PRACTICAL LESSONS

IN

ELEMENTARY BIOLOGY,

FOR

JUNIOR STUDENTS.

BY

PEYTON T. B. BEALE, F.R.C.S. ENG.

Demonstrator of Physiology, and Lecturer on Elementary Biology in King's College, London, Assistant Surgeon to King's College Hospital, and Surgeon to Out-patients to the Great Northern Central Hospital.

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PREFACE.

THIS series of practical lessons is one which, with some recent modifications, I have used during the last few years in the classes of practical Elementary Biology at King's College, London, for preparing students for the biological portion of the first examination of the Conjoint Board.

I have endeavoured in each lesson to treat practically of those points in the structure and life history of several organisms, which are of great importance, and which some students have difficulty in mastering.

At the end of each lesson a brief descriptive explanation has been added, as I consider that the student should himself prepare and see the things, make his own drawings, and form his own conclusions, before reading an account of them.

In each lesson I have endeavoured to indicate what may be seen, and how the important points may be practically demonstrated, but the student must not regard the work as taking the place of a biological text-book.

Each lesson will occupy about two hours, and upon another day, a lecture should be given at which it is desirable that the teacher should draw his own diagrams upon the board.

I have not given the exact measurements of the various objects, because I think it better that junior students, when using the high power, should draw the specimen and compare its diameter with that of a human coloured blood corpuscle, with which they become acquainted in Lesson II.

I am indebted to a number of text-books of Biology, Zoology, and Botany, and especially to the "Practical Zoology" of Marshall and Hurst, and Prof. Ward's "Diseases of Plants."

I desire to thank my friends and colleagues, Dr. James Galloway, Pathologist and Physician to the Great Northern Central Hospital, and Dr. R. T. Hewlett, Demonstrator of Bacteriology in King's College, London, for many valuable suggestions in the lessons upon Sporozoa and the Fungi, and Dr. Hewlett for his kindness in revising the manuscript.

I shall esteem it a favour if any reader who may notice errors, will be so kind as to draw my attention to them.

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PRACTICAL LESSONS

IN

ELEMENTARY BIOLOGY.

INTRODUCTION.

It is not intended that these lessons should be worked in the order in which they are placed. Experience shows that the student progresses better if he begins by dissecting a higher animal first, and becomes acquainted with the general arrangement of its parts and organs.

I would therefore advise that the student should begin by dissecting a rabbit or cat, and then pass on to the microscopic work, the general idea being to keep the microscopic work going with the practical dissection as far as is possible in each lesson.

Too much stress cannot be laid upon drawing; the student must draw everything he sees; everybody can draw sufficiently to make outlines of things which he sees, and as a rule those who have never learnt drawing are found to make the best drawings.

The student must not look upon Biology merely as a subject to be "crammed up," and as quickly forgotten; it is found to afford the best training for the eyes and hands in order to prepare him for those sciences which he has afterwards to learn; moreover, it teaches him to think for himself. Let him therefore do the work set forth in this book carefully and thoroughly, never going on to another section until he is sure he has grasped the facts in the one before him. There are sure to be errors in the various lessons, let the student be the first to find them out, and he is sure to do so if only he does the work thoroughly and in earnest.

Each student must be provided with the following:—

1. A **microscope**, with two powers magnifying respectively about 50 and 400 diameters.

   There are very many good microscopes at reasonable prices to be obtained now: I would recommend the student to obtain one which will be serviceable to him in the later courses of practical histology and pathology.

   Such are the microscopes manufactured in England by Swift, of Tottenham Court Road, London, or those manufactured in Germany by Leitz of Wetzlar (agent, Baker, of High Holborn, London).

2. Microscope **slides**, glass slips three inches long by one inch broad.
3. **Cover glasses** known as "No. 1," square or round.
4. **Two scalpels**; a strong one, and a small thin one, or a razor instead of the small one.
5. Two pairs of **scissors**; a strong pair, and a small pair; both sharp-pointed.
6. A pair of **forceps**.
7. Two **mounted needles**; large sewing needles, the eye ends fixed into wooden handles.
8. Two **watch glasses**.
9. A **book**, in which to make drawings.

   The sheets should not be ruled.

10. A good H.B. **pencil**, and three **coloured pencils**, red, blue, and brown.

11. A **dissecting tray**; a pie dish with a layer of hard paraffin, half an inch thick, at the bottom, is as good as anything.
12. A **hand lens,** magnifying from three to six diameters.
13. **Pins**; black steel pins, and a few blanket pins.

The various reagents, iodine, acetic acid, etc., can be handed round when required.

Lesson I.

The microscope: Method of using it for the examination of objects; drawing; examination of cotton and woollen fibres, starch, milk.

Microscopes are of two kinds, simple and compound.

The **simple microscope** is a bi-convex or plano-convex lens, which may be held in the fingers, and can be used to magnify objects only a few diameters.

The greater the magnifying power of the instrument, the nearer must the latter be to the object when focussed.

The **compound microscope** consists of a tube from six to ten inches long, at the lower end of which can be screwed a set of lenses constituting the objective, object glass, or **power**; at the upper end is inserted a set of lenses, fixed in a short tube, constituting the ocular or **eyepiece.**

There should be two powers, a **low power** and a **high power**; the former magnifying when in use from 40 to 80 diameters, the latter from 250 to 400 diameters. The tube which carries the power and eyepiece may slide in an outer tube which may be rigidly connected with the stand of the microscope, or may be attached to the stand by a rack and pinion.

To the **stand** is fixed the **stage,** upon which the object to be examined is placed, and beneath the stage there is an adjustable **mirror,** which reflects light through the aperture in the stage, so that it may be transmitted through the object (which must be transparent) the power, the tube and eyepiece, to the observer's eye.

The amount of light can be regulated by a **diaphragm,** the aperture in which can be varied, and which is placed immediately beneath the stage.

The object is brought approximately into **focus** by moving the tube with its attached lenses up or down; **coarse adjustment.**

The focussing is completed and rendered perfect by turning a milled screw attached to some part of the stand. The motion of this screw is imparted to the outer tube directly or by a system of levers; **fine adjustment.**

When the object is in focus, in the case of the high power, the distance between the lens and the object is much less than in the case of the low power.

We cannot enter into the optical principles involved in the compound microscope: suffice it to say that an image of the object is formed in the tube by the power, and this image is again magnified by the eyepiece.

The student should place the microscope before him upon the table, and, before attaching the power, look down the tube through the eyepiece, at the same time moving the mirror until he sees the light reflected from a neighbouring window or lamp.

The object to be examined should be placed upon a glass **slide** (3 inches by 1 inch) in a drop of fluid, and covered by a small piece of very thin glass, the **cover slip.** The slide is then placed upon the stage, the object being immediately over the aperture in the latter, through which the light is reflected from the mirror. The low power should then be screwed on to the tube, and the tube pushed down by a twisting motion, or lowered by the rack and pinion until the object is brought into view. The focussing should then be completed by using the fine adjustment screw. The whole of the illuminated disc which is then seen is called the **field of the microscope.** It is of the utmost importance to keep both eyes open when using the microscope, and to be able to use either eye.

If a sheet of paper be placed on a level with the stage on the right of and touching the stand, and the left eye be used to examine the object, then the right eye will see a magnified image of the object projected upon the paper, and its outlines can be drawn with a pencil held in the right hand. This may not be accomplished for some time, but should be attempted from the beginning.

The actual measurement of objects under the microscope will be explained in the next lesson.

1. Place upon a slide in a drop of water a few fibres of cotton wool, cover with a thin cover-glass, and examine first under the low power, and then under the high power. Note

> The elongated **cotton fibres**, each twisted upon itself at intervals, and with a line running down its centre—in reality a thick walled, flattened tube, with occasional thickenings upon it. Draw.

2. Mount in the same manner a few fibres from the edge of a piece of white flannel or worsted, and examine under the low and then under the high power. Note

> The elongated **wool fibres**, with zigzag transverse markings upon them.
>
> Each is, in reality, a tube covered with small scale like cells overlapping one another. Draw.

3. Mount in the same manner a scraping with a knife from the freshly cut surface of a raw potato; cover, and examine under the low power. Note

> The **starch granules**, a number of clear ovoid bodies of unequal size, each with a distinct outline, and with a number of fine concentric lines around a small spot situated near one of the ends. Draw.

4. Allow a drop or two of dilute iodine solution to diffuse under the cover-glass. (Appendix 1.) Note

> The granules become blue, due to the formation of iodide of starch.

5. Place on a slide a drop of **milk** diluted with three volumes of water. Cover and examine first under the low, and then under the high power. Note

a. The spherical clear globules of unequal size and devoid of structure; each globule is a **globule of oil** (fat). Draw.

b. **Air bubbles** of various sizes; contrast these carefully with the fat globules. Draw.

Lesson II.

The Cell, living matter, the cell wall, the nucleus. Examination of vegetable cells, movements of living matter within vegetable cells.

1. Place upon a slide in a drop of water a stamen of *Tradescantia Virginica*, with the fine hairs attached to it. Cover, and examine under the low power. Note

The **hairs**, made up of rows of violet coloured ovoid **cells.** Draw.

Examine one of the hairs under the high power. Note

a. The **cell wall** surrounding each of the cells.

b. The **living matter, "primordial utricle,"** colourless, or nearly so, and granular, lining the cell wall and sending fine prolongations to the centre of the cell.

c. The **nucleus,** embedded in a small amount of the living matter near the centre of the cell.

d. Spaces containing **cell sap** between the prolongations of the living matter. Draw.

Allow a drop of iodine solution to diffuse under the cover-glass. Note

e. The whole of the living matter, including the nucleus, is stained brown, the nucleus being darker.

The transparent cell wall remains colourless; it may appear brownish, because the stained matter is seen through it.

2. Cut with a razor, or sharp thin scalpel, very thin sections of the white stalk of a lettuce or cabbage.

Mount the thinnest upon a slide in a drop of water, cover, and examine under the low power. Note

The large, oblong, colourless, **cell walls**, one cell wall separating any two cells. Draw.

Allow a drop of iodine solution to diffuse under the cover-glass, and examine the thinnest part of the section under the high power. Note

a. As in 1, *e*, the living matter stained brown, but **starch** grains which are present in it will become **blue**.

b. The cell wall remains colourless or becomes only slightly brown.

Follow the iodine by a drop or two of sulphuric acid (70 per cent.).

The **cell wall** becomes **blue**.

The cell wall is composed of **cellulose**. Sulphuric acid converts cellulose into a kind of starch, and the starch thus formed becomes blue in the presence of iodine.

(See Lesson I, Starch.)

This reaction of cellulose may not be seen in all of the cell walls. It can also be shown by soaking a little cotton wool in iodine solution, and then adding sulphuric acid.

3. Mount a stamen of *Tradescantia* as in 1, and examine one of the cells of which the hair is composed under the high power. Note

a. The **streaming movements of the granular living matter**, especially in its prolongations stretching from beneath the cell wall to the nucleus.

b. The direction of the movements, in some cells to, and in others from, the nucleus, or in different directions in parts of the same cell. This is known as the **circulation of living matter** in the cell.

The actual living matter is quite clear; its movements are rendered visible by the movements of the granules contained in it. Draw, indicating the direction of the movement by means of arrows.

4. Mount in the same way the tip of a leaf of *Anacharis*, the American pond weed, and examine under the low power. Note

The green cells closely packed together. Draw. Examine under the high power. Note

a. The green colouring matter, **chlorophyll,** in the form of spheres embedded in the living matter beneath the cell wall. Draw.

b. The movement of the living matter round and round the margin of the cell carrying the spheres with it. This is known as **rotation of living matter** in the cell.

The movement is not seen in all the cells.

If none of them exhibit it, dip the leaf in water at 30° C. for a minute and examine again.

5. Examine a drop of blood obtained by pricking your finger in Lesson VI, 1), under the high power. Note

The **coloured corpuscles** of the blood; each is a slightly yellowish disc; its diameter is $\frac{1}{3200}$th of an inch. Draw several in outline carefully.

This will serve as a standard wherewith to compare any other object which you draw under the same power.

If the object drawn is equal in diameter

to one coloured corpuscle, its diameter will be ... $\frac{1}{3200}$ths of an inch.
if to two coloured corpuscles its diameter will be $\frac{2}{3200}$ or $\frac{1}{1600}$,, ,, ,,
,, four ,, ,, ,, ,, ,, $\frac{4}{3200}$ or $\frac{1}{800}$,, ,, ,,
,, eight ,, ,, ,, ,, ,, $\frac{8}{3200}$ or $\frac{1}{400}$,, ,, ,,

and so on.

The student will learn a more exact method of measuring microscopic objects when he comes to the class in Practical Histology later on in his career.

Biology is the science which treats of living things.

Vegetable biology treats of the structure, and of the changes taking place during the life of vegetable cells and organisms.

Animal biology, of the structure, and of the changes taking place during the life of animal cells and organisms.

The elementary part of all living things is **the cell.**

The lowest organisms consist of a single cell only, higher organisms of more than one cell.

The **cell,** or elementary part, consists of a mass of living matter generally containing a nucleus, and often surrounded by formed matter which sometimes takes the form of a cell wall, and varies much in thickness and structure.

Living matter ("Bioplasm," "Protoplasm") is essentially a perfectly clear, homogeneous, semifluid substance, endowed with a power enabling it to alter its form, to grow, to divide, and to perform various functions. This power is **vital force,** and is quite distinct from chemical, mechanical, and electrical forces. Living matter is often found exhibiting "a granular condition," and has been described as consisting of two parts, a network or "**spongioplasm,**" which contains a fluid substance "**hyaloplasm,**" within its meshes; but as living matter is frequently found without these "differentiations," and yet exhibits all the characteristics to be presently described, the "granules" or "network" cannot be said to be a necessary part of living matter.

Within living matter there is generally found a nucleus.

The **nucleus** also consists of living matter which is slightly denser than that surrounding it. It is described as consisting of two parts, a "network of fine threads," composed of "**chromatin,**" so called because it stains readily with certain dyes, and a "clear material within the meshes," "**achromatin,**" which does not stain with the same dyes.

Where the threads of this so-called "intranuclear network" cross one another we get the appearance of small dark dots; these, together with small dark bodies contained within the meshes of the network, have been called "**nucleoli.**"

The nucleus plays an important part in presiding over the nutrition of the cell and in its reproduction.

It cannot, however, be essential to the living matter in all cells during the whole of their existence, because we sometimes find small portions of a cell breaking off, leading a free existence, growing and then developing a nucleus within themselves, as in some amœbiform bodies. (See Appendix 22.)

Living matter contains carbon, oxygen, hydrogen, nitrogen, and sulphur, and often in addition phosphorus, potassium, magnesium, calcium, sodium, and iron. In what state these elements are, and how related to one another, it is impossible to say. We know that they are present, because we find them on analysing matter when it has ceased to live, but which was living. "Any attempt to analyse living matter fails, because its life is destroyed. It no longer lives and we operate upon the lifeless products resulting from its death." (L. S. Beale, 1860.)

Living matter exhibits :

1. **Spontaneous movement.** It is continually changing its form, independently of any external stimulus.
2. **Irritability.** It responds, by movement, to various stimuli, light, heat, mechanical and electrical.
3. **Instability.** The arrangement of its molecules is continually altering.
4. **Deoxidising power.** The power of removing oxygen from the medium in which it is placed and of giving off carbonic acid gas to the medium.
5. **Growth.** It tends to increase in size, up to a certain point. New living matter is always produced in the substance of already existing living matter, and often in its central part. The food comes from without, and passes through the living matter and any formed material which may be around it, *i.e.* upon its surface.

   If the food is not already dissolved when it enters, it soon becomes soluble and diffusible owing to changes taking place as it traverses the matter already living. The properties of the already existing living matter are communicated to the new particles, as they become living.

   In all vital change the elements of the materials which constitute the food become re-arranged, and are made to take up positions with respect to one another until, at the moment they cease to be influenced by vital power, they differ much in composition in different organisms, and in different parts of the same organism; generally, new living matter is produced in the central part of already existing living matter, the outer part of which is pushed outwards. In all "cells" the oldest layers are most external, the youngest within.

"The separation of elements from their combination in the substances constituting the food, and their re-arrangement before they unite to form new substances, are effected by **vital force or power.** Assimilation, growth, formation of tissue and various substances characteristic of living things having structure or being structureless, are due to the influence of this living power which is handed down from particle to particle and cannot be produced anew." (L. S. Beale, 1860.)

All non-living matter, if it increases in size, does so by the deposition of new material outside that previously deposited, as occurs in the increase in size of crystals and various concretions.

6. **Division,** *i.e.* production of similar living matter. Division does not always occur. To some extent it depends upon the amount and kind of nourishment which the living matter is able to obtain, and upon the condition of the medium which surrounds it. Living matter always proceeds from pre-existing living matter.

Active movement of living matter may not lead to change in form in all cells, for the living matter of adult vegetable cells is surrounded by a cell wall composed of cellulose which limits its movement, but in this case streaming movements of the living matter can often be detected.

In animal cells no such cell wall exists as a rule, so that its change of form is often easily seen; but there may be a cell membrane around the living matter. Moreover, when several cells are closely packed together, mutual pressure may prevent the movement being manifest.

The cell wall or cell membrane is in all cases primarily formed by the activity of the living matter; "it is material formed from living matter but is not itself living."

Lesson III.

The Algæ: Protococcus, Spirogyra.

Protococcus.

1. Place upon a slide, in a drop of water, some of the green material found at the edges of the water in a water tank or gutter; cover and examine first under the low, and then under the high power. Note

   a. The unicellular, green, or green and red spherical **protococcus** in its resting stage.

   b. The **chromatophores** (small spherical masses containing the chlorophyll or cclouring matter) embedded in the **living matter** just beneath the cell wall.

   c. The **cell wall** of varying thickness. Draw.

2. Allow a drop of iodine solution to diffuse under the cover-glass. Note

   a. The living matter stained brown (and killed).

   b. The cell wall unstained—prove this by pressing sharply on the cover-glass and so rupturing the cell wall. Draw.

3. Follow the iodine by a drop of sulphuric acid (50 per cent.). Note

   The ruptured cell wall becomes blue, proving that it is composed of cellulose.

Every cell wall is sure not to be ruptured, so it is as well to examine under the low power first in order to find one.

The amount of acid necessary to bring about this reaction depends upon the quantity of water present in the specimen.

4. Mount and examine (as in 1) some water containing protococcus in the motile stage. Note

The **ovoid cells**, with cell walls varying in thickness, moving freely by a zigzag motion.

5. Treat with iodine as in 2. Note

The two **flagella**, whip like processes of living matter, attached to one pole of the cell, motionless and stained brown. Draw.

6. Search in a fresh drop of fluid, (as in 4), for protococci undergoing reproduction. Note

a. The **zoospores**, devoid of any cell wall, free, and moving rapidly through the water by means of their flagella. Draw.

b. Zoospores within the parent cell wall—as a rule, 2, 4, or 8 in number.

Protococcus can be readily grown in "Wolff's nutrient fluid," and be observed at all seasons of the year and in all stages. (See Appendix 2.)

Spirogyra.

1. Place upon a slide in a drop of water some pieces of filaments of **spirogyra**; cover, and examine under the low power. Note

The **filament** composed of **cylindrical cells** placed end to end and separated from one another by a common thin cell wall. Draw.

2. Examine a part of one filament under the high power. Note

a. The **granular living matter** in the cell (primordial utricle) lining the cell wall and stretching in bands towards the centre.

b. The **nucleus** in the centre of the cell with some living matter surrounding it.

c. The spaces between the bands of living matter filled with **cell sap.**

d. The spirally arranged **band of chlorophyll** with irregular margins, lying in the living matter. In some species two bands are found.

e. **Starch** grains and small **globules** of oil in the chlorophyll band. Draw.

3. Search for a cell in which the living matter is exhibiting streaming movement. (See Lesson II, 3.)

4. Mount as in 1 some filaments which have been kept in the dark for two or three days, examine under the low power and search for cells of contiguous filaments, showing various stages in the process of conjugation.

The Algæ with the Fungi form the group of Thallophytes. They include the lowest forms of vegetable life, and contain a great number of species which may be unicellular or multicellular, varying much in size, colour, and form. The **Algæ** all contain a green or red colouring matter, **chlorophyll,** and are all inhabitants either of salt or fresh water, or live on moist surfaces. Like other living organisms they require oxygen, and give off carbonic acid gas, but in addition, like all other organisms containing chlorophyll, they are able, in the presence of sunlight, to decompose carbonic acid, to "fix" the carbon, and to give off the oxygen as free gas. This latter process is carried on with great activity in sunlight, and far outweighs the ordinary respiratory process, but in the dark it ceases to take place.

