portions** of the **diaphragm.** The oesophagus and the aorta and posterior vena cava pass through it.

(5) THE REPRODUCTIVE SYSTEM

(i) Male

It will probably be necessary to remove a great deal of fat before the organs and blood vessels are clearly exposed. Find the scrotal sacs and cut along the ventral surface.

It has already been noted that the two scrotal sacs are enclosed in a single fold of skin externally. Each contains an ovoid **testis** with a large mass of coiled tubes, the **epididymis,** on its inner surface, the upper part of which is the **caput epididymis** and the lower part the
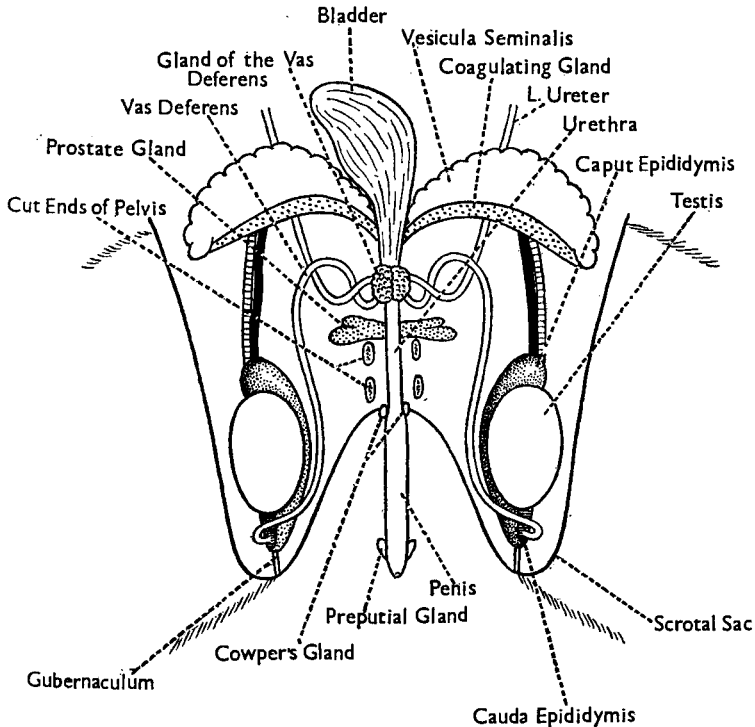
FIG. 177. Rattus. Male Reproductive System.

cauda epididymis, the connection between the two parts being narrow. A cord, the gubernaculum, joins the cauda epididymis to the scrotal sac. Also arising from the cauda is a duct, the vas deferens (modified mesonephric duct) which passes into the abdomen, loops round the ureter and then passes dorsal to·the bladder to enter the urethra. Note the two large hook-shaped sacculated vesiculae seminales and the two coagulating glands alongside them.

Carefully cut longitudinally through the ischium and pubis on each side of the pelvis, i.e., on each side of the symphysis pubis, and remove the central part of the girdle. Deflect the bladder and the vesiculae seminales and the coagulating glands to one side.

There are two prostate glands, each subdivided into two, lying at the neck of the bladder. Deflect them with the structures above.

From the neck of the bladder the urethra arises and passes through the erectile tissue of the penis to its free end, the glans penis, enclosed in a loose sheath, the prepuce. In the ventral wall of the penis is a cartilaginous process. Two Cowper's glands will be found alongside

the urethra near the bladder. The **prostate glands** are attached to the neck of the bladder and there is a gland around the neck known as the **gland of the vas deferens.** The blood vessels associated with these organs will be seen later.

(ii) Female

Carefully cut through the pelvic girdle and separate the two halves as indicated for the male.

Note the two small, irregularly-shaped **ovaries** on the dorsal body wall lateral to the kidneys and the very short, narrow and coiled **Fallopian tubes** which arise close to the ovaries. These lead posteriorly into the thick-walled **uteri** in which the embryos develop. From the uteri a wide median tube, the **vagina,** leads to the exterior, opening into the slit-like **vulva.** Unlike the rabbit, this opening is
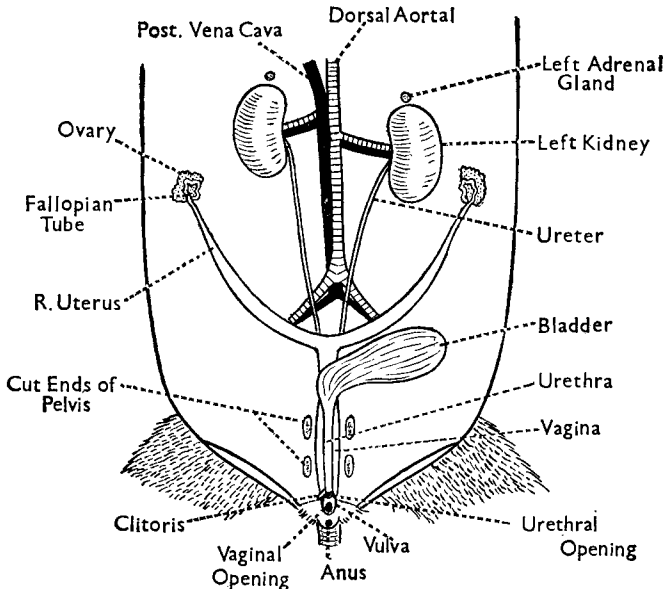
FIG. 178. **Rattus. Female Reproductive System.**

separate from and dorsal to that of the urethra. In the ventral wall of the vulva is a small rod-shaped structure, the **clitoris** (the homologue of the male penis). The blood vessels associated with these organs will also be examined later.

(6) THE KIDNEY

Examine a kidney in situ. Removal of surrounding fat will be necessary.

Note that it is of typical "kidney" or bean shape.

The inner concave edge is known as the **hilus.** The **renal artery** and **vein** will be seen entering and leaving at this point, from which the **ureter** also emerges.

Remove one of the kidneys and cut a longitudinal section with a scalpel. Examine the cut half with a hand lens.

Note that it consists of (i) an outer rind, or **cortex,** in which is a number of **Malpighian bodies,** appearing as specks, and (ii) an inner **medulla.** The ureter widens after entering the hilus as the **pelvis** of the kidney into which the conical **pyramid** opens. The tubules of the kidney open on to the pyramid.

(7) THE ABDOMINAL BLOOD VESSELS

The abdomen is now ready for examination of the blood vessels. *After removal of obscuring fat, including the large fat bodies at the posterior end of the abdomen, deflect out of the way any organs and structures (such as the bladder and rectum, the seminal vesicles in the male and the vagina in the female) which hide the blood vessels and pin them down. Remove any mesentery which may also be obscuring the vessels and cut through the ureters near the kidneys and again near the bladder and remove the portion between. The arteries and veins will then be exposed.*

It should be noted that there is considerable variation in the position and arrangement of some of the blood vessels and they may not, therefore, appear quite as described below.

Find the **dorsal aorta** and the **posterior vena cava** in the mid-line on the dorsal body wall. Now find and identify the following arteries:—

The single **coeliac artery** arises from the dorsal aorta just posterior to the diaphragm and divides into a **gastric artery** to the stomach (already seen), a **splenic artery** to the spleen and an **hepatic artery** to the liver. Behind it arises a single **anterior mesenteric artery** which supplies the greater part of the intestine. Next the paired **renal arteries** leading to the kidneys will be found. These are followed by the **genital arteries (spermatic** in the male and **ovarian** in the female) but the left genital artery may arise from the renal artery on that side. A pair of **ilio-lumbar arteries** then arise from the aorta at slightly different levels, the left being slightly anterior to the right.

Towards the posterior end of the abdomen the aorta bifurcates and at this point an unpaired **posterior mesenteric artery** which supplies

the rectum will be found. The bifurcations are the **common iliac arteries** which pass into the hind limbs where each divides into an **external iliac (femoral) artery** and an **internal iliac artery.** *These should be traced into the legs by careful separation of the muscles and connective tissue.* A small **vesical artery** to the bladder arises from each common iliac. There is much variation in the branching of the iliacs and they may even be asymmetrical.
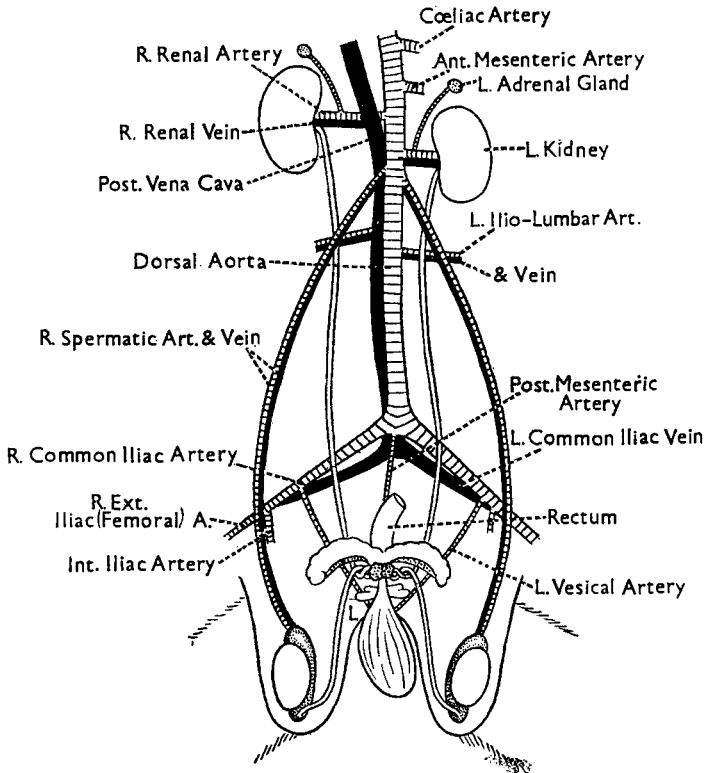
FIG. 179. **Rattus. Abdominal Blood Vessels. Male.**

The following veins should now be found and traced:—

In some specimens two **common iliac veins** formed by the union of the **external iliac veins** (continuations of the **femoral veins**) and **internal iliac veins,** both from the legs, join to form the posterior vena cava. In other specimens it seems the vena cava is formed by the union of the external iliacs and the internal iliacs occur as a series of small veins which enter the external iliacs. There appears to
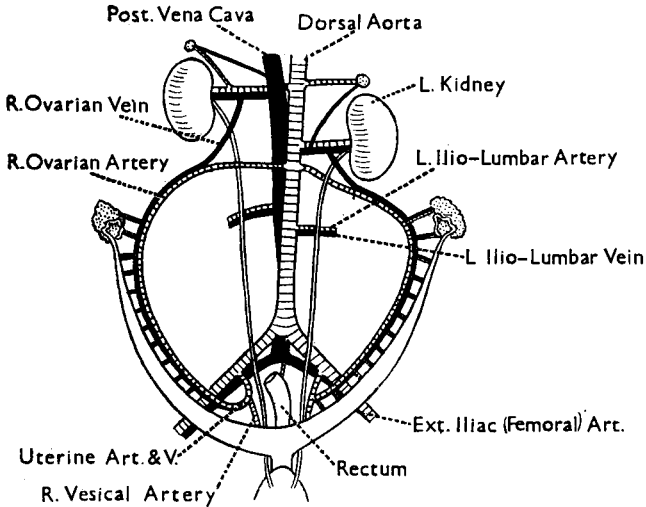
FIG. 180. **Rattus. Abdominal Blood Vessels. Female.**

be variation here and some confusion over nomenclature. On the whole, it is perhaps better to name the two veins which form the posterior vena cava the **common iliac veins.** The **ilio-lumbar, genital** and **renal veins** should now be identified alongside the corresponding arteries. The portal vein has already been seen in an earlier dissection: it is formed from veins from the alimentary canal. Finally the **hepatic veins** from the liver will be seen entering the posterior vena cava just below the diaphragm.

II. THE THORAX

Remove the ventral wall and as much as possible of the lateral walls of the thorax. It is not as easy to leave the diaphragm in its normal position as is the case when dissecting the rabbit but the method suggested for the rabbit may be tried if desired.

Insert the scissors below the last rib on one side and well down towards the side. Take care not to damage the organs inside the thorax with the lower blade of the scissors. Cut upwards and inwards through the ribs towards the xiphoid cartilage. Continue to cut above the cartilage through the xiphisternum and then down through the ribs on the opposite side to a position corresponding to the original cut on the other side. Now cut upwards through the ribs, well down towards the outside, first on this side and then on the other, right to the top of the thorax so that the two cuts meet. Be careful at the anterior end not

to cut the anterior vena cava. The ventral wall of the thorax can then be removed and the diaphragm pinned down to expose its upper surface.
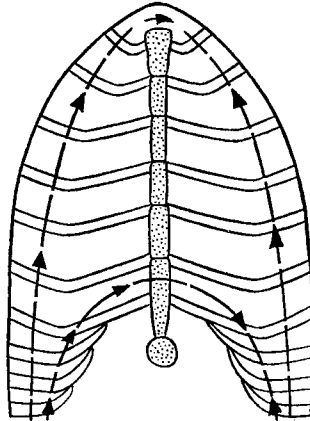
FIG. 181. Rattus.
Method of Removal of Ventral Thoracic Wall.

(1) THE THORACIC VISCERA IN SITU

Note the **thymus gland** which partly covers the **heart,** lying in its **pericardium.** On each side of it is a **lung** enclosed in **pleura** but the latter will most probably have been removed when opening the thoracic cavity. The **mediastinum** will also have been removed. This lies between the pleural cavities from the diaphragm to the pericardium and encloses the lower part of the trachea and oesophagus (which is dorsal to the trachea) and the main blood vessels in this part, such as the posterior vena cava. More of the **trachea** will also be visible anterior to the heart.

(2) THE HEART AND GREAT VESSELS

Carefully remove the thymus gland and free the heart from the pericardium. Remove any fat which obscures the blood vessels and deflect the heart to the animal's right and pin it down.

The **heart** consists of thin-walled **right** and **left auricles** anteriorly and thick-walled **right** and **left ventricles** posteriorly. The right auricle will probably be at least partially hidden in its deflected position. Immediately anterior and dorsal to the heart you will see the **aortic arch** and the **innominate artery** arising from it. To the left of this the **left common carotid artery** will be found. Further to the

left is the **left subclavian artery.** The **pulmonary artery** from the right ventricle of the heart runs across to the lungs and between it and the aortic arch is a ligament known as the **ductus arteriosus.** The right and left **anterior venae cavae** and the **posterior vena cava** will also be found. These enter the right auricle.

The **azygos vein** from the intercostal muscles between the ribs should also be seen entering the left anterior vena cava near to the heart.

(3) THE HEART OF THE SHEEP

The sheep's heart, being much larger than that of the rat, is more suitable for the study of the internal structure. It differs little from that of the rat. Instructions for the dissection will be found on page 236.

III. THE NECK

Owing to the fact that the neck is short and that the nerves are very slender, this dissection needs considerable care. A lens will be found helpful for identification of the nerves and blood vessels.

See that the head is well extended and pin it down. Use a small scalpel and remove fat and connective tissue and so expose the two very large **external jugular veins** *which run forwards from the anterior venae cavae. Cut through the sternohyoid muscles longitudinally, taking care not to cut beneath them. Then sever the two halves at each end and remove them. Now carefully remove the muscles on either side and thus expose the blood vessels, nerves and trachea.*

On either side of the **trachea** the slender **internal jugular veins** will be found. Note the **thyroid gland** at the anterior end of the trachea. It is composed of two lobes, one on each side of the trachea and joined on the ventral side. Anterior to this gland is the **larynx.** *Careful separation of the following blood vessels and nerves running more or less parallel with the trachea will now be necessary if they are to be identified.* Find the **common carotid artery,** the origin of which was seen earlier. It divides into an **internal carotid artery** and an **external carotid artery** at the level of the thyroid gland. Now find the **vagus nerve (X)** which runs alongside and the **recurrent laryngeal nerves** which lie on the surface of the trachea. Trace them backwards to their origins from the main branch of the vagus. The **left recurrent laryngeal nerve** arises just below the aortic arch, loops round the ductus arteriosus and then passes up the neck. The **right recurrent laryngeal nerve** arises a little further forward and then follows a similar course upwards. Near the larynx the main branch of the vagus gives off a small branch to that organ known as the **anterior**

laryngeal nerve. This in turn gives off a slender **cardiac depressor nerve** to the heart: it is difficult to find.

A ganglion, the **anterior cervical sympathetic ganglion,** will be found alongside the origin of the internal carotid artery and a **sympathetic nerve** runs backwards from it. **Middle** and **posterior**
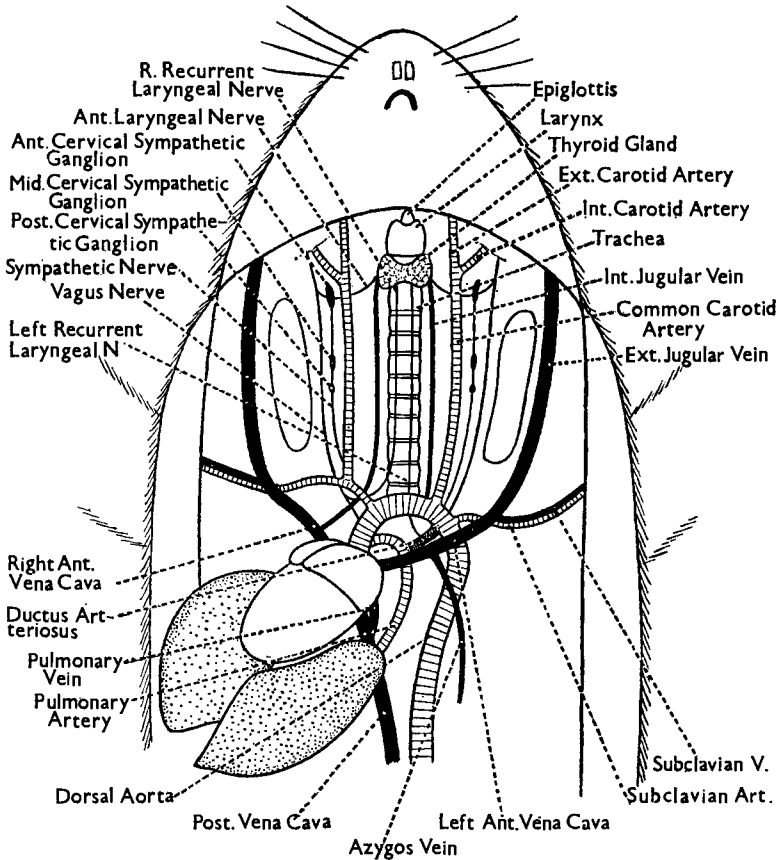
FIG. 182. **Rattus. Neck Dissection.**

cervical sympathetic ganglia will be found close to each other further back. Note the **phrenic nerve** which lies outside the external jugular vein and supplies the diaphragm. Finally find the **hypoglossal nerve (XII)** which runs across the carotid arteries at the anterior end, level with the larynx and then along the posterior cornua of the hyoid to the tongue.

IV. THE RESPIRATORY SYSTEM

Remove and examine the complete respiratory organs of the neck and thorax.

As already seen the **larynx** is situated at the anterior end of the **trachea.** Ventrally and laterally it is composed of the **thyroid cartilage**
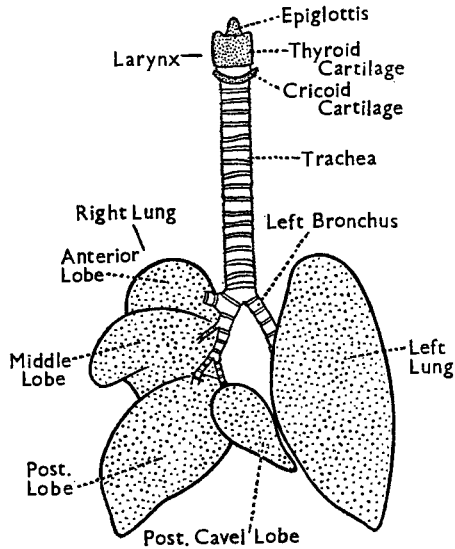
FIG. 183. **Rattus. Respiratory System.**

with the ring-like **cricoid cartilage** posterior to this. The trachea is composed of rings of cartilage, incomplete dorsally, and divides posteriorly into two **bronchi,** one to each lung. The **right lung** is divided into four lobes, the **anterior, middle, posterior** and **post-caval** lobes while the left lung is undivided.

V. THE VASCULAR SYSTEM

Now that all the blood-vessels have been seen *you should do separate dissections of the complete* **Arterial** *and* **Venous Systems.** *You should also draw connected diagrams of them.*

IV. THE HEAD

The only part of the head which will be studied is the brain.

THE BRAIN

The brain must be examined in a freshly killed rat or in which it has been preserved in accordance with the instructions on p. 236,

because it decomposes rapidly. It is soft and delicate and very gentle and careful dissection is necessary. *Cut through the skin in the mid-line on the dorsal side of the head and pull the two portions apart. The roof of the cranium is thin and the brain can be seen through the bone.* (No decalcification is therefore necessary as is the case with the rabbit.) *The cranium roof must now be removed. The brain is small and, as already pointed out, great care is necessary owing to the thinness of the bone.*

Holding a very small scalpel horizontally, insert its point into the posterior end of the interparietal bone at the back of the skull, taking care not to penetrate so as to damage the brain. This will loosen the roof of the cranium which should now be removed gradually and carefully. Now remove the rest of the bone, again very carefully, so as to expose the brain completely from end to end. The brain should now be removed from the cranium as follows:—

Remove the surfaces of the first few vertebrae and cut through the spinal cord thus exposed. Now place the scalpel under the cranium at its posterior end and, working forwards and cutting through the nerves on the ventral side, gradually free the brain from the cranium. Great care must be exercised in the region of the cerebellum as part of it, the paraflocculi on the sides, are encased in bone. On no account touch the brain with the scalpel or other metal instruments or it will be damaged. Gently place the brain in a small dish (petri dish or large watch-glass). It should be preserved in 70 per cent. alcohol or 4 per cent. formaldehyde if required on a future occasion.
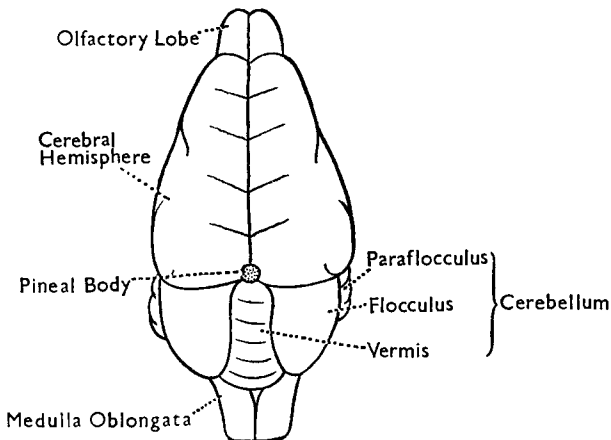
Fig. 184. Rattus. Brain. Dorsal View.

(a) Dorsal View

The **Fore-Brain** is composed of two **cerebral hemispheres** with a few slight convolutions anterior to which are the proportionately large **olfactory lobes** or **bulbs.** The posterior part of the fore-brain, the thalamencephalon, is hidden in this view by the cerebral hemispheres. The rounded structure between the hemispheres at their posterior end (if it has not been removed) is the **pineal body** (an endocrine gland of which little is known).

The **Mid-Brain** consists of the two optic lobes but these are not visible in the dorsal view.

The **Hind-Brain** is composed of the **medulla oblongata** with the cerebellum on its dorsal side. This consists of a central **vermis** on each side of which is a **flocculus** bearing a small **paraflocculus** externally towards its anterior end. The spinal cord is continuous with the medulla.

(b) Ventral View

Again note the **olfactory lobes** and **cerebral hemispheres** also the **olfactory tracts** running backwards in the centre from the olfactory lobes. In the mid-line, a little further back, will be seen the **optic chiasma,** formed by the crossing over of the **optic nerves (II).** Behind this is the **pituitary body** followed by the **medulla oblongata.** Parts of the **flocculi** and the **paraflocculi** will also be visible in this view.
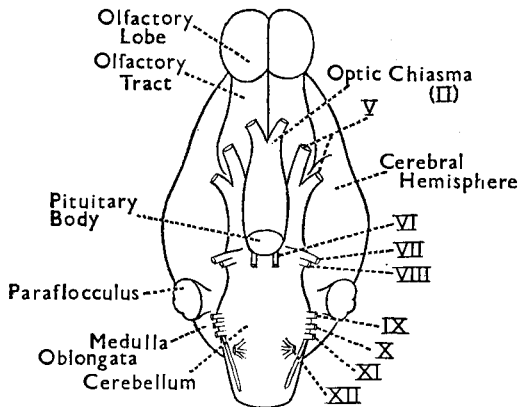
FIG. 185. **Rattus. Brain. Ventral View.**

The roots of the **trigeminal nerves (V)** run alongside the posterior part of the optic chiasma. *It is necessary to remove the roots of the trigeminal on one side to reveal the* **oculo-motor nerve (III)** and the **trochlear nerve (IV)** beneath. Immediately posterior to the trigeminal

nerve, the roots of the **facial nerve (VII)** and the **auditory nerve (VIII)** will be seen and, a little further back still, those of the **glossopharyngeal nerve (IX),** the **vagus nerve (X)** and the **spinal accessory nerve (XI).** The roots of the **abducens nerve (VI)** and of the **hypoglossal (XII)** are more ventrally situated, the former being just behind the pituitary body and the latter posterior to the spinal accessory nerve.

(c) Longitudinal Section

This must be done on a brain hardened in alcohol. Make a longitudinal vertical cut through the centre of the brain with a razor blade and examine the cut surface. Retain the other half.

Identify the **olfactory lobes,** the **cerebral hemispheres,** the cut edge of the **corpus callosum** and the **anterior choroid plexus** immediately behind and below it. The large rounded structure ventral to this is the **middle commisure** (or **massa intermedia**). The small **anterior commissure** lies just anterior to this. The **body of the fornix** is to be found just dorsal to the anterior commissure, continuous with the
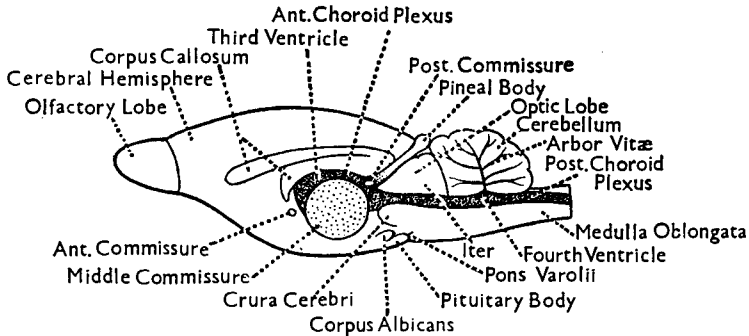
FIG. 186. Rattus. Brain. Longitudinal Section.

corpus callosum. The **posterior commissure** lies dorsal and posterior to the middle commissure. The **optic lobes** (or **corpora quadrigemina**) can now be seen (it will be remembered that they were not visible in the external views of the brain). They lie between the cerebral hemispheres and the **cerebellum** which is easily identified, with its tree-like **arbor vitae** internally, on the dorsal side of the **medulla oblongata.** The thick **crura cerebri** form the floor of the mid-brain and beneath this lies the **pons varolii.** The **fourth ventricle,** roofed over by the **posterior choroid plexus,** will be seen in the medulla and the **iter** connecting it to the **third ventricle** in the **thalamencephalon.** The **pineal body** on its long **pineal stalk** (if either is still present) and

the **pituitary body** will be found in their respective places. Just anterior to the latter the **corpus albicans** will be found.
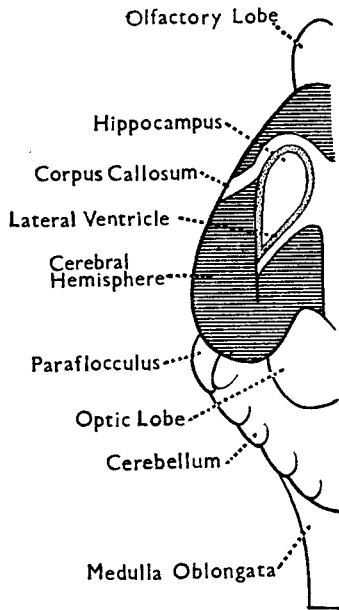
FIG. 187. **Rattus.**
Brain. Horizontal Section.

(*d*) Horizontal Section

Now take the other half of the brain and cut a horizontal section a little above half way down, again using a razor blade.

Note the **olfactory lobe** and the **cerebral hemisphere.** In the latter will be found the wide **hippocampus** and the narrow **lateral ventricle.** The **optic lobe, cerebellum** and **medulla oblongata** will also be seen.

PART III

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* but in animal cells this is absent and the protoplast has a protoplasmic membrane known as the *plasmalemma*. 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 and animal 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 Protozoa and 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 AND ANIMAL 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 and in* **Ascaris** *or other suitable animal. Search for different stages of mitosis in different nuclei. You may not succeed in finding them all.*

(i) **The Prophase.** In *animal cells* the **centrosome** (a small spherical structure just outside the nucleus, containing a small body, the **centroile**) divides into two and later these move to opposite poles of the nucleus, a **nuclear spindle** developing between them. Centrosomes are not found *in plant cells* and the spindle is formed without them. Fibrils radiate from the centrosomes forming **asters** which form the poles of the spindle. 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. The centrosomes reach opposite ends of the nucleus with the nuclear spindle between them, the mitochondria becoming arranged around the spindle.

*The shape and number of chromosomes varies with different plants and animals but is constant for any one species, *e.g.*, in man 23 pairs, in the rabbit 11 pairs and in the frog 12 pairs while in Ascaris there is but one pair in one species and up to 24 pairs in different species of this animal. In the higher plants the number varies between 6 and 65 pairs. In *Cheiranthus* (Wallflower), for example, there are 7 pairs and in *Primula* 9 pairs, while *Triticum* (Wheat) has 21 pairs.

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(ii) **The Metaphase.** The chromosomes clearly show their double structure and these chromatids arrange themselves on the equator of the spindle at points of them known as **centromeres.**
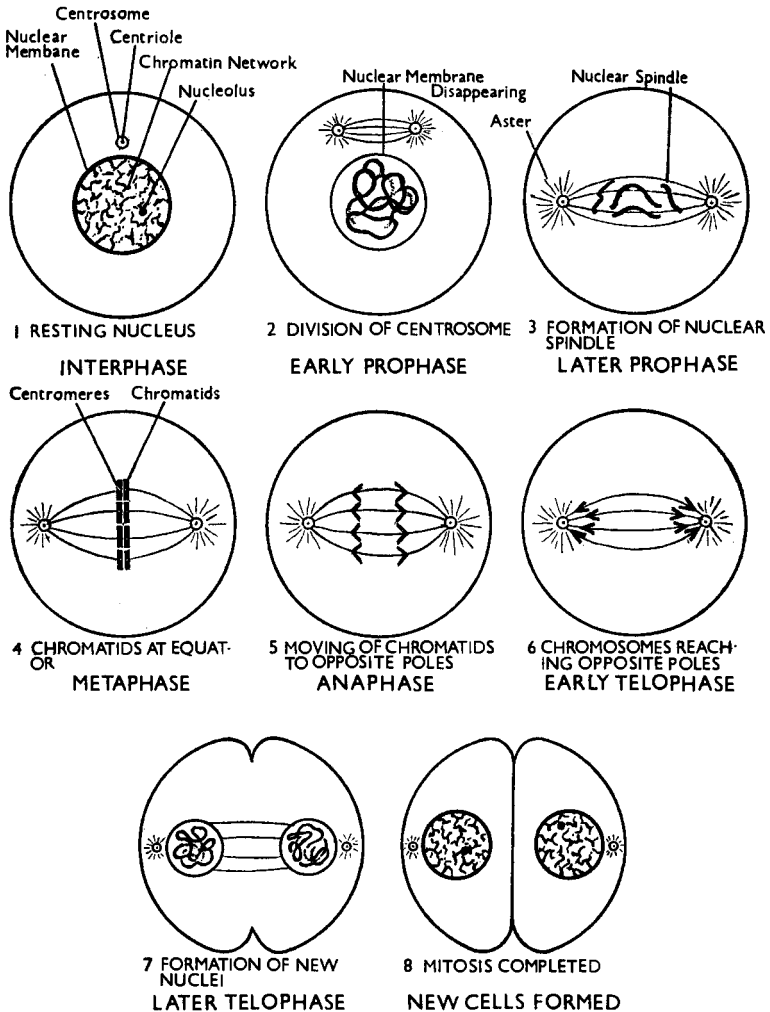
FIG. 188. **Mitosis in Animal Cells.**

(iii) **The Anaphase.** The chromatids begin to move towards the opposite poles of the nucleus, guided by the spindle and being pulled into V-shaped loops in the process.
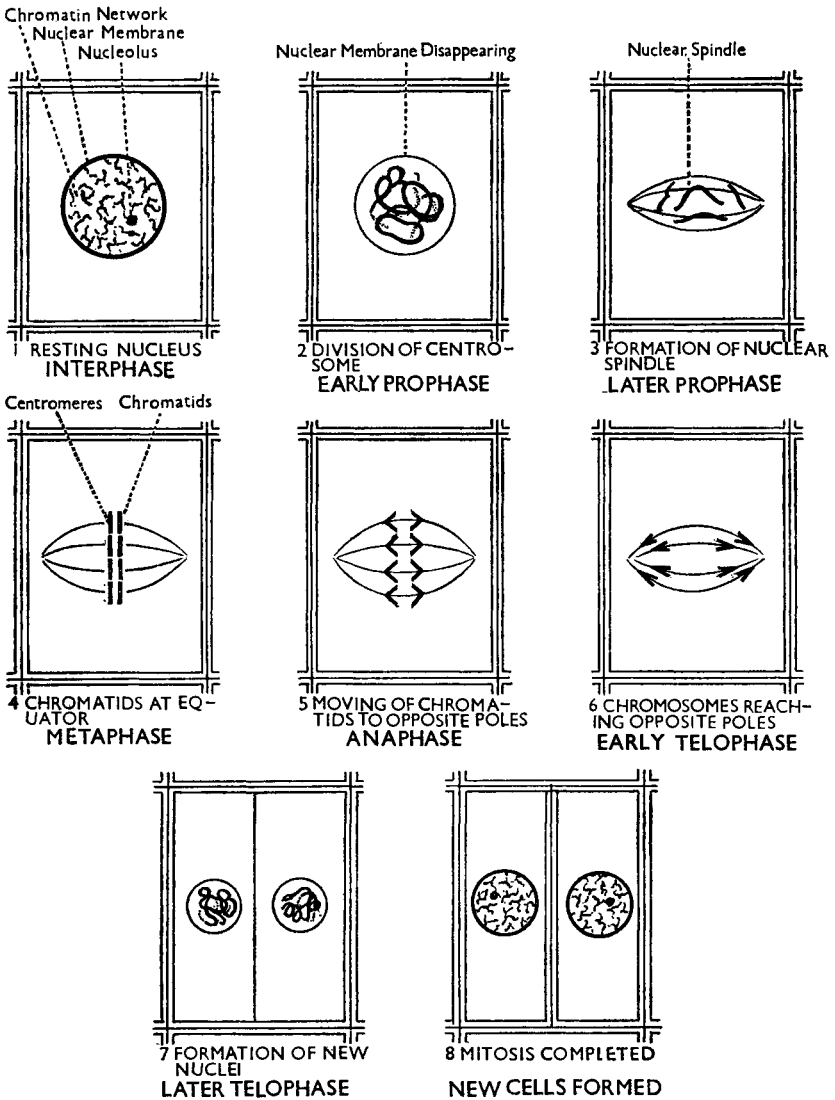
FIG. 189. **Mitosis in Plant Cells.**

(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. A nuclear

membrane develops, the asters disappear and in animal cells a single centrosome is left outside each nucleus.

Cell division follows. In *animal cells* a constriction develops in the cytoplasm between the nuclei. This deepens and forms a cell membrane. 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 laid down on either side of the middle lamella and so a new cell wall is formed. Thus in both plants and animals two new cells are formed, each with its own nucleus containing the original number of chromosomes.

I. PLANT CYTOLOGY AND HISTOLOGY

INTRODUCTORY NOTES

The histological structure of the tissues of which plants organs are composed and the structure of the cells in these tissues will be seen in the study of the histology of the root, stem, leaf and flower in this section. Before this study can be properly understood, however, it is desirable to know the kind of tissues which are found. These are therefore summarised first.

Refer to Part I for instructions on *the making of microscopical slides* and to *directions for drawing microscopical preparations* on p. 2.

Meristematic Tissues

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

(1) **Primary Meristem** will be seen in the apical growing points of the root and stem and in the fasicular 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.**

PARENCHYMA T.S. COLLENCHYMA T.S. FIBRES L.S. "STONE CELLS" SCLERIDES SCLERENCHYMA
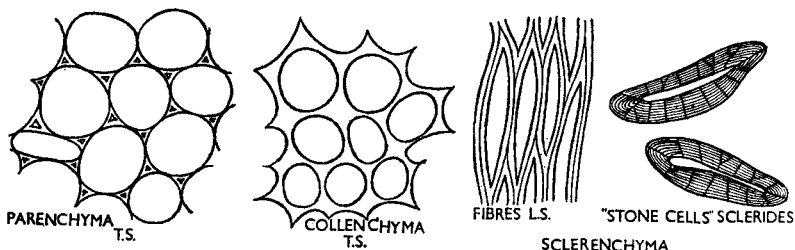
FIG. 190. Spermatophyta. Ground Tissue and Supporting Tissue.

(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 annus*) *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.**
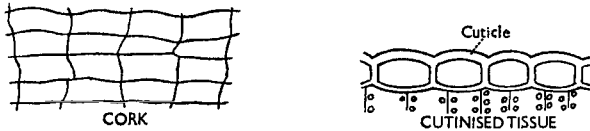
FIG. 191. **Spermatophyta Suberised and Cutinised Tissue.**

(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.

XYLEM—FORMS OF THICKENING

ANNULAR   SPIRAL   RETICULATE   SIMPLE PITS   BORDERED PITS   SCALARIFORM

Companion Cell

Companion Cell

SIEVE PLATE T.S.

Sieve Plate

SIEVE TUBE L.S.
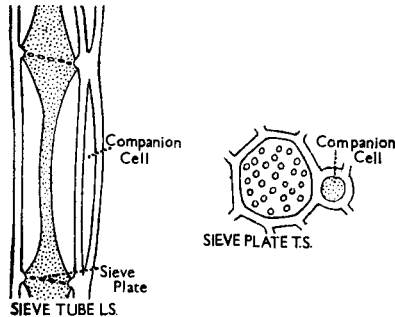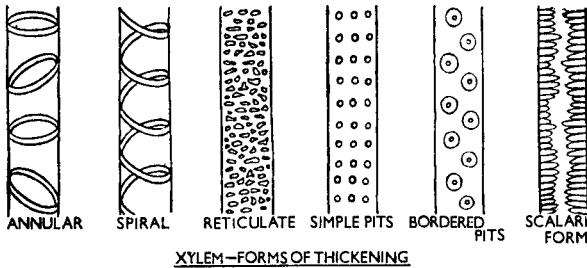
FIG. 192. Spermatophyta. Vascular Tissue.

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 unlignified 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 secretion and, in some cases, 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.

(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.
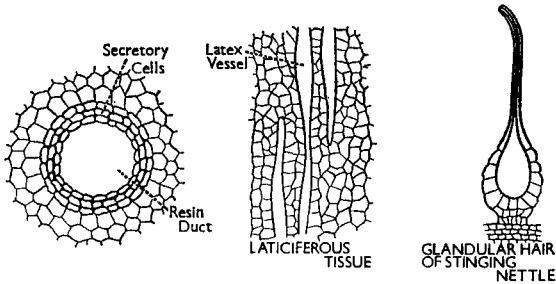
FIG. 193. Spermatophyta. Glandular Tissue.

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

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

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

the gum or resin. The glandular hairs on the leaf of the Sundew secretes 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.

I. THE HISTOLOGY OF THE ROOT

(1) DICOTYLEDONOUS ROOT

(i) Apical Meristem

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

Under *low power* note the **calyptra** or **root-cap,** protecting the tip and behind it the **calyptrogen** 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 sections separately in Schultze's solution and observe the results.*

(1) Under *low power draw a diagram* of the regions of the root.* On the outside is the **piliferous layer** (epidermis), 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 this is usually single (sometimes double though it may not be continuously so). 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*

* See Introduction pp. 2 *seq.*
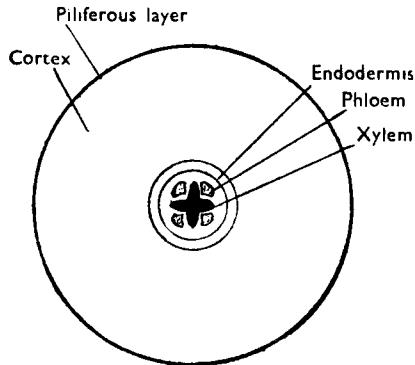
UNDER LOW POWER
(DIAGRAMMATIC)
FIG. 194. T.S. Dicotyledonous Root.
Low Power Diagram.

xylem groups. (Dicotyledonous roots vary normally from two to five and are described as di-, tri-, tetr- or pentarch.)

(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,**
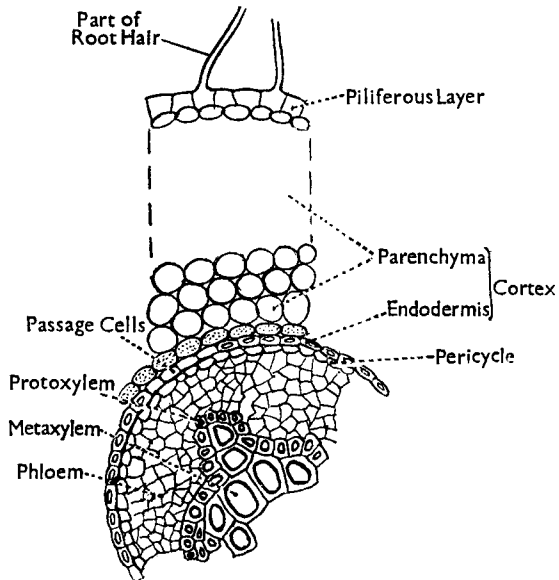
FIG. 195. T.S. Portion of Dicotyledonous Root.
High Power Drawing.
* See Introduction pp. 2 *seq.*

cortical parenchyma, endodermis, pericycle, protoxylem and meta-xylem; 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.

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

(iii) Transverse Section of Old Dicotyledonous Root (in the lateral root region) showing Secondary Growth

Examine a T.S. *of the root of a* broad bean *in the region where the lateral roots are found. Mount as before.*

(1) Under *low power* note the 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 traversed by parenchymatous medullary rays 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. When considerable secondary growth has taken place 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) *Examine a* transverse section through a lateral root.

Note that the secondary roots arise from the pericycle usually opposite the protoxylem. Because they arise internally they are said to be *endogenous.*

(3) *Cut and stain* a transverse section of an old root *with safranin or haematoxylin and make a permanent preparation.*

(2) MONOCOTYLEDONOUS ROOT

Transverse Section of Monocotyledonous Root

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

*See Part I. (Microscopical Technique) pp. 21 *seq.*

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, 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 high power drawing of a vascular bundle.*
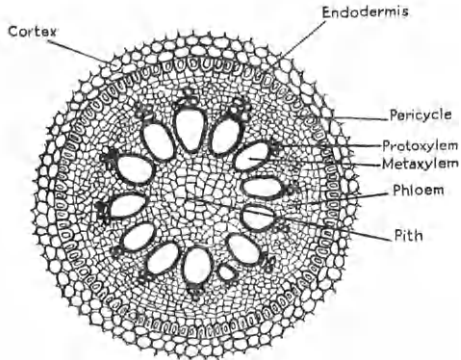
FIG. 196. T.S. Monocotyledonous Root—Central Portion.

II. THE HISTOLOGY OF THE STEM

(1) DICOTYLEDONOUS STEM

(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 and observe the results.*

(a) Under *low power* note the outermost layer, a single row of cells, the **epidermis,** with multicellular **hairs,** then the narrow but many-layered **cortex,** the innermost row of which form the **endodermis,** 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 same radius on the *inside* (and therefore said to be *collateral*), the two separated by a primary meristem, several cells thick, known as the **fasicular cambium** (the bundle is therefore said to be *open*). Look for the **inter-fasicular cambium,** a secondary meristem 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.
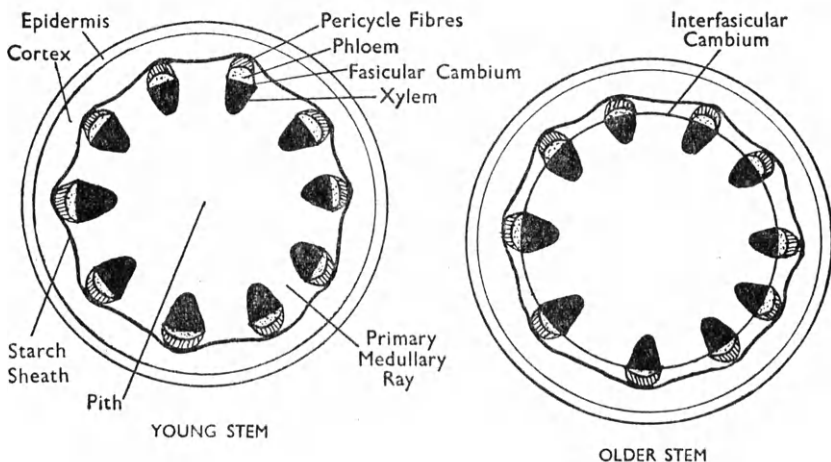
FIG. 197. T.S. Dicotyledonous Stem. Low power diagram.

