UNIVERSAL
LIBRARY

OU_158688

UNIVERSAL
LIBRARY

OSMANIA UNIVERSITY LIBRARY

Call No. 570/H 98 C Accession No.

Author Huxley, & Martin.

Title Course ..... bio

This book should be returned on or before the date last marked below.

A COURSE

OF

ELEMENTARY INSTRUCTION

IN

PRACTICAL BIOLOGY.



A COURSE OF ELEMENTARY INSTRUCTION IN PRACTICAL BIOLOGY

BY

T. H. HUXLEY, LL.D., F.R.S.,

ASSISTED BY

H. N. MARTIN, M.A., M.D., D.Sc., F.R.S.

REVISED EDITION

EXTENDED AND EDITED

BY

G. B. HOWES

ASSISTANT PROFESSOR OF ZOOLOGY, ROYAL COLLEGE OF SCIENCE, LONDON,

AND

D. H. SCOTT, M.A., PH. D.,

ASSISTANT PROFESSOR OF BOTANY, ROYAL COLLEGE OF SCIENCE, LONDON.

WITH A PREFACE BY

PROFESSOR HUXLEY, F.R.S.

London

MACMILLAN AND CO., LIMITED

NEW YORK: THE MACMILLAN COMPANY

1902

First Edition Printed 1875. *Second, Printed and Stereotyped* 1876. *Reprinted March and July* 1877, *February and December* 1879, 1881, 1882, 1883, 1885, *March, September, December*, 1886, *May* 1887. *Third, December* 1887. (*This Edition was edited with the assistance of Messrs Howes and Scott.*) *Reprinted with additions and alterations* 1889, 1892, 1898, 1902

PREFACE TO THE REVISED EDITION.

THE first edition of the Course of Practical Instruction in Elementary Biology appeared twelve years ago, and the motives which led to its publication are fully explained in the original preface, which is subjoined. The present edition has been carefully revised and, where necessary, enlarged by my colleagues Mr Howes and Dr Scott, assistant Professors in Zoology and Botany in the Normal School of Science and Royal School of Mines, and such additions and improvements are entirely their work. But besides these changes, the reader who compares the two editions will observe that the order in which the subjects are presented is completely changed. In the first edition the lowest forms of life were first dealt with; the series of plants followed in ascending order; and then the series of animals, from the Bell animalcule upwards to the Frog.

No doubt there is much to be said for the principle of this arrangement, which leads the student from the study of simple to that of complex phenomena; but the experience of the Lecture-room and the Laboratory taught me that

philosophical as it might be in theory, it had defects in practice.

All the simplest forms of life, which are easily accessible, are of very minute size and their study involves the use of high microscopic powers. The student who begins with them is therefore not merely introduced suddenly into a region in which everything is new and strange, but he has to familiarize himself with the use of unwonted means of exploration. By taking this road, the teacher (to whom the world of the microscope is so familiar that he is apt to forget its strangeness to students) sets himself against one of the soundest canons of instruction, which is to proceed from the known to the unknown, and from familiar methods of learning to those which are strange.

After two or three years' trial of the road from the simple to the complex, I became so thoroughly convinced that the way from the known to the unknown was easier for students, that I reversed my course, and began with such animals as a Rabbit or a Frog, about which everybody knows something, while their anatomy and physiology is illustrated by innumerable analogies with those of our own bodies. From this starting point we proceeded further and further into the unfamiliar regions of invertebrate organisation until we reached the border region between animals and plants, whence there was a natural and easy ascent to the most complicated vegetable organisms.

This order is followed in the present edition; which is greatly improved by the addition of the Earthworm and the Snail in the series of animal, and of *Spirogyra* in the series of vegetable, types.

I have every reason to believe that our course of instruction in Elementary Biology has been found useful by many learners and teachers. But whatever the value of our attempt to carry out a certain method of instruction, I am more than ever convinced that the method itself is one which will eventually be universally adopted, not only by teachers of the biological sciences as such, but by the teachers of so much of those sciences as constitute the foundation of medicine.

No man can be competent to deal with the greater problems of biology as they are now presented to us, unless he has made a survey, at once comprehensive and thorough, of the whole field of biological investigation. The animal and the vegetable worlds are only two aspects of the same fundamental series of phenomena, and each is capable of throwing a flood of light upon the other. I know of no way by which such a broad, but not superficial, survey can be effected except the method adopted in this work.

Again, while to my mind, nothing is more to be deprecated than the compulsory waste of the invaluable time of students of medicine, upon topics so remote from the serious business of their lives as are systematic Zoology and Botany, there is no preparatory discipline so well calculated to serve as a practical introduction to the study of Human Anatomy and Physiology, as that afforded by a proper laboratory course of Elementary Biology.

Sundry experiments have left no doubt upon my mind that, by following such a course of three or four months' duration, the medical neophyte is enabled to enter upon his proper studies, provided with a practical knowledge of

Anatomy, of Histology, and of the Elements of Embryology and of Physiology, such as under the present system is either not acquired at all, or is gained at the expense of time and labour which can be ill spared from practical subjects.

T. H. HUXLEY.

November, 1887.

PREFACE TO THE FIRST EDITION.

VERY soon after I began to teach Natural History, or what we now call Biology, at the Royal School of Mines, some twenty years ago, I arrived at the conviction that the study of living bodies is really one discipline, which is divided into Zoology and Botany simply as a matter of convenience; and that the scientific Zoologist should no more be ignorant of the fundamental phenomena of vegetable life, than the scientific Botanist of those of animal existence.

Moreover, it was obvious that the road to a sound and thorough knowledge of Zoology and Botany lay through Morphology and Physiology; and that, as in the case of all other physical sciences, so in these, sound and thorough knowledge was only to be obtained by practical work in the laboratory.

The thing to be done, therefore, was to organize a course of practical instruction in Elementary Biology, as a first step towards the special work of the Zoologist and Botanist. But this was forbidden, so far as I was concerned, by the limitations of space in the building in Jermyn Street, which possessed no room applicable to the purpose of a labora-

tory; and I was obliged to content myself, for many years, with what seemed the next best thing, namely, as full an exposition as I could give of the characters of certain plants and animals, selected as types of vegetable and animal organization, by way of introduction to systematic Zoology and Palæontology.

In 1870, my friend Professor Rolleston, of Oxford, published his "*Forms of Animal Life.*" It appears to me that this exact and thorough book, in conjunction with the splendid appliances of the University Museum, leaves the Oxford student of the fundamental facts of Zoology little to desire. But the Linacre Professor wrote for the student of Animal life only, and, naturally, with an especial eye to the conditions which obtain in his own University; so that there was still room left for a Manual of wider scope, for the use of learners less happily situated.

In 1872 I was, for the first time, enabled to carry my own notions on this subject into practice, in the excellent rooms provided for biological instruction in the New Buildings at South Kensington. In the short course of Lectures given to Science Teachers on this occasion, I had the great advantage of being aided by my friends Dr Foster, F.R.S., Prof. Rutherford, F.R.S., and Prof. Lankester, F.R.S., whose assistance in getting the laboratory work into practical shape was invaluable.

Since that time, the biological teaching of the Royal School of Mines having been transferred to South Kensington, I have been enabled to model my ordinary course of instruction upon substantially the same plan.

The object of the present book is to serve as a laboratory

guide to those who are inclined to follow upon the same road. A number of common and readily obtainable plants and animals have been selected in such a manner as to exemplify the leading modifications of structure which are met with in the vegetable and animal worlds. A brief description of each is given; and the description is followed by such detailed instructions as, it is hoped, will enable the student to know, of his own knowledge, the chief facts mentioned in the account of the animal or plant. The terms used in Biology will thus be represented by clear and definite images of the things to which they apply; a comprehensive, and yet not vague, conception of the phenomena of Life will be obtained; and a firm foundation upon which to build up special knowledge will be laid.

The chief labour in drawing up these instructions has fallen upon Dr Martin. For the general plan used, and the descriptions of the several plants and animals, I am responsible; but I am indebted for many valuable suggestions and criticisms from the botanical side to my friend Prof. Thiselton Dyer.

T. H. H.
T. H. Huxley.

LONDON,
September, 1875.

CONTENTS.

I.

Frog.

General characters—Development—Specific characters of *Rana temporaria* and *R. esculenta*—Splanchnic (pleuro-peritoneal) cavity and the organs of the viscera—Neural cavity and the cerebro-spinal axis—Comparison of transverse sections across the head and trunk—General comparison with Lobster and Crayfish—Skeleton—Digestive system—Lymph and blood vascular systems—Heart—Respiratory organs and respiration—Urinogenital system—Ductless glands—Nervous system—Sense organs and integument—Laboratory work . . pp. 1—172.

II.

Fresh-water Crayfish and Lobster.

Habitat—General structure—Exoskeleton and appendages—Digestive system—Circulatory system and heart—Respiratory system—Excretory organs—Nervous system and sense organs—Reproductive organs—Development—Ecdysis—Laboratory work. . . pp. 173—239.

III.

Earthworm.

Habitat—General external characters—Exoskeleton and setæ—Digestive system—General structure of body wall and alimentary canal—Body cavity and mesenteries—Excretory system—Circulatory system—Neurosensiferous system—Reproductive organs—Development—Clitellum—Laboratory work. . . . . pp. 240—271.

IV.

Common Snail.

Specific characters of *Helix aspersa* and *H. hortensis*—Habitat—General characters—Shell or exoskeleton—Digestive system—Pulmonary sac and excretory organ—Heart, compared with that of Crayfish—Circulatory system—Nervous system and sense organs—Reproductive system—Development of Pond Snail (*Lymnæus stagnalis*)—Polar bodies—Laboratory work. . . . . pp. 272—304.

V.

Fresh-water Mussel.

Habitat—General characters—Branchiæ and alimentary canal, in relation to the branchial chambers and the exterior—Circulatory system and heart—Excretory organs—Digestive gland and glycogenous tissues—Nervous system and sense organs—Reproductive organs—Development—Laboratory work. . . . . pp. 305—341.

VI.

Fresh-water Polypes.

Habitat and movements—Mode of feeding—Reproduction and reproductive organs—Histological structure—Specific characters of *Hydra fusca* and *H. viridis*—Symbiosis probably exemplified by—Comparison with Amœba—Neurosensiferous apparatus—Comparison with development of higher animals—Laboratory work. . . pp. 342—358.

VII.

Bell-Animalcule.

Infusoria, compared with other animals—Bell-animalcule—Habitat and movements—Histological structure—Physiology—Green varieties and chlorophyll—Reproduction—Encystation—Infusoria associated with Frog—Laboratory work. . . . . pp. 359—368.

VIII.

PROTEUS ANIMALCULE. COLOURLESS BLOOD CORPUSCLES.

AMŒBA. Habitat—Movements—Histological structure—Physical constitution—Physiology—Chemical composition—Effects of temperature and electric shocks. COLOURLESS BLOOD CORPUSCLE. Facts which show Amœba to be an animal—Reproduction of Amœba—Laboratory work. . . . . pp. 369—376

IX.

YEAST.

General characters—Fermentation—Appearance under the microscope—Structure of cells—Chemical composition—Mode of multiplication—Gemmation—Spores—Physiology—Nutrition—Metabolism—Laboratory work. . . . . pp. 377—388.

X.

PROTOCOCCUS.

Habitat—Histological structure—Physiology—Chlorophyll—Dependence on light—Mode of multiplication—Macrozoospores—Microzoospores—Laboratory work. . . . . pp. 389—395.

XI.

SPIROGYRA.

Habitat—External characters—Histological structure—Cell-division—Sexual reproduction by conjugation—Zygospore—Physiology—Laboratory work. . . . . pp. 396—407.

XII.

BACTERIA.

General characters—Structure—Movements—Zoogloea stage—Spores—Physiology—Relation to putrefaction—Life-history—Laboratory work. . . . . pp. 408—414.

XIII.

MOULDS.

Fungi — Physiology — Spores — PENICILLIUM — Habitat — General characters—Histological structure—Asexual reproduction—Conidia—Development—EUROTIUM—Habitat—General characters—Reproduction by ascospores—MUCOR—Habitat—General characters—Histological structure—Asexual reproduction—Development—Conjugation—Germination of zygospore—Laboratory work. . . pp. 415—429.

XIV.

STONEWORTS.

Habitat—External characters—Histological structure—Mode of growth—Sexual reproduction—Development—Physiology—Laboratory work—Protoplasmic movements. . . . . pp. 430—442.

XV.

BRACKEN FERN.

External characters—Histological structure—Stem—Leaf—Root—Physiology—Asexual reproduction—Spores—Germination—Prothallus—Sexual reproduction—Embryo—Alternation of generations—Laboratory work. . . . . pp. 443—459.

XVI.

BEAN-PLANT.

External characters—Reproductive organs—Development—Histological structure—Stem—Leaf—Root—Homology of the reproductive organs with those of the Fern—Physiology—Laboratory work. pp. 460—481.

APPENDIX. General—On dissecting—On injecting—Microscope and Microscopic examination—Preparation and use of reagents and culture solutions—Sections and section cutting. . . . pp. 482—498.

INDEX . . . . . pp. 499—512.

I.

THE FROG (*Rana temporaria and Rana esculenta*).

THE only species of Frog indigenous in Britain is that termed the 'common' or 'Grass Frog' (*Rana temporaria*). On the Continent, there is, in addition to this, another no less abundant species, the hind-limbs of which are considered a delicacy, whence it has received the name of the 'Edible Frog' (*Rana esculenta*). This species occurs only in a few localities in Britain, into most of which it is known to have been introduced. Unless the contrary be expressly stated, the description here given applies to both species. The Edible Frog is usually larger than the other, and is therefore more convenient for most anatomical and physiological purposes.

In the body of the Frog the head and trunk are readily distinguishable; but there is no tail and no neck; the contours of the head pass gradually into those of the body, the fore-limbs being situated immediately behind the former. There are two pairs of limbs, one anterior and one posterior.

The whole body is invested by a smooth moist integument. The yellowish ground-colour of the skin is diversified by patches of a more or less intense black, brown, greenish, or reddish-yellow colour, and, in the Grass Frog, there is a large, deep brown or black patch on each side of the head, behind the eyes, which is very characteristic of the species. The coloration of different frogs of the same species differs widely; and the same frog will be found to change its colour,

becoming dark in a dark place, and light if exposed to the light.

The body of the Frog presents only two median apertures, the wide mouth and the small cloacal aperture. The latter is situated at the posterior end of the body, but rather on its upper side than at its actual termination. It is commonly termed the *anus*, but it must be recollected that it does not exactly correspond with the aperture so termed in the Mammalia.

The two nostrils, or *external nares*, are seen at some distance from one another upon the dorsal aspect of the head, between the eyes and its anterior contour. The *eyes* are large and projecting, with well-developed lids, which shut over them when they are retracted. Behind the eye, on each side of the head, there is a broad circular area of integument, somewhat different in colour and texture from that which surrounds it; this is the outer layer of the membrane of the *tympanum*, or drum of the ear.

The fore-legs are very much shorter than the hind-legs. Each fore-limb is divided into a *brachium*, *antebrachium* and *manus*, which correspond with the arm, fore-arm and hand in Man. The manus possesses four visible digits which answer to the second, third, fourth, and fifth fingers in Man. There is no web between the digits of the manus.

The hind-legs are similarly marked out into three divisions, *femur*, *crus*, and *pes*, of which the femur answers to the thigh, the crus to the leg, and the pes to the foot, in Man. The pes is remarkable not only for its great relative size as a whole, but for the elongation of the region which answers to the tarsus in Man. It will be observed, however, that there is no projecting heel. There are five long and slender digits, which correspond with the five toes in Man, and are united together by thin extensions of the integu-

ment constituting the web. The innermost and shortest answers to the *hallux*, or great toe, in Man.

At the base of the hallux, the integument of the sole presents a small horny prominence, which overlies a bony *calcar;* sometimes there is a similar but smaller elevation on the outer side of the foot: but there are no nails upon the ends of any of the digits of either the pes or the manus. Thickenings, or *callosities*, of the integument, however, occur beneath the joints of the digits, both in the pes and the manus.

During the breeding season, the integument on the palmar surface of the innermost digit of the manus, in the male, becomes converted into a rough and swollen cushion. This, in the Grass Frog, acquires a dark-brown or black colour.

The Frog, when at rest, habitually assumes a sitting posture much like that of a dog or cat. Under these circumstances the back appears humped, the posterior half being inclined at a sharp angle with the anterior half. The vertebral column, however, will be found to be straight, and the apparent hump-back arises, not from any bend in the vertebral column, but from the manner in which the bones of the hip-girdle are set on to the same.

The walk of the Frog is slow and awkward, but it leaps with great force, by the sudden extension of the hind-limbs, and it is an admirable swimmer.

In a living Frog, the nostrils will be seen to be alternately opened and shut, while the integument covering the under side of the throat is swollen out and flattened. The sucking in and circulation of the air needed for the Frog's respiration is connected with these movements.

The upper eyelid of the Frog is large and covered with ordinary pigmented integument, and it has very little

mobility. That which performs the function of the lower eyelid in Man, is a fold of the integument little pigmented and, for the most part, semi-transparent, resembling the nictitating membrane of a bird rather than an ordinary lower lid. If the surface of the cornea be touched, the eyeball is drawn inwards under the upper lid, which descends a little, at the same time as the lower lid ascends over the ball, to meet the upper lid and close the eye.

As is well known, Frogs emit a peculiar croaking sound, their vocal powers being more especially manifested in the breeding season, when they collect together at the surface of ponds, pools and sluggish streams, in great numbers. At this season, which commences in the early spring for the Grass Frog, but much later on in the year for the Edible Frog, the male seeks the female and, clasping her body tightly with his fore-limbs, remains in this position for days or even weeks, until her ova are discharged, when he fecundates them by a simultaneous out-pouring of the seminal fluid. Shortly after the eggs pass into the water, the thin layer of mucus secreted by the oviduct, with which each egg is surrounded, swells up by imbibition and, with that which surrounds the others, it gives rise to a swollen mass, in which the eggs remain imbedded during the early stages of their development.

The process of fecundation above alluded to, usually results in the fusion between each egg and one of the spermatozoa contained in the seminal fluid, and it is only when this has been effected that the egg, which is then said to be fertilized, is competent to reproduce the species. It follows that the *fertilized ovum* is a compound of the egg of the female and a spermatozoid of the male; and it has been accordingly termed the *oosperm*, by way of distinction from

the unfertilized egg (*ovarian ovum*) as it leaves the body of the female.

The development of the eggs is closely dependent upon temperature, being greatly accelerated by warmth and retarded by cold. The process of yelk-division, or *segmentation* which commences within a few hours of impregnation, can be readily observed when the eggs are examined as opaque objects under a lower power of the microscope. It is ushered in by the appearance, on the surface of the egg, of a furrow which passes completely round the same, and, gradually deepening, constricts it into two equal halves. Each of these again becomes subdivided, and, the process being repeated, there results a mulberry-like mass of *embryo-cells*, from which the formative tissues of the body are ultimately derived. Segmentation such as this, in which the first furrow completely cleaves the whole egg into two, each successive one similarly subdividing that cell with which it is related, is termed complete or *holoblastic*.

While still within the mucous investment the embryo assumes the form of a minute fish, devoid of limbs and with only rudiments of gills, but provided with two adhesive discs on the ventral side of the head behind the mouth.

After leaving the egg, the larva acquires three pairs of *external branchiæ* having the form of branched filaments, attached to the sides of the hinder part of the head. Narrow clefts in the skin at the roots of the branchiæ lead into the back of the throat or *pharynx*. Water taken in at the mouth passes out by these *branchial clefts*. The animal crops the aquatic plants on which it browses, by means of the horny plates with which its jaws are provided.

In the *Tadpole*, as the larval Frog is called, the intestine, which is relatively longer than in the adult, is coiled up like a watch-spring in the body cavity. A membranous lip,

the surface of which is beset with numerous horny papillæ, surrounds the mouth, and the muscular tail acquires a large relative size. The eyes, the nasal and auditory organs, become distinct, but no limbs are at first visible.

A fold of the integument in the hyoidean region, called the *opercular membrane*, now grows back over the external gills and unites with the integument covering the trunk, leaving only a small aperture or *spiracle* on the left side, through which the ends of the external gills may, for some time, be seen to protrude. The external gills atrophy, and are succeeded functionally by short processes developed from the opposing faces of the branchial clefts—the *internal branchiæ*. The rudiments of the limbs appear, rapidly elongate and take on their characteristic shape, the hind pair only being at first visible on account of the anterior pair being hidden under the opercular membrane. The lungs are developed and, for a time, the tadpole breathes both by these and by its internal gills.

As the legs grow the tail shortens and, at last, is represented merely by the pointed end of the body; the gape elongates until the angle of the mouth lies behind the eye, instead of a long way in front of it, as in the tadpole; the labial membrane and the horny armature of the mouth disappear, while teeth are developed in the upper jaw and on the roof of the mouth; the intestine becomes less and less coiled as, not growing at the same rate as the body, it becomes relatively shorter; and the animal gradually relinquishes its mixed diet for one of animal matter—the perfect Frog being mostly insectivorous.

The two species *Rana temporaria* and *Rana esculenta*, are distinguishable by the following external characters. In *Rana temporaria*, the interspace between the eyes is flat or slightly convex, and its breadth is usually greater than, or

at least equal to, that of one of the upper eyelids. The diameter of the tympanic membrane is less than that of the eye, often much less. The horny elevation on the outer side of the pes is small or absent, and that on the inner is flattened and has a rounded margin. A patch of dark colour extends from the eye backwards over the tympanic membrane. The males have the cushion on the radial side of the manus black, and they are devoid of vocal sacs.

In *Rana esculenta*, on the other hand, the interspace between the eyes is usually concave and narrower than the breadth of one of the eyelids. The diameter of the tympanic membrane is as great as that of the eye. The horny elevation on the inner side of the pes is elongated, compressed and brought to a blunt edge, so as almost to resemble a spur, and a small outer elevation is constantly present. There is no patch of colour at the sides of the head, such as exists in *Rana temporaria*, and the cushion of the inner digit in the male is not black. The males of both species have a pouch on each side of the head, behind the angle of the jaw, communicating with the cavity of the mouth, and, when they croak, these pouches, becoming dilated, assume the form of spherical sacs.

Having thus become acquainted with the general characters and life-history of the Frog, and with those features of its organization which are visible to the naked eye and without dissection, its structure may next be studied in detail.

If the trunk be laid open, it will be found to enclose a cavity in which some of the most important viscera—the stomach and intestine, the liver, the pancreas, the spleen, the lungs, the kidneys and urinary bladder, and the reproductive organs—are contained. As this cavity answers to

those of the pleuræ and of the peritoneum in the higher animals, it is termed the *pleuroperitoneal cavity;* and the soft smooth membrane which lines it and covers the contained viscera is the *pleuroperitoneal membrane.*

The vertebral column traverses the middle of the roof of this cavity, and the layer of pleuroperitoneal membrane which lines each lateral wall of the cavity, passes downwards on each side of the vertebral column and joins its fellow in the middle line to form a thin sheet, the *mesentery*, which suspends the alimentary canal. In the triangular interval left between these two layers before they unite, a wide canal (*subvertebral lymph sinus*), the dorsal aorta and the chain of sympathetic ganglia, are situated.

The antero-dorsal moiety of the pleuroperitoneal cavity is occupied by the gullet, which places the mouth in communication with the stomach. Beneath the gullet the pleuroperitoneal cavity is separated only by a thin partition from a chamber, the *pericardium*, which contains the heart. The posterior face of the partition is constituted by the pleuroperitoneum, its anterior face by a membrane of similar character, the *pericardial membrane*, which lines the pericardium and is reflected on to the heart, in the same way as the pleuroperitoneum lines the pleuroperitoneal cavity and is reflected on to the intestine. The exposed surfaces of both the pericardial and pleuroperitoneal membranes are kept permanently moist by a fluid (*serous fluid*) which more or less completely fills the cavities which they enclose; hence they are termed *serous membranes.*

A layer of the muscular fibres which enter into the body-wall is continued inwards at the anterior boundary of the pleuroperitoneal cavity and is attached to the sides of the œsophagus and to those of the pericardium, thus constituting a so-called diaphragm; which, it will be observed, is

situated in front of the lungs, and not behind them, as in the higher animals.

Thus, in the trunk, on the ventral side of the vertebral column, the body presents two cavities, a large posterior pleuroperitoneal cavity, and a small anterior pericardial cavity; while neither of these communicates directly with the exterior, there is in the female an indirect communication by the oviducts.

On the ventral side of the head, the very wide mouth opens into a spacious buccal cavity, the roof of which is hard and firm, while the floor is soft and flexible, except so far as the middle of it is occupied by a broad, flat, for the most part gristly plate, the body of the *hyoid* bone. Within the lips the upper jaw is beset with numerous sharp small teeth, and two clusters of similar teeth are to be seen in the fore part of the roof of the mouth; the latter, being attached to the bones termed the *vomers*, are the *vomerine* teeth, while the former, attached to the *premaxillæ* and *maxillæ*, are *maxillary* teeth. The lower jaw or *mandible* is edentulous.

At the sides of the clusters of vomerine teeth are the apertures termed *posterior nares*, by which the nasal chambers communicate with the mouth. At the sides of the back part of the throat two wide passages, the *Eustachian recesses*, lead into the *tympanic* cavities, which are closed externally by the tympanic membranes. In the male the small apertures of the vocal sacs are seen on the inner side of each ramus of the jaw, close to the angle of the gape below and in front of the Eustachian recesses. In the middle of the back of the throat is the opening of the œsophagus, closed by the approximation of its sides except during deglutition, while in the median line of the hinder part of its floor lies a longitudinal slit, the *glottis*. A fleshy tongue, bifurcated and free at its posterior end, is attached anteriorly to the middle part

of the lower jaw. In a state of rest, therefore, it lies on the floor of the mouth with its free end turned backwards, and one point on each side of the glottis.

The gullet, after traversing the so-called diaphragm, passes into the elongated stomach. At its posterior end this narrows and joins the slender *small intestine*. Though short, this is too long relatively to the length of the pleuroperitoneal cavity to lie straight in it. It is, therefore, thrown into sundry folds which are suspended to the dorsal wall of that cavity in the manner before described. Finally, the small intestine enters the dilated short *large intestine*, and this opens into a chamber with muscular walls, the *cloaca*, the external aperture of which has been already mentioned.

Thus the alimentary canal is a tube which traverses the body from the oral to the anal apertures; and the heart, enclosed in the pericardium, is situated in the middle line on the ventral side of the same.

Separated from the pleuroperitoneal and buccal cavities by the bodies of the vertebræ and the hard roof of the buccal chamber which continues the direction of these forwards, is an elongated cavity, widest in the head but becoming very narrow posteriorly, which is closed on all sides by the bony and other elements of the head and spinal column. This is the *neural* cavity and contains the brain and spinal cord, which together constitute the *cerebro-spinal nervous axis*. The neural cavity is lined by a serous membrane resembling the peritoneum and the pericardium, and this *arachnoid membrane* is reflected on to and covers the contained cerebro-spinal axis, so that the latter is related to it as the heart is to the pericardial membrane.

The *cerebro-spinal nerves* which are given off from the brain and spinal cord pass to their destination through the boundary walls of the neural cavity.

A transverse section of the head in the region of the eyes will shew, in the middle line, a dorsal cavity in which the anterior part of the cerebro-spinal axis, the *brain*, is contained, separated by the solid floor of the skull from a ventral cavity, the mouth.

A transverse section of the trunk will shew a dorsal cavity containing the posterior part of the cerebro-spinal axis, the *spinal cord*, separated by the solid floor of the vertebral column from a ventral cavity enclosed by the alimentary canal and continuous with that of the mouth. But the backward continuation of the alimentary canal lies within the large pleuroperitoneal chamber, of which there is no indication in the head.

If a transverse section of the trunk of the Frog be compared with one across the middle of the body of the Crayfish or Lobster (Chapter II.) it will be seen that while the chief nervous centre is on one side of the alimentary canal and the heart on the opposite side in both cases, the face of the body on which the nervous centre lies is that on which the Crayfish or Lobster naturally rests, while in the Frog it is the reverse. The limbs are turned towards the neural side in the Crayfish and away from it in the Frog, and the like difference obtains between all *Arthropoda* and all *Vertebrata*.

Using the term *skeleton*, in its broadest sense, for the framework which protects, supports and connects the various parts of the organism, it consists in the Frog of four kinds of tissue; the Horny, the Osseous, the Cartilaginous and the Connective. Moreover, the hard parts are either developed in the integument, constituting an *exoskeleton*, or they are deeper seated and belong to an *endoskeleton*.

The exoskeleton in the Frog is almost absent, being represented only by the horny coating of the calcar.

The endoskeleton, on the contrary, is well developed and, as in all the higher Vertebrata, may be resolved into an axial and an appendicular portion.

The adult *axial endoskeleton* is represented by the spinal column and the skull.

The *appendicular endoskeleton* consists of the limb-bones and the pectoral and pelvic arches to which they are attached.

In the order of development, the endoskeleton is at first represented by a membranous rod or *notochord* alone; secondly, nascent connective tissue and cartilage are superadded to the notochord; thirdly, these acquire their special characters; fourthly, they become replaced by bone, wholly or in part.

The processes of conversion and replacement indicated under the last head are very incomplete, even in the adult Frog, for remains of the notochord are to be found in the centres of the vertebræ (*intra-vertebral bodies*); while the cartilage, of which the greater part of the skeleton was at one period of larval existence composed, to a great extent persists.

Such cartilage is found forming the free surfaces of the bodies of the vertebræ, the extremities of the caudal style (*urostyle*) and the ends of the transverse processes; and it enters largely into the girdles. In the skull, nearly all the bony elements may be removed, leaving behind the primitive cartilaginous skull, or *Chondro-cranium*, altered only so far as parts of it have been replaced by bone.

It furnishes a floor, side walls and roof to the brain-case, interrupted only by three spaces (*fontanelles*) in its roof covered in by membrane, and by the foramina for exit of the cranial nerves. In front it is continued forward

between the two nasal sacs, as a cartilaginous partition (*septum narium*), from which are given off, dorsally and ventrally, transverse alæ of cartilage which furnish a roof and a floor, respectively, to the nasal chambers. These pass into one another where the chondro-cranium ends anteriorly and give rise to a truncated terminal face, the lateral angles of which are produced outwards and forwards into two flattened *præ-nasal processes;* these widen externally and support the adjacent portions of the premaxillæ and maxillæ. From the ventral face, just behind the truncated anterior end of the chondro-cranium, spring two slender cartilages, the *rhinal processes*. Each of these inclines towards the middle line and ends against the middle of the posterior face of the ascending process of the premaxilla by a vertically elongated extremity. An oval nodule of cartilage is attached to the posterior face of the above-named process, and serves to connect it with the rhinal process. On the dorsal face of the chondro-cranium, just above the point of attachment of the rhinal processes, the external nasal apertures are situated, and the outer and posterior margins of each of these apertures are surrounded and supported by a curious curved process of the cartilaginous ala—the *alinasal process*. Where the sphenoidal and the ethmoidal portions of the sphenethmoid meet, a stout, transverse, partly osseous and partly cartilaginous bar is given off, which is perforated at its origin by the canal for the orbito-nasal nerve. It then narrows, but becoming flattened from above downwards, rapidly widens again, and its axe-head-like extremity abuts against the inner face of the maxilla. The anterior angle of the axe-head is free; the posterior angle is continued back into a slender cartilaginous *pterygoid* rod which bifurcates posteriorly; this (the *suspensorium*) furnishes the articulation for *Meckel's carti-*

lage which forms the core of the ramus of the lower-jaw or mandible.

The floor of the mouth is supported by a plate of cartilage (the *hyoid*), this is attached to the skull by a slender cartilaginous band or *cornu* which is connected with the auditory capsule.

The pectoral and pelvic arches (see Laboratory work) are, in the young state, paired undivided cartilages, and the development of bone in and upon them does not really destroy this continuity, the cartilage persisting at the ends of the bones, and between them in the cavities for articulation of the limb bones.

In like manner, the bones of the limbs consist originally of cartilaginous models of the perfect bone; but, as development proceeds, the middle of the cartilaginous model commonly becomes invested by a sheath of true bone, while calcareous deposits take place in the cartilage close to its growing extremities. As the bone grows, the superadded sheath invades the middle of the cartilage and more or less replaces it; while the terminal portions of cartilage continue to grow and enlarge, and the calcareous deposit within them increases, without however reaching their surfaces. Thus one of the larger adult limb bones (say the femur) consists of a median portion or *shaft*, and of two terminal caps of cartilage containing calcified *epiphyses* overlying, and more or less enclosed within, the hollow ends of the shaft.

Seeing that the bony elements of the adult skeleton arise by superaddition to and replacement of a cartilaginous predecessor, it follows that those portions of it which remain permanently cartilaginous are persistent representatives of latter or embryonic endoskeleton.

The general disposition of the parts which are seen in the mouth has already been described.

Teeth are present only in the upper jaw and roof of the mouth. They are small, with recurved and pointed crowns. New teeth are constantly being developed in the gum to replace those which are worn out or broken away, and as they attain their full size these teeth become ankylosed to processes of the subjacent bone. Their distribution and characters are such as to render mastication an impossibility; they are prehensile and utilized as hooks by means of which the animal as it were gets outside its prey. The eversible tongue is utilized as a means of capture of smaller creatures, chiefly insects.

The gullet passes without change of diameter into the stomach, which lies on the left side of the pleuroperitoneal cavity and is nearly as long as it. The stomach narrows posteriorly and the almost tubular terminal portion bends round sharply and passes into the small intestine. A slight constriction (*pylorus*) marks their point of junction. The small intestine runs forwards parallel with the stomach, so that with the latter it forms a sort of loop; it is continued on coiled up into a sort of packet which lies to the right side of the pleuroperitoneal cavity, being held in its place by a mesenteric fold of the peritoneum. Thence the small intestine proceeds backwards in the middle line and opens into the anterior end of the dilated large intestine.

The inner wall of the stomach is raised up into a number of strong longitudinal folds which project into its cavity and give it a stellate appearance in transverse section. Similar but more delicate folds are met with in the small intestine, the lining-membrane of which is produced into parallel series of semi-lunar folds whose free edges project backwards.

The opening of the ileum into the large intestine is valvular, its edges projecting backwards into the cavity of the

latter. On the dorsal aspect the large intestine presents a slight forward dilatation, which may be regarded as a rudiment of a cæcum.

The liver is very large, and incompletely divided into two lobes, the left of which is further subdivided into two. The gall bladder is attached to the posterior face of the right lobe. The bile duct opens into the duodenum, at some distance behind the pylorus, running through the pancreas and receiving the duct of that organ on its way.

The rounded spleen lies in the mesentery, projecting more to the left than to the right side, near the point at which the small intestine first becomes coiled.

The apparatus of circulation in the Frog consists of the blood and lymph vessels and their contents.

The lymph is a colourless fluid containing colourless nucleated corpuscles which exhibit amœboid movements: it is contained partly in large spaces immediately beneath the integument; in the pleuroperitoneal cavity and probably in the other serous cavities; and, partly, in capillaries and larger trunks which are interlaced with and accompany the blood-vessels. The largest of the trunks is the great sub-vertebral lymph-sinus, which lies between the layers of the root of the mesentery and communicates by small pores with the pleuroperitoneal cavity.

The blood consists of a colourless plasma which contains colourless corpuscles, similar to those of the lymph, and in addition a great number of oval nucleated red corpuscles. It is contained in the blood-vessels, which consist of capillaries, arteries and veins, the two latter being connected on the one side by the capillaries and, on the other, by the heart into which they open. The lymphatics and the blood-vessels are brought into connexion with one another

at opposite ends of the body, through the agency of two pairs of contractile muscular sacs (*lymph-hearts*), which pump the lymph contained in the wide lymphatic vessels and in the pleuroperitoneal cavity into the great veins in their neighhourhood.

The blood and lymph systems may thus be regarded as subdivisions of a common *circulatory system*.

The heart is connected with the walls of the pericardium, by the vessels which enter and leave it, and by a slender band which passes from the dorsal face of the base of the ventricle to the posterior dorsal wall of the pericardial chamber.

The heart consists of four readily distinguishable segments, (1) the *sinus venosus*, (2) the *atrium*, (3) the *ventricle*, and (4) the *truncus arteriosus*, disposed in such a manner that the sinus venosus, which is the hindermost division, lies in the middle line on the dorsal aspect of the heart: the atrium is also median and on the dorsal side but is in front of the sinus venosus; the ventricle is median, ventral and posterior; and the truncus passes obliquely forwards from the right side of the ventricle and is ventral and anterior. The heart therefore may be compared to a tube divided by constriction into four portions and bent somewhat into the shape of an S.

The *sinus venosus* receives on each side, in front, a large vein, the *vena cava superior;* while behind the, usually single, *vena cava inferior* opens into it. It opens by a valvular aperture into the atrium. The latter shews no signs of division externally, but internally, it is divided by a delicate partition, the *septum of the auricles*, into a smaller *left auricle* and a larger *right auricle*. The sinus venosus opens into the atrium, to the right of the septum and therefore

into the right auricle. Into the left auricle, the *common pulmonary vein*, formed by the junction of the veins from the right and left lungs, opens.

At its posterior end the atrium opens by the auriculo-ventricular aperture into the ventricle.

A small valve, prevented from flapping back by fine tendinous cords, exists on each side of this aperture, and the septum of the auricles is continued back upon the faces of these valves and ends by a free edge between them, thus dividing the auriculo-ventricular aperture itself into two openings.

The walls of the sinus and of the atrium are very thin. Those of the ventricle, on the other hand, are thick and spongy, only a comparatively small, transversely elongated, cavity being left at its anterior end or base. At the right-hand extremity of this is the aperture which leads into the truncus arteriosus. Three semilunar valves, which open from the ventricle into the truncus, surround this opening.

The walls of the *truncus arteriosus* are thick and muscular, though not nearly so thick as those of the ventricle. At its anterior end it appears to divide into two trunks, which diverge and immediately leave the pericardium to pass on to the sides of the gullet. The elongated undivided part is the *pylangium*, the terminal part common to the divergent trunks is the *synangium*. The former is divided throughout its length by a sort of fold which is attached to the dorsal wall while its opposite edge is free. Three semilunar valves separate the pylangium from the synangium, in which are the openings, posteriorly, of the *pulmonary* arteries, anteriorly of the *carotid trunks;* while, at the sides, the cavity of the synangium opens into those of the right and left aortic arches. The apparently simple

branches into which the *truncus arteriosus* divides, are, in fact, each made up of three separate trunks, the *pulmo-cutaneous trunk* behind, the *aortic arch* in the middle and the *carotid trunk* in front.

When the heart is in action, the sinus venosus, the atrium, the ventricle and the truncus arteriosus contract in the order in which they have just been named. Each contracts as a whole, so that the two auricles are emptied simultaneously. The blood from each is forced into the corresponding half of the spongy cavity of the ventricle, so that the right half of the ventricle contains venous blood and the left arterial blood. When the systole of the ventricle takes place, the blood which is first driven into the truncus arteriosus (the opening of which is, as has been seen, at the right end of the cavity) is therefore venous. It fills the conus arteriosus and, finding least resistance in the short and wide pulmonary vessels, passes along the left side of the median valve into them. But as they become distended the next portion of blood, consisting of the venous and arterial blood which have become mixed in the middle of the ventricle, passes on the right side of the longitudinal valve into the aortic arches. And, as the truncus becomes more and more distended, the longitudinal valve, flapping over, tends more and more completely to shut off the openings of the pulmonary arteries and to prevent any blood from flowing into them.

Finally, the last portion of blood from the ventricle, representing the completely arterialized blood of the left auricle which is the last to arrive at the opening of the truncus, passes into the carotid trunks and is distributed to the head.

The principal vessels of the Frog are disposed as follows :—

A. *Arteries.* *Efferent* in relation to the heart, breaking up into lesser *branches.*

1. The system of the *anterior aortic arch* (*carotid* trunks), distributing blood to the parts of the head generally and to the brain.
2. The system of the *middle aortic arch* (*aortic* trunk), distributing blood to the body and limbs, together with the organs of the viscera other than the lungs.
3. The system of the *posterior aortic arch* (*pulmo-cutaneous* trunks), distributing blood to the lungs and skin.

B. *Veins.* *Afferent* in relation to the heart, formed by the union of lesser *factors* (they break up only in the case of the *portal* veins).

1. The system of the *superior cava*, formed on each side by the union of the veins bringing back the blood from the head, fore-limbs, and parts adjacent. Special veins (*great cutaneous*) are concerned in the return of the blood from the skin.
2. The system of the *inferior cava*, formed by the union of the *efferent-renal*, *genital* and *hepatic* veins.
3. The system of the *anterior abdominal* and *renal-portal veins;* formed by the bifurcation of the veins coming in from the hind-limbs. The former receives blood from the urinary bladder and the body-wall, and at its anterior end divides into two branches which go to the corresponding lobes of the liver. The latter gives off branches (*afferent renal veins*) which break up in the substance of the kidney.
4. The system of the *vena portæ* (*hepatic-portal vein*) formed by the union of the veins which bring back

the blood from the alimentary-canal and its appended glands, with the spleen. This vessel enters the liver on the left side, breaking up within that organ; before doing so, it enters into a direct anastomosis with the anterior abdominal vein.

5. The system of the *pulmonary vein*, formed by the union of the veins of the right and left lungs.

The slit-like glottis of the Frog is formed by the apposition of two longitudinal folds of the mucous membrane of the mouth, each of which contains a cartilage of similar form. These cartilages are the *arytenoid* cartilages. They are articulated with an annular cartilage (*cricoid*) which supports the wall of the very short laryngeal chamber. When the two folds of the glottis are divaricated, there are seen between them two membranous pouches, the free edges of which meet in the middle line, while anteriorly and posteriorly they pass into the mucous membrane which lines the faces of the longitudinal folds. These are the *vocal ligaments*, and the slit between them is what answers to the glottis in Man. It is by their vibration that the croak of the Frog is produced.

Laterally the laryngeal chamber opens into the lung of each side. The lung is a transparent oval sac, somewhat pointed posteriorly, which lies at the side of the œsophagus in the anterior region of the pleuroperitoneal cavity. It is covered by a layer of the pleuroperitoneal membrane which represents the visceral layer of the pleura in the higher animals. The wall of the pulmonary sac is produced inwards so as to give rise to a network, which is much more prominent in the anterior than in the posterior part of the lung and divides the periphery of the cavity

into numerous air-cells, on the walls of which the ramifications of the pulmonary vessels are distributed.

The lungs are elastic, the distended lung collapsing suddenly when it is pricked, and they contain abundant muscular fibres.

It is essential to respiration that the mouth should be shut, and it is said that frogs may be asphyxiated by keeping their mouths open.

Inspiration is effected by a buccal force-pump. The mouth being shut and the external nostrils open, the floor of the mouth is depressed, and the buccal cavity fills with air. The muscles of the flank next come into play, exercising a pressure upon the surrounding viscera which suffices to expel the air (expiration).

The nostrils being then shut, the hyoid, and with it the floor of the mouth, is raised, and, the aperture of the gullet being at the same time closed, air is forced through the glottis, distending the lungs.

Expiration is doubtless aided by the contraction of the intrinsic muscular fibres of the lungs; and it may be that those fibres which form the so-called diaphragm also tend to diminish the capacity of the lungs.

In addition to its principal pulmonary apparatus of respiration, the Frog has a secondary respiratory apparatus in its moist and delicate skin. A considerable amount of venous blood is, in fact, constantly supplied to this organ by the large cutaneous branch of the pulmo-cutaneous artery. It has been experimentally ascertained that frogs in which the lungs have been extirpated will continue to live and respire for a considerable time, especially at a low temperature, by means of the skin.

The kidneys are elongated and flattened from side to side, and are kept in position by the continuation of the peri-

toneum over their ventral faces. The ducts of the kidneys pass along their outer edges and, approaching as they pass backwards, open by two slit-like apertures in the posterior wall of the cloaca.

The urinary bladder (*urocyst*) is a large bilobed sac, opening posteriorly, by a wide median aperture, into the antero-ventral end of the cloaca.

The *testes* are ovoidal yellowish bodies situated in front of the kidneys and enveloped in peritoneum, a fold of which, forming a sort of testicular mesentery or *mesorchium*, is derived from that which covers the ventral face of the kidney. The delicate *vasa efferentia* of the testes may be seen traversing this fold to enter the substance of the kidney. They communicate with the urinary tubules, and thus the duct of the kidney serves not only as the duct of the urinary secretion but as the *vas deferens*.