Chlorophyll is found in certain bodies called chromatophores, which may be spherical or in the form of bands, etc. The material of which they are composed, rather than the chlorophyll itself, may be the means by which the carbonic acid gas is split up.

These plants require simple salts only for their growth.

The salts must contain potassium, magnesium, calcium, sulphur, phosphorus, and iron. They require in addition carbon, oxygen, hydrogen and nitrogen.

The nitrogen is obtained from nitrates or nitrites in solution in water.

In the algæ we have three methods of reproduction: 1 Cell division, 2 Rejuvenescence, 3 Conjugation.

In **cell division** the living matter of the cell divides into two, this being preceded by the division of the nucleus. (See Lesson XX.)

The nucleus may divide into two, and each of these into two, and so on, each being surrounded by some of the living matter of the cell, without a new cell wall being formed around it, so that there may be many such daughter cells within one common cell wall.

Intimately connected with this process is that in which the living matter breaks up into a number of small masses called **spores**, the process being called **endogenous spore-formation.**

In **rejuvenescence** the whole of the living matter of the cell contracts, and reconstitutes itself as a new living daughter cell, which subsequently becomes surrounded with a new cell wall.

In **conjugation** two neighbouring cells approach one another. An aperture appears in the wall of each, and the living matter of one becomes fused with that of the other, forming a "**zygospore,**" which then develops a new cell wall.

Protococcus in its adult form is a unicellular vegetable organism varying in size from $\frac{1}{200}$ to $\frac{1}{4000}$ of an inch in diameter. It is found in root-gutters, water-tanks, &c., frequently in groups, suggesting the idea that the cells have been formed by rapid division. There are many species, some being green, some red, and some green with a red centre. Protococcus consists of a mass of **living matter** containing a **nucleus** in which may be found a **nucleolus.** The living matter is surrounded by a more or less thick **cell wall** of cellulose, and on its outer zone, *i.e.* close beneath the cell wall, there are a number of small globular masses containing chlorophyll, called **chromatophores.** Before reproduction takes place, the cell wall usually becomes much thickened, forming a kind of cyst. Reproduction may take place by repeated **cell division,** or in some species by the formation of **zoospores.**

In this latter process the living matter divides into 2, and each of these into 2, making 4 pear shaped masses, each of which has at its small end two vibratile **flagella**, and is devoid of any cell wall. These four spores are known as **macrospores.**

The cell wall of the parent cell is then absorbed, or bursts and the macrospores are set free and move freely about in the water by means of their flagella. Each then develops a cell wall and continues to move freely for some time. Eventually the flagella disappear, and the cell assumes its resting condition. In some cells the division is carried further and leads to the formation of eight spores, "**microspores.**"

It will thus be seen that in some species there is a **motile** as well as a **resting** adult stage.

Spirogyra is one of the multicellular algæ.

It consists of a number of fine green filaments about as thick as human hairs, composed of cells joined end to end, and often found covered with a viscid or slimy sheath. It is found in ponds during the summer, and is very widely distributed.

Each cell contains granular **living matter,** which is arranged very like that in the cells of the tradescantia hairs (Lesson II), containing spaces filled with **cell-sap.** It is surrounded by a cellulose **cell wall.** A **nucleus** is found in the living matter, usually near the centre of the cell.

The outer zone of living matter contains **chlorophyll** arranged in the form of a **spiral band,** giving the plant a very characteristic appearance under the microscope.

There are several species of spirogyra differing from one another in the closeness of the turns of the band and in the size of the cells.

Reproduction takes place generally during the night :

1. By conjugation.
2. By cell division.

In **conjugation** two cells of different filaments lying close together conjugate, leading to the formation of a **zygospore.** The living matter of each of these cells pushes its cell wall outwards at a certain point. The two cells then meet and burst at the point where they touch one another, the living matter in each of the cells having previously accumulated around its own nucleus, and become separated from its cell wall. The living matter of one then passes into that of the other. The mass so formed becomes oval in shape, and forms a thick cell wall around itself; it is termed a **zygospore.**

The living matter of the zygospore gradually becomes of a brownish-red colour, and very granular, and remains dormant for some months, then it starts into activity and begins to form a new filament by rapid cell division.

Cell division.

Cells may also be found exhibiting cell division. The nucleus first divides into two parts and the living matter then collects around each part. A cell wall is formed between the two masses of living matter, and thus two new cells are produced. New cells may be formed by cell division at any part of the filament, often about its centre.

Both these reproductive processes occur during the night, or when spirogyra is kept in the dark; conjugation, when it is scantily supplied with nutrient materials, cell division when it can obtain plenty of nourishment.

Lesson IV.

The Fungi: Fermentation, Saccharomyces Cerevisiæ.

1. Place a drop of brewer's yeast, or of Pasteur's fluid (see Appendix 7) to which a little German yeast has been added and kept at 35° C. for two or three hours, upon a slide; cover and examine under the low power. Note

A great number of minute, ovoid and spherical bodies suspended in the fluid. Each of these is a **yeast plant, Saccharomyces Cerevisiæ.**

Examine under the high power.
In each of the yeast cells: Note

a. The granular **living matter.**
b. The **vacuoles,** one or more cavities containing fluid. (Vacuoles are not always present.)
c. The **cell wall** (cellulose) surrounding it. Draw.

2. Allow a drop of iodine solution to diffuse under the cover-glass. Note

a. The living matter becomes **stained brown** (and is killed). In some cells a **nucleus** more deeply stained may also be seen.

b. The vacuole and cell wall remain colourless. Both may appear brown, because they are seen through the stained living matter.

3. Remove the specimen from the microscope, tap the centre of the cover-glass firmly with the point of a pencil and examine again. Note

a. The torn and colourless cell walls.

b. The scattered brown granular *débris*. Draw.

4. Mount another specimen as in 1, and examine under the high power. Note

The yeast cells **budding**, a chain of from three to six yeast cells is often seen. Draw.

5. Place upon a slide a little yeast which has been spread upon a disc of plaster or porous china, and kept moist for five or six days under a bell glass; cover and examine under the high power. Note

Here and there a yeast cell containing two or four small round bodies; these are spores, **asco-spores.** Draw.

6. Examine prepared and stained specimens showing asco-spores. (See Appendix 8.)

The **Fungi** may be uni- or multicellular, and do not contain any chlorophyll or green colouring matter. As therefore they are unable to obtain their carbon from carbonic acid gas in the air, they must obtain it from organic compounds, either from those occurring in the remains of dead organisms, or from those previously formed by living organisms. The two groups belonging to the fungi with which we are concerned are the "**Saccharomycetes**" and the "**Schizomycetes.**" In these multiplication takes place by budding, cell-division, or by the formation of spores; closely connected with the life of these are two processes, "**fermentation**" and "**putrefaction.**"

A **ferment** is a body which can bring about chemical changes in other bodies, without undergoing any appreciable change itself. For the proper action of a ferment, the following **conditions** must be fulfilled :

1. A given temperature generally not lower than 0° C or above 75° C.

   As a rule the best temperature is about 40° C.
2. The presence of moisture.
3. The product, or products formed by the activity of the ferment must be removed, and not allowed to accumulate in any great quantity.
4. An acid **or** alkaline medium; most ferments require acid or alkaline, few act in neutral fluids.

Ferments have been shown to be, or to be akin to, **proteids** (highly complex compounds of carbon, oxygen, hydrogen, nitrogen, and sulphur). (See "Chemical Physiology," Halliburton.)

Ferments are classified as organic, and inorganic, or better as **organised** and **unorganised**; the former are living cells, (Bacteria, yeast plant, etc.); the latter, chemical compounds formed by the activity of living cells, (pepsin, etc.) Probably the real difference between the two lies in the fact that the former are contained within living cells, and are unable to diffuse out during the life of the cells, the latter easily diffuse out of the cells.

Ferments are also classified according to the effects they produce, *e.g.*, alcoholic, those converting sugar into alcohol and carbonic acid gas; amylolytic, starch into sugar, and others.

Yeast, as obtained from the brewer, is a thick, light brown soft mass or fluid, with a frothy surface and a characteristic odour.

It consists of **water**, with **salts** in solution, and a vast number of minute **cells**.

Each cell is a **yeast plant**, Saccharomyces Cerevisiæ, and averages $\frac{1}{3500}$ of an inch in diameter.

If **yeast** is added to a solution of **sugar** it causes fermentation to take place with the production of **alcohol** and the evolution of **carbonic acid gas.** If the yeast is separated from the sugar solution by a porous earthenware surface (such as a battery porous pot), no fermentation occurs, although the fluid portion of the yeast passes through into the solution.

The **yeast cells** are therefore the **cause** of the **fermentation.**

Yeast can be kept dried for a long period, and if then moistened and kept at the proper temperature and under proper conditions, will cause fermentation.

German yeast is yeast deprived of most of its water, so that it is of the consistency of putty.

If yeast be **burned** in the air, the remaining **ash** is found to contain carbon, oxygen, hydrogen, nitrogen, phosphorus, sulphur, potassium, calcium, and magnesium.

Yeast will grow in a fluid containing these elements in certain proportions. Such a fluid is **Pasteur's solution,** which contains water, sugar, ammonium tartrate, potassium phosphate, calcium phosphate, and magnesium sulphate. (See Appendix 7.)

If **yeast,** or the fluid containing it, be **boiled** for a few minutes, it no longer possesses the power of setting up fermentation, because the living matter of the cells has been killed. The yeast plant or Saccharomyces is therefore an **organised ferment.**

There are a great many species of saccharomyces, many being found in a dried state, as dust in the air ; if these settle upon fruits, etc., they begin to grow and cause fermentation in the juice of the fruit.

Multiplication takes place very rapidly by **budding,** and ocasionally by endogenous spore formation. (Ascopores, Endogonidia.)

The latter process only occurs when yeast is sparingly supplied with its proper nutrient material.

Lesson V.

Micro-organisms : their mode of life and growth, their growth in artificial media, putrefaction.

Pour some hot water on to a handful of hay in a vessel, and filter the brown infusion. Put some of the clear brown filtered fluid in a glass vessel, cover it with a glass plate and keep it at 35° C until it becomes dull and turbid and a scum forms upon the surface.

1. Place a drop of the turbid fluid upon a slide, cover and examine under the high power. Note

The whole field covered with minute, living, moving **micro-organisms** of various shapes.

a. **Bacteria**, rod shaped bodies, each constricted at its centre, about $\frac{1}{12000}$ of an inch in length, and averaging $\frac{1}{50000}$ of an inch in diameter.

b. **Bacilli**, longer rod shaped bodies, about $\frac{1}{3000}$ of an inch in length (*i.e.* slightly longer than the diameter of a human coloured blood corpuscle).

They are often seen forming long chains, produced by rapid division.

They move about by a rapid side to side motion.

c. **Vibriones**, slightly bent rod-shaped bodies, often in pairs joined end to end, moving by a serpentine motion, and about the same size as *b.*

d. **Spirilla**, spirally twisted rod-shaped bodies, moving by a rapid rotatory motion round a central long axis; they are often as much as $\frac{1}{1000}$th of an inch in length. (These vital movements are caused in many forms of micro-organisms, by a **flagellum** attached to one or both ends of the organism. The flagellum is a prolongation of the living matter covered with a prolongation of the cellulose cell wall.)

e. All exhibit in addition, rotatory and swaying movements round one another known as **Brownian movement.** Draw.

2. Examine some of the largest bacilli very carefully under the highest power available. Note

The **structure** of the organism.

a. A minute mass of **living matter** surrounded by a **cell wall** (cellulose).

b. Within the living matter perhaps **vacuoles, granules, oil globules,** and **spores.** No visible nucleus. Draw.

Spores, which are sometimes present, are small oval bodies situated in the living matter generally at each end of the organism.

3. Mount in the same manner and examine under the high power a drop of putrefying animal fluid such as blood or urine. Note

In addition to the various organisms above described.

a. Numbers of very minute spherical organisms or **cocci**, exhibiting vital and Brownian movements.

b. Micrococci, single organisms.

c. Diplococci, organisms in pairs.

d. Streptococci, organisms arranged in long rows. Draw.

4. Examine in the same manner a little of the scum from the surface of the fluid. Note

The various **organisms** motionless and **embedded** in a mass of gelatinous material; **zooglea stage.** Draw.

5. Examine in the same manner a drop of the fluid or of the hay infusion which has recently been boiled for one minute. Note

a. The **dead organisms** exhibiting **Brownian movement** only; contrast this carefully with the vital movement previously seen.

6. Place upon a slide a drop of water containing a little powdered **gamboge** (it should have been previously boiled, to destroy any organisms which might be present), and examine under the high power. Note

The particles of gamboge exhibiting **Brownian movement.**

7. The student should also examine stained prepared specimens of micro-organisms under a $\frac{1}{12}$th inch objective, carefully note their structure, spores, &c., and draw them. (See Appendix, 5.)

Putrefaction or putrefactive fermentation accompanies the disintegration of dead animal and vegetable matter.

It is brought about by the vital activity of bacteria or other micro-organisms which find in these materials suitable nourishment for their growth.

If dead animal or vegetable matter be left in contact with the air, the organisms which are present in the air settle upon it and grow and multiply very rapidly. They obtain oxygen from the air; carbon, hydrogen, nitrogen and various salts containing sulphur, potassium, magnesium, phosphorus and calcium, from the decomposing tissues. Their living matter separates these elements which are combined in the dead matter and causes them to combine again to form more living matter, and also certain compounds which are set free; these latter consist of ammonia, carbonic acid gas, and foul smelling gases, of which sulphuretted hydrogen is the chief, and of nitrogenous com pounds, **ptomaines**, which are virulent poisons when introduced into the tissues or into the circulatory fluids of some animals.

If, on the contrary, the air be excluded from the dead animal or vegetable matter, then the organisms which have already gained access to it, grow and multiply, obtaining their oxygen from the decomposing matter, forming ptomaines as before, carbonic acid gas in smaller quantity, and practically no foul smelling gases. The disintegration also takes place more slowly, and after a time the organisms may cease to multiply, and be even killed by the poisons they have produced during their growth. The production of foul smelling gases and ptomaines depends also largely upon the kind of organism which is present in great quantities. Again, if some antiseptic, such as carbolic acid, thymol, perchloride of mercury, or boracic acid has been added to the dead matter, or the latter be exposed to air which has been filtered through a solution of one of these or through cotton wool, then no organisms will be able to grow and the dead matter will not undergo any putrefactive changes. The same effect can be produced by keeping the dead matter at 0° C. or at 100° C. If any spores were present before these precautions were taken they may not be killed, but will be prevented from developing so long as these conditions are observed.

Schizomycetes are minute unicellular vegetable organisms, classified according to their shape or form as cocci, bacilli, bacteria, vibriones and spirilla.

There are hundreds of species of these various genera, and as many of them cause by their growth and spread, or by the chemical products produced by their growth, various diseases n man, in the lower animals, and in plants, they have also been classified according to the diseases so produced. In order to prove that any one of these organisms is the actual cause of a disease, Koch has laid down the following postulates.

1. The organism must be found in the blood, lymph, or diseased tissues of the animal or plant suffering from, or dead of the disease.
2. The organisms must be isolated from such blood, lymph or diseased tissue, and cultivated outside the body in suitable media.

   Such "pure cultivations" must then be carried on through successive generations of the organism.
3. A pure cultivation thus obtained, must when introduced into the tissues of a healthy animal or plant, produce the disease in question.
4. In the inoculated animal the same organism must again be found.

Bacteria can be dried and, being smaller than yeast cells, are more readily disseminated through the air. They can be collected from the air upon glass plates coated with "a nutrient medium" such as a solution of meat extract and gelatin which has been previously boiled for two hours so as to destroy any organisms which might be present in it. This latter process is known as "**sterilisation.**"

When the dried organisms settle upon the surface of the medium they grow and multiply, forming visible masses, "colonies." These can be recognised as consisting of a certain species of organism, either by the colour produced during its growth, or, microscopically by the arrangement of the cells in the "colony."

The colours produced by some organisms can be well shown by growing them upon the surface of a potato, previously sterilised by the aid of steam.

In surgical procedures, operations and dressing of wounds, care has to be taken to arrest the growth of, or to destroy by sterilisation and by the use of "antiseptic" solutions, any organisms which may be present in, or upon instruments, dressings etc., and the skin.

Unless these precautions be taken, certain organisms may grow and multiply in the wound, and by their growth, or by the passage into the tissues and blood stream of the chemical bodies formed during their vital activity, may prevent the healing of the wound, or cause a general and sometimes fatal disease.

Lesson VI.

Human Blood : Frog's Blood, Clotted Blood.

Human Blood.

1. Place a drop of your own blood upon a slide, and cover quickly. (To obtain blood for examination, wind a handkerchief tightly round a finger, from base to tip, flex the terminal phalanx, and run the point of a clean needle into the skin at the base of the nail.) Examine first under the low power, and then under the high power where the film of blood appears thinnest. Note

a. The innumerable **coloured corpuscles**, their shape, especially when seen on edge, and yellowish colour.

b. The coloured corpuscles heaped up in **rouleaux** like piles of coins.

c. The **colourless corpuscles**, spherical, granular, colourless cells, few in number. Draw.

2. Allow a drop of **distilled water** to diffuse under the cover glass, and examine under the high power. Note

a. The coloured corpuscles becoming **spherical** and almost invisible. The water dissolves out the colouring matter, **hæmoglobin**, which is contained in the coloured corpuscles.

b. The colourless corpuscles ; they **swell** and eventually **burst**.

3. Mount another drop of blood, as in 1, but allow a drop

of dilute **acetic acid** to diffuse under the cover glass, and examine under the high power. Note

a. The coloured corpuscles ; much the same as in 2 *a*.

b. The **colourless corpuscles** ; the **nucleus** of each appears visible (it often consists apparently of two or three nuclei close together). Draw. The dilute acid kills the living matter, and makes it transparent, and so causes the nucleus to become visible.

4. Mount another drop of blood as before but add a drop of syrup or strong salt solution, and examine under the high power. Note

a. The coloured corpuscles, they shrivel up and become **crenated.** Draw.

Owing to the constancy of the size of the coloured corpuscle of human blood ($\frac{1}{3200}$ of an inch in diameter) it affords a ready method of measuring the size of other objects under the same magnifying power ; the student is recommended to measure all objects which he sees under the high power in terms of a coloured blood corpuscle, *i e.* equal in diameter to 1, 2, 4, etc., coloured corpuscles, as explained in Lesson II.

Frog's Blood.

1. Mount a drop of frog's blood on a slide, cover and examine first under the lower power, and then under the high power. Note

a. The **coloured corpuscles** ; their shape, especially when seen on edge ; the indistinct oval **nucleus** contained in each. Draw.

b. The **colourless corpuscles** ; very irregular in shape, and exhibiting **amœboid** movement. This may not be seen until the blood has been mounted for some minutes. Draw one at intervals of two minutes.

2. Allow a drop of water to diffuse under the cover-glass. Note

a. The coloured corpuscles become spherical, colourless and almost invisible; the nucleus remains visible.

b. The colourless corpuscles; the amœboid movements cease, they become **spherical** and eventually burst.

3. Mount another drop of frog's blood and allow a drop of dilute acetic acid to diffuse under the cover glass, examine under the high power. Note

a. The coloured corpuscles, the same as 2, *a*, only the nucleus becomes very distinct.

b. The colourless corpuscles, the same as in the case of human blood 3, *b*.

4. Examine under the high power stained prepared specimens of frog's blood. (See Appendix 6.)

Blood Clot.

Examine some blood which has been obtained from the slaughter house in a jar or other vessel.

1. Turn the mass out on to a plate. Note

a. The mass of **clotted blood.**

b. The **serum.** A clear straw coloured, but more often reddish, fluid which has been squeezed out of the clot.

2. Cut the mass of clot in half. Note

a. The **surface, bright** red.

b. The **deeper parts, dark** red.

The **hæmoglobin** in the coloured corpuscles upon the surfaces of the clot has combined with the oxygen of the air to form **oxyhæmoglobin** which is of a bright red colour.

The air has not been in contact with the deeper parts of the clot, hence the dark red colour.