(b) Under *high power* note that the **epidermis** has thickened cutinised outer walls forming a **cuticle** and bears a few multi-cellular **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 it are the **pericycle fibres,** the **phloem** composed of **sieve tubes with sieve plates, companion cells** and **phloem parenchyma.** Next comes the thin **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.
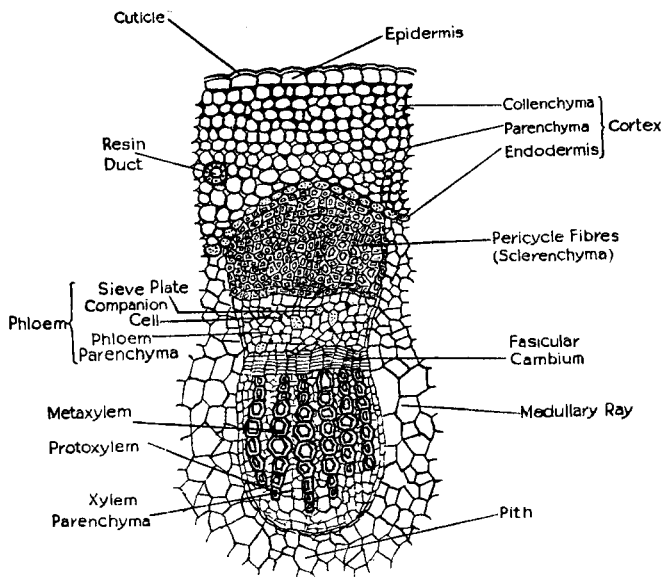
FIG. 198. T.S. Portion of Dicotyledonous Stem. High Power Drawing.

(*c*) *Stain a section singly with haematoxylin or safranin 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 **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 **pitted vessels** of the **metaxylem** and the **spiral** and **annular vessels** of the **protoxylem** and the **inner phloem.**

(vi) 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,** consisting of rows of flattened cells and the **phellogen** or **cork cambium**
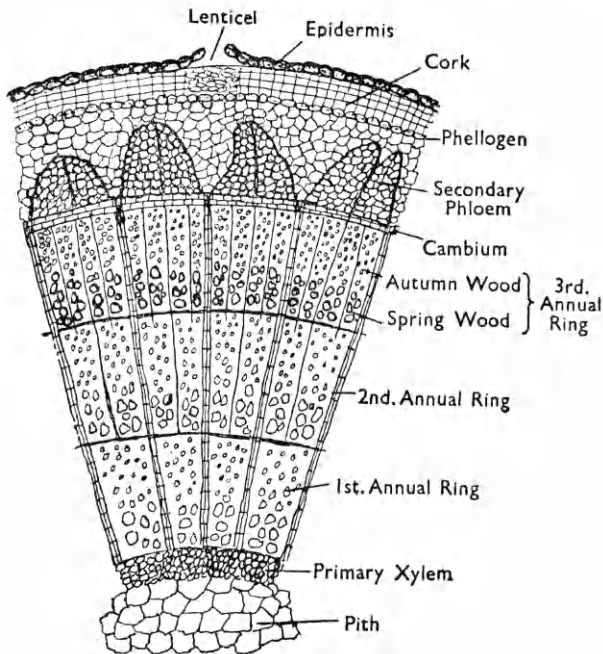
FIG. 199. **T.S. Part of Three Years Old Woody Stem. Under low power.**

inside this. Next comes the **phelloderm** or secondary cortex, followed by the **cortex** (parenchyma). Cork, phellogen and phelloderm are collectively known as **periderm**. The **secondary phloem** is divided into wedges roughly triangular in shape due to 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** or **autumn wood** external and large, and less thickened elements of **early wood** or **spring wood** internally. The **primary xylem** is in the centre projecting into the
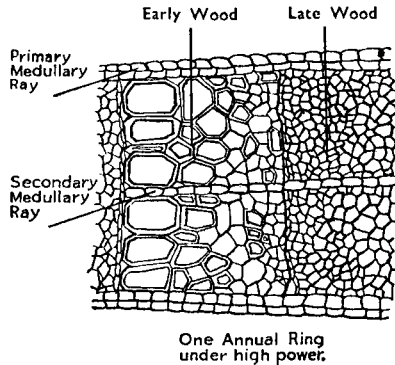
FIG. 200. **Annual Ring. Under high power.**

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.

(vii) **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.**

(2) MONOCOTYLEDONOUS STEM

(i) **Transverse Section of Monocotyledonous Stem**

Cut a **T.S.** *of the* **stem** *of* **maize** *(Zea mais) or other monocotyle-donous stem.*

(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.
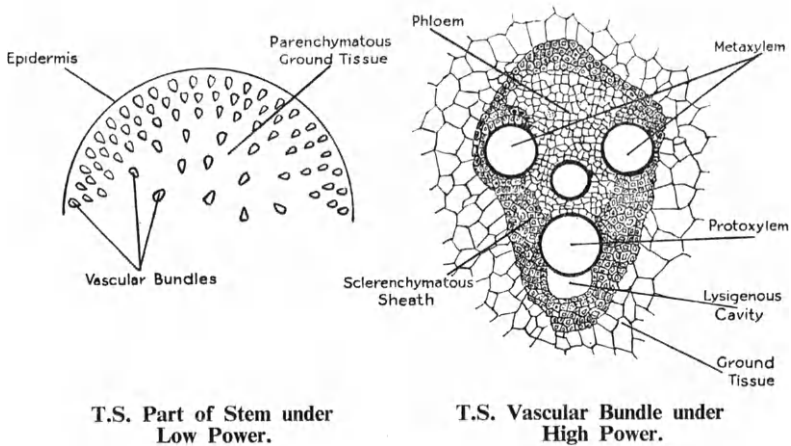
Fig. 201. T.S. Monocotyledonous Stem.

(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 with 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.

III. 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.** Between these layers will be
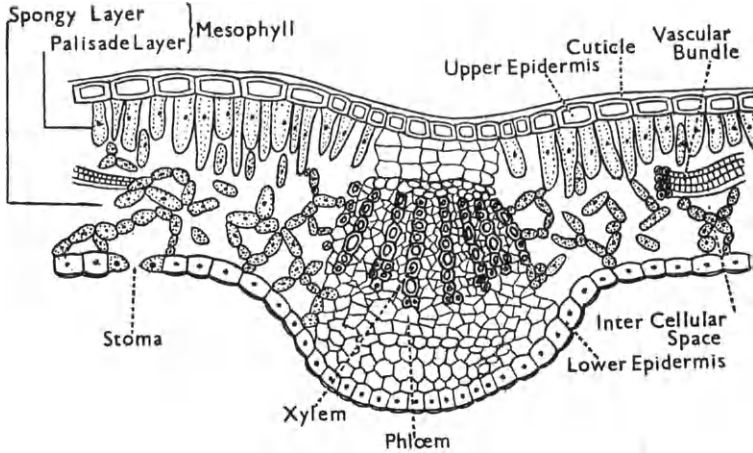
Fig. 202. **T.S. Part of Dicotyledonous Leaf.**

seen the veins or **vascular bundles** (in transverse section in the mid-rib 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** (with **guard cells).**

(ii) Surface View of Stomata

Macerate a leaf by boiling 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.**
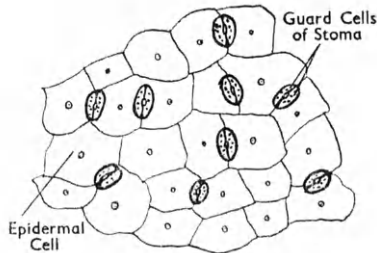
Fig. 203. **Epidermis of Leaf.**
Surface view.

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

IV. HISTOLOGY OF THE FLOWER

(1) **Structure of the 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*) *Examine a* **T.S. of a dehisced anther.** Compare this with the previous slide.

(2) **Structure of the Ovary**

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

Note the **ovary wall,** the **mid-rib,** the **vascular bundle** and the **placenta** to which is attached the **ovules.**

II. ANIMAL CYTOLOGY AND HISTOLOGY

INTRODUCTORY NOTES

Before examining the histological structure of organs, it is necessary to study the nature of the various tissues of which they are composed.

The frog provides good material for the study of animal histology, though in some cases it will be necessary to use tissues from other animals, while in others only prepared slides should be examined. A freshly killed animal dissected dry should be used for most preparations though the preserved dogfish or frog can be used for cartilage. *Make a few permanent preparations from the animals as you dissect them. In all cases permanent prepared slides should be examined.*

Refer to Part I for instructions on the making of slides and to the directions for drawing microscopical preparations on p. 2.

(I) THE STRUCTURE OF TISSUES

There are five chief kinds of animal tissue:—

        (1) Epithelial tissue.
        (2) Connective tissue.
        (3) Blood.
        (4) Muscular tissue.
        (5) Nervous tissue.

(1) EPITHELIAL TISSUES

Epithelium is the tissue which forms either the external covering of the body and its organs or the lining of such structures as the coelom, the alimentary

canal and the blood vessels. The latter are known as **endothelia.** Epithelium may consist of one layer of cells—**simple epithelium,** or of several layers—**compound epithelium.** The shape, structure and function of epithelial cells vary and the tissue may be classified as follows:—

Simple Epithelia	Compound Epithelia
Columnar	Transitional
Cubical	Stratified
Ciliated	Pseudo stratified
Squamous (or Pavement)	
Glandular	
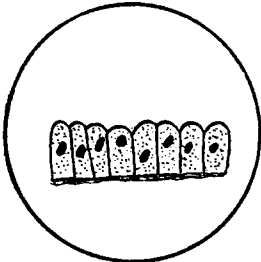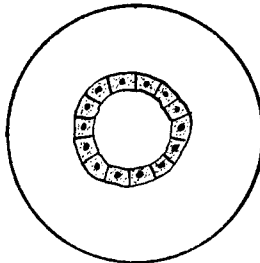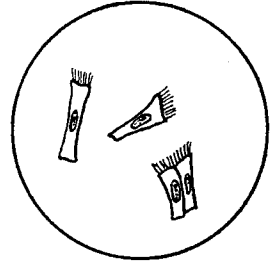
COLUMNAR   CUBICAL   CILIATED
                                                    (Isolated Cells)
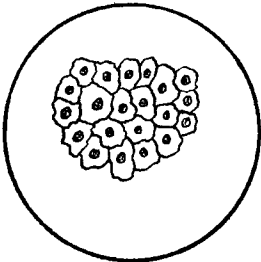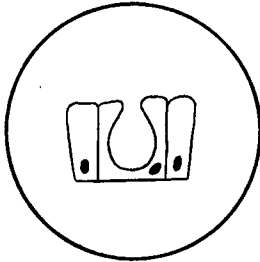
SQUAMOUS   GOBLET CELL   SIMPLE SACCULAR
                                                    GLAND
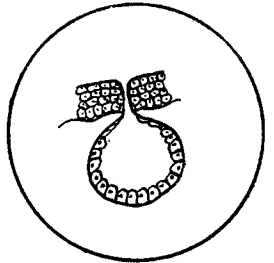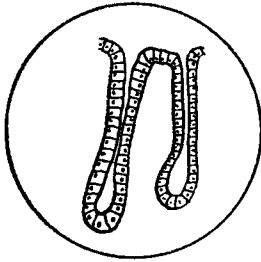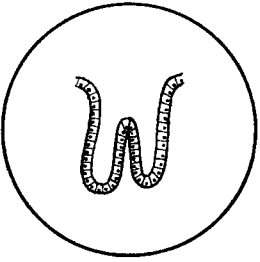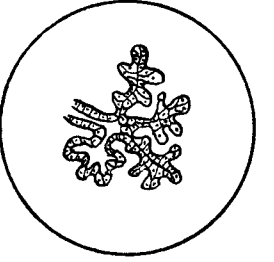
SIMPLE TUBULAR   BRANCHED TUBULAR GLAND   RACEMOSE GLAND
   GLAND

FIG. 204. **Simple Epithelia.**

SIMPLE EPITHELIA

(1) COLUMNAR EPITHELIUM

(i) *Take a small piece of the lining of the small intestine of the frog. Place in 2 per cent. osmium tetroxide for a few hours and then leave in in Ranvier's alcohol for twenty-four hours. Stain with picro-carmine. Mount in dilute glycerine and examine under the high power.*

Note the elongated column-like cells and their rather elongated **nuclei,** the cells standing on a **basement membrane.**

(ii) *Examine a prepared slide of* **T.S. stomach** or **intestine.**

In the **mucous membrane** note the single layer of elongated cells close together in columns and at right angles to the surface, which it lines, and resting on a non-cellular **basement membrane.** The **nuclei** are somewhat elongated.

(2) CUBICAL EPITHELIUM

Examine a prepared slide of a **T.S. of the kidney.**

Note the cubical cells and their central rounded **nuclei** lining the uriniferous tubules.

This kind of tissue also occurs as the lining of sweat and other glands.

(3) CILIATED EPITHELIUM

Examine under the high power a prepared slide of (*a*) *the* **membrane lining the roof of the mouth of the frog,** (*b*) *the* **trachea or bronchus of a mammal,** *or* (*c*) **isolated ciliated epithelial cells.**

The cells are columnar and bear on their free edges hair-like protoplasmic processes called **cilia.** Note also their **nuclei.** This tissue occurs characteristically in the bronchial passages.

(4) SQUAMOUS (or PAVEMENT) EPITHELIUM

(i) *Examine a prepared slide of* **squamous epithelium** (*e.g., from the* **mesentery of a mammal** *which has been treated with* 1 *per cent. silver nitrate and reduced in sunlight*).

Note the blackened outlines of the thin, flat cells which fit into one another like "crazy paving" and which have rather large **nuclei.**

(ii) *Carefully scrape the inside of your cheek with the back of a scalpel. Put the scrapings on a slide. Stain with methylene blue and examine under the high power. These squamous epithelial cells form the superficial layer of a stratified epithelium.*

When the edges of the cells are wavy as found in the peritoneum, the tissue is said to be **tessellated.**

(5) GLANDULAR EPITHELIUM

(i) *Examine a prepared slide of the* **T.S. of the ileum of a mammal.**

Look for cup-shaped **goblet cells** formed by the infolding of the walls of the columnar epithelial cells here and there in the mucous membrane.

(ii) *Examine a prepared slide of the* **T.S. of the frog's skin.**

Note the somewhat flask-shaped **simple saccular glands** opening on to the surface.

(iii) *Examine a prepared slide of the* **T.S. of the small intestine of the frog** *or* **mammal** *under the high power.*

Note the **simple tubular glands** lined by **columnar epithelial** and **secretory cells** in the mucous membrane.

(iv) *Examine a prepared slide of the* **T.S. of the stomach (pyloric end)** of a frog or mammal.

Note the **branched tubular glands** lined by columnar epithelial cells in the mucous membrane.

(v) *Examine a prepared slide of a* **T.S. of the gland of a salivary mammal.**

Observe the very much branched tubules constituting the compound saccular or **racemose gland** which are lined by **secretory cells.**

COMPOUND EPITHELIA

(6) TRANSITIONAL EPITHELIUM

Examine a prepared slide of a **section of the wall of the urinary bladder of a mammal.**

Note that the tissue consists of three or four apparent layers of cells, those on the surface being large and flattened. This tissue is capable of considerable stretching, when the number of layers is reduced, sometimes to a single layer.
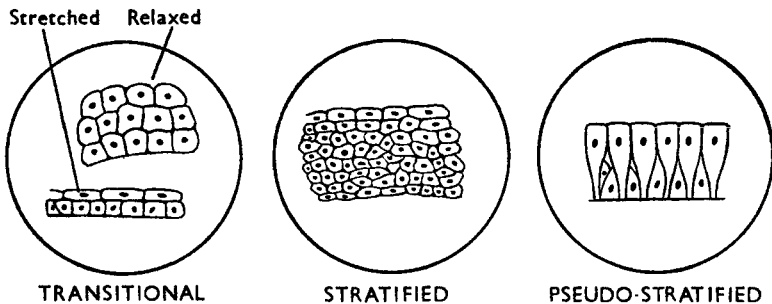
Fig. 205. Compound Epithelia.

(7) STRATIFIED EPITHELIUM

Examine again the prepared slide of the **T.S. of the skin of a frog** *or the* **T.S. of the skin of a mammal.**

Note that there are several layers of cells in the outer region (or **epidermis**). The dermis beneath is composed mainly of connective tissue. In the frog's dermis a number of black pigment cells of **melanophores** will also be seen.

(8) PSEUDO-STRATIFIED EPITHELIUM

Examine the prepared slide of the **T.S. of a mammalian salivary gland** *again. Examine one of the larger ducts.*

Note that although the cells lining it appear to be in several layers they all, in fact, lie on a basement membrane, though some do not reach up to the free surface.

(2) CONNECTIVE TISSUES

Connective Tissue joins various organs and structures together and its composition and histological structure varies with its function and location. In some cases it is purely skeletal. A characteristic feature is the presence of a non-cellular matrix in which the cells are embedded and which is secreted by the cells. The tissue is classified as follows:—

Fibrous Tissue	Adipose Tissue
Elastic Tissue	Cartilage
Areloar Tissue	Bone

(1) WHITE FIBROUS TISSUE

(i) *Pull up the connective tissue between the muscles of the thigh of a frog. Remove with small scissors and mount in physiological saline (0·75 per cent.). Examine under the high power.*

Note the white wavy bundles of thin fibres crossing each other in all directions. These are non-elastic.

Irrigate with 1 per cent. acetic acid.

The fibrillae disappear and the **connective tissue cells** become visible.

(ii) *Remove one of the white cords* **(tendons)** *attaching the leg muscles of the frog to the bone. Mount in physiological saline and tease out the fibres from one end with a mounted needle, holding the other end with another needle. Examine under the high power.*

Note the **white fibres** in parallel bundles and the **tendon cells** between them.

Irrigate with 1 per cent. acetic acid.

Note the **nuclei** of the tendon cells. The fibres swell up into a mass.

(2) YELLOW ELASTIC TISSUE

Examine a prepared slide of **elastic tissue from the ligamentum nuchae of the ox or other mammal.**

Note the coarse fibres which branch and anastomose at intervals.

This tissue rarely occurs pure but is found in abundance in these powerful ligaments of the neck.

FIBRILLAE

WHITE FIBROUS · YELLOW ELASTIC · AREOLAR (Yellow Fibres, White Fibres)

ADIPOSE · HYALINE CARTILAGE (Cartilage Cells, Matrix) · FIBRO CARTILAGE

ELASTIC CARTILAGE · BONE T.S. (Lacunae, Canaliculi, Lamella, Haversian Canals) · BONE L.S
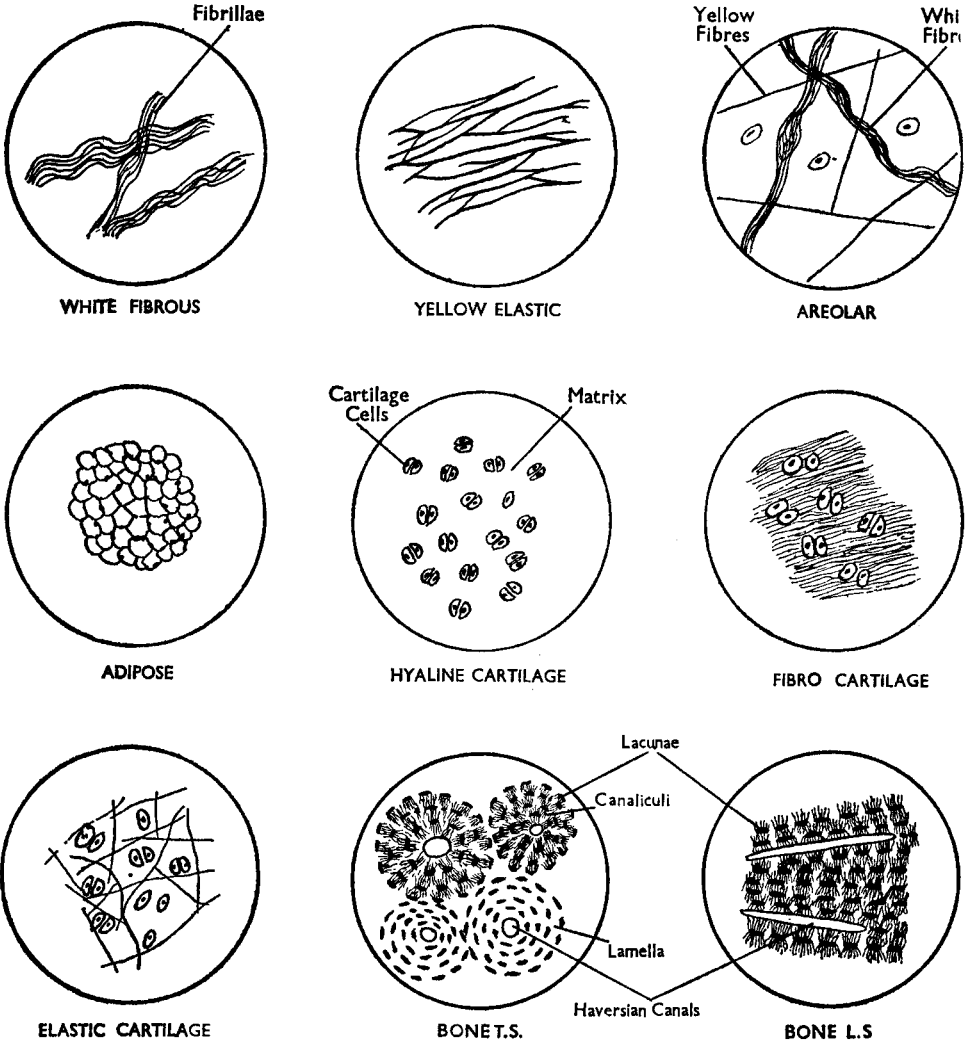
FIG. 206. Connective Tissues.

(3) AREOLAR TISSUE

This is the most frequently occurring tissue found in the body.

(i) *Examine a prepared slide of the* **subcutaneous tissue of a rabbit or rat** or (ii) *make your own preparation of this tissue, mounting it in physiological saline* (0·9 *per cent.*).

Note that it is composed of bundles of **white fibres** and **yellow elastic fibres,** the latter being lesser in quantity and thinner than those found in the pure tissue.

Fix in Bouin's fluid and stain with van Gieson's stain.

The white fibres are stained red and the elastic fibres dark brown.

(4) ADIPOSE TISSUE

(i) *Examine a prepared slide of the* **mesentery of a rat or other mammal,** or (ii) *Examine a prepared slide of the* **T.S. mammalin skin.** Note the concentration of rounded **fat cells** *or* (iii) *Take a small piece of* **mesentery** *or* **subcutaneous connective tissue.** *Fix in Bouin's fluid, stain with Sudan III or* 1 *per cent. osmium tetroxide.* The **fat cells** are stained red (Sudan III) or black (osmium tetroxide).

Note the connective tissue forming a meshwork between two layers of pavement epithelium in the spaces of which lie a number of rounded **fat cells.**

(5) HYALINE CARTILAGE

(i) *Remove one of the thin cartilages from the sternum of a frog or cut a thin section of cartilage from the chondrocranium of the dogfish. Mount in physiological saline and examine under the high power.*

Note the **cartilage cells** or **chondroblasts** with their **nuclei** lying in spaces called **lacunae,** some occurring singly, others in groups of two or four, embedded in a clear **matrix** composed of chondrin. It occurs in the costal cartilages, in the larynx and nose and, as articular cartilage, covering the ends of bones where they form joints.

Irrigate with 1 *per cent. acetic acid.*

The **nuclei** become more visible.

(ii) *Make a permanent preparation. Fix in formalin. Stain with Delafield's or, better, Ehrlich's haematoxylin. Differentiate in acid alcohol. Wash in water. Counterstain with Eosin (aq.) if desired, and wash in water. Dehydrate, clear and mount in balsam.*

(6) FIBRO-CARTILAGE

Examine a prepared slide of **fibro-cartilage.**

Note that it is a modification of hyaline cartilage in which **white fibres** are found in the matrix. It occurs in the intervertebral discs.

(7) ELASTIC CARTILAGE

Examine a prepared slide of **elastic cartilage.**

This is a modification of hyaline cartilage, in the matrix of which **yellow elastic fibres** abound. It occurs in the epiglottis, the pinna of the ear and in the septum at the free end of the nose.

(8) BONE

Bones formed by the deposition of bone substance in connective tissue are called **membrane bones** while those formed in cartilage are called **cartilage bones.** This is a difference in origin only; histologically, they are identical. The formation of bone in these tissues is known as **ossification.**

(i) *Examine a* **T.S. of bone.**

Note the large circular **Haversian canals** (which in the living animal contain blood vessels) surrounded by concentric rings, **lamellae** (in which calcium salts are deposited). Between each pair of lamellae are small cavities, **lacunae,** which contain bone cells, osteo-blasts, in the living animal. Tiny channels, **canaliculi,** run across and connect the lacunae. The Haversian canals and their lamellae consti-ute what are known as **Haversian systems.**

(ii) *Examine a* **L.S. of bone.**
Identify the structures in (i).

(iii) *Examine a section of* **membrane bone in process of ossification.** Look for (*a*) bundles of **osteogenic fibres** around which (*b*) bone cells or **osteoblasts** may be seen.

(iv) *Examine* **longitudinal sections of a long cartilage bone in process of ossification.** Look for (*a*) **unchanged cartilage,** (*b*) **cartilage undergoing calcification and disintegration,** the cartilage cells becoming smaller, large amoeboid, multinucleate **osteoblasts** effecting this erosion, and bone cells, **osteoblasts,** which lay down the bone substance. Note that bone *replaces* the cartilage which has been destroyed.

(3) BLOOD*

(1) AMPHIBIAN BLOOD

To make successful blood films, it is essential that slides and coverslips are free from grease.

(i) *Take a drop of frog's blood on a coverslip. Dilute with a drop of physiological saline* (0·6 *per cent.*†) *and examine under the high power.*

Note the numerous **erythrocytes** (or **red corpuscles**), pale red or

*Sometimes classified as a Connective Tissue, the plasma corresponding with the matrix.
†Some prefer to use 0·75 per cent. saline.

yellowish in colour, oval in shape and nucleated. Those seen from their edges show that they are biconvex.

Irrigate with 1 per cent. acetic acid.

The **nuclei** become more easily visible. Look for **leucocytes** (or **colourless corpuscles**). They are much fewer in number, and are smaller than the red. They are granular, nucleated and amoeboid. The red corpuscles and leucocytes are suspended in a colourless fluid, the **plasma.**
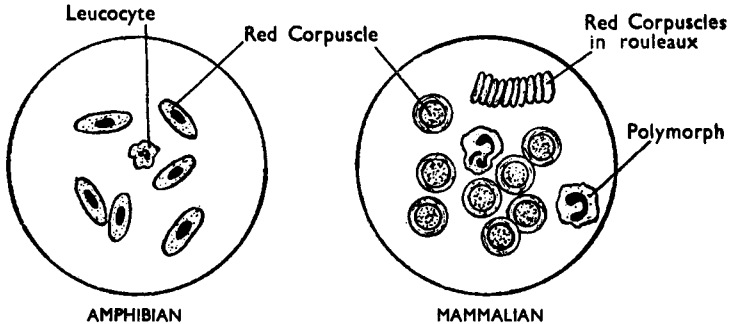
FIG. 207. **Blood.**

(ii) *Put a drop of frog's blood on a coverslip and make a smear. Fix in absolute alcohol and stain for three or four minutes in alcoholic methylene blue. Wash off the excess of stain and mount.*

The **nuclei** of the **red corpuscles** and **leucocytes** will be readily seen.

(2) MAMMALIAN BLOOD

(i) *Clean the tip of your left fore-finger with spirit and sterilise a sharp mounted needle in the flame. Quickly prick the tip of the finger. Squeeze a drop of blood on to a coverslip and invert it quickly on a slide. Examine under the high power.*

Note the circular **erythrocytes (red corpuscles),** smaller than those of the frog, occurring singly and **in rouleaux** (*i.e.,* like piles of coins). They are not nucleated, but, being biconcave discs, the central part appears light. Some **leucocytes (white corpuscles)** may be seen. They are larger and fewer than the erythrocytes, are nucleated and exhibit amoeboid movement. There are about 600 or 700 red corpuscles to every leucocyte.

(ii) *Make another preparation as in* (i) *but dilute with physiological saline* (0·6 *per cent.**).

The red corpuscles will be more easily examined.

*Some prefer to use 0·75 per cent. saline.

(iii) *Prepare a smear of human blood, fix at once by waving rapidly in the air, and stain with methylene blue or Leishman's stain.*

The nucleated **leucocytes** will be visible. There are several kinds of leucocytes. *Search for the various types. You are certain to find* **polymorphs** *because they are the most numerous, and probably one or two* **lymphocytes** (see below), *but you may be unable to identify other types. The colours given below are given with Leishman's stain.*

(*a*) **Polymorphs.** The greater proportion of the leucocytes (65-70 per cent.), somewhat larger than the erythrocytes. The nucleus, stained purplish red, is composed of several lobes or is roughly U-shaped with the free ends lobose. Their chief function is phagocytosis.

(*b*) **Lymphocytes.** About the same size as the erythrocytes and form 20-25 per cent. of the leucocytes. The nucleus, stained blue, is round.

(*c*) **Acidophils** (or **eosinophils**). Somewhat larger than polymorphs, they form only about 3 per cent. of the leucocytes. The nucleus, stained red, may consist of two or three lobes.

(*d*) **Basophils**, distinctly larger than polymorphs, form a mere 0·5 per cent. of the total leucocytes and have a bi-lobed nucleus which stains a purplish colour.

(*e*) **Monocytes** are the largest of the leucocytes and form about 3 per cent. of them. The nucleus, which is unstained, is usually round. These are the most actively motile of the leucocytes and their function is phagocytosis.

The **Erythrocytes** will be stained an orange-pink colour.

Blood platelets (or **thrombocytes**), small, rounded structures, stained purplish (colourless when unstained), about a quarter of the size of the erythrocytes *may* be visible in the plasma. *A specially prepared stained slide should be examined in order to see these.* They are concerned with coagulation.

The average number of erythrocytes in man is 5,000,000, per cu. mm. (4,500,000 in woman) and their size is about $7 \cdot 5\mu$. In the rabbit they are somewhat small (about $6 \cdot 5\mu$) while in the frog they measure about $22 \cdot 3\mu \times 15 \cdot 7\mu$.

The average number of leucocytes is man is 7,000–8,000 per cu. mm., but the number varies at different ages, at different times and under different conditions. Thus the proportion of red corpuscles to leucocytes is about 600 or 700:1. In size they vary between 9μ and 20μ.

(4) MUSCULAR TISSUES

Muscles are composed of **fibres** which are of three kinds:—

(1) **Striated** (or **striped**) found in the voluntary muscles.

(2) **Unstriated** (**unstriped, plain** or **smooth**) found in the involuntary muscles in the wall of the alimentary canal, blood vessels, urinary bladder, ureters, etc.

(3) **Cardiac** found only in the walls of the heart.

(1) STRIATED MUSCLE

(i) *Remove a small piece of one of the muscles of the frog's leg. Tease out the fibres with mounted needles on a slide and crush some of them. Mount in physiological saline (0·75 per cent.). Examine under the high power.*

Note the long cylindrical **fibres** which show alternate light and dark transverse bands. Each is enclosed in a membranous sheath, the **sarcolemma,** which will be better seen where the fibres have been crushed.

Irrigate with 1 per cent. acetic acid.

STRIATED   UNSTRIATED   CARDIAC

FIG. 208. **Muscular Tissues.**

The striations will be less distinct but the oval **nuclei** are now rendered more visible, scattered in the fibre, showing that it is not a single cell but a **syncytium.** Actually, each fibre is composed of a series of longitudinally running **myofibrillae,** between which is the cytoplasmic **sarcoplasm.**

(ii) *Remove one of the leg muscles of a cockroach. Tease out the fibres and mount in physiological saline (0·6 per cent.). Examine.*

It will be found to show a similar structure to that of the frog, though the striations are wider.

(iii) *Make a permanent preparation of striated muscle from the frog, the cockroach or the dogfish. Fix in formaldehyde. Stain with borax-carmine.*

(2) UNSTRIATED MUSCLE

Spread a piece of the frog's urinary bladder on a slide. Rub off the epithelium from the inside with a camel's hair brush or with your finger. Fix in formaldehyde and stain with haematoxylin or eosin. Make a permanent mount. Examine under the high power.

Note the spindle-shaped **fibres** dove-tailing into one another, and their centrally placed **nuclei.** They are devoid of sarcolemma.

(3) CARDIAC MUSCLE

Examine a prepared slide of **cardiac muscle.**

Note the striated fibres with central nuclei, the striations being less distinct than in striated muscle, the fibres being devoid of any sarcolemma and being shorter than the fibres of voluntary muscle. Also observe that the fibres branch and join neighbouring fibres.

(5) NERVOUS TISSUES

Nervous tissue is composed of:—

(1) **Nerve Cells** or **Neurones** found in the brain and spinal cord and in ganglia.

(2) **Nerve Fibres,** which are of two kinds:—

    (*a*) **Medullated fibres** found in bundles in the cranial and spinal nerves.

    (*b*) **Non-medullated fibres** which occur in the terminal portions of the sympathetic nerves though they are also found in some of the peripheral nerves.
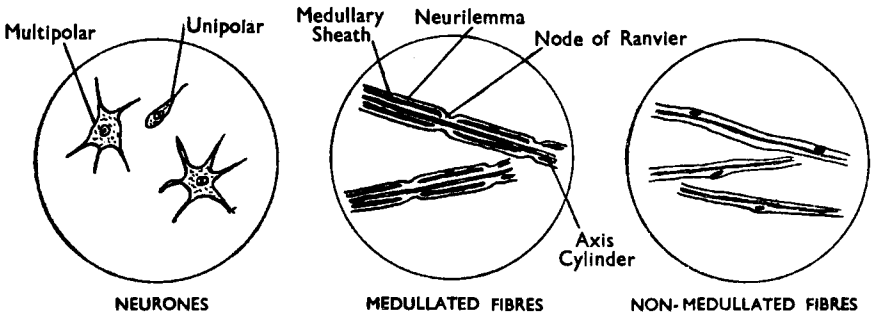
FIG. 209. Nervous Tissues.

(1) NERVE CELLS (NEURONES)

Examine a prepared slide of **nerve cells from the brain or spinal cord.**

Note the large **multipolar nerve cells** or **neurones,** each composed of a **cell-body** containing a large **nucleus** in which will be seen a **nucleolus,** with small elongated bodies, **Nissl's granules,** in the cytoplasm. The cell body bears long non-branching processes, **axons,** (which become the axis cylinders of nerve fibres) and short processes called **dendrons,** which bear small branches called **dendrites.** These form synapses with the dendrites of neighbouring neurones. The cells around the neurones which serve as packing tissue between them are known as **neuroglia.**

(2) MEDULLATED NERVE FIBRES

Cut out a short piece of the sciatic nerve from the leg of a frog. Put on a slide and tease out one end with a mounted needle. Add physiological saline (0·75 per cent.). Examine.

Note the long unbranched **nerve fibres.**

Irrigate with 1 per cent. osmium tetroxide and examine a quarter of an hour later.

Note the **axis cylinder** in the centre (a continuation of the axon of a neurone) surrounded by the fatty **medullary sheath** (stained black), outside which is the thin **neurilemma,** or primitive sheath. The medullary sheath shows interruptions at intervals, known as **nodes of Ranvier.** The **sheath nuclei** in the cytoplasm lining the neurilemma in the internodes may not be visible.

(3) NON-MEDULLATED NERVE FIBRES

Examine a prepared slide of **non-medullated nerve fibres from the sympathetic nervous system.**

They are similar to the medullated nerve fibres except that they have no medullary sheath, they have several nuclei and they often branch and anastomose.

(4) NERVE ENDINGS IN MUSCLE

Examine a prepared slide showing nerve endings in striated muscle.

Note the **medullated fibres** which branch two or three times, each branch running to a **muscle fibre** where they terminate in **end-plates.** In unstriated and cardiac muscle, the fibres, non-medullated at their terminations, branch and end in plexuses.

(II). THE STRUCTURE OF ORGANS

A microscopical examination of the structure of certain mammalian organs should now be made. They will be found to consist of more than one kind of tissue.

In the various sections examined, blood vessels and lymphatics will be seen, mostly in transverse section. The wall of an **artery** is composed of (*a*) an **external coat** of **areolar tissue,** (*b*) a **middle coat** of **unstriated muscle fibres** and, in the case of the larger arteries such as the aorta and the carotids, **elastic tissue** and (*c*) an **inner coat** of **elastic tissue** lined by an **endothelium** of pavement epithelium.

The wall of a **vein** is similar but thinner, there being less muscular and elastic tissue in the middle coat and less elastic tissue in the inner coat. The valves which occur at intervals are strengthened infoldings of the inner coat.

The walls of **capillaries** are thin and are composed of endothelium only in which the cells are flattened.

The walls of **lymphatics** are in general similar to those of veins but are much thinner.

(1) THE SKIN

(i) *Examine a* **T.S. of mammalian skin.**

The external layer, the **epidermis,** is composed of stratified epithelium, with the **stratum corneum** (or horny layer) consisting of rows of flattened cells on the outside and, beneath it, in some parts of the skin, a layer of granulated appearance, the **stratum granulosum.** This is followed by a somewhat wavy layer, the **stratum Malpighii.** There are no blood vessels in the epidermis. The stratum corneum consists mostly of epithelial "cells" called squames, which are devoid
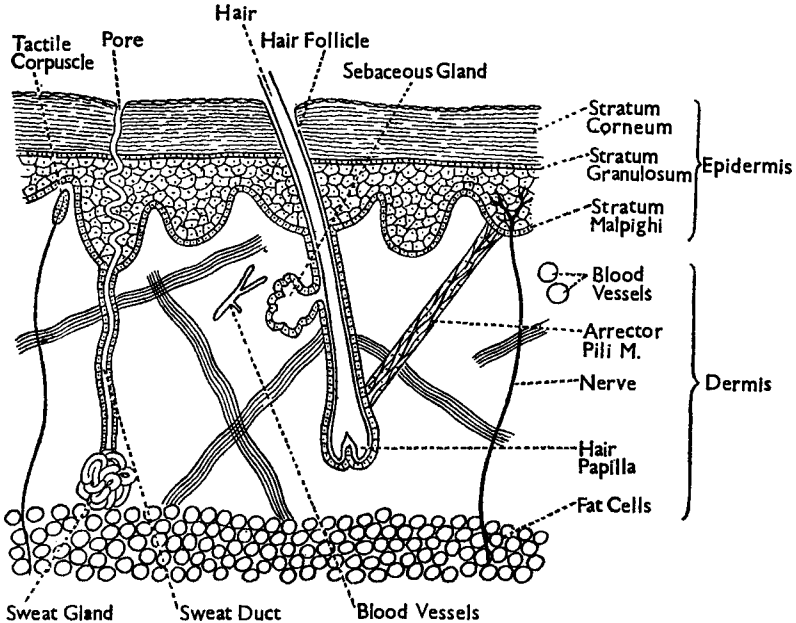
FIG. 210. **Mammalian Skin. T.S. Semi-diagrammatic.**

of nuclei. The stratum Malpighii is the growing region and the cells in its lower layers show mitosis. The deepest cells of this layer contain the pigment melanin abundantly in dark-skinned races. The thickness of the epidermis and particularly of the stratum corneum varies considerably in different parts of the body.

Beneath the epidermis is the true skin or **dermis.** This is a much thicker layer composed of **connective tissue,** denser towards the outside where it projects into **papillae** containing elastic tissue which cause the Malpighian layer to have its wavy appearance. Blood **capillaries** will be seen in the dermis with loops from them passing into the papillae. **Nerve fibres** will also be found terminating in sense organs in some of the papillae. These sense organs are the bulbous **tactile corpuscles,** though in certain parts such as the deeper layers of the hands and feet, the larger **Pacinian bodies** are found. Situated deep in the dermis are the **sudorific** or **sweat glands** composed of coiled tubes each provided with a duct, straight at first but spiral in the epidermis. It opens on the surface as one of the **pores** of the skin. Parts of the ducts only will be visible, of course. In the deepest layers of the dermis where the tissue is less dense is a quantity of **fat cells.**

(ii) *Examine a* **T.S. of the skin from the scalp.**

Note the **hairs,** which are epidermal in origin, each consisting of (*a*) a bulbous **root** into the base of which projects, like the bottom of a wine bottle, a small vascular **hair papilla** (this is the growing point), and (*b*) the **hair shaft.** Each hair is multicellular and hollow, and consists of an outer **cuticle** and an inner **medulla,** and is embedded in a pit-like **hair follicle** epidermal in origin. Each hair follicle is provided with (*a*) an **arrector pili** muscle near its base, composed of unstriated fibres and (*b*) one or more **sebaceous glands,** which secrete the oily sebum into the hair follicle.

Nails, claws and hoofs are thickenings of the stratum corneum and originate from the Malpighian layer. They contain a substance known as *keratin.*

Examine a slide of the **T.S. of the skin of the dogfish.**

As in the mammal the skin consists of epidermis and dermis. In the lower region of the **epidermis** is the Malpighian Layer, external to which are the cells to which it gives rise, the outermost ones being flattened and dead. The dermis is composed chiefly of connective
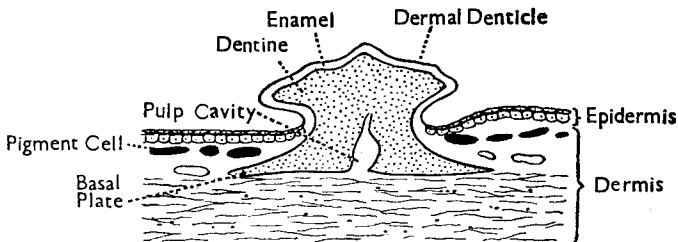
FIG. 211. **Dogfish Skin. T.S.**

tissue containing blood vessels and nerves, and pigment cells are found in its upper region immediately beneath the epidermis. **Dermal denticles** are seen with their **basal plates** in the dermis and from these the backwardly directed **spines** arise and project through the epidermis. Each dermal denticle is composed mainly of **dentine** which in the **spine** is covered with **enamel** and there is a **pulp cavity** in the centre.

Examine a slide of the **T.S. of the frog's skin.**

The **epidermis** consists of a Malpighian layer, followed by the cells arising from it, the outermost cells being flattened and periodically cast off. The **dermis** is composed of **connective tissue** and bundles of **unstriated muscle fibres** and contains blood vessels and
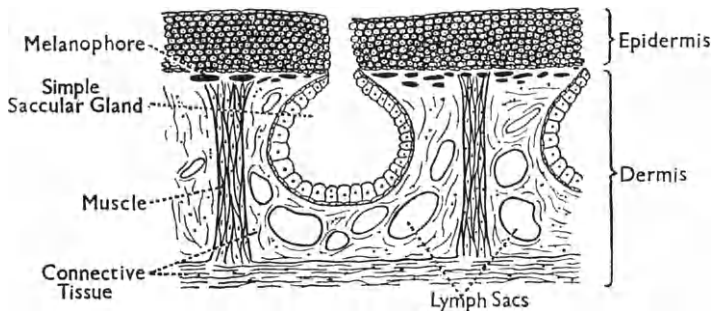
FIG. 212. **Frog Skin. T.S.**

nerves. Pigment cells or **melanophores** occur immediately below the epidermis. **Simple saccular glands**, which are epidermal in origin, abound and these secrete the watery mucus which keeps the surface of the skin moist for purposes of respiration. Some of the glands secrete a bitter fluid which serves to protect the animal against capture by its enemies. Also in the dermis is a number of **lymph sacs** and it will be remembered that large sub-cutaneous lymph spaces lie between the skin and the muscular body wall.

(2) A TOOTH

Examine a **L.S. of a tooth.**

Note the **crown** separated from the **root** by the **neck**. The crown is covered by **enamel** and the root by **cement**, somewhat similar to bone in that it contains lacunae and canaliculi but deficient of Haversian canals. The greater part of the tooth is composed of **dentine** also somewhat similar to bone but devoid of Haversian canals. The **pulp**

cavity in the centre contains pulp which is composed of connective tissue and contains blood vessels and nerve fibres which enter the tooth through a small hole in the root.
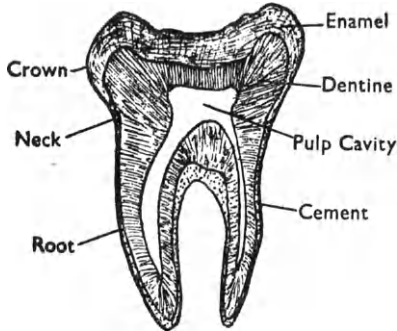
FIG. 213. L.S. Mammalian Tooth.