The spermatozoa of the Frog are filiform bodies with cylindrical, more or less linear, nucleus bearing heads.

The *ovaries* are broad lamellar organs, very large and much folded and plaited in the breeding season. Innumerable ovisacs, or follicles, containing dark-coloured ova, are scattered through the substance of the ovary and give rise to projections on its surface as they become fully developed. The ova are finally thrown off by dehiscence.

The *oviducts* are long convoluted tubes situated on each side of the dorsal wall of the abdominal cavity, to which they are connected by peritoneal folds; each curves over the outer face of the root of the lung. Their anterior ends are very slender, and terminate by open mouths at the sides of the pericardium, between the attachment of the so-called diaphragm and the lobe of the liver. For the greater part of their length their walls are thick and glandular, and

swell up when placed in water. Posteriorly, the oviducts dilate into capacious thin-walled chambers and end, close together, by openings which are situated in the dorsal wall of the cloaca immediately in front of the apertures of the ureters. Remnants of the oviducts are usually present in the male.

Each ovum, when ripe, consists of a protoplasmic yolk-laden mass or *vitellus*, enclosed in a structureless vitelline membrane, and containing a germinal vesicle, within which are several 'germinal spots.' One half of the vitellus is deeply coloured, the other pale.

The so-called *ductless glands* other than the spleen already described, are three in number. The *Thymus;* a small rounded body situated immediately behind the suspensorium, in a position corresponding to the dorsal ends of the obliterated branchial arches. The *Thyroid;* represented by two or more oval bodies, which are found attached to the bases of the aortic arches. The *Adrenals;* yellow bodies imbedded in the ventral face of the kidney.

The actions of the different parts of the organism of the Frog are coordinated with one another and brought into relation with the external world by means of the muscular and nervous systems and the organs of sense.

The *muscles* consist partly of striped and partly of unstriped fibres, the former being confined to the muscles of the head, trunk and limbs and the heart, while the latter are found in the viscera and vessels. The former are usually arranged in sets, the actions of which may be antagonistic and simple or coordinated and complex. An account of the disposition of the muscles in the hind-limb will be found in the Laboratory work.

The *nervous system* is conveniently divisible into two parts, the *cerebro-spinal* and the *sympathetic*. The cerebro-spinal nervous system again consists of the brain, or *encepha-*

lon, with its nerves, and the spinal cord, or *myelon*, with its nerves.

The encephalon lies in the cranial cavity, which it nearly fills, and is divisible into the *hind-brain*, the *mid-brain* and the *fore-brain*, which last again comprises three divisions; the median thalamencephalon, and the paired cerebral hemispheres and olfactory lobes.

The greater part of the hind-brain is formed by the *medulla oblongata*, which is the continuation of the myelon forwards and presents, when laid open from its dorsal aspect, a triangular cavity, the apex of which is directed backwards. The cavity is the *fourth ventricle*; it communicates behind with the central canal of the myelon, while, in front, it narrows into a passage which connects the fourth ventricle with the brain cavities anterior to it. The thick lateral ridges of nervous substance at the sides of the fourth ventricle, which represent the *restiform bodies*, pass, in front, into the outer extremities of a short broad tongue-shaped plate, convex ventrally and concave dorsally, which overhangs the anterior part of the fourth ventricle, and is the *cerebellum*.

In front of this, the dorsal moiety of the mid-brain is formed by two oval bodies, the *optic lobes*. When laid open, each is seen to contain a cavity or ventricle which communicates with the *iter a tertio ad quartum ventriculum*, as the ventricle of the mid-brain is termed. The floor of this canal is formed by the thick principal mass of the cerebro-spinal axis. It exhibits a median longitudinal depression or raphe, and in this region represents the *crura cerebri*.

In front of the mid-brain comes the hinder division of the fore-brain, or *thalamencephalon*, which is very distinct in the Frog and contains a median cavity, the *third ventricle*.

On each side, the cavity of the third ventricle is bounded by a thick mass of nervous matter into which the crura cerebri pass. These are the *optic thalami*. The roof of the third ventricle is very thin and easily torn through; its fore part is prolonged up to form a delicate process in connexion with an ovate body, which is lodged between the posterior parts of the cerebral hemispheres and represents a portion of the *pineal gland*. The front part of the floor of the ventricle, on the other hand, is produced into a backwardly directed process, the *infundibulum*. This is connected below with a highly vascular mass, the *pituitary body*.

The hemispheres are elongated bodies, broader behind than in front, where they are marked off only by a slight constriction from the olfactory lobes.

Anteriorly, the wall of the third ventricle is thickened to form the *lamina terminalis;* on each side, between this and the peduncle of the pineal gland, is a small aperture, the *foramen of Monro*, which places the median third-ventricle in communication with the paired *lateral ventricle* of the cerebral hemisphere.

The lateral ventricle is continued on either side into the base of the olfactory lobe to form the *olfactory ventricle*. In front these lobes become nerve-like cords, which leave the skull and spread out on the posterior faces of the olfactory sacs.

The inner faces of the olfactory lobes are confluent with each other, and there pass between corresponding parts of opposite halves of the brain bands of fibres or *commissures* (see Laboratory work) the most important of which runs through the substance of the lamina terminalis and represents the *corpus callosum*.

The entire cerebro-spinal axis is invested in a highly

vascular membrane or *pia mater*. This becomes immensely thickened, above the hind-brain and on the inner faces of the hemispheres, to constitute the *choroid plexuses*. The former of these is median and overlies the greater part of the fourth ventricle. The latter is paired; in its growth each half forces its way into the lateral ventricle, pushing the thin inner wall of the same in front of it; while therefore it lies, in a sense, within that cavity, it is in reality outside it.

There are ten pairs of cranial nerves ordinarily so-called, the first two of which are proved, by their development, to be lobes of the brain.

1. *Olfactorii.*

   The olfactory lobes. Their nerves are distributed exclusively to the olfactory sacs.

2. *Optici.*

   These diverge from the base of the brain in front of the infundibulum. They are originally outgrowths of the thalamencephalon which secondarily become connected with the optic lobes. They are distributed exclusively to the retina of the eye.

Of the remaining cranial nerves five pairs leave the skull in front of the auditory capsules, while one pair enters those capsules and two pairs pass out behind the same.

The *Præauditory nerves* are the following.

3. *Motores oculorum*

   arise from the front part of the floor of the mid-brain and are distributed to all the muscles of the eye except the external rectus, the superior oblique and the retractor bulbi.

4 *Pathetici*

arise from the floor of the mid-brain and pass out, on the dorsal aspect of the brain, between the cerebellum and the optic lobes. They are distributed to the superior oblique muscles of the eye.

5. *Trigemini*

take their origin in the front part of the floor of the hind-brain and, passing out at its sides, each dilates into a yellow enlargement—the *Gasserian ganglion*—which lies in front of the auditory capsule, in the foramen by which the nerve, after leaving the ganglion, passes out of the skull.

This ganglion is connected with the trunk of the sixth and seventh nerves and with the anterior end of the sympathetic (some of the branches which appear to be given off from it really belong to the sixth and the seventh nerves, see *infra*). Beyond the ganglion, the nerve divides into two main branches, the *orbito-nasal*, and the *maxillo-mandibular*.

i. The *orbito nasal* (usually termed the first division of the fifth nerve); passing through the antorbital process of the skull, it is finally distributed to the nasal mucous membrane and to the integument of the nose. It anastomoses with the fourth nerve, and sends a branch to the Harderian gland.

ii. The *maxillo-mandibular;* forks over the mouth-cavity into two trunks, usually termed the second and third divisions of the fifth nerve.

a. Preoral or *Maxillary*, passes outside the eye and is distributed to the integument of the upper jaw.

b. Postoral or *Mandibular*, passes between the temporal and pterygoid muscles, over the articulation of the mandible and along the inner face of the latter, to the symphysis, giving off branches to the integument, muscles, and tongue.

6. *Abducentes*

arise from the floor of the hind-brain and leave the ventral surface of the medulla oblongata close to the middle line. Each then unites so closely with the Gasserian ganglion and with the orbito-nasal division of the fifth as to appear to be only a subdivision of the latter. Its fibres are distributed to the external rectus and retractor bulbi muscles of the eye.

7. *The Faciales*

take their origin from the floor of the hind-brain, behind the fifth and in common with the eighth; and, leaving the hind-brain, enter into close connexion with the Gasserian ganglion. Each then divides into two branches, an anterior and a posterior.

i. The anterior or *palatine nerve;* distributed to the roof of the mouth and palate.
This nerve is in anastomosis with the maxillary branch of the fifth.

ii. The posterior branch. It forks over the tympanic cavity into two trunks; a smaller pre-tympanic one, and a larger post-tympanic or *hyoid nerve*, which supplies the muscles of the hyoid and the floor of the mouth.

8. The *Auditorii*

arise in common with the foregoing. Each divides

into two branches which enter the auditory capsule to reach the organ of hearing.

The *Post-auditory nerves* are:

9. The *Glossopharyngei.*

   These nerves arise, side by side with the next, from the medulla oblongata; and the roots of both leave the skull by an aperture behind the auditory capsule on each side, and form a common ganglion. Each passes downwards and forwards to the root of the tongue, which organ it finally supplies. Moreover, it gives off muscular branches and forms an anastomosis with the seventh.

10. The *Pneumogastrici* or *Vagi.*

    Immediately after leaving the ganglia these nerves separate from the glossopharyngeal and each gives off a cutaneous branch to the dorsal integument of the head and trunk: it then divides into two branches, one of which is distributed to the larynx, the other to the heart, lungs, and stomach. (Sympathetic fibres are in part bound up with these.)

The *myelon* or spinal cord is continued back from the hind-brain as a subcylindrical cord, which lessens somewhat rapidly towards its apparent end at the level of the seventh vertebra. It does not really end here, however, but is continued back as a slender filament, the *filum terminale*, to the commencement of the canal of the urostyle. The diameter of the cord is somewhat enlarged opposite the origin of the nerves for the limbs. In transverse sections, the cord is seen to be not truly cylindrical but to be indented by two longitudinal grooves, one dorsal and one ventral, which leave but a small connecting bridge between its two halves.

In the centre of this is a canal, the *canalis centralis*, the cavity of which is continued forwards into the axial ventricles of the brain.

Ten symmetrically disposed pairs of nerves come off from the sides of the cord, each nerve having two roots, one from the dorsal surface of the lateral half of the cord and one from the ventral surface. The dorsal root presents a small ganglionic enlargement, beyond which it is bound up in a common sheath with the ventral root to form the common trunk of the spinal nerve. The roots of the hinder spinal nerves are very long and lie, side by side, in the spinal canal.

The first spinal nerve leaves the neural canal by the interspace between the arches of the first and second vertebræ, so that there is no nerve in the Frog answering to the suboccipital. It gives a branch to the muscles which move the head upon the backbone, but the main trunk of it descends behind the mandible, along with the glossopharyngeal nerve, and is distributed to the muscles of the tongue. Its distribution therefore answers to that of the *hypoglossal nerve* in the higher Vertebrata.

The second and third spinal nerves unite to form a '*brachial plexus*,' and are distributed chiefly to the fore-limb.

The fourth, fifth and sixth spinal nerves go to the middle parietes of the body.

The seventh, eighth and ninth, are large nerves which unite to form the *lumbosacral plexus*, whence nerves are given off to the posterior parietes of the body, and to the hind-limb.

The tenth spinal nerve leaves the neural canal by the coccygeal foramen, and is distributed to the adjacent parts.

Sympathetic.

The *sympathetic* system consists of ganglia, usually ten in number on each side, connected by longitudinal commissures, and situated on the ventral face of the vertebral column; in the region of the dorsal aorta they come into close relation with it. Each sympathetic ganglion is joined by a communicating filament or ramus with one of the spinal nerves, and the most anterior ganglia are connected, in the same way, with the ganglia of the ninth and tenth cerebral nerves. Thence a delicate cord passes into the cranial cavity on the inner side of the periotic capsule, and unites with the Gasserian ganglion.

The branches of the sympathetic accompany the vessels, and large branches are given off to the viscera.

The sheath of the ganglion of each spinal nerve encloses a milk-white body (*perigangliouic gland*) whose function is unknown. Each consists of a tubular gland, lined by a single-layered epithelium and containing calcareous matter in a finely crystalline state. When fully formed these glands force their way through the inter-vertebral foramina, appearing as a series of concretionary masses lying around the spinal nerves at their points of exit from the column, and alternating with the bases of the transverse processes of the vertebræ. (Cf. next page.)

The *Olfactory organs* occupy all the space between the mesethmoid cartilage, the antorbital processes, and the premaxillæ and maxillæ, and open in front and externally by the external nares, behind and internally, into the mouth-cavity, by the posterior nares. Each consists of an essential part or *olfactory sac*, the inner face of which is lined by a peculiar epithelium, which receives the olfactory branches of the trigeminal nerves, and an accessory part consisting of a partly cartilaginous, partly bony, *capsule*.

The *Eyeball* is lodged in the orbit and protected by the eyelids. It has four *recti* and two *oblique* muscles (which proceed from the orbit to the circumference of the globe) and one *retractor* muscle (cf. p. 113). In addition, a fine tendon passes between the outer end of the lower eyelid, or nictitating membrane, and the fibres of the *retractor*, so that when the bulb is retracted the nictitating membrane is raised over the eye. The upper lid has no muscles. A secretory organ, termed the *Harderian gland*, is situated in the anterior part of the orbit beneath the superior oblique muscle.

The essential part of the eye is the inner lining or *retina*, which receives the fibres of the optic nerve; to this there are superadded a vascular pigmented *choroid* and a cartilaginous *sclerotic*, which together constitute an accessory capsule. The lens is nearly spherical.

The *Ear* consists of an essential part—the *membranous labyrinth*—receiving the fibres of the auditory nerve, lodged in an accessory partly osseous, partly cartilaginous, perioticcapsule; to the latter are superadded the *columella auris* and the *tympanic membrane*.

The labyrinth consists of three semicircular canals which open into a vestibule divided into *utriculus* and *sacculus*. The latter, especially, contains a great quantity of white crystalline calcareous otoliths. There is developed intercranially a sac-like derivative of the labyrinth, the *saccus endolymphaticus*, lying about the brain and filled with otolith-like crystals. It is from this that the periganglionic glands referred to on p. 32 are derived.

On the outer side of the vestibule is a small dilatation which is possibly a rudimentary *cochlea*.

The membranous labyrinth is filled with a fluid (*endolymph*),

and contained, as has been stated, in the periotic capsule into which it fits but loosely; the interval between the two being filled with a fluid, the *perilymph*. In the outer face of the periotic capsule is an opening, the *fenestra ovalis*, into which the end of the *columella auris* fits. This columella is shaped like a pestle, the end of the handle of which is fitted with a cross-piece. The rounded inner end of the pestle, which is fixed by fibrous tissue into the fenestra ovalis, is cartilaginous. The middle of the handle is ensheathed in bone, while the outer part is cartilaginous. The cross-piece is fixed into the inner face of the membrana tympani, which is covered externally by the integument, and lined internally by the mucous membrane of the tympanic cavity, which is continuous with that of the mouth through the Eustachian recess, and wraps round the ventral face of the columella. A sound wave, impinging upon the drum of the ear, is transmitted through the agency of the columella to the perilymph and endolymph, auditory epithelium and nerve, to the brain.

The *Tongue*. This organ, as has been seen, is fixed only in front to the mandible, and by the anterior half of its ventral aspect to the floor of the mouth; the posterior half being free and bifid at the extremity. Narrow-ended and broad-ended papillæ (*papillæ filiformes* and *fungiformes*) are scattered over the whole dorsal aspect of the tongue and are largest in front; small glands lie between these papillæ.

The fungiform papillæ contain the ultimate ramifications of the glossopharyngeal nerve, and the epithelium covering their summits is peculiarly modified.

The *Integument*. No special organs of touch have been observed, but the integument is remarkable for the immense number of close-set simple glandular cæca (*cutaneous glands*) which open upon its surface. In the swollen integument

which covers the base of the inner digit in the males, large papillæ with interposed glands are developed.

A singular spheroidal sac-like body known as the *browspot* or *inter-ocular* gland, occurs in the integument of the fronto-parietal region of the head. In the young animal it is in connection with the pineal gland, and represents the rudiment of the median or pineal eye—a structure which is more fully developed in some Fishes and Lizards.

Cells containing pigment abound in the integument and undergo remarkable changes of form, the pigment being sometimes drawn together into a spheroidal mass—at other times distributed in a radiating fashion.

LABORATORY WORK.

Frogs may best be killed with chloroform. Place the animal under an inverted tumbler together with a square inch of cotton-wool saturated with chloroform; cover with a cloth and leave for 10—15 m

A. **General external characters.**

1. Its division into *head*, *trunk*, two pairs of *limbs* or *appendages*.
2. Its *anterior* or head end; pointed and bearing the mouth, eyes, and other organs of the higher senses. Its *posterior* or tail end. Its *dorsal* or back region; clad in a darkly coloured integument. Its *ventral* or belly region; clad in a whitish integument. Its *lateral* or side area; bearing the two pairs of limbs. (Work over the specific characters given on pp. 6, 7.)

a. • *The head.*

Somewhat triangular, with the blunted apex turned forwards and passing, without any neck-constriction, into the trunk.

α. The prominent *eyes* with their *eyelids*.

β. The *membrana tympani*, a thin part of the integument stretched over a hard ring, on each side, behind and somewhat below the eyes.

γ. The *anterior nares;* two small apertures near the middle line at the end of the snout.

δ. The *mouth-opening:* note its boundaries and extent.

ε. The *browspot;* lying in the mid dorsal integument of the head on a level with the front of the eyes. Clearly visible under a hand lens.

ζ. Open the mouth widely; the fleshy tongue will be seen, with its bifurcated free end turned backwards; teeth are present only in the upper jaw and on the roof of the mouth.

b. *The trunk.*

Tapering towards the hinder end, and allowing the hard parts of the skeleton to be felt beneath the soft integument on the dorsal side, and in the anterior half of the ventral aspect. Note the *cloacal aperture* near the dorsal surface of its posterior end.

c. *The limbs.*

α. The anterior pair; their three subdivisions, *brachium*, *antebrachium*, and *manus;* the four *digits*.

β. The posterior pair; their length as compared with that of the anterior; their subdivision into *femur*, *crus*, and *pes:* the five long *digits;* the well-developed *web;* the horny prominence on the inner side.

B. **Preliminary, and general disposition of the viscera.**

1. Place the animal on its back and pin down high and dry, inserting the pins through the limbs and point of snout. Raise the ventral integument with forceps and slit it up with scissors along the middle line from the lower jaw to the origin of the hind limbs; pin back the two flaps and note :—

a. The *skin;* it is but loosely connected with the subjacent muscular wall of the trunk, the two being separated by spacious chambers (*lymph cavities*, cf. Sect. I. *a*).

b. A large vein (*great-cutaneous*) on the under-surface of each flap of skin.

c. Some of the muscles of the body wall; covered by a thin sheath or aponeurosis, through which they can be seen—

α. The *rectus abdominis;* running from pelvis to sternum along the middle line, and divided into a number of segments by transverse tendinous intersections.

A large vein (*anterior-abdominal*) will be seen, running along the ventral middle line, immediately under cover of this.

β. The *pectoralis;* an immense tract of tissue arising from the ventral wall of the trunk externally, and in front ventrally, to α. It is subdivided into a series of lesser muscles which converge towards the base of the fore-limb. (Note that it is confluent posteriorly with α, and, in front, subdivided up by tendinous intersections.)

γ. The *mylohyoid* or *sub-maxillaris;* a sheet of muscle, tendinous in the middle line, whose

fibres pass transversely across the floor of the mouth. It is subdivided into a larger fasciculus which supports the floor of the mouth, and a smaller ribbon-shaped one which passes back on either side to the angle of the lower jaw.

δ. The *external oblique;* underlying and external to β; forming an investment for the body wall laterally. Its fibres run obliquely upwards and forwards.

ε. The *internal oblique;* seen on removing a portion of δ. Its fibres pass obliquely downwards and backwards.

ζ. The *transversalis;* seen on removing a portion of ε. It forms the inner layer of the muscular constituent of the body-wall; its fibres run transversely, so as to encircle the trunk ventro-laterally.

2. Raise the tissues of the body-wall with a pair of forceps and carefully divide them a little to the right of the median line, so as to open the underlying body-cavity without injuring its contents; prolong the incision from the pelvis to the posterior end of the breast-bone. Make a transverse incision close to the pelvis and throw back the flap on each side: on the under side of the left flap will be seen the *anterior abdominal* vein (cf. 1 *c.* α).

Seize the posterior border of the sternum with a pair of forceps and raise it up; then, with a strong pair of scissors, cut through the hard parts a little to one side of the middle line, being very careful not to injure the organs beneath them. Raise each half of

the shoulder girdle thus liberated and remove it, with care, together with the rest of the ventral portion of the body-wall.

A spacious cavity (*pleuro-peritoneal cavity*) will be laid bare. Note the general characters and mutual relations of the undermentioned organs of the viscera which lie within it, as seen in the undisturbed state. They will be found severally connected with the body-wall and each other by a delicate membrane or *mesentery*.

a. The *liver:* a great brown two-lobed mass, its left lobe the larger and subdivided into two, lying in the anterior end of the cavity.

b. The *lungs:* the posterior ends of these may be seen as sacculated pouches, one on each side of the liver, but they are frequently not visible until the latter organ has been displaced.

c. The *stomach:* a small portion of this is seen projecting beyond the lower left border of the liver. Note that it lies exclusively to the left side.

d. The *intestine:* a convoluted tube, continuous with the stomach, and occupying the middle third of the cavity. It can be resolved into—

α. The *small intestine;* a yellowish-white tube, convoluted and situated wholly to the right side.

β. The *large intestine;* a short straight greyish-green tube, of greater calibre than α, passing obliquely backwards in the middle line.

e. The *urinary bladder* (*urocyst*), a thin-walled bilobed sac (which may or may not be distended) lying just in front of the pelvis, immediately above (ventral to) β.

In *R. temporaria*, the urinary bladder is larger proportionately and more deeply lobed than in *R. esculenta*.

f. The *spleen:* a small red body lying in the middle line, immediately in front and to your right of the large intestine.

g. The *kidneys;* two dark red lentil-shaped bodies, seen, on displacing the intestine, lying close together in the middle line.

h. The *genital glands;* immediately adjacent (ventral and external) to *g*.

α. In the male. *The testes:* a pair of yellowish bodies lying near the anterior ends of the kidneys.

β. In the female. The *ovaries;* a pair of blackish yellow bodies, coincident in position with, but much more extensive than, *α*. Each is folded, and seen to be composed of an immense number of spherical *ova*.

i. The *fat masses* (*corpora adiposa*); long filiform deep-yellow processes attached to the anterior ends of the genital glands. They are much the larger in the male.

j. The *genital ducts;* obvious in the female as highly convoluted dead-white tubes (*oviducts*) lying immediately beneath *β*. (Those of the male may be better studied later on.)

k. The *heart;* seen lying within a delicate sac (the *pericardium*) immediately in front of the liver.

Slit open the pericardium. It will be found to contain a fluid (*serous fluid*) which bathes the enclosed viscus (heart). Examine the pleuro-peri-

toneal cavity; a similar fluid will be found to be present, but to a lesser degree, imparting a moistness to the organs therein contained.

C. **The study of transverse sections.** Especially with a view to ascertaining the limits and mutual relationships of the larger cavities and the body-wall, together with the mode of suspension of the viscera.

Obtain a frog (preferably one which has been for 24 hours or longer in spirit) and make transverse sections across its body, as directed below. Examine under water.

1. Across the mid region of the trunk; to pass through the kidneys, genital glands, and large intestine.

   a. The *body-wall;* especially its muscular constituent, which is thickened dorsally in the region of the back.

   b. The *splanchnic* (*pleuro-peritoneal*) *cavity;* ventral and spacious; it lodges the organs of the viscera.

   c. The *neural cavity;* relatively small, situated in the dorsal middle line and enclosed on all sides by the bony vertebral column. It lodges the central nervous system, seen, in section, as a delicate white cord.

   d. The *alimentary canal;* cut across at various points. Remove those portions of it which do not happen to be connected by mesentery with the body-wall or other parts. The *large intestine* will be seen in section in the ventral middle line; take note of its position and relationships.

   e. The *kidneys;* lying close together, dorsally to all the other organs and obliquely disposed.

f. The *genital glands;* immediately external and ventral to *e.* (cf. Sect. B. 2. *h.*)

g. The *pleuro-peritoneal membrane.* This will be most readily seen stretching from the dorsal border of the kidney to the upper angle of the body-wall; having found it, proceed as under.

α. Start from one kidney and follow it upwards; on reaching the body-wall it becomes closely applied thereto, lining it, in the form of a dense pigmented membrane (*parietal-layer* or *peritoneum*).

β. Follow it downwards on the same side. It passes over the outer face of the kidney and then gives rise to a fold which suspends the genital gland (*mesorchium* ♂, *mesoarium* ♀); the folds of opposite sides then meet in the middle line and pass down as a double membrane (*mesentery*) which suspends and enwraps the intestine.

Little difficulty will be found with the above, if, starting from the kidney, the membrane be carefully torn away as followed.

γ. The *cisterna magna* (*sub-vertebral lymph sinus*); a spacious cavity into which the kidneys project, enclosed between the folds of the mesentery dorsal to the alimentary canal. The cut ends of a large blood-vessel (*dorsal aorta*) and of certain small nerves will be seen lying within it.

2. Obliquely forwards, across the anterior end of the body cavity; to pass through the heart, the anterior border of the liver and the roots of the lungs. Cf. generally with 1. and note in addition.

a. The *œsophagus;* a thick walled tube lying at or near the middle of the whole section.

b. The *lungs;* thin walled spongy sacs (tubular in section) right and left of *a.*

c. The *liver;* occupying the greater portion of the pleuro-peritoneal cavity and lying immediately below *a.* and *b.*

d. The *heart;* situated in the middle ventral line.

e. The *neural canal and central nervous system;* relatively much larger than in Sect. 1.

f. The *body-wall;* in part inflected, giving rise to a sheet of muscle (so-called *diaphragm*) which is attached, on opposite sides, to the œsophagus.

g. The *aorta;* here paired (*aortic arches*).

h. The *pericardium;* a double-walled sac enclosing the heart, and related to it as is the pleuro-peritoneal membrane to the body-wall and its contained viscera.

i. The *pleuro-peritoneal membrane.* (Cf. generally with previous section.) Note that having suspended the organs above-named, it passes round the pericardium, giving rise to a fold (*falciform ligament*) which is reflected on to the body-wall below, there being thus formed a septum which completely subdivides the pleuro-peritoneal cavity, in this region, into two.

Its special folds are:

α. The *ligamentum latum;* suspending the lung to the œsophagus.

β. The *lesser omentum;* passing between the œsophagus and liver in the middle line.

γ. The *falciform ligament* (suspensory ligament of the liver). Cf. *supra*. If the section has passed through the anterior border of the liver, this will also be seen to pass between its outer edges and the body-wall.

j. The *sub-cutaneous lymph-spaces;* seen (in this and the preceding section) as a series of four spacious chambers, lying between the body-wall and integument, and separated from each other by membranous partitions.

k. The *anterior abdominal vein;* this will probably be seen, enclosed within the folds of the mesentery immediately below the liver.

3 Across the head; to pass through the mouth-cavity and eyes.

a. Note the absence of body cavity and the relatively great size of the neural canal and central nervous system (*brain*).

b. The *mouth cavity;* spacious, and enclosed on all sides in a soft mucous membrane.

c. The *tongue;* a median up-growth of the floor of the mouth, supported at its base by a cartilaginous plate, the *hyoid*.

d. The *eyes;* spherical sacs with firmly resistant capsules, closely applied to the outer integument. Note, in connection with them,

α. The *eye-muscles;* passing backwards and downwards, between the eye-ball and cranial wall.

β. The *eye-lids;* folds of integument above and below the eyes, continuous at their bases with a delicate fold of skin (*conjunctiva*) which invests

the outer face of the eye-ball. The upper lid is thick and fleshy; the lower (*nictitating membrane*) is thin and transparent.

c. The *sub-cutaneous lymph spaces:* present only above and below.

D. **The alimentary system.**

1. Obtain a male frog, and pin down upon its back under water. Remove the whole of the ventral integument and body-wall and dissect away the heart. Next remove the dorso-lateral portion of the body-wall, between the back of the head and the level of the hinder third of the large intestine, and with it the kidneys and testes. Carefully dissect away the mesenteric folds which support the liver and bladder, and wash clean.

a. The *general disposition* of the parts. Cf. Sect. B. 2 *a—f.*

b. The *pancreas:* a Y-shaped compact yellow mass lying in the mesentery between the stomach and head of the intestine.

c. The *gall-bladder:* a small green sac, lying between the right lobe of the liver and the head of the pancreas.

d. The *small intestine;* of uniform calibre throughout. It may be resolved into

α. The *duodenum;* a short straight segment running parallel with the stomach and related to the pancreas.

β. The *ileum;* the terminal coiled portion, destitute of connection with the pancreas.

e. The *large intestine;* cf. Sect. B. 2. *d.*

f. The *lungs;* apparent as sac-like diverticula of the gullet, immediately in front of the liver (leave them in place).

2. Lay open the interior of the stomach and duodenum. Wash carefully and examine in water under a hand lens.

a. The *stomach:* its lining membrane (*mucous membrane*) is smooth and thrown (if the viscus be not distended with food) into a number of irregular longitudinal folds or *rugæ.*

Note the nature of its contents; consisting of worms, insects, or other small animals in a partially digested state.

b. The *duodenum.* Note the shaggy nature of its lining membrane; it is sharply marked off from that of the stomach at

c. the *pylorus;* a valve-like fold, obvious as a constriction at the point of junction between *a.* and *b.*

d. The *ileum;* slit it open and examine under a hand lens.

α. Its *contents;* food material in a highly emulsified assimilable condition.

β. Its *lining membrane;* beset by numerous semilunar folds (*intestinal valves*) arranged in parallel series. These may be ill defined.

e. Open up, in like manner, the large intestine.

α. Its *contents;* food material in a dry state, little assimilable and green (*fæces*). Note that the colour of the same is identical with that of the bile seen in the gall-bladder.

β. Its *lining membrane;* smooth and comparatively thin.

3. *The liver, bile and pancreatic ducts.* Cf. Sect. B. 2. *a.* Gently squeeze the gall-bladder between the finger and thumb of the left hand, keeping your attention fixed on the duodenum and pancreas. The bile-duct will be thereby injected and the bile will be seen to enter the duodenum by a small orifice situated at about its middle.

a. The *common bile-duct;* its terminal third is plainly visible, as a delicate tube (now injected green) emerging from the pancreas to enter the duodenum. Follow it up; it runs through the pancreas towards the gall-bladder.

b. The *hepatic ducts;* variable in number. Seen, on moving the gall-bladder about, to pass from the liver and unite to form the main duct *a.*

c. The *gall bladder;* a blind sac, set on at the extreme end of the above system of ducts. Open it, and note that it communicates with the liver through the agency of the hepatic ducts alone.

d. *The pancreatic ducts;* one or more in number; seen, on careful examination, to enter the bile-duct as it courses through the pancreas.

4. *The cloaca and bladder.* Cut away the front of the pelvis with a stout pair of scissors, taking care not to injure the bladder: remove the front wall of the latter, together with a portion of that of the large intestine. Pin the bladder aside and examine under water.

a. The *cloaca;* the terminal portion of the alimentary

canal, at that point at which it receives the urino-genital ducts.

Examine its lining membrane; it is for the most part identical with that of the large intestine; that of its terminal third has the characters of the integument.

b. The *ureters* (*urinogenital ducts* ♂. Cf. Sect. F. 3); their openings will be seen as two minute orifices, on the dorsal wall of *a.*, situated close together and surrounded by tumid lips.

c. The *bladder;* a median ventral diverticulum of *a.*, having no direct communication with the ureters.

E. The cavity of the mouth and the respiratory organs.

1. Use the specimen dissected above. Cut the alimentary canal across immediately in front of the liver, and retain the anterior portion. Pin down under water ventral surface uppermost; enlarge the mouth opening by cutting through the sides of the buccal cavity with a pair of scissors: pull down the lower jaw and pin it back.

a. The two internal openings of the nasal cavities (*posterior nares*); near the anterior end of the roof of the mouth.

b. The openings of the *Eustachian recesses;* situated far back near the angles of the jaw.

c. The *teeth;* confined to the upper jaw and roof of the mouth.

a. The *maxillary teeth;* forming a parallel series, set along the inner face of the whole upper jaw.

β. The *vomerine teeth;* two oval patches situated close together near the middle line, immediately internal to the posterior nares.

d. The *tongue;* heart-shaped and projecting freely backwards; its free end is prolonged back into two fleshy cornua. Note its mode of attachment.

Examine its surface under a hand lens; it is beset by a number of elevations (*papillæ*).

e. *The glottis;* a median longitudinal slit, situated immediately behind the tongue, on the summit of an elevation of the floor of the mouth (*larynx*). Pass a probe down it and note that it enters the lungs.

Occasionally, the mucous membrane at its anterior end is prolonged into two short papillæ, which project forwards and probably represent rudiments of the *epiglottis*.

2. Close the mouth; dissect away the mylohyoid muscle and examine from beneath.

a. The *larynx:* forming a hard prominence in the middle line, between and in front of the lungs.

b. A series of small muscles will be seen arising on all sides and converging immediately in front of *a*. Dissect them away *on one side;* there will thus be laid bare the *hyoid* (cf. Sect. C. 3. *c*.). Examine this, in relation to the larynx.

α. Its *body;* a plate of cartilage closely applied to the floor of the mouth, immediately in front of the larynx.

β. Its *posterior cornua.* That of the exposed side will be seen as a bony rod (*thyro-hyal*) in close apposition with the outer border of the larynx,

immediately under cover of a small muscle (*constrictor laryngis*).

c. Examine the above-named muscles.

α. The *geniohyoid;* a band of tissue arising from the body and cornu of the hyoid and passing forwards to be inserted into the lower jaw.

β. The *hyoglossus;* arising from the posterior cornu and passing forwards in the middle line beneath *α.* to enter the substance of the tongue.

γ. The *sterno-hyoid;* its cut end will be seen passing up from behind to be attached along the outer border of the body and cornu of the hyoid.

δ. The *omohyoid;* its cut end will probably be found attached to the outer anterior border of the body of the hyoid (it arises from the shoulder girdle).

ε. The *petrohyoides;* a series of small slips lying immediately beneath *δ.* and passing between the hyoid and hind region of the skull.

3. Remove the ventral face of the right lung, and examine its interior under a hand lens.

a. The *lung sac;* thin-walled and distensible; highly vascular and thickened around the larger vessels which ramify within it.

b. The *bronchi;* short membranous tubes placing the lungs in communication with the laryngo-tracheal cavity.

4. Remove the respiratory organs together with the floor of the mouth, and pin the whole down on its side, left lung uppermost. Dissect away the floor of

the mouth to the level of the larynx and examine the *laryngeal cartilages.*

a. The *cricoid cartilage;* an annular tract, embracing the base of the larynx. It sends a spur down towards the base of each lung.

b. The *arytenoids.* That of the left side will be seen as a hood-shaped mass, surmounting *a.* and lying within the wall of the larynx.

Muscular fibres (*constrictors*) will be seen to pass between *a.* and *b.*

5. Turn the specimen over and remove all that remains of the right half, cutting to the level of the middle line.

a. The *laryngeal cavity;* spacious above and lined by a soft mucous membrane.

b. The *vocal cords.* That of the left side will be seen as a tense fold of the lining membrane, running antero-posteriorly. Note its intimate connection with the arytenoid cartilage.

c. Examine the relations of the laryngeal cartilages, as seen by following their cut edges.

d. Strip off the mucous membrane with care, so as fully to expose the cartilages for detailed examination.

These, and similar cartilaginous structures, may be isolated with ease by maceration in $\frac{1}{2}$ per cent. nitric acid solution, the excess of acid being afterwards removed by repeated washings in water.

F. **The Urinogenital organs.**

Dissect from the ventral aspect, proceeding as directed in Sect. B. Having laid bare the viscera, re-

move the alimentary canal between the base of the œsophagus and posterior third of the large intestine. Next open up the latter, as directed in Sect. D. 4; wash and examine under water.

1. In both sexes.

   a. The *kidneys;* symmetrically disposed on opposite sides; relatively largest in the male. Each is convex dorsally (outer border), incompletely lobed on its inner border.

   b. The *adrenal body;* a band of yellow tissue running along the ventral face of each kidney.

   c. The duct—*ureter* (female) or *genito-urinary canal* (male)—running from the outer side of the posterior part of each kidney to the cloaca. Open the cloaca and pass a bristle into one of their openings.

      In the male *R. esculenta* each duct is somewhat dilated after leaving the kidney: it then narrows again and opens on the dorsal wall of the cloaca by an oblique slit with sharply defined edges. In *R. temporaria* the duct does not dilate, or only very slightly; but on its outer side lies a glandular mass (*vesicula seminalis*), from the inner side of which a number of minute ducts open into the genito-urinary canal. The aperture of the latter in the cloaca is round and has tumid edges. In the female of both species the ureters are very slender.

   d. The *bladder;* (cf. Sect. D. 4. *c.*)

2. In the female.

   a. The *genital glands* (*ovaries*) (cf. Sect. B. 2. *h.* β.); varying much in size with the season of the year.

They overlie the kidneys as seen, each being suspended by a fold of mesentery (*mesoarium*).

b. The *genital ducts* (*oviducts*); convoluted tubes, not continuous with the ovaries, and running back to open into the cloaca. Each can be resolved into three segments.

α. A middle or *glandular segment;* forming the conspicuous coiled portion of the tube. It will be found to swell rapidly under imbibition of water.

β. A terminal or *uterine segment;* thin-walled and lying immediately below and external to the kidney. Examine it in relation to the cloaca; it will be found to open on the apex of a papilla, lying side by side with its fellow, immediately in front of the apertures of the ureters.

Inflate it; it is highly distensible.

If the Frog be killed during the breeding season, this segment will be seen to act as a receptacle for the eggs prior to oviposition.

γ. An anterior or *thin-walled segment;* short and straight; it passes behind (dorsal to) the lung and courses over the base of the same to open into the pleuro-peritoneal cavity antero-ventrally by a wide funnel-shaped mouth.

3. In the male.

a. The *genital glands* (*testes*), (cf. Sect. B. 2. *h. a*); varying in size with the season of the year, and each suspended by a fold of mesentery (*mesorchium*).

b. The genital ducts (*vasa efferentia*); delicate tubes lying within the mesorchium and placing each testis in communication with the inner border of the kidney of the same side.

c. The *genito-urinary canal* (*ureter*). Cf. Sect. 1. *c.*

Examine the seminal vesicle (*R. temporaria*) under a hand lens; it will appear to be continued forwards as an excessively delicate filament which can be traced, within the mesentery, as far as the base of the lung. Try to isolate this and follow it back; it will be found to skirt the outer border of the vesicula seminalis, becoming lost in the base of the ureter.

Not unfrequently one or more watery vesicles or *cysts* are set along the course of the above-named filament. More rarely it may be wholly or in part well defined and tubular, having the appearance of an immature oviduct, a vestige of which it really represents.

d. The *fat body* (cf. Sect. B. 2. *i.*). This is, in the male, very large. Rarely its basal portion may be swollen and pigmented, giving rise to an organ (*Bidder's organ*) having much the appearance of the ovary of the female and confluent with the head of the testis.

4. Remove a kidney and pin down under water, ventral face uppermost. Carefully dissect off the mesentery and examine under a lens; its whole surface will be seen to be studded by a number of minute orifices (*nephrostomes*), placing the interior of the kidney in communication with the pleuro-peritoneal cavity.

G. The skeleton.

The skeleton of a Frog may be prepared for examination by removing the viscera from the body, and roughly dissecting away the muscles and other soft parts. Then place the remainder in water and let it macerate for about a week; afterwards carefully pick away the soft parts, with forceps, from the bones and cartilages.

Obtain two such skeletons; allow one to dry slowly; preserve the other in weak spirit.

1. Its general arrangement.

a. The *axial skeleton*; consisting of the *vertebral column* and *skull*, both of which lie in the same antero-posterior plane.

b. The *appendicular skeleton*, lateral parts (*limbs* and *limb-girdles*), supported, directly or indirectly, by the axis.

a. The *fore-limbs*: their supporting *shoulder-girdle* or *pectoral arch*, not directly attached to the axial column; the *limb proper*; its main divisions, *humerus*, *radius* and *ulna* (the two latter ankylosed), *carpus* and *digits*.

b. The *hind-limbs*: their supporting *hip-girdle* or *pelvic arch*, directly attached to the vertebral column; the *limb proper*; its main divisions, *os femoris*, *tibia* and *fibula* (ankylosed), *tarsus*, *digits*.

2. The vertebral column; dried preparation.

It consists of an anterior segmented portion (each segment being a *vertebra*) and of a posterior unsegmented portion (*the urostyle*).

a. Examine carefully and draw various aspects of a detached *vertebra*, say the third.

α. Its solid flattened ventral part (*centrum*), with an anterior concave and a posterior convex surface (*procœlous*).

β. The *neural arch:* an arch of bone arising from the centrum dorso-laterally. It furnishes the undermentioned.

The *transverse process:* a bony bar arising on each side from the base of the arch and passing outwards and a little downwards.

The *articular processes* (*zygapophyses*); an anterior and posterior pair, springing from the sides of the arch; the *anterior* (*pre-zygapophyses*) having their smooth articular surfaces directed upwards; the *posterior* (*post-zygapophyses*) with similar surfaces directed downwards.

The short *spinous process;* springing from the dorsal aspect of the arch and directed backwards.

γ. The *neural canal;* bounded, above and at the sides by the arch, below by the centrum.

b. Examine the remaining vertebræ.

α. The first vertebra: its *body*, produced forwards into a wedge-shaped process (*odontoid process*) which lies between the occipital condyles of the skull: its *arch*, usually incompletely ossified in the region of the spinous process, which is rudimentary; posterior zygapophyses alone present: the large concave anterior facets (*condylar facets*), partly on the arch and partly on the centrum, for articulation with the skull.

β. The 2nd, 4th, 5th, 6th and 7th vertebræ: closely resembling the third, the chief differences being found in the varying size and direction of the transverse processes, all of which are smaller than those of the third vertebra.

γ. The 8th vertebra: concave at each end of its *centrum* (*amphicœlous*).

δ. The 9th vertebra (*sacrum*): its *centrum*; convex in front and with two convex tubercles behind: its powerful *transverse processes* directed upwards and backwards and expanded for articulation of the hip-girdle.

The characters of γ. and δ. are liable to variation.

Occasionally the vertebræ may be 8 or 10 in number.

c. Examine any two vertebræ from the side, in their natural positions.

α. The *zygapophyses*; cf. *supra*; note their modes of articulation.

β. The *neural spines*: short and backwardly directed.

γ. The *transverse processes*: sloping upwards and backwards.

δ. The *inter-vertebral foramina* (for exit of spinal nerves); interspaces left between each pair of neural arches, immediately below the zygapophyses.

Note that there is no corresponding passage between the skull and first vertebra.

d. The unsegmented portion of the column (*urostyle*).

α. Its anterior end, enlarged and bearing two articular concavities.

This region is liable to variation.

β. Its posterior end; tubular in the dried skeleton, but in the fresh state filled with cartilage which projects beyond it.

γ. The prominent arch along its dorsal surface; getting ridge-like and disappearing posteriorly.

δ. The small neural canal, enclosed by *γ*.

ε. The two minute foramina (for exit of the tenth pair of spinal nerves) at its extreme anterior end.

3. **The vertebral column, wet preparation.**

Cf. supra, and note in addition.

a. The great mobility of the segments upon each other. Parts in articulation will be found to be united by sheets (*capsules*) or bands (*ligaments*) of glistening white fibrous tissue.

b. Make a longitudinal vertical section of the entire vertebral column, and examine the cut surface.

α. The *vertebral bodies* (*centra*). Note the shapes and mode of articulation of their applied ends (*articular surfaces*); each will be seen to be capped in cartilage, the apposed faces being kept moist during life.

β. The *intra-vertebral bodies;* small gelatinous masses lying within the substance of *a*. They are most marked in young specimens.

γ. The *urostyle;* its body is cylindrical in section and largely filled with a gristly core, identical with β, which is continued back as a free termination to the whole axial skeleton.

δ. The *neural canal:* note especially its termination in a pointed extremity, lying within the arch of the urostyle.

c. Isolate the third vertebra, and examine—

α. Its *zygapophyses:* cf. Sect. 2. *c.* Each is capped in cartilage, in common with the other articular faces.