Human blood is an **opaque red** fluid. Its colour varies from bright red if obtained from an artery, to dark red if from a vein. Blood consists of a clear straw-coloured fluid, **plasma** or **liquor sanguinis** containing floating about in it a great number of **corpuscles** both **coloured** and **colourless.**

The **coloured corpuscles** are non-nucleated bi-concave discs with rounded edges, the diameter of each is $\frac{1}{3200}$th of an inch, its thickness averages $\frac{1}{12000}$th of an inch. Each corpuscle consists of a structureless homogeneous **stroma** pervaded with a colouring matter, hæmcglobin ; it is this which causes a single corpuscle to appear yellowish, and masses of corpuscles to appear red ; **hæmoglobin** has a great affinity for oxygen, with which it combines chemically to form oxyhæmoglobin.

The dark red colour of venous blood is therefore due to hæmoglobin, the bright red of arterial blood to oxyhæmoglobin pervading the stroma of the corpuscles.

The **colourless corpuscles** are much fewer in number, one to about three or four hundred coloured.

They are, when withdrawn from the blood vessels and at the temperature of the air, spherical colourless cells, of from $\frac{1}{3000}$ to $\frac{1}{3500}$th of an inch in diameter.

Each consists of a mass of living matter, richly granular, containing an irregular nucleus ; at a temperature of 40° C it exhibits amœboid movement ; the colourless corpuscle is a typical animal cell.

When **blood** is withdrawn from a blood vessel and left exposed to the air it **clots** in a few minutes. The clot consists of a fine network of a material called **fibrin** and the corpuscles, coloured and colourless, enclosed in its meshes ; the clot gradually shrinks and squeezes out a clear straw-coloured fluid called **serum.**

Blood in blood vessels of a living animal consists of :

1. Plasma.
2. Corpuscles { coloured. / colourless.

Clotted blood consists of :

1. Serum.
2. Clot { corpuscles { coloured. / colourless. ; fibrin

Serum is therefore plasma from which the elements which go to make fibrin, and the corpuscles, have been removed. Serum is generally reddish, owing to the fact that it contains some hæmoglobin in it, dissolved out of the coloured corpuscles.

Frog's blood consists also of **plasma**, and **corpuscles** coloured and colourless. Each **coloured corpuscle** is an **elliptical disc** with rounded edges, and consists of a homogeneous **stroma** pervaded with **hæmoglobin**. It contains an **oval nucleus**, so that if seen on edge the corpuscle appears **bi-convex**. Each corpuscle is about $\frac{1}{900}$th of an inch in its long diameter. The **colourless corpuscles** are like those of human blood but slightly larger, they exhibit **amœboid** movement at the ordinary room temperature, this being higher than the temperature of the frog's body.

Lesson VII.

The Protozoa, the Amœba : differences between animals and plants. (See Appendix 11.)

1. Place upon a slide a drop of water containing amœbæ, cover, interposing a hair between the slide and cover-glass.

Search for an amœba under the low power, and when you have found one examine it under the high power. Note

a. The **living matter**, its clear outer part, so called **ectosarc**, and its granular inner part, so called **endosarc**.

b. The **nucleus** in some part of the "endosarc." It may be quite obscured by the granules.

c. The **contractile vacuole** in the living matter. Watch it for some minutes and see it gradually dilating and then suddenly collapse.

d. The **food-vacuoles** in the living matter.

e. The absence of any cell wall around the living matter. Draw

f. The **movements** of the living matter leading to change in shape and change in position of the amœba.

Watch the **pseudopodia**, processes of living matter protruded first at one part, then at another ; each protrusion at first consists of the clear living matter, "ectosarc," only, then the granular living matter, "endosarc," flows into it gradually.

2. Try and find an amœba feeding ; two pseudopodia may be seen to surround some small vegetable organism and coalesce. The organism is thus enclosed by the living matter together with a little water, forming a food vacuole.

3. Search for amœbæ exhibiting one or other form of **reproduction.**

a. A pseudopodium may be seen to become constricted at its base and then to separate, and begin amœboid movements.
b. An amœba may be seen containing two nuclei and undergoing fission.
c. Two amœbæ may be seen to fuse together, generally a large one with a small one.

4. The student should, if possible, look at, and draw stained prepared specimens. (See Appendix 4.)

The **protozoa** form the lowest group of the animal kingdom.

They are single-celled animals, microscopic in size, or only just visible to the unaided eye.

They are found in water, free, or attached to various solid substances or to other organisms, amongst decomposing animal and vegetable matter, and as parasites, that is living upon or within other organisms and obtaining from these their nourishment, either from their tissues, or from the various fluids within their organs.

The members of the protozoa differ in form and structure according to the situations in which they live, and may be divided in two main groups :

1. Those which have no membrane surrounding the living matter, these are subdivided into

a. Rhizopoda (*e.g.* Amœba).
[*b*. Mycetozoa (*e.g.* Plasmodiophora).]

2. Those which have a definite membrane surrounding the living matter, which membrane has been formed from the outermost part of the living matter.

These are subdivided into

a. Sporozoa (*e.g.* Coccidium, Gregarine).
b. Ciliata (*e.g.* Vorticella).

The **amœba** belongs to the Rhizopoda.

It is found in the ooze at the bottom of ponds, roof gutters, etc.

The amœba is a single animal cell, composed of **living matter** containing a **nucleus**, and, in most specimens, a **contractile vacuole** and food vacuoles. There is no cell wall.

The living matter is granular around the nucleus ("endosarc"); perfectly clear and homogeneous externally ("ectosarc"). The outer portion of the "ectosarc" is firmer than the rest.

In size the amœba varies from the $\frac{1}{1000}$th to the $\frac{1}{50}$th of an inch in diameter, the largest species (amœba princeps) being just visible to the naked eye.

Its shape is, during its period of activity, continually changing owing to the living matter being constantly protruded at some points, and withdrawn at others. The "ectosarc," is first quickly protruded and into this the granular "endosarc" gradually flows.

These protrusions are termed **pseudopodia**, and by means of them the animal is able to change its form and position.

When at rest the amœba is more or less spherical, and during the winter the outer layer of the "ectosarc" becomes considerably firmer and thicker, thus enclosing and protecting the living matter within. This thickening of the "ectosarc" is made of a material which is formed by the living matter, and as the water in which the amœba lives becomes warmer, during the spring, the material may be absorbed by the living matter, and active movements by means of pseudopodia may again commence.

The amœba feeds upon minute living organisms, mostly vegetables, which it surrounds by two of its pseudopodia; the living matter practically "flows around" and encloses the particle of food.

The food is accompanied by some water and forms a **food vacuole**; it is then gradually assimilated. (See Lessons II and VIII, intracellular digestion.)

The amœba like all living cells takes in **oxygen** which is dissolved in the water, and gives off carbonic acid gas to the water.

Water is a **chemical combination** of oxygen and hydrogen, no living cell can separate these two gases from water, but in water there is always some air in a state of solution; **air** is a **mechanical mixture** of nitrogen and oxygen (100 vols. air contain about 79 vols. N, 21 vols. O, ·04 vol. CO_2). It is some of this oxygen of the dissolved air which cells living in water take in.

The living matter of the amœba causes some of its carbon to unite chemically with some of this oxygen to form carbonic acid gas. This process is called intracellular **respiration.**

The living matter gets rid of certain parts of the food which are not required (the cellulose cell walls of the organisms upon which it feeds and probably some material containing nitrogen) by "flowing away" from them just as it flowed round the food particle.

The **contractile vacuole** is a space within the living matter probably containing water. At intervals of a few minutes the living matter suddenly contracts upon, and forces the water contained in the vacuole into its substance. The contractile vacuole is often near the surface of the living matter, and the fluid which it forces out may contain some of the nitrogenous waste products formed within the living matter.

The amœba reproduces;

1. By one of the pseudopodia becoming constricted and severing itself from the rest of the cell, forming a new amœba.
2. By fission; the nucleus divides, and this is

followed by the division of the surrounding living matter into two equal parts.

3. By conjugation; two amœbæ fuse together and become "encysted," resembling the condition of the amœba during the winter; this is followed by the methods 1 or 2 or both.

We see that the amœba (an animal) differs from protococcus and saccharomyces (vegetables) in two important respects.

1. Amœba requires for its food the immediate products of recently existing living matter, which it takes into itself and assimilates. Protococcus requires only simple salts in solution, and carbonic acid gas. Saccharomyces requires simple salts in solution, and some organic compound, sugar. They all require oxygen, but saccharomyces can obtain it from organic compounds containing oxygen. Amœba can only obtain that oxygen which is dissolved in water.
2. Amœba has no cellulose cell wall. Protococcus and yeast both have a cellulose cell wall, though the zoospores of protococcus have at first no cell wall. (See Lesson III, page 17.)

There is no great difficulty in distinguishing between the higher animals and plants, for instance, a dog and a geranium; but when we come to the lower members the difficulty increases enormously, and in the lowest there is no one definite character distinguishing the animal from the plant, indeed, some of the mycetozoa resemble plants at one stage, and animals at another stage of their existence.

The essential part of all living things is living matter. What we do know for certain is that the living matter of a vegetable cell will not produce an animal cell, and *vice versâ*. But if a certain mass of living matter be given to us, we cannot tell whether it is going to form a vegetable or an animal cell.

The difference between the two kinds of living matter is vital, and is not demonstrable.

Speaking broadly, we may say that plants, as a rule, require simple salts in solution, oxygen, and if they contain chlorophyll, carbonic acid gas; from these simple substances the living matter which plant cells contain is able to make complex bodies, viz., more living matter and starch.

Animals require just those substances which the plant living matter has made, viz., previously existing living matter and starch, and in addition, oxygen.

Lesson VIII.

Vorticella.

(See Appendix 11.)

1. Place some algæ or duck-weed roots, which have Vorticellæ attached to them (this can be determined by examination with a powerful hand lens) upon a slide in a drop of water, cover and examine under the low power. Note

   a. The bell-shaped unicellular animals, **Vorticellæ,** each attached by a long stalk to the weeds, etc.; other species may be found with a single stalk bearing many bell-shaped cells.

   b. A kind of vortex or whirlpool in the water opposite the mouth of each bell. Draw.

2. Tap the cover glass. Note

   a. Each stalk **suddenly contracts** and is thrown into a closely-wound spiral.

   b. The bell becomes **spherical,** and the water near it quiescent. Draw.

   c. The conditions in 1 *a* and *b* become again evident within a short space of time.

3. Examine one of the Vorticellæ under the high power. Note

   a. The granular **living matter** within the bell, its outer part, sometimes striated longitudinally (**"myophan striation"**).

b. Several small spherical spaces containing colourless fluid and perhaps also particles of food, **food vacuoles.**

c. One large vacuole containing a colourless fluid only, the **contractile or pulsating vacuole**; if this be watched, it will be seen to dilate gradually, then suddenly collapse and gradually dilate again, and so on.

d. An elongated **nucleus,** situated generally at the bottom of the bell.

b, *c*, and *d*, all within the living matter.

e. The thin **membrane,** sometimes transversely striated, which encloses the living matter and gives rise to the bell shape. At the mouth of the bell this membrane is everted, and being folded upon itself (with a little living matter within the fold), forms the **peristome.**

f. The **cilia** upon the inner aspect of the peristome; (the living matter of the bell is continued into each cilium.)

g. The fine membrane, bulged outwards, which covers the mouth of the bell, **the disc.**

h. A groove between the disc and the peristome, which deepens, forming the **vestibule,** and is continued as a narrow tube leading into the living matter of the bell. The vestibule is lined with cilia.

(The whole bell has been described as consisting of "protoplasm," the inner more fluid part being "endosarc," and the thicker external part "ectosarc," the "ectosarc" being covered by the "cuticle.")

k. The **stalk.** It has an outer membranous covering (similar to the bell membrane), which contains some living matter (probably continuous with the living matter within the bell). Draw.

4. Allow a drop of iodine solution to diffuse under the cover-glass, and examine first under the low, and then under the high power. Note

a. The Vorticellæ are killed. They assume and retain the form seen in 2 *a* and *b*.
b. The **living matter** of the bell and of the stalk **stained** brown.
c. The **nucleus stained** most deeply.
d. The fine membrane covering bell and stalk unstained. Draw.

5. Search under the low power for **free-swimming** forms; these somewhat resemble the adult, but are smaller, have no stalk, and possess a ring of cilia, **aboral cilia**, near the base of the bell. Draw.

6. Search also for Vorticellæ showing stages in reproductive processes.

a. Undergoing longitudinal **fission**. The bell divides into two unequal parts; one, the larger, remains fixed to the stalk, the smaller develops aboral cilia and becomes a free swimming form.
b. **Conjugation**. A free swimming form becomes attached to, and fuses with a stalked form.
c. **Encystment**. The bell is detached from its stalk, the living matter becomes spherical and forms a thick cyst around itself.

Vorticella belongs to the **ciliata** of the **protozoa**. (See classification of animals.)

Various kinds are met with both in fresh and salt water, some with a single bell-shaped cell, others with many such cells, upon a single stalk which is attached to weeds, stones, limbs of aquatic animals, etc.

The **bell-shaped cell** is a mass of **living matter** containing **granules**, a **nucleus**, pulsating or **contractile vacuole**, **food vacuoles**, and is surrounded by a very thin cell **membrane**.

There is a ring of very fine hair-like processes, each containing some living matter, **cilia**, around the everted mouth of the bell or **peristome**, which by their rapid, rhythmical, lashing movement set up a whirlpool in the water, and so drive the water together with small organisms contained in it into an aperture in the **disc**-like membrane which fills in the mouth of the bell. This aperture is the outer end of a groove, the **vesti-**

bule which exists on one side between the peristome and the disc; it is lined with cilia and curves downwards, gradually narrowing until it leads into the living matter of the bell. The small particles of food, together with some water, are wafted down the vestibule by the motion of its cilia, and collect in a mass which is suddenly received and enclosed by the living matter, forming a **food vacuole.** Here the food gradually undergoes intracellular digestion. The food vacuoles have been shown to contain some acid, probably resulting from changes in the living matter around them.

The undigested portions of the food pass out of the living matter at intervals in small masses and escape to the exterior, sometimes through the vestibule, sometimes through a small slit-like opening in the membranous covering of the bell, situated just below the peristome.

Reproduction takes place—

1. **Asexually,** by fission.

The fission is unequal and occurs longitudinally. The nucleus divides first, and this is followed by the division of the living matter into two parts: the larger remains adherent to the stalk, the smaller develops aboral cilia and becomes a free swimming form. Some of these smaller forms become fixed, develop a stalk, and grow up into stalked forms.

2. **Sexually,** by conjugation.

One of the free-swimming forms attaches itself by its base to one of the fixed forms, probably to one which has already divided. The two fuse together, and a tough membrane is formed by the living matter around the whole. The encysted mass then falls to the bottom of the water and remains there during the winter. In the spring the greater part of the membrane disappears, and the organism begins to exhibit amœboid movement. It then develops cilia at both ends, and becomes a free swimming form. This attaches itself to some weed or stone and a stalk gradually grows until the adult stalked stage is reached. It then probably undergoes fission asexually. This process of conjugation is of great interest, as it occurs in an organism so low in the animal scale, and is one of the earliest evidences of conjugation between two dissimilar forms. It has been said that the small free swimming form represents the male, and the larger stalked form, the female.

Lesson IX.

[This lesson should be worked towards the end of the course, when the student has become familiar with the structure of higher plants and animals.]

The Mycetozoa, Plasmodiophora Brassicæ: the Sporozoa, Coccidium Oviforme.

Plasmodiophora.

1. Obtain the root of a cabbage suffering from club-root. Wash in water to get rid of the earth. Note

a. The **nodular swellings** connected with almost every rootlet.

b. Parts of the nodules undergoing decomposition. Draw.

Compare this with a healthy cabbage root, and, if possible, compare the appearances presented by a stunted cabbage plant growing from a club root, and a plant growing from a normal healthy root. Draw.

2. Cut thin transverse sections of one of the younger nodules, place the thinnest upon a slide, in a drop of normal salt solution ·65 per cent.), cover, and examine under the low power. Note

a. The normal cells of the **fundamental tissue** of the root.

b. The mass of **fibrovascular bundle** systems forming a ring within the fundamental tissue and separating.

c. The **cortex**, outside from

d. The **pith**, inside.

e. Certain masses, generally granular, some of which seem to occupy spaces between the cells of the fundamental tissue, some to occupy the cells themselves. Draw.

3. Examine one of these masses under the high power. Note

a. The mass of **living matter,** irregular in shape, **Plasmodiophora,** which has invaded several of the cells, and may be seen invading others, and absorbing the cell walls.
b. If carefully watched, it will be seen to exhibit very slow, but decided **amœboid** movements.
c. The **granules, drops of water,** and **foreign bodies** within the Plasmodiophora. Draw.

4. Examine other parts of the section. Note

a. Larger irregular masses composed of innumerable minute spherical bodies. These are **spores,** the whole mass of living matter having broken up into these minute spores.
b. Each spore is surrounded by a cell wall of cellulose. Draw.

5. Stain a section with carmine fluid, and mount it in glycerine. (See Appendix 21.) Examine first under the low and then under the high power. Note

a. The **healthy cells** of the root living matter pink, their nuclei red.
b. The **Plasmodiophoræ** ; intensely pink or red, with the droplets and bodies contained within each.

The **mycetozoa** comprise a very large number of organisms, about which little was known until quite recently, and which will, as time goes on, probably become of the greatest importance.

They are of great interest to the student, because they form a kind of connecting link between plants and animals, some of them resembling plants at one stage, and animals at another stage of their existence.

The mycetozoa (myxomycetes) or "slime fungi" for the present cannot be definitely classified with vegetables or animals, but occupy a place by themselves. They are placed temporarily near the protozoa. (See classification of animals.)

The example which we have chosen, **Plasmodiphora,** is very widely distributed, and at one stage is found in the roots of cabbages and allied plants, at another in the soil in which these plants grow.

It will be convenient to start at the stage in which we have seen masses of minute spherical bodies within large spaces between and within the cells of the root tissue.

Each of these minute bodies is a **spore** and has a cellulose cell wall. As the root rots in the soil or upon it, after being pulled up, these spores are set free. From each one issues a minute mass of living matter very like a minute amœba without any nucleus, this is an amœbula or **myxamœba.** It exhibits amœboid movements, and develops a process, an elongated pseudopodium, by which it moves about freely.

If young cabbages be planted in the soil containing these organisms, the latter enter the tissues of the young roots and increase in size, feeding upon the nutrient materials formed by the activity of the vegetable living matter within the cells of the root.

Then several of these organisms unite forming a **plasmodium.** This also increases in size, and gradually breaks down the cell walls of the root cells, and thus forms the mass of frothy vacuolated living matter, the Plasmodiophora, which we have seen in 3 *a*, *b*, and *c*.

The living matter of the plasmodiphora eventually breaks up into a mass of spores, and thus the cycle is again continued.

Coccidium Oviforme.

1. Cut out the liver of a recently killed young rabbit, which is thought to be suffering from the disease known as "wet snout," and place it in normal salt solution. Examine the surface of the liver for irregular yellowish nodules. If none be seen cut the liver in various directions until one is found; remove the nodule and squeeze a little of its contents on to a slide, add a

drop of salt solution, cover and examine under the low power Note

a. Innumerable small **ovoid capsules** with granular contents. Each is a **Coccidium Oviforme**.

b. Débris, consisting of broken down cells, oil globules, etc. Draw.

Examine under the high power. Note

Each ovoid capsule contains

a. A mass of **living matter** which completely fills the capsule and appears to be **finely granular**, and contains small highly refractive **spherical bodies** generally collected at one part, perhaps due to disintegration of a part of the living matter. Other capsules, generally more numerous, will be seen in which the living matter consists of a spherical mass containing as before fine granules throughout, and larger granules collected at one part.

All the capsules, if seen on end, appear of a circular outline. Draw.

Coccidium Oviforme* belongs to the sporozoa of the protozoa. It is an organism which causes the disease in young rabbits known as "wet snout."

The organism passes through two distinct stages during its life, one passed within the body of the rabbit, in the intestine or liver, the other outside the body, generally in water or amongst the damp grass in meadows.