(3) THE TONGUE

Examine **transverse sections of the tongue.**

Note (*a*) the **filiform papillae,** conical in shape and edged (in man) with filaments of epithelium, (*b*) **the fungiform papillae** amongst the filiform variety and larger in size and (*c*) the **circumvallate papillae,** large and circular and surrounded by a narrow moat. (These are not found in the rabbit's tongue, **foliate papillae** being present.) They form a V-shape on the tongue of man with the apex directed backwards and two small oval patches on each side at the back of the tongue in the rabbit. The fungiform and circumvallate papillae consist of **connective tissue** enclosed in **stratified epithelium** in which are ovoid **taste-buds.** These are composed of groups of cells of two kinds, the long spindle-shaped **gustatory cells** in the centre, each with a **taste hairlet** on its free end and opening in the narrow **gustatory pore,** and the long flattened **sustentacular cells** around them which give them support.
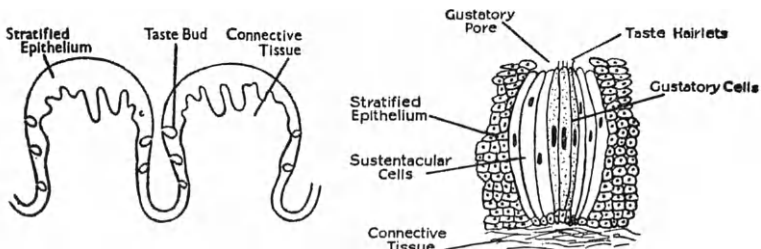
FIG. 214. T.S. Tongue.

(4) SALIVARY GLANDS

Examine a **T.S. of one of the salivary glands.**

As already seen, it is a racemose gland. The **lobules** of which it is composed consist of small sac-like or tubular structures called **alveoli,** lined by **secretory cells,** and from which small ducts lined by cubical epithelium arise which later join others to form the main duct of the gland. The lobules are bound together by **connective tissue.** The secretory cells are mainly of two kinds—mucous cells which secrete mucin and serous cells which secrete the enzyme ptyalin. The submaxillary and sublingual glands are mixed, but the parotids contain only serous cells.
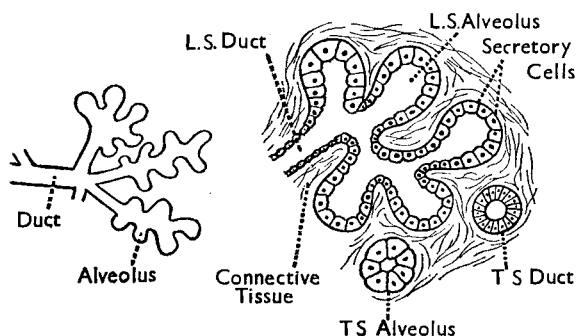
FIG. 215.   T.S. Salivary Gland.

(5) THE OESOPHAGUS

Examine a **T.S. of the oesophagus.**

It is composed of an outer coat of **connective tissue,** inside which is a **muscular layer** composed of striated fibres in the upper end of the oesophagus and unstriated fibres in the lower end. Next comes a **submucous layer** consisting of areolar tissue and finally the **mucous membrane.**

(6) THE STOMACH

Examine **transverse sections of the wall of the stomach in the cardial and pyloric regions.**

This consists of four coats. (*a*) The outer coat is the **serous coat** (or **peritoneal layer**). (*b*) The **muscular coat** is composed of an outer layer of **longitudinal fibres,** a middle layer of **circular fibres** and an inner layer of **oblique fibres.** These are all unstriated. (*c*) The **submucous coat** is composed of areolar tissue and contains **blood vessels**

and **lymphatics.** (*d*) The **mucous membrane** is lined by **columnar epithelium** which dips down to form the ducts of the **gastric glands.** These are simple or branched tubular glands and the epithelial cells secrete mucus. The **peptic cells** in these glands secrete pepsin and the
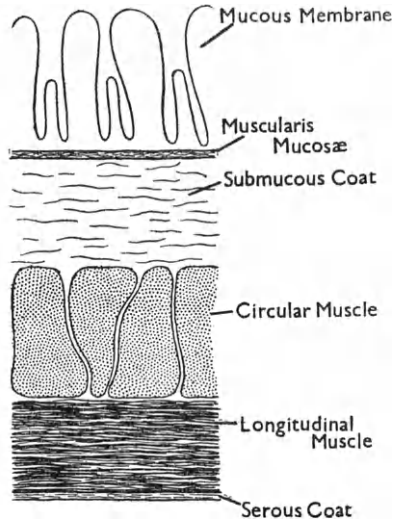
FIG. 216.   **T.S.   Stomach.**

ovoid **oxyntic cells** scattered amongst them secrete hydrochloric acid. The latter are not found in sections taken from the pyloric end of the stomach where the glands have longer glandular portions but shorter
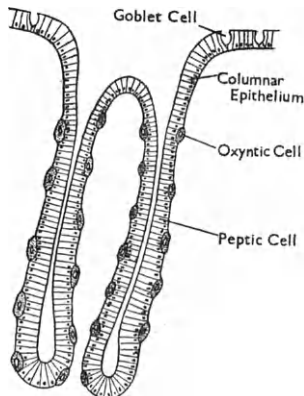
FIG. 217.   **Gastric Gland.**

ducts. The outermost region of the mucous membrane next to the sub-mucous coat consists of longitudinal and circular unstriated muscle fibres and this is known as the **muscularis mucosae.**

(7) THE DUODENUM

Examine a **T.S. of the duodenum.**

It is composed of (*a*) an outer **serous coat** (*b*) a **muscular coat** composed of two layers only, the outer being narrow and consisting of **longitudinal fibres** while the inner is thick and contains only **circular fibres** (*c*) a **submucous coat** composed of areolar tissue and containing blood vessels and lymphatics and (*d*) the **mucous membrane.** The outer region of the mucous membrane next to the submucous coat is a thin layer of muscle fibres, the external one being longitudinal and the internal circular. This is known as the **muscularis mucosae.**

The mucous membrane is extended into the cavity of the duodenum in finger-like processes called **villi** between which are simple tubular glands, the **crypts of Lieberkühn,** lined by columnar epithelium and containing **goblet cells.** Each villus contains blood capillaries and a lymphatic known as a **lacteal.**

In addition small racemose glands will be found in the submucous coat; these are **Brünner's glands.** Their ducts lead either into or between the crypts of Lieberkühn.
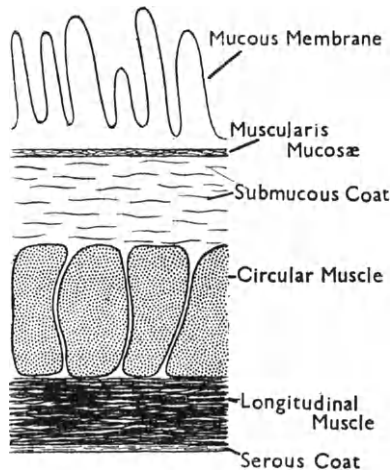
FIG. 218.   T.S. Duodenum.
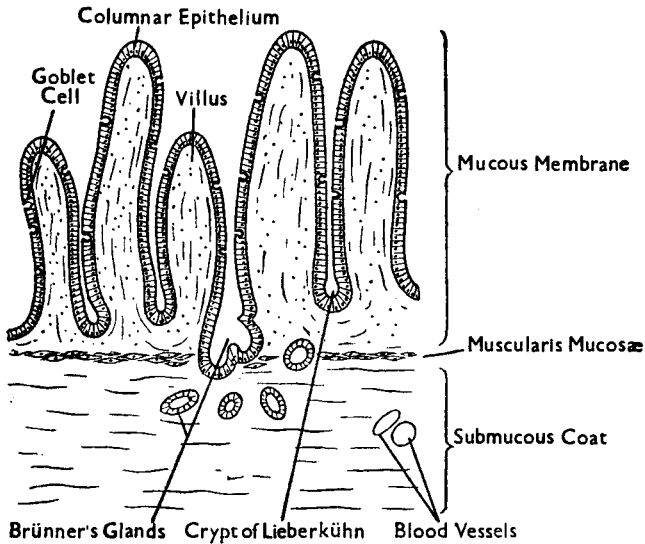
FIG. 219. T.S. Duodenum. Mucous Membrane and Submucous Coat.

(8) THE ILEUM

Examine a **T.S. of the ileum.**

In general structure this is similar to the duodenum consisting of **serous, muscular** and **submucous coats** and **mucous membrane. Villi**
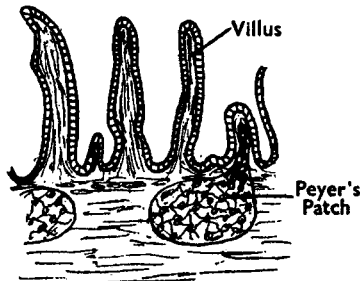
FIG. 220. T.S. Ileum.

and **crypts of Lieberkühn** are present, but there are no Brünner's glands. In addition, small nodules of lymphoid tissue (connective tissue in which the intercellular portions contain lymphocytes from the blood) called **Peyer's patches,** will be found in the mucous membrane below the crypts of Lieberkühn.

(11) THE PANCREAS

Examine a **T.S. of the pancreas.**

This is a racemose gland somewhat similar to the salivary glands but with longer **alveoli**. It also contains isolated rounded groups of small cells, endocrine in function, which secrete insulin and which are known as the islets **of Langerhans.**
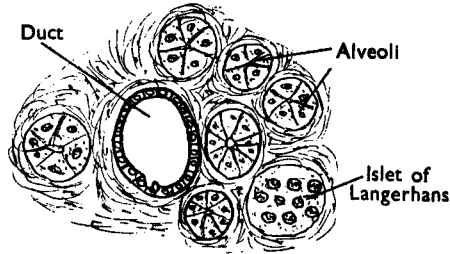
FIG. 223. T.S. Pancreas.

(12) THYROID GLAND

Examine a prepared slide of **a T.S. of the thyroid gland.**

Note the oval or rounded **vesicles** lined by **cubical epithelium** and supported by **connective tissue.** The vesicles contain a colloid which may be stained. Plexuses of **blood capillaries** enclose the vesicles and may be seen throughout the section.
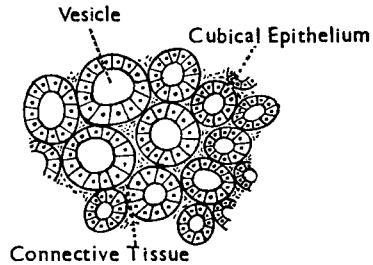
FIG. 224. T.S. Thyroid Gland.

(13) THE KIDNEY

Examine a **L.S. of the kidney.**

This compound tubular gland consists of an outer **cortex** and an inner **medulla** both containing the **uriniferous tubules,** which are lined for the greater part by cubical epithelium. These originate in the **Malpighian bodies** in the cortex, each Malpighian body consisting of a curved widened cup which is the beginning of the tubule and known as **Bowman's capsule.** It surrounds and encloses a tuft of blood capillaries known as **glomerulus.** Leaving the capsule, the tubule is at first convoluted and then passes as an almost straight tube, the **descending limb,** into the medulla. Here it bends back on

itself forming the **loop of Henle** and then runs, almost parallel with the descending limb, back into the cortex as the **ascending limb.** When it reaches the level of the Malpighian body, on the side opposite to
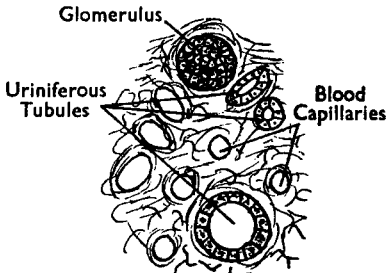
FIG. 225. **Kidney. Section under high power.**

that by which it left it, it again becomes convoluted and then passes across to join a straight **collecting tube** which runs down into the medulla. This is joined by other collecting tubules and then becomes wider to form the **duct of Bellini,** which opens into the **pelvis** of the kidney. The collecting tubules are bound together to form the **pyramids** of the kidney which bulge into the pelvis. In the rabbit there is only one pyramid, but in man there are several.

(14) THE TESTIS

(i) *Examine a* **T.S. of the testis.**

Note the larger number of **seminiferous tubules** lined by **germinal epithelium** and bound together by connective tissue in which may be
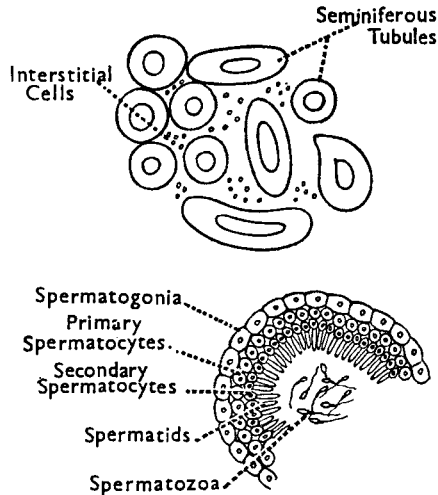
FIG. 226. **T.S. Testis.**

seen **interstitial cells** which secrete the hormones testosterone and androkinin. *If a tubule is carefully examined under the high power or, better, the* $\frac{1}{12}$ *O.I. objective*, stages in **spermatogenesis** may be found. Next to the germinal epithelium are the **spermatogonia.** These divide to give rise to **primary spermatocytes** and these by meiosis to **secondary spermatocytes** which will be in the next layer. Nearer the lumen of the tubule are the small **spermatids**, formed by division of the secondary spermatocytes and, finally, in the cavity of the tubule itself, **spermatozoa** into which the spermatids develop. All stages are unlikely to be seen as they do not necessarily occur at the same time.

(ii) *Examine a slide of* **spermatozoa.** *under high power or, better, under* $\frac{1}{12}''$ *O.I. objective.*

Note that each consists of a **head** (ovoid and pointed in man and the monkey, but rod-like in the earthworm and frog—there is considerable variation of shape in different animals), a **middle-piece** (or **body**) and a long tail.
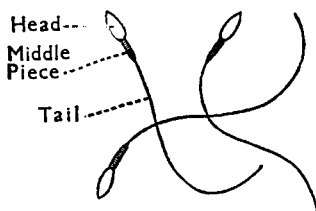
Fig. 227. **Spermatozoa.**

(15) THE OVARY

(i) *Examine a* **T.S. of the ovary.**

The peritoneal covering is known as **germinal epithelium.** Inside this is mostly connective tissue constituting the **stroma** in which will be seen **Graafian follicles** in various stages of development. These arise from the germinal epithelium and those towards the outside will be small. If a larger and more mature follicle is examined it will be seen to contain two layers of stratified epithelium, one lining the cavity and the follicle, the **membrana granulosa,** the other, the **discus proligerus,** surrounding an **ovum.** Stages in **oogenesis** *may be seen if examined under high power*. The **oogonia** arise from the **follicle cells** and divide into **primary oocytes** and each of these by meiotic division into a **second oocyte** and a **first polar body.** The secondary oocyte divides unequally into an **ovum** and a **second polar body,** while the first polar body also divides. However, complete maturation of the ovum usually takes place after liberation by the bursting of the follicle when the oocyte passes into the Fallopian tube. After the discharge of the oocyte, the follicle cells form a yellow body known as the **corpus luteum.**
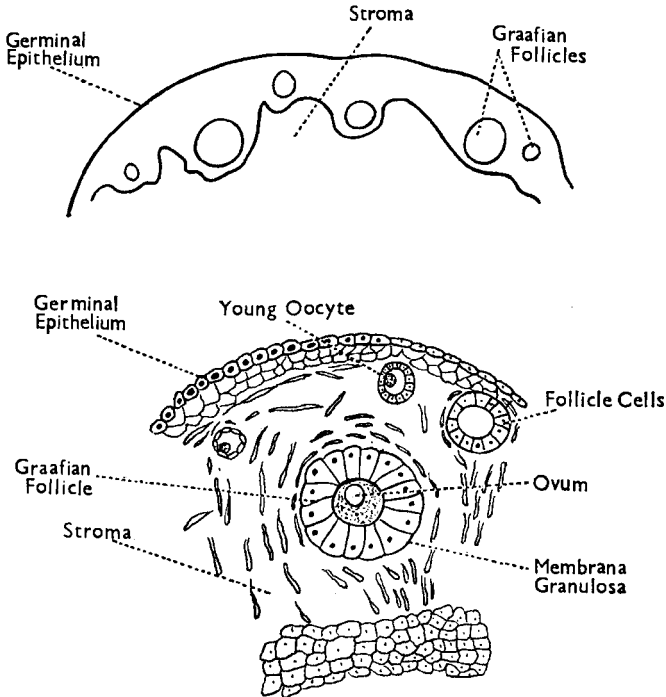
FIG. 228. T.S. Ovary.

(ii) *Examine a slide of* **a mature ovum.**
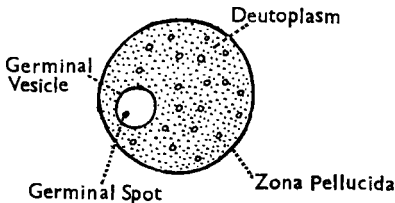
FIG. 229. Mammalian Ovum.

The cell is spherical and is enclosed in a thick transparent membrane, the **zona pellucida.** In the enclosed cytoplasm are **yolk spherules** or **deutoplasm** (not in the rabbit) and a large nucleus called the **germinal vesicle** in which is a distinct nucleolus known as the **germinal spot.**

(16) MAMMARY GLAND

Examine a **T.S. of a mammary gland.**

This is a compound racemose gland and in most mammals it is a modified sweat gland. The saccular **alveoli** are lined by columnar epithelium when milk is being secreted and **milk globules** may be seen

in the alveoli. After secretion, however, these cells become flattened and fill up the alveoli where they may be seen. These are the conditions found in a lactating animal, otherwise the alveoli are fewer in number and smaller. **Ducts** lead from the alveoli to the surface of the teat (which contains unstriated muscle fibres) and near their outer terminations before entering the teat each swells into a small sac, the **sinus lactiferus.**

(17) MUSCLE

Examine a **T.S. of a voluntary muscle.**

Note the bundles or **fasciculi** of **muscle fibres** surrounded and bound together by connective tissue called the **perimysium.** The tissue between the fibres is called the **endomysium** and the external covering of the muscle itself the **epimysium.**

(18) NERVE

Examine a **T.S. of the sciatic nerve of a frog or mammal.**

Note the bundles, **fasciculi** (or **funiculi**), of **nerve fibres** bounded by connective tissue · called the **perineurium.** Between the fibres in the bundles is connective tissue called the **endoneurium** while the external coat of the whole nerve is known as the **epineurium.** The **axis cylinders** of the nerve fibres will be seen in their centres.
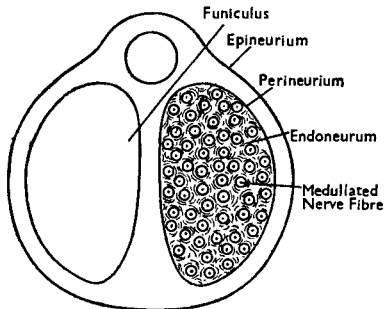
FIG. 230. T.S. Medullated Nerve. (Frog).

(19) SPINAL CORD

Examine a **T.S. of the spinal cord** *under the low power.*

Note the outer **white matter** surrounding the central **grey matter** which is in the shape of a rough letter "H". In the centre of the cross-piece of the H is the **central canal.** The deep **ventral fissure** and the narrower **dorsal fissure** will be recognised in the white matter.

The dorsal limbs of the H-shaped grey matter are known as the **dorsal horns** (or **cornua**), and the ventral limbs as the **ventral horns** (or **cornua**). From them originate the spinal nerves. Outside the spinal cord, the **dorsal root** bears a **ganglion,** but the **ventral root** does not. Just after the ganglion, the two roots join to form the **spinal nerve.**

Examine under the high power.

The white matter is composed of **medullated nerve fibres** (seen in T.S.) amongst which is a tissue composed of cells and fibres and known as **neuroglia.**

In the grey matter, **neurones** and **neuroglia** are found.

The extent to which these structures are visible will depend on the method of staining used in the preparation of the slide.

The whole spinal cord, like the brain, is invested in three membranes, the **meninges.** The thin vascular **pia mater** is the innermost and closely invests the cord, the **arachnoid,** separated from the pia mater by the subarachnoid space, is the middle membrane, while the thick **dura mater** on the outside lines the neural canal of the vertebral column. The only one likely to be present in the specimen is the pia mater.

PART IV

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 biology, (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 the next part of the book.

(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 and animal tissues early in his studies. 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.

(5) Further biochemical experiments are included in Part V (Physiology).

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 heterogenous 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 true solutions diffuse through membranes, the process being known as **osmosis,** but 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** and the diffusion of a solvent through a semi-permeable membrane is known as **osmosis.**

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.**

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*The size of colloidal particles varies between $1m\mu$ and 1μ ($\mu = 1$ *micron* = 0·001 mm. $m\mu = 1$ *millimicron* = 0·000001 mm. $\mu\mu = 1$ *micromicron* = 0·0000000001 mm.^{2})

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.
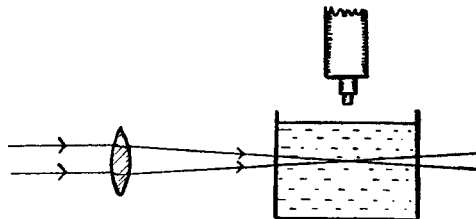
FIG. 231. The Tyndall Effect. Principle of the Ultra-microscope.

CRYSTALLOIDS AND COLLOIDS

EXPERIMENT 1. *Cover a few small 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 until the solution cools. 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.*
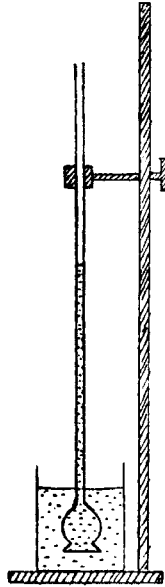
FIG. 232. **Apparatus to demonstrate Osmosis.**

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. Using a pipette carefully run some sugar solution (which may 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.

*This is not a perfect semi-permeable membrane for sugar and water as it allows a small quantity of sugar to pass through it.

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.*
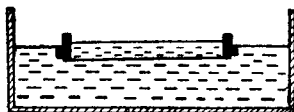
FIG.   233. Dialyser.

II. THE CHEMICAL PROPERTIES OF BIOCHEMICAL COMPOUNDS

PROTEINS

Proteins are compounds of carbon, oxygen, hydrogen and nitrogen. Many of them also contain sulphur and some contain phosphorus. They 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 **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 molecule. All give the "biuret" reaction (Experiment 11 below). This is due to the NH—CO group. Those containing tyrosine also 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 11. **"Biuret" Test:** (*a*) *Add excess of sodium hydroxide solution and then a few drops of weak* (1 *per cent.*) *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

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*An amino-acid is an organic acid in which a hydrogen atom attached to the carbon atom is replaced by an amino group (—NH$_2$). Amino-acetic acid or *glycine* (CH$_2$NH$_2$·COOH) is the simplest. Amino-propionic acid or *alanine* is CH$_3$CH·NH$_2$COOH. *Tyrosine* and *cystine* are further examples.

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 12. **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 13. **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.

CARBOHYDRATES

Carbohydrates are compounds of carbon, hydrogen and oxygen, the hydrogen and oxygen being 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 monosaccharide hexose 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 $(C_6H_{10}O_5)_n$.

(a) 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 14. **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.

(b) Monosaccharides (Hexoses—sugars with six carbon atoms in the molecule)

General Test for Monosaccharides

EXPERIMENT 15. **Barfoed's Test:** *Add about* 1 *ml. of Barfoed's solution and boil.* A red precipitate of cuprous oxide is formed.

Glucose

EXPERIMENT 16. **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 17. **Benedict's Test:** (Used instead of Fehling's reaction for detecting the presence of glucose in urine because ammonia and creatinine, invariably present in urine, dissolve suprous oxide and so render Fehling's test less sensitive.) *Add a few millilitres of Benedict's qualitative reagent to about 1 ml. of glucose solution and boil for two or three minutes.* A yellow precipitate is formed and the solution turns green.

Fructose

EXPERIMENT 18. *Perform (a) Barfoed's and (b) Fehling's Tests with a solution of fructose.*

(c) Disaccharides*

Maltose

EXPERIMENT 19. *Carry out (a) Barfoed's and (b) Fehling's Tests with a solution of maltose.*

Lactose

EXPERIMENT 20. *Carry out (a) Barfoed's and (b) Fehling's Tests with a solution of lactose.*

Sucrose

EXPERIMENT 21. *Perform (a) Barfoed's and (b) Fehling's Tests with a solution of sucrose.*

EXPERIMENT 22. *Hydrolyse some sucrose by boiling a solution with dilute hydrochloric or sulphuric acid for a few minutes and allowing it to stand for five minutes or so. Then neutralise by adding some caustic soda solution (test with litmus paper) and perform Fehling's Test.*

(d) 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.

EXPERIMENT 23. *Perform Fehling's Test.*

EXPERIMENT 24. *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 25. *Hydrolyse some starch by boiling with dilute sulphuric acid for a few minutes: neutralise by adding caustic soda*

*The reducing property of this and other sugars is due to the presence of an aldehyde (—CHO) radicle in the molecule.
†Negative results will be obtained in some of the following experiments,

solution (test with litmus paper). Divide the 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 26. *Perform Fehling's Test.*

EXPERIMENT 27. *Add iodine solution.* Observe that a red colour is obtained.

EXPERIMENT 28. *Hydrolise 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.*

Inulin (Dahlia Starch)
EXPERIMENT 29. *To an aqueous solution of inulin, add some iodine solution.*

EXPERIMENT 30. *Add a few drops of a saturated alcoholic solution of orcein followed by concentrated hydrochloric acid and then boil.*

EXPERIMENT 31. *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 32. *Put a few small pieces of filter-paper (almost pure cellulose) into Schweitzer's Reagent (Cuprammonia), prepared as follows:—Add some caustic soda solution to a solution of copper sulphate. Filter and dissolve the precipitate in ammonium hydroxide.*
Leave the cellulose in the reagent for a while and note the effect.

EXPERIMENT 33. *Put some filter paper in strong iodine solution and after a few minutes' immersion, examine under the microscope.*

EXPERIMENT 34. *Mount some filter paper in Schultze's solution and note the effect.*

Lignin
EXPERIMENT 35. *Cut sections of a woody stem or use a piece of wooden match. 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°C. On hydrolysis, a fat decomposes into glycerol and the fatty acid thus:—

$$(C_{15}H_{31}COO)_3C_3H_5 + 3H_2O = 3C_{15}H_{31}COOH + C_3H_5(OH)_3$$

Tripalmitin   Palmitic acid   Glycerol
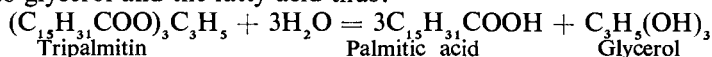
The so-called "essential", "ethereal" or "volatile" oils such as oil of turpentine, oil of cloves, oil of lavender are not lipides though some of their reactions are similar.

Fats and Oils

EXPERIMENT 36. (*a*) *Add a little 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 37. *Add some ether to some olive oil in a test tube and shake. Note that the oil dissolves. Then pour some of this liquid on to a piece of filter paper and examine again when the ether has evaporated.*

EXPERIMENT 38. *Add two drops of* 1 *per cent. solution of osmium tetroxide ("Osmic Acid") to a few drops of olive oil in a watch glass. Leave for a few minutes when* a black colour will develop.

EXPERIMENT 39. *Repeat Experiments 37 and 38 with some oil of cloves or other volatile oil.*

TO TEST FOR THE PRESENCE OF PROTEINS, CARBO-HYDRATES AND LIPIDES IN SOLID SUBSTANCES OR LIQUIDS

EXPERIMENT 40. *Tests for proteins, carbohydrates and fats or oils should now be carried out with substances provided by the laboratory.*

The following scheme for their detection is suggested but any other convenient method can, of course, 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 or Lactose.**† NO PRECIPITATE = **Sucrose** or **Polysaccharide.**
(c) **Test for Lactose** *Add conc. nitric acid. Evaporate to small bulk in porcelain basin in fume chamber.*	SEPARATION OF CRYSTALS = **Lactose.** NO SEPARATION OF CRYSTALS = **Maltose.**

(d) **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 concentrated 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 to 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 these 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.

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. 312.

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 43. **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 44. **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	Axerophtol	Fat	Liver oils of fish (e.g., cod, halibut), milk, eggs.	Promotes growth prevents night-blindness and protects epitherlia against infection.	Derivative of caro-tene ($C_{40}H_{56}$) which is therefore known as provitamin A. Not destroyed by heat.
B	A Complex of several vitamins. About twelve are known. The most important are given below:—				
B_1	Aneurin (Thiamin)	Water	Germ - layer of seeds, fruits and vegetables.	Anti-neuritic. Prevents beri-beri in man and poly-neuritis in birds. Also concerned with release of energy from car-bohydrates.	Removed by milling and therefore ab-sent from white flour but present in whole meal flour. Absent from polished rice.
B_2	Riboflavin	Water	⎫	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	Eggs, yeast, fruits,	Prevents dermatitis.	
B_7	Nicotinic Acid	Water	vegetables, liver, milk and cheese.	Concerned with growth and the prevention of pellegra.	
B_{12}	Cyanoco-balamin	Water		Necessary for the formation of blood corpuscles and used in the treatment of per-nicious anaemia, also for growth and reproduction.	
C	Ascorbic Acid	Water	Fresh fruits and vegetables, par-ticularly in citrus fruits and black currants.	Anti-infective. Anti-scorbutic. Deficiency causes scurvy.	Can be synthesised by mammals. De-stroyed 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 or ergo-sterol ($C_{37}H_{41}OH$) in ergot and yeast. There are at least two other D vita-mins. Can be syn-thesised 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 fer-tility in women and certain lower animals, e.g., cows and rats. Defi-ciency causes sterility.	
H	Biotin	Water	Liver, milk, eggs, vegetables.	Prevents dermati-tis.	
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 en-able blood capilla-ries 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 V

PHYSIOLOGY

INTRODUCTORY NOTES

Experiments should be performed to illustrate the physiology of plants and animals. The following experiments are not elaborate and can be carried out at any suitable time. It is suggested, however, that the most appropriate time for plants is during the study of the flowering plant and for animals during the study of the mammal.

It is not suggested that the experiments explain fully the details of the physiological processes. They are adequate, however, for the purpose for which they are intended. Those in small type may be omitted if time does not allow them to be performed.

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. 239, p. 324). 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 or animal, 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.

The physiological processes which will be studied are *nutrition, respiration, excretion, growth, irritability* and *movement.*

The various methods of transport of substances in the plant and animal body, the properties and action of hormones, pigments and other substances and the properties and part played by the inorganic environment, the air and, in plants, the soil, are included under this heading.

I. 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.

(I.) NUTRITION IN GREEN PLANTS

HOLOPHYTIC NUTRITION

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.

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.

This process is not by any means explained by the simple equation

$$6CO_2 + 6H_2O + Energy = C_6H_{12}O_6 + 6H_2O$$

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.

(1) 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 into boiling water and then transfer them to hot alcohol. The chlorophyll will dissolve in the alcohol so that the leaves become white. 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).*

(2) 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.*

(3) 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 pieces of black (light-proof) paper, out of which identical letters, figures or a design has been cut. Fix the two pieces of paper together with the cut portions over one another, using paper clips. 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. Place the plant in the light. After several hours remove the paper or cork, decolourise the leaf and test with iodine.*
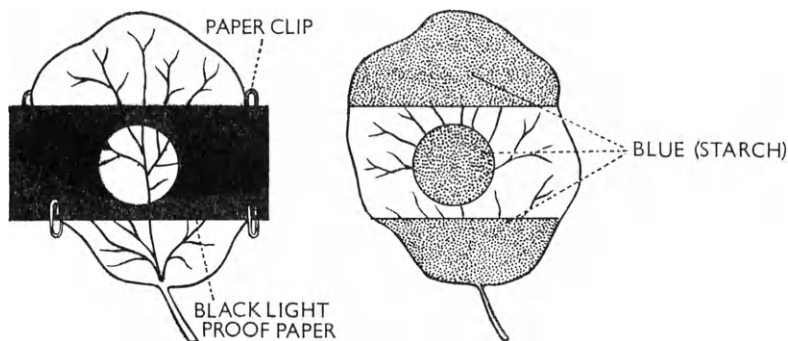
FIG. 234. Starch print.

(4) 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.

(5) 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.*

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.

(6) 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 *to be performed later*, it will be seen that when the food of the plant lacks iron, the plant also lacks chlorophyll.)

(7) 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 few hours (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 in the air in the jar or formed in respiration *if the experiment is left overnight. As a "control" set up a similar*
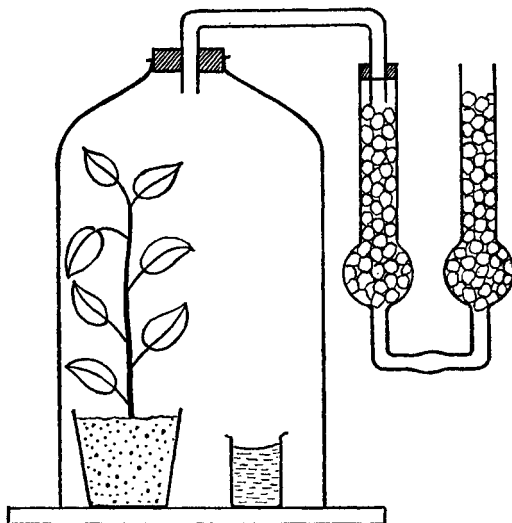
FIG. 235. **Apparatus to demonstrate the necessity of Carbon Dioxide for Photosynthesis.**

experiment omitting the caustic soda and soda-lime or substituting water and pumice respectively. Expose the plants to sunlight and a few hours later remove a leaf from each, decolourise and test for starch.

(8) 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 sun-light 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 left in the light, bubbles rising from the leaves. *When there is sufficient gas test it with a glowing splint.*
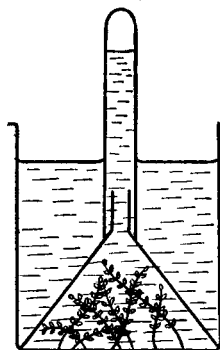
FIG. 236. **Apparatus to show that Oxygen is given off in Photosynthesis.**

EXPERIMENT 9. *Prepare some reduced indigo-carmine by adding a solution of sodium hydrosulphite to a solution of indigo-carmine (see Appendix I, p. 461) until the blue colour is discharged. Do not shake. Put some Canadian pond weed (Elodea) into a clean test-tube, then, using a pipette, run about 20 ml. of the reduced indigo-carmine into the test-tube, taking care to avoid contact with the atmosphere as much as possible. Should any blue colour appear add more sodium hydro-sulphite until it disappears. Make sure that the test-tube is filled with the liquid. Carefully pour some reduced indigo-sulphate into a clean beaker and invert the test-tube in the liquid in the beaker. Place the experiment in bright light. As a control place a similar apparatus in the dark. Very soon a blue colour will appear coming from the leaves of the plant left in the light. This is due to the oxidation of the indigo-sulphate brought about by oxygen liberated from the leaves. No such colour change occurs in the control.*

(9) To demonstrate the action of Diastase

EXPERIMENT 10. *Prepare some starch sol as indicated in Part IV (Biochemistry), p. 307 (d). Polysaccharides. Add a small quantity of the enzyme diastase to some starch sol in a test-tube. Put some iodine solution in the cavities of a cavity tile. Later at intervals add a drop of the contents of the test-tube to the iodine in the cavities. Eventually no blue colour will be obtained. Then carry out Fehling's and Barfoed's*

Tests with the liquid in the test-tube. The results will show that a disaccharide sugar is present. The starch has been hydrolysed by the diastase to maltose.

MICROCHEMICAL TESTS FOR FOOD RESERVES

EXPERIMENT 11. *Cut sections of various plant organs such as seeds (e.g., sunflower, maize, broad bean and castor oil) and fruits and (a) perform tests for proteins, sugars, starches and oils in test-tubes or on microscopical slides. Then examine the sections under the microscope. (b) Mount new sections in Schultze's solution and examine the cell contents.* Proteins are stained yellow, and starch blue. (Note, incidentally, that the cell walls are also stained—cellulose blue or violet and lignin yellow.) (c) *Mount fresh sections in Sudan III.* Oil is stained red.

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 0·002mm.

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.

(1) To make a Mechanical Analysis of the Soil

EXPERIMENT 1. *Either (a) take a large 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.*
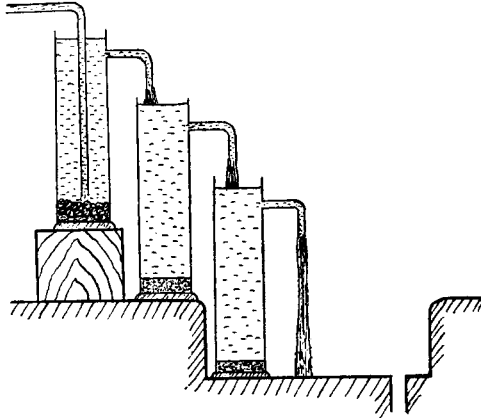
FIG. 237. Apparatus for the Mechanical Analysis of Soil.

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 most
probably have been washed over into the sink.

(2) To find the Percentage Weight of Water in a Sample of Soil

EXPERIMENT 2. *Counterbalance two pieces of dry 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.*

(3) 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. Note the colour of the soil after heating. It is
terra cotta (which means burnt earth).*

(4) 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.*

(5) 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. Pour water on to the sand.*

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

FIG. 238. **Apparatus to show the permeability to air of sand and clay.**

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. This indicates the extent to which air is passing through the three samples and therefore the extent of permeability to air. After a fixed time, measure the volumes of water in each beaker.

(6) 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.

(II.) NUTRITION IN ANIMALS

HOLOZOIC NUTRITION

Animals are unable to synthesis the proteins, carbohydrates and fats which they require and therefore feed on organic material, *i.e.*, on plants or other animals or both. This is known as **holozoic nutrition.** The organic food must be changed from its insoluble solid form into a liquid condition in which it can be absorbed and assimilated. This is effected by the enzymes in the process of *digestion*. It must then be transported to other parts of the animal.

DIGESTION

THE ACTION OF ENZYMES

Enzymes are complex substances produced by living organisms which induce certain chemical reactions essential to the life of the organism. They survive after the reaction and may therefore be called *biological catalysts.* Furthermore, they can function outside the organism of their origin. The chief characteristics of enzymes may be summarised as follows:—

(1) A small quantity of the enzyme will effect change in a large amount of the substrate.

(2) They act only within a certain range of temperature and are destroyed above 60°C.

(3) They act only in certain H-ion concentrations and each has its optimum pH.

(4) They are specific in their action.

(5) Some are inactive until combined with a co-enzyme.

(6) Their action varies with the concentration of the substrate.

The nomenclature of enzymes is rather confused by lack of co-ordination in practice, but the principle intended to be followed by biochemists is to add the suffix **-ase** to the name of the substrate: thus:—

Proteinases act upon proteins.
Amylases act upon starch.   } These are found in plants as well as in animals.
Lipases act on fats.

But many names which do not follow this rule are retained owing to long-established usage, for example:—

Ptyalin in saliva is an amylase, **pepsin** and **rennin** in gastric juice, **trypsin** from pancreatic juice and **erepsin** in intestinal juice are

proteinases. **Invertase** hydrolyses cane sugar to invert sugar. **Diastase** is an amylase found in plants, while **zymase,** found in yeast, is an enzyme complex responsible for the fermentation of sugar.

Perform the following experiments in clean test-tubes, label them, plug them with cotton wool and place them in a thermostat (see Appendix II) kept at body temperature (38° to 40°C.). From the results you will discover which substances are digested by the different juices and the conditions under which the enzymes act.

(1) DIGESTION IN THE MOUTH
Saliva

Chew a piece of rubber tubing for a few minutes: this will produce a copious supply of saliva. Transfer some to a test-tube and dilute with an equal volume of distilled water (it will act more quickly). Filter a portion.

EXPERIMENT 1. *Test the reaction of saliva with a piece of litmus paper.*

EXPERIMENT 2. *(a) To some filtered saliva add a drop of dilute acetic acid.* Mucin, a viscous muco-protein, is precipitated. *(b) Perform Millon's test to show the presence of the protein, mucin.*

The Action of Ptyalin

EXPERIMENT 3. *Add some diluted saliva to (a) some starch sol, (b) a small quantity of solid albumin or finely chopped lean meat, (c) a few drops of olive oil. Label the tubes and place them in the thermostat (see Appendix II) kept at 38°–40°C. Shake periodically. Later examine and test samples of (a) with iodine every five minutes on a white cavity tile until no colour change is produced, then test the residue with Fehling's solution.*

(2) DIGESTION IN THE STOMACH
Gastric Juice

In the following experiments use Artificial Gastric Juice (see Appendix I).

EXPERIMENT 4. *Find the reaction of gastric juice with litmus.*

The Action of Pepsin and Chymase (Rennin)

EXPERIMENT 5. *Add a large quantity of gastric juice to a small quantity of (a) finely chopped lean meat or solid albumin, (b) starch sol, (c) a few drops of oil. Label the tubes and place them in the thermostat. Shake periodically. Examine a few hours later.*

Then carry out the biuret test with the residue in (a).

EXPERIMENT 6. *Repeat experiment 5 (a) with gastric juice which has been made alkaline with a little caustic soda.*

EXPERIMENT 7. *(a) Add a little chymase (rennin) to some milk, diluted with an equal volume of water. Place in the thermostat and examine a few minutes later. (b) Replace the tube in the thermostat, leave it for an hour and then examine again.*

EXPERIMENT 8. *Repeat Experiment 5 (a) or 7 but first heat the enzymes to about 70°C. for a few minutes. Note the effect of this temperature.*

(3) DIGESTION IN THE DUODENUM
Pancreatic Juice

In the following experiments use Artificial Pancreatic Juice (see Appendix I).

EXPERIMENT 9. *Test the reaction of pancreatic juice with litmus.*

The Action of Trypsin, Amylase and Lipase

EXPERIMENT 10. *Repeat Experiments 5 (a), (b) and (c), using pancreatic juice.*

Test (b) with iodine at intervals of five minutes on a white cavity tile. When no colour change is observed, test the residual solution with Fehling's solution. Test the reaction of (c) with litmus solution when digestion appears to be complete.

EXPERIMENT 11. *Acidify some pancreatic juice with dilute hydrochloric acid and repeat Experiment 10.*

Bile Pigments

The two chief pigments of bile, which is not a digestive juice because it is devoid of enzymes, are breakdown products of haemoglobin. They are **bilirubin** ($C_{33}N_{36}O_6N_4$) which is reddish-brown and **biliverdin** ($C_{33}H_{31}O_8N_4$), an oxidation product of bilirubin, which is green.

EXPERIMENT 12. **Gmelin's Test for Bile Pigments.** To some *ox bile, diluted with an equal volume of water, slowly add some fuming nitric acid, sloping the test tube so that the acid forms a lower layer.* A play of colours results at the junction of the two liquids. *Note these colours in the order of their appearance.*

(4) TO TEST FOR THE PRESENCE OF PROTEINS, CARBO-HYDRATES AND LIPIDES IN FOODSTUFFS

EXPERIMENT 13. *Tests for proteins, carbohydrates and lipides should now be performed with various foodstuffs such as meat, fish, vegetables, cereals, fruits, cheese and milk.*

Milk

EXPERIMENT 14. *Examine a few drops of milk under the low and then the high power of the microscope. Note that it is an emulsion.*

EXPERIMENT 15. *To a few millilitres of milk add an equal volume of water and a few drops of glacial acetic acid. Shake thoroughly. The* **casein** *is precipitated, together with fat globules. Filter. (a) Dissolve the residue in dilute caustic soda and test for* **protein.** *(b) Boil the filtrate. The* **lactalbumin** *coagulates. Filter. (c) Make a suspension of the residue in water (it is insoluble) and test for* **protein.** *(d) Test the filtrate for* **lactose** *by applying Fehling's Test.*

II. RESPIRATION

(1) RESPIRATION IN PLANTS

Respiration may be defined as the breaking down of sugar into simpler substances for the purpose of liberating energy for the organism.

(1) **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. Into the other (B) put an equal quantity of pea seeds previously killed by boiling and add a little formalin to prevent decomposition: this is the "Control." 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.*
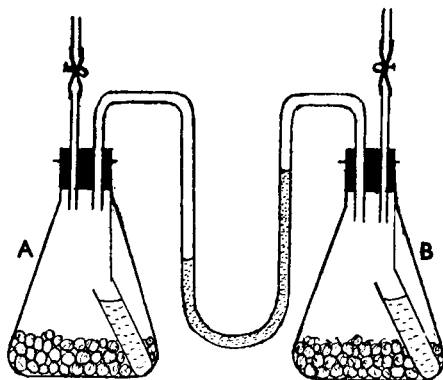
FIG. 239. **Apparatus to demonstrate the Aërobic Respiration of Germinating Seeds.**

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.*

(2) 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 with black paper to prevent photosynthesis from taking place. Make all joints air-tight and set the pump working to draw air slowly through the apparatus.*
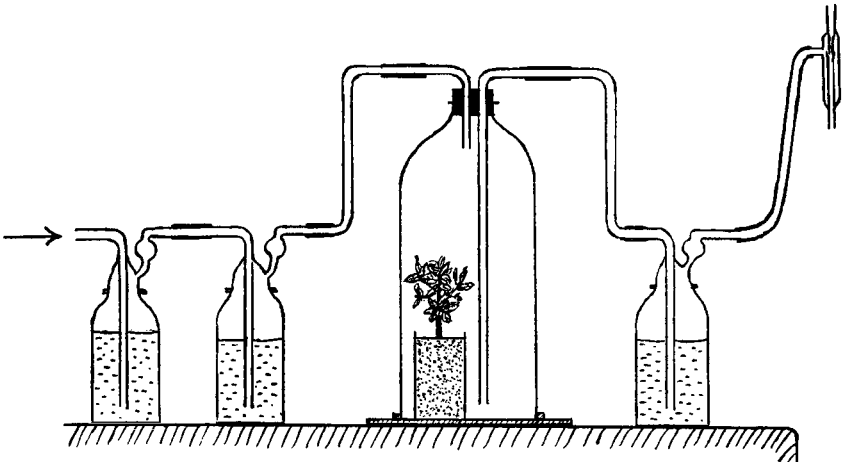
FIG. 240. Apparatus to demonstrate the Aërobic Respiration of a Green Plant.