β. Its *transverse processes;* each terminating in a backwardly prolonged cartilaginous expansion.

4. **The skull; dried preparation.**

a. *General.* Examine from the dorsal aspect; it embraces

α. The *cranium;* median and enclosing the brain, sometimes termed the *brain-case.*

β. The *facial apparatus;* the system of outstanding bars, conspicuous as the jaw-apparatus.

γ. The *sense capsules.* The *auditory* capsules, paired postero-lateral expansions; the *olfactory* capsules, similar but less obvious antero-lateral expansions (they are better seen in the wet state. See *infra*).

b. Examine the *posterior end of the skull.*

α. The large aperture (*foramen magnum*) in the middle line, leading into the *cranial cavity.*

β. The convex surface (*occipital condyle*) on each side of the foramen magnum, for articulation

upon the corresponding concave facet on the front of the first vertebra.

γ. The *exoccipitals;* bearing the condyle on each side, and bounding the foramen magnum laterally.

δ. The *pro-otics;* lying immediately in front of γ. and running outwards on each side, forming the roof and front wall of the auditory capsule.

ε. The *squamosal;* a hammer-shaped bone, extending from the outer edge of δ. downwards and backwards towards the articulation for the lower jaw.

ζ. The *columella auris;* seen, at this stage, as a bony rod which underlies the pro-otic and stands out immediately behind the head of the squamosal. (It is best studied later on.)

c. The roof of the skull.

α. The *exoccipitals*, *pro-otics* and *squamosal.* Cf. supra.

β. The *fronto-parietals;* two long bones united by suture in the middle line, roofing in the cranium.

γ. The *nasals;* two triangular bones immediately in front of β. set transversely and a little obliquely.

δ. The *pre-maxillæ;* two small bones in front of γ., meeting in the middle line and bounding the front of the gape. Each sends up an *ascending process* which is directed towards the inner end of the nasal bone.

ε. The *maxillæ;* on either side a long bone bounding the greater part of the gape, and abutting against δ. in front. Each maxilla sends up an *ascending process*, which abuts against the outer end of the nasal.

ζ. The *quadrato-jugal* (*jugal?*); a delicate bone, on either side, bounding the hinder third of the gape and passing between the maxilla and squamosal.

d. The base of the skull.

α. The *exoccipitals*, *pro-otics*, *pre-maxillæ*, *maxillæ*, and *quadrato-jugals*. Cf. supra.

Note that each pre-maxilla sends back a short *palatine-process.*

β. The *parasphenoid;* a dagger-shaped bone running along the greater part of the floor of the cranial cavity.

γ. The *sphenethmoid* or *girdle-bone;* a large bone underlying the anterior portion of β. and enclosing the anterior third of the brain-case.

δ. The *palatines;* slender bones, one on each side, passing out transversely from near the anterior end of the parasphenoid to the body of the maxilla. Each underlies the corresponding nasal bone.

ε. The *vomers;* two broad irregular shaped bones, immediately in front of δ. These, in common with the maxillæ and pre-maxillæ, bear teeth. (Cf. Sect. E.)

ζ. The *pterygoids;* large triradiate bones, one on either side. Each abuts against the antero-ventral border of the auditory-capsule internally;

anteriorly it is prolonged forwards, flanking the maxilla, to meet the outer end of the palatine; posteriorly it runs downwards and backwards, parallel with the squamosal.

c. *The side of the skull.*

α. Compare generally with the foregoing; note especially the backward rotation of the squamosal, and the mutual relationships of the maxillo-jugal bones.

β. The *mandible* or lower jaw; it consists of two distinct halves, or *rami*, which meet in the middle line in front.

In each *ramus* three pieces may be made out —a main piece, which runs nearly to the middle line in front (*articulare*); a thin scale-like piece (*dentary*) flanking the outer anterior face of the former; and a small nodular one (*mento-Meckelian*) meeting with its fellow in the symphysial line.

5. **The skull; wet preparation.**

Remove the lower jaw and hyoid, and carefully dissect off from the rest of the skull, under water, the undermentioned bones. This may be done with comparative ease, if, holding the whole down with the left hand the bones of one side be removed, either by inserting the point of a scalpel beneath them or by the aid of forceps.

The bones to be removed are—*dorsal series*, fronto-parietals (one or both), nasal, squamosal, maxilla, pre-maxilla; *ventral series*, parasphenoid, vomer, palatine, pterygoid; care must be taken that none of the underlying cartilage is brought away with the latter.

There will thus be laid bare the **chondro-cranium** and its related bones.

a. *Dorsal aspect.*

α. The *chondro-cranium;* a cartilaginous mass enclosing the brain and olfactory and auditory sense organs, and bearing, laterally, an outstanding cartilage which is flanked by the palatine and pterygoid bones (*palato-quadrate cartilage* or *sub-ocular arch*).

β. The *sphenethmoid*, *pro-otics* and *exoccipitals;* seen to be formed as replacements of the chondrocranium in bone; they are inseparable from it, being termed therefore *cartilage bones*, by way of distinction from those superadded ones which have been stripped off (*membrane bones*).

Examine in detail.

γ. The *cranium;* its roof will be seen to be incompletely cartilaginous, bearing three membranous areas or *fontanelles*, all of which underlie the fronto-parietals. They are—a larger median one in front, extending forwards to the posterior border of the sphenethmoid; smaller paired ones behind, extending outwards to the inner border of the pro-otics.

δ. The *sub-ocular arch;* confluent, in front with the olfactory capsule, behind with the auditory capsule. Its *palatine bar;* standing out at right angles to the long axis, and expanded externally to form a plate (*orbital process*) for support of the maxilla (cf. opposite side). Its *pterygoid bar;* produced backwards, outwards

and downwards, into a stout rod or *suspensorium*, which lies between the pterygoid and squamosal and furnishes the articulation for the lower jaw.

ε. The *olfactory capsules;* confluent with each other and the cranium, and incompletely separated above by a median longitudinal furrow. The dorsal wall of each will be seen to be scroll-shaped and produced—in front, into a blunted *pre-nasal process*, which abuts against the inner face of the maxilla (cf. opposite side); behind, into a laminate *ali-nasal process*, which encircles the outer and hinder margins of the anterior nostril.

If the preparation be made with great care, the front wall of the narial passage will be seen to lodge a couple of minute *labial cartilages*, which overlie the base of the pre-nasal process.

b. Ventral aspect.

α. The *cranium;* completely cartilaginous below and at the sides. Note the limits of the cartilage bones.

Certain apertures (exits for cranial nerves) will be seen; they are best studied later.

β. The *sub-ocular arch;* in front, confluent with the expanded floor of the olfactory capsule; behind, in articulation with the ventral outer border of the auditory capsule.

γ. The *rhinal processes.* That of the dissected side will be seen as a small hammer-shaped cartilage lying near the middle-line in front, ventral to

the olfactory capsule. It projects freely forwards and inwards.

c. *Lateral aspect.*

Compare generally with *a.* and *b.* and note especially.

α. The *suspensorium;* its backward rotation; its rounded free end, for articulation of the lower jaw.

β. The *quadrate bone;* represented by an insignificant nodule lying within the substance of *a.* near its articular end. It may or may not be confluent with the quadrato-jugal.

d. *Posterior aspect.*

α. The *exoccipitals;* separated above and below by cartilage (i.e. there are no median occipital bones).

β. The *columella auris.* Remove this on the dissected side; and note that its inner end plugs a large aperture (*fenestra ovalis*) in the outer wall of the periotic capsule.

γ. The *suspensorium;* now seen, on the same side, in its entirety. Note; its *pedicle*, the main bar, prolonged down to give articulation to the lower jaw; its *dorsal cross*, confluent with the outer wall of the auditory capsule, and expanded, to form a ledge (*tegmen tympani*) which overlies the columella; its *ventral cross* (cf. *b.* *β.*) rounded, and in definite articulation with the antero-ventral border of the auditory capsule.

e. *Inner aspect.*

Remove all that remains of the chondro-cranium

on the side which has been dissected, cutting a little to one side of the middle line, thus reducing the whole to a condition of *vertical longitudinal section*. Examine from within.

α. The *cranial* and *nasal cavities*; note their limits and mutual relationships.

β. The *septum nasi*; a median plate of cartilage, separating the two nasal chambers in the middle-line (examine from the front).

γ. The *sphenethmoid*. Seen to consist of a posterior cranial portion which embraces the anterior third of the brain-case, and an anterior paired portion, which is similarly related to the bases of the olfactory capsules.

δ. The *exoccipital* and *pro-otic*. Examine these in relation, as seen from within (cf. § 4 *b*.).

ε. The *fronto-parietal*, *nasal*, *vomer*, and *parasphenoid* (if in place). Examine, in relation to the chondro-cranium.

ζ. The *rhinal process* (cf. § *b*. γ.); seen to form a support for the pre-maxilla, abutting against its inner face.

η. The *foramina for exit of the cranial nerves*—as under, on one side.

i. The *olfactory foramen* (I. Cranial); a perforation of the partition between the two portions of the sphenethmoid.

ii. The *optic foramen* (II. Cranial); obvious as a perforation of an oval membraneous area of the side wall a short distance behind the sphenethmoid.

iii. The *foramen of exit for V. VI. VII.*; a large aperture, below and in front of the pro-otic.

iv. The foramen for IX. and X.; perforating the body of the exoccipital.

v. The *auditory foramen* (VIII.); a small perforation of the cartilaginous wall of the auditory capsule just behind iii.

vi. The foramen for III.; small and situated immediately in front of iii.

vii. The foramen for IV. (*n. patheticus*) very small, above and a little in front of the optic foramen.

f. *The mandible.* See 4. *e.* β.

Strip off the dentary with care; there will be found, in close apposition with the outer face of the angulo-splenial,

a. *Meckel's cartilage;* a gristly rod, forming a core for the lower jaw. Examine its posterior end; it is alone concerned in articulation.

β. The *mento-Meckelian bone*; a small nodule arising as an ossification of the symphysial end of *a*.

6. **The hyoid.**

a. Its broad somewhat tetragonal central part (*body*), bearing a number of outstanding processes, for attachment of muscles (cf. Sect. E. 2. *c.*). This is usually cartilaginous, being ossified only in old individuals.

b. Its *posterior cornua*, or *thyro-hyals;* short bony rods, sloping obliquely backwards and outwards.

c. Its *anterior cornua,* slender cartilaginous rods, arising from the front of the body on either side: each is long and curved, running at first forwards, then backwards and outwards, and finally forwards and upwards, to become attached to the periotic capsule below the fenestra ovalis.

7. **The limbs and limb-girdles.**

These are best studied in the wet state. In dealing with the carpus and tarsus, no difficulty will be found if treated as follows. Isolate sufficient of the limb-skeleton to embrace the carpus or tarsus and pick away any fragments of the soft parts which may remain; immerse in spirit for a few hours; transfer to absolute alcohol for 1 hour, thence into oil of cloves. The latter medium will thoroughly clarify the preparation, and it may be kept in it for an indefinite period.

The shoulder-girdle and its related structures.

a. *Their general arrangement:* they form an incomplete ring round the fore part of the trunk, composed partly of bone, partly of cartilage. Note the cavity (*glenoid fossa*) with which the fore-limb articulates.

Examine from the ventral aspect; the whole is seen to consist of

α. *Paired elements;* symmetrical on opposite sides and meeting each other in the mid-ventral line.

β. *Median elements;* in corresponding relationship anteriorly and posteriorly with the applied ventral ends of *a.*

b. The *paired elements.* Flatten out the whole on one side, so as to obtain a lateral view. In the region of the glenoid fossa there will be seen a sharp line of demarcation between a dorsal *scapula segment;* and a ventral *coracoid segment*, the middle area of which is fenestrate (*coracoid fontanelle*).

α. The *scapula segment;* subdivided into a thin expanded partially ossified dorsal portion (*supra scapula*) and a stouter bony piece (the *scapula*) which furnishes the dorsal half of the glenoid fossa.

β. The *coracoid segment;* largely ossified, and composed of a smaller anterior *clavicle*, and a stouter posterior *coracoid*, the latter furnishing the ventral half of the glenoid fossa.

c. *The median elements.*

α. The *xiphisternum;* a median cartilage, ensheathed in bone, abutting against the applied ends of the coracoids. It expands posteriorly to form a heart-shaped plate, notched from behind (*xiphoid process*).

β. The *omosternum*, a slender cartilage ensheathed in bone, similarly related in front. It also ends in a cartilaginous expansion.

It is doubtful if the above really represent sternal elements, especially in the case of the anterior one.

The skeleton of the fore-limb. (Cf. Sect. A. 2. *c.*)

a. The *arm-bone* (*humerus*).

α. A somewhat cylindrical bone, composed of a middle *shaft* with an *articular expansion* at each end.

β. The great ridge (*deltoid crest*) on its antero-internal surface, to which a muscle (deltoid) was attached.

The development of this crest is greater in the male than in the female.

b. The *bone of the forearm.*

α. Excavated above to receive the lower end of the humerus.

β. Shewing below two articular heads separated by a notch, which is the last trace of division between the two bones of which it is made up; viz. the *radius* and the *ulna*.

γ. Pull the limb out straight, palmar surface downwards, so that it comes to be situated at right angles to the long axis of the body. The radius and pollex lie on the anterior or *pre-axial* side, the *ulna* and little finger on the posterior or *post-axial* side, of a line drawn through the axis of the whole limb.

c. *The digits.*

Five in number, the first (radial one) rudimentary: beginning at the outer or ulna side, we find—

α. The fifth digit: it presents a cylindrical proximal bone (*metacarpal*) followed by three others (*phalanges*), each shorter than its predecessor.

β. The fourth digit: a *metacarpal bone* and three *phalanges.*

γ. The third digit: a *metacarpal bone* with two *phalanges.*

δ. The second digit: a *metacarpal bone* with two *phalanges.*

e. The first digit (*pollex*) supported only by a small *metacarpal bone*.

d. The *carpus*. Proceed as directed at the outset, and examine under a low power. Its elements can be resolved into three sets.

α. *Proximal elements;* two in number, articulating, side by side, upon the radius and ulna respectively.

β. *Distal elements;* three in number. The outer one gives articulation to the three outermost digits; and is mainly connected with the outer element of the proximal series. The two inner ones give articulation to the pollex and second metacarpal respectively.

γ. The *central element* (*centrale*); pre-axial in position, and wedged in between the two innermost elements of the distal series and the corresponding proximal piece.

The hip girdle.

a. Its general form: V-shaped, with the apex directed backwards. It consists of paired elements which unite in the middle line and are in direct articulation with the vertebral column (sacrum).

b. The fossa (*acetabulum*) on each side, for articulation of the hind-limb.

c. Examine from the side. The whole will be seen to be subdivided, by a triradiate fissure running through the acetabulum, into three pieces, viz.—

α. The *pubis;* a small triangular wedge-shaped piece (cartilaginous except in old frogs); ventral

in position and furnishing the middle third of the acetabulum.

β. An anterior elongated piece (*ilium*); subcylindrical in front, behind, laterally compressed (*crista ilii*); it furnishes rather more than the anterior third of the acetabulum. Its articulation with the sacrum is effected through the agency of a cartilaginous *supra-ilium;* related to the ilium, as is the supra-scapula to the scapula in the shoulder-girdle (cf. p. 69).

γ. A posterior, laterally compressed piece (*ischium*); united with its fellow in the middle line. It meets the ilium above, below it is separated from it by the pubis; it furnishes rather less than the posterior third of the acetabulum.

The skeleton of the hind limb.

a. The *thigh-bone* (*os femoris*); its cylindrical shaft and rounded articular ends.

b. The *leg-bone* (*os cruris*).

α. A long cylindrical bone, expanded at its articular extremities. Its upper end; excavated for articulation upon the femur. Its lower end; rounded and notched to give passage to the tendon of the tibialis posticus muscle (Sect. H. 7. α. β.).

β. The longitudinal grooves on it; indicating that it is really made up of two united bones, the *tibia* and the *fibula.*

γ. The *nutritive foramen* (for passage of nerves and blood-vessels into its interior); a small slit-like perforation of the middle of the shaft.

Look for similar perforations in the other bones examined.

δ. Pull the limb out straight, plantar surface downwards, as directed for the anterior appendage. The tibia and hallux are pre-axial; the fibula and little-toe post-axial.

c. The *tarsus.*

Its proximal elements are seen as two elongated bones (separate in the middle but confluent at their cartilaginous extremities) in articulation with the ankylosed tibia and fibula; the pre-axial, or tibial, of these is the *astragalus;* the post-axial or fibular, the *calcaneum.*

d. The *digits.* Five in number; the internal one the shortest, the fourth the longest. Their composition—

α. The first, or *hallux* (the most internal); a *metatarsal bone,* followed by two *phalanges.*

β. The second: same as α, but longer.

γ. The third: a metatarsal bone with three phalanges.

δ. The fourth: a metatarsal bone and four phalanges.

ε. The fifth: like the third, but a little shorter.

e. The *tarsus* and *calcar.*

Remove the middle portion of the foot-skeleton, to include the apposed ends of the astragalus and calcaneum and the metatarsals; treat with alcohol and oil of cloves as previously directed, and examine under a low power.

α. The *distal tarsal series;* two in number in the adult and imperfectly ossified; viz.—a larger compressed post-axial element, giving articulation to the second and third digits; a smaller nodular pre-axial cartilage (*hallux tarsal*), lying in the interspace between the heads of metatarsalia 1 and 2 (it may be absent).

β. The *central element* (*naviculare*); triangular and preaxial, interposed between the astragalus, and hallux metatarsal.

γ. The *calcar* (*pre-hallux*); seen to be composed, except in very old frogs, of three pieces, identical, in their characters and relationships, with the component parts of a digit. It is carried by β. In very young specimens a fourth segment may be detected.

H. **Myology; as illustrated in the hind limb.**

(For the following dissection it is desirable to have a frog which has been lying some time in spirit.)

Pin the animal down on its back, and remove the skin from the hind limbs.

1. **General.**

In dissecting the muscles, separate them gently from one another, tearing through the *connective tissue* which unites them.

a. Each is chiefly made up of a main mass, or *belly*.

b. At both ends, in most cases, the belly is replaced by dense shiny tissue forming a *tendon*.

c. The tendons are fixed directly or indirectly to some of the neighbouring bones, the less moveable attachment is termed the *origin* of a given muscle; the more moveable its *insertion*.

2. **The superficial muscles on the front of the thigh.**

a. The *sartorius:* a thin ribbon-like muscle running down the middle; it *arises* from the symphysis pubis and is *inserted* into a tendinous expansion (*aponeurosis*) on the inner side of the knee-joint.

b. The *rectus internus major:* a large muscle running along the whole inner side of the thigh; arises from the symphysis pubis below the sartorius and is inserted into the same aponeurosis as that muscle.

c. The *rectus internus minor:* a thin muscle lying inside and rather behind the rectus internus major. It arises from the pelvis close to the anus and is inserted into the aponeurosis about the knee-joint.

d. The *triceps femoris:* a powerful muscle on the outer side of the thigh (it may be best studied later).

3. **The deep muscles of the front of the thigh.** Cut across the belly of the sartorius, and turn its ends out of the way; there will thus be laid bare—

a. The *adductor magnus:* a powerful muscle lying immediately above the rectus internus; it arises from the pelvis, between the origin of the sartorius and that of the rectus internus major. Its fibres are inserted directly (*i.e.* without the intervention of a specialised tendon) into the inner side of the distal half of the femur.

b. The *adductor longus:* a thin band immediately internal to the sartorius; it arises from the anterior border of the symphysis pubis and joins the adductor magnus at its insertion.

Force the adductors longus and magnus well apart (noting the insertion of the latter) and pin them back if needful; there will thus be exposed—

c. The *pectineus:* a small muscle, arising from the front of the pelvis, close to the symphysis, and inserted into the anterior surface of the distal half of the femur.

d. The *adductor brevis:* a small muscle lying on the inner side of the pectineus, close to which it arises and is inserted.

Sever the rectus internus major and r.i. minor and turn their cut ends back; thrust the adductor magnus forwards; there will thus be laid bare—

e. The *semitendinosus:* this is a long slender muscle bifurcated at its upper end: its two *heads*, thus formed, arise, one (*anterior head*) from the pelvis between the ischial symphysis and the acetabulum; the other (*posterior head*) from the ischial symphysis: the muscle terminates below in a rounded tendon which is inserted along with the sartorius.

Its anterior head enters into a close relationship with the adductor magnus.

4. **The superficial muscles on the back of the thigh.**

Turn the frog over on to its belly, and remove the skin from the back of the limb.

a. The *triceps femoris:* a very large muscle on the outer side, divided above into three heads, which are often regarded as separate muscles, viz.

α. The *vastus externus:* its outer head; it arises

from the hinder edge of the iliac bone. Cut it across and reflect the two halves.

β. The *vastus internus:* its inner head; a very large muscle on the outer anterior aspect of the thigh, arising from the pelvis close to the hip-joint; it unites with *a.* to form a common tendon of insertion into the aponeurosis on the front of the os cruris.

γ. The *rectus femoris anticus:* its middle head; a small muscle arising from the postero-ventral border of the iliac bone and inserted along with the above.

b. The *biceps femoris:* a long thin muscle, lying along the inner side of the vastus externus; it arises from the iliac bone above the acetabulum; below it divides into two slips, one of which is inserted into the middle of the shaft of the femur, while the other ends in a rounded tendon which is inserted into the back of the distal end of the same bone.

c. The *semimembranosus:* a large muscle immediately below the biceps; it arises from the upper posterior part of the iliac symphysis and is inserted into the aponeurosis round the knee-joint.

5. **The deep muscles of the back of the thigh.**

Divide and reflect the biceps femoris and semimembranosus; there will thus be exposed:—

a. The *glutæus:* a short thick muscle arising from the hinder two-thirds of the external border of the ilium; it runs down between the vastus externus and the rectus anticus to be inserted, by a

powerful tendon, into the back of the head of the femur.

b. The *ileo-psoas:* it arises from the internal surface of the posterior end of the ilium and is inserted into the upper and outer border of the shaft of the femur, immediately above the lesser slip of the biceps.

c. The *pyriformis*. This arises from the hinder end of the urostyle and, passing inside the vastus externus and biceps femoris, is inserted into the shaft of the femur.

d. The *adductor brevis* (cf. § 3. *d.*); a small muscle lying immediately beneath *c.*

e. The *adductor magnus* (cf. § 3. *a.*); a fleshy mass lying immediately beneath the semimembranosus.

6. **The rotator muscles of the femur.**

Pin the whole down on one side, and remove all the muscles hitherto examined with the exception of the gluteus (5. *a.*), leaving their cut ends. Turn these well back, noting their relations; there will thus be laid bare:—

a. The *gluteus* (cf. *supra*); its whole course can now be followed.

b. The *quadratus femoris*, arising from the postero-dorsal border of the ilium and inserted into the posterior upper border of the shaft of the femur, side by side with the pyriformis (5. *c.*).

c. The *obturatorius:* this is best seen on reflection of *b.* It arises from the postero-ventral area of the hip-girdle to which it is closely applied; its

tendon of insertion passes obliquely forwards and downwards immediately beneath that of the gluteus, to reach the head of the femur.

7. **The muscles of the leg.**

a. Turn the frog on its back, and remove the skin from the foot: fasten the same down, dorsal surface upwards. The os cruris will now be seen running down the middle of the leg. Lying on its inner side are two muscles, viz.:—

α. The *gastrocnemius:* a muscle with a great fleshy belly; it arises above by two tendons; one (much the larger) starts in the region of the knee-joint partly from the femur, partly from the os cruris; the other arises from the aponeurosis adjacent. Below, the muscle ends in a great tendon (*tendo Achillis*), which terminates in an aponeurosis on the plantar surface of the foot.

β. The *tibialis posticus:* a slender muscle covered in the main by the gastrocnemius; it arises from the posterior surface of the os cruris and, passing to the inner side of and through the ankle-joint (cf. p. 72, *leg-bone*), is inserted into the astragalus.

b. α. and β. act as *flexors* of the foot; on the opposite side of the leg lie the following *extensors.*

α. The *peroneus:* the largest and most external; it arises from the outer side of the distal articular end of the femur and, running across the ankle-joint, is inserted into the calcaneum.

This muscle is best seen from the dorsal aspect.

β. The *tibialis anticus:* a small muscle beneath and dorsal to the peroneus; it arises from the front of the lower end of the femur and from the capsule of the knee-joint; below it divides into two slips which are inserted into the dorsal surface of the astragalus and calcaneum respectively.

γ. The *extensor cruris brevis:* this lies internal to the upper part of the last muscle; it arises from the front of the distal articular end of the femur and is inserted into the middle third of the os cruris.

δ. The *flexor tarsi anterior:* a small muscle, best seen on reflecting the tibialis anticus; it arises where the extensor cruris ends, and is inserted into the dorsal face of the astragalus.

I. The blood-vascular system.

The dissection of the blood-vessels may be facilitated by previous injection, as directed in the Appendix C. If a permanent preparation be desired gelatine or plaister of Paris may be preferably employed. For ordinary working purposes, there is nothing more convenient than a mixture of French blue, injected wherever desirable with a medicine dropper (see Appendix).

1. The heart.

Obtain a freshly-chloroformed Frog and pin down on its back; slit up the ventral integument along the middle line as directed in Section B, and pin back the two flaps, avoiding stretching. Next raise

the xiphisternum with forceps, and remove the entire ventral portion of the shoulder-girdle, being very careful not to injure the underlying blood-vessels. Raise the pericardium in like manner and remove sufficient of it to expose the heart.

a. *External anatomy of the heart.*

α. Its posterior conical thick-walled portion (*ventricle*) with the apex directed backwards.

β. The *truncus arteriosus;* a sub-cylindrical part, arising from the right side of the base of the ventricle and dividing anteriorly into two (*aortic arches*).

γ. The *atrium;* thin-walled, rounded, lies on the dorsal aspect of the truncus, in front of the ventricle. Its subdivision into the two auricles is not visible from this aspect.

δ. Carefully raise the ventricle: lying beneath it (that is, on its dorsal side) will be seen the *sinus venosus;* an elongated thin-walled sac, seen to be in immediate relationship to the atrium and in connexion with the great veins, which enter it through the dorsal wall of the pericardium.

b. *The pulsation of the heart.*

α. Watch the movement carefully; it is a regularly alternating series of contractions and dilatations.

β. It will be seen that the two auricles contract together; immediately after them, the ventricle; and then, instantly, the truncus arteriosus.

γ. Raise the ventricle so as to see the sinus venosus; it will be found to contract immediately before the auricles.

2. **The anterior abdominal vein.** (Cf. Sect. B. 1. *c.* a.)

This is seen to receive a series of paired *epigastric veins*, which run in the tendinous intersections of the ventral muscles.

Carefully dissect the body-wall away from it, until liberated for its whole course. Note—

a. Its *origin;* by the fusion in the middle line of a couple of short veins (*pelvic veins*), lying at the base of the pleuro-peritoneal cavity and bringing back blood from the hind-limbs.

b. Its *distribution.* Trace it forwards; it runs beneath (dorsal to) the ventricle: on raising the latter it will be found to divide into two branches, one of which goes to each lobe of the liver (*afferent hepatic veins*).

c. Its *lesser factors.*

α. The *epigastric veins;* described above.

β. The *vesical vein;* entering its base, and bringing in the blood from the urinary bladder and intestinal wall immediately adjacent.

γ. The *cystic vein;* a small vessel, usually entering the right afferent hepatic branch, bringing back the blood from the gall-bladder.

δ. The *cardiac vein;* a minute vessel, seen, on displacing the ventricle, bound up in a special fold of the pericardium. It brings back the blood from the wall of the truncus arteriosus.

3. **The hepatic portal vein.**

Raise the left lobe of the liver; the main trunk of the above will be seen running through the head of the pancreas to enter the same; it communicates

with the anterior abdominal vein by a large vessel (*systemico-portal anastomosis*).

Note that it is formed by the union of those vessels bringing back the blood from the alimentary canal and its appended glands (its factors may be better studied in detail later on).

Frequently, but not invariably, some of the veins (*gastric veins*) which carry back the blood from the stomach, enter the liver independently of the rest.

4. **The renal portal veins.**

Remove sufficient of the alimentary canal to fully expose the kidneys. The above-named veins will be seen skirting the outer borders of the same; that of one side may be examined in detail.

a. Its *origin;* when traced back it is seen to arise, side by side with the pelvic vein (1. *a.*) from the bifurcation of a vessel which skirts the outer border of the thigh (*femoral vein*).

b. Its *distribution;* on reaching the kidney it is seen to break up into a number of *afferent renal veins*, for distribution to that organ.

c. Its *remaining factors.*

α. The *sciatic vein;* a large vessel seen, on carefully dissecting away the hip-girdle, to bring in the blood from the deeper parts of the hind-limb.

β. The *dorso-lumbar vein;* a well-defined vessel joining it anteriorly on a level with the kidney; it brings back the blood from the body-wall adjacent.

γ. The *oviducal veins;* two or three vessels entering it (in the female) immediately behind *β*.

5. **The inferior vena cava.**

Remove the stomach and intestine, being careful not to injure the liver. The above-named vein will be obvious as a large vessel lying between the kidneys; follow it forwards; it will be seen to pass behind (dorsal to) the liver, to enter the sinus venosus. Its factors are—

a. The *efferent renal veins;* a series of vessels arising from the ventral faces of the kidneys.

b. The *genital veins;* variable in number and detailed relationships, bringing in the blood from the genital glands.

c. The *hepatic veins* (*efferent hepatic vessels*); two or more in number, seen, on turning the liver over, to enter the vena cava just before it reaches the heart.

6. **The veins of the superior caval system.**

Little trouble will be found in following these if the ventral portion of the shoulder-girdle and its related pectoral muscles be carefully removed.

a. The *superior venæ cavæ;* short vessels seen to enter the sinus venosus on either side, immediately beneath the atrium. Each is formed by the union of the three following factors.

a. The *external jugular vein,* running down the under side of the throat and bringing back blood from the superficial parts of the head. It is formed by the confluence of the *lingual vein* from the tongue, and the *inferior maxillary vein* which skirts the outer side of the lower jaw.

β. The *innominate vein;* formed by the union of the *internal jugular vein*, seen just behind the angle of the jaw, and bringing back the blood from the brain, spinal cord, and deep-seated parts of the head, with the *subscapular vein* returning the blood from the muscles of the shoulder.

γ. The *axillary vein;* formed by the union of the *subclavian vein* which returns the blood from the arm, with a large vessel (*great cutaneous*) bringing back the blood from the integument.

7. **The pulmonary veins.**

These are exceedingly conspicuous in spite of their small size, on account of the black densely pigmented nature of their walls. On raising the ventricle they are seen arising along the inner faces of the lungs and uniting just before entering the left side of the atrium (l. auricle); the right one is, of necessity, the longer of the two.

8. **The system of the aortic arches.**

These may be dissected in the same animal; there are three sets of vessels on each side, arising from the truncus arteriosus. They may be readily distinguished from the great veins by their pinkish colour, due to the presence of a small quantity of blood, and by the thickness of their walls, the latter being very obvious in the case of any one of the larger arteries as whitish contour lines.

α. The *anterior or carotid arch*, concerned with the blood supply to the head; it arises on each side as a short trunk (*common carotid*) which early subdivides into

α. The *external carotid* (*lingual artery*) to the tongue and floor of the mouth; it and the common carotid together accompany the external jugular vein.

β. The *internal carotid;* passing outwards and upwards to reach the deep-seated parts of the head. Its branches accompany most of the factors of the internal jugular vein.

γ. The *carotid gland;* a pigmented enlargement at the base of β.

b. The *middle or aortic arch:* this is the largest of the three: it runs round the throat towards the vertebral column, and unites with its fellow to form the *dorsal aorta* (this may best be followed in detail later). It gives off, on either side, at the level of the arm.

α. The *subclavian artery:* mainly distributed to the fore-limb.

β. The *vertebral artery:* arising immediately in the front of α, and distributed to the vertebral column and the back, together with the superficial parts of the head and face (occipital branch).

c. The *posterior or pulmonary arch;* it runs to the root of the lung, giving off on its way a *cutaneous branch* which passes out to the integument of the trunk, and supplies most of the blood which is carried back by the great cutaneous vein.

9. **The dorsal aorta and its branches.**

Follow the aorta back; it will be found to run along the middle line beneath the vertebral column giving off in order

a. The *cœliaco-mesenteric artery*, to the alimentary organs; the cut end of this will be seen arising either at the point of union of the two aortic arches or, more generally, from the base of the left arch.

Very rarely this is represented by distinct cœliac and mesenteric trunks.

b. The *renal arteries*, to the kidneys; variable in number, arising from the aorta as it passes between the kidneys.

c. The *genital arteries*, to the reproductive glands; usually arising from the anterior renal vessels.

d. The *inferior mesenteric artery;* a small vessel arising far back and supplying the base of the large intestine.

e. The *common iliac arteries;* arising as an apparent bifurcation of the dorsal aorta.

Very rarely, the aorta is continued back, beyond the point of origin of the above, as a delicate *median sacral* or *caudal artery*.

a. The *hypogastric arteries;* arising, one on each side, from the iliacs, just before they leave the body-cavity, and distributing branches to the urinary bladder and body-wall adjacent.

10. **The hepatic-portal system in detail.**

Pin a newly-killed Frog down upon its left side, and remove the body-wall and arm of the right one. Reflect the skin of the back and remove the generative organs; dissect off the pericardium and liberate the right lung. Displace the alimentary canal turning the stomach and liver forwards and the large intestine well back; and pin down under water.

i. The *hepatic portal vein* will now be seen emerging from the head of the pancreas to enter the left lobe of the liver (cf. § 3); it is formed by the union of the following factors.

a. The *gastric vein;* bringing back the blood from the stomach.

b. The *superior mesenteric vein:* a very short vessel running through the head of the pancreas and receiving

α. The *duodenal veins;* bringing in the blood from the duodenum and pancreas.

β. The *lieno intestinal vein;* formed by the union of the *ileal* from the ileum, the *hæmorrhoidal* from the large intestine, and the *splenic* or *lienal* from the spleen.

ii. The *cœliaco-mesenteric artery* may now be traced to its ultimate distribution. Its origin has been described at § 9 *a*; it breaks up near the head of the pancreas into *a* and *b*.

a. The *cœliac artery;* distributing branches to the stomach, liver and pancreas,

b. The *superior mesenteric artery:* supplying the intestine and spleen. The branches of this vessel accompany the factors of the superior mesenteric vein.

c. The *inferior mesenteric artery;* arising from the dorsal aorta far back and supplying the base of the large intestine (cf. § 9 *d*).

iii. Remove the hinder half of the hip-girdle and the head of the femur. Scrape away the liver to the

level of the vena cava inferior, and dissect away the veins of the head.

The dissection, as it now stands, presents a general view of the blood-vascular system; especially noticeable are the following.

a. The *renal portal system* (§ 4). The femoral and sciatic veins will be seen to be united by an anastomosing trunk, which encircles the outer side of the thigh and receives veins from the adjacent integument.

b. The whole course of the *vena cava inferior* (§ 5).

c. The *aortic arches* (§ 8). Examine these with care; the middle one (aorta) may or may not be connected with the other two, one or both but most generally with the carotid arch, by a longitudinal cord-like *ductus Botalli*.

That portion of the ductus which connects the aortic and pulmonary arches, and which is least constant in the Frog, is also known as the *ductus arteriosus*.

11. **The anatomy of the heart.**

The examination of the heart requires a good deal of care and the use of a lens of low magnifying power, and it may be greatly facilitated by working as follows. In a chloroformed Frog the heart is distended with blood when it ceases to beat. When all signs of contractility have disappeared, the distended heart should be removed from the body together with the lungs and sufficient of the adjacent parts to leave the terminations of the great veins and the origins of the aortic trunks intact. The whole should

then be placed in tolerably strong spirit until thoroughly set.

i. When sufficiently hardened, remove the lungs and adjacent parts, so as to isolate the heart together with the cut ends of the great vessels. Examine as under.

a. *Ventral aspect.* Work over the external characters described in § 1.

b. *Dorsal aspect.* Note especially—

α. The *sinus venosus;* spacious and elongated, receiving the three caval veins.

β. The *atrium;* if the heart be well distended a slight constriction will be seen subdividing this into a larger *right auricle* and a smaller *left auricle.*

γ. The *pulmonary veins* (cf. § 7); seen to converge immediately in front of the sinus venosus, to enter the inner border of the left auricle.

δ. The *sinu-auricular aperture;* seen on opening up the sinus venosus and removing its contents. It communicates with the cavity of the right auricle and is guarded by two membranous flaps (*sinu-auricular valves*).

ii. *General dissection from the front.*

Pin down ventral surface uppermost, and dissect with great care, under water. Remove the front wall of the ventricle, atrium and truncus arteriosus, and lay open the bases of the aortic arches; carefully remove the coagulated blood which fills them and wash clean.

a. The *ventricle;* its single ventricular cavity and thick spongy wall.

b. The *auriculo-ventricular aperture;* situated to the extreme left (right of the preparation) of the ventricle, and bounded by two *auriculo-ventricular valves.* Raise the nearest of these, if not already removed, and dissect it away.

c. The *atrium;* its thin wall; its spacious cavity completely subdivided into two chambers (r. and l. auricles) by a longitudinal *inter-auricular septum,* lying well to the animal's left side. Examine the relations of this to the auriculo-ventricular aperture; it is prolonged down on to the valves which guard it, subdividing it into two.

α. The *sinu-auricular aperture;* large and situated near the middle of the right auricle immediately adjacent to the inter-auricular septum. Note its valvular margins.

β. The *aperture of the pulmonary veins;* small and rounded, opening near the top of the left auricle, close to the septum.

γ. The *auriculo-ventricular valve;* note its characters and mode of attachment.

d. The *truncus arteriosus;* arising from the extreme right-hand corner of the ventricle and largely subdivided into two by a *longitudinal valve* (cf. p. 18). Examine the aortic arches in relation, passing bristles into them; they arise as under

α. The *aortic and carotid arches;* from its anterior end, immediately in front of the longitudinal septum, the calibre of the aortic arch being much the greater of the two.

β. The *pulmonary arches;* the pair arise by a single aperture lying immediately to the animal's left of the anterior end of the longitudinal valve.

iii. A companion dissection may be profitably made from the left side, for general comparison with the foregoing, especially with respect to the relations of the auriculo-ventricular valves and inter-auricular septum.

12. **The circulation of the blood in the web of the foot.**

i. Get a piece of thin board, about 5 inches long and 2½ broad; in the middle of one end of it cut a V-shaped notch about the size of the expanded web: place the frog on the board, belly downwards, and fix it by passing round it two or three turns of tape: next tie threads round the toes of one foot, and by means of them spread out the web over the notch, taking great care that it is only very lightly stretched. The animal should be kept moist by spreading a piece of wet blotting-paper over its back.

ii. Examine the web with 1 inch obj.: Note—

a. The black *pigment-cells* in the skin; sometimes irregularly branched; sometimes more compact.

b. The close *network of blood-vessels*, lying deeper than the pigment-bearing layer.

α. The *arteries*, running mainly towards the free edge of the web, and constantly diminishing in size as they break up into branches; the blood-flow, from larger to smaller branches.

β. The *capillaries*, in which the arterial branches end: small vessels forming a close network and frequently branching or anastomosing without much alteration in calibre.

γ. The *veins*, formed by the ultimate union of the capillaries, and increasing in size as they unite with one another; the blood-flow, from smaller to larger factors.

c. The *nature of the blood-flow;* the current is marked by the solid bodies (*corpuscles*) carried along in the blood-fluid: it is most rapid in the arteries; slowest, and most uniform, in the capillaries.

iii. Place a small drop of water on a bit of thin mica or of a thin cover-slip, and place the same, water downwards, gently on the web: then examine with $\frac{1}{4}$ or $\frac{1}{8}$ obj.; note—

a. The *walls of arteries, capillaries, and veins.*

α. The arterial walls, tolerably thick, seen as a clear well-defined line on each side of the blood-stream.

β. The capillary walls; difficult to see; apparent as thin slightly transparent boundary lines.

γ. The venous walls; much like the arterial.

b. The *blood-flow in the small arteries.*

α. The rapid stream in the middle, containing most of the red corpuscles.

β. The slower stream along the edge (*inert layer*), containing many granular-looking colourless corpuscles.

c. The *flow in the capillaries;* much slower than in the arteries; the frequent distortion of the red corpuscles in the capillaries under pressure; the elasticity of the corpuscles, as indicated by the readiness with which they recover their shape when the cause of distortion is removed; the manner in which the white corpuscles creep along, and their tendency to stick to the capillary wall.

J. **The lymphatic system.** The full study of this system is beyond the scope of this work; the undermentioned may however be made out with comparative ease.

a. The *sub-cutaneous lymph-spaces;* cf. Sect. C. 2. *j*.

b. The *cisterna magna;* cf. Sect. C. 1. *g*. γ.

c. The *circum-œsophageal lymph sinus;* a loose fold of mesentery embracing the base of the gullet, opalescent in appearance. It becomes exceedingly conspicuous if inflated, by introducing the point of a blow-pipe beneath the adjacent peritoneum.

d. The *lymph-hearts;* small thin-walled sacs, paired and pulsatile; to be sought for while still beating, immediately on the death of the Frog. They are, on either side—

α. The *posterior lymph-heart;* to be seen on removing the skin of the back, lying a short distance in advance of the cloacal aperture

and immediately in front of the pyriformis muscle (Sect. H. 5. *c*).

β. The *anterior lymph-heart*; lying in an interspace between the small muscles (*intertransversi*) which pass between the transverse processes of the third and fourth vertebræ. Best seen on slitting open the body-wall ventrolaterally and cutting into the cisterna magna.

The cartilaginous free end of the transverse process of the third vertebra is expanded (cf. Sect. G. 3*c*) for protection of the above.

K. **The nervous system.**

1. The *cerebro-spinal axis and neural canal.* Take a recently-killed frog and divide the skin along the dorsal middle line and reflect it on each side; remove the muscles which overlie the arches of the vertebræ, and open the neural canal as follows. Divide the membrane (*vertebra-occipital ligament*) which passes between the 1st vertebra and occiput; then introduce one blade of a small, but strong, pair of scissors into the cranial cavity, and cut away bit by bit the bones which form the roof of the skull, taking care that the point of the scissors does not injure the brain. Next remove, in a similar manner, the tops of the vertebral arches and the head of the urostyle. In all probability a chalky fluid will be met with clouding the water and concealing the underlying organs (secretion of the periganglionic glands (cf, p. 32); if so, wash carefully until clean. There will thus be exposed

a. The *cerebro-spinal axis*; filling the greater part of the neural canal; it can be resolved into

α. The *encephalon or brain;* that portion which lies within the cranium, its constituents in part paired.

β. The *myelon or spinal cord;* axial and lying within the neural canal of the vertebral column.

Its form: wide in front, but narrowing rapidly about the 5th or 6th vertebra, and thence continued back as a slender tapering *filum terminale.*

The groove (*dorsal fissure*) running along its middle line.

b. The investing membranes and the origin of the spinal nerves.

α. The *pia mater;* a delicate densely pigmented membrane, investing the whole nervous axis. It is thickened and highly vascular above the hind portion of the brain (*choroid plexus*).

β. The *dura mater;* a similar but denser membrane lining the wall of the neural canal.

γ. The *roots of the spinal nerves;* conspicuous in the case of the second and eighth and ninth pairs of nerves. The former are very stout, and pass out from the myelon at right angles to the axis, to reach the arm: the latter are long and delicate, passing back on opposite sides of the hinder half of the myelon.

c. Obtain a second Frog (preferably one hardened in alcohol) and make a transverse section across the entire nervous axis and neural canal im-

mediately behind the above-named second spinal nerve. Examine in water, under a hand lens.

α. The *myelon;* subdivided, dorsally and ventrally, by median *longitudinal fissures;* enclosing a small *central canal.*

β. The *nerve roots;* arising dorsally and ventrally, and passing outwards and downwards through the inter-vertebral foramina to form, on each side, the trunk of the nerve. The dorsal root bears an enlargement or *ganglion of the root,* immediately on leaving the neural canal.

γ. The *investing membranes* (cf. supra); seen to merge into each other and the periosteum, in the vicinity of the inter-vertebral foramina.

2. *The anatomy of the brain.*

i. Expose from the *dorsal aspect,* as directed in § 1, preferably in a frog which has been previously hardened in spirit. Examine in situ.