As we have seen, the organism as it exists in the liver of the rabbit consists of an ovoid or spherical mass of **living matter** containing **granules**, within an **ovoid capsule** or cyst. It is found in this condition even more plentifully in the intestine, from which it escapes to the exterior with the fæces of the animal. The living matter then contracts from the cyst wall, forming a **spherical mass**, and under suitable conditions this divides into

* "Morton Lecture" on Cancer, by James Galloway, M.D., F.R.C.S., M.R.C.P. : Royal College of Surgeons of England, 1893.

four spheres. Some of these changes may be seen while the organism is still within the liver or intestine. Each of these spheres then divides into **two** crescentic masses or **sporules,** each pair of sporules enclosed in a cell wall, so that there are **eight** crescentic **sporules** all within the one oval cyst wall. In this condition the organism may remain for at least six months. If it be now eaten by a young rabbit along with its food, the cyst walls become dissolved by the intestinal juices and the eight sporules are set free in the alimentary canal. They then become **amœboid** and each one may again break up into crescentic sporules. This process may be many times repeated. Eventually each sporule enters an epithelial cell of the intestine or creeps up the bile duct and gains access to one of its lining cells; here it grows, becoming oval and developing a thick cyst wall. It is during this growth of the parasite in the epithelial cells that the vitality of the latter is impaired, and the disease produced which may cause the rabbit's death. The epithelial cells eventually burst and the parasites are set free and pass to the exterior. The cells may recover, but when many of them contain Coccidia, some are sure to be destroyed by the growth of the parasite.

The development of the Coccidia outside the body can be easily studied by placing a little of the curdy material from one of the nodules in the liver upon thin glass plates and keeping them moist at the ordinary temperature of the room.

The liver of a rabbit in which the disease is well advanced, containing Coccidia, is seen to be studded with a number of irregularly shaped yellowish masses.

If these be cut out each will be seen to be surrounded by a capsule, so that it separates fairly easily from the surrounding liver substance.

When the nodule is cut open a quantity of curdy yellowish material escapes. This consists of great numbers of Coccidia and broken down cells.

In an earlier stage of the disease, greyish nodules are found in the liver substance, not so definitely encapsuled, and at a later stage the resulting yellowish mass shrivels up leaving a small spot, and a little scar if it be upon the surface of the liver.

A great many young rabbits, especially in some warrens, will be found to be infected, indeed it is rather the exception to find one whose liver does not exhibit the nodules in one or other stage.

Owing to the fact that structures resembling Coccidia have been observed in cancerous growths in the human subject, within the cancer cells, it is of importance that the student should know something of the appearances presented by, and the life history of, the Coccidium Oviforme of the rabbit, especially as it is so very easily obtainable.

Lesson X.

The Metazoa ; Cœlenterata ; Hydra ; division of physiological labour.

Hydra.

1. Place upon a slide in some water a Hydra with a piece of water weed to protect it from pressure: cover and examine first under a hand lens. Note

a. The **colour** of the animal, green, Hydra viridis ; brown, Hydra fusca.

b. The (tubular) **body**, from $\frac{1}{4}$ to $\frac{1}{2}$ an inch in length, closed and fixed at one end forming the base.

c. The other end conical (with an opening, the mouth, in it).

d. The **tentacles**, six to eight in number, arranged around the mouth.

e. The contractions and relaxations of the body, and the movements of the tentacles.

2. Examine under the low power. Note

a. The cavity in the body, **enteron**, which is prolonged into each tentacle. This can be seen by observing the motion of particles in the fluid contained in the cavity, during the movements of the animal.

b. The **ectoderm** cells, forming the outer layer of the body and tentacles, arranged in one layer like a mosaic pattern.

c. The **endoderm** cells forming the inner layer of the body and tentacles, lining the enteron and its prolongations. Draw

3. Examine a tentacle under the high power. Note

a. Certain cells or **cnidoblasts** situated between some of the ectoderm cells. Each has a little free projecting process, **cnidocil**, and contains an oval capsule, **nematocyst** or thread cell, within which there is a coiled, barbed thread.

b. The endoderm cells forming the inner layer of the tentacle, and lining its prolongation of the enteron.

c. **Flagella**, small hair-like processes projecting from the endoderm cells into the enteron. By the rapid lashing movements of these flagella currents are set up in the fluid within the enteron and its prolongations.

d. Clusters of small **interstitial** cells, between the ectodermic and endodermic cells, lying upon a thin membrane, the **supporting lamella**, which separates the two layers.

The structure of a tentacle is, in the main, representative of the structure of the body.

4. In some specimens of hydra under the low power. Note

a. **Buds** growing from the body wall near its base.

b. One or more rounded masses of cells, **ovaries**, projecting externally from the middle or lower part of the body wall. Each contains one cell the **ovum**, which has grown at the expense of the others; its size depends upon the stage which the ovary has reached in its development. As the ovum approaches maturity it becomes amœboid.

c. One or more conical masses of cells, the **testes**, smaller than the fully developed ovary, similarly situated, but higher up, *i.e.* just below the tentacles.

5. Focus carefully a tentacle under the high power, and allow a drop of acetic acid to run under the cover-glass. Note

a. The **retraction** of the tentacle.

b. The discharge of the **nematocysts** by a process of evagination. Some will be found quite separated from, others still attached to the ectoderm, with their barbed threads projecting. Draw.

6. Tease on a slide with needles, in a drop of glycerine, a piece of the body wall of a hydra which has been stained with carmine (see Appendix 9). Cover and examine under the low, and then under the high power. Note

a. The large conical **ectoderm cells**, each containing a nucleus in its outer broader end, and several fine processes projecting from its inner narrower end; these are the **neuro-muscular processes.**

b. The granular **endoderm cells**, variable in shape each containing a nucleus, vacuoles, and perhaps provided with a flagellum; in Hydra viridis, chlorophyll corpuscles are found in these cells.

c. Small **interstitial cells** usually in groups.

d. Oval **nematocysts.**

e. Portions of the **supporting lamella.** Draw.

7. Examine stained prepared transverse sections of Hydra. (See Appendix 10.)

Place the section, consisting of paraffin with the section of hydra in it, upon a slide with a drop of spirit and water (equal parts); place the slide in an oven or chamber, kept at the temperature of melting paraffin, for twenty minutes; then remove the slide, add a drop of turpentine to dissolve the paraffin, wipe up the excess of turpentine, add a drop of canada balsam, and cover;

examine first under the low power, and then under the high power. Note

a. The nucleated wedge-shaped ectoderm cells with neuro-muscular processes appearing as dots in section, forming an outer ring or layer.

b. The nucleated irregular endoderm cells, forming an inner ring or layer.

c. The supporting lamella between the two layers of cells, with

d. The groups of small nucleated interstitial cells upon it.

e. The median cavity, **enteron,** in the centre of the section.

In some sections the endoderm cells project so far into the enteron as to almost obliterate it ; their flagella are seldom seen. Draw.

The student will keep this section and compare it with that of the earthworm later on.

The group of animals known as the **metazoa** includes all multicellular animals, both invertebrate and vertebrate.

The invertebrate metazoa are divided into the **cœlenterata** and **cœlomata.** (See classification of animals.)

The cells of which a **metazoon** is comprised may be similar in form, and each one may carry on those functions which we saw were carried on by a protozoon like the amœba, or, on the other hand, the metazoon may be made up of cells dissimilar in form, or of dissimilar groups of cells, each group of cells being set apart to perform some one definite function, respiration, digestion, etc. ; this is known as **division of physiological labour.** In some animals this is carried to such an extent that we find a common stalk bearing many buds or heads, the cells in one head being adapted for respiration, those of another for digestion, of another for reproduction, and so on. (See Lesson XI, page 56.)

The **cœlenterata** are radially symmetrical animals consisting of two definite layers of cells, the **ectoderm** and the **endoderm,** the latter lining the single cavity, **enteron,** within the animal.

In this enteron, and therefore between the endoderm cells lining it, food is digested, and water containing air in solution circulates. The endoderm cells take up the oxygen from the water, and give off carbonic acid gas to it; this interchange of gases constitutes respiration.

Such digestion and respiration are therefore **intercellular**, in contradistinction to **intracellular** functions which take place within the cells.

The **hydra** belongs to the **cœlenterata**.

It is found attached to water weeds in ponds and lakes, and is either green, H. viridis, or brown, H. fusca; both varieties are common in England.

When fully extended it measures from $\frac{1}{4}$ to $\frac{1}{2}$ an inch in length, and when contracted appears as a rounded gelatinous mass about $\frac{1}{20}$th of an inch in diameter. There are six to eight tentacles arranged round the mouth, which is situated upon a conical papilla at the free end of the cylindrical body. The cavity within the body is called the **enteron**, and it is prolonged into each tentacle.

The hydra occasionally moves about by a crawling motion resembling that of a looped caterpillar. When extended, the tentacles wave about in the water, and if any small free swimming animal touches one of them, it is at first struck and paralysed by a number of fine threads which are shot out of special cells, **nematocysts**, and then surrounded and held by the tentacles, and conveyed by them to the mouth. It then passes into the enteron, where it is killed and digested by a special fluid which is poured out by the cells lining the enteron. The undigested portions of the food are eventually ejected from the enteron through the mouth. Water is continually passing in and out of the enteron; the lining cells take up the oxygen of the air which is dissolved in the water, and give off carbonic acid gas to it.

Hydra reproduces 1, **asexually**.

a. By **budding**, if well fed.

b. By **fission**; if a portion of a hydra be broken or cut off, it develops into a new individual.

2. **Sexually**.

During the summer conical projections are formed upon the body, just below the insertion of the tentacles. These are

testes, and contain great numbers of minute bodies, each being provided with a vibratile cilium, or tail, **Spermatozoa**, which are eventually set free in the water. One or more rounded projections, **ovaries**, are also formed nearer the attached end of the body, and each contains a single large nucleated cell, the **ovum**, or egg cell. This is at first "amœboid," but when mature becomes ovoid. The ovum becomes fertilised by its union with one or more of the spermatozoa, undergoes rapid division or **segmentation**, and drops to the bottom of the pond. Thus there is formed a mass of small cells from which a young hydra is eventually developed.

(For the structure of an ovum see Lesson XX.)

Lesson XI.

Plant tissues : Structure of higher plants ; alternation of generations.

1. Strip off from the under surface of some smooth leaf, such as holly, or laurel, a fragment of the thin **epidermis**.

Mount it upon a slide in a drop of spirit and glycerine (equal parts). cover, and examine first under the low and then under the high power. Note

a. The **epidermal cells** devoid of chlorophyll, with their more or less irregular cellulose cell walls.

b. Between some of them, small elliptical openings, **stomata**.

c. The bean-shaped granular cells, one on either side of each of the stomata, **guard cells**. Draw.

2. Cut several thin transverse sections of such a leaf, with a very sharp, thin, scalpel ; mount the thinnest as before in a drop of spirit and glycerine, and examine under the low, and then under the high power. Note

a. The colourless **epidermal cells** on both surfaces of the section.

b. The **stomata**, upon the under surface of the section of the leaf.

c. The shape of the **guard cells**.

d. Thin walled variously shaped cells beneath the epidermis, **parenchymatous** cells; these and the guard cells contain **chlorophyll** and **starch.** Draw.

The stomata enable air to permeate between the cells of the leaf, thus bringing the carbonic acid gas in the air into close proximity with the chlorophyll-containing cells beneath the epidermis.

3. Cut transverse sections of the stem of a young sunflower of about a quarter of an inch in diameter, fresh or preserved in spirit. Mount the thinnest in a drop of glycerine and spirit as before, and examine under the low power. Note

a. The layer of **epidermal cells** outside, with the multicellular **hairs** attached to it.

b. The thin walled **parenchymatous cells** within, forming the **fundamental tissue** of the stem.

c. Certain wedge-shaped masses, composed of cells varying in shape and size with round or oval spaces between some of them, arranged in the form of a ring and embedded in the fundamental tissue of the stem; the **fibrovascular bundles.**

d. Some fundamental tissue, between the fibrovascular bundles, forming the **medullary rays.** Draw.

This ring of fibrovascular bundles divides the whole stem into an inner part, medulla, and an outer part, cortex.

4. Examine one of the fibrovascular bundles under the high power. Note

a. In the centre of each bundle; several layers of oblong thin walled cells forming the **fascicular cambium.** These may be seen to extend beyond the bundles into the medullary rays, there constituting the **interfascicular cambium.**

b. Internal to the cambium; large thick walled spaces, **vessels** seen in section, and smaller thick walled cells, **prosenchymatous cells,** constituting the **xylem** or wood.

c. External to the cambium; thin and thick-walled cells of different sizes, and large round spaces, **vessels** seen in section, constituting the **phloem.**

Some of the vessels in the phloem may, if the section be thin, be seen here and there to contain thin discs perforated by small holes. These discs are **sieve plates,** and owing to the existence of these plates, some of the vessels are known as **sieve tubes.** Draw.

In the fresh state the xylem vessels contain air, the sieve tubes, sap.

5. Cut longitudinal sections of the sunflower stem passing through the centre of the medulla. Mount the thinnest as before, and examine under the low power.

Draw: Noting the appearances in longitudinal section of those parts already seen in transverse section in 3.

6. Examine one of the fibrovascular bundles, seen in longitudinal section, under the high power.

Draw: Noting the appearances in longitudinal section of those parts seen in transverse section in 4.

Some of the vessels in the xylem are seen to have spiral thickenings in their walls and are known as **spiral vessels.**

In the phloem, the sieve plates, with the perforations in them, are well seen, situated at intervals within the sieve tubes.

We have seen in the examination of the leaf and stem that vegetable cells differ from one another in shape, size, contents, etc.

They also differ in that certain groups of cells perform certain definite functions, some form vessels which contain sap; others, cambium cells, which divide rapidly, forming new cells; others contain the green colouring matter chlorophyll. Thus a higher plant exhibits that **division of physiological labour** which we saw in the hydra, a metazoic animal.

The tissues of the higher plants fall into two main groups:

1. **Meristem** or growing tissue (cambium).
2. **Permanent tissue** (fundamental tissue, xylem, and phloem).

Cambium is found chiefly

1. Within and between the fibrovascular bundles as we have seen.
2. At the growing apex of the plant stem.
3. At the extremity of the root.

It thus forms two cones placed base to base, the apex of one being at the top of the stem, that of the other at the free end of the root.

Some stems (exogenous, perennial) increase in thickness during successive seasons by the cambium cells forming xylem or wood internally, and phloem or bark externally, and are known as **open stems.** These are characteristic of dicotyledonous plants. Some meristem tissue is found beneath the epidermis and is known as **sub-epidermal** or **cork cambium.** It is by the division of the cells in this situation that new "bark" is formed in the stems of many trees to take the place of that which is being split off by the yearly increase in thickness of the stem.

In the stems of monocotyledonous plants we find no true cambium like that previously described between the xylem and phloem of the fibrovascular bundles; moreover the xylem is found in the middle of the bundle and the phloem surrounding it. Such stems therefore, if they increase in thickness, do so mainly by the formation of new fibrovascular bundles in the centre of the stem which gradually push outwards those previously formed; these are known as **closed** stems.

The fibrovascular bundles are continued into the leaf stalks and leaves, giving rise to the "ribs" and "veins" of the latter. When a "skeleton" of a leaf is prepared by maceration in water, it is these bundles only which remain and form the reticulated "skeleton."

The physiological processes which go on in the higher plant are much the same as those which go on in protococcus and spirogyra, but as chlorophyll is only present in some of the cells of the higher plant, it is to these that the air and the carbonic acid gas in it can gain access through the intercellular spaces which communicate with the stomata.

The carbon is used to make new living matter, and to form starch and various other organic bodies found in the cells, these being produced by the living matter itself, and stored up in the cells. The oxygen is given off as free gas. Water containing the necessary salts in solution (see Lesson III) is absorbed by the roots, and conveyed up the vessels of the fibro-vascular bundles contained therein and in the stem and leaf ribs, to the leaves and flowers. This upward movement is produced partly by the evaporation of water constantly going on from all the exposed surfaces of the plant.

Why is it that when a ripe seed is placed in the earth under suitable conditions, the primary root always grows downwards, and the primary stem always upwards towards the light?

All we can say is that the new particles of living matter which are produced by the vital activity of the pre-existing living matter of the cells, are formed, in the case of the stem cells, in the upper portion of each cell, and in the case of the root cells, in the lower portion of each cell. In the root of course the nourishment (salts in solution) comes from the soil and water, *i.e.* from below, and in the stem the light and air come from above. To a certain extent the stem and root grow in those directions where the materials required by each are to be found. Unfortunately the stem does not always grow upwards; in some of the orchids (cattleya citrina) the new leaves always grow downwards, and the roots grow where-ever they find water. But we can say that the stem nearly always grows in a direction contrary to the law of gravitation, and the root occasionally does the same.

A **flowering plant,** such as the white dead nettle (lamium album) consists of:

1. **Primary root,** with rootlets springing from it.
2. **Primary erect stem,** with lateral shoots.
3. **Leaves.**
4. **Flowers** containing the reproductive organs.

The **gynœcium,** or **female reproductive organ,** consists of the **ovary,** containing one or more **ovules,** surmounted by the style and **stigma.**

The **andrœcium,** or **male organ,** consists of **stamens,** bearing at their free ends, **anthers,** which contain the **pollen.** Roughly speaking the ovules represent the ova, and the pollen the spermatozoa, of the higher animals.

The ovules, when fertilised, become the seeds, which, when ripe and under suitable conditions, germinate and grow up into adult plants.

In the plants below the flowering plants, the ferns and mosses, we find that there is an intermediate stage.

The **fern frond** produces **spores** upon its under surface. These, under suitable conditions, germinate, and each grows into a small green flattened body, **the prothallus.** The prothallus develops male and female reproductive organs, **antheridia** and **archegonia** respectively.

The antheridia develop **antherozoids**, which correspond to spermatozoa of animals.

The archegonia develop **oospheres**, which correspond to ova.

The oosphere, after fertilisation by the antherozoid, develops into a young frond-bearing fern, which, when adult, again produces fronds with spores.

Thus in the life history of the fern we have two separate stages:

1. The **sporophore, asexual generation**, which is as a rule, large and conspicuous, consisting of fronds bearing spores.
2. The **oophore, sexual generation**, small and inconspicuous, consisting of the prothallus bearing reproductive organs.

Such a life history exhibits **alternation of generations.** The sporophore generation alternating with the oophore generation.

In the mosses we also find alternation of generations, but the well known and conspicuous **moss plant** is the **oophore generation**, bearing male and female organs, often on separate plants.

The **oosphere**, when fertilised, grows up into the **sporophore generation**, which, however, is not separate from the oophore generation, but develops upon it.

The ordinary moss plant then represents the fern prothallus.

In the animal kingdom we find numerous examples of alternation of generations. Amongst these perhaps the best is that afforded by some of the **hydrozoa** of the cœlenterata.

We find a **colony** of individuals upon one common stalk, exhibiting division of physiological labour, some being adapted for respiration, some for catching food and digestion, and some for reproduction; all these being produced upon the parent stalk by budding. The reproductive buds form animals which are free swimming, and develop sexual organs; these become the sexual generation and their ova develop into free swimming forms which then become fixed, develop a stalk, and grow into the asexual form, again multiplying by budding.

The student should examine practically, under the guidance of the teacher, a flowering plant such as the white dead nettle, which is nearly always obtainable. The practical work relating to it is not introduced here, because it is believed that the parts are only to be learned by the junior student by means of practical demonstrations accompanied by diagrams.

Lesson XII.

The Cœlomata Invertebrata. The earthworm (Lumbricus), external characters, the cœlom, digestive, excretory, and reproductive systems.

A. Kill the worm (as large a one as can be obtained) by placing it in dilute spirit, or in a jar with a few drops of chloroform. When dead, wash it well and examine the external characters, using when necessary a hand lens. Note

1. The **cylindrical body,** flattened posteriorly.
    - *a.* The upper, **dorsal,** darker surface.
    - *b.* The lower, **ventral,** paler surface.
    - *c.* The **anterior,** pointed end.
    - *d.* The **posterior,** blunt end.
    - *e.* The rings, or **annuli,** into which the body is divided by grooves; each ring corresponds to a **segment** or metamere, of which there are between 200 and 300.

f. The band of thickened skin in front of the middle of the body, the **clitellum**. Its position varies a good deal in different species of earthworm.

g. Two pairs of little spines, **setæ**, on either side of each segment, except the first and last.

2. External apertures in the body wall.

a. The **mouth**, on the ventral aspect of the first segment.

b. The **anus**, at the posterior end of the body.

c. The openings of the male reproductive organs:

One pair on the ventral aspect of segment 15, the openings of the **vasa deferentia**.

d. The openings of the female reproductive organs:

One pair on the ventral aspect of segment 14, **oviducts.**

e. The openings of the **spermathecæ** on the ventral aspect: one pair between segments 9 and 10, and one pair between segments 10 and 11.

f. The openings of the excretory organs, **nephridia**; minute apertures one pair on the ventral aspect of each segment, from the 4th to the last but one.

g. The **dorsal pores**, median dorsal apertures one in the groove between each segment from the 10th and 11th to the last.