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 turn milky showing that carbon has been given off by the plant.

(3) To show that Oxygen is necessary for Respiration

EXPERIMENT 3. *Take a small retort (respiroscope) 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*). *This absorbs oxygen from the air. Alternatively use an inverted U-tube (respiroscope) with the soaked seeds with some wet cotton wool in one limb closed with a cork, while the other stands in the solution. Observe whether the seeds germinate and draw your conclusion.*

(4) 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.*

(5) 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 experiment,*
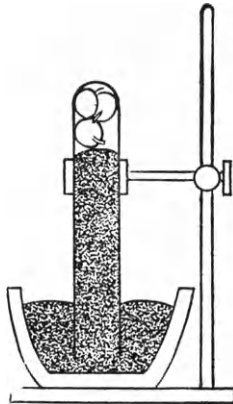
FIG. 242. Apparatus to Demonstrate Anaërobic Respiration.

using killed seeds as a "control". Examine the tubes later. The mercury level will have fallen in the tube containing the living seeds. *Insert a few pellets of caustic potash into the tube or remove the tube with your finger over the open end, invert and insert a rod with a drop of lime-water on the end into the gas. Observe what happens.*

(6) To show that Heat is set free 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.*

FIG. 243. Apparatus to show that Heat is liberated in Respiration.

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 over two or three days.

(7) 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 $\dfrac{\text{output of } CO_2}{\text{intake of } O_2}$ which is known as the **Respiratory Quotient.**
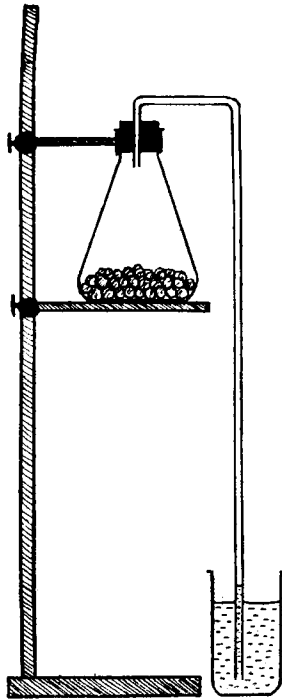
Fig. 244.   Apparatus to Measure the Respiratory Quotient.

(2) RESPIRATION IN ANIMALS

(1) TO DEMONSTRATE THE RESPIRATION
OF ANIMALS

(1) Expiration of Carbon Dioxide

EXPERIMENT 1. *Connect a Dreschel bottle containing caustic potash solution to a second Dreschel bottle containing lime-water. Then connect the second bottle to the inlet-tube of a bell-jar or flask. This should be fitted with a short inlet-tube and a long outlet-tube reaching almost to the bottom. If a bell-jar is used, the flange should be well greased with vaseline and it should stand on a ground-glass plate. Connect the outlet-tube to a Dreschel bottle of lime-water and join this to a water-pump by means of pressure tubing.*

Now place a small animal (e.g., a frog) under the bell-jar or several large earthworms in the flask and set the pump working.
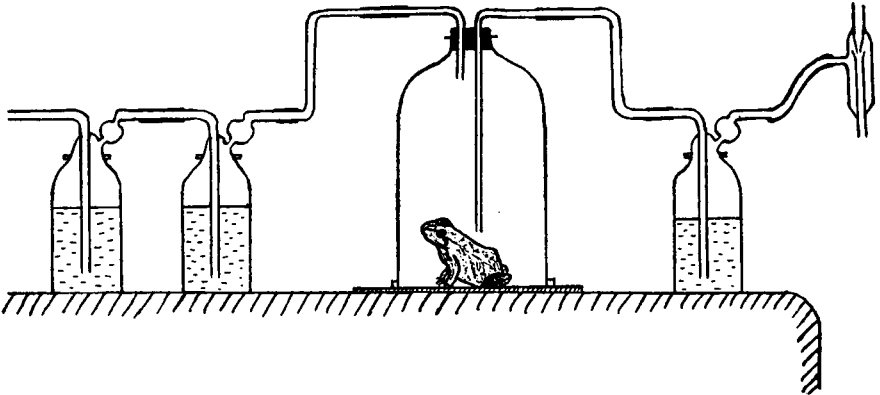
FIG. 245. **Apparatus to Demonstrate the Expiration of Carbon Dioxide by an Animal.**

The caustic potash in the first Dreschel bottle absorbs the carbon dioxide from the air which enters, as shown by the fact that the lime-water in the next bottle remains clear. *Examine the lime-water in the last Dreschel bottle and draw your conclusion.*

(2) Inspiration of Oxygen

EXPERIMENT 2. *Fit a conical flask with a cork through which passes a short piece of glass fitted with a short rubber tube provided with a clip and a double right-angle tube bent further to form a U-tube manometer.*
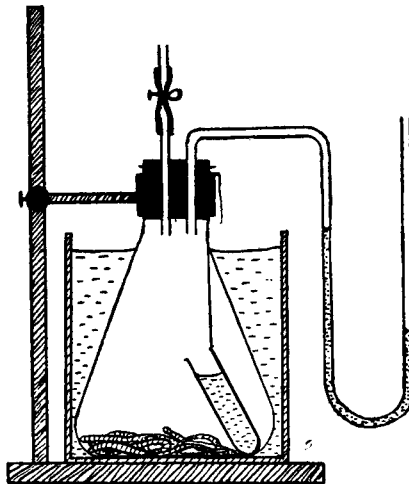
FIG. 246. **Apparatus to Demonstrate the Inspiration of Oxygen by an Animal.**

Put some coloured water into the U-tube. Place a few large earthworms in the flask and lower a small tube of caustic potash solution into it. Replace the cork and stand the flask in a vessel of cold water to keep the temperature constant. Equalise the water levels in the manometer by opening the clip, closing it immediately.

The carbon dioxide expired by the worms is absorbed by the caustic potash and the movement of the water in the manometer indicates a reduction in pressure due to the absorption of oxygen.

(3) TO MEASURE THE RATE OF RESPIRATION AND TO FIND THE RESPIRATORY QUOTIENT

EXPERIMENT 3. *Connect a U-tube* (A) *containing soda-lime to a second U-tube* (B) *containing pumice soaked in concentrated sulphuric acid. This, in turn, should be connected to a light lipless beaker fitted with a cork* (C) *into which the animal will be placed and should be large enough to take the animal at rest. This is the animal chamber. It should be connected to a pair of light U-tubes* (D, E) *containing pumice soaked in concentrated sulphuric acid. The latter should be connected to another pair of light U-tubes,* (F) *containing soda-lime and* (G) *pumice soaked in concentrated sulphuric acid. The last U-tube must then be fitted with pressure tubing connected to a water pump. The animal chamber* (C) *and the U-tubes* D, E, F *and* G *should be provided with light wire handles so that they need not be touched by hand.*

The soda-lime in A will absorb the carbon dioxide from the air which enters and the sulphuric acid in B will absorb water. The sulphuric acid in D and E will absorb the water given off by the animal and the soda-lime in F the carbon dioxide expired, while the sulphuric acid in G will absorb the water given off the soda-lime in F.

Weigh the beaker C empty; then place a small animal in the beaker and weigh again in order to find the weight of the animal.

Weigh the U-tubes D and E together and F and G together. In all cases take care not to touch the glass by hand. Then connect up the apparatus and immerse the animal chamber in a vessel of cold water to keep the temperature constant. Place a thermometer in the water.

Set the pump working for about a quarter of an hour. Then disconnect as before and dry C and weigh it again. Weigh the U-tubes together as before. Record your weighing and calculate:—

(i) **Weight of water given off by the animal** = the increase in weight of D and E.

(ii) **Weight of carbon dioxide expired by the animal** = the increase in weight of
                                        F and G.

A	B	C	D	E	F	G
Soda-lime	H_2SO_4 on Pumice	Animal Chamber	H_2SO_4 on Pumice	H_2SO_4 on Pumice	Soda-lime	H_2SO_4 on Pumice
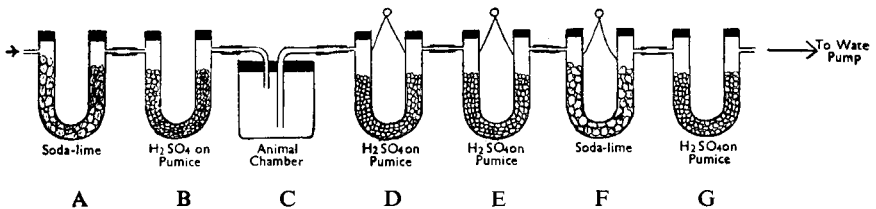
FIG. 247. **Apparatus to Measure the Rate of Respiration and the Respiratory Quotient.**

(iii) **Loss of weight of the animal** = the loss of weight of C.

(iv) **The weight of oxygen absorbed** = weight of water + weight of carbon dioxide expired — the loss of weight of the animal.

(v) **The respiratory quotient** = $\dfrac{\text{volume of } CO_2 \text{ expired}}{\text{volume of } O_2 \text{ inspired}}$

which may obviously be calculated as follows:—

$$\frac{\text{Weight of } CO_2 \text{ expired} \times 32}{\text{Weight of } O_2 \text{ inspired} \times 44}$$

Record the temperature, the time during which the experiment was in progress, the weight of the animal and whether it was active or at rest.

(4) TO DEMONSTRATE THE ACTION OF THE DIAPHRAGM

Securely tie small rubber balloons on the limbs of a glass Y-tube. Fix the tube through the cork of a bell-jar so that the balloons are inside the jar. Now cover the base of the bell-jar with sheet rubber and tie it securely in position. A piece of string or tape should then be stuck to the centre of the rubber sheet on the outside.

The glass tube represents the trachea and bronchi, the rubber balloons the lungs, the bell-jar the wall of the thorax (unfortunately inexpansible) and the sheet rubber base the diaphragm.

Lower the diaphragm by gently pulling the string or tape and observe the effect on the "lungs". Then allow the diaphragm to return to its original position and again observe the effect. Explain the causes of the changes you see.

III. THE TRANSLOCATION OF SUBSTANCES

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 stem.* Note that the red liquid has travelled up the vascular bundles. *Cut a section and examine under the microscope.* Note that the dye has only passed up the xylem.

(*b*) *Leave a seedling under similar conditions for a longer period.* Note that the eosin 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 experiments to demonstrate the process of osmosis* (Part IV (Biochemistry) Experiments 8 and 9, p. 304) if not 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 manometre into the rubber tubing. If the type shown in Fig. 241B 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.*
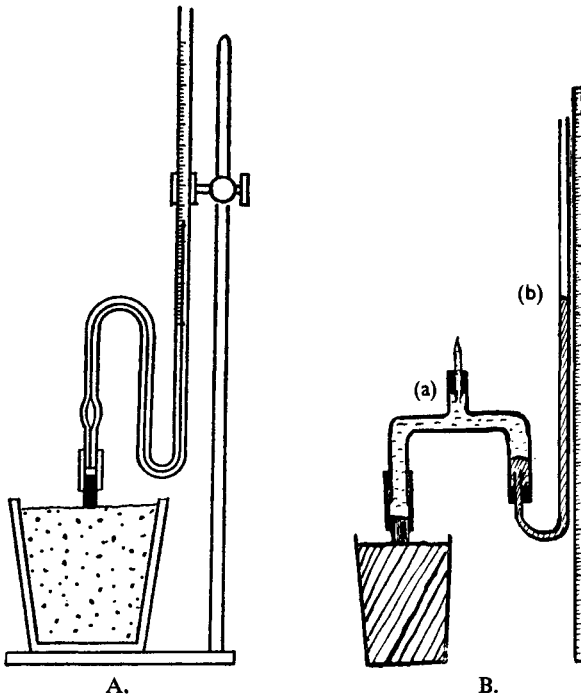
FIG. 248. **Root Pressure Manometers.**

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 mano-meter *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 1. *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. Sew or 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 Transpiraton per hour
			t_1	t_2	Difference	

To find the Relation between Absorption and Transpiration

EXPERIMENT 2. *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 ≡ 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.
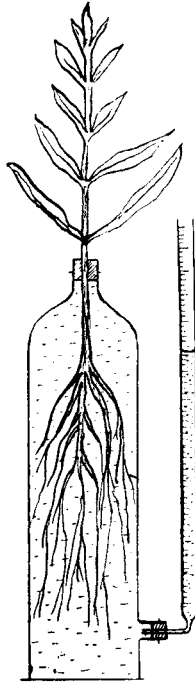
FIG. 249.   Apparatus to show Relation between Absorption and Transpiration.

To Measure the Rate of Absorption and Transpiration

EXPERIMENT 3. *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, then close the tap. 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. 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.*
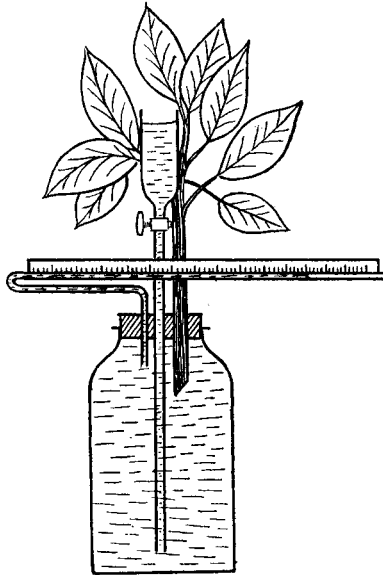
FIG. 250.   Farmer's Potometer.

Enter up your results as follows:—

Atmospheric Conditions	Time			Tube Readings			Rate of Transpiration per min.
	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.
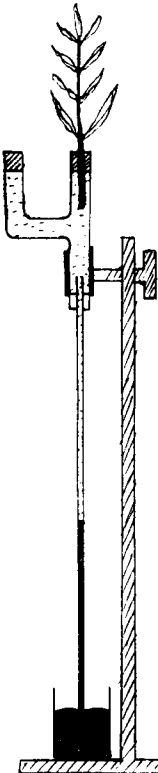
FIG. 251.
Apparatus to show Sucking Force of Transpiration.

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 4. *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 red, 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 5. *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 tubing 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.

(4) 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.* Note the coloured cell sap in the parenchymatous cells. *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 *and 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.

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 = $\dfrac{C \times 22\cdot4}{MW}$ 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.*

IV. CIRCULATION AND PROPERTIES OF BLOOD

(1) TO EXAMINE HEART BEAT

EXPERIMENT 1. *Pith a frog as directed in Appendix II and make a ventral dissection without ligaturing the anterior abdominal vein. Either cut through the centre of the pectoral girdle, exercising care so that the point of the scissors does not injure the heart, or, better, cut through each side of the girdle afterwards removing the central portion. In either case be very careful not to cut any blood vessels. Pull out the*

fore limbs so as to separate the two parts of the girdle and thus expose the heart as much as possible. Now remove the pericardium with great care in order to free the heart.

Examine the heart beat, noting the sequence of the contractions. Count the number of ventricular contractions (systole) in one minute.

(2) TO DEMONSTRATE THE CIRCULATION OF THE BLOOD

EXPERIMENT 2. *Pith a frog, place it on a frog-plate (see Appendix II), stretch the web of the foot over the hole and fix the toes in position. Now put the frog-plate on the stage of the microscope and examine the web under the low power.*

Note the thin-walled **capillaries** and the **red blood corpuscles** flowing through them from the larger **arterioles** to the smaller capillaries and from these smaller capillaries to the larger **venules.**

(3) TO EXAMINE THE PROPERTIES OF HAEMOGLOBIN

Haemoglobin is a chromoprotein and is composed of globin combined with a base called **haematin** or **haeme** which contains ferrous iron $(C_{34}H_{32}N_4O_4FeOH)$. It readily combines with oxygen to form the additional compound, **oxyhaemoglobin** (HbO_2).

EXPERIMENT 3. *Make an aqueous solution of crystalline haemoglobin (obtained from the blood of animals) and note the colour. Now shake with plenty of air and note the colour change.*

EXPERIMENT 4. *To an aqueous solution of oxyhaemoglobin, prepared as in Experiment 3, add some ammonium sulphate, a reducing agent. Note the effect.*

(4) TO INVESTIGATE THE CLOTTING OF BLOOD

EXPERIMENT 5. (*a*) *Prick the finger with a sterilised needle and place a drop of blood on a microscopical slide. Leave it uncovered and examine later under the microscope.*

Note that the blood has clotted.

(*b*) *Put a few drops of 10 per cent. sodium citrate solution or 1 per cent. potassium oxalate on a microscopical slide. Introduce a drop of fresh blood from the finger. Put aside and examine under the microscope later.*

The sodium citrate or potassium oxalate, by removal of the calcium salts, will have prevented clotting from taking place.

(*c*) *To the "salted" blood from (b) add a few drops of calcium chloride solution.*

Observe that the blood clots, showing that clotting depends on the presence of calcium salts.

EXPERIMENT 6. (*a*) *If some freshly drawn blood is available, place it in a test-tube and examine about twenty minutes later.*

Note that the blood has clotted, a reddish jelly-like mass of red corpuscles, the **clot** being suspended in a yellowish fluid called **serum.**

(*b*) *Whip up some freshly drawn blood with twigs for a few minutes.*

Note that the stringy substance collects on the twigs. This is **fibrin.**

Leave the blood for about twenty minutes and examine again.

It will be seen that this defibrinated blood has not clotted.

(5) TO TEST FOR BLOOD

Dilute a few drops of blood with water, boil to destroy any oxidising enzymes and add about a couple of drops of an alcoholic solution of guaiacum. A pre-cipitate is formed. Add sufficient alcohol to dissolve this and add a little hydrogen peroxide. A blue colour is produced.

Blood Groups

When the blood of two vertebrate animals of different species is mixed, the erythrocytes collect together in clumps and are said to **agglutinate.** This also occurs between certain animals of the same species but not in all cases. The blood of human beings falls into four **groups** and agglutination occurs only between certain groups. These groups are inherited. Agglutination is caused by the interaction of a substance in the erythrocytes called **agglutinogen** and a substance in the plasma called **agglutinin.** One group contains no agglutinogen in the corpuscles and this blood is therefore never agglutinated; this group is designated **O.** Of the other groups, one contains agglutinogen **A,** another **B** and the third contains both **A** and **B.** These groups are designated **A, B** and **AB** respectively. In the plasma of Group A is an agglutinin **b,** in the plasma of **B** is an agglutinin **a,** in the plasma of **O** is an agglutinin **ab,** but the plasma of **AB** contains no agglutinin. Consequently,

AGGLUTINATION TAKES PLACE BETWEEN—

the plasma of **O** and the corpuscles of **A, B** and **AB**
,, ,, ,, **A** ,, ,, ,, ,, **B** and **AB**
,, ,, ,, **B** ,, ,, ,, ,, **A** and **AB**
and

NO AGGLUTINATION TAKES PLACE BETWEEN—

the plasma of **A** and **AB** and the corpuscles of **A**
,, ,, ,, **B** and **AB** ,, ,, ,, ,, **B**
,, ,, ,, **AB** ,, ,, ,, ,, any **Group**
,, ,, ,, any **Group** ,, ,, ,, ,, **O**

On account of the fact that the corpuscles of **O** are not agglutinated by the plasma of any group, individuals in this group are **universal donors,** and because the plasma of **AB** cannot agglutinate the corpuscles of any group, individuals in this group are **universal recipients.**

It has been found that, regardless of group, in the case of 85 per cent. of human beings the blood is agglutinated by the serum of rabbits previously injected with the blood of the rhesus monkey. This is due to what is known as the **Rhesus factor (Rh.)** Such blood is said to be **Rh positive.** The other 15 per cent. is **Rh negative.** The condition is inheritable.

These facts form the basis of **blood transfusion,** in which agglutination must, of course, be avoided. **A person's blood group is determined** by mixing drops of his blood with test serums derived from people belonging to groups **A** and **B** respectively. The method is as follows:—

(i) A drop of Serum of Group **A** and (ii) a drop of Serum of Group **B** are placed on a white tile. A drop of the subject's blood under investigation is then added to each. *No agglutination* occurs between the blood of a person in Group **A** and (i), or Group **B** and (ii), or Group **O** and (i) or (ii). *Agglutination* occurs between the blood of a person in Group **A** and (ii), or Group **B** and (i), or Group **AB** and (i) or (ii). Thus the Blood Group is determined.

EXPERIMENT 1. *Put a few drops of the blood of various pairs of individuals to each of which a few ml. of sodium citrate have been added (to prevent clotting) on a slide and mix them by means of a mounted needle. Examine to see whether agglutination takes place or not.*

EXPERIMENT 2. *Prepare a blood film on a coverslip, invert on a slide and irrigate with distilled water.*

Note the effect on the erythrocytes which swell up by absorption of the water and either burst or become so distended that the haemoglobin escapes into the plasma. This is called **haemolysis.**

EXPERIMENT 3. *Prepare another blood film as in (2) and irrigate with a hypertonic solution of salt (0·9 per cent.).*

Water diffuses out of the erythrocytes which shrink and become crenated.

V. EXCRETION

Excretion is the elimination of the waste products of metabolism from an organism.

(I.) EXCRETION IN PLANTS

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.

EXPERIMENT 1. *Examine a section of one of the scale leaves of the Hyacinth.*

Bundles of needle-shaped crystals lying parallel to each other will be seen in some of the larger cells. These bundles are called **raphides** and consist of crystals of **calcium oxalate.**

(II.) EXCRETION IN ANIMALS

The most important excretory products in animals are water, carbon dioxide, urea, creatinine, uric acid and hippuric acid.

(1) UREA

Urea $CO(NH_2)_2$ is an amide and is derived from the protein in the diet. It is the chief nitrogenous constituent in mammalisn urine and is soluble in water.

EXPERIMENT 1. *Heat some crystals of urea in a test-tube.* The substance melts and then decomposes with the evolution of ammonia, *which can be detected by its odour and its action on red litmus paper. Carry out the biuret test (as used for proteins) with the residue.* Biuret is formed, giving a violet colour.
$$2CO(NH_2)_2 = NH_3 \cdot CO \cdot NH \cdot CO \cdot NH_2 + NH_3.$$

EXPERIMENT 2. *Boil a solution of urea.* It is hydrolysed to carbon dioxide and ammonia. *Test for these gases.*
$$CO(NH_2)_2 + H_2O = CO_2 + 2NH_3.$$
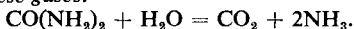
EXPERIMENT 3. *Add some alkaline sodium hypobromite (prepared by adding 2 c.c. of bromide to 23 c.c. of N. sodium hydroxide) to some urea.* A violent effervescence takes place with the liberation of nitrogen.
$$CO(NH_2)_2 + 3NaOBr = 3NaBr + CO_2 + N_2 + 2H_2O.$$
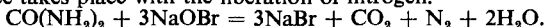
EXPERIMENT 4. *To a few millilitres of urea solution in a watch glass add a few drops of concentrated nitric acid.* Urea nitrate $(CO(NH_2)_2 \cdot HNO_3)$ crystallises out. *Examine the crystals under the microscope.*

(2) CREATININE

$$HN = C \underset{\diagdown N-CH_2}{\overset{\diagup NH-CO}{\big|}}$$
$$\underset{CH_3}{|}$$
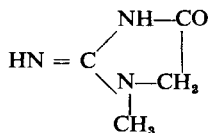
This originates from the phosphagen in muscle which is converted into creatine in muscular activity. Creatine is the precursor and the anhydride of the creatinine in urine.

EXPERIMENT 5. *Add a few drops of freshly prepared dilute solution of sodium nitroprusside to an aqueous solution of creatinine or a few ml. of urine, then add a few ml. of sodium hydroxide.* Note that a deep red colour is formed and that this quickly changes to yellow. *Add dilute acetic acid and boil: a greenish-blue colour is obtained.*

(3) URIC ACID

Uric acid, $C_5H_4N_4O_3$, is the chief nitrogenous excretory product of reptiles and birds. There is little in mammalian urine.

EXPERIMENT 6. **Murexide Test.** This test cannot be performed with urine: It is necessary to extract the uric acid from it. *Add a few drops of concentrated nitric acid to a few drops of uric acid. Evaporate slowly in a fume chamber until no further fumes of nitric acid are evolved. When the yellowish-red residue is cool add a drop of very dilute ammonia.* Note a bright red colour is produced. *Now add some caustic soda and note that the colour changes to purple.*

(4) HIPPURIC ACID

Hippuric acid or benzoyl glycine, $C_6H_5 \cdot CO \cdot NH \cdot CH_2 \cdot COOH$, is a normal constituent of the urine of herbivorous animals. Aromatic substances in the plant material are oxidised to benzoic acid in the animal and, in the liver, this undergoes condensation with glycine to hippuric acid.

EXPERIMENT 7. *Heat a few crystals of hippuric acid. Note that they melt and turn red.* The white sublimate is benzoic acid.

(5) URINE

EXPERIMENT 8. (*a*) *Note the colour and transparency of normal urine.* (*b*) *Find the specific gravity of urine by means of a hydrometer or urinometer.*

EXPERIMENT 9. *Find the reaction of fresh urine.*

EXPERIMENT 10. *Test for the presence of* **urea** *in urine by the sodium hypobromide test* (Experiment 3).

EXPERIMENT 11. *Test for* **creatinine** *in urine by the sodium nitroprusside test* (Experiment 5). (N.B.—Acetone in urine will also give this reaction but it does not turn yellow and on the addition of acetic acid a purple colour is obtained. *Perform this test with acetone and verify this result.*)

EXPERIMENT 12. *Test for inorganic salts in urine as follows:*—

(i) **Chlorides.** *Add a few drops of concentrated nitric acid and then some silver nitrate solution. Note that a white precipitate is obtained. Add some ammonium hydroxide and the precipitate dissolves.*

(ii) **Phosphates.** *Acidify some urine with concentrated nitric acid and boil. Add this to some ammonium molybdate also acidified with nitric acid and boil. A yellow precipitate is formed.*

(iii) **Sulphates.** *Add about two millilitres of concentrated hydrochloric acid to a little urine and then add excess of barium chloride solution. A white precipitate is obtained.*

VI. GROWTH

(1.) GROWTH IN PLANTS

(1) THE GERMINATION OF SEEDS

Conditions Necessary for Germination

To show the Presence of Water in Unsoaked Seeds

EXPERIMENT 1. *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 Effect of Extremes of Temperature on Seeds.

EXPERIMENT 2. (*a*) **Heat.** *Immerse two boiling-tubes containing a few* (i) *soaked and* (ii) *unsoaked pea seeds 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 3. *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 4. *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.*

2. GROWTH OF THE ROOT AND SHOOT

To find the Growing Region of the Root

EXPERIMENT 1. *Dry the root of a bean or pea seedling when it has grown to about 5 cm. in length. Mark it transversely with indian ink lines 2 mm. apart from the tip to the base. This is best done with a piece of thread tightly stretched across a piece of bent wire. Place the bean seedling in a tall germination jar or put the pea 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 pea 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 wooden beam
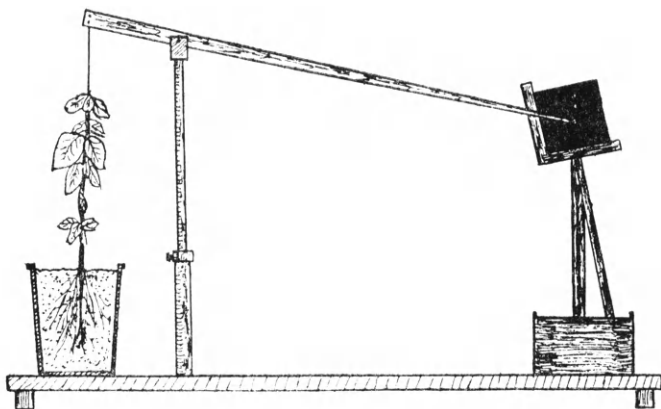
FIG. 252.   **Auxanometer.**

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.

To find the Influence of Light on Growth

EXPERIMENT 5. *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 unless they are transferred to light, when the stem and leaves turn green and the plants continue to grow.

FIG. 253. **Apparatus for Water Culture Experiments.**

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 present in mineral salts in the soil on the growth of a plant, plants should be grown in solutions of salts containing all the elements and in a series of solutions each devoid of one element. These solutions are called **culture solutions.** (These experiments also demonstrate the fact that roots absorb mineral substances 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:—

(a) Complete solution   (e) minus Fe
(b) minus Ca   (f) minus N
(c) minus K   (g) minus S
(d) minus Mg   (h) minus P

and insert the plants. Also set up (i) 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 absorbed or lost by evaporation. Replace the culture 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 the solutions each day in order 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.

(II.) GROWTH IN ANIMALS

A simple experiment on growth in animals and the effect of external conditions on their growth can be performed with tadpoles.

EXPERIMENT 1. *Collect some frog spawn and keep it in the water in which it was found. When tadpoles have hatched from the fertilised eggs, separate them into four equal batches and keep them in separate dishes in the same water in which the spawn was found. Arrange the dishes as follows:—*

  (i) *in a warm but not hot situation, e.g., in a thermostat*
 (ii) *in a cold situation, e.g., in a cold lighted shed or cupboard*
(iii) *in light (not direct sunlight)*
(iv) *in a dark cupboard.*

Leave the tadpoles to grow and develop and keep a record of their growth and development. From your results deduce the effect of the conditions provided on their growth.

VII. IRRITABILITY AND MOVEMENT

(I.) IRRITABILITY AND MOVEMENT IN PLANTS

(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.

(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.

(i) 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 slit or small circular aperture on one side only. Germinate another set in a completely dark chamber as a "control". Examine a few days later when the plants have germinated. It will be seen that in the plants exposed to unilateral illumination, the shoots have grown towards the light. They are therefore said to be positively phototropic. In the control, the shoots grow straight up.*

Note the direction taken by the leaves: they are **dia-phototropic.**
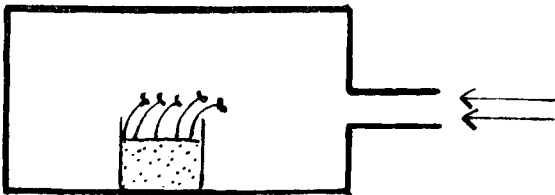
FIG. 254. **Phototropic Chamber.**

To find the Region of Phototropic 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 by the method used to find the growing region (growth experiment No. 1). 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 in a germination jar, 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.*

(ii) 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. Also note the direction of the lateral roots:* they are **dia-geotropic.**

To find the Region of Geotropic 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 as with the stem. 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.
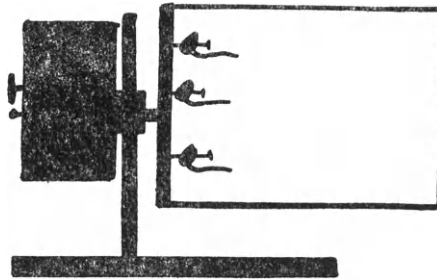
FIG. 255.   **Clinostat.**

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 rotated once an hour by clockwork or an electric motor. A cylindrical celluloid cover fits on to the cork disc.

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 celluloid cover on the disc. Start the clock or motor and leave the apparatus in the dark. All sides of the root and shoot are thus equally subjected to gravity. *Examine after two or three days, winding the clock, if necessary, in between times. Observe the direction of growth of the roots and shoots.* (The instrument can also be arranged with the disc in a horizontal position for the elimination of phototropism.)

(iii) 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.*
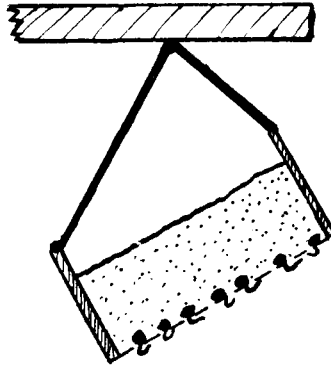
FIG. 256. **Apparatus to demonstrate the hydrotropism of the root.**

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.*

(iv) 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, the surface of*

which has been roughened by coarse emery paper. Note that the curve becomes much greater, forming a complete circle in a very short time.

(v) 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 the 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 tube one half of which is covered with a black light-proof paper while the other is well illuminated. If water containing a sufficient number of Volvox globator is put into the tube, 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.*

Photonastic movements are often affected by other influences, such as changes in turgor, and plants such as these assume these positions at night. This response is therefore referred to as "sleep movement" or **nyctinasty.**

II. IRRITABILITY AND MOVEMENT IN ANIMALS

NERVOUS RESPONSE

(1) TO DEMONSTRATE REFLEX ACTION

Reflex actions are of two kinds:—

(i) **Unconditional reflexes,** which are natural, inherited reflex actions.

(ii) **Conditioned reflexes,** which are acquired during life as the result of frequently repeated stimuli.

The former only need be considered here.

EXPERIMENT 1. *Sit on a chair and cross one leg over the other. Strike the free leg with the edge of the hand just below the knee-cap.*

Note the response, over which you have no control.

EXPERIMENT 2. *Get someone to close his eyes for a minute or two and on opening them, shine a torch on them.*

Observe the effect on the pupils.

EXPERIMENT 3. *Pith a frog* (see Appendix II) *and suspend it by its fore-limbs from the rod of a retort stand. Now apply as a stimulus to one of the legs or to the skin of the trunk a hot wire or a rod which has been dipped in acid.* Note the response. It should be noted that the brain having been destroyed, the animal is not conscious and that the response is entirely reflex.

(2) TO SHOW THAT THE NERVES CONVEY IMPULSES

EXPERIMENT 4. *Take the frog used in the last experiment and dissect one leg so as to expose a short length of the sciatic nerve. Sever the nerve. Now suspend the frog and stimulate as in* Experiment 3.

Observe that the leg in which the nerve has been severed remains motionless. The other will still respond.

VI. 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 hormone 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.

I. PLANT 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.

II. ANIMAL HORMONES

In animals **autocoids** are the secretions of the ductless glands or **endocrine organs.** As the endocrine organs are also known as internally-secreting glands, hormones are also known as **internal secretions.** They are carried by the blood stream to other parts of the body and are responsible for chemical co-ordination and the control of metabolic activities generally. The chemical composition of some of the hormones is known as they have been isolated from the body (some have, in fact, been synthesised). They are of comparatively low molecular weight compared with vitamins. Like vitamins, minute quantities only are necessary for the organism, but unlike most vitamins they are produced within the animal.

* **Auxin** is indole-3–acetic acid or β-indol acetric acid.

Like enzymes, many of them are specific in their action, but unlike them they are not destroyed by heat and are simpler in structure. Furthermore, most of them are destroyed by the digestive enzymes and they act in a different part of the body from that in which they are secreted and which they reach by means of the blood stream. It should be understood that the endocrine organs through the blood stream work together as a co-ordinated whole, thus constituting the **endocrine system.** Deficiency or superfluity of hormones may set up pathological conditions.

It will be obvious from the nature of the actions shown on pp. 355–6 that simple practical work on autocoids is not possible, but the following experiments will illustrate the actions of two of them.

EXPERIMENT 1. **To find the effect of pituitrin on the Skin of the Frog.**

Examine a demonstration specimen, in which a light-coloured frog has been injected with pituitary extract. (See Appendix II.)

Note the dark colour of the skin, caused by the expansion of the melano-phores (black pigment cells). This is due to the action of **pituitrin,** the hormone of the secretion of the posterior lobe of the pituitary gland. *Compare this specimen with an uninjected frog similar in colour to the other frog before injection.*

EXPERIMENT 2. **To find the effect of Thyroid Extract on the Development of the Tadpole.**

Examine a demonstration in which tadpoles which are fully developed and therefore about to metamorphose are kept in two separate aquaria, the one group being kept on their normal diet, while the other group is fed on thyroid extract. (See Appendix II.) *Examine after twenty-four hours or so.*

The tadpoles fed on thyroid metamorphose into minute frogs very quickly. This is due to the **thyroxin.**

EXPERIMENT 3. **To demonstrate the presence of iodone in thyroxin.**

To an alcoholic solution of thyroxin, add a few millilitres of chloroform and a few drops of chlorine water. Shake and allow to stand. The chloroform layer is coloured violet.

The sources and effects of hormones may be summarised as follows though their effects vary in some cases in different animals:—

Endocrine Organ (Source)	Hormone (Active principle in secretion)	Action	Remarks and Effect of	
			Hypersecretion*	Hyposecretion*
Thyroid.	Thryroxin.	In young animals—growth. In adult—control of basal metabolism.	Exophthalmic goitre.	At birth—cretinism. In adult—myxoedema. Endemic goitre due to lack of iodine in food.
Parathyroids.	Parathormone.	Control of calcium and phosphate in blood and (together with vitamin D) bone formation.	Osteitis fibrosa.	Tetany
Adrenals: Cortex	Cortin.	Controls balance of salts in blood and resistance to many diseases	Abnormalities in embryo cause intersexual conditions.	Addison's disease.
Medulla.	Adrenalin.	Increases muscular tone, heart beat, etc. Secretion increased during emotional stress.	Sexual precocity.	
Islets of Langerhans (in pancreas).	Insulin.	Stimulates liver and muscles to store glycogen.	Spontaneous hypoglycaemia. (Sugar deficiency in blood).	Diabetes mellitus with hyperglaemia (excess of sugar in blood).
Stomach (Mucous membrane).	Gastrin.	Stimulates flow of gastric juice during digestion.		
Duodenum (Mucous membrane).	Secretin.	Stimulates flow of pancreatic juice during digestion.		
Testis (Interstitial cells).	Testosterone.	Secondary sexual characters.	Sexual immaturity.	Under certain conditions development of secondary sexual characters of opposite sex.

Endocrine Organ (Source)	Hormone (Active principle in secretion)	Action	Remarks and Effect of	
			Hypersecretion*	Hyposecretion*
Ovary: Interstitial cells.	Oestrin.	Secondary sexual characters. Controls oestrous cycle and promotes sexual maturity.	Sexual immaturity.	Under certain conditions development of secondary sexual characters of opposite sex.
Corpus luteum (from follicle cells after discharge of oocyte).	Progesterone.	In pregnancy, induces changes in lining of uterus to prepare it for reception of zygote and, in conjunction with oestrin and prolactin from the pituitary causes mammary glands to enlarge and secrete milk.		
Thymus.		Maturing of sexual organs.	Disappears at puberty. Its abnormal persistence renders animal liable to death on receipt of sudden shock.	
Pituitary: Posterior lobe.	Pituitrin. Vasopressin.	Raises blood pressure.	High blood pressure.	Abnormal loss of water from body (diabetes insipidus).
	Oxytocin.	Stimulates uterine contraction.		
Anterior lobe.	Somatotropic hormone, Thyrotropic hormone. Parathyrotropic hormone. Adrenotropic hormone. Pancreaticotropic hormone. Gonadotropic hormone.	Growth. Secretion of thyroid. Secretion of parathyroids. Secretion of adrenals. Secretion of islets of Langerhans. Secretion of endocrine tissue in testes and ovaries.	Gigantism, acromegaly. These hormones control the activities of other glands as shown and thus the anterior lobe acts as "master gland". Complete loss of anterior lobe secretions causes Simmond's disease with premature senility.	Dwarfism.
	Mammotropic hormone. Prolactin.	Secretion of corpus luteum to induce mammary glands to secrete milk.		

*The symptoms of the diseases caused by over- and under-secretion can be found in a medical dictionary. These diseases may be treated sometimes by therapeutic administration of the appropriate hormones.

PART VI

EMBRYOLOGY AND DEVELOPMENT

I. PLANT EMBRYOLOGY AND DEVELOPMENT

THE STRUCTURE AND GERMINATION OF POLLEN

(1) *Mount some* **pollen grains** *from a flower in dilute glycerin.*

Under high power, note the outer wall or **exine,** the inner wall or **intine,** and the granular **cytoplasm.**

(2) *Examine a prepared slide of stained* **germinating pollen grains.**

Note the two male **gametes** and the larger **vegetative nucleus** in the **pollen-tube.**

STRUCTURE OF THE OVULE

(3) *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 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).
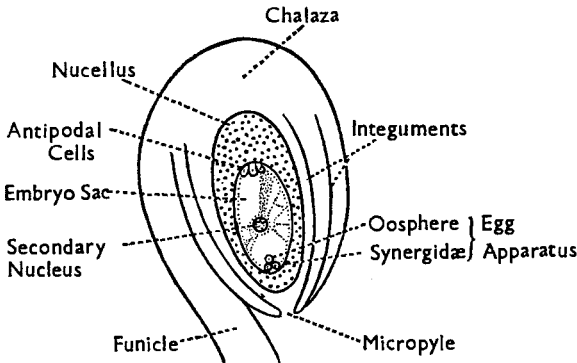
FIG. 257. **L.S. Anatropous Ovule.**

At the micropylar end is a large cell, the **megaspore** (sometimes referred to as the **embryo sac**) containing vacuolated cytoplasm, and in its centre is the large **secondary nucleus** (often 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 the **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.**

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.

THE DEVELOPMENT OF THE EMBRYO

Examine a slide of **L.S. of ovules after fertilisation.**

The zygote (fertilised oosphere) develops a cellulose wall and is then called an **oospore.**

Under *high power* look for various **stages in the development of the embryo.** The following may be seen: Division of the **zygote,** 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** and plumule, radicle and cotyledon(s) constitute the **embryo.** The ovary wall becomes the fruit wall or **pericarp.**

Note on the Flower
The Flowering Plant is the **sporophyte** (2n chromosomes).

♂	**Sporophyte**	♀

Stamen = microsporophyll (2n). Carpel = megasporophyll (2n).
Pollen sac ⎫ = microsporangium (2n). Nucellus ⎫ = megasporangium (2n).
of anther ⎭ of ovule ⎭

Gametophyte

Pollen grain = microspore (n). Megaspore (n).
Pollen tube = male prothallus (n). Embryo sac = female prothallus (n).
Generative cell (n). Oosphere (n).

↘ ↙

Zygote (2n)
= **Sporophyte.**

THE GERMINATION OF THE SEED

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,** **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**:—

The **primary root** (elongated radicle) and the **secondary** (lateral) **roots: root hairs** growing on the surface of the primary and secondary roots: the **hypocotyl,** which by its elongation in the *epigeal* types raises the cotyledons (which turn green) but which does not elongate in *hyogeal* germination so that the cotyledons remain below ground: the **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.

II. ANIMAL EMBRYOLOGY

VERTEBRATE EMBRYOLOGY

INTRODUCTORY NOTES

It should be remembered that during the development of the embryo undifferentiated cells during multiplication undergo specialisation and differentiation into structures which will ultimately be found in the adult. Consequently the appearance of the cells and developing organs in the embryo are quite different from those in the adult. It will be observed that the nuclei of embryonic cells are larger than in the adult and stages in mitosis may be visible. Furthermore it must be understood that, except in the earliest stages, various processes are going on concurrently.

In the study of embryology it is advisable to do the practical work with the aid of a text-book and plenty of diagrams and it must be borne in mind that owing to the very large number of sections cut through any particular region of an embryo, the specimens examined may not be identical with the diagrams studied.

Examination of embryological models, if available, is of great assistance but this must *not* replace the study of slides and specimens. Prepared slides will have to be used, of course, in all cases.

In drawing sections it is not necessary to fill in the individual cellular structure except where it is advisable in order to make a particular structure clearer. Outlines of the structures, adequately labelled, will suffice.

SEQUENCE OF DEVELOPMENT

The following sequence of stages occurs in the development of the embryo from the fertilised ovum:—

(1) **Segmentation** or **cleavage** of the zygote.

*(2) Formation of the **blastula,** a spherical structure composed of a single wall of cells enclosing a cavity, the **blastocoele.**

*(3) Formation of the **gastrula,** an elongated structure containing a fresh cavity, the **archenteron** or primitive gut, the blastocoele being obliterated. The wall is composed of two layers of cells, the **ectoderm** (which, in some cases, takes part in the formation of extra-embryonal structures) externally and what is often called the **"endoderm"** internally. It is really better to refer to the latter as the **"inner layer"** since the endoderm and also the mesoderm and the notochord are derived from it.

(4) Formation of the **mesoderm,** the third of the germ-layers.

(5) Formation of the **neural tube** giving rise to the nervous system. This stage is referred to as the **neurala.**

(6) Formation of the **notochord** which, except in primitive Chordata in which it persists, is replaced by the vertebral column which develops around it from mesodermal cells.

(7) Formation of the **gut** from the archenteron.

(8) Development of **organs** and **systems.**

But it must be understood that, except in the earliest stages, many structures are developing at the same time.

The presence of yolk, however, considerably modifies these stages. Ova, such as that of Amphioxus, which contains a small amount of yolk, evenly distributed are said to be **microlecithal** (or, better, **isolecithal).** But in the majority of the Chordata the yolk is concentrated towards one pole of the ovum known as the **vegetative pole** to distinguish it from the **animal pole,** where the nucleus of the ovum is situated more or less free from yolk. Ova of this type are said to be **telolecithal.** In some ova, however, such as those of the invertebrate Astacus, the yolk is situated in the centre and these are known as **centrolecithal** ova.