The anterior half of the brain consists of two elongated masses, each marked off by a slight transverse depression into a smaller anterior and a larger posterior portion.

a. The *cerebral hemispheres* (*prosencephalon*); the posterior of the above-named, separated by a deep cleft.

b. The *olfactory lobes* (*rhinencephalon*); the anterior of the above-named, confluent with each other at their bases. Each passes into a rounded trunk (commonly termed the *olfactory nerve*), which leaves the skull, and ramifies in the lining membrane of the nose.

c. The *thalamencephalon:* a thickened mass immediately posterior to the cerebral hemispheres.

Note, in connection with it,

α. The *thalami optici;* its lateral walls, constituting its main portion as here seen.

β. Its thin roof; transparent and very readily torn.

γ. The *pineal gland;* represented by a small pinkish body, lying in the bay enclosed by the hinder ends of the hemispheres.

d. The *optic lobes* (*mesencephalon*): a pair of rounded eminences lying behind the thalamencephalon.

e. The *cerebellum* (*metencephalon*): a narrow transverse band next in order of succession.

f. The *medulla oblongata* (*myeloncephalon*): the portion of the brain lying behind the cerebellum and passing into the spinal cord. Its roof is covered by the *choroid plexus* (cf. 1. *b.* α).

ii. Divide the olfactory lobes, and raise the front end of the brain; turning it back gradually, divide with a sharp scalpel any nerves that may be observed passing from it to the cranial walls: most of these, being small, will probably be torn across unobserved, but the large optic nerves will at any rate be seen. Remove the brain, together with a small portion of the spinal cord, and examine from the *ventral aspect.*

a. The *hemispheres and olfactory lobes* (cf. supra). The outer walls of the latter are thickened

ventrally to form two cord-like tracts (*ventral roots of the olf. lobes*).

b. The *lamina terminalis;* a median bilobed eminence, interposed between the bases of the hemispheres.

c. The *optic lobes;* seen to project laterally, behind the hemispheres.

d. The *optic tracts;* band-like masses arising in connection with *c*; that of the left side giving off the optic nerve to the right eye and *vice versa*. The interlacing of the two at the point of decussation, to form the *optic chiasma*.

e. Lying in the ventral middle line immediately behind the optic chiasma there will be seen, in the order named

α. The *tuber cinereum;* a large tongue-shaped mass, bilobed posteriorly.

β. The *infundibulum;* a small stalk-like body giving attachment to

γ. the *pituitary body;* a rounded pinkish mass (highly vascular in the fresh state).

iii. Examine from one side (*lateral aspect*), and compare generally with i. and ii.

iv. Slice away, with a small scalpel, the dorsal walls of the cerebral hemispheres and olfactory and optic lobes, and remove in like manner the greater portion of the cerebellum and roof of the thalamencephalon. The *central cavities or ventricles* will thus be laid bare; they can be resolved into two sets, as under:

a. *Median or axial* (forward continuations of the central canal of the spinal cord); in order of succession from behind forwards—

α. The *fourth ventricle;* tongue-shaped and expanded in front; enclosed by the medulla and cerebellum.

β. The *iter* or *aqueduct of Sylvius;* between and below the optic lobes.

γ. The *third ventricle;* lying within the thalamencephalon and bounded in front by the *lamina terminalis* (front wall of the median portion of the cerebro-spinal axis).

b. *Paired.*

α. The *optic ventricles;* lying within the optic lobes and derivative of the iter.

β The *lateral ventricles* (central cavities of the hemispheres) in communication, on either side, with the third ventricle through a wide passage (*foramen of Monro*). Each is seen to lodge a thickened fold of pia-mater (*choroid plexus*) which enters it immediately in front of the above-named foramen. (Cf. p. 27.)

γ. The *olfactory ventricles;* forward prolongations of *β*, extending into the bases of the olfactory lobes.

v. *Longitudinal vertical section.* Obtain the brain of the largest frog accessible, and, when fully hardened in spirit, remove one half *cutting to the near side of the middle line.*

Examine in water under a hand lens, and note the following

a. The *median ventricles* (§ iv. *a*); note their limits and their relationships to the several constituents of the brain. Take especial note of the *third ventricle;* its walls are modified as follows

- *α.* Front wall; greatly thickened to form the *lamina terminalis* (iv. *a.* γ).
- *β.* Roof; thin and delicate, prolonged upwards and forwards to give attachment to the "pineal body."
- γ. Its floor; for the most part thin prolonged downwards and backwards as the *infundibulum* (ii. *e.* β) to meet the pituitary body; thickened at its middle around the optic chiasma (ii. *d*).
- δ. The *foramen of Monro;* lying immediately above the lamina terminalis and large enough to admit a good-sized bristle.

b. The *commissures;* small but definite tracts passing between the opposite halves of the brain; relatively whitish in colour. Look for their cut edges with a powerful hand lens, as under—

- *α.* The *posterior commissure;* lying in the substance of the roof, immediately in front of the optic lobe.
- *β.* The *corpus callosum;* represented by a well-defined tract traversing the head of the lamina terminalis, immediately below and behind the foramen of Monro.
- γ. The *anterior commissure;* smaller than the above and running through the middle of the lamina terminalis.

3. The *spinal nerves.*

Pin a frog down upon its back and remove the ventral integument and body-wall, together with one-half of the floor of the mouth. Carefully dissect away the viscera and dorsal aorta and remove the centra of the vertebræ, cutting well down to the level of the roots of the spinal nerves. Wash, and examine under water,

a. The *spinal cord;* in form cylindrical; enlarged at the points of origin of the nerves for the limbs (*brachial* and *lumbo-sacral swellings*).

b. The *spinal nerves* arising from it. Ten on each side; the first one passing out between the first and second vertebræ, the last through the head of the urostyle (cf. Sect. G. 2. *d.* ε). Each leaves the neural canal behind that vertebra with which it corresponds.

α. Their *origin*; each arising by two *roots* (*anterior* and *posterior*) as is most easily seen in the 7th, 8th and 9th nerves, in which they are much the longer. Sometimes the fibres of the anterior roots are bound up into two or more fasciculi.

β. The *direction of their roots:* outwards in the anterior nerves; obliquely backwards in the 4th, 5th and 6th; almost directly backwards, and running for a considerable distance in the neural canal, in the 7th, 8th, 9th and 10th.

γ. The *ganglia of their dorsal roots* (cf. § 1. *c.* β); very conspicuous in the 7th, 8th, and 9th; lying, for the most part, outside the spinal

canal and largely surrounded by the *perigan-glionic glands* (cf. p. 32).

c. *Distribution of the spinal nerves.* Follow this on one side.

α. The 1st; examine it on that side upon which the floor of the mouth is retained. It passes at first outwards and then forwards and inwards, beneath the mylohyoid muscle, to be distributed to the muscles of the tongue (*hypoglossal nerve*).

β. The 2nd and 3rd; meeting, on a level with the end of the transverse process of the third vertebra, to form the *brachial nerve.* Follow it to the arm.

γ. The 4th, 5th, and 6th; each forming a long trunk which passes back for a considerable distance, for distribution to the body wall and integument.

δ. The 7th, 8th, and 9th; running parallel with each other and the urostyle (*lumbo-sacral plexus*), and finally becoming bound together to form the *iliac nerve* for distribution mainly to the hind limb. Follow this to the leg.

This plexus is liable to variation.

ε. The 10th, a small nerve lying immediately internal to *δ* and close against the urostyle. It enters into connection with the lumbo-sacral plexus, and is distributed to some of the lesser muscles of the posterior extremity and parts adjacent.

ζ. Examine one of the middle spinal nerves with care, under a hand lens if necessary.

There will be found to arise immediately external to its ganglion a small *dorsal ramus*; note that this is distributed to the muscles and other parts of the body dorsally, much as is the main trunk or *ventral ramus* ventrally.

4. The *cranial nerves*.

The study of these may be greatly facilitated by placing the animal when dead in $\frac{1}{2}$ per cent. chromic acid solution, the same to be frequently changed until decalcification is complete. This done; wash well under running water and preserve in methylated spirit, slightly dilute.

i. Dissect from the ventral aspect as directed at § 3, leaving the floor of the mouth intact.

a. The *9th or glossopharyngeal nerve*. Its *trunk*; seen, on removing the middle portion of the mylohyoid muscle (Sect. B. *c*. γ) lying immediately external to that of the hypoglossal nerve (§ 3. *c*. *a*); its *branches*, seen, on dissecting the genio-hyoid, to be distributed to the tongue and some of its muscles.

ii. Remove the floor of the mouth together with the heart and gullet, leaving the mandible and a small portion of the mylohyoid muscle on one side. Raise the mucous membrane of the roof of the mouth with forceps and dissect it away with the utmost care, being especially cautious to avoid removing certain nerves which lie in close apposition with it. Next slice away the floor of the cranium and auditory capsules so as to expose their internal cavities; wash clean and examine in water under a hand lens.

a. The *1st or olfactory nerve;* passing forwards in front (cf. § 2. *b*). Remove the vomer of one side and cut down to its level, following it out; it will be seen to ramify on the base of the olfactory sac.

b. The *2nd or optic nerve;* arising from the optic chiasma (§ 2. *d*); it runs forwards and upwards to enter the eye-ball from below.

c. The *3rd or oculo-motor nerve* (described, in common with the 4th and 6th, in connection with the eye-muscles. Sect. I. 3. p. 116).

d. The *5th or trigeminal nerve.* Remove the eye-ball on one side; lying internally to it, deeply seated and in close apposition with the cranial wall, will be seen the *ophthalmic or orbito-nasal nerve* (1st division of the 5th); trace it back, it will be found to enter, immediately internal to the front end of the auditory capsule.

α. the *Gasserian ganglion;* a comparatively large oval pigmented body, seen to be in connection with other nerves also.

β. The *maxillo-mandibular nerve;* arising from *a* and passing outwards immediately behind the eye. It early subdivides into the two following; the *mandibular nerve* (3rd division of the 5th) to the lower jaw and its integument (follow it on that side in which these still remain); the *maxillary nerve* (2nd division of the 5th) seen, on removing the hinder third of the upper jaw, to be somewhat similarly distributed to the maxillary region.

γ. The *ophthalmic or orbito-nasal;* its distribution may now be studied by turning the animal over and examining from the dorsal aspect. Remove the nasal and sphenethmoid bones to its level; and note its branches to the antero-dorsal integument of the head.

e. The *7th or facial;* intimately connected with the Gasserian ganglion and the trigeminal. Examine it, from the ventral aspect, on that side at which the eye remains.

α. Its *root;* arising, side by side with that of the 5th, from the side wall of the medulla; the two entering the Gasserian ganglion.

β. Its anterior branch (*palatine nerve*); leaving the Gasserian ganglion immediately external to the ophthalmic branch of the 5th, and passing forwards, perforating the vomer, to be distributed to the mucous membrane of the roof of the mouth.

γ. The *maxillo-palatine commissure;* connecting the palatine and maxillary nerves; on a level with the front end of the eye-ball.

δ. The posterior branch of the 7th; leaving the outer side of the Gasserian ganglion, immediately behind the maxillo-mandibular nerve, and passing outwards and backwards dorsal to the columella auris (beneath it as here seen). Its distribution may best be studied later.

f. The *9th and 10th* (*post-auditory nerves*); arising close together from the medulla posteriorly to

the 5th and 7th, and passing behind the auditory capsule, much as do the 5th and 7th in front of it.

a. The 10*th or pneumogastric* (*vagus*); its cut end will alone be seen. Soon after leaving the skull it bears a large ganglion (*g. trunci vagi*).

β. The 9*th or glossopharyngeal;* it arises immediately in front of *a*, and is largely confluent with the above-named ganglion. It is commissurally connected with the posterior branch of the 7th (*commissura ad facialem*). Its distribution has been studied (§ i. *a*).

g. The 8*th or auditory;* arising from the medulla along with the 7th, part of which it appears to be. Dissect to its level and trace it into the auditory capsule.

5. The *sympathetic system.*

Dissect from the ventral aspect as before, but leave the aorta, the cut end of the cœliaco-mesenteric artery and the base of the large intestine with the bladder, in place. On raising the aorta the sympathetic will be found immediately beneath (dorsal to) it.

a. The *main trunk of the sympathetic;* paired and longitudinal, accompanying the arches and trunk of the aorta.

b. The *splanchnic nerve;* arising from *a* at the point of junction of the aortic arches and accompanying the cœliaco-mesenteric artery.

c. The *sympathetic ganglia;* enlargements on *a*, variable in size and number; usually one for each

spinal nerve. Best seen on turning the aorta to one side.

d. The *rami communicantes;* relatively long commissures between *c* and the trunks of the spinal nerves.

e. The *distribution* of the sympathetic; best studied in the pelvic region, and rendered the more conspicuous if a little acetic acid be added to the water. *Vaso-motor nerves;* arising from the ganglia and passing to the aorta. *Viscero-motor nerves;* seen to arise from both sympathetic and lumbo-sacral plexus for distribution to the pelvic viscera.

f. The *cranial sympathetic.* Remove the aortic arches with care and follow the sympathetic forwards, dissecting as at § 4. ii. With a little care the above can be traced to the Gasserian ganglion; passing internally to the auditory capsule and entering into connection, *en route*, with the post-auditory nerves.

6. The *further course of the facial and vagus nerves* (7th and 10th cranial).

Pin a frog down upon its side and remove the integument of the head, together with the arm and shoulder-girdle and body-wall adjacent. Pull down the stomach and pin it back, so as to put the mesentery on the stretch and fully to expose the near lung and the heart.

a. Note the course of the *mandibular nerve* (§ 4. *d.* β). It will be seen to run backwards and downwards immediately in front of the squamosal

and under the jugal, to reach the outer side of the mandible.

b. The *facial nerve.* Its *hyoid branch;* seen on dissecting away the membrana tympani and outer end of the columella auris. It leaves the skull immediately behind the squamosal and passes downwards and backwards, giving off—

α. A small branch to the integument of the cheek (in close apposition with the jugal bone).

β. A *mandibulo-hyoid branch;* continued along the inner face of the mandible, and giving off nerves to the muscles and skin adjacent. (Be careful not to confuse these with the branches of the mandibular nerve.)

c. The 9th and 12th (cf. § 4); the former will be seen to pass internally, the latter externally, to the anterior cornu of the hyoid. Remove the hypoglossal with care, thus laying bare–

d. The 10th (*vagus*). Seen to pass downwards and backwards, externally to the posterior cornu of the hyoid. At the side of the gullet it breaks up into the under-mentioned

α. The *gastric rami;* two or more in number, distributed to the stomach. They pass through the so-called diaphragm.

β. The *pulmonary ramus*, to the lung; accompanying the pulmonary artery.

γ. The *cardiac ramus*, to the heart; arising in front of *β* and coursing along the dorsal wall of the atrium. (Sympathetic fibres are bound up with this, if not with *α* and *β* also.)

δ. The *recurrent laryngeal;* a long nerve arising in front of and above α—γ and coursing round the base of the pulmonary aortic arch to reach the larynx.

ε The *dorsal ramus;* arising from the main trunk immediately after it leaves the skull, and distributed to the adjacent dorsal integument.

e. Incidental.

α. The course of the 5th and 7th (cf. § 4. ii. *d.* ε. et seq.). Remove the eye and the squamosal and quadrato-jugal bones and follow the course of the above nerves, as seen from the side.

Note especially the relations of the 5th to the mouth-cavity and of the 7th to the tympano-Eustachian passage (Eustachian recess). See pp. 28, 29.

β. The *sympathetic.* Note especially the further course of the *splanchnic nerve* (§ 5. β). It bears a large ganglion in close proximity with the cœliaco-mesenteric artery; add a little acetic acid; offshoots will be found to proceed from it to the alimentary canal, accompanying, at their outset, the branches of the artery.

L. The anatomy of the sense organs.

1. **The gustatory organ.**

The shape and arrangement of the tongue have already been described (Sect. F. § 1 *d.*). Snip off a bit of mucous membrane from the upper surface of the tongue of a recently killed frog, mount in salt solution and cover in plenty of the fluid with a large coverslip: examine with one inch obj.

a. On the surface of the fragment and especially around its edges numerous minute elevations will be seen: these are the *papillæ*: some (*filiform papillæ*) are pointed at the free end and others (*fungiform papillæ*) are flattened. Note the loops which the blood capillaries make in many of them.

b. Examine one of the thinner bits of the specimen with a high power: a flickering motion, due to the presence of *cilia*, will be observed. Some of the papillæ however will be seen to have no cilia except a narrow belt around the somewhat truncated apex: it is on these that the *gustatory discs* are placed, and in fortunate specimens nerve-fibres can be seen entering them.

2. **The olfactory organ.**

The general relations of this have already been described (Sects. A and E).

a. Take a frog which has been preserved in spirit; insert the point of a small pair of scissors into the external nostril of one side and cut away the roof of the nasal cavity. A chamber is thus exposed which has a somewhat triangular form, its apex being anterior.

α. The walls of the cavity are slightly folded, and there is a well-marked hemispherical eminence on its floor which overlies the vomer.

β. The *posterior nostril;* situated some distance in front of the hinder boundary of the nasal sac (i.e. the sac is prolonged back behind it).

b. Open the other nasal cavity in a similar way:

α. The *septum narium;* a median longitudinal par-

tition separating the apposed sacs of opposite sides.

β. The boundaries of the olfactory organ. It lies within an area enclosed by the premaxilla and front end of the maxilla and the palatine bones, being wholly in front of the latter.

c. Obtain a second frog, and lay open the olfactory sac from the side, cutting obliquely from the anterior nostril across the outer anterior border of the eyeball, in order that the line of section may pass through both anterior and posterior nares.

α. *General.* Cf. supra.

β. The *anterior nostril;* its upper lip, relatively small and constricted; its lower lip, produced antero-laterally into a deep fold around the ali-nasal cartilage (cf. Sect. G. 5. *a.* ε).

3. **The visual organ.**

i. *General.*

Take an uninjured frog and examine its eye. It will be found to project considerably above the top of the head, but if touched it is withdrawn into a sort of socket. If the animal's mouth be opened, a depression, caused by the eye-ball, will be seen on its roof, and this is more prominent when the eye is retracted. When the eye is open observe from without.

a. The *eyelids;* folds of skin developed round the margin of the eye-ball. The *upper lid* is thick and swollen, the *lower lid* thin and transparent.

Gently touch the eye and observe that it is closed, by the pulling over it of the lower eye-lid. The upper lid is hardly moveable.

b. When the eye is open, observe the following—

α. The *cornea;* the transparent covering for its exposed surface.

β. The *iris*, a membrane, seen through *α*, coloured by brown and golden pigment, the latter forming a very brilliant ring around its inner margin. Below it is interrupted by a faint dark line which can be traced downwards through the whole iris.

γ. The *pupil;* elliptical and lying within the iris, its long axis directed antero-posteriorly.

ii. **The eye-muscles and their nerves.** These can be most satisfactorily made out, notwithstanding their small size, in a frog preserved in chromic acid as directed in Sect. K. 4. Examine under a hand lens.

a. *Dorsal aspect.* Remove the head from the body, cutting well back so as to include the first two vertebræ, and pin down in as small a dish as may be convenient. Dissect off the integument of the top of the head together with the upper eyelids, but be very careful to leave the lower lids uninjured.

α. The *eyeball;* completely filling the orbit.

β. The *superior oblique muscle;* ribbon-shaped and passing obliquely forwards between the antero-dorsal face of *α.* and the orbit.

γ. The *superior rectus;* a trifle larger than *β.* and arising from the dorsal face of the eyeball. It passes backwards and inwards, to be attached to the cranial wall.

δ. The *4th cranial nerve* (*n. patheticus*); seen, on slightly pulling the eye-ball outwards, skirting the inner wall of the orbit to reach the superior oblique muscle.

ε. The *3rd cranial nerve* (*oculomotor*). A branch of this may be seen breaking up in the superior rectus muscle, if that be cut across near its point of attachment to the eye-ball and turned back.

ζ. The *lower eyelid*; follow it forwards, it will be found to be continuous with a strong tendon which passes through a loop immediately external to the superior oblique muscle.

b. *Ventral aspect.* Turn the head over and pin down ventral surface uppermost; remove the entire lower jaw and floor of the mouth, and dissect off the mucous membrane of its roof with care.

α. The *levator bulbi muscle*; a sheet-like mass underlying (overlying as seen) the whole eye-ball; its fibres pass obliquely outwards and backwards.

> Compare a transverse section such as is described at Sect. C. 3. The fibres will be found to pass between the upper jaw and the internal dorsal wall of the orbit.

β. The *inferior oblique*; a small muscle passing upwards and inwards between the antero-internal face of the eye-ball and the orbit.

γ. The *Harderian gland*; a nodular mass immediately behind β, interposed between the eye-ball and orbit.

δ. The *recti muscles* other than the superior one; seen on removal of the levator bulbi, to converge postero-internally. The *inferior rectus*, arising from the ventral face of the eye-ball and passing obliquely backwards; the *external rectus*, arising from its posterior face and passing inwards and upwards; the *internal rectus*, arising in front under cover of the inferior oblique, and passing backwards along the inner face of the eye-ball.

ε. The *3rd* (*oculomotor*) *nerve*; seen to break up, immediately in front of the superior rectus, for distribution to β. and δ. with the exception of the external rectus.

ζ. The *6th cranial nerve* (*n. abducens*). A small muscle (*pterygoid*) will be seen lying in the posterior region of the orbit; remove this and lay bare the *Gasserian ganglion* (Sect. K. p. 105); the 6th nerve will be found passing from the outer face of the latter to the postero-ventral border of the external rectus. It is very short and may best be seen by gently drawing the muscle forwards.

η. The *retractor bulbi muscle*; a considerable mass of tissue, seen, on removing β. and δ. It surrounds the optic nerve.

θ. The *tendon of the lower lid* (Cf. α. ζ.); shining and thread-like, passing round and intimately connected with the outer edge of η. It is inserted behind into the adjacent integument.

c. The *origins of the 3rd, 4th and 6th cranial nerves.*

Lay bare the cerebro-spinal axis from the ventral aspect, as directed in Sect. K. § 3, and expose the Gasserian ganglion with the utmost care.

α. The *3rd* (*oculomotor*) *nerve;* arising, on each side, from the ventral surface of the brain on a level with the pituitary body. It passes upwards and outwards to leave the skull in front of the 5th nerve (cf. p. 67, vi.).

β. The *6th nerves* (*n. abducentes*); extremely delicate, arising close together in the middle line from the floor of the medulla, on a level with the 9th and 10th. Each passes upwards and outwards along with the trunk of the 5th to enter the Gasserian ganglion.

γ. The *4th* (*n. patheticus*); best seen on removing one optic nerve and turning the brain a little to one side. It leaves the dorsal surface of the brain in front of the cerebellum; and passes upwards and forwards to leave the skull above and in front of the optic nerve (cf. p. 67, vii.).

iii. **The structure of the eye-ball.** Obtain a freshly-killed frog and remove the head from the trunk; bisect the former longitudinally and examine the eye-ball while still in position, as under.

Make two sections; that of one side (*a.*) to bisect the eye-ball transversely to the long axis of the body (to pass through the crystalline lens if possible); that of the other (*b.*) to bisect it equatorially, at right angles to *a.* Examine under a hand-lens.

Section a.

i. Examine high and dry and note –

α. The *eye-ball;* firm and resistant, enclosing a central cavity.

β. The *crystalline lens;* a relatively large transparent body filling a considerable portion of *a*, globular, its outer face somewhat flattened. It is held firmly in position.

γ. The *vitreous or inner chamber;* that portion of the cavity of the eye-ball internal to the lens. It is filled with a dense gelatinous *vitreous humour*, which may be raised *en masse* with a forceps.

δ. The *aqueous or outer chamber;* relatively much smaller than γ. and external to the lens. It lodges a more fluid *aqueous humour*.

ii. Dissect the inner portion, under water, to the level of the optic nerve, so as to get the same into longitudinal section. Examine the coats of the eye, following their cut edges.

α. The *sclerotic;* the outermost coat, dense and cartilaginous; it serves to give consistency to the whole and to furnish attachment for the muscles. Externally it is continued over the outer face of the eye-ball as the thin and transparent *cornea;* internally it forms the *sheath of the optic nerve*.

β. The *choroid;* internal to *a*, vascular and spongy and blackened by pigment. It is prolonged forwards in front of the lens to form the *iris* (cf. § 1. *b*).

γ. The *retina;* a thin filmy layer, greyish and transparent, internal to β. It stops short at the base of the iris (*ora serrata*); internally it passes into the optic nerve.

If the vitreous humour be much disturbed this layer will be puckered and torn, or otherwise displaced.

δ. The *conjunctiva and eyelids* (cf. Sect. C. § 3. *d.*). The former can only be distinguished from the cornea with difficulty.

Section b.

i. Examine the inner half from within, under water.

α. The *retina.* Gently raise it; it will be found to adhere firmly at the point of entrance of the optic nerve (*blind spot*). Note the position of same.

β. The *choroid;* strip off the whole retina and examine this, noting its texture and great vascularity.

γ. The *sclerotic;* examine this in like manner, after having removed β.

ii. Examine the outer half from within, and note especially the relations of the lens and iris.

4. **The auditory organ.**

a. Examine the tympanic membrane from the outside (cf. Sect. A. *a.* β.). Dissect off its outer or 'tegumentary layer'; there will thus be brought into view:

α. The *annulus tympanicus;* a cartilaginous ring supporting the edge of the membrane.

β. The *columella auris*: its head will be seen as an opaque white patch in the middle of the deeper layers of the tympanic membrane, now laid bare. Cut away the latter around it and note its relations to the *Eustachian recess* (Sect. E.). Follow the cut edge of the mucous membrane; it will be found to wrap round the columella, suspending it much as the mesentery suspends the alimentary canal (i.e. the columella is outside it).

b. The *internal ear*. Remove the skin from the top of the head of a large frog which has been preserved in alcohol, and scrape the roof of the auditory capsules quite clean. Isolate the capsule of one side and hold it between the finger and thumb of the left hand. If examined with care, a couple of greyish streaks will be seen on the inner side of its roof, diverging outwards. Carefully slice away the bone, being especially cautious to cut quite superficially along these streaks; there will thus be laid bare:

α. The cavity of the *internal ear*; enclosed on all sides by the auditory capsule and filled with a fluid (*perilymph*).

β. The *membranous labyrinth*; a portion of this is now visible as two delicate pigmented canals, coincident in position with the divergent streaks afore-named.

c. Still holding the capsule as before, slice away, bit by bit, its outer wall and the remainder of its roof. A third membranous canal will be seen on the outer side; when this is liberated, transfer the

whole to a watch-glass or small vessel filled with water (preferably one having a white bottom). The *membranous labyrinth* will probably float out; if not, it may be liberated with a camel's hair brush. It consists of a greyish pigmented structure of the size of a small pea: Note

α. The *vestibule;* sac-like and constricted into two—an upper portion or *utriculus*, and a lower one or *sacculus*. It is largely filled during life by a milk-white *otolithic mass*.

β. The *semicircular canals;* three in number and connected with the utriculus. The *anterior* and *posterior vertical canals*, the two divergent ones referred to at *b;* their inner ends unite before entering the utriculus. The *horizontal canal;* situated externally, on a level with the bases of the other two.

γ. The *ampullæ;* enlargements of the bases of the canals. Those of the anterior and horizontal canals are situated close together at the anterior end of the utriculus.

δ. The *ultimate ramifications of the auditory nerve;* visible on the inner face of the labyrinth.

d. General dissection of the whole auditory organ. Obtain the head of a frog which has been preserved in spirit, and dissect from behind.

Scrape the occipital region of the skull quite clean and then pin the whole down under water. Carefully remove the wall of the auditory capsule —externally, to the level of the columella auris— internally, to that of the auditory nerve; cut away

the hinder half of the tympanic membrane, to the level of the head of the columella.

Work over the mutual relations of the following, all of which have been previously described.

α. The *columella auris;* to the tympanic membrane and fenestra ovalis.

β. The *membranous labyrinth;* to the periotic capsule and its enclosed cavity.

γ. The *auditory nerve:* to the membranous labyrinth and brain.

δ. The *Eustachian recess;* to the mouth cavity, and that of its lining membrane to the columella.

ε. The *annulus tympanicus;* to the membrana tympani.

M. **Histology.**

In the undermentioned directions, the reagents which may preferably be employed in each case are alone enumerated; details as to preparation and methods of treatment will be found in the Appendix.

1. **The blood.**

a. *Freshly drawn blood.* Obtain a drop of the same from a freshly-killed frog, and examine with a low power, under a cover-glass.

α. The *plasma;* thin, watery and colourless.

β. The *corpuscles;* exceedingly numerous solid bodies, freely suspended in *a.* Two kinds will be seen: larger and more numerous *red corpuscles;* smaller and less numerous *white corpuscles*, irregular in shape, greyish in colour, and

about $\frac{1}{3}$ the size of the red ones. Examine under a high power.

b. *The red corpuscles.*

α. *Their form;* oval when seen *en face;* almost linear in profile but slightly swollen at the centre.

β. *Their size;* their length, breadth, and thickness; measure.

γ. *Their colour;* pale yellow, when seen individually; redder when seen in the aggregate.

δ. *Their structure;* homogeneous for the most part, but possessed of a central oval or rounded *nucleus.*

ε. Treat with water; they swell up and become more spherical; their colouring matter (*hæmoglobin*) is gradually dissolved out, leaving behind a colourless *protoplasm.* The nucleus is rendered very evident, and ultimately all the rest of the corpuscle disappears.

ζ. Treat with dilute acetic acid; results same as with water, but produced more rapidly.

η. Treat with alcohol and borax-carmine successively. The nucleus stains with great intensity, the rest of the corpuscle remaining unaffected.

c. *The white corpuscles.*

Obtain a drop of fresh blood and examine as before under your highest power.

α *Their form;* very irregular, the surface being produced out into a number of blunted processes or *pseudopodia.*

β. *Their movements;* best seen if the slide be gently warmed, by contact with a lighted match or other heating agent. They creep about in a sluggish manner through the agency of the above-named pseudopodia (*amœboid movement*).

γ. *Their size;* cf. generally with the red corpuscles.

δ. *Their structure;* granular centrally, clear and transparent peripherally: usually lodging one or more clear round *nuclei*.

ε. Treat with acetic acid and magenta; the nucleus alone will be stained. It will be found to lodge one or more small granular bodies (*nucleoli*).

d. *The microcytes* or *blood platelets.* Very small bodies, for the most part colourless, freely suspended in the plasma: in shape variable; generally fusiform or ovoidal, more rarely irregular.

e. *Coagulating blood.* Allow a drop of blood to coagulate upon a glass slide, taking care that it does not dry up. Examine under a high power.

α. The *plasma;* transformed into a colourless fluid (*serum*) which is permeated by a coagulate material, *fibrin*.

β. The *corpuscles;* the red ones show a marked tendency to arrange themselves in radiate series; the white ones are largely to be found, together with microcytes, in the foci of the radii.

γ. Wash with water. The colouring matter of the red corpuscles will be dissolved; note the *fibrin filaments*, for the most part coincident with the above-named radii.

2. **Epithelia.**

An epithelium consists of a layer of cells which

ing the skin and the epithelium of the alimentary canal, with which it is continuous at the buccal and cloacal orifices, may be cited as examples. There are several main types of epithelium, viz.—

a. *Ciliated epithelium.* Gently scrape the mucous membrane of the tongue or roof of the mouth of a recently-killed frog and transfer that which is obtained to a slide; mount in salt solution, avoiding pressure, and examine under a high power.

α. The *cells;* occurring singly or in aggregates. A shimmering appearance (*ciliary action*) will be seen along their free edges, produced by the rapidly moving cilia.

As the cilia die their movements slacken. Watch an individual cilium as this happens; it moves in a definite direction from a point of rest and does not oscillate.

Place a freshly-killed frog upon its back and open the mouth to its utmost. If a fragment of pith or cork be placed on the roof of the mouth, it will be carried back to the gullet by the action of the cilia.

β. The *cell protoplasm.* Stain with eosin or magenta, and examine the individual cells. *Shape;* flattened at the free surface, rounded or elongated at the base. *Structure;* granular for the most part (*endoplasm*); clear and transparent peripherally (*ectoplasm*), especially so at the free border. *Nucleus;* usually central and rounded, containing one or more nucleoli.

b. *Columnar epithelium.* Scrape gently the inner surface of the mucous membrane of the intestine

of a frog, and mount the detached fragments in water. Examine with a high power.

α. Numerous elongated cells will be seen, somewhat resembling those of the ciliated epithelium in shape, but more uniform in size. Each is flat at one end and somewhat pointed at the other, and has a well-marked oval nucleus. Not unfrequently aggregates of them may be seen: look for such, and note that the cells are closely applied and arranged in a single layer.

β. *Goblet cells;* scattered among α; characterised by the presence of a watery-looking globule (*mucus drop*) which distends the free end or greater part of the cell body. Note the position of the nucleus.

c. *Scaly or tesselated epithelium.* Open the body cavity of a recently-killed frog; carefully remove the viscera and lay bare the cisterna magna. Cut away its thin wall as carefully as possible, taking great care not to drag or pull it. Place the fragment in 0·5% solution of silver nitrate for three minutes or longer: then remove, wash well in distilled water, and finally leave the specimen in the same fully exposed to the sunlight. When it has assumed a well-marked brown colour stain with eosin or hæmatoxylin and examine with a high power.

ω. The *matrix* (*intercellular cementing substance*); rendered highly conspicuous as a network of black lines, irregularly disposed on the free surfaces of the mesentery.

β. The *cells*; irregular, flat, and close fitting.

γ. The *cell structure* and *nucleus*.

δ. Look for *lymph stomata*; perforations of the membrane, each surrounded by small more deeply stained cells.

Should the silver nitrate reaction fail, the methylene blue process may be tried, as follows. Place for 10-15 minutes in 4 per cent. solution methylene blue. Transfer to saturated solution of picrate of ammonia for half an hour; wash in the same and examine in weak glycerine.

d. *Stratified epithelium*; see epidermis § 15. iii.

3. **Connective tissue.**

Of this there are two main varieties, *a*. and *b*.

a. *White fibrous tissue*. This occurs typically in tendons, but is mixed with the other tissues. Tease out a bit of fresh tendon in water: examine with a high power.

α. It is chiefly made up of very fine wavy fibres which, in the aggregate, impart a glistening white colour to the tissue (*white fibres*); they run in parallel bundles and do not branch.

β. Treat with dilute acetic acid. Most of the above disappear, but a few well-defined curled fibres (*yellow elastic fibres*) remain. Besides these small irregular protoplasmic masses are brought into view (*connective-tissue corpuscles*).

b. *Areolar tissue*. Lay bare the muscles of the hind-limb; sheets of areolar tissue will be seen passing between them and the integument. Remove one of these, being careful to avoid undue stretching and transfer to a slide: examine in water.

α. *White fibres;* more or less numerous and disposed in wavy bundles.

β. The *matrix;* a transparent imbedding mass, of sufficient density to resist the pressure of the cover-slip.

γ. The *yellow elastic fibres* (*α. β.*). Treat with acetic acid; the white fibres disappear, the yellow ones remaining. Note that they occur singly and anastomose, taking a very irregular course.

δ. Stain with magenta. The yellow fibres stain slowly but intensely; their torn ends will frequently be seen rolled into a spiral or otherwise contorted, as the result of their elasticity.

The *connective-tissue corpuscles;* small nucleated cells, variable in size and shape, scattered throughout the whole.

ε. Submit a piece of areolar tissue to the action of silver nitrate solution. When it has assumed a deep brown colour, examine in weak glycerine under a high power. The methylene blue process may be here advantageously employed as directed for the tesselated epithelium (§ 2. *c.*).

α. The *matrix;* stained a rich brown, having reduced the silver salt, as did the cementing substance of the epithelium.

β. The *connective-tissue corpuscles;* little if at all stained, appearing as a series of irregular white patches (*cell spaces*). Examine these with care; each is an irregular branching corpuscle, generally in organic continuity with one or more of its fellows (i. e. the whole tissue is permeated by protoplasmic matter).

4. **Hyaline cartilage.**

Dissect out the xiphisternal cartilage of a recently-killed frog and remove its membranous investment (*perichondrium*); mount in salt solution and examine under a high power.

a. The *matrix*; dense, structureless or finely granulated.

b. The *cartilage corpuscles*; large cells occurring singly, or in sets of two's to four's, their apposed faces being flattened. Examine the individual cells.

α. *Cell-protoplasm*; finely granulated and usually containing one or many minute refractive particles (*fat drops*).

β. *Nucleus*; round and sharply defined, containing a variable number of nucleoli. Two nuclei may not unfrequently be present.

γ. If the preparation be made and examined soon after death each cell will completely fill the cavity of the matrix in which it lies: but if it be kept some time or be treated with distilled water the cells contract; thereupon the cavities (*cell spaces*) become obvious as transparent halos around the individual cells, or groups of cells if recently formed.

c. *Cell division.* The initial phases in this process can be nowhere more favourably made out than here. The undermentioned may generally be found on carefully searching the field; note that in all, division of the nucleus precedes that of the cell.

α. Single cells (those filling an entire cell space);

one nucleus may be present (*resting cell*) or two (*dividing cell*).

β. A two-celled nest; one or both of the cells may lodge two nuclei.

γ. A three-celled nest. Usually one cell will be found to be the larger of the three; its nucleus may or may not show traces of division.

5. **Bone.**

a. Cleave the femur of a recently-killed frog longitudinally in two, and examine while still fresh.

α. The *bone;* its central cylindroidal *shaft* and terminal *epiphyses* (cf. p. 14).

β. The *marrow;* a fatty vascular mass, filling the central cavity.

γ. The *periosteum;* a tough vascular connective-tissue sheath, closely investing the shaft.

δ. The *nutritive foramina*. Carefully remove the marrow and examine from within; the torn ends of the periosteum will be seen passing in through the above, for communication with the marrow.

b. Decalcify the middle third of the femur of a freshly-killed frog in $\frac{1}{2}$ per cent. chromic acid solution, stain with borax-carmine and cut into *transverse sections* as directed in the Appendix. Mount in Canada balsam, and examine under a low power.

α. The *bony shaft;* seen to be divided, by a highly refractive *middle lamella*, into an outer more deeply stained portion and an inner thinner and less deeply stained one.

β. The *periosteum*; a thin layer closely applied to the outer face of *a.*, and staining with great intensity.

γ. The *marrow*; composed of larger vacuolated (fat-laden) cells, and smaller rounded deeply-stained ones (*red marrow cells*).

δ The *nutrient vessels*; in section as follows:—a larger ovoidal and thin-walled *vein*, and a smaller cylindroidal and thick-walled *artery*, lying close together near the middle of the marrow.

ε. Search your sections for any which may have passed through a nutritive foramen; if present, note the relations of the periosteum to the marrow.

c. Examine your thinnest section under a high power.

α. The *bony shaft*; seen to consist of a number of concentric layers or *lamellæ*, rendered distinct under the action of the reagent.

Find the middle lamella (*b.* α); it marks the boundary line between an inner, more lightly stained series (*concentric or peri-medullary lamellæ*) and an outer, more deeply stained series (*circumferential or sub-periosteal lamellæ*).

β. The *bone corpuscles*; minute fusiform or branching cells, set along the lines of the above lamellæ. Each clear and little stained, with a deeply stained nucleus. (Cf. connective tissue corpuscle.)

γ. The *periosteum*; if the section be a good one, its innermost layer will be seen to consist of a row of small flattened cells (*osteoblasts*).

δ. The *marrow*; note the aggregation of small cells on the inner face of the shaft.

d. Make a transverse section of the dried shaft of the femur, by grinding it down upon a bone as directed in the Appendix (E.). Mount in Canada balsam and examine under a high power.

α. *The lamellæ*: cf. *supra*.

β. *The lacunæ*; oval spots between the lamellæ; black, as they become filled with dirt in grinding. Each originally lodged a bone-corpuscle.

γ. *The canaliculi*; minute black lines radiating from the lacunæ. Those of adjacent lacunæ frequently anastomose.

δ. The *Haversian canals*; present only in the neighbourhood of the nutritive foramina. Obvious as spaces in the substance of the shaft, each surrounded with its own lamellæ.

ε. Examine a longitudinal section of the dried femur, for comparison with the above. The *Haversian canals* are seen to be channels running for the most part longitudinally, and communicating with one another by cross branches. The *lacunæ* and *canaliculi* appear much as in the transverse section.

The study of the dried bone may advantageously be supplemented by that of the long bone of a mammal.

6. **Adipose tissue.**

Tease up portions of the corpus adiposum, as under.

a. Fresh, in salt solution. Examine under a low power; the following will be met with.

α. *Connective tissue elements.*

β. *Ripe fat cells;* obvious as aggregates of large highly refractive globules (*fat globules*) of which the tissue is chiefly composed.

Note their optical characters, under different levels of focus.

γ. *Immature fat cells;* smaller irregular or rounded cells, each lodging one or more *fat drops.*

b. Preserve in spirit; stain with hæmatoxylin. Examine the ripe cells under a high power; each consists of--

α. The *fat globule;* apparent as a clear space, which may or may not contain crystals.

β. The *cell membrane;* deeply stained and more or less granular, forming a thin envelope for *α.*

γ. The *nucleus;* oval and compressed, buried up in the cell membrane and usually surrounded by granular protoplasm.

δ. *Immature fat cells;* look for those showing stages in the formation of the fat globule.

7. **Unstriped muscle.**

a. Place a small piece of the muscular coat of the intestine in Müller's fluid for 2—3 days. Tease up in hæmatoxylin solution and examine under a high power.

α. The *muscle-cells;* elongated and fusiform, in close apposition with each other; protoplasm granular.

β. *Nucleus;* fusiform or ovoidal, generally situated near the middle of the cell.

b. Obtain a similar but thin piece from the intestine of a recently-killed frog. Treat with silver nitrate as directed at § 2. *c.*

α. The *cementing substance;* obvious as a series of dark lines which cross each other at long intervals.

β. Look for fragments showing two sets of lines which cross at right angles (i.e. cell-contours of longitudinal and circular layers).

8. **Striped muscle.**

a. Preserve the middle third of the belly of a limb-muscle (say the gastrocnemius) in spirit; stain with borax-carmine and cut into transverse sections. Mount in Canada balsam and examine under a low power.

α. The *muscle:* composed of a great number of large *fibres*, each oval, or angulated and irregular as the result of mutual compression. Note their transverse diameter, variable in proportion as the section passes through their middle or extremities.

β. The *perimysium* (*fascia*); a deeply staining sheath for the whole muscle, carrying blood-vessels whose cut ends will be seen (cf. periosteum, § 5.). It will be found to dip into the interior of the muscle as a delicate black pigmented membrane, subdividing it into a number of *muscle bundles* or *fasciculi*.

γ. The *muscular fibres*. Examine the thinnest parts of your sections with a high power; each fibre consists for the most part of a granular pro-

toplasm lodging a number of fusiform deeply-staining nucleate bodies (*muscle corpuscles*).

b. The muscular fibre; structural analysis.

i. Tease out gently a bit of muscle from a freshly-killed animal, and examine in salt solution with 1 inch obj.

α. Composed of elongated *fibres*, which exhibit, unless the fibre be quite fresh, a tendency to split up into finer filaments (so-called *fibrillæ*).

β. The *sarcolemma;* visible as a superficial clear layer of the fibre; expansions of it may be seen at points where the continuity of the fibre has been interrupted by pressure, or other cause of rupture.

ii. Examine with a high power.

α. The alternate lighter and darker bands placed transversely to the long axis of the fibre (*transverse striation*).

β. The *sarcolemma:* enveloping the fibre; seen here and there as a delicate film, where the fibre is twisted or bruised.

γ. The *muscle corpuscles:* visible as elongated and slightly refractive fusiform bodies, disposed longitudinally.

iii. Treat with acetic acid; the striation is rendered very indistinct, the sarcolemma and muscle corpuscles becoming very conspicuous. Add a drop of magenta; the nuclei stain deeply.

iv. Transfer a fragment of fresh muscle fibre to a slide bearing a drop of salt solution, and tear it into small shreds with a couple of needles.

Examine the thinnest pieces under your highest power.

α. The fibre is seen to be marked out into a close-set series of alternately light and dark *transverse striæ*, the former being in all probability the narrower.

β. Keep your eye steadily fixed on a given piece while you alter the focus—parts which were originally dark now become light and *vice versa*.

γ. Examine a fragment in which the cross striæ are very distinct, still more minutely. When in exact focus the following will be seen.

The *septal zones*: obvious as the aforenamed clear striæ.

The *interseptal zones*; alternating with and of greater thickness than α. Each is longitudinally striated.

The *septal lines*; dark lines of great delicacy, one in the middle of each septal zone; seen, on close examination, to appear as a parallel series of minute dots.