B. Fix the worm extended under water, to a board, or to the paraffin in the bottom of the dissecting tray, by means of a pin at each end, dorsal surface uppermost.

Cut through the body wall by a median dorsal incision from end to end. Dissect up the lateral flaps and pin them down. Note

1. The body cavity or **cœlom** exposed, divided into a number of spaces by membranous septa; each space represents a segment:

a. The absence of septa in front of the 4th segment.

b. The contents of the cœlom exposed. Draw.

2. The **digestive system.** The alimentary canal, a straight tube consisting of, from before backwards :

   a. The **mouth.**
   b. The **buccal cavity** in segments 1, 2, and 3.
   c. The **pharynx** in segments 3, 4, and 5.
   d. The **œsophagus** in segments 6 to 14.
   e. The **crop** in segments 14 to 16.
   f. The **gizzard** in segments 16 to 20.
   g. The **intestine,** extending from the gizzard to the anus.

Draw : Slit open the intestine laterally and wash out its contents ; notice a longitudinal fold of its dorsal wall, the **typhlosole.**

3. The **excretory system.**

   Note, with the aid of a hand lens,

   a. In each segment, except the first three and the last, suspended in the cœlom, two white loops close to the body wall, one on each side ; the **nephridia** or segmental organs.
   b. Each is a long coiled tube and commences by,
   c. A minute mouth, **nephrostome,** opening into the cœlom.
   d. The looped part of the tube lies in the segment behind that containing the nephrostome.
   e. It ends by opening on the exterior of this segment as seen in **A**, 2, *f.*
   f. A nephridium should be carefully excised, and placed upon a slide in a drop of glycerine, covered, and examined with the low power.
   g. Stained prepared specimens of nephridia should also be examined under the low power and carefully drawn.

C. The **reproductive system.**

The earthworm is **hermaphrodite,** *i.e.* each worm contains both male and female reproductive organs.

The alimentary canal in segments 13 to 16 should be carefully removed.

1. The **female organs.**

Note:

a. A pair of **ovaries,** minute white bodies, in segment 13, attached to its anterior wall or septum and lying in the cœlom ventrally to the œsophagus.

b. The **oviducts,** two slender tubes opening into segment 13 internally, perforating its posterior wall or septum and opening externally on the ventral aspect of segment 14.

c. The receptacula seminis or **spermathecæ,** two pairs of sacs which receive the male secretion (seminal fluid) of another worm; one pair situated in segment 9 and one pair in segment 10; short tubes from them open to the exterior on the ventral aspect of the grooves between segments 9—10 and 10—11. Draw.

2. The **male organs.**

Note:

a. Two **median vesiculæ** seminales, one in segment 10 and one in segment 11.

(*b.* Within each, a pair of minute **testes**).

c. A pair of **anterior lateral vesiculæ** seminales in segment 9 attached to its posterior wall.

d. A pair of large **posterior lateral vesiculæ** seminales in segment 10.

e. A **similar pair** in segment 11.

f. The **vasa deferentia,** a pair of fine tubes opening to the exterior on the ventral aspect of segment 15.

Each vas deferens is formed by the union of two vasa efferentia in segment 12.

Each of the two vasa efferentia is continued anteriorly as a seminal funnel.

Each pair of seminal funnels is situated within one of the median vesiculæ seminales, and each funnel opens near each testis. Draw.

Lesson XIII.

The earthworm—*continued.* **Circulatory and nervous systems. Examination of transverse sections.**

D. The **circulatory system.**

1. A recently killed worm should be taken and pinned down in the dissecting tray, as before, under weak spirit. Make a longitudinal dorsal incision a little to one side of the middle line. Dissect and pin down the lateral flaps so formed. Note

a. The large **median dorsal blood vessel**, lying dorsally to, and along the whole length of the alimentary canal, giving off branches to the pharynx.

In the living worm the blood flows in it from behind forwards.

b. The **ventral blood vessel**, lying ventrally to, and along the whole length of the alimentary canal.

The blood in this vessel flows from before backwards.

c. Five pairs of large vertical blood vessels, **the hearts,** connecting the dorsal and ventral vessels in segments 7 to 11. In the living worm rhythmical contractions take place in them commencing in the posterior pair and travelling forwards and downwards.

d. Neural blood vessels; the chief being a **sub-neural** vessel lying ventral to the nerve cord in the middle line.

e. Nephridial blood vessels; a pair in each segment supplying the nephridia. They receive blood from the ventral vessel and return it to the sub-neural vessel.

f. In each segment a pair of blood vessels joining the sub-neural to the dorsal vessel.

E. The **nervous system.**

1. The **nerve collar** in the third segment surrounding the buccal cavity.

   a. A pair of **supra-œsophageal ganglia**, small masses of nervous matter, upon the nerve collar, dorsal to the œsophagus.
   b. A pair of **sub-œsophageal ganglia**, ventral to the œsophagus.

2. The **ventral nerve chain**; this is seen by removing the whole of the alimentary canal carefully, leaving the nerve collar uninjured.

   a. The **nerve cord** running longitudinally close to the ventral wall of the cœlom, in the middle line.
   b. A slight swelling upon the nerve cord in **each segment, ganglion.**
   c. Three pairs of nerves from each ganglion supplying the walls of the segment and its contained organs.

F. Examination of **transverse sections** of the earthworm.

(For preparation see Appendix 12.)

Place a section upon a slide, with a drop of spirit and water, equal parts; place the slide in an oven or chamber kept at the melting point of paraffin for twenty minutes; remove the slide, add a drop of turpentine to dissolve the paraffin, wipe up the excess of turpentine, add a drop of canada balsam, cover and examine under the low power. Note

The circular section, the **nerve cord** being seen on its **ventral** aspect.

1. The skin and its appendages.

   a. The **epidermis**, consisting of a layer of columnar nucleated cells, covered superficially by
   b. The **cuticle**, a thin structureless layer.
   c. The **dermis** or true skin, a thin layer just beneath the epidermis.
   d. The **setæ** within small sacs extending deeply into the body wall.

2. The layers of **muscles** beneath the skin.

   a. An external **circular** layer.
   b. An internal **longitudinal** layer.

3. The **cœlom,** or body cavity, a space between the body wall and the alimentary canal.

The contents of the cœlom.

4. The **alimentary canal** in the centre.

   a. The longitudinal fold of its dorsal wall, **typhlosole.**
   b. Large granular cells, **hepatic cells,** found between the two layers of the typhlosole.
   c. The layer of columnar nucleated cells, possessing cilia, lining the canal.

5. The **nephridia** at the sides of the alimentary canal. Their shape depends upon the part of the tube through which the section happens to pass.
6. The **nerve cord,** enclosed in a sheath, on the ventral aspect of the cœlom and partly surrounded by some of the muscular layers of the body wall.
7. The **blood vessels.**

   a. The **dorsal vessel** above the alimentary canal surrounded by hepatic cells.
   b. The **ventral vessel** between the alimentary canal and the nerve cord.

Draw the above 1 to 7, and compare the section with that of hydra (page 47).

The earthworm belongs to the Vermes, which are cœlomate invertebrate animals.

The chief characteristics of invertebrate animals :

1. The firmer part of the body which gives it its shape and helps to support the various organs, is situated on the outside, and if hard or horny, constitutes the exoskeleton.
2. The nerve cord (if present) is a solid rod situated ventrally or laterally, but never dorsally.
3. The main part of the circulatory apparatus (if present) is situated dorsally.

The chief charcteristics of the cœlomata :

1. There are three layers of the body :

    a. Ectoderm ; skin, and appendages.
    b. Mesoderm ; muscles, vessels, etc.
    c. Endoderm ; lining of alimentary canal.

2 There is a cœlom, or body cavity between the body wall and the alimentary canal. Connected with the cœlom, or in it, are the excretory, reproductive, and circulatory organs.

3. They are more or less bilaterally symmetrical.

The **earthworm** inhabits burrows near the surface of the earth, which it makes for itself by alternate contractions and elongations of the body.

It feeds upon vegetable matter which is taken in at the mouth in great quantities.

It absorbs oxygen from the air and gives off carbonic acid gas to it over the whole surface of the body, which is kept moist by a slimy secretion.

The **blood** which circulates in the blood vessels is a red fluid containing colourless living cells, and is pumped through the vessels to the skin where it becomes oxygenated. It then circulates in vessels which traverse the various parts and organs of the body.

The **nephridia** or segmental organs open on the one hand into the cœlom or body cavity, and on the other to the exterior ; they are richly supplied with blood vessels, and remove from the blood and from the cœlom those waste products containing nitrogen which are formed by the activity of the living matter in the cells of the body, and are not required by the animal.

Digestion is **intercellular,** brought about by fluids secreted by various glands in close relation to the alimentary canal, and also **intracellular,** taking place in the cells lining the alimentary canal.

During the breeding season two worms copulate by means of a slimy secretion formed by the clitellum of each, which binds them together so that the openings of the vasa deferentia of the one are closely applied to the openings of the spermathecæ of the other.

The spermatozoa formed in the testes of each, thus pass into the spermathecæ of the other, and from them into a kind of sac or coccoon in which the eggs are laid.

The spermatozoa of one worm do not fertilise the eggs of the same individual.

Lesson XIV.

Cœlomata vertebrata. The dog-fish (Scyllium Canicula) : external characters, the cœlom and its contents, the digestive, respiratory, and circulatory systems.

(See Appendix 19.)

During the steps in the dissection of the dog-fish it is advisable that the student should refer occasionally to a prepared skeleton.

A. Place the dog-fish, upon the table before you. Note

1. The nearly cylindrical **body** with the head and blunt snout anteriorly, gradually tapering to the upturned tail posteriorly.
   - *a.* The upper or **dorsal** surface.
   - *b.* The lower or **ventral** surface.
   - *c.* The sides or **lateral** surfaces.
2. The **skin**, or outer layer of the body wall, covered with hard, closely set **scales**, each projecting backwards, and forming a kind of **exoskeleton.**
3. The appendages of the body covered with skin, and termed **fins.**
   - *a.* The two median **dorsal fins.**
   - *b.* The one median **ventral fin.**
   - *c.* The median upturned tail or **caudal fin.**
   - *d.* A pair of large lateral ventral fins, the **pectoral fins.**
   - *e.* A pair of smaller lateral ventral fins, the **pelvic fins.**

In the male dog-fish the inner aspects of the pelvic fins are separated off, forming backwardly directed grooved rods, the **claspers.**

These pectoral and pelvic fins are rudiments of the fore and hind limbs of higher vertebrate animals.

4. The apertures in the skin and body wall.

a. The **mouth,** on the ventral aspect of the head and bounded by two **jaws,** an upper or anterior, and a lower or posterior.

b. The **nostrils** or anterior nares, a pair of apertures partly covered by a fold of skin just in front of the mouth, and connected with it by grooves, on the ventral aspect of the head.

c. The **gill clefts** or slits, five lateral vertical slits on each side, the most anterior being about one inch behind the angle of the mouth.

d. The **spiracles,** a pair of rudimentary gill slits, one placed just behind each eye.

e. The **cloacal aperture** or vent, on the ventral surface of the body in the middle line, between the pelvic fins.

f. The **abdominal pores,** a pair of small apertures, one on each side of the cloaca, which communicate with the abdominal cavity or cœlom.

B. Fix the dog-fish down to a dissecting tray or board, ventral surface uppermost, by means of strong "blanket" pins passed through the paired fins. Make a median ventral incision through the skin and body wall, beginning between the pectoral fins, and ending just in front of the cloaca. In this region the hard pelvic girdle, which can be felt through the skin, will have to be divided. Make lateral incisions at the posterior end of the median incision, and pin down the flaps so formed. Note

1. The body cavity or **cœlom** thus exposed; it communicates with the exterior by means of the two abdominal pores before referred to. Prove this by inserting bristles.

a. The smooth shining membrane, the **parietal peritoneum,** lining the cœlom.

b. The contents of the cœlom enveloped by a thin membrane, the **visceral peritoneum,** which is reflected from the parietal peritoneum. (See p. 78.)
Draw the contents in sitû.

C. The **digestive system.**

1. (The structures *a* to *d* will be better seen during the dissection of the respiratory system.) Note

a. The **mouth,** leading into the **buccal cavity.**
b. The **teeth,** on the jaws bounding the mouth.

The teeth are modified scales of the skin.

c. A tongue-like body in the floor of the mouth.

This is the **basi-hyal** cartilage which gives attachment to muscles, and is covered with mucous membrane.

d. The wide **pharynx,** or throat, the posterior continuation of the buccal cavity.
e. The short **œsophagus,** or gullet, which leads from the pharynx to
f. The **stomach,** a large **U**-shaped organ lying in the cœlom or body cavity.
g. The intestinal tube, consisting of, from before backwards, **intestine, colon, rectum,** all continuous with one another.
h. The **cloaca,** a cavity into which the rectum and other tubes open.
k. The **cloacal aperture** or vent, the external opening of the cloaca.

2. Open the intestine along its whole length on its right side; remove the ventral part of its wall, and wash it out. Note

The **spiral valve,** a membranous fold inside the intestine attached by one edge to its inner aspect, forming eight or nine spiral turns, and thereby increasing the extent of surface over which the food has to travel.

D. **Glands.**

a. The **liver,** a large brown bilobed organ, nearly filling the anterior and ventral parts of the abdominal cavity. The lobes are connected anteriorly.

b. The **gall bladder,** embedded in the left lobe of the liver near its median aspect.

c. The **pancreas,** situated between the **U**-shaped stomach and the intestine.

Ducts from the liver and pancreas lead into the intestine. The duct of the liver is about three inches long.

d. The **spleen,** a reddish body attached to the loop of the stomach.

e. The **rectal gland,** a thick tube nearly an inch in length, on the dorsal aspect of the rectum and opening into it.

E. The **respiratory system.**

Enlarge the gill clefts on one side of the body by cutting upwards and downwards with scissors. Note

a. The five **gill cavities** ; each opening internally into the pharynx, and externally directly to the exterior at the gill clefts.

b. The **gills.** The lateral wall of each cavity is thrown into a number of folds, within each of which there is a large plexus of blood vessels.

The gills are supported upon firm cartilaginous rods, the **gill arches,** which form part of the visceral skeleton of the dog-fish.

Cut through the angle of the mouth with strong scissors on the same side as that on which the gill clefts have been enlarged, so as to fully open up the internal openings of the gill clefts. Do this carefully, so as not to injure anything behind the last cleft.

By this means the cavity of the mouth and the pharynx will be fully exposed, and can be very thoroughly examined.

F. The **circulatory system.**

Extend the median ventral incision forwards, cutting through the pectoral girdle, which can be felt through the skin, and removing its ventral portion. Note

1. The **pericardial cavity** exposed; this is the anterior part of the cœlom or body cavity; it is separated from the abdominal cavity by a strong fibrous partition representing the diaphragm of higher animals.

2. The **heart** nearly filling the cavity; the heart is a single tube twisted upon itself in an S shape, and dilated to form the following cavities.

   a. The **sinus venosus,** situated dorsally and transversely, the most posterior cavity of the heart; it receives veins returning blood from the body, it leads into

   b. The **auricle,** a large triangular sac situated dorsally, its posterior angles forming lateral projecting processes; it leads into

   c. The **ventricle,** a thick walled rounded sac situated ventrally; it is continued forwards as

   d. The **conus arteriosus,** which is really the commencement of the **cardiac aorta,** a large artery which gives off branches conveying blood to the gills.

The vessels which convey blood from the gills unite together to form the **dorsal aorta,** which, by numerous branches, conveys blood to the various parts of the body. The blood is returned to the sinus venosus by large veins, dilated in places to form sinuses Draw.

The student should refer to specimens which have been injected and then dissected to show,

a. Vessels leading to the gills, (afferent branchial vessels).

b. Vessels leading from the gills, (efferent branchial vessels).

c. Veins and sinuses.

(See Appendix 23.)

3. The sinus venosus and the veins opening into it.

Open the sinus venosus with scissors. Note

a. A pair of **hepatic sinuses** opening into it on its posterior aspect. They return blood from the liver.

b. A pair of **Cuvierian sinuses** forming the lateral continuations of the sinus venosus.

Each Cuvierian sinus receives three main sinuses which bring back venous blood from the greater part of the body.

c. The **renal portal system.**

If a section be made transversely through the tail, the hæmal arch of the vertebra will be seen to contain two vessels. The one, just below the body or centrum of the vertebra is the **caudal artery,** the one again below this is the **caudal vein.** (See page 74.) Draw.

The caudal vein from the tail divides into a right and left **renal portal vein,** which bring blood to each kidney, along its dorsal edge.

The blood is collected from the kidneys by renal veins, and is eventually returned to the Cuvierian sinuses.

d. The **hepatic portal system.**

The **hepatic portal vein** is formed by the union of veins from the intestine and spleen, and receives veins from the pancreas and stomach, it then enters the liver. The blood from the liver passes into the sinus venosus by the hepatic sinuses before mentioned *(a).*

(For a description of "portal systems" see Lesson XVII., the frog.)

Lesson XV.

The Dog-fish — *continued.* The genito-urinary and nervous systems, the skeleton.

G. The genito-urinary system.

1. In the Female. Note

a. The **ovary**, an elongated nodular mass two to three inches long attached to the dorsal wall of the abdominal cavity by a fold of peritoneum. The nodules are the **ova**.

b. The **oviducts** or pronephric ducts, a pair of tubes running along the dorsal wall of the abdominal cavity and near the middle line. Anteriorly they unite in front of the liver and open into the abdominal cavity. A little posteriorly to their point of union each dilates to form a thick walled **oviductal gland**. Posteriorly the oviducts unite and open into the dorsal wall of the cloaca.

c. The **kidneys**, one on each side of the vertebral column and dorsal to the peritoneum.

Strip the peritoneum off the kidneys. Note

d. Each kidney extends nearly the whole length of the abdominal cavity. Its anterior half, **mesonephros**, is rudimentary.

e. Its posterior half or **metanephros**, a brownish lobulated mass.

f. A narrow tube, the **mesonephric** or **Wolffian duct**, on the ventral surface of each kidney.

The posterior part of each duct dilates forming the **urinary sinus**. The two urinary sinuses unite and open into the cloaca.

g. Five or six slender tubes, the **metanephric ducts or ureters**, running from each metanephros and opening into each urinary sinus. Draw.

The cloaca receives:

The rectum.
The genital aperture (united oviducts).
The urinary sinuses.

2. **In the male.** Note

a. The **testes,** a pair of elongated bodies lying along the dorsal wall of the abdominal cavity, each attached to the body wall by a fold of peritoneum, and, in the adult, united to its fellow posteriorly.

b. The **vasa efferentia,** a number of slender ducts leading from each testis to the mesonephros of its own side.

c. The **kidneys,** each divided as in the female into meso- and metanephros.

The **mesonephros** is large and well developed. Strip the peritoneum off the kidneys. Note

d. The **mesonephric or Wolffian duct,** running along the ventral surface of each mesonephros, and receiving tubules from it; near the metanephros it dilates forming the **vesicula seminalis.** This duct acts as a vas deferens and as an excretory duct for the mesonephros, and opens into the urino-genital sinus.

e. The **sperm sac,** closely attached to the vesicula seminalis, closed anteriorly, and dilating posteriorly to form the urino-genital sinus.

f. The mesonephric or Wolffian duct opening into the urino-genital sinus.

g. The **urino-genital sinus,** uniting with its fellow of the opposite side and opening into the cloaca.

h. The **metanephric ducts,** four or five slender tubes leading from the metanephros, and uniting to form the **ureter** which opens into the urino-genital sinus. Draw.

The cloaca receives:

The rectum.
The urino-genital sinuses.

Embryonic Structure.	Female.	Male.
Pronephros...	Absent	Absent.
Pronephric duct (Müllerian duct)	Oviduct.. ...	Rudimentary.
Mesonephros	Rudimentary ...	Mesonephros.
Mesonephric duct (Wolffian duct)	Urinary sinus	Vas deferens. Urino-genital sinus. Sperm sac.
Metanephros	Kidney	Kidney.
Metanephric ducts	Ureters	Ureters.

Contrast this with table in Lesson XIX.