There is no such thing as a completely yolkless ovum but the study of the cleavage and subsequent development of the zygote of Amphioxus (the ovum of which is microlecithal) serves as a basis from which the influence of yolk in higher types can be seen.

From the three primitive **germ layers** all the tissues and organs of the body are derived as shown on the next page *but it must be understood that many organs arise from more than one germ layer.*

*These stages are considerably altered in the chick and mammal.

Ectoderm	Mesoderm	Endoderm
1. Epidermis and structures derived from it.	1. Dermis.	1. Pharynx.
2. Nervous system.	2. Muscles.	2. Respiratory tract.
3. Sensory epithelia—retina of the eye, membranous labyrinths of the ear, olfactory cavities and the lens of the eye.	3. Skeleton.	3. Alimentary canal (except the two extremities) and its associated organs.
	4. Connective tissues.	
	5. Blood.	
	6. Blood vessels.	4. Bladder.
	7. Peritoneum. Pleura and pericardium.	
4. Epithelium of the anterior and posterior ends of the alimentary canal.	8. Urino-genital organs.	
	9. Sclerotic of eye	

In a late blastula and therefore before the formation of the gastrula (see below), certain **presumptive areas** from which parts of the adult animal arise, become evident in the frog and chick. Furthermore, certain embryonic structures, such as the dorsal lip of the blastopore, appear to be responsible for the development of the cells adjacent to them. These structures are known as **primary organisers.** Later in development **secondary organisers** appear, such as the optic cup which is responsible for the development of the lens of the eye. This will be better understood as study progresses.

DEVELOPMENT OF THE FROG

(1) FERTILISED OVUM

Examine **Ova of Rana** *with a hand lens.*

Note the spherical **telolecithal ovum** enclosed in a thin **vitelline membrane,** with **yolk** at the lower **vegetative pole** which is light in colour, the **animal pole** above being dark owing to the presence of pigment. The whole egg is enclosed in an **albuminous envelope.** This is thin immediately after laying but it swells up considerably during the first few hours after exposure to water and binds the eggs together to form the familiar frog spawn.

(2) LARVAL DEVELOPMENT

Examine freshly laid **Frog's Spawn** *and study the* **Development of the Embryo** *(kept in a suitable aquarium) from day to day with a hand lens, keeping a record of the length of time for the development of the various features.*

Note the **elongation of the embryo** (this occurs after gastrulation is completed) and the **hatching of the larva** (tadpole) about two weeks after fertilisation by a wriggling movement which frees it from the albuminous envelope. The larva attaches itself to a weed by a **cement**

(or **mucous**) **gland** and continues to feed on yolk. Note the two pairs of branched **external gills,** followed soon by a third pair. By the end of the third week the stomodaeum opens into the pharynx and the **mouth** has developed. The tadpole, now shaped rather like a globe, swims about actively by means of a well-developed tail. Later (about four weeks), note the disappearance of the external gills and the development of the **operculum,** a fold of skin over the gill-slits which completely fuses with the body wall except on the left side, where an aperture, the **branchial aperture** or **spout,** is left. The development of internal gills is now complete.

The **hind-limb buds** appear at the base of the tail some weeks later (at about six or seven weeks), and it is not until the **hind-limbs** are almost fully developed (at about ten weeks) that the **fore-limbs** are visible, their development having been hidden by the operculum.

During this period of development, the larva feeds actively on a vegetable diet and grows considerably in size.

(3) METAMORPHOSIS

Continue your examination of the **Larval Development.**

When the limbs are fairly well developed (about ten weeks), note that the larvae frequently rise to the surface of the water for air; they now begin to respire by lungs. Feeding ceases and the skin is shed, the mouth and eyes enlarge and the shape of the body changes and becomes more frog-like. After about a fortnight when these changes are complete, the miniature frog with a short tail leaves the water. Finally, the tail is absorbed.

Feeding having been resumed (and this on an animal diet, chiefly insects), growth continues until, after about three years, the frog reaches its maximum size and is fully mature.

DEVELOPMENT OF THE CHICK (GALLUS)

THE OVUM

The **ovum** is large and is **telolecithal.** The orange-coloured **yolk** is enclosed in a **vitelline membrane** and is suspended in **dense albumen** surrounded by **clear albumen** by means of the twisted **chalaza** which extends to the opposite ends of the egg. The whole is enclosed in two **shell membranes,** separating at the rounded end of the outer **shell** and enclosing the **air-sac.** On the upper side of the yolk is a clear protoplasmic area, the **germinal disc** (or **blastodisc**) in the centre of which is the nucleus or **germinal vesicle.**

INCUBATION

After laying, development will continue only if the egg is incubated at a suitable temperature (about 40°C.), but the rate of development (and thus the number of somites) is affected by the temperature of incubation, and this may vary slightly.

The age of the embryo after laying may be *roughly* estimated by counting the number of mesodermal somites present (see (iii) below). *The following table is therefore approximate:—*

Hours of Incubation	Number of Somites (Pairs)
20	2
24	6
28	9
33	12
38	16
48	27
72	36

(1) FIRST DAY OF INCUBATION
(TWO TO SIX PAIRS OF SOMITES)

The influence of yolk will be still more apparent in this study of the development of Gallus.

(i) *Examine prepared slides of* the **Blastoderm of the Chick showing the Segmentation of the Zygote in various early stages,** if available.

Segmentation takes place only in the germinal disc, and is therefore said to be **meroblastic.**

1. First Cleavage   2. Second Cleavage   3. Later Cleavage
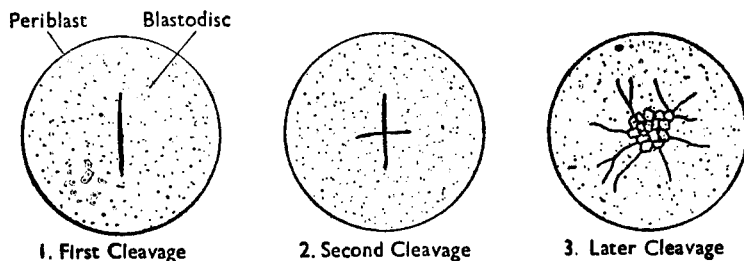
FIG. 258. Gallus. Cleavage of Zygote.

Owing to the large amount of yolk, the division of the nucleus is not accompanied by complete division of the germinal disc. The first cleavage is shown by the appearance of a narrow groove or furrow and the second by a furrow at right angles to the first, neither

of which extend right across the germinal disc. Cleavage continues in an irregular manner in the centre of the **blastodisc,** the outer part being referred to as the **periblast.**

(ii) *Examine a* **T.S. through the Blastoderm at a Late Stage of Segmentation.**

Identify the **ectoderm, endoderm** and the **subgerminal cavity** as described above.

Soon a cavity appears below the central cells, separating the **blastoderm** (as the blastodisc is now called) from the yolk. This is the **subgerminal cavity** and it corresponds with the blastocoele of the simple types already studied, so we may consider this stage of development as the blastula stage.

A little later, the upper layer of cells divides into two layers, the **ectoderm** above and the **endoderm** below.

(iii) *Examine a slide of the* **Blastoderm with 2 pairs of somites (about 20 hours).**
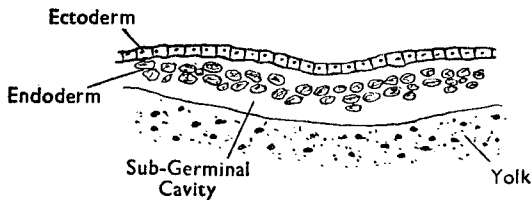
FIG. 259. Gallus. T.S. Early Blastoderm.

It will be observed that there is a comparatively clear region in the centre of the blastoderm; this is known as the **area pellucida** and it lies over the subgerminal cavity. Around it is the **area opaca,** darker in appearance and lying over the yolk. In the former will be seen a row of cells extending from about a quarter of the distance from the future anterior end almost to the posterior end in the midline at right angles to the long axis of the egg. This is the **primitive streak:** it swells at its anterior end to form the **primitive knot.** In the streak is a shallow groove, the **primitive groove,** which widens into the **primitive pit** in the knot. In front of the primitive streak the **notochord** will be seen.

A further development takes place from the anterior end of the primitive knot. This is the **notochordal process** from which the notochord develops. Observe also the block-like **mesodermal somites** on each side of the primitive streak. At this stage there are about two pairs.

It will be seen that the **mesoderm** extends along each side of the primitive streak and forwards on each side of the notochordal process and beyond it when the mesoderm of the two sides join. Anterior to this is a clearer region devoid of mesoderm and called the **pro-amnion.**

(iv) *Examine a slide showing* **Sections of the Blastoderm through the Primitive Streak with two pairs of Somites (about 20 hours).**

The cells have divided off from primitive streak and they spread out between the ectoderm and endoderm and thus form the **mesoderm.**
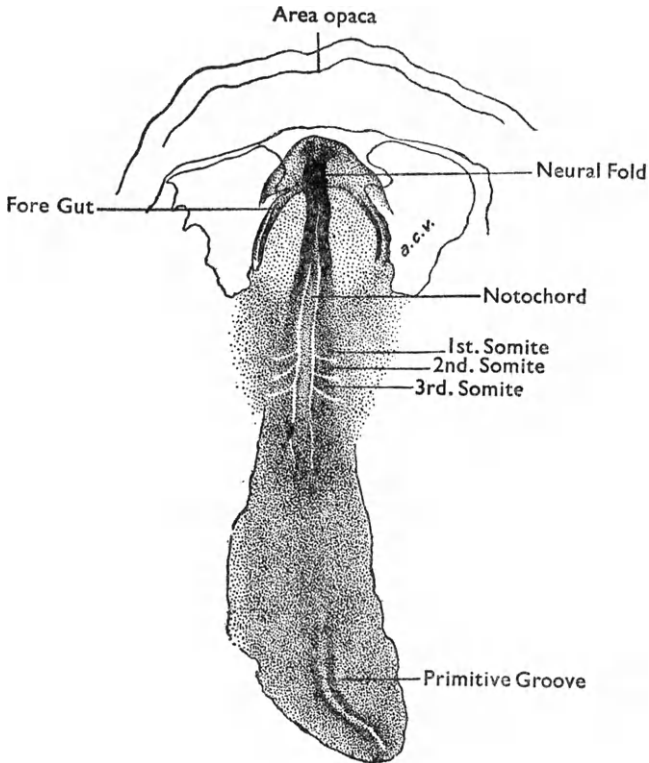
Fig. 260. **Gallus. Blastoderm of 3-4 Somites (about 23 hours).**
(*From Lillie's "Development of the Chick."*)

Note the formation of the **neural** or **medullary plate** (in the region anterior to the notochordal process) and the **neural groove** formed by the **neural folds** which join to form the **neural tube.**

(v) *Examine a* **Blastoderm with six pairs of Somites (about 24 hours).**

Note that the primitive streak is shorter and identify the **head fold,** a curved structure between the **pro-amniotic area** and the **cerebral vesicles.** Observe again the blocks of cells originating from the mesoderm of the primitive streak and visible on either side of the mid-line. These are the **mesodermal somites.**
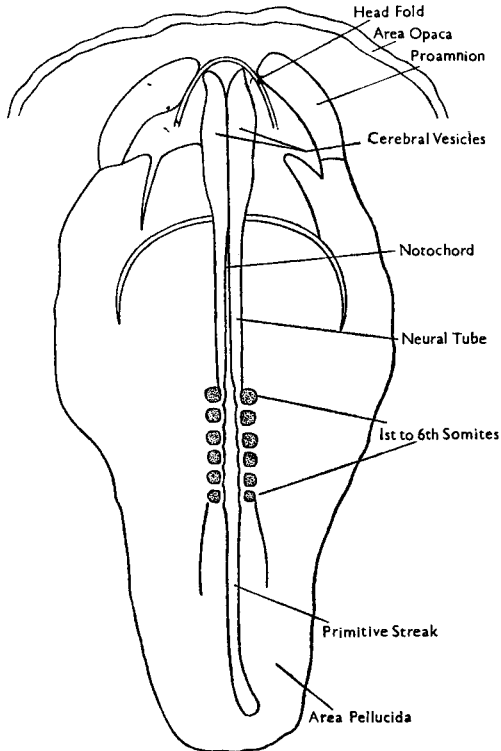
FIG. 261. **Gallus Embryo with 6 Somites (24 hours).**

Note also the plexus of small **blood vessels** in the central portion of the area opaca (formed by the union of groups of cells called **blood islands** which soon coalesce to form the **area vasculosa;** it is mesodermal in origin. Small vessels may be seen in the area pellucida running from the area vasculosa and as the blastoderm gets older, these spread very considerably.

(vi) *Examine a* **T.S. through an embryo with six pairs of Somites (about 24 hours).**

Note the **ectoderm, endoderm and mesoderm;** also the **neural plate, neural groove** and **notochord.**
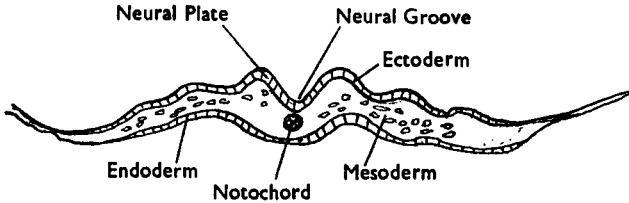
FIG. 262. **Gallus. T.S. Embryo with 6 Somites (24 hours).**

(vii) *Examine a* **Transverse Section of the Blastoderm of about seven Somites.**

The **mesoderm** has split into an upper **somatic layer** and a lower **splanchnic layer** forming a schizocoelic **coelom** between them. The somatic layer with the ectoderm forms the **somatopleure** and the splanchnic layer with the endoderm forms the **splanchnopleure.**
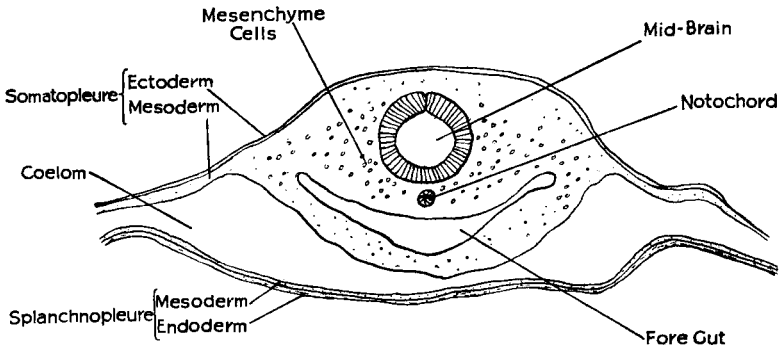
FIG. 263. **Gallus. T.S. through Mid-brain Region of Embryo with 7 Somites (about 25 hours).**

The **fore-gut** is formed as a pocket in the splanchnopleure. The **neural tube,** easily recognised by its dorsal position and its hollow form with thick walls, and the **notochord,** a small circular structure ventral to the neural tube, will also be seen. The loosely packed mesodermal cells are called **mesenchyme.**

(2) SECOND DAY OF INCUBATION
(ABOUT SIX TO TWENTY-SEVEN PAIRS OF
SOMITES)
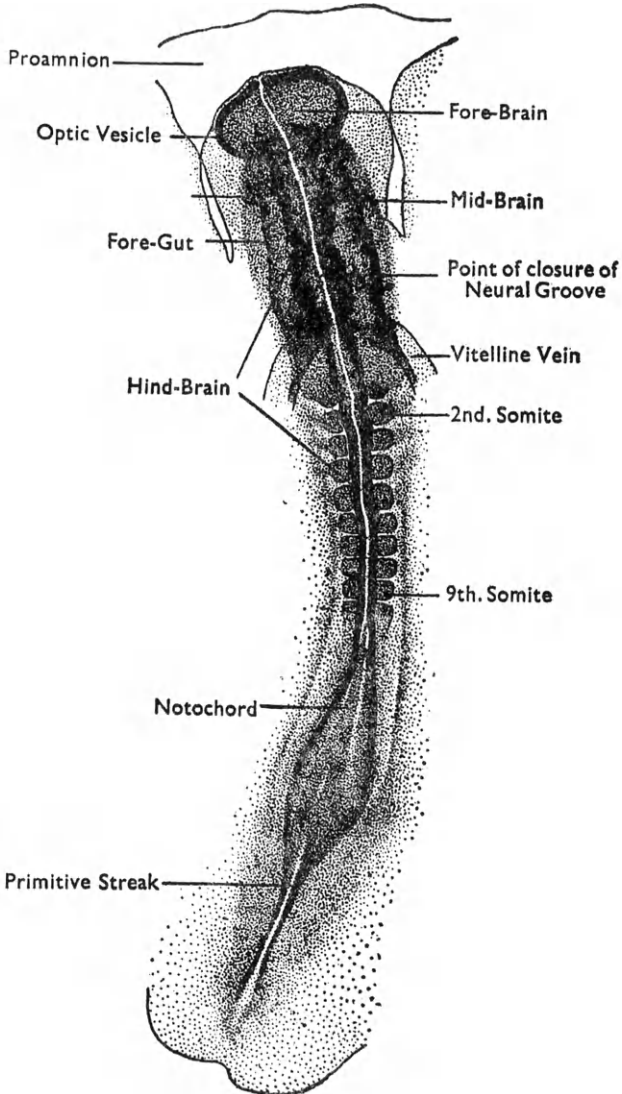
FIG. 264. Gallus. Embryo with 10 Somites (Early Second Day).
(*From Lillie's "Development of the Chick."*)

(i) *Examine* **Blastoderms of about ten to fifteen pairs of Somites (30 hours and 36 hours).**

The blastoderm is now much elongated.

The three **cerebral vesicles** are now clearly visible. Of these the **fore-brain vesicle (prosencephalon)** is the largest, swelling out on each
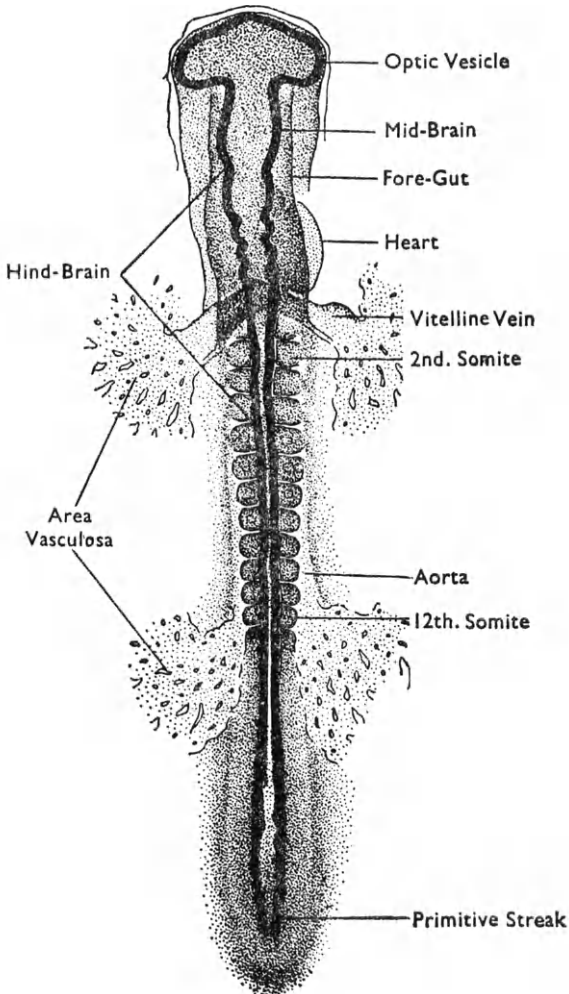
FIG. 265. Gallus. Embryo with 12 Somites (about 33 hours).
(From Lillie's "Development of the Chick.")

side to form the **primary optic vesicle.** The other vesicles are the **mid-brain vesicle (mesencephalon)** and the **hind-brain vesicle (rhomben-cephalon).** These are followed by the **spinal cord.** The **notochord** has matured from the notochordal process. The ventricle of the **heart** will now be visible bulging out to the right (it was formed from two endocardial tubes which develop at about twenty-five hours when there are seven somites and which fuse to form a single tube). From the posterior end of the heart two **vitelline veins** diverge while from from the anterior end the two **aortic arches** arise, each at a later stage giving off a **vitelline artery** at a posterior trunk level. The blood vessels lead to and from the area vasculosa. *Count the* **mesodermal somites.**

In the later blastoderm, a small invagination, the **auditory** (or **otic**) **pit,** will be found level with the hindbrain. The head will be seen to have enlarged considerably and will be beginning to bend at the level of the mid-brain towards the right side.

(ii) *Examine a* **Longitudinal Section through the Anterior End of an Embryo of about fifteen somites.**
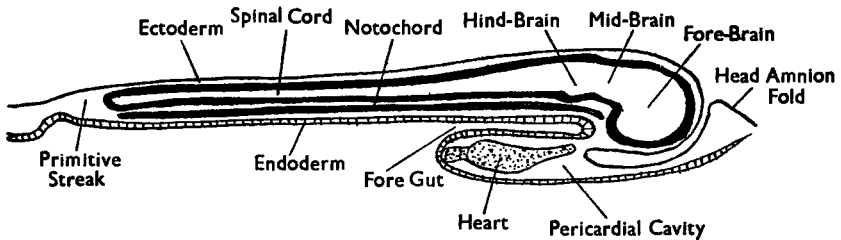
FIG. 266. Gallus. Embryo of about 15 Somites (36 hours).

Identify the **fore-brain** and, covering it, the **head amnion fold,** also the **mid-brain, hind-brain** and **spinal cord.** Find the **notochord** and below it, towards the anterior end, an invagination which is the **foregut,** while beneath this the **heart** will be seen in the **pericardial cavity.** Note the curved form of the head.

(iii) *Examine a* **Transverse Section through the mid-brain of an Embryo of about fifteen somites.**

Note the two edges of the **amniotic fold** which have not yet met, and the **amnion** enclosing the **mid-brain** and, beneath it, the **noto-chord,** below which are the **dorsal aortae** and **ventral aortae.** These aortae surround the pharynx. The **chorion,** composed of ectoderm externally and mesoderm internally, is the outer layer of the head amnion fold while the **amnion,** already noted, composed of mesoderm externally and ectoderm internally, forms the inner layer. Between the two mesodermal layers is the **extra-embryonal coelom.**
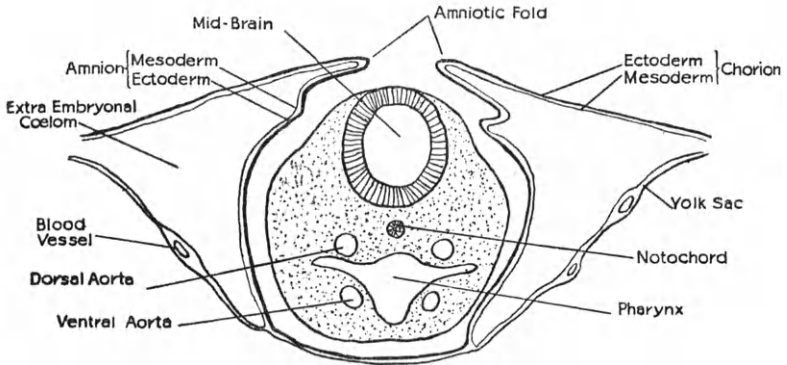
FIG. 267. Gallus. T.S. through Mid-brain Region of Embryo with about 15 Somites (36 Hours).
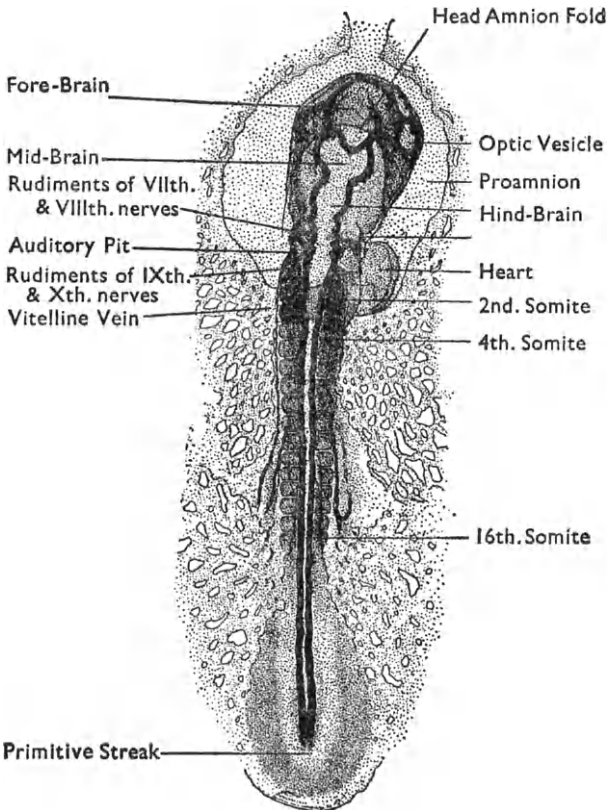
FIG. 268. Gallus. Embryo with 16 Somites (about 38 hours).
(*From Lillie's "Development of the Chick."*)

(iv) *Examine an* **Embryo of about 48 hours (Twenty-seven pairs of Somites).**
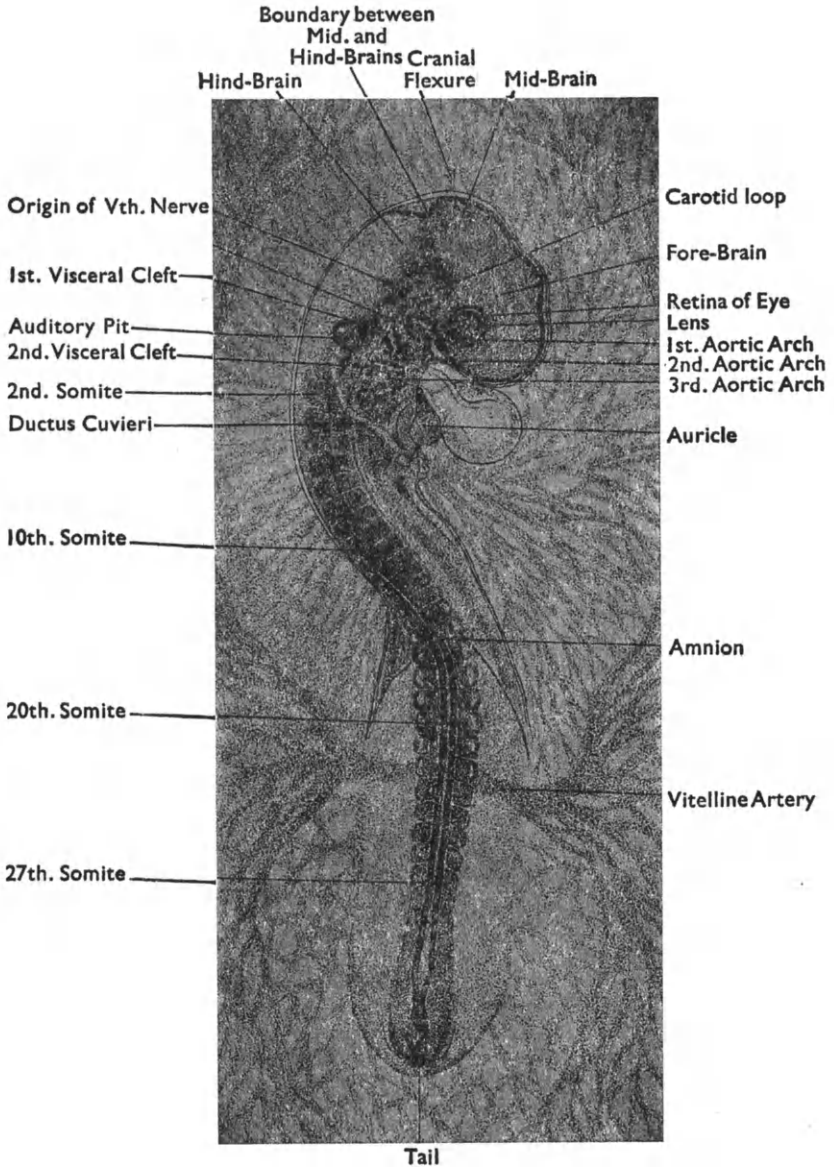
FIG. 269. Gallus. Embryo with 27 Somites (about 48 hours).
(*From Lillie's "Development of the Chick."*)

The structures already seen will be visible and it will be observed that the **amnion** stretches back to about the sixteenth somite. The **cranial flexure** is very marked and another is beginning to develop level with the posterior part of the hind-brain; this is the **cervical flexure.** At the posterior end of the embryo will be seen the **tail fold** behind which is the **tail amnion fold.** The primitive streak has almost disappeared. Examination of the fore-brain shows that it is now subdivided into an anterior **telencephalon** and a posterior **thalamencephalon** by a slight depression. Dorsal to the **heart,** three **visceral pouches** will be seen.

(v) *Examine* **Transverse Sections of the Embryo at 48 hours.**

In **Sections through the Anterior End** one or more parts of the **brain** will be seen (remember that the head is curved downwards). The **optic cup** (or **secondary optic vesicle**), formed by invagination of the outer part of the primary optic vesicle is now quite deeply invaginated and the **lens,** epidermal in origin, will be visible situated in the cavity. Identify the **notochord,** the **aortic arches,** the **amnion** and the **chorion,** and also what can be seen of the **yolk-sac,** a membrane which separates the embryo from the yolk and easily distinguished from the chorion by its processing blood vessels.

In **Sections through the Trunk Region,** identify the **amnion,** the **chorion,** the **yolk-sac,** the **spinal cord,** the **notochord,** the **dorsal aorta** beneath the notochord, and the **gut** beneath the dorsal aorta. On either side of the spinal cord a **mesodermal somite** may be seen.

Anterior and **posterior liver diverticula** may be seen beneath the gut with part of the **vitelline veins** between them at the sides.

(3) THIRD DAY OF INCUBATION (ABOUT TWENTY-SEVEN TO THIRTY-SIX PAIRS OF SOMITES)

(i) *Examine an* **Embryo of about thirty pairs of Somites (about 60 hours).**

The **cranial flexure** will be seen as before and the **cervical flexure** is more marked. Note the general enlargement of the **embryo,** the prominent **ventricle of the heart** with the **bulbus cordis** running forward from it and the **sinus venosus** alongside it leading anteriorly into the **atrium.** The **dorsal aorta** curves round alongside the somites. The **head amnion fold** is now behind the level of the vitelline arteries and continuous with it are the **lateral amnion folds** and the **tail amnion fold.** In front of the latter is part of another membrane, the **allantois.** It arises from the gut and is the third of the foetal membranes.
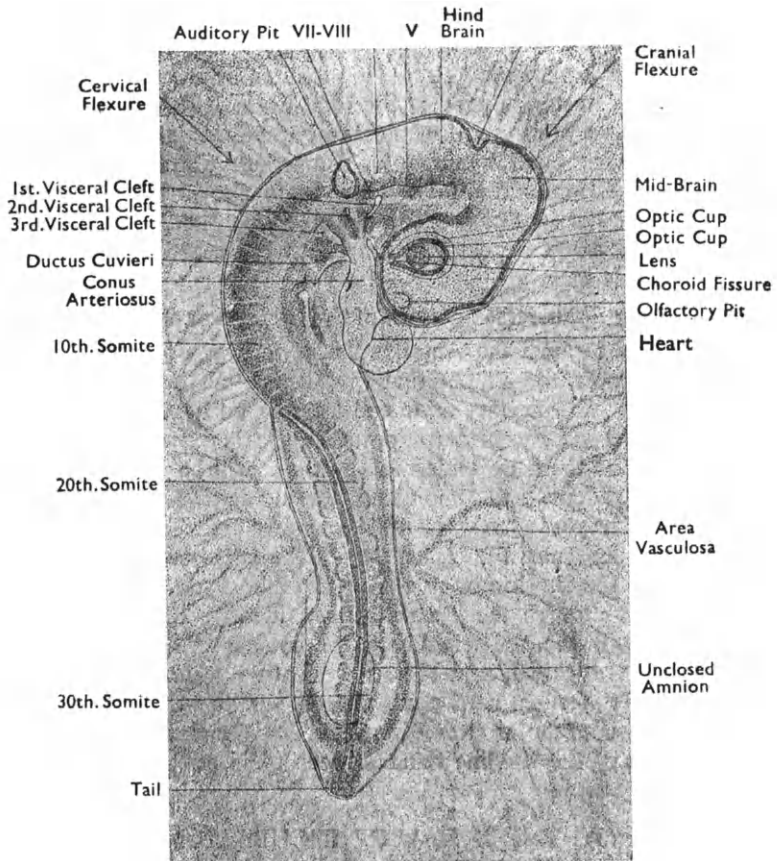
FIG. 270. Gallus. Embryo of 31 Somites (about Two and a Half Days).
(*From Lillie's "Development of the Chick."*)

(ii) *Examine a* **Transverse Section through the Hind Brain of an embryo with about thirty-four somites.**

At the anterior end of this section the **hind brain** will be seen and at the other end of the **spinal cord**. Between them are the **notochord** and the **dorsal aorta** and, on each side between the aorta and the hind brain, the **anterior cardinal veins**. The **auditory vesicles** (formed from the auditory pits seen earlier and which later in development became auditory sacs) are situated on either side of the hind brain at its posterior end. The enveloping membranes, **chorion, amnion** and **yolk-sac,** will be seen in an earlier section.
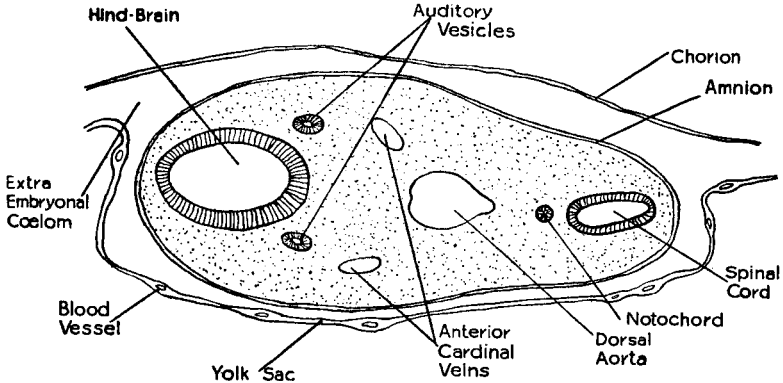
FIG. 271. Gallus. T.S. through Hind-brain Region of Embryo with 34 Somites
(about End of Third Day).

(iii) *Examine* a **Transverse Section through the Pharyngeal Region
of an embryo with about thirty-four somites.**

Again the **hind brain** and **spinal cord** will be seen at the two
extremities and the **notochord, dorsal aorta** and **anterior cardinal
veins** as in the last section. In the centre of this section is a large
cavity, the **pharynx,** with four **visceral pouches** at the edges. The
elongated **laryngo-tracheal** groove (which gives rise to the larynx and
trachea) extends from the pharynx posteriorly. The **amnion,** enclosing
the **amniotic cavity,** will also be seen; likewise the **chorion** and **yolk-
sac.**
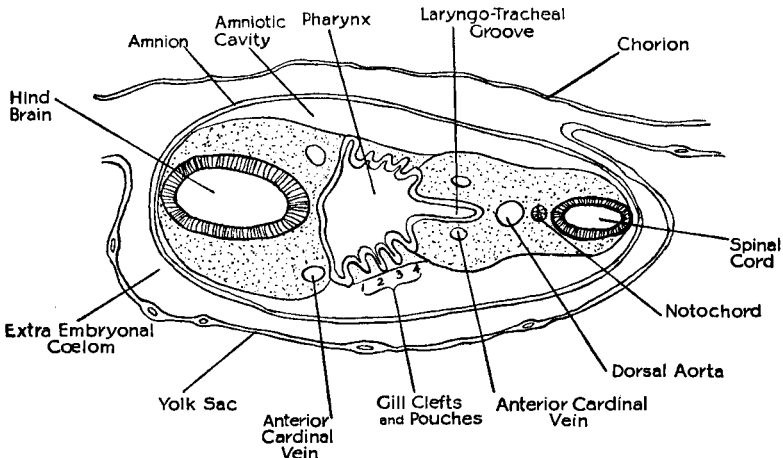
FIG. 272. Gallus T.S. through Pharyngeal Region of Embryo of about 34 Somites.

(iv) *Examine a* **Transverse Section through the Heart Region of an embryo of about thirty-four somites.**

The large **ventricle** occupies the centre of the section with the smaller **auricle** alongside it. Between the aorta and the ventricle is the **oesophagus** and, on either side of the aorta, the **posterior cardinal veins.** Part of the **coelom** will be visible. The **spinal cord** and **notochord** will also be seen at one end. At the opposite end lies the **forebrain** (owing to the cranial flexure) and on either side of it the **optic cup** (a secondary organiser developed from the optic vesicles seen previously) inside which the hollow **lens** of the eye will probably be seen. The **amnion** and **amniotic cavity** should also be noted.
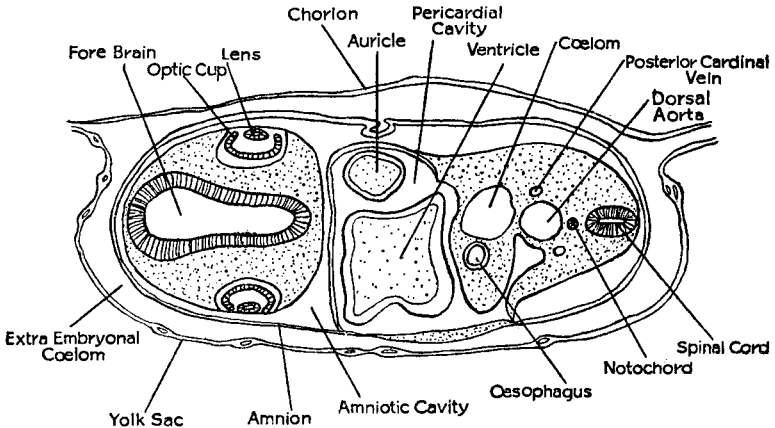
Fig. 273. Gallus T.S. through Heart Region of Embryo of about 34 Somites.

(v) *Examine a* **Transverse Section through the Trunk Region of an Embryo with about thirty-four somites.**

Having identified the **amnion, chorion** and **yolk-sac,** note the large cavity in the centre of the embryo. This is the **hind-gut** and it bears an outgrowth on its ventral side, the **allantois.** The **spinal cord, notochord** and **aorta** will also be seen.

(vi) *Examine an* **Embryo of about thirty-six pairs of Somites (about 72 hours).**

Identify the structures and organs previously seen and note their greater development. There are now four **visceral pouches.**

(4) LATER DEVELOPMENT

Examine **Preserved Mounted Specimens of later Stages in Development.**

Fourth Day. The **urino-genital** system has developed and the other organs already seen have continued to develop. **Wing** and **leg buds** have appeared.

Fifth and Sixth Days. The cartilaginous skeleton develops.

Seventh to Ninth Days. The trunk has developed considerably, bringing it more into its final proportion with the head.

Tenth to Twentieth Days. Note the further development of the organs and systems and the diminution of the **yolk-sac** with the growth of the embryo in size. The **allantois** becomes a highly vascular sac enveloping the embryo, and both allantois and yolk-sac develop stalks which, with associated blood vessels, form the **umbilical stalk.** This ultimately narrows and constricts at its base, thus sealing off the connection with the ventral wall of the embryo.

By the nineteenth or twentieth day, the head of the embryo is situated close to the air sac formed by the **shell membranes** and the yolk-sac has been absorbed.

Hatching occurs on the twenty-first day.

THE MAMMAL

(1) OVUM

Examine a **T.S. of Ovary of a Mammal containing Mature Ova.**

It will be recalled from histological studies that the **ovum** develops in a Graafian follicle in the stroma beneath the germinal epithelium of the ovary and that it is a spherical structure enclosed in the **zona pellucida** and is composed of **deutoplasm** or yolk (absent in the rabbit's ovum) in which is a large nucleus, the **germinal vesicle,** containing a nucleolus known as the **germinal spot.**

(2) LATE FOETUS

(1) *Examine a preserved mounted specimen of a* **Late Foetus of a Rabbit attached to the Uterus.**

Note the vascular **allantoic placenta,** the organ of foetal nutrition, respiration and excretion, to which the foetus is attached by the **umbilical cord** containing two **umbilical arteries** and one **umbilical vein.**

(2) *Examine a slide of a* **T.S. of a Mammalian Placenta.**

It will be remembered that after the ovum has been fertilised in the Fallopian tube, it descends into the uterus where it becomes embedded in the uterine wall, the allanto-chorion of the embryo being attached to thickened mucous membrane of the uterus. The allanto-chorion is covered by a thick syncytium known as the *trophoblast*. From this structure branched finger-like *chorionic villi* containing foetal blood capillaries arise and grow into the blood sinuses
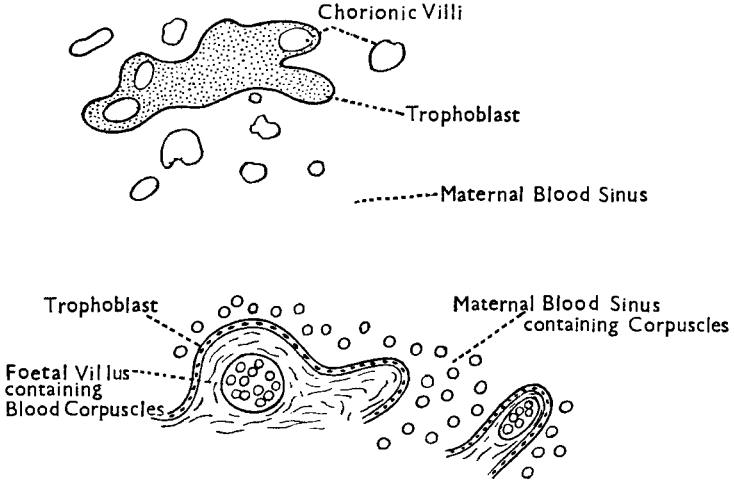
Fig. 274. T.S. Mammalian Placenta.

in the maternal tissue. Thus the *placenta* is formed. Eventually the epithelium breaks down and the tissue containing the capillaries in the villi is separated from the maternal blood by a thin *syncytium* and so the two blood systems are put into intimate contact but they never join and the two bloods never mix. In this way, oxygen and food diffuse from the maternal blood into that of the foetus and carbon dioxide and other excretory matter diffuse from the foetal blood into that of the mother.

The transverse section of the **placenta** therefore shows transverse sections of **chorionic villi** lined by an epithelial layer or a thin **syncytium,** according to the stage of development. These villi contain **foetal blood capillaries.** Surrounding the villi **maternal blood capillaries** will be seen in the **maternal tissue** and corpuscles will be visible in both kinds of capillaries.

PART VII

OTHER FORMS AND MODES OF LIFE

INTRODUCTORY NOTES

Some forms of life differ from typical plants and animals both in structure and in the manner in which they perform some of thei: functions. These differences in form and function are reflected in their manner of living. They are organisms which have become adapted structurally and functionally to particular modes of life. Included under this heading are **insectivorous plants, saprophytes, symbionts, commensals** and **parasites.** In addition to these are **epiphytes** and **epizoites.** These are plants or animals which obtain external support from other plants or animals on which they grow but which show no preference for any particular plant or animal to give this support nor is any benefit derived by either organism other than support by one of them. They cannot therefore be classified under any of the previous types. Examples of epiphytes are the lichens which grow on trees. Epizoites are less common.

These forms of life are dealt with below with the exception of epiphytes and epizoites. Bacteria which fall into more than one of these groups and which play an extremely important part in the lives of plants and animals, including man, are treated in some detail.

1. 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.

1. 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. Down-wardly 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.

2. The Sundew (*Drosera*)

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

Examine a **Drosera 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") ,ınd 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.
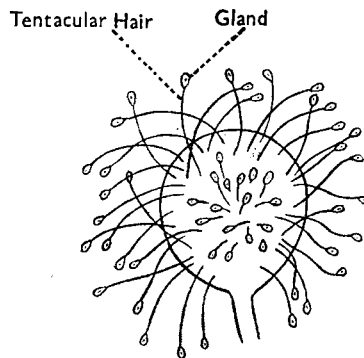
FIG. 275. Drosera Leaf.

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

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

2. FUNGI

As the Fungi, like the Bacteria, fall into some of the subsequent groups in this part of the book, their general characteristics and classification is given below. They, like the Bacteria, could with some justification be classified in a separate Kingdom but they are included in the Plant Kingdom in the DIVISION *Thallophyta* SUB-DIVISION *Fungi*. They are devoid of chlorophyll and therefore incapable of photosynthesis. Their nutrition is therefore *heterotrophic*, either *saprophytic* or *parasitic*. The plant body is called a **mycelium** and the filaments of which the most mycelia are composed are known as **hyphae.** Though the majority are terrestial in habitat, some occur in water such as *Saprolegnia* (fish-fungus, which is a facultative parasite). Moulds and Mildews are Fungi and mushrooms and toadstools are also included in this SUB-DIVISION.