Very generally the fibre is constricted at each septal line, having thus a beaded appearance.

δ. Repeat the operation described at β. and note the different optical effects which are produced at different levels of focus.

v. Melland's method. Place some fragments of fresh muscle in 1 per cent. acetic acid solu. for 10 seconds; transfer to 1 per cent. gold chloride solu. for 1 hour and then to 25 per cent. formic acid solu. for 48 hours or longer (to be kept in

the dark). Finally tear up with a couple of needles in glycerine.

α. *Longitudinal striæ;* parallel lines, differentiated under the above treatment, which can be traced throughout the entire length of the fibre, traversing clear and dark zones alike.

β. *Transverse networks;* seen, in the regions of the septal lines, traversing the fibre at right angles to *a* and thickened at their points of intersection with the same. When looked at *en face* they present a honeycomb-like appearance.

§ iv. and v. may be profitably repeated upon the muscle fibre of an insect.

9. **Nerve.**

a. Preserve a portion of the iliac nerve in spirit of increasing strengths; stain with hæmatoxylin, imbed and cut transverse sections. Mount in Canada balsam and examine with a low power.

α. The *nerve trunk;* composed of an immense number of small rounded *nerve fibres.*

β. The *perineurium;* a deeply staining sheath, seen to carry blood-vessels (cf. perimysium and periosteum). It may be in part fat laden.

γ. The *nerve bundles;* lesser divisions of the trunk, each bound up in an ingrowth of the perineurium (cf. muscle bundles).

b. Examine the thinnest portion of a nerve bundle under a high power; it will be found to contain—

α. *Medullated nerve fibres;* in section round, each with a central deep-stained portion (*axis fibre*)

and a peripheral clear portion (*medullary sheath*); the whole invested in a delicate *primitive sheath*, obvious as a darkly stained outline.

β. *Non-medullated nerve fibres;* irregularly dispersed among α.; each consisting of an axis-fibre alone.

c. *The medullated nerve-fibre; structural analysis.*

i. Tease out a bit of a fresh spinal nerve in salt solution. Examine with a low power.

α. Composed of well-defined fibres (*medullated nerve-fibres*) mixed with white fibrous tissue.

β. The *appearance* of the nerve-fibres; each has a double contour and a highly refractive border. Some fibres are regular in outline, others contorted and irregular.

ii. Examine, under a high power, selecting the least contorted fibres.

α. The *primitive sheath:* look for it on bruised nerve-fibres or at the ends of torn ones.

β. The highly refractive border (*medullary sheath*) within the primitive sheath.

γ. The central homogeneous axis (*axis fibre*); look for it projecting beyond the medullary sheath of torn fibres.

δ. Treat with chloroform; the medullary sheath will be dissolved out, and the axis fibre plainly seen.

ε. *Ranvier's nodes;* apparent breaks (constrictions) in the medullary sheath, seen at comparatively long intervals along the course of the individual fibre.

ζ. *Schmidt's nodes.* Examine the medullary sheath of the least distorted fibres with great care; the above will be found as interruptions of the same at short and fairly regular intervals, the sheath appearing to consist of a series of imbricated segments.

η. Compare a fibre with irregular contour. Note that the irregularity is due to shrinking of the medullary sheath under coagulation. The coagulate masses may often be seen to correspond with the above-named segments.

iii. Tease up a piece of the trunk of a spinal nerve in a drop of 1 per cent. osmic acid solu. After 3—4 hours immersion in the same, wash well and mount in weak glycerine. Examine under a high power.

α. The *medullary sheath;* rendered blackish and highly conspicuous by the above method. Examine it with care, noting especially the nodes of Ranvier and Schmidt.

β. The *axis fibre;* greyish and well defined, seen on focussing through α.

γ. The *nerve corpuscles;* apparent as thickenings of the sheath which bulge inwards, usually of a reddish brown colour under treatment with the above acid. They correspond in number with the nodes of Ranvier, and each consists of an oval refractive nucleus, embedded in a small amount of granular protoplasm.

10. **Nerve-cells.**

a. *Unipolar cells.*

i. Take a spinal ganglion from a recently-killed frog and tease up under a low power in eosin solution. Among the pigment cells and other small cells present, the above-named will be seen as large round or oval pale granular cells, each with a conspicuous large round nucleus. Examine under a high power.

α. The *cell-body;* granular, and connected at its base with a single nerve-fibre (unipolar-cell).

β. The *nucleus;* generally central in position; invariably containing one large nucleolus.

γ. The *cell capsule;* a delicate nucleated investment which may or may not be torn away.

ii. Tease up a second spinal ganglion, and treat with osmic acid and glycerine, as directed at 9. iii. Examine under a high power.

α. *General.* Cf. supra.

β. The *polar plate;* consisting of two or more clear cells, situated at the base of the unipolar cell and in direct connection with the ultimate termination of the nerve fibre.

b. *Bipolar cells.*

Take a sympathetic ganglion from a recently-killed frog and tease it up in eosin as directed above. Examine under a low power. Among the pigment and other cells present, will be seen numerous large pale granular cells somewhat like the unipolar ones. Examine under a high power.

α. The *cell-body;* composed of granular protoplasm, with a large round or oval nucleus. Cf. generally with that of the unipolar cell.

β. The *cell capsule;* like that of the unipolar cell but less conspicuously nucleated.

γ. The *related nerve fibres*, of these there are two (bipolar cell): one passes straight from the cell base like that of the unipolar cell; the other (*processus spiralis*) leaves the cell at a higher level, and, in passing downwards, winds round the base of the cell and the straight fibre.

c. *Multipolar cells.* See infra § 11.

11. **The spinal cord.**

Place some pieces of the fresh cord in potassium bichromate solution (2 to 3 weeks), wash well and afterwards harden in alcohol of increasing strengths. Stain with borax-carmine and mount in Canada balsam.

i. Examine under a low power.

a. The *cord;* bilaterally symmetrical; composed of a superficial lighter portion (*white matter*) and a deeper more deeply staining portion (*grey matter*).

b. *Nerve roots;* seen as bundles of fibres running out dorsally and ventrally from prolongations (*cornua*) of the grey matter.

c. The *canalis centralis;* oval, lined by a deep staining columnar epithelium.

d. The *fissures;* median dorsal and ventral, cleaving the white matter only.

c. The *pia mater;* obvious as a darkly stained investment for the whole. It dips in, especially at the fissures, to form a supporting framework for the cord. Prolongations of it into the substance of the cord may be readily seen, in the vicinity of the ventral fissure.

ii. Examine under a high power.

a. The *white matter;* chiefly composed of medullated fibres, cut across and lying within a supporting meshwork (*neuroglia*). Non-medullated fibres may be detected here and there.

b. The *grey matter.*

α. Composed, for the most part, of non-medullated fibres lodged in a granular matrix.

Note the course of the fibres, especially of those which pass from side to side.

β. *Multipolar nerve-cells.* A cluster of these will be seen in each ventral grey cornu, as large irregular cells, deeply staining and each with a round nucleus. Nerve fibres will be seen to enter them at several points.

γ. *Ventral nerve root.* Examine with care; continuity may often be traced between its individual fibres and the multipolar nerve cells.

12. **The retina.**

Highly satisfactory preparations of this organ can be obtained as follows. Take perfectly fresh eyes from a frog; remove a small portion of each, and lay the eyes for an hour in corrosive sublimate, or for

3 to 4 hrs. in picric acid; wash and transfer them to alcohol of increasing strengths.

Finally, stain with borax-carmine, imbed and cut into longitudinal vertical sections. Mount in Canada balsam.

i. Examine with a low power, the whole eyeball will be seen in section.

a. The *sclerotic;* a cartilaginous outer capsule.

b. The *choroid;* recognizable by the straggling nature of its pigment cells, which form a sort of loose network.

c. The *retina;* seen to be composed of a number of layers, differentiated under the action of the staining reagent. Note the *pigment layer;* reddish brown and regularly disposed, immediately internal to the choroid, one-third the thickness of the whole.

ii. Examine your thinnest section under a high power. Work from within outwards; the following will be seen, in order of enumeration.

a. *The internal limiting membrane*, a thin structureless layer.

b. *The nerve-fibre layer;* thin and granular, with delicate fibres.
[α. and β. are sometimes difficult to make out.]

c. *The nerve-cell layer;* composed mainly of large irregular cells with round nuclei. From some, branches can be traced into the next layer.

d. *The inner molecular layer;* this is thicker than any of the preceding, and has a finely

punctated appearance. It remains unstained under the above method of treatment.

e. *The inner nuclear layer;* nearly as thick as *d.*, and deeply stained. It is made up of a number of nuclei, around each of which is collected a very small amount of protoplasm; and of fine fibres, some of which can be traced into the same.

f. *The outer molecular (fenestrated) layer.* Narrow and unstained, somewhat resembling *d.*

g. *The outer nuclear layer.* Much thinner than the inner nuclear layer and more closely packed. It is composed of distinct fibres (*rod-* and *cone-fibres*), each of which swells out and has a nucleus developed in the enlargement.

h. *The external limiting membrane.* A thin homogeneous layer like *a*, obvious as a hard line which not unfrequently overhangs the outer nuclear layer.

i. *The rod- and cone-layer.* Usually stained a faint pink colour under the above treatment.

α. The *rods;* parallel and ending in blunted free ends; each subdivided transversely into two segments.

β. The *cones;* few in number and shorter than the rods; each ending in a pointed free end. Look for them among the bases of the rods.

k. The *pigment layer;* seen to consist of a close set series of elongated cells (pigment epithelium) forming a cap-like investment for the free ends of the rods and cones.

α. The *cell bases;* closely applied, each containing a round or oval clear nucleus.

β. Their *free ends;* fimbriated and prolonged down among the rods and cones.

l. *The fibres of Müller;* highly refractive supporting fibres, often traceable with ease from the internal limiting membrane to the fenestrated layer.

iii. Obtain, if possible, similar sections which shall pass through the entrance of the optic nerve. Examine under a high power, and follow the course and relations of the nerve-fibres.

iv. Take a fresh frog's eye: prick its cornea and collect the aqueous humour on a slide. Then open the eye, remove a bit of the retina and tease it out in the aqueous humour, mount and examine with a high power.

a. Numerous rods will be seen floating about, many broken but some intact and showing the boundary line between their two segments very plainly. At first both segments are homogeneous, but very soon they begin to alter; the outer layer frequently then gets transversely striated and shows a tendency to split up into several pieces: gradually these rods curl up, swell out, and entirely disintegrate.

13. **The olfactory epithelium.**

Open the nasal sac of a frog; remove a portion of the epithelial lining and transfer to 1 p. c. osmic acid solution for 2—3 hours; tease up in weak glycerine and examine with your highest power.

Numerous mutilated cells will be found, and, among them, more or less perfect representatives of the following.

a. *Epithelial cells;* columnar and elongated, each with an oval nucleus, an unbranched peripheral process and a branched basal one.

b. *Sensiferous cells;* more slender and often somewhat shorter than *a.*: generally to be recognized by their swollen bases (enlarged around an enclosed nucleus). Each terminates at its free end in a cluster of delicate, hair-like, stiff processes, and receives at its base a thread-like nerve-fibre.

14. **The auditory epithelium.**

Place sufficient of the membranous labyrinth to embrace one of the ampullæ (Sect. I. 4. *c.* γ) in 1. p. c. osmic acid solution for 3—4 hours; transfer to weak glycerine and tease up its epithelium with a couple of fine needles. Examine under your highest power, and look for—

a. *Hair cells* (sensiferous cells of auditory epithelium). Each is pear-shaped and composed of a granular protoplasm with a large round or oval nucleus: from its flattened free end there stands out the *auditory hair;* a long pointed structure (from 3 to 4 times the length of the cell-body) seen, on careful examination, to consist of several delicate fibrils bound together.

15. **The skin.**

i. Cut out a piece of skin from the back of the thigh of a recently killed frog: spread it out in water, cover, and examine with a low power: note—

a. *The pigment-cells;* black irregular patches; some compact, others more or less branched.

b. *The mouths of the cutaneous glands;* seen as clear round spots, although their openings are really triradiate: their number.

ii. Preserve a piece of skin in alcohol of increasing strengths, and stain with borax-carmine; imbed and cut sections perpendicular to its surface: mount in Canada balsam. Examine under a low power.

a. The *epidermis;* the superficial thinner and deep stained portion.

b. The *dermis;* the deeper main mass, for the most part lightly stained.

c. *Pigment;* most marked as a conspicuous irregular black layer, in the superficial stratum of the dermis.

d. *Cutaneous glands;* seen in section as numerous large oval, rounded, or flask-shaped spaces, lying within the dermis. Many can be seen to open on the free surface by a narrow neck.

iii. Examine under a high power.

a. The *epidermis* (*stratified epithelium*). Work from within outwards; there will be found in order:

α. The *Malpighian layer;* composed of a usually single basal row of close-fitting columnar cells, each with an oval nucleus; and of a superficial main mass, consisting of several rows of rounded or oval cells whose long axes are parallel with the free surface. Small pigment granules are frequently aggregated around the nuclei of the latter.

β. The *horny layer;* usually of a yellowish colour; made up of flattened cells whose boundary lines are rarely distinguishable. Nuclei for the most part absent; pigment granules present at intervals.

b. The *dermis;* work inwards from the epidermis; the following elements will be seen.

α. *Connective tissue;* forming the main mass, its cellular elements well marked, fibres for the most part in bundles.

β. *Unstriped muscle;* most marked in the deeper portion where it forms a thick *muscular stratum*, lying beneath the cutaneous glands.

γ. *Pigment;* deposited for the most part in two well-defined strata of irregular branching cells; rarely diffused throughout the connective-tissue mass.

The above-named strata are: a superficial one lying beneath the epidermis (cf. ii. *c.*), generally black and highly conspicuous; a deeper one, immediately internal to the muscular stratum, of somewhat lighter colour than the other.

δ. *Fat;* rarely present: when it is deposited, like the pigment, within individual cells of the connective-tissue mass.

ε. *Blood vessels;* inconspicuous in uninjected preparations. Their cut ends may however be seen among the cells of the inner pigment layer; thence vessels may sometimes be traced passing up, in a pigmented sheath, towards the bases of the cutaneous glands.

c. The *cutaneous glands.* Look for thin sections which pass through their necks.

α. The *gland;* usually lined by a single layer of flattened epithelial cells (more rarely by two diversely differentiated ones) occasionally surrounded by pigment. Its secretory product may often be seen within it, as a pale finely granular mass.

β. The *duct;* subdivided into two segments: an inner enlarged one, lined by a single layer of cells which graduate into the deeper layer of the epidermis; an outer much constricted one, the epithelium of which graduates into the horny layer of the epidermis.

γ. Look for sections showing the glands *en face.* Note the boundaries and characters of their epithelial cells.

16. **The intestine.**

i. Preserve some pieces of the small intestine, first washing out the contents with care, in ½ per cent. solution of chromic acid; transfer to alcohol of increasing strengths; stain with borax-carmine, imbed and cut transverse sections. Mount in Canada balsam and examine with a low power.

a. The *intestinal wall;* smooth externally, much folded internally. Its outer layer; uniformly thick (*muscular layer*): its inner layer; much folded and more deeply stained (*epithelium*).

b. Examine the thinnest portion under a higher power. Work from without inwards; there will be found in the order of enumeration—

α. *Serous layer* (peritoneum); obvious as a faint

contour line. Look for places where it may have been separated in preparation.

β. *Longitudinal muscular layer;* thin and deeply stained; seen to consist of the cut ends of close-set cells.

γ. *Circular muscular layer;* relatively thicker than β; cells close-set and parallel with the free surface (cf. § 7. *b.* β).

δ *Submucous layer;* faint and composed of vascular connective tissue. Large blood-vessels will be seen ramifying within it.

ϵ. *Mucous membrane;* composed of three layers. A *muscular layer*, forming a thin stratum immediately internal to δ; a *lymphoidal layer*, somewhat like the submucous layer, but containing numerous rounded or irregular cells (lymphoidal cells); the *epithelium*, composed of close-set columnar cells (cf. § 2. *b.*) whose inner ends for the most part stain very deeply.

c. Compare similar sections across an intestine the blood-vessels of which have been injected with gelatine (see Appendix C). Note the course of the vessels, and especially the relations of their ultimate ramifications.

17. **The pancreas.**

Preserve small portions in alcohol of increasing strengths; stain with borax-carmine; imbed and cut transverse sections; mount in Canada balsam.

a. Examine under a low power.

a. The *gland;* composed of groups of cells (*acini*)

diffused throughout a connective-tissue meshwork (*stroma*).

β. The *tunic;* a dense connective-tissue investment for the whole, continuous with the stroma. Look for the cut ends of blood-vessels (cf. perimysium, perineurium, periosteum).

b. Examine under a high power

α. The *acini;* each composed of a single layer of large epithelial cells, set around a small irregular central cavity.

β. The *ducts;* packed among α, characterized by the large size and regularity of their central cavities; in section rounded or oval, each lined by an epithelium of squarish cells.

Look for portions showing the transition of gland into duct.

18. **The liver.**

Preserve some small pieces of fresh liver in alcohol, and prepare sections as directed for the pancreas.

a. Examine under a low power.

The *gland cells* will be found to be arranged in groups, around a ramifying system of clear spaces with granular-looking contents (*blood-vessels*).

b. Examine under a high power.

α. The *gland cells;* for the most part squarish or hexagonal, each lodging a large round nucleus with one larger and several smaller nucleoli.

β. Look for sections of longitudinal series cells; note their arrangement in parallel rows.

γ. *Blood capillaries;* cf. supra. The granular appearance seen under the low power will be found

to be due to the presence of red corpuscles whose nuclei alone stain with great intensity, the bodies of the corpuscles remaining faint and yellow.

δ. *Bile capillaries;* apparent as a system of minute inter-cellular passages. Look for them in your thinnest sections; their cut ends appear sometimes as round or oval spaces, at others as deeply stained dots, between applied cells, much smaller than the nuclei of the same.

They may often be demonstrated if, prior to removal of the liver from the body, the bile-duct be ligatured and the bile injected into them by gently squeezing the gall-bladder.

c. Compare sections of injected liver, prepared as directed for the intestine § 16. *c.*

d. Tease up a fragment of fresh liver in salt solution and examine with $\frac{1}{8}$ objective.

α. The *hepatic cells;* polygonal and granular, often containing oil-drops (cf. osmic acid reaction).

β. Treat with acetic acid: a nucleus, or sometimes two, will be rendered apparent in each of the cells.

e. Tease up a similar fragment in iodine solution. Many of the cells will be seen to contain a product (*glycogen*) which stains a deep reddish brown.

19. **The kidney.**

Preserve a frog's kidney in alcohol of increasing strengths; stain with hæmatoxylin or borax-carmine; imbed it and cut into longitudinal sections parallel with its flat surface. Mount in Canada balsam.

a. Examine with a low power.

α. Note the numerous *uriniferous tubules* of which the organ is mainly composed; they twist about in several directions, and are consequently cut, some transversely, some obliquely, and others more or less longitudinally. The latter are most conspicuous at the outer (convex) border.

β. The clear round spaces, scattered about; these are sections of *Malpighian capsules*. Some may be seen to lodge a granular mass (*glomerulus*).

b. Examine with a higher power—

α. The *epithelium of the tubules:* composed for the most part of a single layer of large squarish cells, each with a large round deep-staining nucleus.

β. The *epithelium of the capsules;* flattened and squamous, its nuclei well defined, seen to be reflected over the glomeruli (i. e. the latter are outside it). Look for sections showing the connection of tubules with capsules.

γ. *Blood capillaries;* scattered among the tubules, conspicuous in borax-carmine preparations by the refractive deep-staining nuclei of the red corpuscles (cf. § 18. *b*. γ).

c. Compare sections of the fresh kidney treated with silver nitrate, as directed at § 2. *c*. Examine under a high power.

α. The *capsules*. The boundary lines of their epithelial cells will be distinctly seen; they are those of a tesselated epithelium.

β. The *smaller blood-vessels.* In those immediately related to the glomeruli, longitudinal and transverse striations are generally obvious under the above treatment. Examine with care; the longitudinal striæ will be seen to be the deeper (boundary lines of epithelial cells); the transverse ones are more superficial (boundary lines of unstriped muscle cells).

d. Compare sections of injected kidney. Examine under a low power; two sets of capillary vessels will be seen—

α. Around and among the tubules.

β. The *glomeruli;* vascular tufts indenting the Malpighian capsules (cf. *b.* β). Examine them in detail.

20. **The testis.**

Imbed a testis which has been hardened in alcohol of increasing strengths and stained with borax-carmine. Cut into transverse sections and mount in Canada balsam.

a. Examine with a low power.

α. The organ is chiefly made up of large tubules, radially disposed at the periphery, more tortuous internally.

Spermatozoa may or may not be present; if so, they will probably be aggregated into bundles with their heads (deeply stained) in close relation with the walls of the tubules.

b. Examine with a high power.

α. Note the epithelium (*germinal epithelium*) lining the tubules: it varies with the season of the

year, and may be one or more layers of cells deep.

c. *The spermatozoa.*

i. Tease up a small piece of the fresh testis in magenta solution. If spermatozoids are present, each will be seen to consist of

α. *Head;* rod-like, generally straight, more rarely arched, mainly composed of a deep-staining body (*nucleus*).

β. *Tail;* elongated and flagelliform, stains but feebly, if at all. In favourable specimens it may be seen to be continuous with a small amount of clear protoplasm which surrounds the "head."

ii. Compare the aggregates of spermatozoa seen in the prepared section. The applied heads of many of them will be seen to be capped by a large irregular nucleated cell (last remnant of parent cells of the group).

iii. Compare the living spermatozoa, if accessible, obtained from either the testis or vas deferens. Note the nature of their movements.

21. **The ovary.**

Prepare transverse sections of an ovary:—alcohol, borax-carmine, Canada balsam.

i. Examine under a low power.

a. The *ova* (*ovisacs*); variable in size; smaller ones, seen to consist of granular protoplasm with a large germinal vesicle (nucleus): larger ones, pigmented peripherally; germinal vesicle

rarely seen, as it only occasionally lies in the plane of section.

ii. Examine under a high power.

a. The *smaller ovisacs.*

α. The *ovum;* the central large cell; its *protoplasm*, uniformly finely granular and deep-staining.

β. *Germinal vesicle;* often irregular in outline; germinal spots diffused or arranged peripherally in a single row.

γ. *Ovarian follicle;* a single-layered epithelial investment for the ovum.

b. Compare the *larger ovisacs*

α. *Protoplasm of ovum.* Peripherally, black and pigmented, otherwise converted, for the most part, into an immense aggregate of small ellipsoidal or irregular refractive *yolk granules.*

β. Germinal vesicle. Look for specimens in which this is visible; the germinal spots are mostly, if not wholly, aggregated in the middle of the same.

γ. *Ovarian follicle;* still recognizable, but generally flattened.

δ. *Vitelline membrane;* obvious as a thin faint line, interposed between the ovum and the follicle. Look for it in ova whose investing structures may have been ruptured.

c. The *germinal epithelium;* a cellular mass immediately beneath the investing membrane of the ovary. Look for young ovisacs still in connection with it.

N. **The physiological properties of muscle and nerve.**

Place a frog under a beaker, with a drop or two of chloroform: take it out immediately it becomes unconscious, which will probably be in a few seconds. Now feel with a finger-nail for the depression beneath the skin at the back of the animal's head, which indicates the point of articulation of skull and spinal column: it lies in a line joining the posterior borders of the two tympanic membranes. Divide the skin and muscles at this point until the neural canal is laid open, and then pass a stout wire into the cranium and down the neural canal of the vertebral column. By this process (known as *pithing*) the frog is rendered totally incapable of further consciousness, though most of its tissues will retain their vitality for some time.

a. Remove the skin from one leg, so as to lay bare the muscles: send an interrupted electric current through any one of them (or tap the muscle sharply with the back of a scalpel): it will immediately *contract*, or *alter its form in a definite way;* it becomes *shorter and thicker, gaining in breadth just so much as it loses in length;* in so doing it moves the bones to which it is attached.

b. Very carefully lay bare the sciatic nerve, taking care not to crush or drag it: divide it as high up as possible, and, seizing it with a pair of forceps close to its cut end, lay it over the electrodes of an induction-coil. Probably when the nerve is cut the muscles of the limb will contract: whether or not, they will contract violently while the interrupted current is going through the nerve.

[If an induction-coil is not at hand a bit of clean copper wire twisted round a strip of zinc, with the points of contact moistened with dilute acetic acid, may be used to stimulate the nerve; smart tapping or pinching with a pair of forceps will also excite it, but by such means the nerve is soon killed.]

The above experiments show:

c. That the muscle is *irritable* and *contractile:* certain external agencies (*stimuli*) excite some change in it, the result of which is a muscular contraction.

d. The nerve is *irritable:* certain external agencies excite some change in it, which in this particular case manifests itself by a contraction of the muscles connected with the nerve.

e. The nerve possesses *conductivity:* although it is stimulated at some distance from the muscles, the change excited by the stimulus travels along it to them.

O. **Development.**

Place some freshly-deposited frog's spawn (taken directly after extrusion by the female) in a table aquarium or other glass vessel filled with water. Examine and preserve the eggs and embryos as directed below.

It is necessary for both observation and preservation that the mucus investment which surrounds the egg should be removed; this may best be done with a couple of fine needles, under water in a small saucer, or on any convenient white surface. When liberated, float the objects into a shallow watchmaker's glass, and examine under a low power as opaque objects, on a white ground unless otherwise directed. Preserve in Kleinenberg's picric acid solution, and transfer to alcohol of increasing strengths.

i. **Segmentation of the fertilized ovum (oosperm) and larval metamorphosis.**

The rate of both segmentation and development generally is liable to vary, in accordance with circumstances. The periods enumerated below are approximately those best suited for observation.

The specimens should be examined both in the fresh state and when fully preserved.

a. *One hour after fertilization.*

α. *General characters;* shape and size; the black pigmented upper pole; the yellowish white non-pigmented lower pole.

β. The *first cleavage furrow;* an annular constriction passing right round the egg, longitudinally with respect to the afore-named poles. It constricts the whole into two equal halves (*embryo cells or blastomeres*).

b. *Two hours after fertilization.*

α. The *second cleavage furrow;* longitudinal and at right angles to the first one, subdividing each blastomere into two. There are now four cells of equal size.

c. *The same at* 3—4 *hours.*

α. The *third furrow;* at right angles to the two former and excentric, lying within the pigmented pole. It subdivides the whole into four smaller pigmented *upper layer cells*, and four larger non-pigmented *lower layer cells*. Examine from several aspects.

d. *The same at* 6—8 *hours.*

A mulberry-like mass conspicuous by the irregularity in size of the blastomeres.

α. The *lower layer cells;* forming the lower half of the whole, their boundary lines regular.

β. The *upper layer cells;* forming the upper half, smaller and more numerous than those of the lower layer, their boundary lines in part irregular.

i. e. the upper layer cells are dividing more rapidly than the lower ones.

e. The same at 24 *hours.*

Recognizable by the preponderance of the pigmented area.

α. The *lower layer cells;* forming now the lower third or thereabouts; their boundary lines can only be made out with difficulty.

β. The *upper layer cells;* forming the upper two-thirds at least; their boundary lines no longer clearly definable under a low power, the pigmented portion having a granulated appearance.

i. e. the upper layer cells are growing round the lower ones.

f. The same at 4—5 *days.*

The lower layer cells apparent only as a small white spot (*blastopore*).

α. The *blastomeres;* boundary lines no longer recognizable.

β. The *blastopore.* Examine this with care; its lip, horse-shoe shaped, well defined above and at the sides, ill-defined below.

g. The same at 6—7 *days.*

Signs of the embryo are now beginning to appear,

the whole having undergone a slight increase in total capacity and lost its spherical shape.

α. The *blastopore;* still visible but small and excentric (displaced towards the flattened or dorsal surface).

β. The *neural plate;* obvious as a lyre-shaped flattening, in front of and in a line with the blastopore; its edges thickened and raised up (*neural folds*), its mid region depressed (*neural groove*).

The neural folds will in all probability be seen to unite in front, and die away behind, at the sides of the blastopore. A more or less marked approximation of their hinder halves will be obvious.

h. The same at 12—14 *days.*

The embryo may now be definitely recognized as a pear-shaped body still enclosed within the mucous investment. Remove and examine it.

α. The *body;* head and trunk, very obvious if seen from the dorsal aspect.

β. The *neural folds;* seen, if examined from the dorsal aspect, to be uniting in the middle line.

γ. The *blastopore;* no longer recognizable.

δ. The *suckers;* two oval thick-lipped depressions on the under side of the head (mouth not yet recognizable).

ε. The *visceral arches;* generally to be seen at this stage, as a couple of oblique ridges on either side, above δ.

i. The embryo at 15—18 *days.*

Still enclosed within the mucus mass; recogniz-

able by the presence of the tail, the lashing movements of which are very conspicuous (when at rest it is usually bent round to the right side). Liberate the embryo, and in doing so note that the mucous mass is undergoing disintegration.

α. The *body;* now differentiated into distinct head, trunk and tail.

Examine from the side.

β. The *visceral arches;* four in number on each side, the two hindermost bearing papillate outgrowths (*external branchiæ*).

γ. The *eye;* obvious as a rounded eminence above the interspace between the first two visceral arches.

δ. The *auditory organ;* obvious as a small pit (auditory pit) behind and above *γ*.

Examine from beneath.

ε. The *suckers;* now at their maximum of development and probably confluent posteriorly.

ζ. The *mouth;* a deep oval pit in the middle line immediately in front of *ε*.

η. The *olfactory organs;* apparent as two shallow pits (olfactory pits) in front of the mouth.

θ. The *anus* (*proctodæum*); a minute pore at the base of the tail.

k. The *free swimming larva.* Fish-like and bilaterally symmetrical, tail and external branchiæ much elongated. Introduce some duck-weed or other small plant into the water, and note the movements and habits of the larvæ.

Examine from the side.

α. The *head*; well marked and possessed of functional sense organs.

β. The *external branchiæ*; long and pectinated; three in number on either side, a small third-pair having appeared.

γ. The *tail*; elongated and marked out into a number of well-defined segments (*myomeres*).

δ. The *median fin*; a thin fold of integument encircling the tail and continued forwards—dorsally to the middle of the back—ventrally, for a short distance in front of the anus (cloacal orifice).

Examine from beneath.

ε. The *mouth*; transversely enlarged and surrounded by fleshy lips, within which can be seen the jaws, beset by small horny teeth.

l. *The larva, at a later period.* Look for larvæ in which the external branchiæ of the right side are no longer visible. Examine from beneath.

α. *Head, trunk and tail.* Cf. generally with the foregoing stages, as to relative proportions.

β. The *external branchiæ*; visible only on the left side, projecting out from beneath a fold of the cephalic integument (*operculum*). A similar fold is seen on the opposite side.

γ. The *mouth*; transversely oval and very large; the *lips*, now papillate; *horny teeth*, more marked.

δ. The *suckers*; disappearing, and reduced to the condition of a couple of small tubercles.

Examine from the side.

ε. The *mouth;* surrounded by a protractile bell-shaped *suctorial lip*, and utilized for purposes of adhesion, in place of the suckers which are now disappearing.

Cf. the movements of the living larva.

m. *The development of the operculum.* Look for larvæ intermediate between *k* and *l*. Examine from the side.

The operculum will be seen arising, on either side, immediately in front of the external gills, as a backwardly-directed fold of the second (*hyoid*) visceral arch.

n. The *larva at a later stage than l;* conspicuous by the great length of its tail and the absence of external branchiæ. Examine from beneath.

α. The *body generally;* clad in a thin transparent integument, elegantly pigmented in black and gold.

β. The *mouth;* still increasing in size, the circum-oral papillæ becoming more marked.

γ. The *suckers;* still further reduced and represented by two small vestiges.

δ. The *intestine;* visible through the body-wall as a greyish tube of large calibre, coiled in a reversed spiral.

ε The "*anus*" (*cloacal orifice*); median and ventral, situated at the base of the tail at the posterior-ventral extremity of the trunk (cf. side view).

ζ. The *hind-limbs;* minute papillate outgrowths of the body-wall, at the sides and a little in front of ε.

η. The *branchial pore* (*spiracle*); asymmetrical and on the left side (external branchiæ are no longer present). Remove the ventral portion of the adjacent integument (coalesced opercula); a spacious *branchial chamber* will be exposed. Note its limits.

θ. The *internal branchiæ*; paired vascular folds of the sides of the head, four sets on each side, lying within the branchial chamber. Try and make out their relations. They are borne upon outgrowths of the wall of the pharynx (*branchial visceral arches*), which alternate with perforations of the same (*visceral clefts*) putting the pharyngeal cavity in communication with the branchial chamber.

ι. The *lungs*; seen, on opening up the body-cavity, as small diverticula of the alimentary canal not yet distended with air.

o. The larva on the appearance of hind-limbs.

Examine from beneath.

α. The *body generally*; note the proportions of its several constituents.

β. The *mouth*; its large size; papillate lips and horny teeth well marked.

γ. The absence of suckers and branchial pores.

δ. The *hind-limbs*; small, but fully differentiated; one-third the length of the body and separated in the ventral middle line by the terminal portion of the alimentary canal.

ε. The *fore limbs*; differentiated, but covered by (visible through) the opercular membrane.

ζ. The *tail;* in all probability beginning to shrink.

p. *The young frog shortly after the moult* (loss of the larval skin): characterized by the coexistence of both pairs of limbs and the tail.

Note its frog-like characters and especially

α. The *mouth;* gape-wide, sucking lips gone, horny teeth replaced by true teeth.

β. The *tail;* absolutely shorter than in the late tadpole. Note its relations to the trunk.

γ. The *cloacal orifice.* Although displaced, it still lies at the base of the tail (i.e. it is ventral in position. Cf. the adult).

q. Look for specimens showing the mode of liberation of the fore limbs. The left one almost invariably appears first, forcing its way through the spiraculum; the right one is set free by absorption of the opercular membrane.

ii. The formation of the embryonic layers and certain of the more important organs.

Imbed and cut sections as directed below. Staining is unnecessary.

a. *At the first cleavage;* longitudinal and at right angles to the cleavage furrow.

α. The *blastomeres;* equal in size and separated by the cleavage furrow. Protoplasm; note its pigment and yolk granules.

β. *Nuclei.* These may or may not be visible; each is clear and transparent and generally surrounded by pigment. (If visible, look for indications of division.)

b. *On the formation of the third furrow* (cf. i. *c*). Median longitudinal, as at *a*.

α The *blastomeres*, four in number as seen in section; two smaller upper ones, densely pigmented; two larger lower ones, pigmented only at the periphery and yolk laden.

β. The *cleavage cavity* (*segmentation cavity*); small and central, in a line with the transverse furrow.

c *At* 6—8 *hours.* (Cf. i. *d*.) Longitudinal vertical.

α. The *upper layer cells*; small and pigmented, a single layer deep, nuclei generally visible.

β. The *lower layer cells*; large cells with little or no pigment, nuclei rarely visible.

γ. The *yolk granules*; aggregated in and largely confined to the lower cells (vegetative pole of the oosperm).

δ. The *cleavage cavity*; large and irregular, interposed between α and β.

d. *At* 30—38 *hours.* Longitudinal vertical, to pass through the first trace of the blastopore.

α The *cleavage cavity*; large and excentric, interposed between the cells of the upper and lower layers.

β. The *lower layer cells*; several rows deep, nucleated and yolk laden; forming the main mass.

γ. The *upper layer cells*; now two or three rows deep (*epiblast* or outer germinal layer) and differentiated into two layers.

Follow the course of the epiblast; it largely encloses the lower layer cells and is probably invaginated at the blastopore.

e. *At* 4—5 *days* (typical blastopore stage. Cf. i. *f*). Longitudinal vertical, to pass through the blastopore.

α. The *lower layer cells*; now practically destitute of pigment and almost completely enclosed in epiblast; exposed only in the region of the blastopore.

β. The *archenteron* (primitive alimentary canal); a spacious cavity occupying much the position of the cleavage cavity in Section *d*; in communication with the exterior at the blastopore.

γ. The *cleavage cavity*; small and irregular, situated below β at the end opposite the blastopore.

δ. The *hypoblast* (inner germinal layer or digestive epithelium); a single layer of closely packed cells forming the roof of the archenteron, continuous with the epiblast at the upper lip of the blastopore (i. e. formed as the result of invagination of the latter layer).

ε. The *mesoblast* (middle germinal layer); obvious in median longitudinal section as two series of cells somewhat irregular and more loosely scattered than the rest; an upper series lying in the interspace between the roof of the archenteron and adjacent epiblast; a lower series somewhat similarly interposed below between the lower layer cells and adjacent epiblast, and most numerous at the blastopore.

f. *The same at* 12—14 *days* (cf. i. *h*). Transverse across the middle of the neural plate. Examine under a high power.

α. The *neural plate;* a thickened medio-dorsal sheet of epiblast, partly invaginated and enclosing a deep *neural groove;* its raised edges (*neural folds*).

β. The *archenteron;* a wide cavity situated nearer the upper than the lower pole.

γ. The *hypoblast;* a single layer of cells forming the roof and side walls of β: its cells, somewhat flattened above, graduating into the lower layer cells laterally.

δ. The *notochord;* a median oval mass of small cells, immediately beneath the neural plate, not yet completely constricted off from the hypoblast.

ε. The *mesoblast;* interposed between the epiblast, and hypoblast with the lower layer cells; most marked dorso-laterally and symmetrical on opposite sides.

g. *The same at* 15—18 *days* (cf. i. *i*). Transverse across the middle of the trunk, for comparison with *f*.

α. The *epiblast;* well-defined and pigmented. Examine under a high power, and note the order of succession and characters of its layers.

β. The *hypoblast and lower layer cells.* Cf. *f*. γ.

γ. The *neural tube* (cerebro-spinal axis) pigmented and medio-dorsal, enclosing a *central canal;* it no longer shows any trace of connection with epiblast. (Cf. i. *h*.)

δ. The *notochord;* immediately beneath γ, large and rounded, composed of large vacuolated cells.

ϵ. The *mesoblast;* forming a denser mass (*mesoblastic somite*) on either side of the neural tube and notochord; below that point subdivided into two layers—an outer one (*somatic layer*) which applies itself to the epiblast, and an inner one (*splanchnic layer*) which invests the hypoblast and lower layer cells.

ζ. The *body cavity;* the interspace between the somatic and splanchnic mesoblast; more or less obvious, in accordance with the degree of shrinkage of the preparation.

η. The *leading blood-vessels*, as under. The *dorsal aorta*, median and cylindroidal underlying the sub-notochordal rod; the *posterior cardinal veins*, large vessels, ovoidal or irregular in section, right and left of the aorta. Blood corpuscles will in all probability be seen within them.

Examine under a high power. The above vessels will be seen to be formed as excavations of the mesoblast, each bounded by a single layer of modified cells.

θ. The *pro-renal* (*segmental*) *duct;* a conspicuous thick-walled tube seen, on either side, lying within the somatic mesoblast immediately beneath the posterior cardinal vein.

h. *The larva at* 12—14 *days* (cf. i. *h*). Median longitudinal vertical, to pass through the blastopore (anus). Examine under a low power.

α. The *body;* elongated and enclosing two cavities:—a ventral or *archenteric canal*, in communication with the exterior at the blasto-

pore, and a dorsal or *neural canal*, at this period in communication posteriorly with the archenteron and blastopore by a short narrow passage (*neurenteric canal*).

β. The *notochord;* a median rod of slightly vacuolated cells, lying in the interspace between the archenteric and neural tubes.

γ. The *neural tube;* enlarged in front (brain) and overhanging the notochord.

δ. The *archenteron;* its extent; its *roof*, formed by the hypoblast cells; its floor, still bounded by undifferentiated lower layer cells, now comparatively few in number.

ε. The *mesoblast;* composed of irregular scattered cells and more extensive than in the earlier stages examined.

i. *At* 15—18 *days* (cf. i. *i*); transversely oblique, to pass through the visceral arches and external branchiæ.

α. The *brain;* a large vesicle, situated at the anterior end.

β. The *mesenteron* (archenteron); a large central cavity behind and in a line with α, wide in front, constricted behind.

γ. The *visceral pouches;* paired diverticula of the hypoblast, 4 or 5 in number on either side, their blind ends abutting against the epidermis (if the section be much shrunk their walls may be in close apposition).

δ. The *visceral arches:* mesoblastic aggregates alternating with γ; each lying behind its cor-

responding pouch, and usually lodging a large blood-vessel (*aortic arch*) seen in section.

ε. The *external branchiæ*. These will be seen in some of the sections as filiform outgrowths (from 1 to 3 in number on either side) of the visceral arches.

Each consists of a mesoblastic core invested in epiblast. If the five visceral pouches are seen, the first gill will be found to arise behind the second one (first branchial).

k. Examine the lower sections of the above series and look for the formation of the mouth cavity.

α. The *mesenteron;* cf. supra; it ends blindly in front and is lined by a non-pigmented epithelium (yellow if prepared as directed and not stained).

β. The *stomodæum;* a sac-like involution of the epidermis abutting against α, spacious, and well marked on account of its dense pigmentation. Its enclosed cavity is the mouth cavity.

l. Compare similar sections through the head of an older larva, in which the internal branchiæ are present.

α. The *visceral pouches;* those of the branchial series are now in open communication with the exterior (*visceral clefts*).

β. The *internal branchiæ;* vascular pectinations of the opposite faces of α. Note their number and arrangement.

γ. The *operculum;* a backwardly-directed fold arising behind the first pouch (from the hyoid arch; cf. i. *m*).

m. Across the head of *i.* (15–18 days); transversely to the long axis, to pass through the eyes.

α. The *epidermis;* forming a continuous investment for the whole; thickened below and produced into two inverted cup-shaped folds (*suckers*).

β. The *brain;* dorsal and elongated, pigmented and enclosing a spacious central cavity (3rd ventricle).

γ. The *notochord and archenteron;* occurring in order of succession below β.

δ. The *developing retina;* on either side a cup-shaped outgrowth of the brain (*optic cup*), connected with the same by a narrow neck (*optic stalk*) visible only in a few sections. Examine the cup and note its thick outer wall; its thin inner wall; its central cavity, continuous with that of the 3rd ventricle.

ε. The *lens;* visible either as a thickening or involution of the epiblast, immediately external to δ.

n. Compare similar sections through the eye of a more advanced larva. Examine under a high power.

α. The *optic cup;* its central cavity becoming obliterated, its inner wall densely pigmented (pigment epithelium; cf. Sect. M. 12 *k*).

β. The *lens;* completely constricted off from the epidermis and enclosing a central cavity; its thin outer wall; its thick inner wall.

γ. The remaining constituents of the eye; seen, for the most part, in course of differentiation from the surrounding mesoblast.

II.

THE FRESH-WATER CRAYFISH (*Astacus fluviatilis*) AND THE LOBSTER (*Homarus vulgaris*).

THE Crayfish and the Lobster are inhabitants of the water, the former occurring in many of our rivers and the latter abounding on the rocky parts of the coasts of the European seas. They are bilaterally symmetrical animals, provided with many pairs of limbs, among which the large prehensile 'claws' are conspicuous. They are very active, walking and swimming with equal ease and sometimes propelling themselves backwards or forwards, with great swiftness, by strokes of the broad fin which terminates the body. They have conspicuous eyes, mounted upon moveable stalks, at the anterior end of the head; and two pairs of feelers, one pair of which are as long as the body, while the other pair are much shorter.

The body and limbs are invested by a strong jointed shell, or *exoskeleton*, which is a product of the subjacent epidermis, and consists of layers of membrane which remain soft and flexible in the interspaces between the segments of the body and limbs, but are rendered hard and dense elsewhere by the deposit of calcareous salts; the exoskeleton is deeply tinged with a colouring matter which turns red when exposed to the action of boiling water. The body presents an anterior division—the *cephalothorax*—covered

by a large continuous shield, or *carapace;* and a posterior division—the *abdomen*—divided into a series of segments which are moveable upon one another in the direction of the vertical median plane, so that the abdomen can be straightened out, *in extension;* or bent into a sharp curve, *in flexion.* Of these segments there are seven. The anterior six are the *somites* of the abdomen, and each of them has a pair of appendages attached to its ventral wall. The seventh bears no appendages and is termed the *telson*—it is subdivided into two pieces in the Crayfish. The anus is situated on the ventral aspect, beneath the telson and behind the last limb-bearing somite.