H. The nervous system.

Take a dog-fish which has been hardened in spirit (pa·t of the roof of the skull having been previously removed), place it in a dissecting tray filled with water and spirit equal parts, remove the rest of the roof of the skull so as to fully expose the brain. Note

1. The **brain**, its parts from before, backwards.
   a. The **prosencephalon** or **cerebral hemispheres**, the largest division of the brain.
   b. The **olfactory lobes** arising from the sides of the prosencephalon.
   c. The **thalamencephalon**, a narrow portion containing a cavity, the third ventricle.
   d. The **mesencephalon** or **optic lobes**, their posterior parts covered by
   e. The **cerebellum**, projecting forwards over the optic lobes and backwards over the medulla.
   f. The **medulla** oblongata, behind the cerebellum, having a very thin roof and containing a cavity, the fourth ventricle. Draw.

Make an incision into the prosencephalon on one side so as to open up a space within it, the **lateral ventricle**.

2. Remove the brain from the cranium, examine its under surface, and see the ten pairs of **cranial nerves** on each side. From before backwards.

I. Olfactory.	VI. Abducens.
II. Optic.	VII. Facial.
III. Motor oculi.	VIII. Auditory.
IV. Patheticus.	IX. Glossopharyngeal.
V. Trigeminal.	X. Pneumogastric or vagus.

3. The **spinal cord** and **spinal nerves.**

Remove the roof of the neural canal (see page 74), slicing it away horizontally along its whole length, so as to expose the spinal cord from the dorsal surface. Note

a. The **spinal cord,** lying in the **neural canal** of the vertebral column, and stretching from the medulla, with which it is continuous, to the tail.

b. The pairs of **spinal nerves.**

Each nerve arises from the cord by two roots, a dorsal sensory, and a ventral motor root. These roots leave the vertebral canal through foramina, and join outside to form spinal nerves. Draw.

4. Remove part of the spinal cord and make a transverse cut through it. Note

a. It is nearly divided into two lateral halves by a dorsal and a ventral **longitudinal fissure** or groove.

b. It is **hollow** throughout its whole length. The space in it is called the **central canal** of the cord, and opens anteriorly into the fourth ventricle of the brain. Draw.

K. The skeleton.

To prepare the skeleton of the dog-fish, take a fresh specimen and put it into nearly boiling water for a few minutes, dissect and scrape away the skin, muscles, and viscera, leaving the framework or endoskeleton, which is throughout composed of a gristly material, cartilage ; keep the skeleton in spirit. Note

1. The **vertebral column,** or backbone, composed of a number of separate cartilaginous rings. Note

a. Each ring is thick in the middle and thin at each margin, and forms the **centrum,** or body of a vertebra.

b. Dorsally to each centrum, an arch, the **neural arch,** consisting of two lateral cartilaginous processes, the **neural processes,** united dorsally and surmounted by a neural **spinous process.**

These arches together help to form a longitudinal **neural canal,** which lodges the spinal cord.

c. The spaces existing laterally between the various neural arches are filled in by cartilaginous plates, the **neural plates,** which complete the neural canal.

d. Ventrally to each centrum an arch, the **hæmal arch,** also composed of two lateral cartilaginous processes, **hæmal processes,** which in the tail unite ventrally, and so help to form a canal which lodges blood-vessels.

In the middle of the body of the dog-fish the hæmal processes do not unite ventrally. Draw.

2. Sections, both longitudinal and transverse, should be made through several vertebræ so as to see the shape of the centra, and the arrangement of the neural and hæmal arches.

In longitudinal section each centrum is seen to be shaped like an hour-glass.

Through its middle runs the **notochord,** which is therefore narrow in the middle of each centrum, and wide at each end, *i.e.* at the junction between two centra. Draw.

3. The **skull.** Note

It is a somewhat oblong, cartilaginous box, which lodges the brain, and has depressions and foramina in its walls for the sense organs, and for the passage of the cranial nerves.

a. Posteriorly it presents a large opening, the **foramen magnum,** through which the medulla oblongata passes.

b. Two rounded prominences, one on each side, and ventral to the foramen magnum, the **occipital condyles,** by which the skull articulates with the first vertebra.

c. Between the condyles is the **notochord,** around which, part of the base of the skull is formed.

4. The **pectoral girdle,** immediately posterior to the last gill arch, in the form of a cartilaginous hoop incomplete dorsally; ventrally it helps to form part of the pericardial cavity; laterally it articulates with the pectoral fins.

5. The **pelvic girdle,** a cartilaginous bar just in front of the cloaca, situated transversely and ventrally; laterally it articulates with the pelvic fins.

6. The **visceral skeleton.**

It consists of a series of cartilaginous hoops which partly encircle the pharynx, supporting the gills and forming the **gill arches.**

In front of the gills the first hoop forms both upper and lower jaws.

Vertebrata.

All the vertebrata possess at some time of their existence,

1. A **tubular dorsal nerve cord.**
2. Situated ventrally to the nerve cord, the **notochord,** a rod of fibrous tissue or cartilage.
3. **Gill clefts** or slits.

They may be roughly divided into:

1. The **craniata,** those which have a cranium or brain box enclosing the hollow brain which is the anterior expansion of the tubular dorsal nerve cord (*e.g.* dog-fish).
2. The **acraniata,** those without a cranium and brain (*e.g.* amphioxus).

In the higher vertebrates the centra, or bodies of the vertebræ become formed around the notochord during the development of the embryo, but in the adult state only a trace of the notochord remains. The notochord does not become the vertebral column, it merely acts as a rod around which the bodies of the vertebræ are formed.

In lower vertebrates, which have no vertebral column, the notochord persists throughout life, forming a dorsal longitudinal rod often consisting of fibrous tissue only (amphioxus).

The dog-fish belongs to the craniate vertebrata. The skin exhibits no evidence of segmentation, but during the dissection it will have been noticed that the muscular body wall is divided by dorso-ventral septa of fibrous material into segments or metameres. This segmentation is also seen, to a certain extent, in the skeletal system, in the vertebræ; in the nervous system, in the paired spinal nerves; and in the genito-urinary system, in the kidneys.

The skeleton is cartilaginous throughout, lime salts may be deposited in it here and there, but true bone is never formed.

The dog-fish belongs to the elasmobranch or cartilaginous fishes, in contra-distinction to the teleostean or bony fishes.

The heart is branchial, that is to say, it pumps the contained venous blood to the gills to be oxygenated. Efferent vessels from the gills unite to form the dorsal aorta, from which arteries distribute the blood to the body.

In the gills the blood is brought into close contact with water containing air dissolved in it.

The water passes in at the mouth, then over the gills, and through the gill slits to the exterior.

The ova are fertilised within the body of the female, and become invested with a tough horny capsule formed in the oviductal glands, and provided with long thread-like processes at each corner, which serve to attach the egg to seaweeds, etc., during the development of the embryo.

The organs of special sense consist of **olfactory sacs, eyes,** and **auditory sacs,** one pair of each lodged in depressions of the outer surface of the skull, and connected by cranial nerves with the brain. The olfactory and auditory organs are contained within cartilaginous capsules which, in the adult dog-fish, are fused with the cartilaginous skull, forming lateral expansions in front and behind.

Lesson XVI.

Cœlomata-vertebrata—*continued.* **The Frog: external characters, the cœlom, the digestive and respiratory systems. The elementary animal tissues. Epithelium, white fibrous tissue, fat.**

The frog.

A. External characters.

1. Kill the frog by placing it in a jar with a few drops of chloroform. When dead, wash it well to remove the slimy secretion of the skin, and examine. Note

a. The smooth moist **skin** covering the surface of the animal.

b. The **head**, directly continuous with

c. The **body** or trunk.

d. The **fore limbs**; each consists of the arm, fore-arm, and hand.

The hand has four digits.

The male frog has at the base of the first digit, a hard rounded mass, which is absent in the female.

e. The **hind limbs**; each consists of the thigh, leg, and foot.

The foot has five digits, joined together at their bases by a web.

Draw: Showing the general arrangement of the head, body, and limbs.

2 The **apertures** in the body.

a. The **mouth**, bounded by the jaws, upper and lower.

b. The **cloaca**; an opening at the posterior end of the body towards the dorsal surface.

c. The **nostrils** or anterior nares, a pair of minute apertures one on each side of the snout above the mouth.

B. The cœlom, the alimentary canal, the digestive glands, spleen, and other viscera.

Fix the frog down under water in the dissecting tray by pins passed obliquely through the hands and feet, with the ventral surface uppermost.

Make a median ventral longitudinal incision, through the skin only, from the lower jaw to the cloaca and divide its very loose attachment to the parts beneath and pin it down.

Note :

The spaces, **lymph spaces,** between the skin and muscular body wall. (See lymph hearts, page 86.)

Make a longitudinal incision through the muscular body wall, a little to the right of the middle line, extending from the posterior end of the body to the breast-bone. Cut through the latter with strong scissors, being very careful not to injure the structures beneath it, and prolong the incision anteriorly nearly to the lower jaw.

Make a transverse incision on each side at the posterior termination of the longitudinal incision, and pin out the two ateral flaps so formed.

On the deep aspect of the left-hand flap notice a vein, the **anterior abdominal vein.**

Note :

1. The **cœlom,** body cavity, or pleuroperitoneal cavity thus exposed, its posterior larger portion, the **peritoneal or abdominal cavity.**

   a. The smooth, thin, shining membrane lining it, the **parietal peritoneum.**

   b. A reflection of the peritoneum covering most of the organs in the cavity, the **visceral peritoneum.**

   These organs are thus not strictly speaking in the peritoneal cavity or cœlom ; they project into it, and are covered by peritoneum, the cœlom itself being an empty cavity.

2. The **digestive glands** and **alimentary tract.**

   a. The **liver** ; a large brown mass overlapping some of the other viscera, and divided into lobes by fissures.

   b. On its deep aspect, a small globular green body, the **gall bladder.**

   Draw the liver, showing its relations to other parts, and then remove the greater part of it carefully with scissors.

c. The **stomach** ; a curved whitish sac to the left of the middle line.

Anteriorly the stomach is continuous with the œsophagus.

d. The **small intestine** ; a coiled tube, the posterior continuation of the stomach. The intestine is supported by the mesentery, which consists of two layers of peritoneum reflected from the parietal peritoneum ; the layers separate to envelop the intestine.

e. The **large intestine** ; a wide tube continuous with the small intestine. It opens to the exterior at the cloaca. Draw.

f. The **mouth** or buccal cavity ; open it fully and see the fleshy tongue attached to the anterior part of the floor, and the **teeth, maxillary** teeth round the margin of the upper jaw, **vomerine** teeth in the centre of the roof of the mouth.

g. The **pharynx** ; it begins at the posterior part of the buccal cavity, and is continued into the **œsophagus.**

h. The **pancreas** ; a yellowish mass in the mesentery between the stomach and the first part of the small intestine or **duodenum,** into which its **duct** opens.

3. Other viscera exposed.

a. The **urinary bladder,** situated ventrally to, and near the posterior end of the large intestine. It is a bilobed sac which may be more or less distended.

b. The **kidneys,** a pair of red elongated bodies one on each side of the middle line, lying against the dorsal wall of the posterior part of the abdominal cavity, behind the peritoneum.

c. The **spleen,** a small red globular body suspended by the mesentery near the commencement of the large intestine.

d. The **fat-bodies,** consisting of two masses of digitate yellow processes, one near each kidney.

e. In the male frog. The **testes**, a pair of ovoid yellowish bodies suspended by folds of mesentery, each ventral to the kidney of its own side.

f. In the female frog. The **ovaries**, a pair of dark granular bodies occupying the same positions as the testes in the male.

g. The **oviducts**, a pair of much convoluted tubes, one lying external to each kidney.

In the breeding season the ovaries and oviducts almost fill the peritoneal cavity.

h. The **heart**, a red triangular organ enclosed in a thin bag, the **pericardium**, lying in the anterior portion of the pleuro-peritoneal cavity; its apex is free and projects posteriorly, its base is fixed.

k. The **lungs**, one on each side of the base of the heart, the free apex of each projecting posteriorly. Draw.

Cut through the œsophagus near the stomach; cut through the large intestine and remove the alimentary tract by dividing the mesentery.

C. **The mouth or buccal cavity and respiratory system.**

1. Open the mouth fully.

Note:

a. The two inner apertures of the nostrils, the **posterior nares**, one on each side of the anterior part of the roof of the mouth.

b. Two larger openings on the lateral aspects of the roof and further back, the **Eustachian recesses.**

2. Enlarge the mouth by cutting through its angles with scissors. Note

a. On the floor of the pharynx, a slit-like opening, **the glottis**, situated upon a little papilla.

The glottis leads into a cavity, the **larynx**, which is continued into a short tube, the **trachæa.**

The trachæa leads into the lungs.

b. The **lungs.** These are seen lying in the anterior part of the pleuro-peritoneal cavity, one on each side.

Each is a conical pinkish sac, with its apex directed posteriorly. Draw.

Place a blowpipe in the glottis and blow through it, the lungs will be seen to be inflated with air.

Microscopical examination of the **elementary animal tissues.**

(The student should examine stained prepared specimens of these tissues in addition to the specimens which he makes himself.)

Epithelium, white fibrous tissue, fat.

1. Scrape off with a scalpel from the surface of the skin of the frog, some of the thin epidermis. Place the scrapings upon a slide in a drop of dilute acetic acid, cover, and examine first under the low and then under the high power. Note

a. The squamous or **tesselated epithelium,** made up of epithelial cells closely packed together.

b. Each cell is polygonal and flattened, containing a **nucleus.** It is joined to its fellows by a thin layer of material known as **intercellular substance** (in the fresh state each cell contains living matter). Draw.

2. Open a piece of the small intestine, scrape the velvety looking inner surface lightly with a scalpel, mount the scrapings and examine as before. Note

The isolated **columnar cells.**

Each is a columnar **epithelial cell,** its free surface is distinct and straight, its attached extremity is generally forked. It contains an **oval nucleus** surrounded by granular matter. Draw.

3. Place upon a slide, in a drop of normal salt solution (·65 per cent.), a piece of one of the thinner foot tendons of the frog,

separate the tendon by teasing with needles. Cover, and examine first under the low, and then under the high power. Note

The parallel bundles of wavy fibres, **white fibrous tissue.** (The tendon cells which lie between the bundles cannot be well seen unless stained.) Draw.

4. Tease and mount as before (3) a piece of adipose or fat tissue (from the base of one of the fat bodies of the frog) examine under the low power. Note

The **spherical** or **oval cells** containing nothing but fat (oil). Draw.

Fat cells are formed thus :

Each was originally a cell containing living matter and a nucleus.

Small globules of oil are formed by the living matter, these run together and so enlarge, gradually pushing the living matter and nucleus to one side ; eventually the oil fills the whole cell, the nucleus becoming flattened out.

The latter can then only be seen when suitably stained ; at a later stage the cell consists only of a membrane enclosing an oil globule.

Remove a piece of the roof of the skull carefully, picking it away with forceps, so as to expose the brain. Place the frog in spirit and keep for future examination.

Lesson XVII.

The Frog—*continued.* **The circulatory system.**

Elementary tissues—*continued.* **Striated muscle, non-striated muscle, cartilage.**

D. The circulatory system.

Dissect a recently killed frog as in B, last lesson, exposing the pleuro-peritoneal cavity. Separate the anterior abdominal vein from the left hand flap of the body-wall.

Beneath the breast-bone (already divided longitudinally). Note

1. The **pericardium**, a thin membranous bag partly adherent to the breast-bone, and containing

2. The **heart**.

Open the pericardium and see the divisions of the heart.

a. The single thick-walled **ventricle**, of a pale red colour, conical in shape, its apex posteriorly.
b. The two thin walled **auricles, right** and **left**, of a dark colour, anteriorly to the ventricle.
c. The **truncus arteriosus**. A tube arising from the right anterior border of the ventricle and crossing the auricles obliquely. Draw.

Lift the ventricle and turn it forwards, and see

d. The thin walled **sinus venosus** on the dorsal aspect of the auricles, receiving three large veins, the **venæ cavæ**. Draw.
e. Examine the **pulsation** of the heart carefully: it may go on for some hours after the frog has been killed; it consists of alternate contractions and dilatations of all the divisions of the heart. Their contractions take place in the following order:

Sinus venosus.
Both auricles.
Ventricle.

3. **The venous system.**

The veins are thin walled vessels which convey the blood from the various organs and parts of the body to the heart.
Trace the veins opening into the sinus venosus; two superior venæ cavæ and one inferior vena cava.
The pulmonary vein, opening into the left auricle. Note

a. The **right superior vena cava**; formed by the union of veins from the head, neck and upper extremity, on the right side, and returning blood from those parts to the sinus venosus.

b. The **left superior vena cava** ; its course and formation like that of the right.

c. The **inferior vena cava** ; it commences between the two kidneys by the union of four or five small veins on each side from those organs ; it runs forwards, receiving blood from the liver by the hepatic veins, and thus indirectly from the lower extremities and other organs. It opens into the posterior aspect of the sinus venosus.

d. The **pulmonary vein**, opening into the left auricle, and formed by the union of veins from each lung, returning the blood from those organs to the heart. Draw.

4. The **portal venous system.**

A portal system is formed by the breaking up of a large vein into minute branches and capillaries, within some organ, and the re-union of these to form another vein or veins which convey the blood to the heart.

The **renal portal system :**

a. Trace the anterior abdominal vein backwards.

It is formed by the union of two lateral pelvic veins.

b. The **pelvic vein** of each side is one of the two branches into which the femoral vein divides.

c. The **femoral vein** is the large vein returning blood from one of the lower extremities.

The other branch of the femoral vein is

d. The **renal portal vein**, which runs to the outer part of the kidney on its own side ; it receives some branches from the back of the body and thigh, and then breaks up into a number of minute vessels within the kidney. These, as we have seen, again unite to form the commencement of the inferior vena cava. Draw

The hepatic portal system. Note

a. The **anterior abdominal vein,** formed posteriorly by the union of the two pelvic veins. It runs along the ventral wall of the body cavity in the middle line to the level of the liver, receiving branches chiefly from the body wall. Here it is joined by

b. The **hepatic portal vein,** a large vessel bringing blood from the stomach, intestines, and spleen. It gives off a branch to the liver. The anterior abdominal vein after joining with the hepatic portal vein divides into branches which enter the lobes of the liver.

Within the liver the branches divide up into innumerable minute vessels, which eventually unite together to form the hepatic veins, which, as we have seen, enter the inferior vena cava.

5. The **arterial system.**

The **arteries** are thick walled vessels which convey the blood from the heart to the various organs and parts of the body.

In dissecting the arteries remove the veins where necessary. Note

a. The **truncus arteriosus,** which divides anteriorly into a **right** and a **left** branch, each of these again divides into **three arterial arches.**

b. The **carotid,** the most anterior and internal of the three, divides into arteries which supply the head and neck and part of the brain on its own side.

c. The **systemic** arch, the middle of the three; it runs obliquely round to the dorsal aspect of the œsophagus, and then runs posteriorly along the dorsal body wall beneath the peritoneum. At about the level of the anterior end of the kidney it unites with the systemic arch of the other side to form the **dorsal aorta**. Before and after its union it gives off branches supplying the larynx, sides of the head, muscles of the body wall, upper and lower extremities, the digestive tract, the spleen, and genito-urinary organs.

d. The **pulmo-cutaneous** arch, the most posterior of the three, divides into:

the **cutaneous artery**, which bends backwards and supplies almost the whole of the skin of the back along its own side of the body;

the **pulmonary artery**, which gives off branches supplying the lung of its own side. Draw.

The student should refer to a frog in which the arterial system has been injected. (See Appendix 24.)

6. **The Lymph Hearts.**

These consist of an anterior and a posterior pair. They are small muscular sacs which receive a clear fluid containing colourless corpuscles—practically those parts of the blood which have passed through the walls of the smallest blood vessels (capillaries)—from the various spaces existing in the tissues of the body, especially between the skin and body walls (lymphatic spaces).

The lymph hearts pulsate rhythmically and so pump the lymph into communicating large veins in the vicinity of each pair of hearts.

The **anterior lymph hearts** are situated close to the transverse processes of the third vertebra.

The **posterior**, one on each side of the posterior part of the urostyle beneath the skin, where, in a living frog, their pulsations can be seen through the skin.

Circulation of blood in the frog's web.

Take a frog in which the brain has been destroyed by pithing one hour previously (see Appendix 13).