There are three main CLASSES—(1) *Phycomycetes* (Alga-like Fungi, generally with non-septate, multi-nucleate hyphae, mostly terrestial and reproducing asexually by non-motile spores or motile zoospores. Sexual reproduction may also occur). The Phycomycetes are further classified by some taxonomists into two SUB-CLASSES (i) *Zygomycetes* (lack antheridia or oogonia, have similar gametes and give rise to zygospores) and (ii) *Oomycetes* (in which antheridia and oogonia are developed as the sexual organs though asexual zoospores are readily formed). (2) *Ascomycetes* (Hyphae septate. Reproduction asexual by non-motile spores and an imperfect sexual reproduction by *antheridia* and *ascogonia* with the production of endospores called *ascospores* developing in a structure known as an *ascus*). (3) *Basidio-mycetes* (hyphae non-septate, heterothallic. Four *basidiospores* arise from a structure called a *basidium*. There is also a CLASS known as *Fungi Imperfecti* but many of these have been shown to belong to the Ascomycetes. *Microsporon*, the cause of ringworm *(Tinea)* comes in this class.) The *slime fungi*, which are not true fungi, are classed as **myxomycetes.** The study of fungi is called **mycology.**

3. BACTERIA

Bacteria are very minute micro-organisms and are included under the heading of what are popularly known as "microbes" but it must be understood that not all microbes are bacteria. Protozoa such as those which cause amoebic dysentery and malaria and viruses which are responsible for many plant and animal diseases also come under this description.

Again, **spirochaetes** such as *S.* (or *Treponema*) *pallida*, the cause of syphilis, are not true bacteria though they are classified in one of the Orders of that group.

Though bacteria, like Fungi, could with some justification be placed in a separate kingdom, they are, in fact, classified as plants in the DIVISION *Thallophyta*, SUB-KINGDOM *Bacteria*, and CLASS *Schizomycetes* in which there are several Orders.

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 aerobic **(obligatory aerobes),** others solely anaerobic **(obligatory anaerobes)** while still other species can use either method **(facultative anaerobes).**

A few are **autrotrophic,** 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.*
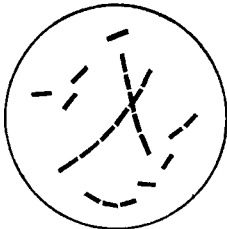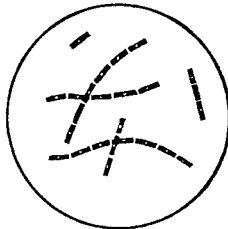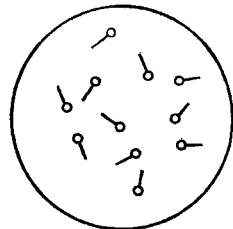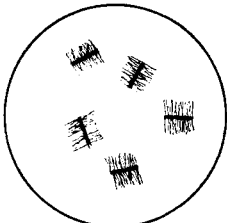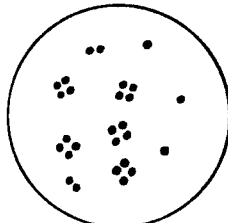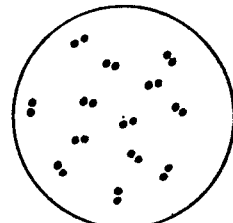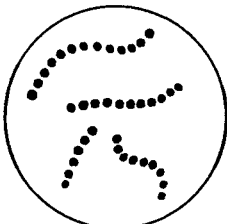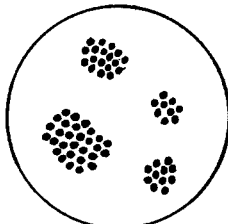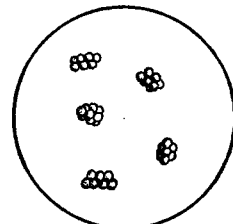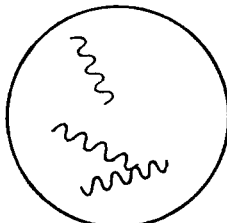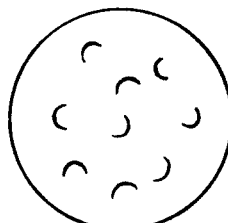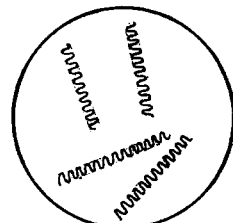
FIG. 276. **Bacteria. Forms.**

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. However, 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 20μ* 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.

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.

Gram's Test. A standard bacteriological test stain is Gram's Iodine (see Appendix IA (2), 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

*$\mu = 0.001$ mm. $m\mu = 0.000001$ mm.

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.

From Above

Nutrient
Agar

Nutrient
Agar

Side View

For Slope
(Surface)
Culture

For Stab
(Eg. Anærobic)
Culture

For
Surface
Cultures

For
Stab
Cultures

PETRI DISH

CULTURE TUBES

PLATINUM WIRES
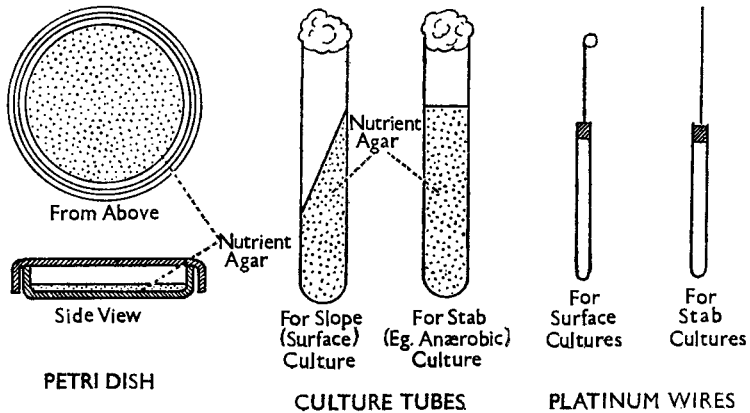
FIG. 277.   **Bacteriological Apparatus.**

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.

(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 a platinum wire or forceps, sterilised in the flame.*

---

*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 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

(i) *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.

(ii) *Scrub your hands thoroughly using an antiseptic soap, rinse and repeat the above experiment.*

(iii) Leave a third petri dish containing nutrient agar untouched as a "control".

(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 scrape 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 from one of the cultures grown in one of the previous experiments. To transfer the material use a platinum needle previously sterilised in the flame (it will cool almost immediately).

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*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.

Apply it to the material and then stroke the surface of the agar with it. Dry the smear with gentle heat. Stain with methylene blue or carbolfuchsin. Cover and examine with a $\frac{1}{12}$ in. O.I. objective or make a permanent mount.

(12) **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.**

(13) **Bacterial Cultures**
Examine some **cultures of various Saprophytic bacteria:—**

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

4. SAPROPHYTISM

Saprophytes are plants which depend for their nutrition on non-living organic material such as the remains of dead organisms or substances derived from them. These plants live on the **substrate,** as it is called, and digest it externally by enzyme action in order to absorb the food in liquid form. Correspondingly in the animal kingdom are **saprozoites** and it will be remembered that some species of Euglena are able to feed saprozoically. Examples of saprophytes are the moulds *Aspergillus, Mucor, and Penicillium, yeast, mushrooms* and *toadstools* and the *soil bacteria* responsible for the nitrogen cycle.

(1) SAPROPHYTIC BACTERIA

Examine slides of **saprophytic bacteria** *such as the following, using a $\frac{1}{12}$ in. O.I. objective:—*

B. nitrosomonas, B. nitrobacter, Azotobacter chroococcum, Clostridium pastorianum.

(2) SAPROPHYTIC FUNGI
MUCOR

This fungus is commonly known as *white mould* or *pin mould.* The commonest species, *M. mucedo,* grows on bread and similar food-stuffs. Another species grows on dung. Mucor belongs to the SUB-DIVISION *Fungi,* the CLASS *Phycomycetes* and the SUB-CLASS *Zygomycetes.* It is an *obligatory saprophyte.*

(1) *Expose a piece of wet bread to the air for about an hour. By this time spores of the white mould* **Mucor mucedo** *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.**

*An olive green mould *Eurotium* (*Aspergillus*) often grows on bread in addition to Mucor.

(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** which are responsible for its **asexual reproduction.** By careful focussing it will be seen that the hypha bulges into the sporangium: this is the **columella.**

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

Look for **gametophores,** short branches on the sides of the hyphae, the apical parts of which **(gametangia)** are cut off by a septum and contain multinucleate gametes, and 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; they are said to be *heterothallic.* 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 **promycelial hypha,** a slender hypha bearing a **sporangium** at its tip but these are usually formed only after a period of rest.

PENICILLIUM

Penicillium glaucum is an *obligatory saprophyte* which will develop on bread. It is a blue green mould and must not be confused with the green mould *Eurotium* (*Aspergillus*) which also grows on bread. *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.

Penicillium is in the CLASS *Ascomycetes*. *P. expansum* is interesting in that it produces an antibiotic, *Patulin*, which inhibits the growth of the fungal parasite *Pythium* which causes the disease known as the "damping off" of seedlings.

(1) *Mount some* **Penicillium** *in dilute glycerine.*
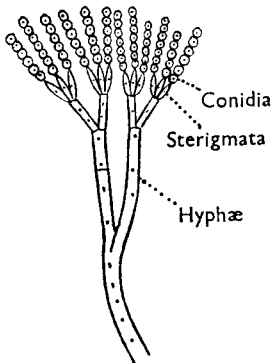
FIG. 278. Penicillium.

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.

YEAST (SACCHAROMYCES)

This fungus occurs naturally on fruits and is found in the air. It is responsible for the fermentation of sugar to ethyl alcohol (**alcoholic fermentation**) in which the yeast cells are able to survive, even in a quite high concentration of alcohol. The cells contain *B vitamins.*

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

The mycelium consists of isolated ovoid cells each with a **cell wall** of polysaccharide material containing granular **cytoplasm,** in which will be found small **vacuoles** and **granules** of **glycogen** and **volutin.** The **nucleus** is also vacuolated and is traversed by a network of **chromatin threads.** Asexual reproduction is by the formation of **buds (gemmation)** and this takes place rapidly so that groups and chains of buds will be seen.

(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 occur in some species and are formed under adverse conditions. The cells containing **ascospores** may be regarded as **asci.**

(6) *Investigate the* **fermentation of sugar by yeast.**

(7) *Fit a large flask with a rubber stopper and double right-angle tube as shown in Fig.* 13. *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.*
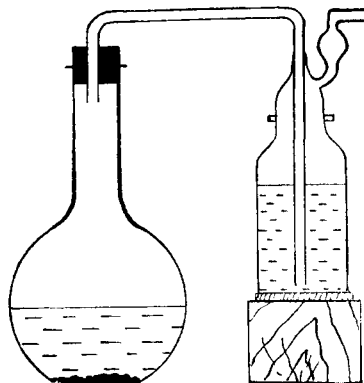
Fig. 279. **Apparatus to demonstrate Fermentation.**

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.* A yellow precipitate of iodoform with a characteristic smell is obtained.

$$C_2H_5OH + 6NaOH + 4I_2 + CHI_3 = HCOONa + 5NaI + 5H_2O.$$

*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.

(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.

$$2C_2H_5OH + 2CH_3COONa + H_2SO_4$$
$$= 2CH_3COOC_2H_5 + Na_2SO_4 + 2H_2O.$$
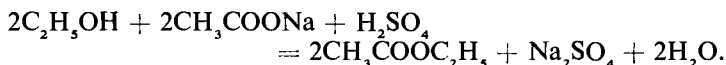
(iii) **Aldehyde Test.** *Add a few crystals of potassium dichromate and a few millilitres of dilute sulphuric acid. Heat.* The liquid turns green and a characteristic smell of acetaldehyde is produced.

$$C_2H_5OH + O = CH_3 \cdot CHO + H_2O.$$
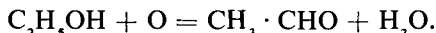
The fermentation of sugar is brought about by the enzyme complex, **zymase.** The action is rather complicated but, briefly, it may be summarised as follows:—

(i) Glucose + phosphoric acid $\xrightarrow{}$ a hexosephosphate

$$C_6H_{12}O_6 + 2H_3PO_4 \xrightarrow{phosphatase} C_6H_{10}O_4(H_2PO_4)_2$$
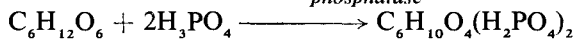
(ii) Hexosephosphate $\xrightarrow{}$ pyruvic acid and phosphoric acid

$$C_6H_{10}O_4(H_2PO_4)_2 + H_3PO_4 \xrightarrow{phosphatase} CH_3 \cdot CO \cdot COOH.$$
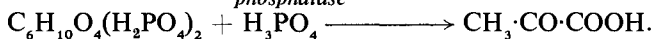
(iii) Pyruvic acid $\xrightarrow{}$ acetaldehyde + carbon dioxide

$$CH_3 \cdot CO \cdot COOH \xrightarrow{carboxylase} CH_3 \cdot CHO + CO_2.$$

(iv) Acetaldehyde $\xrightarrow{}$ ethyl alcohol

$$CH_3 \cdot CHO \xrightarrow{hydrogen} CH_3 \cdot CH_2 \cdot OH.$$

THE MUSHROOM

PSALLIOTA (AGARICUS)

Psalliota (*Agaricus*) *campestris,* the field mushroom, belongs to the CLASS *Basidiomycetes* and is one of the Gill-fungi. It is an *obligatory 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* **sporophore (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 **lamellae** (or **gills**). 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 inter-woven and more densely arranged towards the outside than in the centre.
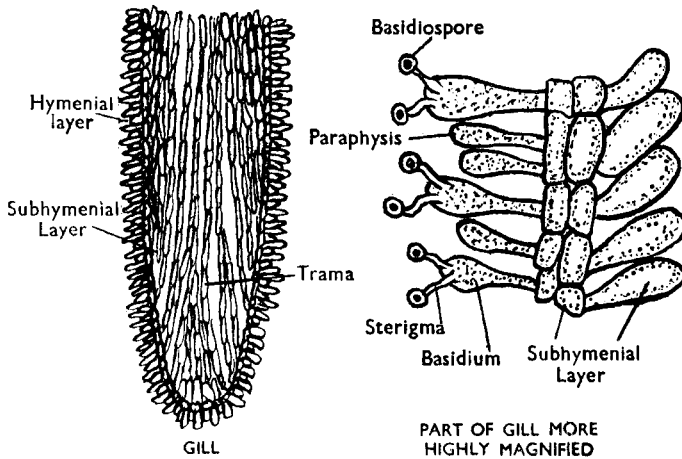
FIG. 280. Psalliota. **Structure of Gill.**

(5) *Examine a* **tangential vertical section of the pileus,** *passing trans-versely through the gills. Mount in dilute glycerine.*

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.**

5. SYMBIOSIS

This is an association between two organisms in which both benefit by the association and in which neither can exist without the other. The organisms concerned are known as **symbionts.**

1. Animal/Plant Symbionts

Examine a slide of a **T.S. of Hydra.**

In the endodermal cells non-cellular green plants known as *Zoochorellae* will be found. (These will have been seen when studying Hydra.) These plants live symbiotically with the animal, obtaining carbon dioxide for photosynthesis and nitrogen from the animal while Hydra provides that nitrogen in its excretory products and obtains oxygen from the photosynthesis of the plant.

2. Plant/Plant Symbionts

(i) Alga/Fungus—Lichens

These plants, which consist of a fungus and an alga living sym-biotically, 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 the foliose Dog's Tooth Lichen (*Peltigera canina*), *Cladonia furcata* or *Xanthoria*.

In this the thallus is flat and of a dull green colour when wet though rather greyish when dry. Underneath the thallus are hair-like **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.**

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.
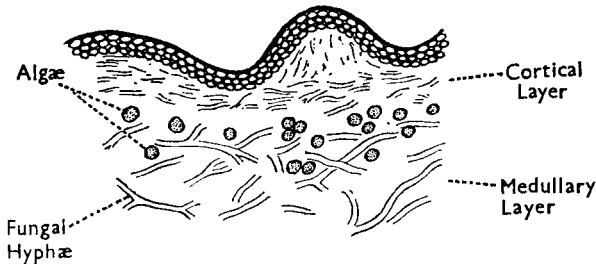
FIG. 281. Lichen. T.S. Thallus.

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.

(ii) **Spermatophyte/Bacteria**

Examine a slide of 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. radicicola* (*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.

(iii) **Spermatophyte/Fungus—Mycorrhiza**

This is an association between *Fungi* and the roots of flowering plants. In **ectotrophic mycorrhiza,** the fungal mycelium grows round the roots externally and functions as root-hairs whereas in **endo-trophic mycorrhiza,** the fungus lives inside the roots. Many toadstools growing in woods are ectotrophic.

Examine a slide of a **T.S. of the root of Bird's Nest Orchid** (*Neottia nidus-avis*).

In some of the outer cells of the cortex **fungal hyphae** will be seen and the plant, which is devoid of chlorophyll, its leaves being reduced to yellow scales, obtains its carbohydrates and nitrogen from the fungus. In some of these cortical cells the hyphae are less evident as

such since they are in the process of being digested by the cells of the root, while the innermost cortical cells are completely devoid of fungal hyphae and contain starch grains. This **endotrophic mycor-rhiza** is typical of this kind of association which is quite commonly found in plants living, as this plant lives, saprophytically in the humus in the soil of woods. The fungus is a saprophyte living in the humus and its hyphae enter the roots of this plant where they live symbi-otically with the flowering plant.

6. COMMENSALISM

When two organisms of different species associate together and share the same food, though they can exist independently of each other, they are known as **commensals.**

Examine a demonstration specimen of **a sea anemone on a hermit crab.**

The **sea anemone** is a sedentary animal and, living on the back of of the **hermit crab,** it obtains its food from particles which float upwards when the crab is feeding. It also gets transported from place to place, of course. The crab obtains protection by the nematoblasts on the tentacles of the anemone.

7. PARASITISM

A **parasite** is an organism which lives on or within another living organism known as the **host** and from which the parasite derives its nutrition to the detriment of the host. Parasites are thus entirely dependent physiologically on their hosts. Some plant parasites have plant hosts while others depend on animal hosts. Likewise while some animal parasites have animal hosts, there are others in which the host is a plant. The structure and life-history of parasites is modified and adapted to this form of existence and since the continuance of the species depends on a suitable host being found, capacity for reproduc-tion is greatly increased.

Parasites such as fleas and bugs which live on the outside of the host's body are known as **ectoparasites** whereas those living internally, like the tapeworm, are **endoparasites.** Some such as *Pythium*, a fungus which causes the "damping off" of seedlings are capable of living a free existence under suitable conditions and these are referred to as **facultative parasites** whereas those which can live only parasitically are **obligatory parasites.**

A number of plant and animals diseases are caused by Fungi though, as already seen, many of these are saprophytes. These

remarks apply equally to Bacteria. Some diseases, both of plants and animals are, however, caused by **viruses.** Animal parasites are found amongst the *Protozoa*, the study of which is known as **protozoology,** and amongst the *Platyhelminthes* (flat worms) and *Nematoda* (round worms). The study of worms is known as **helminthology.** Parasites obviously influence the health and lives of plants and animals to a considerable degree and the study of these organisms, known as **parasitology,** is a very specialised branch of biological investigation. A knowledge of their life-histories enables methods of prevention **(prophylaxis)** and treatment **(therapy)** of the diseases caused by them to be investigated and discovered.

The transmission of parasites to a new host may be through the air, in the soil, in food, in water or by actual contact. On the other hand parasites may require the assistance of another organism as an intermediate host for this purpose. These animal carriers of the parasite are called **vectors.** The female anopheline mosquito, for example, is the vector of the malarial parasite while diseases may be transmitted by the house-fly. The former are clearly *biological vectors* since they play their part in the parasite's life-cycle, whereas the latter which carries parasites on its body without taking any part in the life-cycle of the parasite might be called a *mechanical vector*, a term used for inanimate objects responsible for transmitting parasites. The study of these vectors is included in this study of parasitology.

I. PLANT PARASITES

(1) A PARASITIC ANGIOSPERM

DODDER (CUSCUTA)

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. europæa*) **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.**
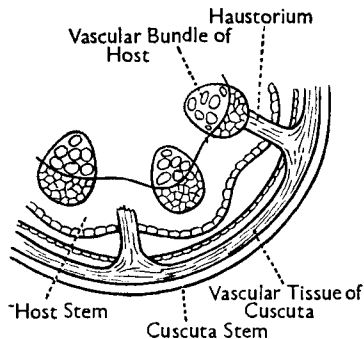
FIG. 282. Cuscuta. T.S. Stem of Infected Host.

These **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.

(2) VIRUSES

Viruses are ultra-microscopic and some have been seen under the electron microscope, their sizes varying between 6 mμ or 10 mμ and 500 mμ* 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.The tobacco mosaic virus has been extracted and it took the form of crystals. When reinjected into the host it became an active virus. This virus is rod-shaped and measures about 500 mμ by 15 mμ. 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 and appear to be entirely dependent on their hosts. The practical study of viruses **(virology)** is clearly beyond the scope of this work.

(3) PATHOGENIC BACTERIA

(1) *Examine preserved killed* **cultures of various pathogenic bacteria** (*if available*) *such as the following:*—
Staphylococcus pyogenes, Bacillus typhosus.

*μ = 0·001 mm. mμ = 0·000001 mm.
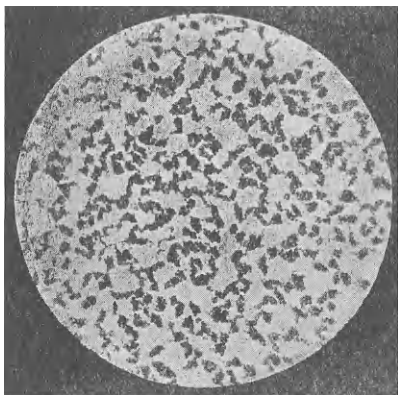
Staphylococcus pyogenes aureus.
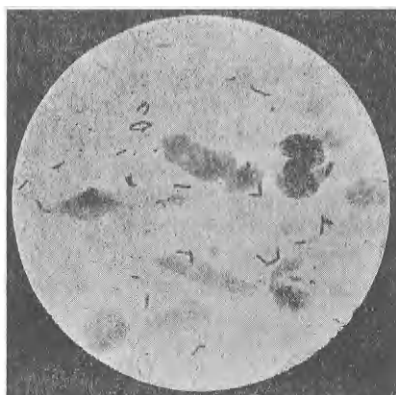
Bacillus tuberculosis.
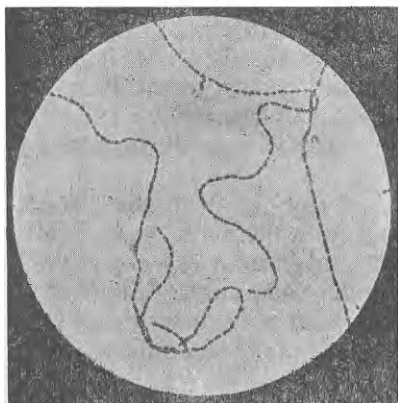
Streptococcus pyogenes longus.
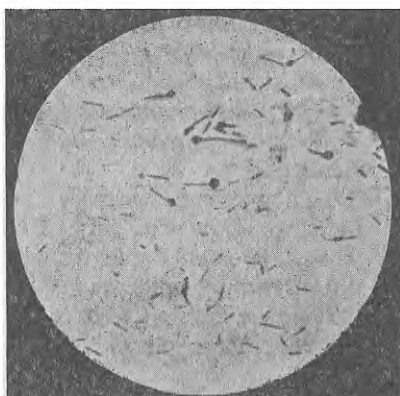
B. totani.
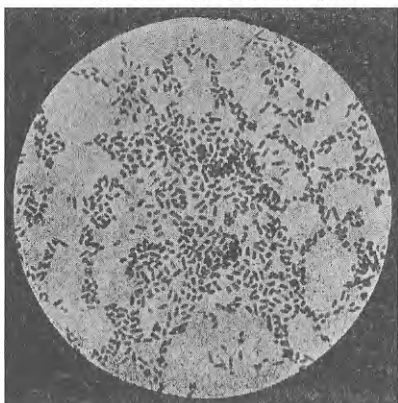
B. coli communis.
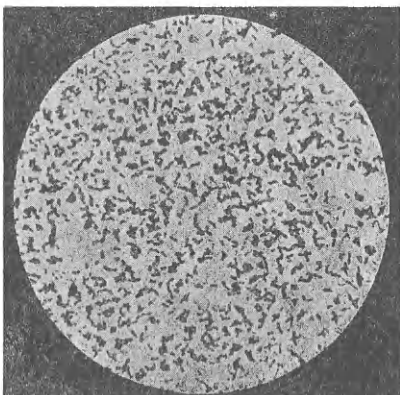
Pneumococcus.

FIG. 283. **Pathogenic Bacteria.**
(*Bulleid "Textbook of Bacteriology for Dental Students." London: Heinemann.*)

(2) *Examine prepared slides of* **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*.

(4) PARASITIC FUNGI

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. It is in the CLASS *Phycomycetes* and the SUB-CLASS *Oomycetes.*

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

The **mycelium** which is composed of non-septate **hyphae,** and therefore *coenocytic,* arises from zoospores which settle on the leaf surface. It 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 branches **(haustoria)** which enter the cells. It is when these cells die that the brown patches appear on the leaves.
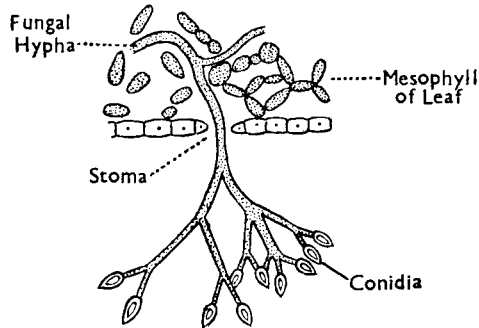
FIG. 284. **Phytophthora infestans on Leaf of Potato.**

Hyphae also grow out through the stomata of the leaves. These hyphae branch and develop **sporangia** and are therefore **sporagiophores. Conidia** are set free in dry conditions and zoospores in wet conditions 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.)

PYTHIUM

This Fungus is the cause of the "damping off" of seedlings. It is a *facultative parasite* and the disease is caused by the plants germinating in very moist and crowded conditions. It belongs to the CLASS *Phycomycetes* and the SUB-CLASS *Oomycetes*.

(1) *Examine with a lens a* **seedling** (*e.g., cress or broad bean*) **which is "damping off", i.e., infected by Pythium de Baryanum.**
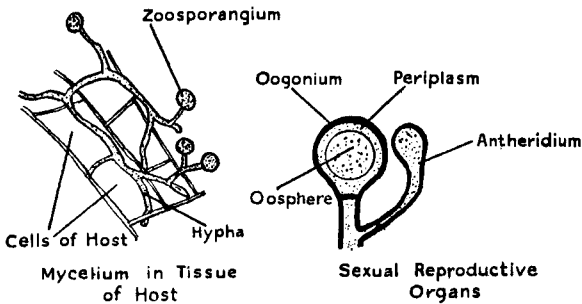
FIG. 285. Pythium.

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.**

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

Note also the **sporangia** which germinate into new mycelia and the contents of which divide to form **conidia** or **zoospores,** each bearing two laterally placed flagella, the former being produced in dry conditions and the latter if conditions are wet.

(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** and **zygotes** and **oospores** (zygotes with resistant coats).

Examine **germinating oospores,** each bearing a **hypha** and a **zoosporangium.**

PERONOSPORA

This mildew is an *obligatory parasite* living within the tissues of the host and is a *selective parasite, i.e.,* it can attack only a specific host. It is one of the "downy mildews", found on cabbages, turnips, beet, etc. It belongs to the CLASS *Phycomycetes* and SUB-CLASS *Oomycetes.*

(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 non-septate **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 which bear **conidia** 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 short narrow **antheridium** on lateral branches of the same or another hypha. Look for **stages in conjugation,** showing **fertilisation tubes. Oospores** are formed from the zygotes.

ALBUGO (CYSTOPUS)

Another *obligatory parasite* which is *selective,* one species attacking Shepherd's Purse. It causes what is known as "white rust". It is in the CLASS *Phycomycetes* and SUB-CLASS *Oomycetes.*

(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.**
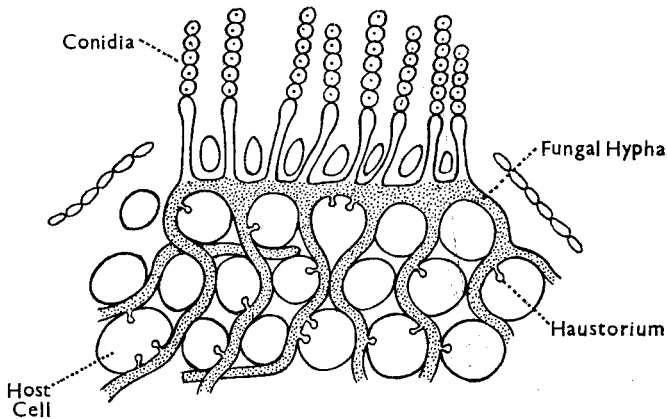
FIG. 286. **Albugo on Host.**

(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, and penetrate through the epidermis of the host or **sporagiophores** 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.

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. It is in the CLASS *Ascomycetes.*

(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.

P.B.—14

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

Note the short unbranched septate **conidiophores** from which large spores called **conidia** are budded off.
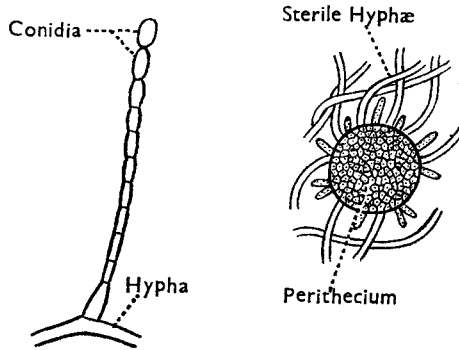
FIG. 287. **Erysiphe.**

(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.

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. It belongs to the CLASS *Basidiomycetes.*

(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 spermo-
gonia are short hyphae which bud off minute spores called **spermatia.**
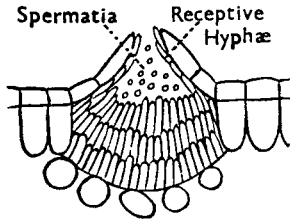
FIG. 288. **Puccinia Spermogonium.**

The spermogonia and therefore the spermatia are of two types, +
and —. They are therefore *heterothallic.* The spermatia are trans-
ferred to receptive hyphae of opposite types by insects. New hyphae
develop and aggregate together to form **aecidia.**
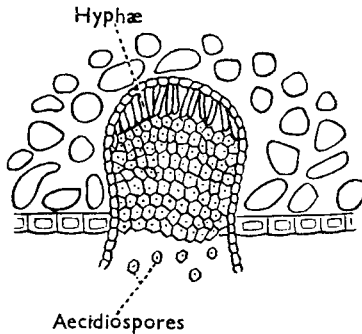
FIG. 289. **Puccinia Aecidium.**

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.
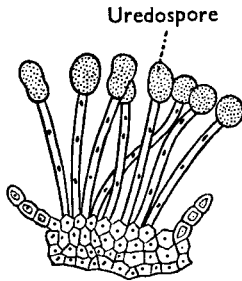
Uredospore

FIG. 290. **Puccinia.**
Uredospores.

(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.**

(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 spermogonia.
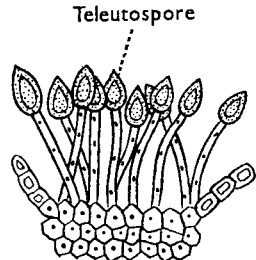
Teleutospore

FIG. 291. **Puccinia.**
Teleutospores

(5) PARASITIC ANIMALS

ENTAMOEBA

There are various species of this parasite which live in the colon and rectum of mammals. *E. histolytica*, which lives in the intestine of man is the cause of amoebic dysentry. It is interesting to note, however, that man may be a carrier of this parasite without suffering from the disease. Like Amoeba, it belongs to the PHYLUM *Protozoa* and to the CLASS *Rhizopoda*.

Examine a slide of **Entamoeba histolytica**

Note the amoeboid form of the organisms. Unlike Amoeba, however, it has no contractile vacuole. It feeds by ingesting solid particles, cells of the epithelium of the intestine and blood corpuscles. It occurs in two different sizes, a **small form** which feeds on bacteria and is probably harmless or even beneficial to the host and a **large form** into which the former develops. Reproduction is by **binary**

fission. In the lumen of the gut it forms numerous **cysts** in which the nucleus divides twice. These cysts are passed out with the faeces. A new host may be infected through water or food containing the cysts and when they reach the intestine the cyst wall is dissolved by enzyme action and the cytoplasm containing the nuclei escapes. It then divides to form new entamoebae.

MONOCYSTIS

This is parasitic in the seminal vesicles of the earthworm feeding on developing spermatozoa in a smear from which they may be obtained. The two commonest species of this parasite are *M. agilis* and *M. magna*. It belongs to the PHYLUM *Protozoa* and the CLASS *Sporozoa*.
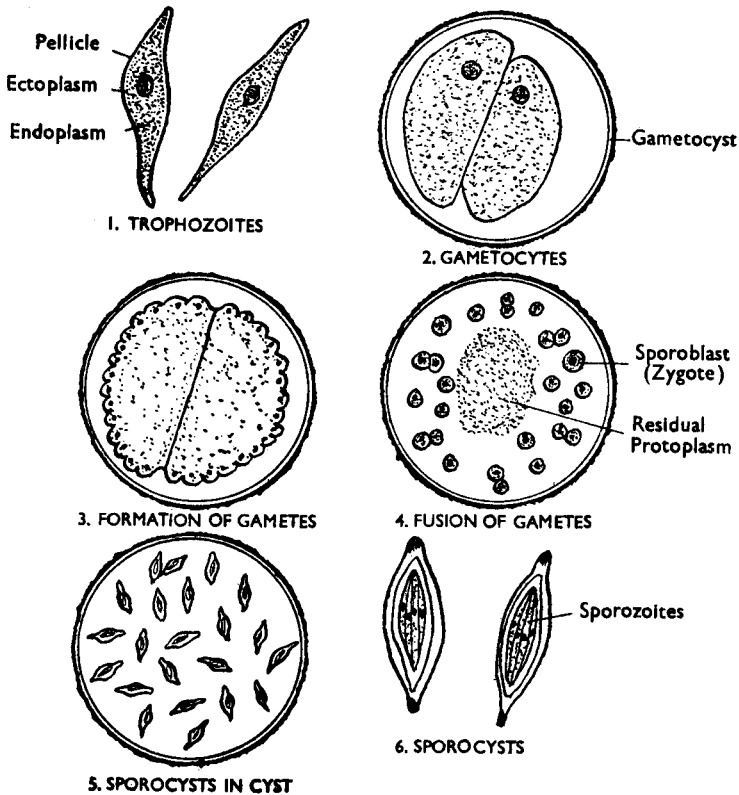
FIG. 292. Monocystis. Stages in Life History.

(1) *Examine a prepared slide of* **Monocystis** *in the* **trophozoite stage** *or make a smear of the contents of the seminal vesicle of the earthworm* (see p. 26 and p. 87).*

Note the cigar-shaped **trophozoite,** surrounded by a **pellicle** and containing a **nucleus.** There is a thin **ectoplasm** not easily recognisable, and a very granular **endoplasm** containing a number of oblong bodies composed of **paramylum** (a carbohydrate allied to starch).

(2) *Examine a prepared slide, showing the* **reproduction of Mono-cystis,** *and find as many of the following stages as possible under the high power.*

Look for **gametocytes,** two spherical cells each containing a **nucleus,** enclosed in a chitinous cell wall or **gametocyst (association cyst).** Look for later stages with the **nuclei** round the periphery of each cell and, still later, the pear-shaped **gametes.** After fusion of the gametes, note the ovoid zygotes or **sporoblasts** and the **residual protoplasm.** Later the sporoblasts are surrounded by a cyst and are known as **sporocysts (pseudo-navicellae,** so-called because of their resemblance to the plant *navicella*): they are boat-shaped. Eight minute **sporozoites** (or **falciform bodies),** shaped like a sickle, are formed by nuclear division in the sporocyst.

THE MALARIAL PARASITE

PLASMODIUM

There are three species of plasmodium, *P. vivax,* the cause of *benign tertian malaria, P. malariae,* the cause of *quartan malaria,* and *P. falciparum,* the cause of *malignant tertian malaria.* It is in the PHYLUM *Protozoa,* CLASS *Sporozoa.*

(1) *Examine prepared slides showing one of these species of* **Plasmodium in the blood of man.** *Using the high power, or better a $\frac{1}{12}$ in. O.I. objective, find as many as possible of the following stages.*

You will probably succeed in seeing but one or two.

In the **red corpuscle** note the amoeboid, spherical or, at a later stage, vacuolated signet-ring-shaped **trophozoite** with the nucleus to one side, which after nuclear division forms a mulberry-like mass, known as the **rosette stage.** This is an asexual process, **schizogony** (or **merogony),** and the **schizozoites** (or **merozoites)** thus formed are set free into the plasma and enter other corpuscles. Look for the smaller male **microgametocytes** with large nuclei and clear cytoplasm and the larger female **megagametocytes** with small nuclei and granular cytoplasm, globular (*P. vivax* and *P. malariae*) or sausage-shaped

*This should certainly be done when dissecting this animal if not done at this stage.

(*P. falciparum*) cells formed at a later stage from trophozoites which do not undergo schizogony.
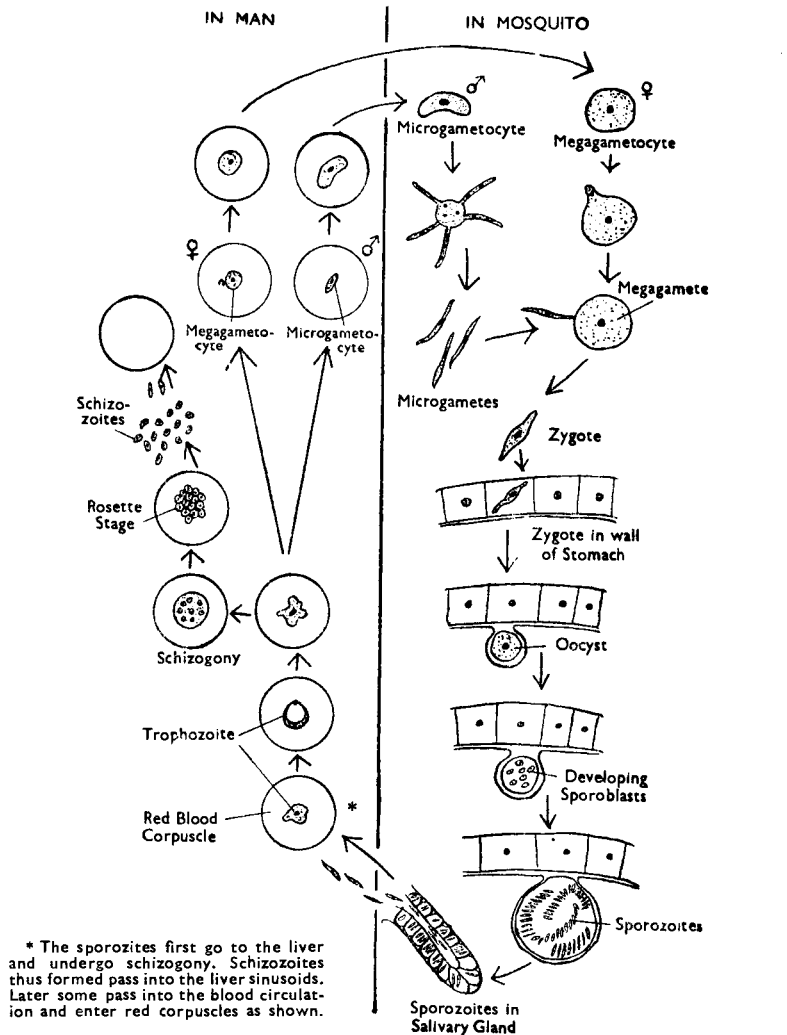
FIG. 293. **Plasmodium. Stages in Life History.**

(2) *Examine prepared slides of* **Plasmodium in the mosquito.** *Find as many as possible of the following stages:—*

Long thin **microgametes** and larger spherical **megagametes** are

formed from the microgametocytes and megagametocytes respec-
tively. These fuse to form zygotes. **Oocysts** are developed from the
zygotes (which cause swellings on the "stomach" of the mosquito).

Examine a slide showing **oocysts.**

Sporoblasts are formed from these by vacuolation and nuclear
division. From the sporoblasts arise large numbers of spindle-
shaped **sporozoites,** by multiple fission.

Examine a slide of salivary gland showing **sporozoites.**

These pass into the blood of a man when he is "bitten" by an
infected mosquito, but they quickly leave it and go to the liver where
schizogony takes place and the schizozoites enter the sinusoids. This
is known as the *pre-erythrocytic stage.* Some of the schizozoites
remain in the liver and undergo further schizogony. This is called
the *exo-erythrocytic stage* and is responsible for the re-occurrence
of the disease after a latent period. Others pass into the blood stream
and enter red corpuscles where they develop into trophozoites. This is
the *erythrocytic stage.* Schizogony then takes place again as described.

(3) *Examine, but do not draw, the ova, larva, pupa, and female
imago and a slide of the mouth parts of* **Anopheles,** *the mosquito* ($\female$)
which carries the malarial parasite.

Note the sucking-tube, or **proboscis,** on the head of the insect.

TRYPANOSOMA

Trypanosoma gambiense is responsible for the tropical disease,
sleeping sickness, and is carried by the tsetse fly. It lives in the blood
plasma of man. It belongs to the PHYLUM *Protozoa* and the CLASS
Mastigophora.

Examine a slide of **Trypanosoma,**
preferably under the $\frac{1}{12}$ in. O.I. objective.

The organism is elongated and
spindle-shaped. Its shape is maintained
by a **pellicle.** Running along the greater
part of its length is an **undulating mem-
brane** and arising from the posterior end
is a long **flagellum** which runs along the
free edge of this membrane and pro-
trudes beyond the anterior end. A small
parabasal body lies near the base of the
flagellum and a granule known as the
blepharoplast at the actual base. The
nucleus is large and ovoid. Reproduction
is by longitudinal binary fission.
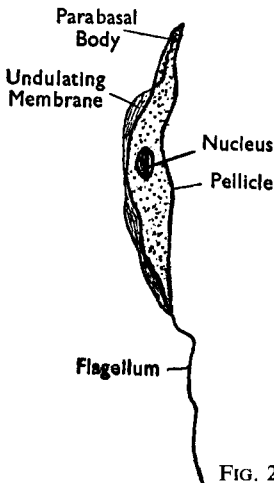
FIG. 294. **Trypanosoma.**

ROUND WORMS

ASCARIS

This Nematode is parasitic in the intestine of mammals and is therefore an *endoparasite*. *A. lumbricoides* occurring in man and the larger *A. megalocephala* in the horse. Small nematodes will often be found when dissecting the earthworm and dogfish. It belongs to the PHYLUM *Nematoda* (triploblastic acoelomatic metazoa, devoid of blood and respiratory systems).

EXTERNAL ANATOMY

Examine a specimen of **Ascaris lumbricoides,** *using a hand-lens as necessary.*

This large Nematode is yellowish-white in colour, unsegmented and cylindrical in shape, tapering to a point at each end, though less so anteriorly. It may attain a length of about 15 or more cm. in the male and as much as 22 cm. or more in the female. The posterior end curves more sharply in the male than in the female. The cuticle which protects the body is smooth and somewhat transparent and white lines will be seen running along the dorsal and ventral sides and a brownish line along the sides. Each of the former encloses a nerve cord and in the latter are canals which unite and open by a so-called excretory pore a millimetre or two behind the anterior end on the ventral side.
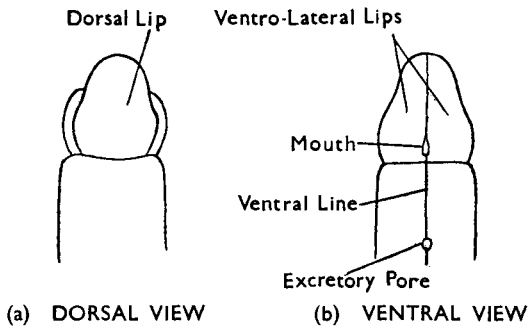
(a) DORSAL VIEW   (b) VENTRAL VIEW
Fig. 295. Ascaris. Anterior End.

At the extreme anterior end is the **mouth** bearing three lips, one **dorsal lip** and two **ventro-lateral lips,** all bearing minute teeth. *Examine the mouth in order to determine the dorsal and ventral sides of the animal.*

On the ventral side, almost at the posterior end in the *male* is the **cloaca,** the common intestinal and reproductive aperture and protruding from it is a pair of **copulatory setae** (or **penial setae**). In the *female* the apertures are separate, the **anus** being in a similar position to that of the cloaca in the male, the **genital pore** being further forward, about a third of the way along the anterior end. In both sexes the so-called **excretory pore** is found a millimetre or two behind the mouth.

(a) MALE   (b) FEMALE
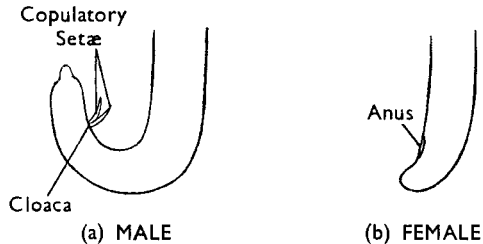
FIG. 296. Ascaris. Posterior End.

HETERODERA

This round-worm is similar in structure to *Ascaris* but it is an *ectoparasite* and its host is a plant.