A groove on the surface of the carapace, which is termed the *cervical suture*, separates an anterior division, which is termed the head or *cephalon*, from a posterior division or *thorax;* and the thoracic division of the carapace further presents wide lateral prolongations, which pass downwards and cover the sides of the thorax, their free ventral edges being applied against the bases of the thoracic limbs. These are the *branchiostegites.* Each roofs over a wide chamber in which the gills are contained and which communicates with the exterior, below and behind, by the narrow interval between the edge of the branchiostegite and the limbs. Anteriorly and inferiorly, the branchial chamber is prolonged into a canal, which opens in front and below at the junction of the head with the thorax, immediately behind the cervical suture. In this canal there lies a flat oval plate—the *scaphognathite*—which is attached to the second pair of maxillæ and which plays a very important part in the performance of the function of respiration. Of the thoracic limbs themselves there are eight pairs, and, on the ventral face of the body, the lines of demarcation between the eight somites to which these limbs belong

may be observed. There is no trace of any corresponding divisions in the cephalothorax of the Lobster; but, in the Crayfish, the last thoracic somite is incompletely united with those which precede it. The four posterior pairs of thoracic limbs are those by which the animal walks and are termed the *ambulatory legs*. The next pair in front is formed by the great claws or *chelæ*. The anterior three pairs are bent up alongside the mouth and are moved to and from the median line so as to play the part of jaws, whence they are termed foot-jaws or *maxillipedes*. The external or third pair of these maxillipedes are much stouter and more like the ambulatory limbs than the rest, and the inner edges of their principal joints are toothed. The innermost or first pair of maxillipedes are broad, foliaceous and soft. When these foot-jaws are taken away, two pairs of soft foliaceous appendages come into view. They are attached to the hinder part of the cephalon and are the jaws or *maxillæ*. The second, or outermost, is produced, externally, into the scaphognathite, which will be seen to lie in a groove separating the head from the thorax laterally and known as the *cervical groove*.

Anterior to these maxillæ lie the two very stout mandibles. Between their inner toothed ends is the wide aperture of the mouth, bounded, in front, by a soft shield-shaped plate, the *labrum*; and behind, by another soft plate, divided by a median fissure into two lobes which simulate appendages—it is termed the *metastoma*. Thus far, the surfaces of the somites to which the appendages are attached look downwards, when the body is straightened out and the carapace is directed upwards. But, in front of the mouth, the wall of the body to which the appendages are attached is bent up, at right angles to its former direction, and consequently looks forwards. This bend of the ventral wall of the body

is the *cephalic flexure*. In correspondence with this change of position of the surface to which they are attached, the three pairs of appendages of the somites which lie in front of the mouth are directed either forwards, or forwards and upwards. The posterior pair consists of the long feelers or *antennæ:* the next, of the short feelers or *antennules ;* and the most anterior is formed by the short subcylindrical stalks (*ophthalmites*), on the ends of which the eyes are situated.

This enumeration shews that the Lobster and Crayfish have six pairs of abdominal appendages—the swimmerets and "tail-fin"; eight pairs of thoracic appendages (four pairs of ambulatory limbs, one pair of chelate prehensile limbs, three pairs of maxillipeds), and six pairs of cephalic appendages (two pairs of maxillæ, one pair of mandibles, one pair of antennæ, one pair of antennules, one pair of eyestalks), making in all twenty pairs of appendages. It may or may not be that the eyestalks are modified appendages ; should they have that value, the body in correspondence with the number of appendages will consist of twenty somites (the telson excepted): of these six remain moveable upon one another to form the abdomen, while the other fourteen are, with the exception of a portion of the last thoracic one in the Crayfish completely united to form the cephalothorax.

The branchiostegite is an outgrowth of the dorso-lateral region of the confluent thoracic somites. The serrated rostrum which ends the carapace is a fixed median prolongation of the dorsal wall of the anterior cephalic somites. The telson is subdivided by a transverse constriction into two portions ; a posterior post-anal one and an anterior one which, like the somites in front of it, transmits the intestine. The labrum and the metastoma are median growths of the sterna of the præ-oral and post-oral somites.

Thus the whole skeleton in these animals may be con-

sidered as at least a twentyfold repetition of the ring-like somite with its pair of appendages, which is seen in its simplest form in one of the abdominal somites. Moreover, notwithstanding the great variety of functions allotted to the various appendages, the study of the details of their structure (see Laboratory work) will shew that they are all reducible to modifications of a fundamental form, consisting of a basal-portion (*protopodite*) with two terminal divisions (*endopodite* and *exopodite*). A third division or *epipodite* is superadded to the appendages concerned in respiration.

Of the twenty pairs of appendages, the three anterior are concerned with sensation and the six posterior with swimming; the mandibles, which bound the mouth, are most efficient in mastication. Of the ten pairs which remain, five are modified to form foot-jaws, the others being functional for walking or climbing; three of the latter however, in that they are chelate and often used to capture prey or to tear up food material, combine the functions of locomotor feet and foot-jaws.

Each of the larger appendages is composed of a number of segments, each movable upon its fellow in a single plane; the various segments are so articulated that the limb, as a whole, is capable of considerable rotation.

As has been already said, the Lobster and Crayfish are bilaterally symmetrical; that is to say, a median vertical plane passing through the mouth and anus divides them into two similar halves. This symmetry is exhibited not merely by the exterior of the body and the correspondence of the paired limbs, but extends to the internal organs; the alimentary canal and its appendages, the heart, the nervous system, the muscles and the reproductive organs, being disposed so as to be symmetrical in relation to the median vertical plane of the body.

The wide gullet leads almost vertically into the spacious stomach, and both are lined by a chitinous continuation of the exoskeleton. The stomach is divided by a transverse constriction into a spacious *cardiac*, and a much smaller *pyloric* division, from which latter the intestine passes. The walls of the anterior half of the cardiac sac are thin and membranous, but, in the posterior half, they become calcified so as to give rise to a *gastric skeleton* of considerable complexity. The chief part of this skeleton consists of a median dorsal T-shaped 'cardiac' ossicle, the cross-piece of which forms a transverse arch, while its long median process extends backwards in the middle line. The ends of the transverse arch are articulated obliquely with two small 'antero-lateral' pieces, the extremities of which again are articulated with postero-lateral pieces, and these with a cross-piece, the 'pyloric' ossicle, which arches over the roof of the pyloric division of the stomach. In this manner a sort of hexagonal frame with moveable joints is formed, and the median process projects backwards so far, as to end below the pyloric piece. It is connected with this, however, by a short 'pre-pyloric' ossicle which ascends obliquely forwards and is articulated with the anterior edge of the pyloric piece. The lower extremity of this is produced into the strong median 'uro-cardiac' tooth; while the postero-lateral pieces are flanged inwards, and, becoming greatly thickened and ridged, form the large 'lateral cardiac' teeth. Two powerful muscles are attached to the cardiac ossicle, and ascend obliquely forwards to be inserted into the under face of the carapace. Two other similar muscular bundles arise from the pyloric ossicle, and, passing obliquely upwards and backwards, are also inserted into the under face of the carapace, in the region of the cervical groove. The disposition of all these parts is such that when these

muscles contract, the dorsal ossicles are divaricated, the urocardiac tooth is thereby thrust forwards and downwards, while the lateral teeth move inwards downwards and backwards, and the three meet in the middle line. The working of these muscles can be readily imitated by seizing the anterior and posterior cross-pieces with forceps and pulling them in the direction in which the muscles act. The three teeth will then be seen to come together with a clash. Thus the food which has been torn by the jaws is submitted to further crushing in this gastric mill. The walls of the pyloric division of the stomach are thick, and project like cushions into its interior, thereby reducing its cavity to a narrow passage. The cushion-like surfaces of the pyloric walls are provided with long hairs which stretch across this narrow passage, and thus convert it into a strainer, which allows of the passage of only very finely divided matter from the gastric sac to the thin and delicate intestine. The intestine, in both Lobster and Crayfish, is made up of an anterior thin-walled segment whose roof is prolonged up into a cœcal process, and a posterior segment which, like the stomach, is lined by a chitinous continuation of the exoskeleton. The latter is spirally folded and papillose in the Crayfish. The anus is bounded by two valve-like thickenings of the exoskeleton, which are connected with the adjacent intestinal wall by a series of small muscles. The alimentary-canal is thus to be resolved into a straight tube of three segments; an anterior *fore-gut* and a posterior *hind-gut*, each lined by an involution of the cuticular exoskeleton, and a non-cuticular *mid-gut* which bears the above-named cœcal process and receives the ducts of the digestive gland. The digestive gland itself is the seat of the formation of a combustible carbo-hydrate oily material which is as it were held in reserve in its constituent cells, as well as

of a digestive ferment; as these physiological activities are frequently relegated respectively to separate liver and pancreas, the single gland which here performs the two functions has been appropriately termed the *hepato-pancreas*.

The heart is a short, thick, somewhat hexagonal, symmetrical organ lodged in the pericardiac sinus, to the walls of which it is attached by fibrous bands. In its anterior half three pairs of apertures are visible, two being placed upon the upper face, two at the sides, and two on the under face. The lateral apertures are the most posterior, the dorsal, the most anterior in position. Each aperture begins in a funnel-shaped depression of the outer face of the organ, which leads obliquely inwards and terminates by a valvular slit in the cavity of the heart. This cavity is very much reduced by the encroachment of the muscular bands which constitute the walls of the heart, so that a transverse or longitudinal section shews only a small median cavity surrounded by a thick and spongy wall.

During life, the heart beats vigorously, the whole of its parietes contracting together. From the dorsal part of its anterior extremity three arteries are given off, one median and two lateral, to the cephalon and its contents, and from the ventral aspect of this end of the heart an *hepatic artery* is given off, on each side, mainly to the liver. At its posterior end, the heart ends in a median dilatation from which two great arterial trunks are given off: one, the *superior abdominal* artery, runs along the dorsal face of the intestine, giving off transverse branches as it goes, in each somite; the other, the *sternal* artery, immediately on leaving the pericardial-sinus distributes branches to the genital gland; it then passes ventrally, to the interspace between the penultimate and antepenultimate thoracic ganglia, passes between their commissures and divides into two

branches, which run, backwards and forwards, between the ganglionic chain and the exoskeleton.

These arteries divide and subdivide and end in what, in some parts of the body at any rate, *e.g.* the liver, is a true capillary system. The veins are irregular channels, or *sinuses*, which lie between the several muscles and viscera. One of the largest of these is situated in the median ventral line, and can be readily laid open by piercing the soft integument which lies between any two of the abdominal sterna. The blood flows out of the aperture with great rapidity, and the quantity shed shews the size of the sinus and its free communication with the rest of the vascular system. By cutting across any one of the limbs and inserting a blow-pipe into the place whence the blood wells forth, this ventral sinus can be readily injected with air. A large and irregular sinus is also to be found in the median dorsal region of the abdomen and is freely connected with the median ventral sinus. The stem of each branchia contains two canals, one running along its outer and the other along its inner face. The outer canal communicates, at its origin, with the thoracic portion of the median ventral sinus. The inner canal opens into a passage which ascends in the lateral wall of the thorax and opens, after meeting with other '*branchio-cardiac*' canals, opposite the lateral aperture of the heart. As the valvular lips of this and the other apertures of the heart open inwards, the blood, when the systole takes place, is driven out of the heart through the various arteries, and a considerable part of the blood thus propelled into the capillaries is collected by the median ventral sinus and thence, passing through the gills, eventually returns to the heart. It is customary to speak of a heart such as this, which propels aerated blood, as *systemic*, by way of distinction from a *branchial* heart, which propels impure venous

blood to the organs of respiration. The central cavity of the carapace is a vascular one, receiving its blood from a great sinus which surrounds the liver and stomach, and in part, it is believed, from the arteries of the anterior extremity; the blood, on leaving the carapace, is collected into two great trunks which enter the pericardium postero-dorsally. It is uncertain whether the so-called pericardium is to be regarded as one cavity, or whether the fibrous bands, which connect the heart with its walls, may not subdivide it into compartments in immediate communication with certain of the cardiac apertures, and not with the rest.

In the Lobster, from which the blood is readily obtained in quantity, it is a nearly colourless fluid, which usually has a faint neutral tint. It readily coagulates, a tolerably firm clot separating from the serum. It contains nucleated corpuscles, which throw out very long pseudopodial prolongations, and thereby take an irregular stellate form.

It has been seen that the respiratory organs, or branchiæ, are lodged in a chamber situated between the branchiostegite externally, the lateral walls of the thoracic somites internally, and the bases of the thoracic limbs below; and that there is a narrow interspace between the free edge of the branchiostegite and the latter. At the anterior end of the chamber, a funnel-shaped passage leads to the anterior opening mentioned above, and, in this passage, the scaphognathite lies like a swing door.

During life, the scaphognathite is in incessant movement forwards and backwards, scooping out the water in the branchial chamber. This action results in the inducing of a current which flows in by the inferior and posterior cleft beneath the free edge of the branchiostegite, and thus a constant circulation over the gills is secured.

Each branchia is somewhat like a bottle-brush, having a stem beset with numerous filaments; and the blood contained in the vessels of the latter, being separated by only a very thin membrane from the air contained in the water, loses carbonic anhydride and gains a corresponding amount of oxygen in its course through the branchiæ.

The branchiæ are exclusively thoracic, being attached partly to the inter-articular membranes between the appendages and the body-wall and partly to the proximal ends of the limbs themselves. The last thoracic appendage is gill-less, and the branchia present in its vicinity in the Crayfish differs from the rest in being attached to the epimeral wall of the thorax; the Lobster has, in addition, three such gills on either side fully developed and functional: all or most of these are represented in the Crayfish by short filamentous rudiments, no longer functional as branchiæ. The epipodites of the limbs ascend between the sets of branchiæ which belong to each somite, and separate them. The branchiæ which are attached to the limbs must necessarily be stirred by the movement of the latter, and hence the exchange of gases between the blood which they contain and the water must be, to a certain extent, increased, in proportion to the muscular contractions which give rise to the movements of the limbs and the consequently increased formation of carbonic anhydride. There is reason to believe that the blood system of the carapace (cf. p. 182), may be concerned in respiration, through the mediation of its thin inner wall.

The excretion of nitrogenous waste goes on in the two large *green glands* which lie in the cephalon. Each gland stands related to a thin-walled muscular sac which opens upon the ventral face of the basal joint of the antenna. The gland itself consists of a series of coiled tubes, abundantly supplied with blood vessels.

The nervous system consists of a chain of thirteen ganglia—united by longitudinal commissures—lodged in the median line of the ventral aspect of the body, from which nerves are given to the organs of sense, to the muscles of the trunk and limbs, and to the integument; and of a *visceral* nervous system, developed chiefly upon the stomach and hinder segment of the intestine.

The ganglia are centres of aggregation of the nerve cells; of the thirteen seen in the adult the most anterior lies in the cephalon, close to the attachments of the three anterior pair of appendages, and gives branches to them and to the visceral nervous system. It is usually termed the brain or the *supraœsophageal* ganglion. It is connected by two commissural cords, which pass on each side of the gullet, with a larger ganglionic mass, which is called the *subœsophageal* ganglion. This occupies the region of the hinder part of the cephalon and the anterior part of the thorax, and gives off nerves to the mandible, maxillæ and the three pairs of maxillipeds. Five other ganglia lie in the five somites which bear the chelæ and the ambulatory limbs, and there is one for each abdominal somite, the last of these being the largest of the six.

The longitudinal commissures are double, and the ganglia themselves shew more or less evident indications of being double also. There is reason to believe that these thirteen apparent ganglia really represent twenty pairs of primitive ganglia, one pair for each somite; the three anterior pairs having coalesced preorally to form the brain; and the six which follow the mouth having united into the subœsophageal mass.

The only organs of special sense which are recognizable in the Lobster and Crayfish are eyes and auditory organs, and a series of specially modified setose appendages which

fringe the exopodite of the antennule, and are thought to perform an olfactory function.

The *eyes* are situated at the extremities of the eyestalks, or ophthalmites, which represent the first pair of appendages of the head. The rounded end of the eyestalk presents a clear smooth area of somewhat crescentic form, divided into a great number of small mostly four-sided facets. This area corresponds with the *cornea*, which is simply the ordinary chitinous layer of the integument become transparent. The inner face of each facet of the cornea corresponds with the outer end of an elongated transparent slightly conical body—the *crystalline cone*—the inner end of which passes into a spindle-shaped transversely striated body—the *striated spindle* or *rhabdome*. The inner extremity of this again is connected by a nerve fibre with the optic bulb, the dilated gangliform termination of the optic nerve. The respective *striated spindles*, and *crystalline cones*, thus radiate from the outer surface of the terminal ganglion to the inner surface of the cornea, and each is separated from its neighbour by a nucleated *sheath*, parts of which are deeply pigmented. Nothing is accurately known as to the manner in which the function of vision is performed by the so-called *compound eye* which has just been described. The inner and outer faces of the corneal facets are flat and parallel. They therefore cannot play the part of lenses; and, if they could, there is no trace of nerve endings so disposed as to be affected by the points of light gathered together in the foci of such lenses. Morphologically, the striated spindles and their nerve fibres and probably the optic bulb itself wholly or in part, are in many ways analogous to those elements of the retina of the Vertebrata which make up the layers of rods and cones and the granular layers. The aforementioned structures are, together with the optic nerve and ganglion, derivatives of the epidermis; in the vertebrate the

rods and cones arise as outgrowths of the optic cup, which is a derivative of the invaginate brain (cf. *ante*, p. 172); morphologically speaking therefore, the free ends of the rods and cones of the vertebrate eye are, as in the crustacean, turned outwards. There is good reason for believing the crystalline cones to be derivatives of the investing epidermis.

The *auditory organ* of the Lobster and Crayfish is situated in the basal joint of the antennule, on the dorsal surface of which its small slit-like opening, protected by numerous setæ, is to be seen. The chitinous layer of the integument is invaginated at the opening, and thus gives rise to a small flattened sac lodged in the interior of the antennule. One side of this sac is in-folded so as to produce a ridge, which projects into the cavity of the sac, and is beset with very fine and delicate hair-like setæ. The auditory nerve enters the fold, and its ultimate filaments pass into the setæ at thēir bases. The sac contains water in which minute particles of sand are suspended in the manner of otolitns.

The sexes are distinct in the Lobster and Crayfish. The external characters of the males and females and the form of the reproductive organs are described in detail in the Laboratory work. One pair of genital ducts is normally present in each sex, but marked indications of their duplication occasionally occur (cf. pp. 191 and 218).

The ovary is median and saccular, and its investing membrane is prolonged backwards to form a paired oviduct whose walls are glandular. Each ovicell is invested, during its maturation, in an epitheloid *follicle* of a single layer of cells; by the rupture of this the ripe ovum is liberated, and, thrown off thus into the interior of the ovary, it makes its way down the oviduct and so to the exterior. The impregnated ova are attached in great numbers, to the hairs of the swimmerets where they undergo their development, by the viscid secretion of a series of "*cement glands*," borne upon

the ventral surfaces of the abdominal somites and bases of the last pair of swimmerets of the female. A Lobster with eggs thus attached, is said by the fishermen to be 'in berry.'

The segmenting egg of the Crayfish differs in some important respects from that of the Frog. The yolk material is aggregated in the central protoplasm, and not at one pole as in the case of the latter animal, and segmentation is restricted to the superficial least yolk-laden protoplasm; the egg is at no period completely cleft into two parts, segmentation is from first to last partial or *meroblastic*, a cellular investment being as it were formed around a central yolk-bearing mass. The investing cells become invaginated at one point to form a small sac, which remains for a time in open communication with the exterior. The embryo at this stage is to be resolved into a double-walled sac or *gastrula*, the interspace between the two walls being filled with yolk. The outer layer or *epiblast* eventually gives rise to the epidermis and its derivatives—the nervous system and sensiferous epithelia; the inner one or *hypoblast* forms the lining membrane of the mid-gut, and from it the digestive gland is formed later as a paired outgrowth—it is from first to last the true digestive epithelium. The cavity enclosed by the hypoblast in its simple sac-like condition is the primitive alimentary canal or *archenteron*, its aperture of invagination being termed the *blastopore*. The remaining constituents of the body are derived from cells which are budded off from one or both of the above layers. Early traces of the embryo are obvious in the development of the cephalo-thoracic appendages, which arise as paired outgrowths of a relatively small area of the surface of the egg known as the *germinal area*; there appear at the same time, at opposite ends of this, two median papillate outgrowths—an anterior one which gives rise to the labrum, and a posterior one which, by subsequent elongation and segmenta-

tion is transformed into the abdomen, for which reason it is termed the *abdominal papilla.* The blastopore meanwhile becomes closed, the archenteric sac being no longer in communication with the exterior; to meet this latter there are instituted, immediately under cover of the labrum and abdominal papilla, median ingrowths of the epidermis which give rise to the lining membranes of the fore and hind gut respectively, their apertures of invagination persisting as the adult mouth and anus.

The yolk does not enter conspicuously into any of the above-mentioned outgrowths; it is enclosed within the cephalo-thoracic region, which becomes thereby much distended. The gills and branchiostegites appear late; the former as simple outgrowths of the body-wall and appendages, the latter as lateral folds of the body wall. The embryo which results from these developmental processes passes through all the stages which are needed to bring it very near to the form of the adult before it leaves the egg: but, in the Lobster, the young, when hatched, are larvæ extremely unlike the parent, which undergo a series of metamorphoses in order to attain their adult condition. The larvæ may frequently be obtained by opening the eggs of a 'hen-lobster' in 'berry.' They have when young a rounded carapace, two large eyes, a jointed abdomen devoid of appendages; and the thoracic limbs are all provided with long exopodites. During the later metamorphoses the abdominal appendages appear, as that region of the body elongates and increases in importance; the growth of the exopodites of the thoracic appendages is at the same time arrested, those of the chela and ambulatory legs vanishing entirely as these appendages become specialised for locomotion.

The ordinary growth, no less than the metamorphoses of the Lobster and Crayfish, are accompanied by periodical

castings of the outer, chitinous, layer of the integument, or whole exoskeleton if calcified. The shedding of this is preceded by a process of disintegration along certain definite lines, such as the edge of the chela and the inter-articular membrane between the cephalo-thorax and abdomen; these become, during the period at which the animal is freeing itself, so many points of least resistance and a consequent rupture of them ensues. After each *ecdysis*, the body is soft, being invested in a continuous chitinous cuticle, and the animal retires into shelter until, by calcification of this, the 'shell' is reproduced.

As the hard parts of the exoskeleton are the result of replacement in earthy matter of portions of an originally continuous chitinous cuticle, it follows that the uncalcified areas, which remain soft and flexible, are but persistent portions of the cuticular predecessor of the whole; they stand related to the calcified portions of the exoskeleton, as do the articular surfaces of the long bones of the Frog's endoskeleton to their ossified shafts. Special thickenings or ingrowths of the exoskeleton take place at all points, for purposes of furnishing the surfaces requisite for the insertion of muscles: these ingrowths or *tendons* are, for the most part, mere tongue-shaped involutions, densest where resistance is greatest. The maximum of specialization is reached in the thoracic region where, from metameric ingrowths of the sternal and epimeral walls, a complicated *endophragmal system* is formed. This is fully described in the Laboratory work.

LABORATORY WORK.

Crayfish are best killed under chloroform, proceeding as with the Frog. (p. 35). They may be best kept alive in a moist atmosphere near running water, fed upon sopped bread. A sink, covered with a sheet of glass, meets all requirements.

A. **General external characters.**

The animal is covered by a continuous *exoskeleton* which is for the most part calcified, the following parts are readily recognised:—

a. The *body proper:*

α. Its anterior unsegmented portion (*cephalothorax*): the great shield-like plate (*carapace*) covering the back and sides of the cephalothorax; the groove across the carapace (*cervical suture*) marking out the line of junction of *head* and *thorax:* the anterior prolongation of the carapace to form the *rostrum* or *frontal spine.*

β. Its posterior segmented portion (*abdomen*): its seven divisions; the anterior six much like one another; the most posterior (*telson*) different from the rest (Cf. pp. 170 and 174).

b. The jointed limbs (*appendages*) attached to the ventral aspect of the body: their varying characters in different regions.

c. The external apertures of the body.

α. The *mouth;* seen on turning aside the appendages beneath the head.

β. The *anus;* a longitudinal slit beneath the telson. It is bounded by two flap-like *anal valves*.

γ. The *paired genital apertures:* in the male, on the basal joints of the last pair of thoracic appendages: in the female, on those of the antepenultimate pair. Examine the bases of the adjacent thoracic appendages, for possible traces of supernumerary genital orifices (cf. p. 186).

The two sexes may be at once distinguished from each other, apart from these apertures, by the following characters. The transverse diameter of the abdomen of the ♀ exceeds, at its widest part, that of the cephalothorax; it is the reverse for the ♂. The first two pairs of abdominal appendages are especially modified for purposes accessory to reproduction, in the ♂; in the ♀ the second pair are normal, the first are either very small or absent.

δ. The *excretory apertures;* each on the summit of a tubercle, borne upon the under surface of the basal joint of the antenna.

ε. The *auditory apertures;* on the flattened upper surfaces of the basal joints of the antennules.

These can be better examined when the appendages on which they are situated have been removed.

B. **The exoskeleton and appendages.**

Note that the exoskeleton forms a continuous investment for the whole body (*axial portion*) and its appendages (*appendicular portion*); it is for the most part calcified, but it remains soft and flexible where freedom of motion is required.

1. **The typical abdominal segment.**

Isolate, by carefully dissecting it away from its fellows, the third abdominal segment and its appendages. Pick away its contents and examine—

a. *The segment proper:* arched above; flattened below. It may be subdivided into

α. a dorsal *tergum*, the anterior smooth portion of which is overlapped by the preceding segment, in extension of the abdomen.

β. The *sternum:* that portion of the ventral surface which lies between the points of attachment of the appendages.

γ. The *epimeron:* the portion of the ventral surface which lies on each side immediately external to the attachment of the appendage.

This region is very short and passes almost directly into the inner wall of

δ. the *pleuron;* a free wing-like downgrowth of each lateral wall of the somite, seen, in section, to be formed by both tergum and epimeron. Note the smooth anterior half of the pleuron; it is overlapped by the one in front during flexion of the abdomen, much as is the tergum during extension.

ε. The *articular facets;* small tubercles developed, one on either side, from the anterior edge of the base of the tergum, immediately above the pleuron. They fit, during life, into corresponding recesses of the posterior border of the tergum in front.

b. *The appendages* or *swimmerets*, one on each side: the structure of each —

α. The short two-jointed basal portion (*protopodite*), consisting of a shorter proximal and a longer distal piece.

β. The flattened lamellæ attached to the distal joint of the protopodite, an inner (*endopodite*) and outer (*exopodite*).

2. **The structure of the cephalothorax.**

a. Note again the carapace, with its frontal spine and cervical suture.

b. Turn the animal over and note the narrow sterna of the thorax; they are laterally compressed, as the result of the great development of the basal joints of the appendages.

The sternum of the last thoracic somite is not completely ankylosed with the one in front in the Crayfish. In the Lobster it is.

c. Raise with a pair of forceps the free edge of the lateral expansion of the carapace which overlies the bases of the thoracic appendages: note that it is formed by the large united pleura of the thoracic segments, and overlaps a chamber in which the gills lie. It is termed the *branchiostegite* accordingly; its ventral edge is smooth in the Crayfish, it is notched metamerically in the Lobster.

d. Make a transverse section across the thorax, immediately behind one of the larger appendages; boil for five minutes in weak solution of Caustic Potash and pick away the soft parts, until quite clean. Examine under water, and note, as compared with the corresponding section across the abdominal segment—

α. The *sternum;* its middle portion is laterally compressed (cf. *b.*). It gives off (for each segment) two ingrowths or *endosternites*, which arise close together, immediately internal to the bases of the appendages.

β. The *tergum;* arched above, prolonged down on either side to form the outer limb of the branchiostegite.

If examined carefully, there will be seen arising from the cervical groove, close together near the middle line, two small tubercles or *endotergites*. They are most fully developed in the Lobster.

γ. The *epimera*. Each is vertically elongated—pushed up as it were upon itself—to form the inner wall of a great chamber (*branchial chamber*) lying under cover of the branchiostegite. From it the inner limb of the branchiostegite is derived.

Note the *endopleurites;* ingrowths of the opposite epimeral walls, which abut against the upper and outer borders of the endosternites. These, with the endosternites and endotergites above mentioned, are, one and all, ingrowths of the exoskeleton, developed in connection with muscular attachment Cf. Sect. C. 2.

δ. The *branchiostegite;* its outer limb is dense and calcified, its inner one is very delicate, largely chitinous and hirsute.

3. **The telson.**

Its whole tergal area is densely calcified, its sternal one is largely chitinous. It bears, in both Lobster

and Crayfish, two lateral spines (*furcal processes*); in the latter animal it is segmented, at the point of origin of these, into two portions—an anterior peri-anal one, the tergal surface of which remains largely chitinous, and a terminal post-anal one, which is completely calcified (cf. p. 176).

The circum-anal cuticle is calcified on opposite sides, to form a couple of *anal plates;* they are most marked in the Lobster.

Note the delicate *setæ*, which fringe the free border of the telson.

4. **The appendages.**

Remove the entire set from one side of the body in the order enumerated below, cutting through the inter-articular membranes close to their points of attachment to the axial skeleton.

Examine under water, posterior face uppermost.

a. The *third abdominal appendage*. Its general characters have been described (1. *b*.); examine in detail—

α. Its *protopodite;* made up of two segments—a shorter proximal hip-segment or *coxopodite*, and a long distal one (*basipodite*) which forms a base of articulation for the exopodite and endopodite.

β. Its *endopodite* (the longer of the two terminal portions). It is subdivided into two segments of equal length—the basal one is continuously calcified, the terminal one is multi-articulate.

γ. Its *exopodite;* relatively shorter than β. It also is subdivided into a single basal and a terminal

multi-articulate segment; the former however is but a fourth the length of the whole.

δ. Examine under a low power, and note that the calcifications of the multi-articulate segments are incomplete; they are restricted to their outer borders. Both exopodite and endopodite will be found to be fringed with delicate setæ.

The above description applies equally to both Lobster and Crayfish, except that in the former animal the whole appendage is more foliaceous and paddle-like.

b. The *second maxillipede* (second appendage in front of the great chela), as compared with *a*.

α. Its *protopodite;* its two segments are short and equal in size. The distal one furnishes the base of articulation for both exopodite and endopodite; the proximal one is prolonged out into a delicate lamella or *epipodite* (not represented in *a*) which bears a well-defined gill.

β. Its *endopodite*. This now forms the main portion of the whole limb, it is subdivided into five segments; a basal *ischiopodite;* an elongated laterally compressed *meropodite;* a small *carpopodite;* an expanded *propodite*, and a short terminal *dactylopodite*. The inner edge of the dactylopodite is beset by a series of sharp spines; the rest of the endopodite is partly fringed in setæ.

γ. Its *exopodite*, long and filamentous; its structural features recall most nearly those of the endopodite of the abdominal appendage.

The above description applies equally to both Lobster and Crayfish, except that in the former animal the epipodite and gill are in no way confluent.

c. The *third maxillipede.* Its *protopodite;* much as in *b*, except that its distal segment is ankylosed with the basal one (ischiopodite) of the endopodite. Its *endopodite;* greatly increased in size and importance, as compared with that of *b*. The five segments enumerated for *b* can be recognized; the ischiopodite is the longer of the series, the others become relatively shorter in proportion as the free end of the appendage is approached. The inner edge of the ischiopodite is beset by a single series of crushing teeth. Its *exopodite;* structurally identical with that of *b*, but considerably shortened up.

Note the presence of a tuft of long setæ (*coxopoditic setæ*) arising from the base of the epipodite.

In the Lobster, the ischiopodite bears two rows of teeth and the meropodite one row.

d. The *great chela;* much larger and more powerful than the last appendage, but resembling it in general structure, and in the ankylosis of its ischiopodite and basipodite; it also carries a gill.

The exopodite is entirely suppressed. The ischiopodite is relatively short, the meropodite and propodite being, as in *b*, the longer of the series of segments. The *propodite;* greatly enlarged and prolonged outwardly to form, with the dactylopodite, an opposable forceps (*chela*).

e. The four posterior thoracic appendages (*ambulatory appendages*).

All are, like *d*, destitute of exopodite. All are elongated, the proportionate lengths of the several segments being identical with those of *d*. The first and second pairs are chelate, and the first three bear both a gill and an epipodite.

The fourth; destitute of both gill and epipodite: when at rest it is backwardly directed.

In the Lobster, the gill borne by each of the above appendages is, like that of *b*, distinct from the epipodite.

The genital orifices are borne upon these appendages. They have been described at A. *c*. γ.

f. The *abdominal appendages*, other than the third pair (described at *a*).

α. The fourth and fifth pairs: closely resembling the third, functional as swimmerets.

β. The sixth pair, modified to form, together with the telson, a *tail-fin*. The protopodite: represented by a single short strong segment. (In the lobster there is a second incomplete basal segment.) The exopodite and endopodite: wide plates fringed with setæ. The *exopodite;* divided into two portions by a transverse joint: the free edges of its proximal portion are markedly serrated. The *endopodite;* continuously calcified; its proximal internal surface is beset, in the Crayfish, by a patch of short setæ, which play, during life, upon a corresponding hirsute area of the under surface of the telson.

γ. The second pair. Cf. Sect. A. *c*. γ.

Closely resembling the third in the female. In the male the *endopodite* is much modified; its

terminal multi-articulate segment being shortened up, in proportion as its basal one is elongated. The latter is here at its maximum for the whole abdominal series; it is produced up into an accessory piece, which is segmented off and modified to form a plate, rolled upon itself so as to enclose a demicanal, concave inwardly.

In the Lobster, this accessory process of the basal segment of the endopodite is still more marked.

δ. The first pair; in the female rudimentary, exceedingly variable in size and not unfrequently absent altogether: except in very rare cases the exopodite is suppressed. In the male the exopodite is invariably absent, the protopodite and endopodite become ankylosed and terminate in a plate rolled upon itself. In the Lobster the terminal division differs slightly from that of the Crayfish.

g. The *first maxillipede*. Its protopodite, flattened and foliaceous, its two segments well defined; its exopodite, substantially identical with that of *a*; its endopodite, reduced to a small two-jointed structure, lying under cover of the basal joint of the exopodite; its epipodite, fully developed but destitute of a gill.

In the Lobster, the reduction of the endopodite is far less marked.

h. The *second maxilla*. Its protopodite, foliaceous like that of *g*, but pectinated internally; its endopodite, elongated and filamentous, its free end is recurved to form a hook-shaped process which,

during life, is received into a recess of the mandible. Its epipodite; well developed, and united in front with a lamina, which probably represents the exopodite, to form a wide oval plate (*scaphognathite*) which lies at the anterior end of the gill-chamber (Cf. Sect. H. *c.* and p. 182).

If the above appendage be examined in the living animal, it will be found that with every movement of the scaphognathite there is a corresponding pull upon its filamentous endopodite.

i. The *first maxilla.* Epipodite and exopodite undeveloped. The endopodite is reduced to the condition of a small squame, its basal segment (*ischiopodite*) being enlarged and foliaceous[1] constituting the main mass of the whole appendage. Its protopodite is foliaceous and single jointed (*basipodite*), blade-like and recurved, with its free end inserted, during life, into the oral aperture. (Cf. Sect. E. 6 *d.*)

In the Lobster, the endopodite is large and segmented.

k. The *mandible.* Its strong toothed basal-joint (*coxopodite*) bearing a small appendage (*the palp*) which represents the basipodite and endopodite[1], epipodite and exopodite unrepresented.

l. The *antenna.* Its two-jointed basal-portion (*protopodite*) bearing a flattened protective squame (the modified *exopodite*) and a long multi-articulate filament (*the endopodite*), the two basal segments of which are greatly enlarged and modified for purposes of articulation. Note the opening of the green gland (Cf. Sect. A. *c.* δ).

[1] As is seen on comparison with the same in certain of the lower crustacea.

The distal segment of the protopodite (basipodite) is subdivided into two; the outer segment is squamous in the Lobster.

m. The *antennule.* Its large three-jointed basal segment (*protopodite*), bearing a pair of multi-articulate filaments (*endopodite* and *exopodite*): the opening of the auditory organ, in the midst of a tuft of setæ on the upper surface of the basal joint. The exopodite, the longer of the two jointed filaments, is carried erect during life (it bears sensory setæ. Cf. Sects. A. *c.* ε. and K. 2).

n. The *ophthalmite* or *eyestalk.* A short two-jointed structure which appears to represent the protopodite of an appendage. (Cf. Sect. K. 3 *c.*)

5. Remove that half of the body from which the appendages have been dissected, thus reducing the whole to the condition of longitudinal vertical section; boil for a few minutes in weak solution of Caustic Potash, pick away the soft parts and examine from within.

a. The *inter-articular membranes;* flexible, persistently uncalcified, portions of the exoskeleton. Note that (in the Crayfish) between the two last thoracic sterna. (Cf. Sect. B. 2 *b.*)

b. The *endophragmal system.* Each set of endosternites and endopleurites of which it is composed (cf. Sect. B. 2 *d.*) is seen to arise in the same plane; each endosternite slopes forwardly, carrying with it the anterior limb of its corresponding endotergite, the posterior limb of the one in front passing back to meet it. The points of apposition of the successive sets of elements alternate with those of their origin.

c. The *pro-cephalic process;* a tongue-shaped ingrowth of the exoskeleton, at the base of the rostrum.

d. The *thoraco-abdominal link work;* a somewhat complicated system of bars, passing between the last two thoracic sterna and a special process of that of the first abdominal segment.

e. The aperture of the *mouth;* bounded by the mandible—note the natural relations of the latter. The upper-lip or *labrum* and the lower-lip or *metastoma* will be seen, as uncalcified prolongations of the sternal skeleton, situated, respectively, in front of and behind the mouth. Compare the same as looked at from beneath, in an uninjured specimen.

α. The *labrum;* a shield-shaped plate calcified marginally.

β. The *metastoma;* composed of a median fold, produced on either side into a blade-like process (*paragnatha*), which simulates the basal joint of the first maxilla.

f. Note the limits of the several segments of the axial skeleton, and the mode of articulation of each upon its fellow and of the appendages upon each.

6. **The histology of the exoskeleton.**

a. Make a thin longitudinal-vertical section of any of the inter-articular membranes, and examine in water under a high power. It consists of a highly elastic membrane, traversed longitudinally by regularly disposed clear striæ; if pressure be applied to the cover-slip, the layers obvious as the above-named striæ will be found to be denser and more resistant than the intervening ones, *i.e.* the whole is composed

of chitinous material, deposited in layers, alternately denser and less dense.

b. Compare a similar section of any calcified portion of one of the appendages, ground down on a hone (see Appendix E.). A similar longitudinal striation is obvious.

Examine the peripheral area; the striæ—but a tenth the diameter of those seen in the main mass—are closely aggregated; the free surface is invested in an uncalcified *epiostracum*. The whole is permeated by a close set series of wavy *pore-canals*, usually filled with air in the process of manipulation, and rendered thereby highly refractive.

Compare tangential sections, made in a similar manner. The pore-canals will be obvious as minute black-dots, closely (in places irregularly) aggregated together.

An aggregation of the striæ, identical with that described above, is frequently met with on the inner side also.

c. Cut a longitudinal vertical section of a piece of the exoskeleton, together with the underlying integument, which has been decalcified by treatment with $1\frac{0}{0}$ chromic acid solution. Note, in addition to the structural features already described—

a. The *setæ;* each is an uncalcified outgrowth, usually arising at the base of a shallow pit. They vary greatly in size and detailed structure in different parts of the body; the commoner forms end in a pointed extremity, fringed with lateral hairlike filaments.

β. The *integument;* largely composed of branched nucleated granular cells: the outermost giving off a large number of short processes which penetrate a short way into the exoskeleton.

Prolongations of it extend into the setæ and into all skeletal outgrowths.

The ischiopodite of the third maxillipede may be preferably utilized, as, in it, all gradations from seta to spine and from spine to tooth can be readily followed.

C. **The muscular system.**

1. Detach one of the great chelæ from the body and lay bare its interior. The enlarged base of the propodite will be found to lodge two muscles, which pass between it and the dactylopodite; they are—

a. A larger *adductor*, arising from the outer side.

b. A smaller *abductor*, arising from the inner side.

2. Remove the superficial portions of the two muscles; there will be found lying within each a central *tendon*—a plate-like ingrowth of the exoskeleton, towards which the muscular fasciculi converge. Remove all the soft parts and examine—

α. The tendons in relation to the dactylopodite; they are ingrowths of the opposite sides of its base.

β. The articular facets and interarticular membranes; so arranged as only to admit of motion in one plane (abduction and adduction).

3. Examine the remaining segments of the appendage. Note that—

a. The relations between any two of them are identical with those described above, motion being possible in only one plane.

b. The individual segments are incapable of rotation upon each other. The limb as a whole can be rotated; owing to the variation in the angles of inclination of its several joints.

4. Make a median longitudinal section of the whole body, similar to that described in Sect. B. 5; pick away the viscera under water, wash and examine

a. The *flexor abdominis muscle;* a powerful mass, extending from the anterior border of the thoracic epimeron and endophragmal system to the base of the telson. It arises by a series of slips, which wind round each other in a highly characteristic manner and are inserted into the abdominal sterna.

b. The *extensor abdominis;* a much less powerful muscle, arising immediately above *a.* Its fasciculi are inserted into the abdominal terga.

c. The *levator abdominis;* arising, immediately above the extensor, from the antero-dorsal border of the thoracic epimeron. It passes obliquely backwards, to be inserted, laterally, into the first abdominal segment.

This muscle is continuous with a series of fibres which skirt the entire anterior border of the thoracic epimeron and attach it to the cervical groove.

Note that the parts of the axis are, like those of the appendages, capable of motion only in one plane.

d. The *adductor muscle of the mandible;* a powerful fan-shaped mass, passing obliquely upwards from

the hinder border of the mandible, to be attached to the cephalic shield, slightly in front of the cervical groove. Note its powerful tendon.

Small muscles will be seen, passing from the mandible and antennary organs to the adjacent exoskeleton.

e. Lay bare the basal portion of the great chela, and note the relations of its muscles. They arise from the endophragmal system, and are attached to tendinous ingrowths of the appendage itself similarly to those described in § 2.

D. **The general disposition of the viscera.**

1. Place the animal on its ventral side and pin down under water, inserting the pins through the tail fin and bases of the chelæ; remove the whole tergal skeleton and with it the extensor abdominis muscle. Note in order—

a. The *alimentary canal;* a straight tube, running the whole length of the body in the middle line. It is greatly enlarged in front to form the so-called stomach, which fills the greater portion of the cephalon.

b. The *heart;* a slightly yellowish pentagonal organ of delicate texture, lying above *a* in the middle thoracic region.

c. The *genital gland.* In the female, a yellowish brown mass; in the male, a whitish tongue-shaped mass; lying immediately beneath *b.*

It varies in size and character with the season of the year. (Cf. Sect. G.)

d. The *genital duct.* In the male; a highly convoluted

dead-white tube, immediately external to *c*. In the female; a short membranous tube, only visible at this stage with difficulty.

e. The *digestive gland;* a yellow pulpy-looking mass, filling up the interspaces in the cephalothorax; it is most obvious in front of the genital apparatus.

f. The *excretory organ* (green-gland); a paired delicate green structure, lying, at a low level, in the extreme anterior end of the cephalic cavity. It can be at once seen on slightly displacing the stomach.

g. Note, incidentally.

α. The *adductor mandibuli muscle;* a large oval fleshy mass, lying immediately external to the stomach (cf. Sect. C. 4 *d*.).

β. The *extensor abdominis muscle;* its cut ends will be found, attached to the thoracic epimera.

γ. The *gastric muscles;* passing from the stomach to the adjacent exoskeleton (for details see Sect. E).

δ. The *integument* (hypodermis); a delicate dark red layer, lying immediately under cover of the exoskeleton.

ε. The *body-cavity;* obvious as an ill-defined chamber, within which the various organs of the viscera are contained.

ζ. The *respiratory organs;* seen lying within the branchial chamber; they are exclusively thoracic. Follow the cut edge of the branchiostegite.

E. The alimentary organs.

1. Dissect from the tergal aspect, as directed for Sect. D., and remove the heart and reproductive apparatus. Examine—

a. The *stomach;* it is marked off by a transverse constriction into a large *cardiac chamber* and a small, posterior, *pyloric* one.

b. The *intestine*, a straight tube leading back to the anus; its wall is thrown into a series of shallow folds, which take a longitudinal and slightly spiral course.

c. The *cæcum;* a median dorsal upgrowth, immediately behind the stomach. It is directed obliquely forwards.