Fix it to a piece of wood or metal in one end of which a **V** shaped notch has been cut.

Fix the web over the notch by means of thread round the toes, moisten the foot with water, and examine the web under the low power. Note

a. The thick walled **arteries.** The blood flowing in them towards the toes, *i.e.* **away from the heart.**

b. The thin walled **veins.** The blood flowing in them from the toes, *i.e.* **towards the heart.**

c. The very thin walled **capillaries**; these are minute vessels forming a network between the arteries and veins. The blood flows in them **from the arteries to the veins.**

Elementary tissues—*continued.*

Striated muscle; non-striated muscle; hyaline cartilage.

1. Tease with needles upon a slide in a drop of normal salt solution (·65 per cent.) a very small piece of one of the muscles forming the ventral aspect of the floor of the mouth from a recently killed frog, cover and examine first under the low and then under the high power. Note

a. The long narrow **muscular fibres.**

b. The regularly arranged transverse striations upon each.

c. The very fine sheath, sarcolemma, which encloses the striated muscular substance in each fibre. This may be seen in some spot where the muscular substance has been torn in the process of teasing, leaving the more tough and elastic sarcolemma uninjured.

Allow a drop of dilute acetic acid to diffuse under the cover-glass, and note

d. The nuclei mostly within the striated muscle substance.

e. The striations rendered more distinct. Draw.

2. Tease upon a slide in a drop of dilute acetic acid a small piece of the wall of the frog's small intestine, cover and examine under the high power. Note

a. The sheet of non-striated muscular tissue, and at its ragged edge, *b.* a few isolated small **fusiform cells.**

Each is a cell of non-striated muscular tissue.

c. The rod-like nucleus in each cell. Draw.

The cells in non-striated muscle are packed closely together, each fitted in between the ends of two others, and joined by a small quantity of intercellular substance, forming sheets of tissue, generally consisting of many layers of cells.

3. Dissect out the femur or thigh bone of the frog ; observe its rounded head which helps to form the hip-joint. With a sharp scalpel cut some thin sections of the pearly substance, **hyaline cartilage,** which covers the head of the bone ; place the thinnest upon a slide in a drop of normal salt solution, cover and examine under the high power. Note

a. The very finely granular ground substance or matrix with oval or round spaces in it.

b. The oval or round nucleated cells, **cartilage cells,** in the spaces, sometimes more than one cell in each space. Draw.

Lesson XVIII.

The Frog—*continued.* **The urinary and reproductive systems ; the nervous system.**

Elementary tissues—*continued.* **Nerve fibres ; nerve cells ; bone.**

E. The urinary (excretory) system.

Take the frog which has been in spirit since Lesson XVI, and pin it down in the dissecting tray under water as before. Note

The parts of the urinary system briefly described in Lesson XVI, A. 3.

a. The **kidneys** ; remove the peritoneum from the ventral surface of each.

b. The **ureters** ; a pair of slender tubes each running from the outer edge of the kidney of its own side and opening into the dorsal aspect of the cloaca. Each orifice has a fold or valve projecting over it from the lining wall of the cloaca.

c. The urinary **bladder**; a bilobed sac opening into the ventral aspect of the cloaca by a median aperture. Draw.

F. The reproductive system.

1. In the male. Note

a. The **testes**; a pair of ovoid yellowish bodies, one on the ventral aspect of each kidney.

b. The **vasa efferentia**; ten or twelve very slender tubes connecting each testis with the kidney of its own side.

c. The **vasa deferentia**; the ureters act as vasa deferentia.

d. The **receptacula seminis**; each is a sac-like dilatation of the outer part of the vas deferens (ureter) just posterior to the kidney. Draw.

2. In the female.

a. The **ovaries**; a pair of dark granular bodies, one on the ventral aspect of each kidney.

b. The **oviducts**; a pair of much convoluted tubes; each is suspended by a fold of peritoneum along the outer border of the kidney of its own side. Draw.

The anterior end of each opens into the cœlom, just behind the lungs.

The posterior part is dilated and opens into the cloaca just anterior to the ureter of its own side. Draw.

See also page 80.

G. The **nervous system.**

The central nervous system (brain and spinal cord), as in the dog-fish, is enclosed within the skull and the neural canal of the vertebral column.

Turn the frog over and pin it out, dorsal surface uppermost.

Remove the skin from the head and back, and the muscles from the sides of the vertebral column. Pick away with forceps what is left of the roof of the skull, then insert one blade of a strong pair of scissors into the neural canal of the vertebral column and cut through the neural arches, one at a time, from before backwards, first on one side, then on the other, and so expose the brain and spinal cord *in sitû*.

1. The **brain.**

Remove a thin membrane, the **pia mater**, covering it.

Note : from before backwards the following parts (dorsal surface of the brain.

a. The **olfactory lobes.**

b. The **cerebral hemispheres** or prosencephalon.

c. The **thalamencephalon,** covering a small cavity, the third ventricle.

d. The **optic lobes** or mesencephalon, two ovoid bodies, obliquely placed, forming the widest part of the brain.

e. The **cerebellum**, or metencephalon, a small mass just behind and between the optic lobes.

f. The **medulla** oblongata, triangular in shape, its base being forwards.

A cavity within it, the fourth ventricle, covered over by a membrane which may have been already removed. Draw.

The medulla is continuous posteriorly with the spinal cord.

Cut through the medulla oblongata and carefully remove the brain from the cranial cavity in which it lies, cutting through the various cranial nerves from before backwards.

Observe the ten pairs of **cranial nerves** enumerated as in the dog-fish (page 73).

The ninth and tenth nerves (glossopharyngeal and vagus) on each side are involved in a common ganglion close to their origin from the medulla oblongata, and deserve special attention.

After leaving the ganglion the two nerves separate.

The **glossopharyngeal** passes downwards and forwards to the tongue.

The **vagus** separates from the glossopharyngeal and runs downwards and backwards round the side of the pharynx, dividing there into two branches,

one, the laryngeal nerve, travels to, and supplies the larynx.

The other divides into three branches :

a. The cardiac branch, travels dorsally by the pulmonary artery, and is distributed to the septum between the two auricles of the heart.

b. Pulmonary filaments to the lung.

c. Gastric filaments to the stomach.

The cardiac branch of the frog's vagus is employed by the physiologist, being stimulated electrically and otherwise, in order to study the changes brought about through its agency in the rhythm and beat of the heart.

2. The **spinal cord.** In structure the spinal cord much resembles that in the dog-fish (page 73). Note

The ten pairs of **spinal nerves,** each pair arising from the cord by two roots, a dorsal and a ventral.

The first leaves the neural canal between the first and second vertebræ.

Its main trunk is distributed to the tongue; it corresponds to the **hypoglossal** cranial nerve of the higher vertebrate animals.

The second, third and part of the fourth form a plexus, the **brachial plexus,** the branches of which are distributed mainly to the upper extremity.

The fourth, fifth and six are distributed to the body walls.

The seventh, eighth and ninth form a plexus, the **lumbosacral plexus.** From it nerves are given off to the lower extremity and the back of the body.

The tenth is distributed to the parts lying near the posterior end of the vertebral column.

3. The **sympathetic** nervous **system.**

Examine the dorsal wall of the cœlom : Note

a. Beneath the peritoneum and on each side of the aorta, a fine greyish cord with swellings, ganglia upon it, the **sympathetic nerve chain.**

Trace it on one side upwards and downwards.

b The ten **ganglia** upon it, one corresponding, to some extent, to each vertebra.

c. Small **communicating nerves** between each of the ganglia and the neighbouring spinal nerve.

Elementary tissues—*continued.* **Nerve fibres ; nerve cells ; bone.**

1. Tease out upon a slide in a drop of normal salt solution a piece of fresh frog's nerve ; cover and examine first under the low power to see the general appearance of the nerve fibres, then under the high power to see the structure of a nerve fibre. Note

The single **nerve fibre** with its double contour, and highly refractive border on each side.

a. A fine tubular membrane enclosing the fibre, the **primitive sheath.**

b. A layer of thick curdled looking matter within the primitive sheath, the **medullary substance.**

c. In the medullary substance, and running down its centre, the **axis cylinder.**

d. The nerve fibre is divided into nodes and internodes ; at each node the medullary substance stops, while the primitive sheath and axis cylinder continue. Draw.

Other more slender nerve fibres may be seen, **non-medullated nerve fibres,** intermingled with the medullated fibres. They have no medullary substance. Draw.

2. Nerve cells.

Tease upon a slide in a drop of normal salt solution one of the ganglia of the sympathetic nerve chain, cover and examine first under the low and then under the high power.

Note : Amongst the dark pigment cells,

a. Numerous granular spherical cells,

Nerve cells. Each contains a conspicuous nucleus with a nucleolus within it, and is connected with one or more nerve fibres. Draw.

3. **Bone.**

The student should examine under the low power a section of dried bone previously prepared and mounted in canada balsam. (See Appendix 14.) Note

a. The numerous series of **concentric rings**, each series having a cavity in its centre (the cavity appears black because it contains air, and the refraction of light differs when it passes through air and through the solid bone substance).

b. This cavity is the **Haversian canal** in section; in the fresh state it contains blood vessels.

c. Small black dots situated upon the concentric rings, the **lacunæ**, which in the dried state contain air, and in the fresh state contain living bone cells.

Lesson XIX.

The Frog—*continued.* **The skeleton; the life history of the tadpole and frog.**

H. The **skeleton.**

The endoskeleton of the frog consists, as in the dog-fish, of an **axial** portion, vertebral column and skull, and an **appendicular** portion, the pectoral and pelvic girdles and the limbs articulating with them.

The skeleton is partly composed of true bone and partly of cartilage, differing from that of the dog-fish, which is cartilaginous throughout.

The frog's skeleton may be prepared by dissecting away as much of the soft parts as possible, then macerating the frog in cold water until the remaining soft tissues can be removed with a stiff brush.

1. The axial skeleton. Note

The **vertebral column,** composed of nine true **vertebræ.** The ninth corresponds to the sacrum in man, and with it articulates posteriorly a long undivided prolongation of the vertebral column, the **urostyle.**

Examine the third vertebra as a type of the others.

It consists of

a. a body or **centrum** ; running through the centre of each body are the remains of the notochord.

b. A **neural arch,** dorsal to the centrum, and ending in a **neural spinous process.**

The neural arches of the various vertebræ are distinct from one another, and the neural canal is therefore not closed laterally along its whole length as in the dog-fish.

c. A **transverse process** on each side springing from the lateral aspect of the neural arch.

d. An anterior and a posterior **articular process** also springing from each side of the arch, and articulating with corresponding processes of the vertebræ in front and behind.

The **skull.**

It consists of

a. a strictly axial part, the **cranium** proper, at the posterior aspect of which is the foramen magnum, through which the medulla oblongata becomes continuous with the spinal cord. Below the foramen magnum are the occipital **condyles,** one on each side, by which the skull articulates with the first vertebra.

The cranium lodges the brain. Parts of the bones forming its roof are membrane bones, that is, they are developed from parts of the skin, and therefore represent a portion of an exoskeleton.

b. "**Sense capsules,**" fused to the front and back of the axial part of the skull, containing the organs of smell, anteriorly, and hearing posteriorly.

c. The framework of the **jaws,** and

d. an arch of cartilage, the **hyoid bone,** which is situated in the floor of the mouth, and serves as a point of attachment for important muscles.

2. The **appendicular skeleton.**

a. The **pectoral girdle.** It consists of two curved portions, one on each side, partly encircling the body just posteriorly to the head; they are joined ventrally with the **sternum.**

Each of the curved portions is made up of three parts,

A **scapular** portion, dorsally.
A **præcoracoid** portion, ventrally and anteriorly.
A **coracoid** portion, ventrally and posteriorly.

At the point of junction of these three portions is the **glenoid cavity,** where the fore limb articulates with the girdle.

Closely connected with the præcoracoid, which is cartilaginous, is a slender bone, the **clavicle.**

b. The **pelvic girdle.**

It is obliquely placed, so as to be nearly parallel with the vertebral column, and is composed like the pectoral girdle of two curved portions, one on each side, joined together ventrally.

Each is attached anteriorly and dorsally to the transverse process of the ninth vertebra (sacrum).

Each curved portion consists of

An **iliac** portion, dorsally.
A **pubic** portion, ventrally and anteriorly.
An **ischial** portion, ventrally and posteriorly.

At the point of junction of these three portions is the **acetabulum**, where the hind limb articulates with the girdle.

In the pectcral girdle of the frog

The scapular portion	=	the scapula of man.
The precoracoid portion	=	the sterno-clavicular and inter clavicular ligaments of man.
The coracoid portion	=	the coracoid process of the scapula of man.

In the pelvic girdle of the frog

The iliac portion	=	the iliac	Portions of the pelvis of man.
The pubic ,,	=	the pubic	
The ischial portion	=	the ischiatic	

Many of the bones of the frog, like those in higher vertebrata, possess at one or both ends small caps of cartilage, **epiphyses**, which act as new centres for growth, the increase in length of the bone taking place between the epiphysis and the diaphysis, or central portion of the bone.

The student should draw a diagrammatic transverse section through the middle of the body of the frog, and compare it with his drawings of transverse sections of the hydra, earthworm, and dog-fish.

Life history of the frog.

In dissecting the reproductive organs of the female frog, we saw that the ovary was not connected with the oviduct. The ova, or eggs, which are formed in the ovary pass into the cœlom. During the breeding season when this occurs, the anterior openings of the oviducts become much enlarged and funnel shaped, and into these the ova pass, aided partly by the contraction of the muscles in the body wall. They then pass down the oviducts.

At this time the male frog clasps the body of the female with his fore-limbs, and as the ova surrounded by a viscid secretion formed in the oviduct escape from the cloaca, the secretion of the testes containing spermatozoa is ejected upon them.

The ova are thus fertilised and begin to undergo segmentation. The ova surrounded by the viscid secretion, which imbibes water and swells up, are found in masses constituting frog's spawn, either free or attached to weeds in the water. This viscid secretion is composed of "mucinogen" a substance which on the addition of water becomes "mucin."

The embryo which is formed from the ovum is called a tadpole. It possesses a sucker at its anterior end, by which it attaches itself at first to the viscid material of the neighbouring ova, and then to water weeds.

A mouth armed with horny jaws then develops, and the tadpole feeds upon weeds and decaying vegetable substances, and swims about freely by means of its laterally flattened tail.

About fourteen days after hatching, it possesses external gills in the form of three pairs of branched filaments attached to the sides of the head; at the bases of these gills are clefts leading into the pharynx. Water is taken in at the mouth, passes over and between the gills, and out at the clefts.

A fold of skin, the opercular membrane, now grows from before backwards, covering in the external gills, and leaving only a small external aperture. This membrane fuses with the skin posteriorly during the fourth week. Then the external gills atrophy and are succeeded by internal gills, developed on the surfaces of the gill clefts or slits. Thus the respiratory organs of the tadpole at this stage represent those of the adult dog-fish.

At the same time rudiments of the anterior and posterior limbs appear as outgrowths from the body wall, and the tail shortens as the legs grow, the arms lying at first beneath the opercular membrane. At the end of the second month lungs are being developed internally, and for a time the tadpole breathes by lungs and gills. This change from the tadpole stage to the frog is known as "metamorphosis," and is accompanied by a shedding of the skin and the horny jaws.

If the intestine of the tadpole be examined it will be found to be much longer in comparison with the size of the animal than in the case of the adult frog.

It is a general rule that in herbivorous animals (vegetable feeders) such as the tadpole, the intestine is very much longer than in carnivorous animals (animal feeders) such as the frog, the intestine in omnivora, such as man, who lives upon animal and vegetable food, being midway in length.

The adult frog feeds upon insects, worms, etc., which are caught and held by the teeth, of which there are two kinds, one set around the upper jaw (maxillary teeth), and one set in the roof of the mouth, vomerine teeth.

With regard to the respiratory system of the adult, air is taken in at the anterior nares, the mouth being kept shut. Some small folds of mucous membrane covering the posterior nares act as valves, so that when the muscles forming the floor of the mouth contract, the air is prevented from passing to the exterior, and is forced through the glottis and trachæa into the lungs, where it is brought into close contact with the blood contained in capillary vessels.

In the higher vertebrata this process of inspiration is mainly effected by the contraction of a muscle known as the diaphragm, forming the lower or posterior boundary of the cavity in which the lungs are placed, the cavity being a vacuum. In the frog there is no diaphragm in this region, but a rudimentary structure is found in front of the lungs.

Expiration takes place in the frog and in higher animals mainly by the elastic recoil of the inflated lungs.

The circulatory system of the frog and tadpole presents features of great interest. In the tadpole the heart pumps the blood to the gills to be oxygenated. The blood is then distributed to the vessels of the body; such a heart is a branchial heart, as in the adult dog-fish; some of the gill or branchial arteries persist in the adult frog and form the arterial arches. In the adult frog the blood from the lungs (oxygenated or arterial) passes by the pulmonary veins to the left auricle.

The blood from the body (venous) passes to the sinus venosus, and from it into the right auricle; both auricles empty into the ventricle; the ventricle therefore contains venous and arterial blood more or less mixed, but by the action of certain valves, the venous blood is sent to the pulmo-cutaneous arterial arches, the arterial chiefly to the carotids, while the mixed blood travels to the systemic arches.

The genito-urinary system.

In the tadpole at the time of hatching the pronephros on each side is found well developed, forming the head kidney; its duct, the segmental duct, leads down to the cloaca.

About the time that the limbs appear, a mass is being developed behind each pronephros; this is the mesonephros or Wolffian body, and it remains as the permanent kidney of the frog. While it is developing the segmental duct acts also as a duct for it.

Now the pronephros or head kidney and the upper portion of its duct atrophy and the lower part of the duct becomes the Wolffian duct or ureter, and remains as the permanent ureter.

Tadpole. **Embryonic Structure.**	Frog.	
	Female.	**Male.**
Pronephros	Atrophies	Atrophies.
Pronephric duct (Müllerian duct).	Oviduct	Atrophies in upper part.
Mesonephros	Permanent kidney	Permanent kidney.
Mesonephric duct (Wolffian duct).	Ureter	Ureter (vas deferens).

This table should be studied with that on p. 72.

The skeleton of the frog is made up of bone and cartilage, the latter remaining permanent in parts of the skull.

The hæmal arches are not developed as in the tail vertebræ of the dog-fish. In the bodies of the vertebræ the notochord has practically disappeared, but portions of it remain in the centre of the soft intervertebral discs, between the bodies.

The girdles and limbs are much more highly developed than in the dog-fish.

Lesson XX.

Division of cells ; karyokinesis ; ova ; spermatozoa.

Karyokinesis or Mitosis.

1. Tease upon a slide the piece of one of the ovules of a tulip which is given to you already stained and prepared. (Appendix 15.) Apply a drop of canada balsam, cover and examine under the high power. Note

a. The masses of vegetable cells, each with a nucleus, generally oval and uniformly stained pink or red.

b. Search amongst the thinnest pieces for a cell containing a **nucleus** which is larger than the rest, and in which the **stained** material is arranged in the form of a variously **coiled thread.**

Find as many as you can and draw them all.

These stained threads are the **chromatin filaments** of the nucleus, and the various arrangements of the filaments, coiled, in the form of a star, etc., are indicative of changes taking place in the nucleus prior to its division. These changes are known as **karyokinesis** or mitosis, and the figures formed by the filaments, (coil, star, etc.), as karyokinetic or mitotic figures.

2. Place upon a slide the small piece of epithelium from a newt's tail, already stained and prepared (Appendix 16). Apply a drop of canada balsam, cover and examine as before under the high power. Note

a. The various cells forming the more superficial layers of the epithelium, arranged in the form of a mosaic pavement, each cell with its generally spherical nucleus stained pink or red.

b. Search for a cell the nucleus of which is larger than the rest, and in which the **chromatin filaments** are arranged in various ways, exhibiting karyokinesis. Draw.

These nuclei are much smaller than those in the vegetable cells already seen.

Animal and vegetable cells multiply most commonly by **fission,** that is by division of the cell, generally into two equal parts.

Such division is always preceded by the division of the nucleus, and in very many cases changes, known as karyokinesis or mitosis, occur in it prior to its division.

A portion of the living matter of which the nucleus is composed seems to appear in the form of a much coiled thread; this is known as the chromatin portion, because it readily stains with certain dyes (*e.g.* saffranin, one of the anilin dyes).