The female larvae, (better known as **juveniles** as they are, in fact, similar to adults though smaller) attach themselves to the roots of sugar-beet, cabbage, tomatoes and other plants and obtain their nutrition from the sap of the plant, growing in a gall. Males visit the plants and after fertilisation the zygotes are released into the soil in cysts. Larvae develop and pass into the soil ready to infect other plants.

FLUKES

These are in the PHYLUM *Platyhelminthes* (triploblastic metazoa, bilaterally symmetrical flatworms with a complex reproductive system and usually hermaphrodite). The CLASS to which they belong is *Trematoda* (parasitic, leaf-like in shape and possessing a thick cuticle with suckers and an enteron).

All have at least two hosts and some, mostly in eastern countries, infect man, the adult fluke living in the liver, intestine or lungs and the intermediate host being snails, molluscs or fish. Small trematodes infect the lungs and bladder of the frog.

THE SHEEP LIVER FLUKE

FASCIOLA HEPATICA

This is a parasite which lives in the bile duct of the sheep causing a disease known as *liver rot*. The intermediate host is the water snail *Limnaea*. Occasionally man is infected by eating water-cress containing encysted larvae.

(1) THE ADULT FLUKE

(i) External Structure

Examine a preserved **liver fluke.**

Note the shape and size of the organism. It is protected by a thick cuticle. At the more rounded anterior end is a small projection on the apex of which is the **anterior** or **oral sucker** within which is the **mouth.** On the ventral side, a shorter distance back from the anterior end and in the mid-line is the **ventral sucker.** Between the anterior
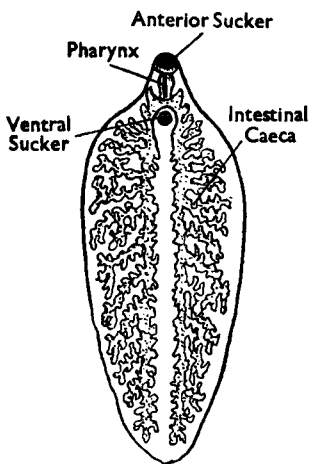
FIG. 297. Fasciola. Alimentary System.
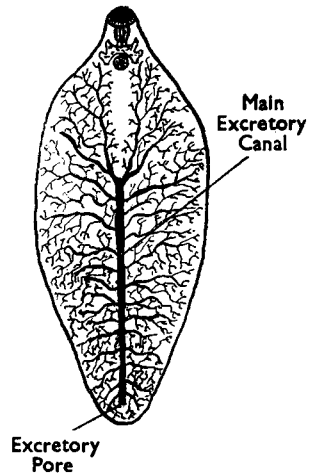
FIG. 298. Fasciola. Excretory System.

sucker and the ventral sucker is the **genital pore** and the posterior end of the organism is the **excretory pore.**

Examine a prepared stained preparation of the **liver fluke.** *It will probably be necessary to examine the following systems on the same microscopical preparation.*

(ii) Alimentary Canal

The **mouth** leads into a muscular **pharynx.** Continuous with this is a very short **oesophagus** which leads into the **intestine.** This is composed of two main branches from which arise a large number of branched blindly-ending **caeca** which reach all parts of the animal.

(iii) Excretory System

There is a **main excretory canal** into which numerous branched canals lead from all parts of the body. These small canals originate in flame bulbs and the main canal leads to the **excretory pore** at the posterior end of the animal.

Examine a slide showing **flame bulbs.**

Note that the cells are large and have several **cytoplasmic processes** prolonged from them. In the centre is a large cavity containing several **flagella.** A **duct** leads from this cavity. The **nucleus** is found in the cytoplasm to one side.

(iv) Nervous System

There are two **cerebral ganglia** at the anterior end of the animal. These are joined by the **nerve ring** round the oesophagus. Two main **lateral nerves** will be seen running backwards from the ganglia on each side of the mid-line. There are no special sense organs.

(v) Reproductive System

The animal is hermaphrodite.
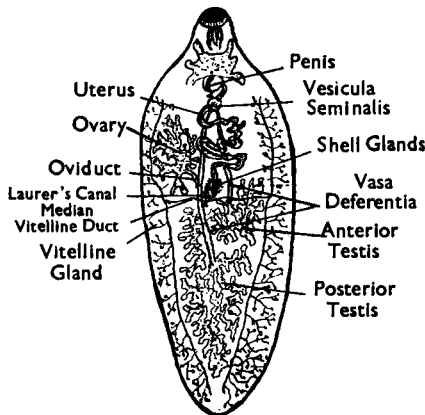
Fig. 299. Fasciola. Reproductive System.

(i) Male Organs

There are two **testes** which occupy the greater part of the centre and posterior part of the body, one being posterior to the other.

Each consists of a series of much branched tubules and from the central part of each arises a **vas deferens** which runs forwards and then joins its fellow to form a wider tube, the **vesicula seminalis.** This leads by a narrow **ejaculatory duct** into the **penis** which is protrusible through the **male genital pore.**

(ii) Female Organs

There is one **ovary,** anterior to the testes and lying towards one side. This, too, consists of branched tubules and from it a short **oviduct** leads inwards to a point where it is joined by the **median vitelline duct** formed by the union of the two transverse ducts from the **vitelline glands.** These are composed of a series of rounded follicles connected together and occupying the sides of the body. From the point where the oviduct and median vitelline duct join is a wider convoluted tube, the **uterus,** which runs forward to the **female genital pore** lying alongside the male pore. At the base of the uterus is a group of **shell glands** which lead into the oviduct. A further duct, **Laurer's canal,** arises from the point where the oviduct and median vitelline duct join and runs to the surface on the dorsal side of the animal. Into this the penis of another fluke is inserted.

(vi) Transverse Section

Examine a **transverse section of a liver fluke.**

Note the external **cuticle** bearing small **spines.** Inside this is a layer of **circular muscle** followed by **longitudinal muscle** and then the **vitelline glands. Portions of the alimentary canal** will be seen in transverse section. Look for **portions of the testes** and **ovary** and
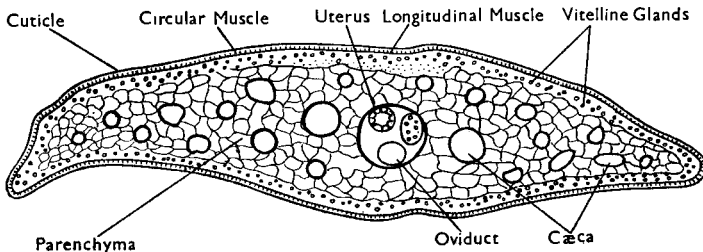
FIG. 300. Fasciola. T.S.

their associated structures, the **vasa deferentia,** the **uterus** and the **oviduct.** Near the outer ends of the sections may be seen the **lateral nerves** and in the centre the **main excretory canal.** Filling up the spaces between these structures and serving as a general packing tissue is **parenchyma.**

(2) Larval Forms

(i) Miracidium

Examine a slide showing **Miracidia.**

These minute larvae hatch out from the fertilised eggs and are roughly conical with a small projection at the anterior end behind which is a pair of **eye-spots.** They bear long **cilia** on the surface.
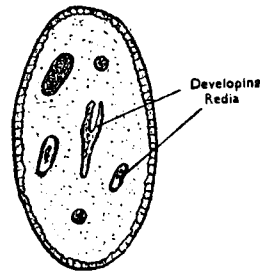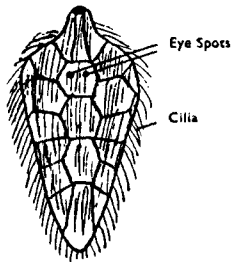
FIG. 301. Fasciola. Miracidium.   FIG. 302. Fasciola. Sporocyst.

(ii) Sporocyst

Examine a slide showing **Sporocysts.**

When the miracidium enters the water snail *Limnaea*, which lives in ponds and damp meadows, it develops into a second larva, the sporocyst. This is simple ovoid structure and may contain **developing rediae,** a third form of larva which eventually leaves the sporocyst.

(iii) Redia

Examine a slide of **Rediae.**

This larva is an elongated structure with a **mouth** at its anterior end leading into a **pharynx** and wide blindly ending **gut. Secondary rediae** develop inside during the summer and a fourth kind of larva called a **Cercaria,** during the winter.
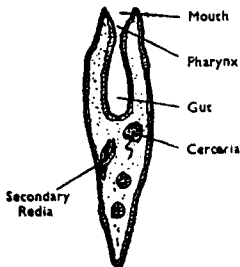
FIG. 303. Fasciola. Redia.   FIG. 304. Fasciola. Cercaria.
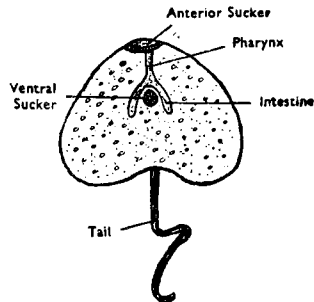
(iv) Cercaria

Examine a slide of **Cercariae.**

The Cercariae are roughly heart-shaped. Each has a small **anterior sucker** at the more pointed end. The mouth in this sucker leads to a short **pharynx** behind which is a simple **intestine** shaped like an inverted V. A **ventral sucker** is also present. At the wider posterior end is a long **tail.** The cercariae leave the snail and encyst in water or on the grass. When eaten by a sheep they develop into adult flukes: otherwise they die.

THE TAPE-WORM
TAENIA

Tape-worms, so-called from their resemblance to a tape-measure, are, like the Flukes, in the PHYLUM *Platyhelminthes* but in a different CLASS, *Cestoda* (endo-parasites devoid of an enteron, most of which bud-off a series of structures known as *proglottides* or *strobili*—*not* segments).

There are several species of *Taenia* which infect different animals and which must have at least two hosts in which to complete their life-history. It will be seen from the following examples that one host, the intermediate host, serves as food for the other.

T. serrata infects the dog and has the rabbit as its secondary host. *T. coenurus* also infects the dog: its secondary host is the sheep in which it reaches the brain causing a disease called "staggers". Another dog tape-worm is *T. echinococcus* which is unusually short: the secondary host is the cow. *T. crassicollis* lives in the cat and the mouse. *T. solium* occurs in man and the pig and *T. saginata* in man and cattle. Dog tape-worms are the commonest and one of these, *Dipylidium caninum,* has the dog flea as its alternative host. *Taenia metotica* lives in birds and earthworms. In an entirely separate genus infecting man is *Diphyllobothrium latum* which has two intermediate hosts, the small crustacean *Cyclops* and the *pike* but this infection of man occurs only in Eastern Europe.

(1) *Examine a* **Tape-worm** *under a hand lens and a slide of the* **scolex** *under the low power.*

The parasite may be several feet in length. Note the minute head or **scolex** bearing on its free end a projection, the **rostellum,** immediately below which is a ring of chitinous **hooks**. On the side of the scolex are four **suckers**. *T. saginata* has suckers but no hooks.)
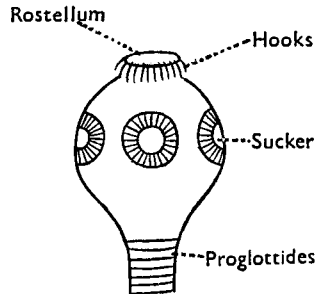
Fig. 305. Taenia. Scolex.

Immediately behind the scolex is the narrow **neck** followed by a chain of flattened structures called **proglottides.*** The newly formed ones near the neck are small, the older ones behind being much larger. *T. echinococcus* is very short and has but three or four proglottides.

(2) *Examine a* **mature proglottis** *from about the middle of the animal under the low power, the dissecting microscope or a strong lens.*

There is no mouth, alimentary canal, vascular or respiratory system, these being unnecessary, but each mature proglottis has complete sexual organs. It is hermaphrodite, though the male organs develop first and so the anterior proglottides contain these alone.

Note the male organs—the small rounded **testes** scattered throughout the proglottis, particularly towards the anterior end, from which tiny ducts, the **vasa efferentia,** lead and join, forming the **vas deferens.** This leads to the **genital atrium** which opens to the exterior on one side of the proglottis. The thicker terminal part of the vas deferens is the **penis.**

Note also the female organs—two **ovaries,** large somewhat oval organs in the posterior part of the proglottis, behind which is the yolk-gland or **vitelline gland.** Anterior to this gland in the centre is the small **shell gland.** A **vitelline duct** and a pair of **oviducts** join behind the shell gland, and from the junction arises the blindly ending **uterus,** which runs up the centre of the proglottis and is at first a simple tube (but later it is much branched) and the **vagina** which runs across to the genital atrium, being wider at the inner end where it is called the **receptaculum seminis.**
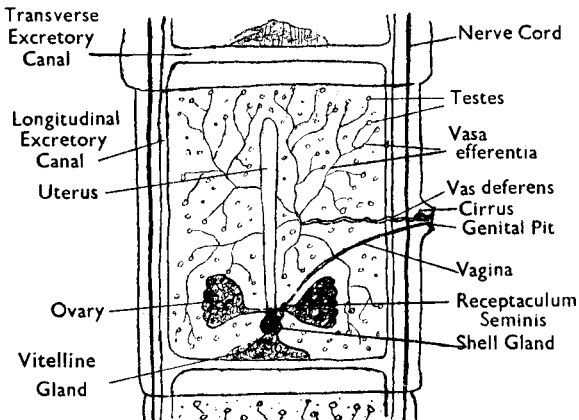
FIG. 306. Taenia. A Mature Proglottis.

*These must not be called segments as this implies metamerism which does not occur in Taenia.

The **longitudinal excretory canal** will be seen running along each side and a **transverse excretory canal** (or **commissural vessel**) uniting the two longitudinal vessels across the posterior end of the proglottis. The **nerve cord** will be found on each side external to the longitudinal excretory duct.

(3) *Examine a* **more mature proglottis** from the posterior end of the animal. Almost the whole of the proglottis is occupied by the much much **branched uterus,** the other reproductive organs having degenerated.

(4) *Examine the* **bladder-worm** *under a hand lens and under the low power.*

Hexacanth embryos develop from the fertilised eggs and these are enclosed in a protective shell. the whole structure being known as an **onchosphere.** These may be found in the mature proglottides at the end of the tapeworm. These proglottides become detached and are passed out with the faeces. If eaten by the alternative host, the shell is dissolved and the liberated embryos are carried by the blood stream to the muscles where they develop into **bladder-worms** or **cysticerci.**These consist of small bladders in which is an invaginated scolex. When the muscle is eaten by the primary host, the bladder is digested and the scolex evaginated. This attaches itself to the wall of the intestine and a new tape-worm develops by strobilation.

Note that the bladder-worm or **cysticercus** is a bladder-like structure which later bears a narrow protruding neck which has been everted from it. This is the **proscolex** and bears suckers and hooks like the scolex of the tape-worm itself.

T. echinococcus may infect man if, like men working in stables, their food becomes contaminated with the ova of this tape-worm. The cysticercus undergoes division internally forming proglottides and reaches an extremely large size in the lungs, liver or even in the brain to which the embryos pass. This forms what is called a *hydatid cyst.* This, too, is what occurs in the brain of sheep infected with *T. coenurus* causing "staggers".

Cysticerci are often seen in the mesentery of the rabbit and liver of the rat when dissecting these animals.

(5) *Examine a* **Transverse Section through a mature proglottis.**

Note the thick external **cuticle** inside which is a layer of **circular muscle** followed by a wider layer of **longitudinal muscle.** The general "packing tissue" is called **parenchyma** and lying across it on each side is a band of **transverse muscle.** In the parenchyma will be found sections of the various parts of the **uterus** and of the **testes** and at each end the **longitudinal excretory canal,** external to which is the

nerve cord. Other structures, such as the **ovary,** will be seen in a section through the posterior end of a proglottis.
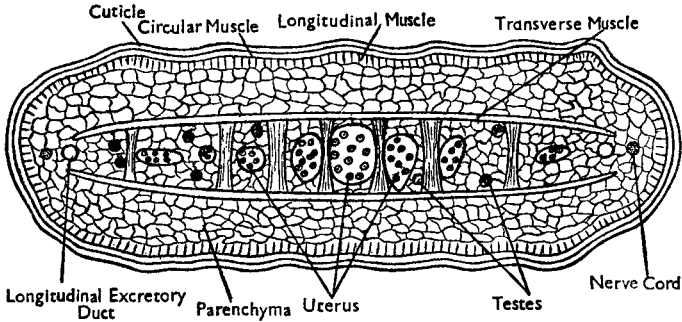
FIG. 307. Taenia. T.S.

Examine a slide of a **flame bulb** unless you have already done so in a previous animal.

Note that the cells are large and have several **cytoplasmic processes** prolonged from them. In the centre is a large cavity containing several **flagella.** A **duct** leads from this cavity. The **nucleus** is found in the cytoplasm to one side.

LIFE HISTORIES OF PARASITIC INSECTS AND VECTORS

These are all in the PHYLUM *Arthropoda* and the CLASS *Insecta*, of course.

Reference should be made, if necessary, to the external structure of the cockroach on pp. 97 *seq.*

THE HOUSE FLY

MUSCA DOMESTICA

The *house-fly* belongs to the ORDER *Diptera* (two-winged insects) and is *holometabolous.* The *Blow Fly* or Blue Bottle (*Calliphora erythrocephala*) is similar to the house fly with minor differences. These are *mechanical vectors* because, owing to their habit of alighting indiscriminately on manure heaps and other sources of infection and on food, they carry parasites on their bodies, particularly on their legs. Eggs are laid in food such as meat as well as on

other organic substrates which serve as food for the larvae. *Tsetse flies* such as *Glossina palpalis* also belong to this Order. These live in tropical countries and may carry the protozoal parasite *Trypanosoma* which causes *Sleeping Sickness* in man.

EGGS

Examine a slide of the **Ova of Musca domestica.**

These are only about 1 mm in length, elongated, rounded at the ends and white in colour.

FIG. 308. **Musca domestica. Egg.**

LARVA

Examine the **larva of M. domestica** *with a hand lens.*
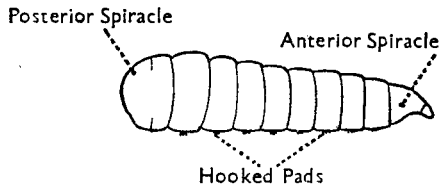
FIG. 309. **Musca domestica. Larva.**

These are limbless grubs composed of twelve **segments**. They have minute **heads** devoid of eyes and with **hooked mandibles. Spiracles** are found only on the second and last segments and **hooked pads** are present on the ventral side of the 6th to 12th segments.

PUPA

Examine the **pupa of M. domestica** *with a hand lens.*

FIG. 310. **Musca domestica. Pupa.**

This is shorter than the larva and is barrel-shaped and brown in colour.

IMAGO

(i) *Examine the* **imago of M. domestica** *with a hand lens.*
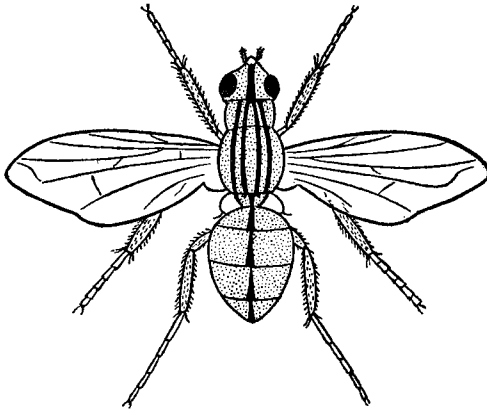
FIG. 311. Musca domestica. Imago.

The **head** bears **compound eyes** and **three ocelli.** The **antennae** are short and feathery. The labium is modified into a proboscis. The **thorax** bears only one pair of **membranous wings,** the anterior pair, and these are large. The posterior wings are modified into dumb-bell shaped structures which serve as balancers and are known as
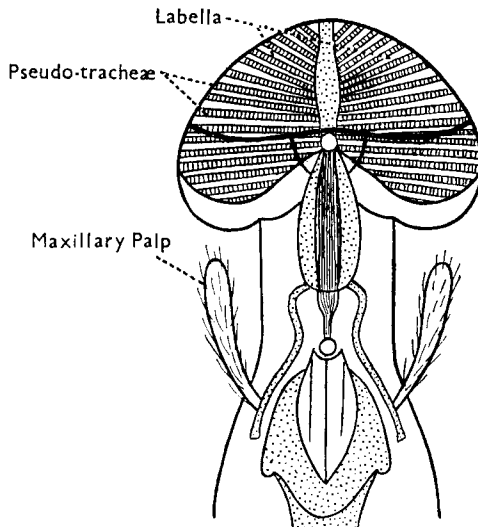
FIG. 312. Musca domestica. Mouth Parts.

halteres. They are hidden by the anterior wings. The **abdomen** shows the usual segmented structure.

(ii) *Examine a slide of the* **mouth parts of the house-fly** *or* **blow-fly.**

These are adapted for sucking and consist of an extensible **proboscis.** Note the two large expanded lobes (the **labella**) on the under side of which are grooves called **pseudotracheae** which open downwards, join and lead into a trunk-like tube which can be protruded. There are no mandibles but **maxillary palps** are present.

THE MOSQUITO

This insect is an *ectoparasite,* belongs to the ORDER *Diptera* (two-winged insects) and is *holometabolous.* Though there are other Genera and many other species, the two chief species are *Culex* (the common house mosquito), and *Anopheles,* the female of which is *vector* of the malarial parasite, *Plasmodium.* Virus diseases such as *Yellow Fever* and *Dengue Fever* may be transmitted by other mosquitoes. The following description applies to *Culex.*

CULEX PIPIENS

OVA

Examine a slide of the **egg raft** *or* **isolated ova of Culex.**

The tiny eggs, which are laid in water, are roughly cigar-shaped and are stuck together to form an egg raft.
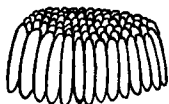
FIG. 313. **Culex. Egg-raft.**   FIG. 314. **Anopheles. Eggs.**

LARVA

Examine a slide of the **larva of Culex.**

This, too, is aquatic. The large head has two **eyes,** a pair of short **antennae,** mandibles and maxillae and two **mouth brushes,** plumed structures for entrapping its food. The three thoracic segments are fused together to form a single large **thorax.** The 8th abdominal segment bears a respiratory structure called the **siphon** which opens to the surface of the water. It has five small valves at its tip. The 9th abdominal segment has four plates which serve as a **rudder** but also

act as gills and contain the tracheae. **Setae** occur on the thorax and the first seven abdominal segments, those on the last segment forming a dense tuft.
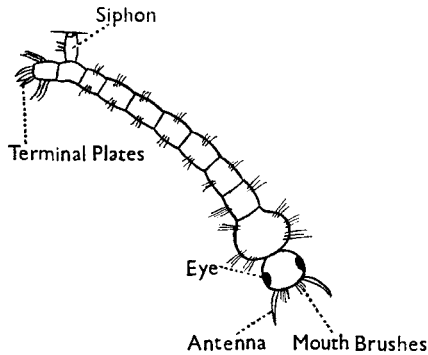
FIG. 315. **Culex. Larva.**

PUPA

Examine a slide of the **pupa of Culex.**

This is also aquatic and, unlike most pupae, it is motile. It is shaped like a large comma. The large rounded anterior portion of the head and thorax has a transparent cuticle through which can be seen the **outlines of the eyes, legs** and **wings of the future imago.** It bears two
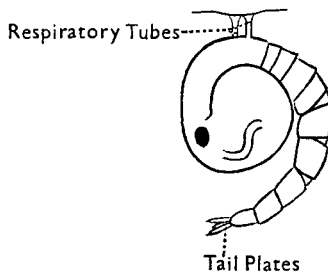
FIG. 316. **Culex. Pupa.**

respiratory tubes on the dorsal side. The curved, segmented, narrow posterior portion of the pupa is the **abdomen** and it terminates in a pair of **tail plates** forming a paddle and used in swimming.

IMAGO

Examine slides of the **male** *and* **female imagines of Culex.**

The **head** is small but the two **compound eyes** are large. The **antennae** are slender in the female but feathery in the male. The mouth parts form a **proboscis,** the female sucking blood, the male plant juices.
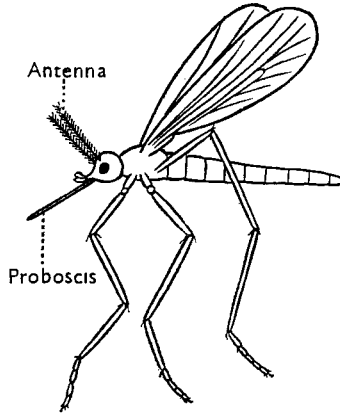
FIG. 317. **Culex. Imago.**

The slender **thorax** bears three pairs of very long, delicate **legs** and one pair of long delicate **wings,** along the edge of which is a fringe of **setae.** The posterior wings are, as in the house-fly, modified into balancers or **halteres.** The segmented **abdomen** is long and very slender.

Examine a slide of the **mouth-parts of the female mosquito.**

The long **proboscis** is adapted for sucking blood and cannot be retracted. It consists of a long, stiff but flexible **labium,** forked at the tip which is known as the **labella.** Inside is a series of pointed structures for piercing the skin and a tube up which the blood is sucked.

In the male, which feeds solely on plant juices, the proboscis is just a slender tube.
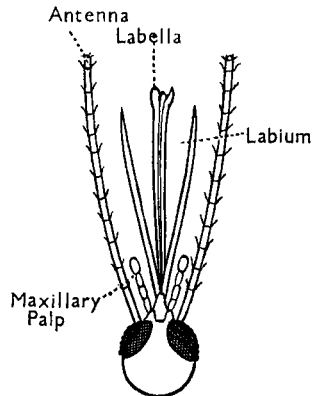
FIG. 318. **Culex. Mouth Parts.**

THE BUG

CIMEX LECTULARIUS

Bugs belong to the ORDER *Hemiptera* and are *hemimetabolous,* the pupal stage being absent. A common species affecting man is the bed bug. This is a night visitant rather than an ectoparasite.

OVA

Examine a slide of the **ova of Cimex.**

The egg is white in colour and ovoid in shape.

LARVA

Examine a slide of the **larva of Cimex.**

The larva is yellowish-white and similar to the adult (see below) but smaller.

IMAGO

Examine a slide of the **imago of Cimex.**

The body is dark brown and is covered with short hairs. It is flattened dorso-ventrally and is about 5 mm. in length. The small
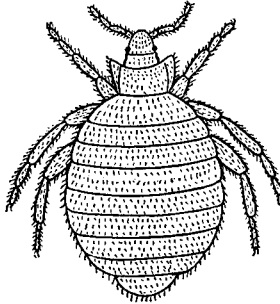
FIG. 319. Cimex lectularis. Imago.

head fits into a notch in the thorax. There are **compound eyes** but no ocelli and two jointed **antennae.** The mouth parts adapted for piercing and sucking. The **thorax,** as already stated, is notched anteriorly and bears only vestigial wings. The metathoracic segment bears stink glands. The **abdomen** is oval and broad, tapering slightly at its posterior end and being slightly narrower in the male than it is in the female.

THE LOUSE
PEDICULUS HUMANUS
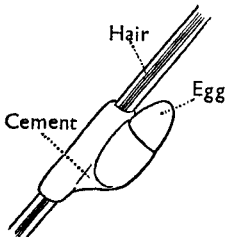
FIG. 320. **Pediculus humanus Capitis. Egg on Hair.**

Lice are *ectoparasites* in the ORDER *Anophura.* They are *hemimetabolous,* there being no pupal stage in their metamorphosis. Different species are parasitic on different animals such as cattle, sheep, poultry and man. *Pediculus humanus* is a parasite on the head (*P. humanus capitis*) or body (*P. humanis corporis*) of man, the latter being slightly larger than the former.

OVA

Examine a slide of the **eggs of Pediculus humanus capitis.**

These minute ovoid structures are yellowish-white in colour and are attached to the hair by **cement.**

LARVA

Examine a slide of the **larva of P. humanus.**

It is similar to the adult (see below) but the **head** and **thorax** are larger in proportion to the **abdomen.**

IMAGO

Examine a slide of the **imago of P. humanus.**

This insect is only about 3 mm. in length, roughly oval in shape and wingless. The body is rather flattened dorso-ventrally. The **head** bears rather inconspicuous **eyes,** short **antennae** and mouth-parts adapted for sucking in head lice and for biting in body lice. The three pairs of **legs** on the thorax terminate in **claws.** There are ten **abdominal segments.**
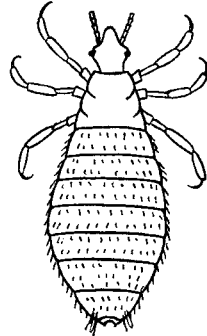
Fig. 321. **Pediculus humanus capitis. Imago.**

THE FLEA

PULEX

The flea is in the ORDER *Aphaniptera* and is *holometabolous* and wingless. Different species are parasitic on man, dogs, rats, mice and poultry. Some of these *ectoparasites* are *vectors* of disease, for example rat fleas may carry the bacillus of bubonic plague (*pasteurella pestis*). The human flea is *Pulex irritans.*

(1) *Examine a slide of the* **ova of Pulex irritans.**

These are minute, ovoid in shape and white in colour.

(2) *Examine a slide of the* **larva of P. irritans.**

The larva, which is not itself parasitic, is about 4 mm. in length and white in colour apart from the head which is brown. The **head** has no eyes but bears single-jointed **antennae** and the mouth-parts

Fig. 322. **Pulex irritans. Larva.**

are adapted for biting. Three **thoracic segments** and ten **abdominal segments** follow the head, each provided with **bristles** which assist movement as the larva is devoid of legs.

(3) *Examine a slide of the* **pupa of P. irritans**.
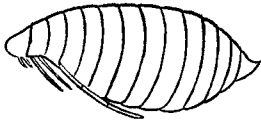
FIG. 323. **Pulex irritans.**
Pupa.

This is soft and whitish in colour and is enclosed in a silken cocoon which must be removed in order to see the pupa itself. In shape this is rather like the imago (see below) and developing appendages can be seen, as is usual with pupae.

(4) *Examine a slide of the* **imago of P. irritans**.

The body is pale brown in colour and compressed laterally. The body wall is tough in texture and is covered with backwardly directed hairs. The **head** is provided with mouth-parts adapted for piercing
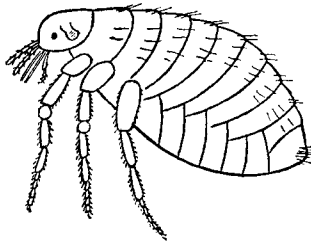
FIG. 324. **Pulex irritans. Imago.**

and sucking and the short, thick **antennae** lie in grooves at the sides of the head. The eyes are very much reduced. The **thorax** has no wings but the **legs** are well developed with a stout femur, and the hind legs are longer than the others. It is thus well adapted to jumping.

APHIS

The *Aphides* are the well-known *green flies* and *black flies* which are parasitic on plum, apple and rose trees and on the bean plant, feeding on the juices of the plant. They usually have a different plant as host during the Winter months. These parasites are bugs and belong to the ORDER *Hemiptera*; they are *hemimetabolous*, the pupal stage being absent. Some Aphides are *vectors* carrying virus diseases to crops. The life-history is unusual and complicated and there are variations in different species. In early Summer winged *viviparous females* from the Winter host fly to new plants and produce offspring *parthenogenetically*. The first generation are *wingless females*. When these mature they produce *winged females* which fly

away to infect new hosts and give rise to new colonies. Ultimately new *sexual forms* appear. In the Autumn the last generation of these winged females fly to the Winter host plant and give rise to *oviparous females.* These are fertilised by *winged males* and the eggs are laid in the Winter host. In the following Spring these fertilised eggs produce the winged females which fly in early Summer to the Summer host plant.

(1) *Examine a slide of an* **Aphis imago.**

These insects are either black (or brownish-(black or green in colour. The **head** bears long **antennae** and **compound eyes** and mouth-parts

FIG. 325. **Aphis.** Imago.

adapted for sucking the plant juices. The three segments of the **thorax** are difficult to distinguish. The **legs** are comparatively long and usually lighter in colour. Both **winged** and **wingless** forms occur during the life-history as stated above. The segments of the **abdomen** are easily identified.

(2) *Examine a slide of the* **mouth-parts of the Aphis imago.**

The labium is modified into a **proboscis** bearing a groove in which lie needle shaped **stylets** when these are not in use. These stylets can be released from the groove and used to penetrate the cells of the plant host.

FIG. 326. **Aphis.** Mouth Parts.

PART VIII

GENETICS

INTRODUCTORY NOTES

Genetics is the study of the variation and inheritance of characters.

Adequate time must be available for the performance of experiments in heredity, particularly in the case of 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*) and the fruit-fly *Drosophila* serve as suitable material for these experiments, though, if time, labour and space permit, the Garden Pea (*Pisum sativum*) will give good results. These all provide suitable alternative allelomorphs and *Drosophila* breeds rapidly, and produces large numbers of offspring. Experiments which involve plant breeding will necessarily take much longer 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, *Drosophila*) can be purchased from biological supply firms (see Appendix V).

EXPLANATION OF TERMS USED IN GENETICS

Chromosomes are paired bodies in the dividing nucleus and are responsible for inheritance. Those responsible for the inheritance of characters other than sex are known as **autosomes.** They occur in identical pairs in somatic cells, which are therefore said to be *diploid*, and these are known as **homologous chromosomes.** Those responsible for the inheritance of sex are known as **sex chromosomes** and generally form an identical pair in one sex, the *homogenetic sex* (usually the female), in which they are referred to as **XX** while those in the other sex, the *heterogametic sex*, form a dissimilar pair referred to as **XY.** In the gametes, of course, only one member of each pair of chromosomes is present and the gametes are therefore *monoploid* (or *haploid*).

Chromosomes are composed of longitudinally arranged particles known as **chromomeres** which are considered to be the locations of the actual units of inheritable material known as **genes** (these are Mendel's "factors"). They consist of D.N.A. (Deoxyribonucleic acid) in a matrix of R.N.A. (Ribonucleic acid).

Alternative characters, such as tallness and dwarfness in plants or yellow body and ebony body in *Drosophila*, are called **allelomorphs** and the genes carrying particular allelomorphs occupy identical loci in the homologous chromosomes and are known as **alleles.** If, after crossing two plants or animals having contrasting allelomorphs, one character appears in the offspring to the exclusion of the other, that character is said to be **dominant** and the one which fails to appear is said to be **recessive.** In this generation, known as the **First Filial** or **F1 Generation,** the zygotes clearly have different alleles on corresponding loci in the paired chromosomes and they are known as **heterozygotes** as opposed to those obtained by crossing identical individuals which always give offspring identical to themselves with regard to a particular character and are known as **homozygotes.** When the F1 generation is crossed inter se, the next generation is referred to as the **Second Filial** or **F2 Generation.** If, instead, the F1 generation is crossed with a homozygote of the parental type, either carrying the dominant or the recessive allelomorph, this is known as a **back-cross.** Any cross-bred organism is a **hybrid.** The inheritance of one pair of genes is called **monohybrid inheritance** while the inheritance of two pairs is called **dihybrid inheritance.**

Dominance is not by any means universal. For example, in the plant *Mirabilis jalapa*, popularly known as the "Four O'clock", there are red-flowered and white-flowered varieties. If these are crossed, all the F1 generation are pink-flowered and if these are self-pollinated, the F2 generation consists of red, pink and white flowers in the ratio 1 : 2 : 1.

Characters are sometimes always inherited together and never separate in the offspring, in contrast to independent assortment; this is called **linkage** and if the characters are associated with one sex, it is known as **sex-linkage.** Obviously the genes responsible in these cases are located on the same chromosome. It often happens that an interchange between corresponding parts of chromatids occurs during the pachytene stage of meiosis in the formation of the gametes. If the genes are different this, of course, produces a different combination of characters in the next generation and is known as **crossing-over.** As the result of very careful and detailed study of crossing-over in *Drosophila*, the actual positions of over four hundred genes in the chromosomes have been identified.

Individuals of different generations may be identical with regard to a particular character though they may be genetically different. Such individuals are said to be **phenotypes.** If their genetic constitution is identical they are referred to as **genotypes.**

Sometimes the sudden inception of an inheritable variation occurs. This is known as **mutation** and the organisms bearing the new character are called **mutants.**

MENDEL'S LAWS

First Law—The Law of Genetic Segregation
The genes for two alternative allelomorphs are separated in the formation of the gametes so that only one appears in each gamete.

Second Law—The Law of Independent Assortment
The behaviour of the genes for any one pair of allelomorphic characters is independent of the distribution of the members of other pairs on different chromosomes and they therefore undergo independent assortment. (But see linkage above.)

I. INHERITANCE IN PLANTS

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 results should work out as follows:—

    Red : white   3 : 1
    Purple : white 1 : 3

This shows that red is dominant in the first pair of allelomorphs and white in the other in the F1 generation and clearly illustrates Mendelian Segregation.

Monohybrid Inheritance

As similar results are produced with the insect Drosophila and in a much shorter time, it is suggested that the experiments in plant genetics be confined to the examination of the maize cobs above.

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 plant (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.

Parents   **YY**   **yy**
Gametes   **Y**   ×   **y**

F_1 **Yy**
(YELLOW)
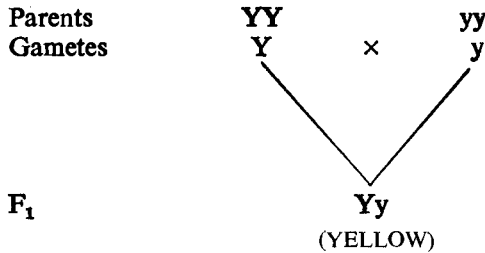
EXPERIMENT 2. *Plant these seeds and allow them to germinate. When flowers have been produced, cross-pollinate as before or allow them to self-pollinate. When seeds have been developed, observe the colour as before and count the number of each.* The result should show both yellow and green seeds and they should be in the ratio of 3 yellow : 1 green.

F_1 Yy   Yy

F_2 YY   Yy   Yy   yy
           YELLOW   GREEN
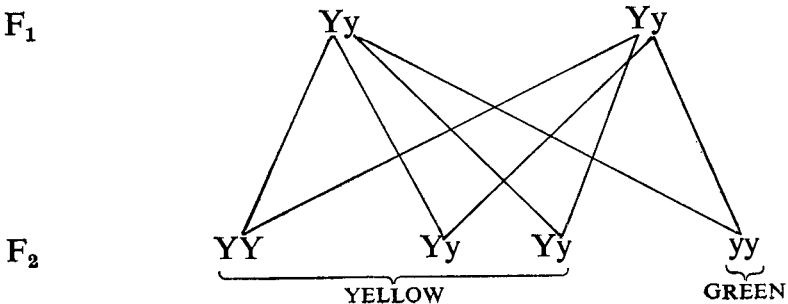
It will have been seen from the text-book studies that one-third of the yellow seeds are homozygotes and two-thirds heterozygotes, and that the green seeds are, of course, homozygotes. To verify this, further experiments will be necessary in which only self-pollination is allowed to take place but this will lengthen the time even more. Back-crosses between the F1 generation and the parental dominant and recessive types could also be carried out.

II. INHERITANCE IN ANIMALS

DROSOPHILA MELANOGASTER

Drosophila melanogaster is a fruit-fly which feeds on decaying fruits. It belongs to the ORDER *Diptera* and is *holometabolous*. There are several species in this Genus but *D. melanogaster* breeds rapidly, produces large numbers of offspring, has a short life-history and shows several contrasting characters. Furthermore the fly is easily cultured and crossing of different types presents little difficulty. It is therefore admirably suited to experimental work in genetics in spite of its small size.

LIFE-HISTORY

The **ova** are minute, white, ovoid and somewhat pointed with a pair of filaments towards one end. They are about 0·5 mm. in length. The **larvae** are transparent, segmented, limbless grubs about 4·5 mm. in length which undergo two ecdyses. The **pupae** are broader and shorter than the larvae, about 3 mm. in length and are brownish in colour (white at first). The whole metamorphosis from the hatching of the larvae from the fertilised ova to the emergence of the imagines from the pupae takes only about eight days to a fortnight, according to conditions.

Examine the **male** *and* **female imagines of the Wild Type** *under a lens on a white tile, under a dissecting microscope or under the low power of the microscope.*

The imago which emerges from the pupa is only about 2 mm. or so in length. The **head** has two red **compound eyes** and three **ocelli** which are dorsally situated. The **antennae** are feathery and the mouth-parts adapted for sucking. The **thorax** consists of a large anterior segment known as the **dorsum** and a smaller posterior **scutellum.** The three pairs of **legs** terminate in claws. In the **male,** the first of the four podomeres of the tarsus of the first legs, the **metatarsus,** bears what is known as a **sex-comb,** a black hairy structure shaped like a comb. The **anterior wings** are large and membranous and extend beyond the posterior end of the insect. As in all *Diptera* the posterior wings are modified into **halteres.**

The **abdomen** differs in the two sexes. In both it is yellowish in colour with dark transverse bands but it is paler in the *female.* Owing to the general effect, the colour is sometimes referred to as "grey". The abdomen of the *female* is also broader than it is in the male. In fact, the *female* as a whole is slightly larger than the *male.*

Furthermore the posterior ends differ in the two sexes. In the *male* where the external genitalia, a penis and two claspers, are situated it is somewhat rounded in side view whereas in the *female* it is more pointed, the external genitalia mostly consisting of a ventrally placed vaginal plate. There are six abdominal segments but, owing to the development of the external genitalia in the male, the last segment is so modified that there appear to be only five. These characteristics, with the sex-comb on the first leg of the male, enable the sexes to be distinguished and this distinction will be required in the experiments.
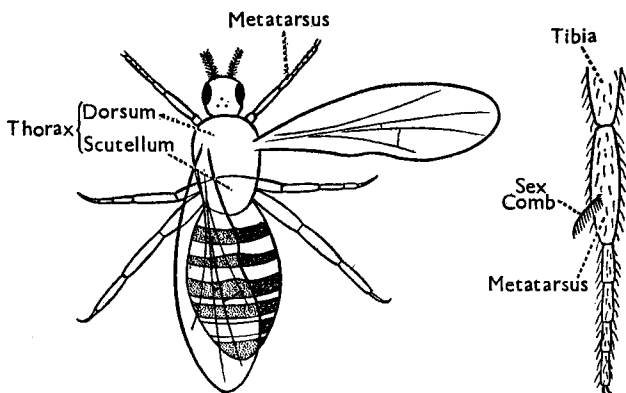
FIG. 327. **Drosophila. Imago.**   First Leg. **Male.**

There are several *mutations* in colour of body and eyes, size, shape and form of wings and so on which lend themselves ideally to interbreeding in experimental work as will be seen below. Cultures of the Wild Type and the various mutants can be purchased from firms supplying biological material (see Appendix V).

It is interesting to note that *Drosophilia* has only four pairs of chromosomes, three pairs of autosomes and a pair of sex chromosomes, XY in the male and XX in the female. The chromosomes are unusually large in the salivary glands and the actual locations of particular genes has been identified.

TECHNIQUE

I. PREPARATION OF APPARATUS AND MATERIALS

1. Culture Bottles

Half-pint milk bottles are the best and most commonly used vessels in which the cultures are kept. Any bottles of similar size and shape will do equally well. In fact large specimen tubes can be used

but they are rather unstable. The bottles must be plugged with cotton wool, preferably wrapped in muslin secured with thread.

2. Sterilisation

It is essential that the containers, the cotton-wool and muslin used as plugs and the paper used to absorb moisture and on which the flies can walk and pupate should be absolutely sterile. The absorbent type of toilet-paper is ideally suitable.

(i) Containers.

These should be thoroughly washed, rinsed and then sterilised in a hot-air oven at about 65°–70°C.

(ii) Cotton-wool and Muslin.

These should be heated in a hot-air oven not above 180°C. to avoid charring.

3. Food for the Cultures

Although *Drosophila* will thrive on rotting banana skins, it is better to provide the flies with a pabulum on which yeast will readily grow since the insect feeds on this. There are several formulae for making such a pabulum but the most suitable is probably the *Maize/ Molasses/Agar Medium* (see p. 458). *The medium is melted by gentle heat and poured into the culture bottles to a depth of a couple of inches or so. Some live yeast is then mixed with a little water and a few drops added to the medium which is then left for twenty-four hours before use.* Warning—Any mould growing on the medium will use the food and starve the flies. Fresh yeast should be added if this appears and if this does not successfully dispose of the infection, the medium should be replaced.

4. Ether Bottles

It will be necessary to *anaesthetise* the flies for examination and an ether bottle should be prepared for this purpose. The bottle should be identical to those used for the cultures but must be fitted with a cork to the inside of which a small cotton-wool pad has been fixed. The pad can be wrapped in muslin and stuck to the cork. When required for use a few drops of ether are put on to the pad.

Ether Pad. Cotton-wool wrapped in Muslin

FIG. 328. **Drosophila. Ether Bottle.**

FIG. 329. **Drosophila. Culture Bottle.**

5. Keeping the Cultures

On receipt of the cultures it is necessary to put them into the prepared culture bottles, keeping each type in a different bottle, suitably labelled. The label should state the Type, sex (if relevant) and the date of insertion into the bottle.

Having sterilised and prepared the culture bottles, insert a length of the sterilised paper, fixing the lower end into the medium its upper end resting against the side of the bottle. The cultures will contain both male and female flies and mating will, of course, take place. It is essential that only virgin females be used in the experiments. The following procedure should therefore be adopted.