In the Lobster, this is situated far back, near the anus.

2. Some small muscles will be seen attached to the roof of the stomach. Remove these. In doing so, note that there comes away with them the soft *cellular wall* of the viscus, under cover of which there is seen a *chitinous lining*. Examine the latter with care; it will be found to be calcified to form a series of *ossicles*, related as under.

a. The *antero-dorsal* (cardiac) ossicle; a transverse bar, extending across the roof of the cardiac chamber.

b. The *postero-dorsal* (pyloric); similarly related to the roof of the pyloric chamber as is *a* to that of the cardiac one.

c. The *antero-lateral* (lateral-cardiac) ossicles; two small hammer-shaped pieces, abutting, one on either side, against *a*.

d. The *postero-lateral* (lateral-pyloric); two long bars, similarly related on either side to *b* as are *c* to *a*.

They extend forwards to meet the antero-lateral ossicles.

e. The *antero-median* (urocardiac) ossicle; a long tongue-shaped bar, passing back, from the antero-dorsal one, in the middle line of the roof of the cardiac chamber.

f. The *postero-median* (prepyloric); a similar but shorter bar, arising from the postero-dorsal piece. When the parts are at rest, it passes obliquely downwards and backwards, meeting the antero-median under cover of the postero-dorsal.

It will be observed that the above-named parts form a repetitional series; the roof and side walls of each chamber are calcified to form a dorsal transverse, a median, and a pair of lateral ossicles, the two latter sets articulating with each other.

g. The *digestive gland;* a paired mass, roughly trilobed, on either side. It is an aggregation of short cœca.

Carefully dissect away its upper half on one side, until a spacious *central duct* is reached, around which the glandular cœca are seen to be arranged. Wash until quite clean, and look for their orifices.

Follow the duct inwardly; it enters the head of the intestine by a wide aperture, immediately in front of the cœcum (1. *c*).

3. Place the animal on its side and remove the exposed half of the body and the digestive gland, so as fully to lay bare the entire alimentary canal.

The cellular wall and certain muscles will be seen as before; remove these, and examine the chitinous lining and its associated structures. Note, in addition to the parts described above, the following.

a. The *gastrolith;* a discoidal stony-mass, interposed between the cellular and cuticular layers of the anterior cardiac wall.

This is developed only in the summer season; an underlying thickening of the chitinous cuticle is invariably present to mark its position.

b. The *postero-ventral ossicles;* delicate vertically elongated bars in the hind wall of the cardiac sac.

c. The *accessory lateral ossicle;* a small bar, extending backwards and downwards from the point of junction between the two main lateral ones.

d. The intestine, the orifice of the digestive duct and the cœcum. (Cf. *supra*.)

4. Reduce the whole gut to the condition of *median longitudinal section*, by carefully removing the exposed half with scissors; wash until quite clean and examine under water.

a. The *gullet;* a spacious tube placing the stomach in communication with the exterior. Note its relations to the mouth.

b. The *median cardio-pyloric valve;* seen, in section, as an upgrowth constricting the passage from the cardiac to the pyloric chambers.

c. Follow the cut edge of the *chitinous lining* of the stomach; note

α. That it is continuous at the oral aperture with the exoskeleton.

β. That it ends abruptly, in front of the orifice of the digestive duct; terminating in a series of valve-like processes, one of which can be very

readily seen to project backwards and downwards immediately in front of the intestinal cœcum.

d. Examine, in like manner, the cuticular lining of the intestine.

α. It is continuous with the exoskeleton, at the anal orifice.

β. It terminates abruptly, in a thickened border, immediately posterior to the cœcum.

γ. Examine it under a lens; it is folded longitudinally and slightly spirally and beset by a number of short papillæ.

e. Examine the middle segment of the alimentary canal (head of the intestine): it is destitute of chitinous lining; it bears the cœcal appendage and receives the digestive ducts.

f. Examine the interior of the stomach and note the characters and relations of the *gastric teeth.*

α. The *median tooth;* a triangular red-brown structure, set on at the point of apposition of the two median ossicles.

It will probably be seen in section.

β. The *lateral tooth;* larger but similar in character, and situated (when at rest) in front of and below *α.* It is carried by the postero-lateral ossicle.

γ. The *accessory lateral tooth;* a small denticle, borne upon the posterior end of the accessory lateral ossicle (§ 3. *c*).

g. The *lateral cardio-pyloric valve;* a thickening of the side wall of the stomach, immediately below

the lateral tooth. Examine it under a powerful hand lens; it is beset by short stiff setæ.

5. Carefully remove the alimentary canal, cutting the gullet through close to the stomach; slit open longitudinally from beneath, and examine the interior under water.

   a. The *crushing surfaces* of the lateral teeth; note that they approximate anteriorly, being obliquely set.

   b. Seize the opposite ends of the stomach between two pairs of forceps and pull them apart. Note that with the divarication of the cardiac and pyloric ossicles the median tooth is elevated (depressed in life) to meet the lateral ones, which are at the same time rotated inwardly and slightly backwardly.

   c. Examine the free border of the chitinous lining; the pyloric valves (4. *c.* β) can be well seen from this aspect.

6. Isolate the stomach of a fresh specimen, together with the adjacent mouth organs. Dissect from the front and remove the anterior half, cutting through the gullet below and the cardiac ossicle above. Wash out the food contents and examine under water. Note—

   a. The median and lateral cardio-pyloric valves (§ 4. *b* and *g*); they form an efficient straining apparatus.

   b. The gastric teeth. Note that they meet, when in action, wholly in front of *a*.

   c. The *lesser cardio-pyloric valves;* three small setose eminences, on either side adjacent to the lateral teeth.

d. Note incidentally—

α. The metastoma; its lateral lobes are seen to be outwardly directed.

β. The basal joints of the first maxillae; two blade-like structures, curving round the bases of *a* to reach the gullet (cf. Sect. B. 4. *i*).

γ. The relations of the gastrolith, if present.

7. **The Gastric muscles.** All are paired, those of one side are alone described. Dissect the cephalo-thoracic region from the side, removing the exoskeleton and body-wall to the level of the pro-cephalic process (p. 202). Examine, in the order given, the *extrinsic muscles*. A spirit specimen may be used with advantage.

a. The *anterior and posterior gastric muscles* (divaricators of the gastric skeleton). Those of the exposed side will be seen—the anterior; arising from the roof of the cardiac chamber (cardiac ossicle) and inserted into the pro-cephalic process: the posterior; arising similarly from the roof of the pyloric chamber (pyloric ossicle) and inserted into the carapace, immediately in front of the cervical groove.

b. The *levator muscles.*

α. A conical mass, arising from the roof of the pyloric chamber, immediately in front of the cœcum. It is attached to the cervical groove (*endotergite*, Sect. B. 2. *d.* *β*).

β. Two thin strips, arising from the side wall of the cardiac sac, and inserted above into the mid-cephalic region of the carapace. They cross each other on nearing their insertions.

c. The *depressor muscles.* Three sets are obvious—

α. Two delicate strips, arising from the cardiac sac, immediately above the gullet. They pass obliquely forwards and downwards, converging as they do so, to be attached to the sternal skeleton immediately in front of the labrum.

β. A small slip, arising from the extreme posterior wall of the cardiac sac, and passing downwards and inwards to unite with its fellow of the opposite side, prior to its attachment to the anterior end of the endophragmal system.

γ. A long slip, arising from the ventral wall of the pyloric sac, and passing between β obliquely forwards and outwards. It is inserted close to the base of the gullet.

d. The *dilator muscles;* small fan-shaped tracts, arising from the gullet and attached—

α. the anterior ones, to the adjacent exoskeleton;

β. the posterior, to the endophragmal system.

The upper anterior one may receive a depressor slip.

The *intrinsic muscles.* Carefully dissect off the levator and depressor fibres described above and examine—

e. The *great constrictor;* a large sheet investing the postero-ventral half of the cardiac sac (a seeker can be readily inserted beneath it). Its fibres are seen to be interrupted by the intervention of the postero-ventral ossicles (3. *b*) to which they are attached.

f. The *lesser constrictors;* of these there are two sets.

α. Two tracts of fibres, arising from the head of the postero-ventral ossicle and attached—the slender ventral one to the base of the antero-lateral ossicle; the fan-shaped dorsal one to the postero-lateral bar.

β. A sheet of fibres, investing the side wall of the pyloric chamber and usually broken up into three fasciculi.

The above-named constrictor muscles exercise a direct control over the cardio-pyloric and other "valves" of the straining apparatus.

γ. The *cardio-pyloric* muscles; two delicate sheets, seen, on carefully removing the cellular roof of the stomach, to pass obliquely forwards and inwards from the lateral-pyloric to the cardiac ossicles.

g. The *anal muscles;* extremely delicate tracts, passing between the anal valves and the immediately adjacent wall of the intestine.

8. **The digestive gland.** Its general characters have been previously described.

a. Tease out a bit in water; it is made up of cœcal tubes, which are seen to be lined by a large celled epithelium.

b. Submit a small piece to the action of 1 % solution of Osmic Acid until well blackened. Examine in water under a high power; many of the cells will be found to contain a fatty product, deposited in globules, blackened under the action of the reagent.

c. Prepare (as directed in the Appendix) transverse sections of the frozen gland; transfer them, as cut,

to a glass slide bearing a drop of Osmic Acid solution; wash, when sufficiently stained, and examine under a high power.

Note that the cells are arranged in aggregates and flattened, as the result of mutual compression. Look for—

a. *Hepatic cells;* characterized by the presence of fatty globules, differentiated as at *b*. Their *nuclei;* rounded or oval, each frequently surrounded by a clear refractive halo.

β. *Ferment cells;* each containing a single large secretory globule, which does not decompose the Osmic Acid. Their *nuclei;* larger than those of *a* and oval, they usually contain a single nucleolus.

The larger of these cells (the older of the series) will be found in or near the middle of each group.

d. Compare similar sections of material, hardened in alcohol and stained with borax-carmine. The nuclei of the hepatic cells stain with great intensity. Look for stages in the elaboration and discharge of the secretory product of the ferment cell.

F. **The excretory-organ or green gland.**

1. Dissect from the left-side, and remove the greater part of the exposed cephalic shield. The entire organ lies in the extreme front part of the cephalo-thoracic cavity, immediately in front of the gullet (cf. Sect. D. *f*.). It consists of—

a. The *glandular segment;* a soft greenish mass, position as above.

b. The *muscular segment;* a distensible sac, overlying

a. It may be rendered the more obvious by inflation from the excretory orifice (Sect. B. 4. *l.*).

Having inflated in, lay open the basal joint of the antenna; on entering this, the neck of the sac becomes constricted and duct-like.

2. Tease up a small portion of the glandular segment in eosin solution; examine under a high-power. It is largely composed of a coiled tube, lined by a square-celled epithelium.

G. **The reproductive organs.**

These differ in detail, in the Crayfish and the Lobster. They lie partly ventral to the heart; their general relationships have been described (Sects. A. and D.). Dissect the cephalo-thoracic region from the tergal aspect, and remove the heart. Examine the genital apparatus *in situ*.

1. The *male organs*.

a. The *testis*. A median greyish-white mass, bilobed anteriorly.

b. The two *vasa deferentia*, arising where the posterior lobe of the testis meets the two anterior. Each is narrow near the gland; its calibre increases as it proceeds back from it, and, becoming extremely convoluted, it finally ends at the genital opening on its own side (Sect. A. *c.* γ).

These ducts generally contain a dense milk-white product, secreted by their glandular lining (*spermatophoral glands*). (Cf. Sect. L. 1. *b.*)

In the Lobster, the testes are two long tubes which extend back into the abdomen. Their posterior portions meet in the middle line, but in front they

diverge, a short transverse branch uniting the two anteriorly. The vas deferens arises a little in front of the middle of each testis and passes without convolution towards the genital opening. Its distal half is dilated.

c. Tease out a bit of the testis in water, and examine with $\frac{1}{6}$ obj.: it will be seen to be composed of glandular tubes terminating in grape-like sacculations. In it or in the vas deferens *ripe spermatozoa* may be found: they are motionless, discoidal, and provided with long radiating processes. Stain with eosin, and note

α. The *annulate corpuscle;* a transparent ring, lying within the middle of cell body.

β. The *oval corpuscle;* a granular mass, lying to one side of *α*.

In the Lobster the spermatozoa are also motionless; each consists of an elongated cell, from one end of which three rigid pointed processes radiate.

2. The *female organs.*

a. The *ovary;* in shape very similar to the testis of the male, being bilobed anteriorly.

b. The *oviducts:* two short ducts which, like the vasa deferentia of the male, are directly continuous with the genital gland. They can be at once seen on displacing the anti-penultimate walking legs to one side (Sect. A. *c.* γ).

A supernumerary oviduct, opening upon the second ambulatory appendage, may be very rarely present (cf. p. 186).

In the Lobster the ovaries are elongated and prolonged into the abdomen. Each is a dark green mass, and near their anterior ends the two meet in the middle line and remain confluent for a short distance. An oviduct arises from each ovary a little in front of its middle, and passes directly to the genital opening of its own side.

3. The genital organs, having been first examined *in situ* as above directed, may be profitably compared side by side, after removal from the body, together with their related appendages and thoracic sterna.

A highly instructive view of them may be obtained from the side, dissecting as directed for Sect. I. 3.

4. Lay open the *ovary* from the dorsal aspect and wash carefully until quite clean. Examine under water

a. Its *wall;* directly continuous with that of the oviduct.

b. Its *central cavity;* similarly continuous with the lumen of the oviduct.

c. The *ovisacs;* spherical masses developed within the wall, they are sometimes irregular in shape owing to mutual compression.

Examine under a hand-lens. They vary in size, the younger being very minute white bodies irregularly scattered in groups throughout the mass; remove a few of these, and transfer to a glass slide. Stain with magenta or eosin and examine under a low power, avoiding pressure.

a. The *ovum;* a single large cell, filling the entire ovisac. Note its highly granular *vitellus;* its large round *germinal vesicle* containing a number of *germinal spots*.

Examine the peripheral portion of the youngest ovisac under a high power. Look for—

β. The *vitelline membrane;* transparent and well defined, closely investing the ovum.

γ. The *follicular epithelium;* a single layer of small clear cells investing the whole; they are slightly flattened.

δ. The *membrana propria;* a delicate cuticle investing γ.

Look for very young ovisacs. Note the relatively small size and simple structure of the ovum, as compared with the older egg-cell; there is greater uniformity in size between it and the cells of the follicular epithelium.

H. **The respiratory organs.** Remove the branchiostegite of one side and examine the *gills:* the functional ones are 18 in number, arranged in three sets.

1. Examine the branchial-chamber; it is bounded in front by the cervical-groove, foremost among its contents are—

a. The *epipodites* of the thoracic appendages; seven in number, all upwardly directed. The most anterior one (that of the first maxillipede) is gill-less; the others bear each a gill (*podobranchia*).

b. The scaphognathite (Sect. B. 4. *h*) lying in the lower anterior region of the branchial chamber, immediately in front of *a*. Examine its base of attachment; compare the living animal.

2. Cut each of the gill-bearing epipodites across at its base and remove thus the *podobranchiæ* as a set. Pin down under water and examine any one in detail.

a. The epipodite; a delicate chitinous *lamina*, folded upon itself and pleated posteriorly.

b. The podobranchia; made up of a series of *gill-filaments* set upon a central *stem.*

c. Examine the same in transverse section. The lamina of the epipodite and the stem of the gill are continuous.

Cf. the Lobster: the gill is free of the epipodite, and the gill-filaments, which are very numerous, are arranged in close set parallel series.

3. Examine the remaining 12 branchiæ *in situ*, each consists of a central stem giving off a number of delicate filaments. There is nothing answering to the epipodite. They are arranged in two sets—

a. The *arthrobranchiæ;* attached to the inter-articular membranes of the thoracic appendages, with the exception of the first maxillipede and the last ambulatory leg. There are eleven of them, the odd one being related to the second maxillipede.

b. The *pleurobranchiæ*—

α. *Functional;* a solitary gill, attached to the inner wall of the branchial chamber, immediately above the last ambulatory appendage.

β. *Vestigial;* two to four delicate filaments (best seen after removal of the arthrobranchiæ) attached anteriorly to and in a line with *a.*

In the Lobster the above are all functional. There are, in that animal, 20 gills on each side; six podobranchiæ and four pleurobranchiæ attached as in the Crayfish, but only ten arthrobranchiæ—there being no such gill related to the second maxillipede.

I. The circulatory organs.

1. Dissect away the greater portion of one branchiostegite, so as to expose the branchial chamber and gills uninjured. Place the animal on its side and drill a small hole in the roof of the carapace near the heart; insert the point of a cannula into the same, inject and examine under water. Upon displacing the branchiæ there will be seen—

   a. The *branchio-cardiac canals;* six well-defined channels, running parallel with each other immediately beneath the inner wall of the branchial chamber.

   b. Remove the podobranchiæ and turn back the gills remaining *in situ.* The stem of each will be seen to lodge a vessel (*efferent branchial vessel*)—those of a given set of branchiæ uniting to form one branchio-cardiac trunk.

   c. Cut all the gills short and follow the branchio-cardiac canals upwards. They converge towards the region of the heart, to enter the *pericardial sinus*—a spacious chamber now largely filled with injection.

   As a rule, the trunks related to the ambulatory appendages 2, 3, and 4 enter the pericardium independently: the last of them receives the posterior trunk of the series, which brings in the blood from the pleurobranchia alone; the upper ends of the three are connected by short anastomoses. The anterior trunk is formed by the union of those related to the second and third maxillipedes and the chela.

2. Dissect a specimen which has been at least 24 hours in alcohol, under water, from the tergal aspect, cutting away carefully with a pair of scissors the mid-thoracic portion of the carapace.

A chamber (*the pericardial sinus*) is thus laid bare in which lies the *heart* (D. 1. *b*).

a. The *alary muscles;* delicate strands passing from the angles of the heart to the adjacent pericardial wall.

b. The two *dorsal cardiac apertures;* oblique openings in the cardiac roof, guarded by *valves*.

c. Carefully dissect off the roof of the heart, and note

α. Its *wall;* thick and spongy, the muscular fibres being arranged in irregular bundles.

β. Its *cavity;* central and single; i.e. there are no auricles.

γ. The *ventral apertures* and *valves;* identical in their characters with *b*; they are situated at the anterior end, transversely to the long axis.

δ. The *lateral apertures;* similar perforations of the middle region of the side walls. Dissect to the level of one and examine its valves; they are pocket-shaped, and so arranged as only to admit of a passage of the blood from the pericardial sinus into the heart.

d. Compare the heart of a similar specimen, after removal from the body.

3. *The great arteries.* Obtain a fresh uninjured specimen and pin down under water on its side; lay bare the pericardial sinus with extreme care, and inject from the lateral cardiac aperture. Work leisurely and apply a steady gentle pressure.

Cut along the post-mandibular portion of the exoskeleton to the level of the middle line (with the

exception of the ambulatory appendage bearing the genital orifice; leave that *in situ*) and remove the skeleton together with its related muscles, being careful not to injure the digestive or reproductive organs.

Cut along the dorsal middle line of the cephalic skeleton, and continue the incision downwards, clear of the green-gland. Remove the hard parts, but leave the adductor muscle of the mandible *in situ*. Dissect away any extraneous tissue and wash carefully.

a. The *superior abdominal artery;* a backward continuation of the heart, immediately above the intestine. It distributes branches metamerically to the intestine (hind-gut) and adjacent parts.

b. The *ophthalmic artery;* a similar forward continuation of the anterior end of the heart. It runs over the stomach and distributes branches to the eye stalks and parts immediately adjacent.

c. The *hepatic artery*. Turn the exposed digestive gland downwards; branches of the above vessel will be seen ramifying in it. Cut its main trunk across and remove the gland, noting the branches to the mid-gut; the main trunk can now be traced beneath the genital gland, to its origin, from the antero-ventral region of the heart.

d. The *sternal artery;* a large trunk, arising from the heart at the base of *a*. It runs vertically downwards towards the ventral surface, passing to one side of the intestine, and perforating the nervous system (obvious as a delicate greyish median-ventral cord. Cf. Sect. J.). It subdivides into

α. The *antero-ventral artery;* a median trunk, extending forwards to the mouth region, and dis-

tributing branches metamerically to the appendages, muscles and parts adjacent.

β. The *postero-ventral* (*inferior-abdominal*) *artery;* a median trunk extending back to the telson. It also distributes branches metamerically to the adjacent parts.

The above trunks furnish the main vessels to the reproductive organs. They are, on either side—a dorsal one, arising from the sternal artery soon after it leaves the heart and distributed to the genital gland and duct; a ventral one, arising from the base of β, and distributed to the terminal segment of the duct alone.

The sternal trunk may pass to either side of the alimentary canal. If the injection be successful, it will be found that the vessels distributed to the ends of the intestine and genital ducts debouching on to the exterior, terminate in special capillary networks.

e. The *antennary artery;* arising from the antero-dorsal region of the heart, immediately external to *b*. Its main trunk runs downwards and forwards, passing round (external to) the adductor mandibuli muscle and immediately under the carapace, to terminate anteriorly in the antenna. It gives off on its course—

α. The *gastric artery;* arising close behind the posterior gastric muscle, and distributed to the stomach (fore-gut).

β. Branches to the gastric muscles, several *en route.*

γ. A *rostro-antennary branch;* arising from the main

trunk at the base of eye-stalk, and distributed to the antennule and rostrum.

δ. The *renal arteries;* two or more branches breaking up on the anterior face of the green gland.

ε. Other minor branches, which are variable.

4. Puncture any one inter-sternal membrane of a freshly-killed animal; blood will escape freely (a blood space has been entered). Treat the inter-articular membrane between the two last thoracic sterna similarly; insert the point of a cannula and inject.

a. Remove the branchiostegite and examine the branchiæ. The injection will have filled some of the *afferent branchial vessels;* large trunks running (one for each gill) along the outer face of the stem externally to the efferent vessel (§ I. *b*.).

b. Make a transverse section across the thoracic region. With a little care the afferent branchial vessels can be traced to a great ventral sinus (*sternal sinus*) now largely injected. It is in free communication with the adjacent blood-spaces.

5. The *blood.* Obtain a drop of blood, from a freshly-killed Crayfish and examine under a high power.

a. The *plasma;* colourless.

b. The *corpuscles;* colourless and amœboid, each with a large round nucleus. Their pseudopodia; filiform and frequently very numerous.

Watch their movements; they exhibit a great tendency to run together into aggregates prior to disintegration, upon exposure, after removal from the body.

J. **The nervous system.**

1. Pin the animal down tergal surface uppermost and lay bare the whole body cavity. Remove the heart, reproductive and renal organs and the digestive gland; sever the alimentary canal across its middle and turn the two halves to one side; cut short the tendon of the adductor mandibuli muscle, and remove the flexor abdominis, together with the endophragmal system.

The whole *nervous axis* will thus be laid bare.

a. The *supraœsophageal ganglia;* two considerable masses, confluent in the middle line, situated at the base of the rostrum immediately behind the eye-stalks.

b. The *circumœsophageal commissures* passing back from *a*.

c. The *longitudinal ventral commissures;* backward continuations of *b*, extending to the base of the telson. They are widely separated in the mid-thoracic region, where they enclose the sternal artery (seen in transverse section. Cf. Sect. I. 3 *d*.); elsewhere they are closely applied.

d. The *abdominal ganglia;* six paired masses, borne upon *c* in the abdominal region. They are equidistant, there being one pair for each somite. The posterior pair are larger than the rest, and distribute fibres to the last somite and the telson. Note, in relation—

α. The *ganglionic nerves;* paired trunks arising from the ganglia.

β. The *inter-ganglionic nerves;* arising from the longitudinal commissures, alternately with *a*.

e. The *subœsophageal ganglion;* a large oval mass, situated immediately behind the gullet. It distributes nerves to the mandibular and five following somites.

f. The *thoracic ganglia;* five pairs, each somewhat larger than the abdominal, distributing nerves to the five posterior thoracic somites.

α. The *posterior pair;* situated in front of their corresponding somite, and consequently far removed from the first abdominal pair. This is seen to be the result of approximation towards the pair in front, the two lying close together immediately behind the passage for the sternal artery.

β. The *second and third pairs;* similarly but less conspicuously approximated, in front of the artery.

γ. The *anterior pair;* equidistant between β and the subœsophageal ganglion.

2. The *nerves of the supraœsophageal ganglion.* Carefully remove one half of the rostrum and lay open the eye-stalk of the same side. Note, passing into the latter—

a. The *optic nerve;* a thick tract, arising from the anterior end of the ganglion and expanding within the eye-stalk to form the *optic bulb.*

b. The *antennulary nerve;* a delicate trunk, arising from the outer border of the ganglion; it passes outwards and forwards to enter the base of the antennule.

c. The *antennary nerve;* a similar trunk, arising from the hinder region of the ganglion; it passes back-

wards and downwards to reach the base of the antenna.

3. Examine the circumœsophageal commissures under a lens; they will be seen to be connected by—

   a. The *postœsophageal commissure;* a delicate transverse band, slightly in front of the subœsophageal ganglion.

   b. The *antœsophageal commissure;* a similar structure to *a*, arising at the sides and passing round the front of the gullet.

   c. The *median-ventral commissure;* a very delicate trunk, passing back from the supraœsophageal ganglia in the middle line, to meet *b*.

   d. The *anterior visceral nerve;* a median nerve arising from the point of union between *b* and *c*; it passes upwards and forwards to reach the roof of the stomach, upon which it subdivides for distribution to its walls.

   e. The *posterior visceral nerves*—arising, one on either side, from the last abdominal ganglia. They pass slightly backwards to reach the base of the intestine, the side walls of which they skirt.

4. A companion dissection may profitably be made, by bisecting the whole body a little to one side of the middle line.

   The real double nature of the entire nervous axis may best be appreciated by removing it and examining it on a blackened surface, under water.

5. *a.* Tease out a bit of perfectly fresh nerve-cord in water and stain with magenta or eosin.

Composed of slender fibres of variable size, each consisting of a structureless outer wall, surrounding a finely granular or obscurely fibrillated central axis. Nuclei; seen at intervals.

b. Tease up in water, a ganglion which has been treated with osmic acid.

Composed of large oval branched cells, each consisting of a granular mass in which lies a clear round nucleus, containing a nucleolus.

K. **The sense organs.**

1. *The auditory organ.* This lies in the basal joint of the antennule and is best examined in the Lobster. The upper surface of this basal joint is flat posteriorly and arched in front. It bears several tufts of setæ: one of these is very small and lies at the inner side of the flattened surface, just at the angle where it meets the arched part; among these setæ is the opening into the auditory sac, through which a bristle can easily be passed.

a. Take a fresh antennule from a Lobster and cut away the under surface of its basal joint, with a scalpel. A transparent chitinous sac will readily be found in it, among the muscles; this is the *auditory sac* and is about $\frac{1}{3}$ of an inch long. Carefully dissect it out.

b. If this sac be held up to the light a little patch of gritty matter will be seen on its under surface near the external aperture. Behind this can be seen a curved opaque line; behind this, and concentric with it, a shorter brownish streak. Cut out carefully the part of the sac which bears these marks: mount

in sea-water or salt solution and examine with one-inch objective.

a. The gritty matter will be seen to consist of a number of irregular earthy particles, largely, if not wholly, foreign to the organism; they are functional as *otoliths*. Carefully remove them with a camel-hair brush.

β. The white line will be seen to answer to a ridge, on the summit of which is a row of large setæ (*auditory setæ* or *hairs*), and both on the brown patch and on the opposite side of the main row will be seen scattered groups of smaller setæ.

c. Examine with $\frac{1}{8}$ obj.

a. Each auditory seta is now seen to be covered over its whole surface with numerous very delicate secondary setæ; these are shortest near the base of the primary one. Towards its base each of the primary setæ is constricted and then dilates into a bulbous enlargement for articulation upon the wall of the sac.

β. The brown patch is seen to owe its colour to a single layer of polygonal epithelial cells containing pigment granules.

γ. By focussing through this epithelial layer a number of parallel fibres will be seen passing up, one to the base of each seta of the main row.

δ. If a perfectly fresh auditory sac be put in 1 per cent. solution of osmic acid for half an hour, and be then well washed in distilled water and examined, each of the fibres mentioned above (ultimate ramifications of the *auditory nerve*) will be

seen to terminate, near the free end of the seta, in an elongated *end organ*.

The auditory sac in the Crayfish is very similar to that in the Lobster, and may be examined in a similar way. It is, on the whole, not so good, chiefly on account of its smaller size.

2. Examine, in the Crayfish, the exopodite of the antennule; it exceeds the endopodite in calibre and length, and is carried, during life, upwardly directed. (Cf. Sect. B. 4 *m*.) Remove it from the rest of the appendage and examine in water, under a low power.

   a. Its *setæ;* short filaments, developed from the anterior borders of the several joints.

   b. Its *sensory setæ;* developed in tufts, from the under face of the filament. There are usually two sets for each segment and they slope obliquely downwards and forwards.

   c. Examine under $\frac{1}{6}$ or $\frac{1}{8}$ obj. Each seta is seen to be subdivided into two segments; a shorter basal one and an elongated terminal one: in the ordinary setæ the latter is pointed, in the sensory ones it is expanded and spatulate.

      More or fewer of the terminal segments of the sensory setæ are, not unfrequently, globular and abnormal.

3. *The visual organ*. Place the eye of a Crayfish for four or five days in 0·5 per cent. solution of chromic acid and then for twenty-four hours or more in alcohol.

   a. Strip off a portion of the *cornea* and examine under a $\frac{1}{6}$ obj. It is marked out into a great number of

minute *facets;* mostly square, more rarely hexagonal or irregular.

b. Tease up in weak glycerine a portion of the contents which underlie the cornea. Look for—

α. *Crystalline cones;* elongated angular transparent bodies, each of which may or may not be in continuity with

β. A *striated spindle* or *rhabdome;* fusiform and about a third the length of *a.* It is striated in appearance and occurs in relation to a cellular pigmented investment (*retinula*).

c. Imbed an eye similarly prepared and cut a number of longitudinal sections from it: mount in glycerine and examine under one inch objective. Look for the sections which have passed through its middle; they will be seen to present a central mass (*optic bulb.* Cf. Sect. J. 2 *a.*) from which a number of lines appear to radiate to the facets on the surface. These radiating lines (which are obscured here and there by concentric pigmented layers) are indications of the *striated spindles* and *crystalline cones.*

α. The *exoskeleton;* calcified to form two segments, a smaller basal annular one and a larger terminal dome-shaped one, united by an inter-articular membrane (Cf. Sect. B. 4 *n.*). That of the terminal segment is thick and dense, except where it overlies the crystalline cones; there it is thin and transparent, forming the *cornea.*

β. The *muscles;* seen on either side, immediately beneath *a*, they pass between the two segments, as between those of any one appendage. (Cf. Sect. C.)

as between those of any one appendage. (Cf. Sect. C.)

γ. The *intermediate mass;* a plug of vacuolated connective tissue, filling up the entire interspace between the optic bulb and the muscles.

d. Examine your thinnest section with a high power. Beginning at the exterior make out successively—

α. The *corneal facet;* its flat outer and slightly excavated inner surface.

β. The *crystalline cones.* Cf. *ante.* Each is in close apposition with the inner face of a corneal facet.

γ. The *striated spindles* or *rhabdomes* with thin *retinulæ;* cf. *ante.* They are disposed lineally with β.

δ. The *sheath* of the above; a nucleated investment for each set of constituents. Pigment is deposited therein, being densest around the outer ends of the cones and the greater portion of the spindles, whence the appearance of the two black zones seen under the low power.

ε. The layer of *nerve fibres;* passing between γ and the optic bulb. Look for them in places where the pigment may have fallen away.

ζ. The *optic bulb;* mainly composed of delicate fibres and small cells with large round nuclei, partly scattered, partly aggregated into two oval

central masses (*gangliform bodies*) which stain deeply.

A zone of pigment is deposited at its outer border.

L. **Development.**

1. Examine the under surface of the body of a living female "in berry."

a. The *fertilized ova;* each is attached to the setæ which fringe the swimmerets, by a delicate peduncle. Make an incision into one: the ovum will be liberated; the peduncle will be seen to be derivative of an *egg-capsule* which remains behind (a secretory product of the abdominal wall and swimmerets (cement glands of) cf. p. 186.

b. Examine the thoracic sterna; aggregates of whitish filiform bodies (*spermatophores*) will in all probability be found adherent to the hinder ones, having been deposited by the male.

Tease one up; it consists of a bundle of spermatozoa, enclosed in a dense milk-white *sperm-capsule* (a secretory product of the vas deferens. Cf. Sect. G. 1 *b*.).

Spermatophores may frequently be found projecting from the reproductive orifices of the male, if killed during the breeding season.

2. Remove the developing ova from time to time from the body of the parent, and dissect off their egg-cases with a couple of needles. Transfer to picric acid solution and harden in alcohol. Look for—

a. The *segmenting ovum* (*oosperm*). Examined under a low power, its exterior appears slightly granulated. Imbed and cut sections; stain with carmine. Note—

a. The *food yolk*: aggregated centrally, and chiefly

deposited in conical masses whose bases look outwardly.

β. The *clear protoplasm;* forming a superficial investment for the whole. *Nuclei* are regularly arranged within it.

γ. The *blastomeres;* segmentations of β, around the nuclei. The furrows which mark them off do not extend into the yolk-laden protoplasm.

b. The same at the *gastrula stage.* Easily recognisable by the large horse-shoe shaped *blastopore.*

Make sections of the area of invagination, through the long axis of the blastopore. Note—

α. The *ectoderm;* now established as a superficial layer investing the whole.

β. The *endoderm;* a small sac, derived from *a* by invagination, completely enclosing the *archenteron.*

c. The same, at a slightly later stage. Look for specimens having on the surface a shoe-shaped white patch (*blastoderm*), immediately in front of the blastopore now greatly reduced in size. Preserve as before, stain and examine *en face.*

α. The *blastoderm;* shoe-shaped or heart-shaped, thickened and expanded in front to form two rounded *procephalic lobes.*

β. The *blastopore;* a small oval aperture, at the extreme hinder end of *a.*

γ. The *abdominal papilla;* a median outgrowth of *a,* immediately in front of the blastopore.

d. The same, at a later stage. Look for specimens in which the blastopore has disappeared. Treat as at *c.*

α. The *appendages;* paired differentiations of the blastodermal area, separated by a median space (more or fewer will be seen).

β. The *labrum;* an oval median outgrowth, situated about on a level with the first pair of appendages.

γ. The *abdominal papilla;* now elongating.

If the specimen be a young one the involution to form the mouth may be seen immediately behind the labrum; that to form the anus, under cover of the abdominal papilla. If it be an advanced one, segmentation of the papilla will be very distinct.

δ. The *optic pits;* two small round differentiations within the procephalic lobes (*c.* α.).

ε. The *carapace;* now appearing as a marginal thickening of the blastoderm, immediately external to the appendages. It is at first paired, the two folds meeting behind the abdominal papilla.

e. The *hatched embryos.* To be found in numbers, fixed to the swimmerets of the mother.

In general they resemble broadly the parent organism. Examine—

α. The *cephalo-thorax;* more arched than that of the adult, owing to distension by the food-yolk, not yet completely absorbed. The rostrum is bent downwards and the cervical groove is, at best, ill defined.

β. The *cephalo-thoracic appendages;* differing but little from those of the adult. Remove and examine the free-end of the *chela;* its pincers

apparatus is spinous and recurved at the tips, for purposes of attachment to the parent.

γ. The *abdomen;* fully segmented and terminating in the telson. Four pairs of appendages are alone free, the first and last pairs being buried beneath the larval integument.

e. Look for and examine stages intermediate between *c* and *d.*

f. The *larval Lobster*, when about half an inch in length. Obtain if possible a specimen in which the abdominal appendages are first appearing. Compare generally with the Crayfish larva and note especially—

α. The *exoskelcton;* like that of the Crayfish larva, a continuous uncalcified cuticle.

β. The *carapace;* terminating in front in the *rostrum*, which assumes the form of an immense protective spine.

γ. The *abdomen;* fully segmented and bearing several smaller protective spines.

δ. The *eye;* its great size at this stage.

ε. The *antennary organs;* both are short and completely under protection of the rostrum.

ζ. The *thoracic appendages.* The third maxillipede, great chela and ambulatory appendages, are more nearly uniform in size than in the adult; they all bear well developed *exopodites.*

g. Remove the second ambulatory appendage and the swimmeret of the third abdominal somite. Examine them side by side, and note in both—

α. The great relative size of the *protopodite*.

β. The increase in length of the *endopodite* over the exopodite; little marked but obvious in the case of the abdominal appendage. (Cf. the corresponding abdominal appendage of the adult Crayfish.)

III.

THE EARTHWORM (*Lumbricus herculeus*).

THE Earthworm is to be found wherever damp earth is accessible, no matter how hard or stony the surface; the presence of moisture is indispensable to its existence. Into this earth it burrows, excavating a tubular habitation to which it repairs during the day time, emerging at night to seek food and to work, or at early morning to reproduce its species. It remains within the burrow throughout both the winter and the dry summer seasons.

The interior of the burrow is smooth and frequently lined with minute stones; its mouth is often surrounded with "castings" and plugged with leaves drawn in by the animal itself, or covered, as with a lid, by stones sometimes of relatively great weight and size.

The body of the worm is fairly uniform in dimensions throughout; it is bilaterally symmetrical, both mouth and anus being situated at opposite ends. The metameric symmetry such as was seen in the abdomen of the Crayfish, is here common to the whole body, which can no longer be subdivided into well-marked regions. It is constricted externally into a number of repetitional segments or *somites*, of which there may be, in a sexually mature animal, as few as 68 or considerably more than 200. For each of these somites there is a similar repetitional series of certain of the internal organs. Each somite is subdivided externally into

[1] There is commonly found in association with this worm another (*Allolobophora* [*? longa*]) indistinguishable from it, so far as concern the requirements of this volume, except for the characters of the peristomium (p. 249), the œsophageal glands (p. 254) and blood vessels (p. 260). The former is in *Allolobophora* partially divided in the dorsal line by two furrows which extend back from the prostomium. In *Lumbricus* this division is complete longitudinally.

at least two lesser divisions or *zonites*, these however are but skin deep and in no way corelated with the internal parts. The body is invested in a continuous uncalcified *exoskeleton* in the form of a delicate iridescent cuticle. No limbs of any sort are present, and locomotion is effected by means of four longitudinal series of bristle-like *setæ*, which project freely from all but the most anterior segment; a powerful muscular apparatus is developed in connection with each of these, and the chitinous setæ themselves are largely buried in tegumental sacs lined by cuticular involutions. The setæ are replaced when cast off.

The posterior terminal segments of the body are, during life, flattened from above downwards, and when the animal on removal from its burrow is observed crawling, a characteristic spatulate appearance is imparted to that region, at the upturned end of which the anus is situated. Under ordinary circumstances the worm, on coming to the surface, retains its hold on its burrow by means of this expanded extremity.

The Earthworm is omnivorous, living mainly upon leaves (for certain kinds of which it has a decided preference), and less conspicuously upon both animal and vegetable organisms ingested with the earth passed through its alimentary canal, in burrowing or otherwise. The alimentary canal is a straight tube, running the entire length of the body. The mouth leads into a thin-walled eversible *buccal sac*, which opens into a spacious muscular suctorial *pharynx*; this, in turn, passes into a long tubular œsophagus, the terminal fifth of which is enlarged to form a distensible *crop*. The crop is, in the common earthworm, succeeded by a whitish thick-walled *gizzard*, so called as it performs a mechanical crushing action. This finally opens into a long sacculated intestine, which is continued on with but slight modification

to the hind end of the body, where it communicates with the exterior by a valvular laterally compressed anus. The absorptive area of the intestine is increased by the development of a conspicuous *intestinal-value* or *typhlosole* which lies along its whole roof, and not by a coiling of the whole viscus as in the case of the Frog.

The intestinal wall is invested in a yellowish-brown tissue of so-called "chloragogen cells." This tissue (originally thought to have been a kind of liver) has no sort of connection with the lumen of the gut; it is intimately associated with the walls of the great blood vessels, many of which it completely surrounds, and there is reason for regarding it as functional in the formation of an excretory product which is discharged into the body-cavity, if not in that of some constituent of the blood also.

The intestinal juice of the Earthworm contains a digestive ferment, and there open into the mouth-cavity a number of small so-called "salivary" glands. Digestion is nevertheless not wholly intra-intestinal, for the animal when feeding vomits a digestive fluid; this is allowed to act upon the raw food material for some time, prior to its ingestion.

Digestive glands other than those just named are unknown, except for the presence of unicellular glands in the typhlosole, and such diverticula of the alimentary canal as remain for consideration are somewhat remarkable, and little understood physiologically. These are usually three pairs of œsophageal pouches, lying midway between the crop and pharynx; they are smallest in winter, and it not unfrequently happens that one or more of them may be absent. Occasionally, their connections with the lumen of the œsophagus may become obliterated, and in rare cases no trace of them is to be found. These œsophageal diverticula are highly vascular, and contain a milk-white product consisting of carbonate of lime either in a finely divided

state or in concretionary masses sometimes reaching a diameter of 1 *millim.* Such being the case they are termed *calciferous glands*, but the purpose served by them is as yet not fully understood.

The body-wall is invested in a single layer of elongated epidermal cells, many of which become converted into small *unicellular cutaneous glands*. This investing epithelium of the body and the cuticle which it secretes (*vide supra*) are continuous respectively, at the mouth and anus, with the epithelial lining of the alimentary canal and its cuticular product. The greater mass of the body-wall is however composed of two sets of muscular fibres, an external circular set and an internal longitudinal one; these also pass, at opposite ends of the body, into an internal circular and an external longitudinal series of fibres entering into the composition of the wall of the digestive tube. The two walls are widely separated by a spacious body-cavity, the alimentary canal being suspended by a series of transverse *mesenteric septa*, instead of by a median longitudinal fold as in the Frog. These septa are metamerically arranged, one for each somitic constriction; circular muscular fibres are developed within them and radial ones pass across them from body-wall to wall of alimentary canal. The body-wall is lined internally by a peritoneum, clad in tessellated epithelium, which can be traced on to both the mesenteric septa and the free surfaces of the various organs contained within the body-cavity. It is from this layer that the chloragogen cells aforenamed take their origin. The walls of the body and alimentary canal are thus both seen to consist of at least three clearly defined layers; a protective cuticular, an epitheloid, and a mechanical muscular one respectively. These layers are continuous at both mouth and anus, and the order of succession is the same

for each except that that which is external in the one wall is internal in the other and *vice versâ*.

The body-cavity is subdivided by the mesenteric septa into a series of somitic compartments, and the metameric symmetry thus established extends to the excretory organs, there being one pair of these attached to each septum, the first three somites excepted. These organs, being thus segmentally arranged, are termed *segmental organs* or *nephridia*. Each consists of a tortuous tube which can be resolved into three segments; a middle glandular one, abundantly supplied with blood-vessels, passing—internally, into a delicate thin-walled loop, which perforates the mesentery and opens into the segment in front by means of a complex ciliated funnel or *nephrostome*—externally, into a vesicular muscular segment, which communicates with the exterior in the vicinity of the ventral pair of setæ. The lining membrane of these excretory tubules is profusely ciliated, and a current is thus induced from within outwardly. In addition to the indirect communication established, through the agency of the nephridia, between the body-cavity and the exterior, a direct one is instituted by means of a metamerically disposed series of median dorsal or *peritoneal pores*. There is reason to believe that the milky fluid ejected through these is functional in facilitating the movements of the animal within its burrow.

The mesenteric septa themselves are incomplete ventrally; they do not subdivide the body-cavity into a series of closed chambers. As this is so, it follows that that cavity is a continuous one in open communication with the surrounding medium. It contains during life a colourless *perivisceral fluid*, in which there are present immense numbers of nucleated amœboid corpuscles.