This chromatin passes through certain figures. The long filament is first irregularly coiled, and then looped; each loop then becomes divided, the whole assuming the form of a star (aster stage), and after passing through several figures, some of the portions of the filament surrounded by some of the material of which the nucleus is composed travel to one end of the nucleus, and some to the other end; each then goes through the same stages in the reversed order, and finally assumes its normal resting condition. Thus two nuclei are formed.

Each of these nuclei then travels to a remote part of the living matter of the cell, and the latter becomes constricted between the two nuclei, and finally divides; in some vegetable cells it appears as if the cell wall dipped in on either side of the cell, and so divided the living matter into two parts, each part containing one of the new nuclei.

Such **division** of cells is known as **indirect.** When the nucleus divides without exhibiting karyokinesis, the process of division is known as **direct.**

Sometimes the living matter of the cell divides into two, and each of these again into two, while still contained within the cell wall (as in protococcus). The cell wall then ruptures, the new cells are set free, and the outer part of the living matter of each then forms a cellulose cell wall.

Ova, or egg cells.

1. Dissect out from a recently killed female frog an ovary, and place it in normal salt solution in a watch glass. Note

It consists of a mass of innumerable spherical masses, varying greatly in size, the smallest being colourless, the largest black at one pole, yellowish at the other.

The appearance of the ovary varies according to the time of the year. In the spring great numbers of the ova are ripe, and the ovary is therefore very large. In the winter all the ova are immature and small.

2. Tease upon a slide in a drop of normal salt solution a small piece of the ovary containing the smallest ova, cover and examine under the low power. Note

The smaller colourless **ova.**

a. Each **ovum** is surrounded by a membrane, the **vitelline membrane.** Within the membrane,

b. The granular **living matter,** containing some yellowish granules, **vitellus,** or yolk substance.

c. The large nucleus or **germinal vesicle** within the living matter, placed eccentrically.

d. One or more nucleoli or **germinal spots** within the germinal vesicle. Draw.

The larger ova, black or brown.

a. Some may be seen showing the darker upper pole and the lower lighter pole. This is easily seen if some ripe ova be examined fresh in the spring, or preserved in spirit.

b. The colour is due to granules of pigment which are in the vitellus or yolk.

3. Tease gently upon a slide in a drop of strong glycerine, or better in glycerine jelly (Appendix 17), a piece of frog's ovary which has been stained with carmine fluid and prepared (Appendix 18), cover, and examine under the low power.

Note

The smaller ova.

a. The living matter, pink, containing the yolk granules ; the yolk often appears to exhibit cracks in it.
b. The germinal vesicle, red.
c. The germinal spots, dark red. Draw.

4. Carefully break the shell of a fresh hen's egg and allow the contents to fall into a basin of water. Note

a. The spherical yellow central mass, "**the yolk.**"
b. Upon the surface of the "yolk," a light spot surrounded by a lighter coloured ring, about $\frac{1}{6}$th of an inch in diameter, the **cicatricula.** The whole "yolk" of the hen's egg is the egg cell, but the living matter is mainly collected at the cicatricula, where also is the germinal vesicle. The cicatricula will be found in that part of the "yolk" which always floats uppermost.
c. The membrane which encloses the yellow "yolk," the **vitelline membrane.**
d. The "**white**" of the egg, a mass of clear or slightly opalescent colourless material (albumin) around the "yolk."
e. A twisted slightly opaque cord suspending the "yolk" in the albumin at each pole, the **chalaza.**

Spermatozoa.

1. Remove one of the **spermathecæ** of an earthworm ; place some of the contained milky fluid upon a slide with a drop of normal salt solution, cover and examine under the high power. Note

a. A great number of small whip-like moving bodies, **spermatozoa.**
b. Each has a thickened anterior part, **the head** or body, composed of living matter, surrounded by a very fine membrane.

c. A long fine posterior part, the **tail**, composed also of a fine process of living matter surrounded by a very fine membrane.

d. The whip like **motion** of the tail. Draw.

2. Examine in the same manner and under the high power the contents of one of the vesiculæ seminales of an earth worm. Note

The spermatozoa in various stages of development.

a. The **spermatospores**; each is a mass of cells packed closely together.

b. The **spermatoblasts**; each is one of the more external cells of the spermatospore.

These spermatoblasts form the spermatozoa, and tufts of them will be seen, the tails projecting outwards from the spermatoblast.

In all the higher metazoa reproduction takes place sexually. Essentially, this is the union of a part of the male element with a part of the female element, to form a single mass of living matter, which then rapidly divides, forming a number of cells massed together, from which the **embryo** is formed.

The female element is the **ovum** or egg cell.

The male element is the **spermatzoon.**

Every animal and plant begins as a single cell.

Many of the lower animals in their adult form represent, in structure, some stage in the development of a higher animal, that is to say, if during the development of a higher animal the process could be stopped at any one stage, the structure of the developing ovum at that stage would represent the adult structure of some lower animal; thus the amœba may be said to represent the ovum of a higher animal, typically, that of the hydra before it is fertilised.

The **ovum** or egg cell must be distinguished from what we call "**an egg.**"

The "yolk" of the hen's egg which we have examined corresponds to the frog's ovum as seen in the ovary.

The "white" of the hen's egg, to the viscid covering of the frog's ovum in the spawn, in o her words if we were to break a number of hens' eggs into a vessel of water, the mass would correspond to a mass of frog's spawn.

The great difference in size is due to the quantity of yolk granules present in each case.

The egg of a rabbit, or a human egg, is about the $\frac{1}{120}$th of an inch in diameter; its structure, independently of the arrangement of yolk granules within the living matter, is identical with that of "the yolk" of the hen's egg.

We shall not consider here the maturation (ripening) of the ovum, its fertilisation or its segmentation.

These processes cannot be practically examined by the junior student, nor can they be learned without the study of very numerous diagrams.

Such diagrams must be made, on a board, by the teacher when he is lecturing upon the subject.

BRIEF CLASSIFICATION OF THE VEGETABLE KINGDOM.

Group I.

Thallophyta—No stem, roots, or fibro-vascular system.

- *a.* those with chlorophyll **algæ.**
- *b.* those without chlorophyll.
  - those in which reproduction takes place **sexually.**
    - The **fungi.**
  - those in which reproduction takes place **asexually.**
    - reproduction by **fission.**
      - **Schizomycetes** (bacteria).
    - reproduction by gemmation.
      - **Saccharomycetes** (yeast plant).

The schizomycetes and saccharomycetes are generally included amongst the fungi. The above classification is a useful one, as it at once differentiates between the true fungi which reproduce sexually, and those so called fungi in which sexual reproduction does not take place (Hewlett).

Group II.

Bryophyta—(mosses).

Group III.

Pteridophyta—(ferns and clubmosses).

Group IV.

Phanerogamia—(flowering plants).

a.
Gymnospermæ,
ovules not enclosed in an ovary (conifers).

b.
Angiospermæ,
ovules enclosed in an ovary.

Monocotyledones,
embryo has one cotyledon.

Dicotyledones,
embryo has two cotyledons.

(The cotyledon is the first or "seed" leaf, produced when the seed germinates.)

BRIEF CLASSIFICATION OF THE ANIMAL KINGDOM.

I. Protozoa.

I*a*. Mycetozoa.

1. Without a cell membrane.
   a. Rhizopoda (amœba).

2. With a cell membrane.
   a. Sporozoa (coccidium and gregarine).
   b. Ciliata (vorticella).

II. Metazoa.

1. Invertebrata.
   a. Cœlenterata. *α*. hydrozoa (hydra). *β*. Actinozoa (sea-anemone).

   b. Cœlomata.
      α. Vermes (worms).
      β. Echinodermata (star-fish, sea-urchin).
      γ. Arthropoda (spider, fly, centipede, lobster).
      δ. Mollusca (cuttle-fish, snail, mussel).

2. Vertebrata, cœlomata.
   a. Acraniata (amphioxus, lamprey).
   b. Craniata.
      α. Pisces (cartilaginous, dog-fish).
      (bony, cod).
      β. Amphibia (frog, newt).
      γ. Reptilia (lizard, snake, tortoise).

δ. Aves (running and flying birds).
ε. Mammalia.

α. Monotremata (duck-bill).
β. Marsupialia (kangaroo).
γ. Placentalia (man, etc.).

The position of the mycetozoa is difficult to determine, they are still often placed amongst the fungi, but we have put them, provisionally, near the protozoa.

The above are only very rough sketches of the classification of living organisms, and are intended merely to show the student the positions of the various organisms which have been examined in relation to the other groups in the animal and vegetable kingdoms.

APPENDIX.

This may be used as a reference for the class demonstrator, who is assumed to be familiar with ordinary histological methods, embedding in paraffin, cutting sections, etc.

1. Iodine solution.

A solution of iodine in potassium iodide solution, of about the colour of sherry.

(See pages 7, 8, 9.)

2. Wolff's nutrient fluid, for protococcus.

Add 300 grains of calcined bone to half a pint of water, and then nitric acid to dissolve the bone ash. Remove excess of nitric acid by adding potassium carbonate until the liquid is slightly turbid. Carbon dioxide is thus formed and dissolved in the fluid. Then add 170 grains of potassium nitrate, 170 grains of magnesium sulphate, and 46 grains of potassium chloride, and make up to a quart with distilled water.

Take one fluid ounce of this and make up to a quart with distilled water, adding a single drop of ferric chloride.

This forms "Wolff's nutrient solution."

(See page 15.)

3. **Dilute acetic acid.**

A two per cent. solution of glacial acetic acid in distilled water.

(See pages, 28, 29.)

4. **Stained and prepared specimens of amœba.**

A very simple and more efficient method than staining and mounting in balsam is as follows :

Obtain some water rich in amœbæ, large ones if possible, and place in a watch-glass before a window for half an hour ; then drop in a few drops of 1 per cent osmic acid, follow this by a little Beale's carmine fluid (see Appendix 21), and leave it from two to four hours ; then pour into a conical glass and allow the débris containing the amœbæ to settle down ; draw off the pink upper fluid with a pipette, and add to the débris some glycerine and water (equal parts) to remove excess of stain, repeat this process until the fluid is colourless or nearly so, and then add a little glycerine and acetic acid (five drops of glacial acetic acid to the ounce) and leave for two or three days ; then mount a little of the débris in pure glycerine, cement the cover-glass down, first with glycerine jelly laid on warm, and then with gold size or other cement.

(See page 32.)

5. **Stained specimens of micro-organisms.**

Cover-glass preparations are made in the usual manner, of various kinds of micro-organisms, such as anthrax in the blood of an infected animal, or in pure cultivation, bacillus megaterium, spirilla from hay infusion, micrococci from any decomposing animal fluid. The cover-glass preparations are stained with $2\frac{1}{4}$ per cent. aqueous solution of gentian violet for two minutes, washed, dried, and mounted in balsam.

(See page 24.)

6. **Stained specimens of frog's blood.**

Mix frog's blood with Hayem's fluid in a beaker in the proportion of one part of blood to a hundred parts of the fluid. Hayem's fluid consists of:

Sodium chloride, 1 grm.
Sodium sulphate, 5 grms.
Bichloride of mercury, ·5 grm.
Distilled water, 200 cc.

Stir the blood and fluid together and leave it for 24 hours, when the corpuscles will be found to have settled to the bottom of the beaker. Draw off the supernatant fluid with a pipette and wash the corpuscles with water several times. (It will save time to centrifugalise after each washing.) The corpuscles can then be stained (eosin hæmatoxylin gives good results), washed and mounted permanently in glycerine or glycerine jelly. They make extremely beautiful preparations. The stained corpuscles can be kept in bulk in glycerine for a long time, and a drop given to each student to mount and keep.

(See page 29.)

7. **Pasteur's fluid for the cultivation of yeast.**

Potassium phosphate	20 parts.
Calcium phosphate	2 parts.
Magnesium sulphate	2 parts.
Ammonium tartrate	100 parts.
Cane sugar	1,500 parts.
Water to make up to	10,000 parts.

(See pages 19, 22.)

8. **Staining ascospores of yeast.**

The ascospores in yeast cells deprived of proper nourishment are not easy to demonstrate to the junior student; it is therefore desirable to have a few stained and mounted specimens which the students can look at. I am indebted to Dr. Hewlett for this method of staining them. Make a cover-glass preparation of yeast which has been kept moist upon a disc of plaster of Paris for some days; dry, and fix by passing three times through a Bunsen flame; stain in Neelsen's fuschsin solution for two minutes; rinse in water; decolorise in alcohol for from $\frac{1}{2}$ to 1 minute, or until the spores only remain stained; rinse in water; counter stain with Löffler's blue for five minutes; wash, dry, and mount in balsam. The spores only are stained red, the remainder of the cells blue.

(See page 20.)

9. **Teased and stained specimens of hydra.**

Kill a hydra with a drop of osmic acid (1 per cent. solution). Tease it up in a watchglass in a few drops of Beale's carmine fluid (see Appendix 21), and treat the pieces as for staining amœbæ (see Appendix 4). Give each student a piece in acid glycerine to be finely teased and mounted in pure glycerine and cemented.

(See page 47.)

10. **Transverse sections of hydra.**

Put one or two of the largest hydras in water in a test-tube and wait till they become extended: then pour in suddenly an equal volume of boiling water, which kills them while fully extended; remove one of them, and keep in Flemming's fluid (see Appendix 20) for ten minutes; wash and stain whole in borax or lithium carmine; put into acid alcohol for a minute or two, dehydrate, clear, and embed in hard paraffin; cut sections by means of the microtome, and give each student a section to mount and keep.

(See page 47.)

11. Obtaining amœbæ, protococcus, vorticella, etc.

I would recommend the simple plan of taking, during the spring or summer, some of the water and the ooze from a roof gutter, or better still from a puddle on a flat lead roof; keep some in a beaker under a bell jar before a window; to the rest add some Wolff's nutrient fluid (see Appendix 2), and keep in a good light, under a bell jar; in this way I have been able to keep all these organisms through the winter, and always to have a good supply.

(See pages 14, 31, 36.)

12. Transverse sections of the earthworm.

Plunge a large worm into a saturated solution of corrosive sublimate and leave it for twelve hours; cut off the posterior half inch of the worm, wash out the intestine through the mouth, and wash the worm in running water for half an hour; then soak in alcohol for some days; cut out about half an inch from the middle of the worm and stain it in bulk in logwood (Delafield's); wash, dehydrate, clear, and embed in paraffin; cut sections with the microtome, and give each student one to mount and keep.

(See page 61.)

13. Pithing a frog.

Run your finger nail along the dorsum of the frog's head, from before backwards, until you feel the depression in front of the atlas; cut the skin here with the point of a scalpel, pass a seeker through the incision vertically, and then forwards into the brain and destroy the latter thoroughly.

(See page 86.)

14. **Preparation of sections of dried bone.**

Small sections of compact bone are cut with a fine saw as thin as possible and then ground between two flat hones moistened with water, until they are quite thin. They are then polished by being rubbed, under the finger, upon plate glass, and mounted, while dry, in a drop of balsam.

(See page 93.)

15. **Preparation of ovules to show karyokinesis.**

Obtain the ovary of one of the following plants just before the petals drop—tulip, fritillary, lily, or practically any liliacious plant (some one of these can be got from the spring to the autumn). Open the ovary and let the ovules drop into Flemming's fluid and leave them for twelve hours; then wash, and stain the ovules whole in saffranin ("XX soluble in alcohol," Grübler). Dehydrate, clear in cedar oil, and give pieces to the students to tease up and mount in balsam, or prepare stock specimens beforehand and give them to the students to draw. This method is simple, and gives beautiful results, if only you get the right saffranin.

(See page 100.)

16. **Preparation of epithelium of newt to show karyokinesis.**

A tail of a salamander larva, newt, or tadpole is prepared as in the preceding case, only the epithelium is scraped off after the fixing, hardening and washing. The karyokinetic figures are not nearly so large as in the vegetable cells. The specimens do not always turn out well; if preferred the tip of the tail may be stained in bulk, embedded, and sections cut. This is more certain, but the figures are not as good as in the teased specimen when it succeeds.

(See page 100.)

17. Glyce ine jelly.

Glycerine, gelatine, and some preservative (creosote) melted together.

It is extremely useful for mounting glycerine specimens in, as it sets and makes the cementing of the cover-glass an easy task. It is a good plan to fix the cover-glass of all glycerine specimens first with a layer of glycerine jelly laid on warm, and when it has set, with the cement (gold size). Glycerine jelly may be obtained from Rimmington and Son, chemists, Bradford.

18. Staining of ova.

Stain a frog's ovary in the summer with Beale's carmine, and treat as before stated (Appendix 4), keeping in acid glycerine until required. All the specimens prepared by this method keep indefinitely, and improve immensely by keeping.

(See page 103.)

19. Dog-fish.

These are to be obtained (in quantities only) fresh, eighteen inches to two feet in length, from "Sinel, Naturalist, Jersey." They should be kept in weak spirit until required, the abdomen having been previously opened, and a deep cut made in the tail end, to allow the spirit to penetrate.

(See page 64.)

20. Flemming's fluid.

Make this as you require it, by mixing in the following proportions :

15 minims of 1 per cent. chromic acid solution.
4 ,, 2 ,, osmic acid solution.
1 minim of glacial acetic acid.

21. **Beale's carmine fluid.**

Carmine ...	...	...	10 grains.
Liq. ammoniæ fort.		...	½ drachm.
Pure glycerine	...	...	2 ounces.
Distilled water	...	...	2 ounces.
Alcohol ...	...	...	½ ounce.

Place the carmine in small fragments in a test tube with the ammonia; shake and warm until the carmine is dissolved, boil for a few minutes and allow it to cool. Add the glycerine and water and leave it in a bottle, uncorked, until there is only just enough ammonia left to keep the carmine in solution. Then add the alcohol. The fluid will keep indefinitely; its efficacy depends upon the quantity of ammonia present. If after standing a long time the carmine is precipitated, add one drop of ammonia. The fluid is used only to stain fresh tissues, and I have found it a good plan to add a drop or two of 1 per cent. osmic acid before putting the tissue in the fluid. For further particulars as to this method, the reader is referred to "How to Work with the Microscope," L. S. Beale, 1880.

(See pages 32, 41, 103.)

22. **Amœbiform bodies.**

To obtain these, place a piece of frog's muscle (about ⅛th of an inch square) in a glass of water, and introduce a very little of the finest cotton wool. Leave the glass in a light part of the room for some days. Examine some of the cotton wool fibres under the high power from day to day. Very minute amœbiform bodies will be found amongst them.

(See page 12.)

23. Injecting blood-vessels of the dog-fish.

A rough injection only is required in order to show the course of the main vessels. I have found a gelatine mass to be the simplest; the best way is to soak some thin sheet gelatine in water for a few minutes; pour off some of the water and then melt it in a water bath, and add the colouring matter at the time you use it.

The efferent branchial vessels may be injected by inserting the nozzle of a brass injecting syringe into the caudal artery, exposed by cutting through the tail of the fish transversely. A little hot normal salt solution should be injected first, and then the hot gelatin, to which a little precipitated carmine has been added (this is made by adding acetic acid to an ammoniacal solution of carmine drop by drop until the carmine is just precipitated).

The afferent branchial vessels may be injected by tying the nozzle into the conus arteriosus, and using gelatine and chromate of lead (this is made by mixing cold saturated solutions of acetate of lead and bi-chromate of potash, and thoroughly washing the precipitate). The veins and sinuses may be injected from the sinus venosus, using gelatine coloured by an aqueous solution of one of the anilin blues. In each case inject a little hot salt solution first, and see that no air enters the vessels.

Dog-fish which have been kept in spirit can be used, as it is not easy always to obtain fresh ones when they are wanted. Use a separate fish for each system of vessels, and directly the injection is finished plunge it into cold water, and then keep in spirit for a few days before dissecting it.

When dissected the fish may be kept in spirit and used for the student to refer to.

If fresh dog-fish can be obtained, plaster of Paris injections are the best, but the plaster is more troublesome to use than the gelatine mass.

(See page 68.)

24. Injecting the arterial system of the frog.

Divide the sternum of a recently chloroformed frog; open the pericardium, snip off the apex of the ventricle, and insert a very fine nozzle, previously filled with salt solution, into the truncus arteriosus, and tie it in with thick cotton (fine cotton will cut through the vessel); use a small brass injecting syringe, and inject a little hot salt solution first and then the hot gelatine and carmine mass referred to above (23). When the injection is completed, plunge the frog into cold water and keep it in spirit for some days before dissecting it.

(See page 86.)

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