Transfer the flies to a culture bottle and keep it at a constant temperature of about 24°C. When the metamorphosis has reached the pupal stage (about 4 days) transfer some of the pupae to a new culture bottle and when the imagines emerge (about 4 days later) they must be transferred to the ether bottle. This is best done as follows:—Remove the cotton-wool plug from the culture bottle and quickly invert the ether bottle on top. If necessary the two bottles must then be turned upside down together and the culture bottle tapped to ensure complete transference of the flies to the ether bottle.

6. Anaesthetising the Flies

Add a few drops of ether to the cotton-wool of the ether bottle and insert the cork into it. Immediately the flies are motionless, remove the cork and transfer the flies to a white tile or into a Petri dish standing on a white tile. Anaesthesia is adequate when the flies are motionless. If they are left too long in the ether they will be killed. Should the wings be folded back and the legs folded or bunched together, anaesthesia has gone too far and the flies will probably be dead.

7. Separating the Sexes

The differences between males and females have already been given in the description of the imago in the account of the life-history.

Examine the flies with a lens and separate the males from the females using a camel-hair brush.

II. THE EXPERIMENTS

1. Mating

Suitable crossings are given below. The method is similar in all cases.

Transfer the females to a new culture bottle and introduce a few males of another type. When the flies have recovered from their anaesthesia mating will take place. Label the bottle stating the types mated and the date. It is important that the labels should not interfere with the observance of the flies. They should not, therefore, be stuck on the bottles. Loose labels tied with string can be attached to the necks of the bottles.

2. Examination of the Results of Mating

When the new imagines have emerged from the pupae, anaesthetise as before. Then remove the flies and examine under a lens. Identify and arrange in batches on separate tiles or in separate Petri dishes according to type. Count the number in each batch. The larger the number of flies used the more accurate will be the result, of course. *Then tabulate your results. In studying sex-linked characters the imagines should be arranged in batches according to sex as well as to the other character.*

3. Suitable Crossings

There are several mutant types of Drosophila of which four are listed below providing suitable alternative allelomorphs.

The normal form of Drosophila with yellow striped body, long wings and red eyes is known as the **Wild Type.** Four of the many mutants are:—

1. **Vestigial Winged** (poorly developed wings, darker body and red eyes)

2. **Ebony body** (black body)

. 3. **White eyes**

4. **Purple eyes**

Experiments can be performed crossing Wild Types with any of these mutants and carrying on further experiments by crossing the offspring. Back-crossings can also be performed. One example is given below and a similar procedure can be followed with the other types.

(1) Monohybrid Ratio

EXPERIMENT 1. (i) *Cross* **Wild Types (long-winged) (L)** *with* **Vestigial-Winged Types (1).**

All the F1 generation will be long-winged, showing that long-wing is dominant to vestigial-wing.

Parents   **LL**   **ll**
Gametes   L   ×   l

F_1 Ll

(ii) *Interbreed the F1 generation.*

The F2 generation will consist of long-winged and vestigial-winged types in the ratio of 3 : 1.

F_1 Ll   ×   Ll

F_2 LL   Ll   Ll   ll

          LONG-WING   VESTIGIAL WING

Clearly the F1 generation was *heterozygous*. Two-thirds of the long-winged types in the F2 generation are also *heterozygous*, as will be seen in the above diagram, the other third being *homozygous* as are the vestigial-winged type. The homozygous types if interbred would breed true while the heterozygous long-winged types would give a similar result to that obtained in the F2 generation.

EXPERIMENT 2. *Back-cross the* **F1 (heterozygous) generation from Experiment 1 (i)** *with the* **homozygous dominant parental type.**

All the offspring will be long-winged but 50 per cent. of these are *homozygotes* and 50 per cent. are *heterozygotes*.

      Ll   ×   LL

      LL   LL   Ll   Ll

EXPERIMENT 3. *Back-cross the* (heterozygous) **F1 generation from Experiment 1 (i)** *with the* **homozygous recessive parental vestigial-winged type.**

Fifty per cent. of the offspring will be long-winged and 50 per cent. will be vestigial-winged.

LI × ll

Ll   Ll   ll   ll
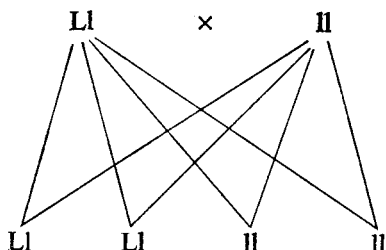
(2) Di-hybrid Ratio

EXPERIMENT 4. (i) *Cross the* **Wild (yellow-bodied) type (Y)** *with the* **mutant ebony-bodied type (y).**

All the F1 generation are yellow-bodied, showing that yellow body (Y) is dominant to ebony body (y). It has already been seen that long-wing is dominant to vestigial-wing.

F_1 Y   y → Yy
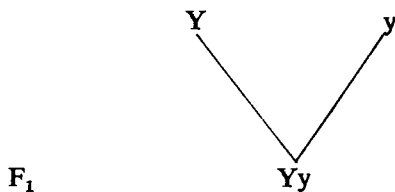
(ii) *Now cross* **yellow body vestigial-winged type (Yl)** *with* **ebony body long-winged type (yL).**

The F1 generation are all yellow-bodied, long-winged types but are, of course, *heterozygotes* (LlYy).

F_1 Yl   yL → Yl y L
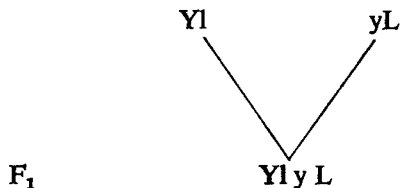
(iii) *Finally interbreed this* **F1 generation.**

If sufficiently large numbers are taken four types will appear and in the following ratio:—

$$\begin{aligned}
&\text{Yellow body long wings (YL)} \ldots \ldots \ldots 9\\
&\text{Yellow body vestigial wings (Yl)} \ldots \ldots 3\\
&\text{Ebony body long wings (yL)} \ldots \ldots \ldots 3\\
&\text{Ebony body vestigial wings (yl)} \ldots \ldots 1
\end{aligned}$$

This ratio can be worked out by the "chess-board" method.

	YL	Yl	yL	yl
YL	YL YL = YL	Yl YL = YL	yL YL = YL	yl YL = YL
Yl	YL Yl = YL	Yl Yl = Yl	yL Yl = YL	yl Yl = Yl
yL	YL yL = YL	Yl yL = YL	yL yL = yL	yl yL = yL
yl	YL yl = YL	Yl yl = Yl	yL yl = yL	yl yl = yl

(3) Linkage

(i) Non-Sex Linked Characters

EXPERIMENT 5. (i) *Cross* **long-winged yellow-bodied (LY) flies** *with* **vestigial-winged ebony-bodied flies (ly).**

All the F1 generation are long-winged yellow-bodied.

(ii) *Interbreed the* **F1 generation.**

The result will be as follows:—

$$\begin{aligned}
&\text{Long-winged yellow bodies} \ldots \ldots \ldots 3\\
&\text{Vestigial-winged ebony body} \ldots \ldots \ldots 1
\end{aligned}$$

Obviously the two alleles for wing formation and body colour are inherited as if they were a single character. This is linkage.

(ii) Sex Linkage

EXPERIMENT 6. (i) *Cross* **red-eyed females** *with* **white-eyed males.**

All the F1 generation are heterozygous red-eyed males and females in equal numbers.

(ii) *Interbreed the F1 generation.*

The F2 generation should be in the following ratio:—

2 red-eyed females: 1 red-eyed male: 1 white-eyed male.

Thus all the males are white-eyed.

(iii) *Cross* **white-eyed females** *with* **pure red-eyed males.**

The result will be

Fifty per cent. white-eyed males.

Fifty per cent. red-eyed females.

(iv) *Interbreed these* **white-eyed males** *with* **red-eyed females.**

The offspring will be in the following ratio:—

    1 red-eyed female   1 white-eyed female

    1 red-eyed male   1 white-eyed male

In these experiments eye colour and sex are linked together and borne on the same chromosomes.

APPENDIX I

THE PREPARATION
AND USES OF REAGENTS

I. MICROSCOPICAL REAGENTS

(1) HARDENING AND FIXING AGENTS

Alcohol (Ethyl), Absolute or **70%**
Use: *Stomach, pancreas, salivary glands.*

Acetic Acid (1 per cent.)

Acetic acid (glacial)	1 ml.
Distilled water	99 ml.

Acetic Alcohol (Carnoy's Fluid)

Acetic acid (glacial)	33 ml.
Alcohol (absolute)	99 ml.

Use: *Animal tissues generally.* Good for mitosis.

Bouin's Fluid (Picro-Formol)

Picric acid (sat. sol. aq.)	75 ml.
Formaldehyde	25 ml.
Acetic acid (glacial)	5 ml.

Use: *Animal tissues generally.*

Chromo-acetic Acid

Chromic acid	0·3 gm
Distilled water	100 ml.
Acetic acid	1 ml.

Use: *Plant tissues, mitosis.*

Corrosive Acetic

Mercuric chloride (sat. sol. aq.)	95 ml.
Acetic acid (glacial)	5 ml.

Use: *Animal tissues and small animals.*

Flemming's Solution

Chromic acid, 1% aq.	90 ml.
Osmic acid, (osmium tetroxide) 2% aq. . .	24 ml.
Acetic acid (glacial)	6 ml.

Use: *Plant tissues.*

Formalin Alcohol
Alcohol (70%) .   .   .   .   100 ml.
Formaldehyde .   .   .   .   6 ml.
Use: *Plant tissues.*

Iodine Alcohol
Iodine   .   .   .   .   2·0 gm.
Alcohol (70%) .   .   .   .   98 ml.
Use: *Washing medium for tissues fixed in corrosive acetic.*

Mercuric Chloride (Corrosive)
See Corrosive Acetic.

Müller's Fluid
Potassium dichromate   .   .   .   .   .   2·5 gm.
Sodium sulphate   .   .   .   .   .   1·0 gm.
Distilled water .   .   .   .   100 ml.
The yellow colour may be removed afterwards by soaking the tissue in 1 per cent chloral hydrate.
Use: *Brain muscle, spleen, liver, kidney.*

Osmium Tetroxide ("Osmic Acid")
Purchase ready prepared.
Osmium tetroxide   .   .   .   .   .   .   0·25 gm.
Distilled water .   .   .   .   100 ml.
Use: *Protozoa.*

Picric Acid
Picric acid   .   .   .   .   .   .   sat. sol. aq.
Use: *Chitinous tissues.*

Picro-formol
See Bouin's Fluid.

Potassium Dichromate
Potassium dichromate   .   .   .   .   .   2·0 gm.
Distilled water .   .   .   .   98 ml.
Use: *As for Müller's fluid. Also for brain and spinal cord.* It is generally quite as effective as Müller's fluid.

Ranvier's Alcohol
Alcohol (90%) .   .   .   .   35 ml.
Distilled water .   .   .   .   70 ml.
Use: *As for absolute alcohol,* but it is a milder fixative.

(2) DECALCIFYING SOLUTIONS

Hydrochloric Acid

      Hydrochloric acid (conc.) .   .   .   .   .   10 ml.

      Distilled water .   .   .   .   90 ml.

Nitric Acid

    For Large Bones:

      Nitric acid (conc.)   .   .   .   .   .   .   10 mi.

      Distilled water .   .   .   .   90 ml.

    For Young Bones:

      Nitric acid (conc.)   .   .   .   .   .   .   1·0 ml.

      Distilled water .   .   .   .   99 ml.

(3) MACERATING FLUID

For Animal Tissues

      Chromic Acid .   .   .   .   0·2%

For Plant Tissues

      Chromic Acid .   .   .   .   5·0%

        See also **Maceration** in Appendix II

(4) 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: *Blood, connective tissue, leucoplasts.*

Alkannin

      Alkanna roots sat. sol. in 96 per cent. alcohol.

    Use: *Fats, cork.*

Aniline Blue (Alcoholic)

      Aniline blue   .   .   .   .   .   .   0·2 gm.

      Alcohol (70%) .   .   .   .   100 ml.

    Use: *Sieve plates, Algoe.*

Aniline Blue (in Lacto-phenol)

      Aniline blue   .   .   .   .   .   .   0·4 gm.

      Lacto-phenol .   .   .   .   100 ml.

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*.

Borax-Carmine (Grenacher's)

Borax	4 gm.
Distilled water	100 ml.
Add Carmine	3 gm.

Apply gentle heat until all dissolved.

Add Alcohol (70%)	100 ml.

Allow to stand for 2 or 3 days. Filter.
Use: *Animal tissues. A good general stain*.

Carbol Fuchsin (Alcoholic)

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 hyphoe*.

Cotton Blue

See Aniline Blue.

Crystal Violet

See Gentian Violet.

Eosin (Aqueous)

Eosin Y	1·0 gm.
Distilled water	100 ml.

Use: *Plant and animal tissues in general*.

*Not readily soluble in water.

Eosin (Alcoholic)
    Eosin Y .   .   .   .   1·0 gm.
    Alcohol (70%) .   .   .   .   100 ml.
Use: *Plant and animal tissues in general.*

Fuchsin (Aqueous)
    Fuchsin .   .   .   .   10 gm.
    Distilled water .   .   .   .   100 ml.
Use: *Counterstain with Gram's iodine.*

Gentian Violet (Crystal Violet)
    Gentian violet .   .   .   .   1·0 gm.
    Distilled water .   .   .   .   100 ml.
Use: *Bacteria, Fungi.*
For Mitosis, use
    Gentian violet .   .   .   .   .   .   sat. sol. aq.

Gram's Iodine
    Potassium iodide   .   .   .   .   .   2·0 gm.
    Distilled water .   .   .   .   100 ml.
Add Iodine   .   .   .   .   1·0 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 and animal tissues.*

Haematoxylin (Delafield)
Best purchased ready prepared
    Haematoxylin .   .   .   .   .   .   4 gm.
    Absolute alcohol   .   .   .   .   .   25 ml.
Add Ammonium alum, sat. sol. aq.   .   .   .   400 ml.
Leave exposed to light for three or four days. Filter.
Add Glycerine   .   .   .   .   .   .   100 ml.
    Methyl alcohol   .   .   .   .   .   100 ml.
Allow to stand for 8 *weeks. Filter.*
Use: *A good general stain for animal tissues.*

Haematoxylin (Ehrlich)
Best purchased ready prepared

Haematoxylin	2 gm.
Absolute alcohol	100 ml.
Add Distilled water	100 ml.
Glycerine	100 ml.
Glacial acetic acid	100 ml.
Alum	in excess

Leave exposed to light for six to eight weeks until it is dark red. Filter.

Use: *Animal tissues general and Monocystis.*

Hoffmann'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.*

Leishman's (Romanowsky) Stain
Best purchased ready prepared.

Methylene blue	1 gm.
Water	100 ml.
Add Sodium carbonate	0·5 gm.
Water	100 ml.

Heat to 65° C. for 12 hours. Allow to stand for 10–12 days.

Add Eosin	0·2 gm.
Water	200 ml.

Allow to stand for 6–12 hours. Filter. Wash the ppt. until the washings are clear. Dry.

Leishman's stain (as above)	0·15 gm.
Methyl alcohol (acetone free)	100 ml.

Use: *Blood.*

Light Green (Alcoholic)
    Light Green   .   .   .   .   sat. sol. in 90% alcohol
  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, Blood.*

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, Blood.*

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)*.

Picro-Carmine (Ranvier)
Picric acid . . . . . . sat. sol. aq.
Add Carmine (sat. sol. in ammonium hydroxide) *until saturated.*
Evaporate to $\frac{1}{5}$ of original volume on a water-bath. Filter. Evaporate
to dryness.
Picrocarmine (as above) . . . . . 1·0 gm.
Distilled water . . . . 100 ml.
Use: *Animal tissues generally*.

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
Use: *Fats (red)*.

Sudan IV (Scharlach Red)
Sudan IV . . . . 5·0 gm.
Alcohol (70%) . . . . 100 ml.
Use: *Fats (red)*.

Van Gieson
Acid fuchsin . . . . 0·25 gm.
Distilled water . . . . 25 ml.
Picric acid (sat. sol. aq.) . . . . . 500 ml.
Use: *Connective tissue, muscle, epithelia*.

(5) DIFFERENTIATING FLUID

Acid Alcohol

Alcohol (70%)	100 ml.
Hydrochloric acid conc.	2·5 ml.

(6) DEHYDRATING AGENTS

(i) Ethyl alcohol

30% Alcohol.	*To prepare dilute solutions from* **Industrial**
50% Alcohol.	**Methylated Spirits (=95% ethyl alcohol),**
70% Alcohol.	*stand the spirit over anhydrous copper*
90% Alcohol.	*sulphate for three or four days, replacing*
Absolute Alcohol.	*the anhydrous copper sulphate as necessary*
	until it is no longer turned blue. Then
	prepare as follows:—

30% Alcohol

Alcohol (95%)	30 ml.
Distilled water	65 ml.

50% Alcohol

Alcohol (95%)	50 ml.
Distilled water	45 ml.

70% Alcohol

Alcohol (95%)	70 ml.
Distilled water	25 ml.

90% Alcohol

Alcohol (95%)	90 ml.
Distilled water	5 ml.

(ii) Cellosolve

= Ethylene glycol monoethyl ether. Purchase ready prepared.

(7) CLEARING AGENTS

(i) Berlese's Medium

Chloral hydrate	16 gm.
Gum arabic	15 gm.
Water	20 ml.
Glucose syrup	10 gm.
Acetic acid (glacial)	5 gm.

Use: *Larvae, chitinous structures and small insects.*

(ii) **Cedar Wood Oil**
Purchase natural oil of cedar wood.
Use: *Best clearing agent for animal tissues.*

(iii) **Chloral hydrate**

Chloral hydrate	250 gm.
Distilled water	100 ml.

Use: *Plant tissues.*

(iv) **Clove Oil**
Purchase as such.
Use: *Animal tissues generally. Best clearing agent for plant tissues.*

(v) **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.*

(vi) **Lacto-phenol**

Lactic acid	20 gm.
Phenol	20 gm.
Glycerine	40 gm.
Distilled water	20 ml.

Use: *Best clearing agent for Algae and Fungi.*

(vii) **Xylene**
Use: *Plant and animal tissues generally.* It is rapid, but it is *not* recommended because it tends to cause shrinkage.

(8) MOUNTING MEDIA

(*a*) **Permanent Mounts**
 (i) **Canada Balsam**
    The best solvent is xylene.
    Purchase ready prepared.

 (ii) **Euparal** and **Euparal Vert**
    Purchase ready prepared.

(iii) **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.
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**

   (i) **Dilute Glycerine**
      Glycerine   .   .   .   .   50 ml.
      Distilled water .   .   .   .   50 ml.
   Add a few crystals of thymol.

   (ii) **Physiological Saline***

      (*a*) **For Invertebrate Tissues (and Vertebrate Blood)**
         Sodium chloride   .   .   .   .   .   0·6 gm.
         Distilled water .   .   .   .   .   .   100 ml.

      (*b*) **For Amphibian Tissues (except Blood)**
         Sodium chloride   .   .   .   .   .   0·75 gm.
         Distilled water .   .   .   .   .   .   100 ml.

      (*c*) **For Mammalian Tissues (except Blood)**
         Sodium chloride   .   .   .   .   .   0·9 gm.
         Distilled water .   .   .   .   .   .   100 ml.
   Use: *Fresh animal tissues.*

   (iii) **Ringer's Solution**
      Sodium chloride   .   .   .   .   .   0·8 gm.
      Calcium chloride   .   .   .   .   .   0·02 gm.
      Potassium chloride   .   .   .   .   .   0·02 gm.
      Sodium bicarbonate   .   .   .   .   0·02 gm.
      Water   .   .   .   .   100 ml.
   Use: *Amphibian tissues.*

*Also called Normal Salt Solution. This does *not* refer to its chemical normality.

(iv) **Locke's Solution**

Sodium chloride	0·9 gm.
Potassium chloride	0·042 gm.
Calcium chloride	0·048 gm.
Sodium bicarbonate	0·02 gm.
Glucose	0·2 gm.
Water	100 ml.

Use: *Mammalian tissues.*

(9) 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.

(10) RINGING CEMENT

Purchase Ringing Cement or Gold Size, Black Varnish or Club Black Enamel.

II. BIOCHEMICAL AND GENERAL REAGENTS, ETC.

Acetic Acid, Dilute

Glacial acetic acid	23 ml.
Water	77 ml.

Adrenalin

Purchase 1 or 5 gm. ampoules.

Albumen

Purchase dried egg or blood albumen.

Ammonium Hydroxide

Ammonium hydroxide S.G. 0·88 . . .	25 ml.
Distilled water	75 ml.

Ammonium Molybdate

Dissolve Ammonium molybdate . . .	15 gm.
in ammonium hydroxide prepared as follows:—	
Ammonium hydroxide, S.G. 0·88 . . .	10 ml.
Distilled water	5 ml.
Add Distilled water	120 ml.

Shake and add this to dilute nitric acid prepared as follows:—

Nitric acid (conc.) . . . . . . 18 ml.
Distilled water . . . . 95 ml.

Ammonium Sulphate

Ammonium sulphate . . Sat. sol. in distilled water

Antimony Chloride (for Vitamin A Test)

Antimony trichloride . . Sat. sol. in chloroform

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.

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 . . . . . . 1·0 gm.
Distilled water . . . . 99 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 (for Transpiration Experiments)

      Cobalt chloride   .   .   .   .   .   . 5·0 gm.

      Distilled water .   .   .   . 95 ml.

Soak filter paper in this solution and dry in an oven. Store in airtight 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.

Minus **Nitrogen**—Substitute chlorides for nitrates.
    „   **Phosphorus**—Omit the potassium phosphate or sub-
        stitute potassium sulphate.
    „   **Potassium**—Substitute calcium nitrate and phosphate
        for potassium nitrate and phosphate.
    „   **Sulphur**—Substitute magnesium chloride for mag-
        nesium 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 sodium hydroxide solution *to* copper sulphate solution *to precipitate cupric hydroxide. Filter. Wash the residue with water and then dissolve it in* ammonium hydroxide. 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.

This can be used immediately.

Diphenylamine

Diphenylamine	20 gm.
Alcohol (95%)	80 ml.

Fehling's Solution
  Solution A.

Copper sulphate	34·64 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 4% (for preservation of material)
Formalin=40% aqueous solution of formaldehyde.
Formalin (40% formaldehyde) . . . . 10 ml.
Water . . . . 90 ml.
(=10% Formalin)

Gastric Juice, Artificial
Pepsin . . . . 0·32 gm.
Distilled water . . . . 99·6 ml.
Hydrochloric acid . . . . . . 0·2 ml.
or use liquor pepticus, purchased ready prepared.

Guaiacum (for Blood Test)
Guaiacum . . . . 2·0 gm.
Alcohol (90%) . . . . 98 ml.

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 ($Na_2S_2O_4$) . . . 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.*

Locke's Solution
See (I) Microscopical Reagents (8) (Mounting Media), p. 45.

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.

Pancreatic Juice

Pancreatin	1·8 gm.
Distilled water	100 ml.

or use liquor pancreatini, purchased ready prepared.

Peptone
Purchase commercial peptone.

Pituitrin
Purchase ampoules.

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 Oxalate

    Potassium oxalate   .   .   .   .   .   .   1·0 gm.

    Distilled water .   .   .   .   99 ml.

  Use: *To prevent blood from clotting.*

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.

Rennin

  Purchase commercial rennin ("rennet").

Ringer's Solution

  See **(1)** Microscopical Reagents (8) (Mounting Media), p. 456.

Saline, Physiological

  See **(1)** Microscopical Reagents (8) (Mounting Media), p. 456.

Schweitzer's Reagent. *See* Cuprammonia

Silver Nitrate

    Silver nitrate   .   .   .   .   .   .   1·0 gm.

    Distilled water .   .   .   .   .   .   99 ml.

  Keep in an amber bottle.

      *The pellets will be found easier to weigh than the sticks.

Sodium Citrate
      Sodium citrate .   .   .   .   10·0 gm.
      Distilled water .   .   .   .   90 ml.
Use: *To prevent blood from clotting.*

Sodium Hydroxide
  (i) **For General Use**
      *Sodium hydroxide   .   .   .   .   .   .   8·0 gm.
      Distilled water .   .   .   .   92 ml.
  (ii) **For Insect Mouth Parts**
      *Sodium hydroxide   .   .   .   .   .   .   2·0 gm.
      Distilled water .   .   .   .   98 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.
with a little cold water to a paste. Add this gradually to
      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%.

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.

Thyroid
  Purchase 1 grain (=0.0648 gm.) tablets.

        *The pellets will be found easier to weigh than the sticks.

APPENDIX II

BIOLOGICAL METHODS

AQUARIA

(1) Use thoroughly washed flat-sided vessels, *e.g.*, good-class battery jars or museum jars and tanks.

(2) Place in a diffuse light, preferably in a north light. If possible light from above and have only one clear side, thus making conditions as natural as possible.

(3) Cover the bottom with fine washed gravel and sand.

(4) Insert a few large stones to provide shaded shelter for the inmates. Some of them should come above the water level if Amphibia are included.

(5) Water-weed, *e.g.*, Elodea, Anacharis, Vallisneria—should be put into the water to provide oxygen, and water snails to keep the sides of the vessels clean and free from Algae.

(6) Keep water-beetles and dragon-fly larvae in vessels by themselves.

(7) Keep aquaria which contain Amphibia or water-beetles covered with perforated zinc.

(8) Do not overcrowd.

(9) Feed the inmates about three times a week with small earthworms or chopped meat. Ant "eggs" (pupae) can be used occasionally. Do not overfeed and on no account allow food to remain in the water and putrefy.

(10) Immediately isolate any fish infected with Saprolegnia ("fish-fungus"). Weak salt solution will sometimes cure this disease.

(11) Keep the water properly aerated.

(12) Do not change the water more often than is necessary.

For Micro-aquaria *see* below.

Aerating Apparatus

Mechanical and electrical aerators can be purchased.

A simple and effective form of aerating apparatus for aquaria can be constructed as follows:—

Fit a 500 ml. 3-necked Woulffe's bottle with rubber stoppers. Through one of the outside necks fix a right-angle tube leading to the bottom of the bottle. This is the *water outlet tube* and should be fitted with rubber tubing leading to the sink. Through the other outside neck fit a short right-angle tube. This is the *air outlet* or *aerating tube* and should be connected by rubber tubing to a glass jet

in the aquarium. The *water and air inlet-tube* is constructed as shown in the diagram, and is fitted in the centre neck. It is, of course, connected to the water-tap. By careful adjustment of the jet in the water inlet-tube sufficient pressure can be maintained in the Woulffe's bottle to aerate several aquaria and to siphon the waste water from the apparatus into the sink.
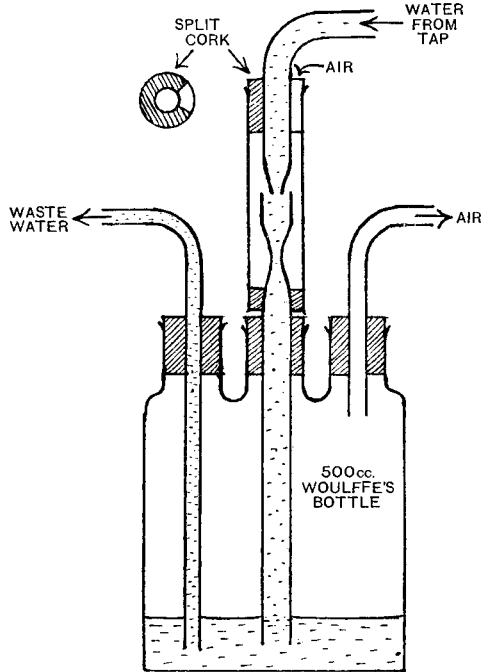
FIG. 330. **Aerating Apparatus for Aquaria.**

Several aquaria can be aerated if T-tubes, to each of which is attached a jet, are fitted where required in the aerating tube. Screw clips should be placed on the rubber connections to give finer adjustment. The advantages of this apparatus are (1) its simplicity and cheapness; (2) its constant efficiency; (3) the non-entry of water with the air into the aquaria.

BLOOD—TO PREVENT CLOTTING

Add blood to $\frac{1}{10}$ of its volume of 10 per cent. sodium citrate or 1 per cent. potassium oxalate.

CLEANING OF GLASS APPARATUS

Wash with commercial hydrochloric acid or aqua regia (HCl, 3 parts: HNO_3, 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.
in 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, above. Appendix I (B), p. 459.

Aspergillus

Dry bread. Cover with bell-jar to exclude dust.

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 VII (Other Forms and Modes of Life), pp. 384, *seq.*

Amoeba

Place one or two boiled (killed) wheat seeds in 100 ml. of water. Inoculate with Amoeba from a culture by means of a pipette. Replace every 2 months. Subculture occasionally if successful.

Daphnia and Cyclops (For Feeding Hydra)

Mix Horlick's malted milk, 0·1 gm., into a paste with boiling water and dilute with 5·0 ml. of cold water. Add weekly to a vessel containing about 500 ml. of water containing Daphnia and Cyclops.

Drosophila

Mix

I. Maize meal	200 ml.
Molasses	100 ml.
Nipagin	0·75 gm.
II. Agar	10·0 gm.

Gradually add

Boiling water	250 ml.
Add (I) to (II) and stir well. Heat until the mixture begins to stiffen then add cold water . . .	150 ml.

Stir. Allow to cool.

Hydra

Put a few decaying leaves or a little pond debris into water. Add Hydra by means of a pipette. Add Daphnia and Cyclops.

Lumbricus

Place earthworms in soil in a large box and add a quantity of leaf mould. Do not let the soil get too dry.

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.

Paramoecium

As for Amoeba. Alternatively chop up some hay, add boiling water and thus prepare hay infusion. Inoculate with Paramoecia when cold. Subculture every six weeks or so.

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 (B), p. 460.

FORMALDEHYDE 40 PER CENT.—TO KEEP

Keep some lumps of calcium carbonate in the bottle to neutralise the formic acid to which the formaldehyde is liable to be oxidised.

FROG PLATE

This can be conveniently made of a rectangular piece of ebonite, wood or brass about 6 in. long and 2 in. wide. A circular hole 1 in. in diameter should be cut with its centre in the mid-line about $1\frac{1}{2}$ in. from one end. Holes about $\frac{3}{16}$ in. in diameter may be drilled round the sides with their centres about $\frac{3}{4}$ in. apart.
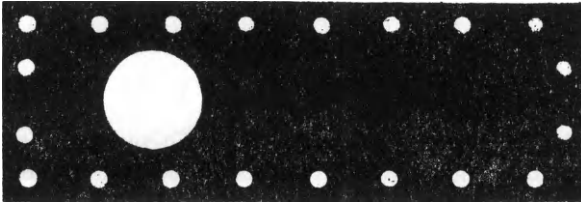
FIG. 331. **Frog Plate.**

The frog plate should be placed on the stage of the microscope with the hole over the hole in the stage and clamped in a retort-stand. A pithed frog can then be placed on the plate, the web of the foot slightly stretched over the hole and kept in position by thread tied round the digits to the small holes at the edge of the plate or to small pins if the frog plate is made of wood.

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.

HORMONE EXPERIMENTS

(1) The Effect of Pituitrin on the Melanophores of the Frog's Skin.
Two similar light-coloured frogs should be lightly anaesthetised and
pithed. Into one inject a subcutaneous injection of 0·2 ml. of "Pitui-
trin" with a hypodermic syringe. When the desired effect is obtained,
i.e., when the melanophores have expanded and the skin consequently
turned dark (this usually takes a little over an hour), the two frogs
can be killed and preserved in 10 per cent. formalin, side by side, in a
museum jar, the uninjected specimen being kept for comparison.

(2) The Effect of Thyroxin on the Metamorphosis of the Frog.
A batch of tadpoles should be isolated in a separate aquarium when
they are about 10 weeks old, and fed on thyroid at the rate of 3·0
grains (= 0·1944 gm.) for each dozen tadpoles. The tablets should
be crushed in a mortar and the powder added to the water in the
aquarium. A comparison with the unfed tadpoles within the next
24 hours to three days will serve as a control. Unsatisfactory results
are obtained if the thyroid feeding is performed at an earlier stage
of development.

INJECTION OF BLOOD-VESSELS

*Successful injection of blood-vessels requires considerable care,
practice and skill.*

It can be done with an ordinary injection syringe, the nozzle of
which should be filled with a piece of rubber tubing into which a
glass cannula or jet can be fitted. Jets of various sizes should be made,
very fine ones being required for small animals.
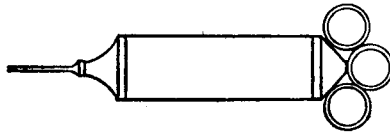
FIG. 332. Injection Syringe.

The operation must be performed on a *freshly killed* animal,
otherwise the blood must first be washed out of the vessels by means
of a solvent such as 10 per cent. sodium citrate. It is important that
a jet of suitable size should be used. The injection masses must be
freshly prepared and used immediately. A gelatine or a plaster of
Paris medium (see below) may be used. If the gelatine method is
adopted, the medium must be kept hot and the animal immersed in
warm physiological saline.

Having inserted the nozzle of the syringe, first making an incision in the blood-vessel if necessary, tie a ligature (not too tightly) round the vessel to keep the nozzle in place and to prevent leakage at this point. Avoid too much pressure on the syringe or bursting of the blood-vessels may occur.

Just before the completion of the operation, the vessels should be ligatured and the pressure maintained for a few minutes. When it is completed the animal should be washed in cold water and then allowed to remain in cold water for an hour or two before placing in the preserving fluid.

Places of injection are as follows:—

Crayfish.	Ostium of heart.	First wash out blood-vessels with physiological saline.
Frog.	Arteries—ventricle. Veins—sinus venosus.	Use red mass. Use blue mass.
Dogfish.	Afferent arteries—conus arteriorus. Efferent and visceral arteries—caudal artery. Visceral veins—caudal vein.	Use blue mass. Use red mass. Cut off the end of the tail to expose the caudal artery. Use blue mass. Insert into the vein in the cut end of the tail.
Rabbit.	Arteries—root of the aortic arch. Veins—sinus venosus.	Use red mass. Use blue mass. Drain the blood from the vessels before injection.

Injection Masses

(1) Gelatine Medium

Soak 5·0 gm. of gelatine in hot water. When melted add sufficient of the colouring matter (see below) to give a bright red or blue colour. Stir well. Use hot.

(2) Plaster of Paris Medium

Rub some plaster of Paris with a little water in a mortar to a thin cream. Add the colouring matter. Stir well. Use immediately.

(3) Colouring Matter

(i) Red.

Rub 2·5 gm. of powdered carmine in water. Add ammonium hydroxide gradually until the carmine is dissolved and the solution transparent.

(ii) Blue.

Dissolve 1·0 gm. of Prussian blue in 50 ml. of water.

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.
Stir.

or Chatterton's Compound supplied in sticks. Melt by gentle heat and apply.

KILLING OF ANIMALS

(1) Lethal Chambers

(a) Small Animals

For small animals such as earthworms and frogs, a chemical desiccator is a suitable piece of apparatus. See that the flange and edge of the lid are greased with vaseline. Place cotton-wool in the bottom of the vessel and add chloroform. Replace the zinc gauze.

Place the animals on the gauze and replace the lid. This should be placed eccentrically at first so that a small space is left to admit air. The air-chloroform mixture will bring about anaesthesia. When active movement ceases, push the lid on completely so as to exclude the entry of air.

This apparatus is particularly advantageous in the case of earthworms as it prevents actual contact with the chloroform, which tends to make them brittle.

(b) Large Animals

For large animals like rabbits and rats, a large metal box with two holes in the lid provided with corks is most suitable.

Place the animal in the lethal chamber, remove both corks and insert a wad of cotton-wool soaked in chloroform through one hole. Alternatively pass coal-gas into the chamber—anaesthesia is rapid and death painless.

The animal will then breathe a mixture of air and chloroform vapour (or coal gas) and will be anaesthetised. When active movement ceases, add more chloroform (or pass more gas) and close both holes with the corks to exclude air. In the case of rats, place the cage containing the animals in the lethal chamber and proceed as above.

Rabbits recover rapidly when returned to the air if they are only anaesthetised by coal-gas and it is therefore essential that they should be left in the chamber for a sufficient length of time (see next page).

(2) Lethal Times

It is obviously essential that the animals should be quite dead. To ensure that this is so, leave the animals for the length of time stated below *after anaesthesia has been obtained:*—

Earthworm	5 minutes
Frog	20 ,,
Rabbit and rat	30 ,,

(3) Methods for Specific Animals

(*a*) Protozoa
1 per cent. acetic acid.

(*b*) Hydra
1 per cent. acetic acid.

(*c*) Earthworm
Chloroform vapour—see (1) (*a*) and (2) above.

(*d*) Cockroach
Either use the lethal chamber for small animals or place the insects into boiling water in a large beaker.

(*e*) Crayfish and Anodonta
Put the animal into boiling water for a few minutes: then transfer to cold water. Do not use chloroform.

(*f*) Frog
Chloroform. See (1) (*a*) and (2) above.

(*g*) Rabbit and Rat
Chloroform or coal gas. See (1) (*b*) and (2) above.

(*h*) Snail
Completely fill a jar with water previously boiled (and cooled) to expel the air. Place the snails in the water, replace the stopper and leave for a few days. This is the only satisfactory method to ensure that the animals are distended when dead. Do not place too many animals in the jar at the same time or they may be distorted.

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.*:

```
┌─────────────────┐
│  ALCOHOL        │
│    50%          │
└─────────────────┘
```

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.

Gummed labels will not stick to plastic vessels. The "Perspex Cement" used to seal lids on plastic museum jars is most suitable for this purpose.

See also microscopical slide labels.

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. 448) and then washed and mounted as above.

MICRO-AQUARIA

Glass vessels such as medium-sized crystallising dishes or beakers prove satisfactory. Petri dishes are suitable for such cultures as Amoeba but they are rather shallow and easily spilled. All vessels must be absolutely clean.

Tap water may be used, but sometimes rain water gives better results.

The pabulum should be added to the water, which should be inoculated with a culture of the organisms by means of a pipette. Pure cultures can only be obtained by sub-culturing.

The vessels should be covered with a piece of glass and suitably labelled.

Some organisms are more difficult to culture successfully than others and in any case some of the cultures may prove to be failures.

For methods for specific organisms see Culture Methods 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 per-manent preparations no longer required can be placed. Leave the slides to soak and keep the jar covered.

Periodically remove, boil in water, with 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 formal-dehyde. 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" pp. 476 *seq.*

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.

Specimens of similar organs of different animals (such as the brains of dogfish, frog and rabbit) may be mounted in the same jar. Dorsal and ventral views should be shown. Dissected systems and animals with injected blood-vessels are most helpful to students.

(2) Bones
Disarticulated small bones, such as those of the frog or rabbit or the entire skeleton of the frog, may be stuck to the bottom of a wooden box made a suitable size, painted a dull black inside and fitted with a glass lid. Black passe-partout can be used to fix the glass in position.

Articulated skeletons (which are very instructive) and larger bones should be kept under glass or in museum cases to protect them from dirt and injury.

(3) Models
Embryological models, though expensive, often enable students to understand the more difficult parts of embryology. Enlarged models of the eye, ear, heart and other organs are also useful.

NERVES—TO SHOW UP DURING DISSECTION
Cover with picric acid (sat. sol. aq.) then wash well with water.

PITHING A FROG
Anaesthetise (but do not kill) a frog with ether.

Cut through the skin and the occipito-atlantal membrane between the skull and spinal column on the dorsal side. Insert a seeker into the cranial cavity and move it about until the brain is completely destroyed.

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) General
70% Alcohol

The material may be softened after preservation by immersing it in the following solution for 24 hours:—

Glycerol—50%
Alcohol—50%.
or   4% Formaldehyde (= 10% Formalin).

For animal material place the organs in the following solution until they are completely decolourised.

Potassium acetate	8·5 gm.
Potassium nitrate	4·5 gm.
Formalin .	80 ml.
Water	400 ml.

Then, after allowing the fluid to drain off, place in 80 per cent. alcohol until the natural colour returns.

Then preserve permanently in the following solution:—

Potassium acetate	200 gm.
Glycerine .	300 ml.
Distilled water .	900 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) Amphioxus

70% Alcohol.

To prepare for dissection soak in—

Nitric acid	20 ml.
Alcohol (70%) .	100 ml.

(d) 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.

(e) Brains

Müller's fluid—see Appendix 1(I), p. 447, Microscopical Reagents.

To remove the yellow colour afterwards soak in 1 per cent. chloral hydrate solution.

or   70% Alcohol,

or   4% Formaldehyde.

(*f*) **Cartilaginous Skeletons**
     Glycerine. .   .   .   .   300 ml.
     Alcohol (70%) .   .   .   .   700 ml.

(*g*) **Cockroaches**
     70% alcohol.

(*h*) **Dogfish**
If the animal is not already injected with formalin *open peritoneal cavity before placing the animal in* 4 per cent. Formaldehyde.

(*i*) **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).

SKELETONS—TO PREPARE

(1) Cut away as much muscle as possible.
(2) Boil gently in water until the muscle and tendon is easily removed.
(3) Soak in chloroform to remove the fat from the bones.
(4) Place in dilute hydrogen peroxide to bleach the bones.
(5) Wash thoroughly in water.
(6) Allow to dry.
Bones may be joined by Durofix or one of the plastic cements. Cover the two surfaces to be joined with a thin layer of the cement and allow it to dry. Then apply another thin layer of cement and press the bones together. Any surplus cement can be removed from the surface when dry by means of a knife.
Bony skeletons may be articulated by Durofix or plastic cement and fine rustless wire, small holes being drilled in the bones where necessary.
Cartilaginous skeletons should be articulated with very fine wire.

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 per cent. aq.) *or* boil in water for ¼ hour.
Used culture-tubes and petri dishes: Boil in water for 1 hour.

Rubber: Steam for ½ hour.

Cotton-wool: Dry heat: not above 180°C.

Instruments: Boil in water containing soda (to prevent rusting) for ¼ hour.

Minor instruments (*e.g.*, platinum needles): Flame: heat to redness.

Nutrient media: Sterilise on three consecutive days by steam for ¼ to ½ hour.

FIG. 333.   **Thermo-regulator.**
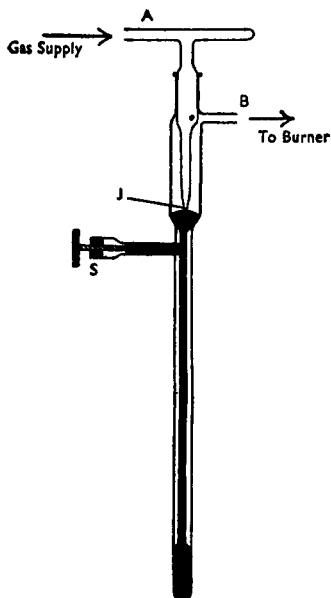
THERMO-REGULATOR

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.

THERMOSTAT

A satisfactory thermostat for experiments on digestion can be made from an ordinary "steamer" as follows:—

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.

Alternatively, a hot water oven fitted with thermo-regulator and thermometer, may be used.
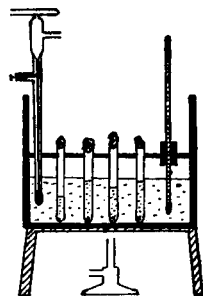
FIG. 334.   **Thermostat.**

TRANSPARENCIES—TO SHOW THE ANIMAL
SKELETON IN SITU

The skeletons of small vertebrate animals can be stained and the other tissues made transparent (thus showing the articulated skeleton *in situ*) by the following method:—

(1) Place in 90 per cent. alcohol for two days.

(2) Soak in 1 per cent. aqueous potassium hydroxide until the tissues are more or less transparent and the skeleton is visible. *Do not leave the animal in this solution longer than is necessary to attain this condition.*

(3) Place in the following solution until the bones are stained deep red:—

Alizarin red	0·1 gm.
Potassium hydroxide (1 per cent. aqueous) .	1000 ml.

(4) Transfer to the following solution to clear:—

Potassium hydroxide	1·0 gm.
Distilled water	79 ml.
Glycerine	20 ml.

(5) When completely cleared, store in pure glycerine.

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.

$1\mu = 0.00003937$ in. $= \dfrac{1}{25,400}$ in. or, more approximately, $\dfrac{1}{25,000}$

Conversion Table

Multiply by

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
°C to °F.	°C $\times \frac{9}{5}$ + 32
°F to °C.	°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 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 per cent. 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 per cent. 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 per cent.) sodium bicarbonate solution.

(ii) **Alkali in the Eye.** Wash with 1 per cent. 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 per cent. 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, MICRO-SCOPICAL SLIDES, STAINS AND REAGENTS, DISSECTING INSTRUMENTS, CHARTS, FILM STRIPS, LANTERN SLIDES, BIOLOGICAL MODELS, CHEMICAL APPARATUS AND REAGENTS, ETC.

Baird & Tatlock (London) Ltd., 14 St. Cross Street, E.C.1.
Flatters & Garnett Ltd., 309 Oxford Road, Manchester.
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.

BIOLOGICAL MATERIAL (Living or Preserved)

Flatters & Garnett Ltd., Address: see above.
Galloway's Biological Agency, Rhydyfelin Aberystwyth, Wales.
T. Gerrard & Co. Ltd., Address: see above.
Marine Biological Station, Citadel Hill, Plymouth.
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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