The true red-blood fluid circulates in a system of vessels,

having, so far as is known, no direct communication with the body-cavity. The larger trunks of this *circulatory system* are five in number; median longitudinal *supra-intestinal* and *supra* and *subneural* vessels respectively, and two small *lateral neural* ones. These are connected metamerically in a manner described in the Laboratory work (p. 261), and highly efficient capillary systems are established in connection with them. In the segments numbering seven to twelve, there exist two sets of vessels not met with elsewhere. These are, firstly, a series of enlarged *circumœsophageal* vessels, 6 pairs in *Allolobophora*, 5 in *Lumbricus*, connecting the supra-intestinal and supra-neural trunks; secondly, a pair of *lateral œsophageal* trunks, which are connected with the supra-intestinal vessel alone (cf. p. 260). The latter vessels are specially concerned with the blood supply to the anterior portion of the œsophagus and its calciferous glands. A condition somewhat exceptional in the animal kingdom is met with in the blood vessels of the *clitellum*, and less conspicuously of the body-wall generally; where the superficial capillaries pass up and ramify among the actual epidermal cells themselves giving rise to an *epidermal blood plexus*. The dorsal vessel contracts from behind forwards and the circumœsophageal ones from above downwards; and there is reason to believe that the blood which sets out to the parietes from both dorsal and ventral median trunks, is returned to the vessels of the alimentary system. The exact seat of origin of blood corpuscles during life, to make good the loss of effete ones, is not yet fully determined; but it is highly probable that certain appendages of the blood vessels known as "*blood glands*" (p. 258) may be concerned in their production.

sists of a watery plasma in which are suspended exceedingly minute transparent non-nucleated corpuscles. These are somewhat variable in shape and size, being usually about the $\frac{1}{3000}$th of an inch in length; their structural features agree closely with those of the nuclei of the epithelial lining of the vessels in which they circulate.

The feature which most clearly distinguishes the red-blood fluid of the worm from that of the Frog, is that its colouring matter is diffused through the plasma and in no way related to the corpuscles.

The nervous system is in an exceedingly simple and interesting condition. It consists of two pear-shaped *supra-œsophageal* ganglia which abut together in the middle line in front; from these there arise two commissures which, like those of the Crayfish, run longitudinally side by side for the whole ventral surface. These commissures are somewhat enlarged for each somite, and there arise from them a metamerically repeated series of nerve trunks. The supra-œsophageal ganglia distribute fibres to the buccal sac and to the anterior end of the body; the latter terminate in modified sensiferous cells borne upon the first segment, which constitute all that the worm has in any way representative of sense organs. One of the most important facts concerning the nervous system of this animal, is the disposition of the nerve-cells. These, instead of being restricted to the ganglia, as they are in the Crayfish, are regularly diffused throughout the entire axis—gangliform enlargements and longitudinal commissures alike. The sheath of the nervous axis is remarkable in being muscular, and especially as concerns the existence of a dorsal *neurochord* made up of three longitudinally disposed "*tubular fibres*," presenting, micro-chemically, leading features in common with medullated nerve fibres.

The proper reproductive organs are restricted to seven of

the anterior segments, and will be found described on p. 268. They are somewhat complicated, chiefly owing to the conditions of maturation of the *seminal vesicles;* but the actual genital glands, though exceedingly small, are well defined. The ripe ovum consists of a round nucleated cell containing a moderate food-yolk, and invested in a vitelline membrane. The sperm-producing cells undergo changes which result in the formation of a number of filiform spermatozoa, each with an elongated nucleus-bearing "*head;*" and the conditions are such as to render observation upon the maturation of these exceedingly easy and instructive.

The worm is hermaphrodite but not self-impregnating. During copulation—which usually takes place at early morning—the bodies of two individuals are brought into apposition, and a transfer of ripe spermatozoa takes place. These are passed into definite *seminal receptacles*, there to await final deposition. During the interval which follows there is secreted by the body-wall an egg capsule or *cocoon*, within which functionally mature ova and spermatozoa are deposited. The developing eggs are ultimately enclosed within an albuminous fluid. The so-called capsulogenous glands (p. 250) are regarded as the seat of its secretion, but there are reasons for believing that they may be concerned in the formation of the cocoon, usually attributed to the clitellum. Segmentation of the fertilized ovum is *holoblastic*, and there result two layers of cells—a more rapidly dividing one, which differs from that described for the Frog mainly in the absence of pigment, and a less rapidly dividing, yolk-laden, one. The smaller cells overgrow the larger ones very rapidly, and there results a simple two-layered sac or *gastrula* which becomes ciliated externally. The embryo early assumes a bilaterally symmetrical form, and as the body elongates there are developed, mainly if not entirely from the archenteric wall, a series of paired cellular

masses which become metamerically arranged. The segmentation of the body is initiated by the appearance of these *mesoblastic somites;* from the central cavities ultimately developed within them the body-cavity is derived, while their walls, coming into apposition antero-posteriorly, give rise to the mesenteric septa.

The nervous system arises by a series of thickenings of the investing epiblastic layer, and the supra-intestinal vessel when first formed is paired.

The clitellum, to which reference has been made, undergoes a periodic enlargement, and may be present in young worms but two inches in length or absent in fully formed ones of six or eight.

LABORATORY WORK.

Displacement of the organs may be entirely obviated by immersing the worm in methylated spirit for two minutes, and allowing it to remain in running water for half an hour.

A. **General external characters.**

1. Examine the living worm and note—
   a. Its *shape.* Anteriorly it tapers off to a point, posteriorly it is flattened and spatulate.
2. Compare the body of the dead animal, killed as above. Note—
   a. Its subdivision by a number of constrictions into a recurring series of body segments or *somites*, each subdivided by a lesser constriction into two *zonites.*

   The zonites of the genital segments (9 to 15) are frequently more numerous.

   b. The *clitellum* or *cingulum* (if present, *vide supra*), a whitish saddle-shaped enlargement usually re-

stricted as a rule to 6 or 7 segments in Lumbricus, 5 to 9 in Allolobophora. It usually commences on segment 26, or on from 1 to 6 segments behind that.

This is best studied in a chloroformed specimen.

The capacity of the whole body is greatest at this point. Posteriorly to the clitellum it is uniform in calibre, except for the postero-terminal region (cf. 1. *a*), where its transverse diameter is greatest. Anteriorly, it is enlarged in the genital region (segments 8 to 12) tapering off to a point in front.

c. The *locomotor organs* or *setæ*, four double rows (two lateral and two ventral) of bristle-like appendages, which project freely from all but the extreme anterior and posterior segments.

The body is flattened ventrally between the two sets of ventral setæ, and laterally between the lateral and ventral ones. The setæ project out at the angles formed by these flattened areas, the lateral ones arising within the limits of the pigmented portion of the body-wall.

d. The *apertures*.

α. The *mouth*, antero-terminal. It perforates the first segment, which is subdivided into a dorsal *prostomium* which overhangs the mouth, and a circumoral *peristomium*[1]. Examine the latter with a lens; its free edge is delicately serrated.

Compare the living animal. The movements of the body clearly show that the first segment is highly sensitive.

β. The *anus*, postero-terminal, and laterally compressed. It perforates the last segment.

Note the valve-like nature of its lips.

[1] Cf. *Lumbricus* and *Allolobophora* (p. 240 foot-note).

γ. The *dorsal* or *peritoneal pores;* small median perforations of the body-wall, lying at the bases of the somitic constrictions between all but some few of the anterior segments. They are best seen in specimens which have been preserved in spirit. If examined in freshly killed worms, their presence can be made manifest by gently squeezing the body, whereupon there will exude from each a drop of perivisceral fluid.

δ. The *sexual apertures.* Examine the worm from beneath, and note the presence of two glandular enlargements immediately external to the ventral setæ of the 15th segment. Each surrounds the slit-like orifice of the *vas deferens* of its own side. Opening, in a line with the above, on to the surface of the 14th segment, there are the smaller apertures of the *oviducts.*

The ventral integument of segments 9 to 11 (more rarely 8 to 12) is generally swollen, like that of the lip of the vas deferens or the clitellum, owing to the presence of the so-called *capsulogenous glands.* (Cf. p. 247.)

The exact function of these is at present unknown (cf. however p. 247).

Examine the somitic constrictions in this region from the side. There will be found, opening upon those which subdivide segments 9—10 and 10—11 in a line with the lateral setæ, the small apertures of the *spermathecæ.*

Not unfrequently one or other of these may open by two orifices, and their presence may easily be demonstrated by gently squeezing the body.

ε. The orifices of the excretory or *segmental organs*, too minute to be examined here. They are best seen in section. (Cf. Sect. H. 1. *c.*)

B. **The setæ and exoskeleton.**

1. Remove one of the setæ from a post-genital segment with a small pair of forceps, and examine in water under a low power. Note—

a. Its *form*, curved and needle-shaped, blunted internally and terminating in a point.

b. Its *structure.* Examine under a high power. It consists of a chitinous rod, a portion of the central axis of which is soft and granular.

The setæ vary somewhat in shape and calibre in different regions of the body, especially so in the clitellar and genital segments.

c. The *exoskeleton*, a delicate iridescent *cuticle*, investing the whole body. Take a freshly killed worm and strip off its cuticle under water; in doing so note that it passes in to line the alimentary canal at both mouth and anus. Transfer a small portion of it to a slide, mount in water and examine under a high power. Note—

α. It consists of a colourless transparent membrane, traversed by delicate lines or *striæ* crossing each other at an angle of 45^0.

β. Examine the same under a low power and note the *cuticular sacs of the setæ*, finger-shaped prolongations of the cuticle which occur in pairs. They embrace, during life, the middle of the setæ.

It not unfrequently happens that the setæ are dragged away with the cuticle or it with them, upon

removal from the body. Examination of a specimen in which this is the case shows that but one-third of the seta projects beyond the body-wall when at rest (Cf. Sect. H. 3).

C. The body-cavity and general disposition of the viscera.

Pin a large worm down under water dorsal side uppermost, and slit up the body-wall along the dorsal middle line. Pin back the two flaps and note—

a. The presence of a spacious *body* or *perivisceral cavity.*

b. The *alimentary canal*, a straight tube running the whole length of the body.

The yellowish-brown tissue investing its walls, renders it especially conspicuous for all but the anterior 20 segments.

c. The *mesenteric septa*, a series of delicate transverse membranous partitions, coincident with the somitic constrictions (Cf. Sect. A. 2. *a*). They attach the alimentary canal to the body-wall.

d. The *segmental organs* or *nephridia*, whitish fluffy-looking appendages, one pair to each mesenteric septum. Examine under a lens, and note that each consists of a delicate convoluted tube, often of a dead-white colour owing to the presence of excretory matter.

e. The *reproductive organs.* Conspicuous among these will now be seen the *seminal vesicles;* three pairs of large white bodies, overlying the alimentary canal in segments 9 to 12.

The relative size of these is variable. (Cf. p. 246.) The reproductive organs are restricted to this

region of the body, but further details concerning them may be left till later.

f. The *perivisceral fluid.* Take a freshly killed worm and remove a small portion of the dorsal body-wall. Insert the point of a capillary tube and draw off a little of the perivisceral fluid. Examine at once under a high power, and note—

α. The watery *serum*, in which float colourless vacuolated *amœboid corpuscles.*

β. Watch the corpuscles. Aggregates of them frequently fuse on exposure, prior to disintegration.

γ. Take a second drop of the fluid and treat with acetic acid and magenta. The *nuclei* of the corpuscles alone stain deeply.

D. **The alimentary organs.**

1. Pin the worm down as before and open it up dorsally. Remove the seminal vesicles and work out in order—

a. The *buccal sac*, a thin-walled sac lying within the first 2—3 segments. Muscular fibres pass from its anterior end to the body-wall. The aperture of the mouth can be seen through its transparent roof.

b. The *pharynx*, a spacious thick-walled structure, extending back to the sixth segment. It is tied down to the body-wall by a fan-shaped series of muscular fibres.

The cut ends of those fibres which attach it to the dorsal surface of the body-wall (removed in dissecting), give it a roughened appearance when viewed from above.

The pharynx is marked off from the buccal sac by a deep recess, in which the pear-shaped supra-œsophageal ganglia are seen to lie.

c. The *œsophagus*, a tube of relatively small calibre, continued back to the 15th segment, where it enlarges to form

d. the *crop*, a spacious thin-walled sac, lying within segments 15 and 16.

e. The *œsophageal diverticula*, 2—3 pairs, conspicuous by their light yellow colour, lying within segments 10 to 12. In Allolobophora the posterior pair are inconspicuous, the anterior pair (*œsophageal pouches*), when examined in detail, opened up, will be found to be reddish in colour and to alone open into the œsophagus, the others (*calciferous glands*) opening into them.

Note the presence of carbonate of lime in the latter (cf. p. 243).

f. The *gizzard*, a pearly-white thick-walled sac, following immediately upon the crop. It usually occupies segments 17 to 19.

g. The *intestine*, a spacious yellow thin-walled tube, extending back to the extreme end of the body. The sacculations of its walls become less conspicuous as its hinder end is reached.

h. Open up the crop, gizzard, and a small portion of the intestine from above, wash out the contents and note—

a. The *crop*. Its walls are thin and membranous.

There is sometimes present a well-marked lateral thickening of a highly vascular nature.

β. The *gizzard*. Note the great thickness of the muscular wall of its anterior half. The cuticular lining of the alimentary canal here attains its maximum of development.

γ. The *intestine*. Note the lateral ingrowths of its walls, developed metamerically at the points of attachment of the mesenteric septa. In transverse section they are seen to be refolded and complex.

2. Take the body of a second worm killed as directed at the outset, but which has been subsequently preserved for 18—24 hours in alcohol. Pin it down and dissect from the side.

Make a median longitudinal section of the alimentary canal, wash out its contents and examine—

α. The *buccal sac* and *pharynx*. The floor of the former and roof and side walls of the latter are both thickened and muscular.

β. The *œsophagus*. Its metameric constrictions are plainly visible. Look for the orifices of the calciferous glands. (Cf. p. 242.)

γ. The *intestine*. Note the *typhlosole*, an immense median dorsal fold of the lining membrane of its roof; it is plicated laterally.

Cf. the same in transverse section.

Examine the lateral intestinal sacculations as seen from within (Cf. 1. *h*. γ).

δ. Work over the relations of the whole to the

mesenteric septa. Those of the crop-gizzard region are liable to considerable variation.

E. **The excretory or segmental organs.**

1. Take the largest worm you can find and open up its anterior third along the dorsal middle line. Pin down the two flaps of the body-wall and remove the alimentary canal, being especially careful to avoid stretching the mesenteric septa more than is necessary. Wash until quite clean and examine in water under a lens. With a little care there will be seen—

   a. The *segmental organ* or *nephridium*, a delicate tube, coiled upon itself and suspended to the mesenteric septum by a special fold or *mesentery* of its own.

   The whole organ is thrown into a series of loops. At its inner end, close to the nervous axis, a very conspicuous *internal loop* will be seen; it projects backwardly and outwardly.

   b. Examine the region of this internal loop with care. The nephridium will be seen to perforate the mesenteric septum with which it is connected, and to open into the somite in front by an enlarged pendant extremity or *nephrostome*.

2. Take a similar but freshly killed worm, and open up as before. Remove as much as possible of a mesenteric septum (preferably from somite 15 or thereabouts, where the calibre of the alimentary tube is least and the reproductive organs do not overcrowd) with one of its related segmental organs. Examine under a low power, and restrict your attention to that portion of the tube in which *ciliary action* is going on. Follow it along until a point is reached at which it

undergoes a bending upon itself and a sudden increase in calibre; substitute a high power and fix your attention on this last-named area. Note—

a. The thick glandular walls of the tubule in which ciliary action is going on (*glandular segment*). Careful examination will reveal the presence of large oval transparent *nuclei*.

b. The *thin-walled segment*, a delicate transparent tube lying immediately adjacent to the lesser limb of *a*. It also is bent upon itself and ciliated.

Its walls appear as mere hard outlines, and the transverse diameter of its two limbs collectively is about equal to that of the lesser one of *a*.

c. The *muscular segment*. Examine under a low power. The position in which this segment will be found varies, as it is generally displaced in the process of manipulation. Like both *a* and *b* it is bent upon itself, and can be at once recognised by its great transverse diameter as compared with the rest of the organ. Follow it along—it will be found to become suddenly constricted at the point at which the glandular segment enters it.

Its aperture of communication with the exterior is best seen in section. (Cf. Sect. H. 1 *c*.)

d. Look for the *internal loop* (1 *a*). Note that both glandular and thin-walled segments enter into its composition.

e. Look for the *nephrostome* (1 *b*). This structure is apt to be more or less hidden under accumulations of effete (? chloragogen) cells (p. 242) in course of disintegration and removal from the body. Having found one, examine it under a high power. It is lined by a *columnar ciliated epithelium*, the individual cells of which are very large and clearly

defined. Follow it back; it is continuous with the thin-walled segment.

The actual course taken by the coils of the segmental organ can only be made out with very considerable trouble. Sufficient is here described to render it clear that three segments are present—an internal thin-walled, a middle glandular and an external muscular one respectively ("internal" and "external" having reference to the fact that these segments communicate respectively with the body-cavity and the exterior). The only source of difficulty which will be found in attempting to unravel the whole, is the internal loop (*d*) formed by a secondary folding of both thin-walled and glandular segments. Eliminate that, and the whole resolves itself into a tube of three segments—each bent upon itself. Cilia are entirely absent for at least the terminal third of the organ, and they elsewhere occur only in isolated spinally disposed series.

f. The *nephridial blood-plexus*. Remove, as directed above, the segmental organ of a worm which has been at least two days in alcohol. Examine in water under a high power. Its sheath carries a complicated series of blood-vessels, conspicuous by their yellow colour. Note—

α. The main *nephridial vessels*: two thin-walled tubes running side by side, parallel with the long loop of the nephridium. They are connected by an *excretory plexus*, the smaller vessels of which form a bold series of loops on the surface of the organ.

β. The "*blood glands*" of the excretory vessels; a series of relatively large globular dilatations, filled with blood and generally crowded with minute colourless non-nucleated *corpuscles*

These structures are sometimes absent, and they are not confined exclusively to the nephridial plexus (cf. p. 245).

F. **The circulatory system.**

Examination of this system is materially facilitated by killing the animal as directed at the outset, and allowing it to remain undisturbed for six or eight hours, in order that the muscular walls of the larger vessels, no less than those of the body itself, may be rigid and well-set prior to dissection.

1. Pin down, at opposite ends, the body of a worm treated as above, and open it up along the dorsal middle line. Reflect the two flaps of the body wall, and note—

   a. The *supra-intestinal* vessel; a large median dorsal vessel, running along the whole length of the roof of the alimentary canal, to which it distributes branches.

   Large *circumœsophageal* vessels will be seen arising from this in segments 6—12. Their detailed connections are described at § 2 *d*.

   b. Dissect away some few of the mesenteric septa of one side, and displace a portion of the alimentary tube thus liberated. Note, overlying the nervous axis, the median longitudinal *supra-neural* vessel.

   The smaller *lateral-neural* vessels may sometimes be observed at this stage. (Cf. Sect. G. *c*. γ.)

2. Take a second worm and pin it down on its side, being careful not to injure the anterior twenty segments. Make an incision with the small scissors along its dorsal middle line, raising the body wall

with the instrument as you cut, in order to avoid the possibility of injuring the underlying vessels. Carefully remove the lateral body wall of the above segments to the level of the middle line, and without dissecting further, note—

a. The *supra-intestinal* and *supra-neural* vessels, together with such of their branches as have been already mentioned.

Look out for the lateral-neural vessels.

b. The *sub-neural* vessel, a delicate median-ventral trunk, seen on raising the nervous axis, to which it is closely applied.

c. The *circumœsophageal vessels* (*hearts* so-called "*pseudo-hearts*"). They lie in segments 6 to 11 in Allolobophora, 7 to 11 in Lumbricus, and connect the supra-intestinal and supra-neural trunks.

d. The *lateral-œsophageal* vessel, a conspicuous usually highly distended trunk, closely applied to the wall of the œsophagus on either side. Large branches of it are seen on the pharynx and œsophageal glands. Trace it to its connection with the dorsal vessel (in Allolobophora in the 12th segment—in Lumbricus in the 10th).

3. Still working from the side, carefully cut (with scissors) into any three or four somitic constrictions of the intestinal region, and without dissecting further manipulate the parts with forceps under water. Note

the relations of the mesenteric septa and nephridia, as seen in the undisturbed condition (cf. Sect. E.) and next examine in order—

a. The *circular commissural vessels;* one on either side for each segment. They lie close under the body-wall (to which they give branches) midway between adjacent mesenteric septa, and connect the supra-intestinal and sub-neural trunks.

b. The *excretory plexus.* The posterior of the two vessels from which this is derived springs from the sub-neural trunk immediately adjacent to the nephridium itself. The anterior one arises from an adjacent circular commissural vessel, and may perforate the mesenteric septum ventro-laterally.

c. Remove a portion of the body-wall of this region, and gently scrape away as much as possible of the yellow-brown tunic of the intestine. There will thus be brought into view the *lateral intestinal vessels;* two in number on either side in each segment. They arise from the supra-intestinal trunk and break up into an *alimentary plexus* within the walls of the digestive canal. A series of small vessels will be seen to pass up towards these from the sub-intestinal trunk.

The blood-vessels described above can all be made out by careful dissection. No mention has been made of the delicate *lateral-neural commissures*, or the smaller branches of the majority of the great

vessels; for the study of these recourse must be had to transverse sections. (Cf. Sect. G. γ.)

d. The *blood fluid.* Lay open, high and dry, the body cavity of a freshly-killed worm, and introduce a fine capillary tube into one of the larger vessels. Transfer the drop of red blood thus obtained to a slide, cover and examine at once under a high power. Note—

α. The blood *serum*, a watery yellowish-red fluid in which float

β. minute colourless *corpuscles.* In shape, they are somewhat irregular (rarely oval); each usually contains a central dark granule.

If unsuccessful by the above method, remove a small portion of one of the larger vessels *en masse* between two pairs of forceps; transfer to a slide and allow the fluid contents to escape.

G. **The nervous system.**

Dissect an entire worm from above, removing the dorsal portion of the body wall, and the alimentary canal from the pharynx backwards. Pin down, wash until quite clean, and dissect under water. Examine under a lens and note—

a. The *supra-œsophageal ganglia;* two pear-shaped masses lying in the third segment in a depression between the buccal-sac and pharynx. (Cf. Sect. D. 1. *b.*) Fibres can be traced from them to the circum-oral integument.

b. The *circumœsophageal commissures;* seen, on displacing the pharynx to one side, to arise from *a.* The nerves arising from them are distributed—externally, to the first two segments—internally, to

the pharynx (*visceral nerves*). On reaching the ventral surface the commissures become firmly united and pass on, to the extreme end of the body, as *longitudinal ventral commissures.* Note the metameric *gangliform enlargements* formed upon the latter

c. The *ganglionic nerves;* two pairs for each somite. They arise from the ganglionic swellings—trace them to the body wall.

d. The *interganglionic nerves;* one pair for each somite, arising from the longitudinal commissures at the bases of the mesenteric septa, to which they are largely distributed.

e. The *histology* of the nervous axis and its associated structures (for method of preparation see Sect. H.).

Examine transverse sections under a high power, note—

α. The *sheath* of the nervous system; a deeply staining investment, mainly composed of the cut ends of muscular fibres.

β. The *neurochord.* It consists of three transparent thick-walled "tubular-fibres" (a larger central and two smaller lateral ones) buried up in the roof of *a.* Note that each bears a well-marked sheath and a coagulable contents. (Cf. p. 246.)

γ. The cut ends of the *sub- and lateral neural blood-vessels*, together with their anastomoses, all of which are buried up in *a.*

δ. The ventral *nervous axis.* Note the close apposition of the two commissures. Each is composed of a transparent *matrix* in which are seen

the cut ends of delicate *nerve-fibres*; and of a denser peripheral ventro-lateral portion, in which large *nerve-cells* are lodged.

Compare sections through the longitudinal commissures and ganglionic enlargements. Nerve-cells are present in both, and except so far as their relative proportions are concerned, there is no important structural difference between them. In sections through the latter, look for the origins of the ganglionic nerves, and the small blood-vessels arising from the lateral neural trunks which accompany them.

II. **The study of transverse sections.**

1. Wash out the contents of the alimentary canal of a freshly killed worm with $\frac{1}{2}$ p. c. chromic acid solution. When all the earthy matter has been removed, cut up into segments of an inch in length and transfer to solutions of chromic acid and alcohol of increasing strength (see appendix E). Stain with borax-carmine and mount in the usual manner in Canada balsam

Examine the thickest sections under a low power, and note—

a. The *body wall*. It is mainly composed of a thick *muscular layer*, external to which is a thin *epidermis* clad in a delicate *cuticle*.

b. The *intestinal wall*; its thick tunic of *yellow-brown tissue*. Internally to this there is a thin *muscular layer*, and the whole is lined by a well-defined *ciliated epithelium*. Examine the minute structure of the *typhlosole*.

c. The *mesenteric septum.* A thin fibrous sheet passing from *a* to *b*. Note its circular and radial *muscular fibres.*

It is perforated ventrally (*circum-neural arcade*) to give passage to the nervous axis and its four accompanying blood-vessels.

Look for the *nephridia*—portions of them will certainly be visible in the thickest sections. Note especially their communication with the exterior, each by a narrow duct passing externally to the ventral setæ.

Look for the *dorsal pore*, for sections of the great blood-vessels and traces of their branches (see Sect. F).

2. Examine a thin portion of the *body-wall* of the above, under a high power. Note —

a. The *epidermis;* composed of a layer of elongated cells. They stain very lightly, and their nuclei are very small.

b. The *unicellular cutaneous glands*, large ovoidal structures buried up in *a.* Each opens on to the exterior by a short neck. They are rendered very conspicuous by the affinity of their granular contents for the staining reagent.

c. The *cuticular exoskeleton*, a delicate investment for *a.* It is structureless, and stains uniformly and with moderate intensity.

It is frequently puckered or otherwise displaced in the process of manipulation.

d. The *circular muscular layer.* It lies immediately beneath, and is two or three times the transverse diameter of, *a.*

Pigment granules will be found scattered among its fibres.

e. The *longitudinal muscular layer*, somewhat thicker than *d*. Each of its several tracts is subdivided into a great number of fasciculi by uniformly arranged radial septa, within which small blood-vessels are carried. The muscular fibres are arranged in close set parallel lamellæ, disposed at right angles to the septa.

f. The *peritoneum*, a delicate membrane immediately internal to *e*.

3. The *setæ, and their associated structures.* When at rest they project inwardly to the level of the peritoneum. Examine in order—

a. The *integumentary sheath* of the seta. Trace its origin from the epidermis- it can be followed to the inner end of the seta.

b. The *cuticular sheath*, carried in with *a*. It can be traced some distance up the side of the seta. (Cf. Sect. B.)

c. The *muscles of the setæ.* Examine any one pair of setæ and note, passing obliquely downwards from their approximated inner ends, the fan-shaped (in section) *protractor fibres*. They pass into the circular layer of the body-wall.

Two sets of *retractor fibres* will be seen arising from each seta at about its middle. By their union there are formed two ribbon-shaped bands which pass right and left of the setæ, upwards and inwards, between the longitudinal fibres of the body-wall and the peritoneum.

d. The *follicle of the setæ;* a small granular protoplasmic mass internal to each pair of functional setæ. It lies close under the peritoneum, and usually bears two conical immature setæ.

4. The wall of the *alimentary canal.* This will be found to vary slightly in detail in different regions,—the intestine is here described.

a. The *epithelium*, a single layer of elongated cells, lined internally by a delicate *cuticular membrane.*

Look for *gland cells*, most prevalent in the typhlosole, and for *cilia*, which may or may not be present (cf. p. 373 foot-note).

b. The *muscular layer*—feebly developed. It consists of an *internal circular* and an *external longitudinal* series of fibres.

The thickening of the wall of the gizzard is due to an excessive development of the circular fibres.

c. The *sub-epithelial* tissue; a vascular layer interposed between *a* and *b.* Look for the cut ends of its small vessels.

d. The *yellow-brown tissue;* a thick investment for the whole, immediately external to *b* (outside the wall of the whole viscus). Its component cells are elongated and club-shaped, each containing a single oval nucleus and crowded with minute highly refractive globules.

Examine the muscular fibres of the mesenteric septum. An aggregation of circular fibres is frequently met with immediately external to the alimentary canal. Many of its radial fibres can be traced into the body-wall.

5. The *central nervous system*, described in Sect. G. *e.*

I. **The reproductive organs.** (Cf. Sect. C. *e.*) Best examined in specimens captured during spring and summer.

1. Obtain the body of the largest worm possible (one having a good clitellum). Open up the anterior twenty segments along the dorsal middle line *under spirit* (stretching the body to the utmost) and remove the alimentary canal with care, leaving the mesenteric septa undisturbed. Look for—

a. The *spermathecæ*, two pairs of small yellowish-white globular bodies, attached to septa 9—10 and 10—11

They are developed within the substance of the septa referred to, and may project forwardly into segments 9 and 10 or backwardly into 10—11.

Their points of exit are invariable, and they may conveniently be used as landmarks in dealing with the remaining organs of the reproductive system.

b. The *seminal vesicles*, 3—4 pairs of soft whitish organs attached to septa 9—10, 10—11 and 11—12. They are internal to *a*.

In Allolobophora there are four pairs, always distinct. In the sexually mature Lumbricus the 3 pairs present unite with each other and their fellows of the opposite side to form a highly conspicuous mass lying beneath the alimentary canal.

c. Remove the seminal vesicles of one side and examine the mouths of the vasa deferentia (*ciliated rosettes*). They are dead-white glairy structures, lying near the middle line in segments 10 and 11; when fully formed exceedingly conspicuous and chalky looking.

d. Trace back the ducts of *c*, on one side. Each perforates the septum behind it and becomes im-

mediately convoluted, uniting in segment 12 with its fellow to form the *vas deferens*. Follow this back, it runs longitudinally midway between the nervous axis and the exterior, passing out on segment 15.

e. The *testes* (visible only in sexually immature specimens), two minute white bodies truncated posteriorly; attached, on either side, to septa 9—10 and 10—11 close to the middle line. They project into segments 10 and 11.

In sexually mature worms these and the ciliated rosettes are both embraced by the enlarged seminal vesicles.

f. The *ovaries*, two small conical bodies, attached by their bases to septum 12—13 in a line with *e*. Their free ends are not unfrequently recurved. They project into segment 13.

g. The *oviducts*. Remove the segmental organs from septum 13—14, seize the latter with forceps and turn it backwards—the mouths of the oviducts will be seen opening into segment 13 in a line with *f*. The ducts themselves are very short and closely bound down to the hinder face of the mesenteric septum. They pass outwards and backwards, opening on to the exterior in a line with *d*.

h. The *receptacula ovorum;* tuft-like appendages of the hind-walls of the oviducts. They project freely into segment 15, and are rendered highly conspicuous by their great vascularity.

i. Remove the entire ovary and transfer it to a slide, stain with eosin and examine under a high power. Commence at its free end, and note—

α. The *ripe ova*, large round bodies completely filling the pointed extremity of the saccular ovary. A granular *vitellus*, a large round *germinal vesicle* and a *vitelline membrane* will be observed for each.

β. The *immature ova*. Work towards the base of the ovary—the ova decrease in size and become more numerous. The younger of them are destitute of a vitelline membrane. Work back towards the attached end, and note graduating into β as β does into α—

γ. the *germinal epithelium*, a solid mass of small undifferentiated cells filling the base of the ovary.

j. Remove a small portion of a testis of the youngest worm obtainable, crush and examine in eosin under a high power. Note the cells of the *germinal epithelium*, irregular in shape, each with a large central nucleus and a single nucleolus.

k. Tease up in a similar manner a small portion of one of the seminal vesicles of an older animal. Treat with acetic acid and magenta—spermatozoa in all stages of development will be seen. Look for the following—

α. Cells of the *germinal epithelium* identical with *j*. They are sometimes to be found only with difficulty.

β. The same during the *early maturation* stages—conspicuous as the predominant mulberry-like masses. Focus to the surface of one of these and work through it. It consists of a large protoplasmic body, differentiated peripherally into

a series of transparent nucleated protuberances—centrally into a large non-nucleated mass.

γ. The same during the *later maturation* stages. Nuclear division has gone on with rapidity, and the central mass is now surrounded by a great number of small nucleate bodies. Examine carefully the protoplasmic investments of these—each is prolonged out into a short filament.

δ. The *ripe spermatozoa;* aggregates of filiform structures resulting from the nucleate bodies above described. Each is enlarged at its attached end to form a 'head,' within which the elongated nucleus is contained. Focus to the centre—the non-nucleated mass has remained passive and unaltered.

ε. Look for free spermatozoa.

IV.

THE COMMON SNAIL (*Helix aspersa*).

THE "Common Snail", *H. aspersa*, and the "Garden Snail", *H. hortensis*, are to be found in abundance in our gardens and hedgerows, and the descriptions here given apply equally to either with the exception of the shell. This, in the 'Garden Snail', can be at once recognized by its delicate texture and predominant whitish-yellow colour, as compared with the rough-surfaced brown-banded shell of its ally. During the summer months the 'Common Snail' is to be met with, leading an active independent existence upon or in the immediate neighbourhood of fruit-bearing shrubs. It is very susceptible to cold, and retires during the later autumn to some recess in a wall or tree, where it usually remains dormant and hibernating until the following spring. It not unfrequently buries itself for the same purpose, and even in the warm season may be induced to hibernate temporarily if starved or submitted to a reduced temperature. During such a period, or during normal hybernation, the body is completely retracted within the shell, the mouth of the latter being sealed by a film of mucus secreted by the animal, which hardens on exposure to the atmosphere; this is perforated to allow of the passage of air during respiration and is best termed for obvious reasons the *hybernaculum*. During the hibernating period the animals are frequently to be encountered huddled together in as-

semblages; hence it is no uncommon thing to find, during the warm season, individuals, to the exterior of whose shells there adhere one or more (often a great number) of these hybernacula, cast off by their fellows on emerging from the dormant state.

The body of the snail is soft and unsegmented, and, unlike that of any other animal dealt with in this work, asymmetrical—inasmuch as the anal, respiratory, excretory and genital orifices all open to the right side, the latter being situated far forwards near the mouth. The ventral surface of the body is thick and fleshy, giving rise to a locomotor *foot*, by the wave-like contractions of which the sluggish movements of the animal are performed. So delicately adjusted are these, that the creature can crawl with ease and comfort over a knife-edged surface. The anterior end of the body is differentiated into a well-marked *head segment* bearing two pairs of *tentacles*—a shorter *labial* pair adjacent to the mouth and a longer *ocular* pair situated above and behind these. The integument covering the apex of each tentacle is especially modified in connection with a nerve supply derived from a large underlying ganglion, whence it follows that the eye-bearing tentacle performs a double function. It may be that the labial one is either tactile or olfactory, but the exact functions of these sensiferous areas have yet to be fully elucidated.

Between the head-segment and the free anterior end of the pedal-disc there is a cleft, at the base of which opens a large mucus secreting *pedal-gland* which extends far back to the hind end of the body.

The dorsal surface of the body is produced into a spirally coiled hump, within which the whole digestive gland and portions of the alimentary and reproductive viscera are lodged—it is hence termed the *visceral sac*. This sac, to-

gether with the wall of the pulmonary chamber which overlies it in front, is invested in the spirally coiled shell, the apex of which lies altogether to the animal's right side. The mouth or *peristome* of the shell overlies the thickened anterior border of the pulmonary sac, from which a constant addition of shelly matter is secreted during the growth of the animal, as is also the hybernaculum during repose.

The aperture of the mouth is bounded by soft fleshy lips, and it leads into a spacious buccal cavity the walls of which are excessively thick and muscular. A denticulate horny upper jaw or *beak* is present, and the floor of the mouth is raised up into a cushion-shaped *odontophore* or tongue, which is in turn surmounted by a dentigerous lingual-ribbon or *radula*. This is thrown into a licking rasp-like motion during feeding, by the activity of an underlying musculo-skeletal apparatus, the odontophoral cartilages connected with which are worthy of note as composing an *endoskeleton*. The mouth itself leads into a long tubular œsophagus, which passes straight back and, on entering the visceral sac, opens into a small stomach which receives the secretion of the digestive gland. The stomach in turn gives origin to a coiled intestine which, on nearing the exterior, skirts the lower right-hand border of the pulmonary sac, terminating in an anus which lies to the right of the respiratory orifice. The middle segment of the œsophagus is enlarged to form a distensible *crop*, applied to which there are a pair of salivary glands, confluent above and pouring their secretion into the mouth by means of two elongated ducts.

The digestive gland is a paired structure; its lobes are asymmetrical—the smaller right one lying altogether within the top whorls of the shell. Microchemical examination shows that it performs a complex function, serving both as a

storehouse of combustible fatty carbo-hydrate material and as a centre for secretion of a digestive ferment.

The pulmonary sac or mantle arises as a fold of the body wall, in which pulmonary vessels appear during development. At the hinder end of the enclosed pulmonary chamber there are situated, side by side, the heart and kidney. The heart is enclosed in a definite pericardium, the floor of which is in open communication with the excretory organ by means of a short ciliated *reno-pericardial duct*. The excretory organ itself lies altogether to the right side of the body and debouches on to the exterior by a long duct, running parallel with the rectum.

The heart consists of a single auricle and ventricle, the valves between them being so disposed as only to admit of a current passing from the lung sac to the body. It therefore transmits only aerated blood, and as it is in no way concerned with the propulsion of the blood to the respiratory organs it is termed—like that of the Crayfish already considered—a *systemic* heart. The ventricle gives origin to a single aorta which, on entering the body-cavity, subdivides into two branches. The anterior of these supplies all parts of the body which lie in front of the heart, and the posterior is restricted to the visceral sac and its contents. These arterial trunks break up into minute ramifications, which pass either into capillary systems or lacunar spaces, all of which converge, directly or indirectly, towards a great sinus which lies at the base of the pulmonary sac. From this, afferent pulmonary vessels arise on all sides; the branches of these, reuniting in the substance of the lung-sac, form a system of efferent pulmonary vessels, which unite to form a large pulmonary vein which enters the heart. The efferent pulmonary vessels of the right side pass, on their way to the heart, through the excretory organ, in the sub-

stance of which they break up into a second (*renal*) capillary network.

The blood contains amoeboid corpuscles, which float in an opalescent serum; it assumes a bluish tinge on exposure to the atmosphere.

The central nervous system is enclosed in a membranous circum-œsophageal sheath. It consists of three yellowish ganglionic masses; the *supra-œsophageal* or cephalic lying above the gullet and giving off nerves to the head segment and related parts; the *pedal* which supplies the foot and body-wall; and the *parieto-splanchnic* which distributes *fibres* to the body-wall and viscera, and all parts lying behind its point of origin, irrespective of the foot. These ganglionic centres are connected together by lateral commissures; and from the cerebral mass there arise a system of *buccal nerves* in relation with the buccal mass and its odontophore, and others distributed to the sense organs. The latter are, a pair of small *auditory vesicles* to be hereafter described (see Sect. J. 3) and visual and tegumental sense organs borne by the tentacles, to which reference has already been made.

The snail is hermaphrodite and the sex-organs are highly complicated. With the exception of the hermaphrodite gland or *ovotestis*, a portion of the duct of the same and its appended albumen-secreting gland—all of which are lodged in the visceral sac, they fill the greater part of the spacious body cavity and can be at once recognized by their dead-white colour. As the hermaphrodite duct approaches the exterior it suddenly divides into distinct oviduct and vas deferens; the base of the latter is enlarged to form a swollen eversible intromittent organ or *penis*, which opens, side by side with the oviduct, into an integumental pit or *genital cloaca*. Appended to the whole apparatus there are several accessory glands and diverticula. Chief among these is a

cœcal diverticulum of the base of the penis which secretes a mucilaginous investment for the spermatozoa; the *spermatophores* or packets of spermatoza thus formed are transferred, during copulation, to a corresponding cœcal diverticulum of the oviduct known as the *receptaculum seminis*. Fleshy valves are developed within the lips of the genital cloaca and at the orifices of the genital ducts which open into it, and the whole condition of the organs is such as to obviate the possibility of self-fertilization.

In the *spiculum amoris*, an accessory to the female portion of the apparatus (see Sect. G. 3 *f*.) we have a structure, almost without parallel in the whole animal kingdom. It reaches maturity during the breeding season, and is forcibly ejected from individual to individual during the amorous overtures, which last for a period of some hours.

The spermatozoa are long filiform bodies, each with an enlarged nucleus-bearing "head." The ova are chiefly noteworthy on account of the absence of a distinct vitelline membrane; they are comparatively large and are provided with a nutritive food-yolk.

The reproductive elements of opposite sexes ripen alternately, the maturation of a given batch of spermatozoa preceding that of the ova they are destined to fertilize, and in view of the facts above related it follows that the spermatozoa must be transferred, during copulation, to the body of the second individual—there to await the descent of the ova.

Fertilization takes place as the ova leave the body, and there are to be found in the haunts of these animals during the summer months, usually beneath some stone or decaying wood or more rarely buried in the earth itself, aggregates of 100 or more eggs, each invested in an albuminous envelope, within which the early developmental phenomena are undergone.

The conditions of development of the "Pond Snail" (*Lymnæus stagnalis*) are much more favourable for observation than those of the "Common Snail," and they are accordingly here dealt with.

The "Pond Snail" is a sluggish carnivorous animal which may be found in abundance during the summer months in ponds and stagnant waters; it is moreover an indispensable acquisition to the aquarium. If well fed, the animals will deposit their eggs upon the vessel, in aggregates, imbedded in the albuminous secretion aforementioned. The more important developmental changes are undergone within this investment.

The transverse diameter of the fertilized ovum is about the $\frac{1}{200}$th of an inch. The segmentation is holoblastic and unequal, and the changes undergone during the early developmental period are substantially such as have been already described for the Earthworm (cf. p. 247), resulting in the formation of a simple two-layered gastrula.

Prior to segmentation there appear on the surface of the egg some two or more minute protuberances, which finally become constricted off and lost[1]. A portion of the nucleus of the egg cell is carried away with each body thus formed. Various interpretations have been put upon these *polar bodies;* they are cellular in nature and of general occurrence in the animal kingdom, but further discussion concerning them is beyond the scope of this work. They are alluded to here, as the conditions for observation are exceedingly favourable.

After the gastrula phase is passed, the embryo assumes a somewhat spherical shape, during which period the mouth is formed as a median involution of the epidermis. There now appears on the surface, immediately in front of the

[1] In this animal they usually remain adherent to the fertilized egg until segmention is far advanced.

mouth, a thickened zone incomplete ventrally. The surface of the body becomes ciliated and especially so this zone, whereupon there results a rapid rotation of the embryo within its albuminous investment. This being so, the zone in question is termed the *trochal ridge*, the larva possessed of it being said to be in the *trochosphere* stage. This, in turn, gives place to a more advanced *veliger* stage, so called on account of the changes undergone by the trochal ridge, which now becomes more marked, being produced out into a hood-shaped pre-oral lobe or *velum*. During this stage the mantle arises as a fold of the body-wall, which, as age advances, takes on the characters of a lung sac.

During the final stages of larval metamorphosis the left side of the body grows much more rapidly than the right one. Thus it is that the originally bilaterally symmetrical larva becomes converted into an asymmetrical adult, a fact which renders clear the displacement of the orifices (other than those of the mouth and pedal gland) and of the organs connected therewith, the suppression of the excretory organ of one side, and the enormous increase in size of the left lobe of the digestive gland, as compared with the smaller right one.

Very early in the history of the larva the locomotor foot arises, as a median ventral outgrowth between the mouth and anus; and as the importance of this structure becomes more marked, the cilia of the velum undergo a reduction. Consequent upon these changes the rotatory movements of the animal, so characteristic of the trochosphere, give place to a sluggish creeping motion. The velum itself does not entirely vanish in Lymnæus, but persists throughout life as a couple of so-called *subtentacular lobes* which lie immediately above the mouth. These are wanting in the "Common Snail."

LABORATORY WORK.

If the Snail be killed, by immersion in water heated to a temperature such as the hand can comfortably bear, the shell will readily part company with the muscles to which it gives attachment. This being the case, no difficulty will be experienced in removing the animal, if, holding it in the left hand, the shell be twisted off by the finger and thumb of the right.

In dissecting the internal organs it is advisable to remove the visceral sac in starting. This may best be done by cutting away its thickened edge with a pair of scissors, and tearing it off with a couple of pairs of strong forceps.

A. **General external characters.**

1. In the living animal observe:

   a. The *body;* produced ventrally into an expanded locomotor disc or *foot;* and dorsally into a spirally coiled *visceral-sac*, invested in the single coiled shell or *exoskeleton.*

   b. The *head segment;* a freely projecting anterior lobe of the body, which overhangs the foot. It bears two pairs of retractile *tentacles;* a smaller lowermost *labial* pair, situated at the sides of the mouth, and a longer dorsally placed *ocular* pair, at the summit of each of which there is borne, when fully extended, a minute black eye.

   c. The *apertures.* Examine the animal *en face*, and note:

α. The *mouth;* surrounded by a thick *circular lip*, externally to which there is, on either side, a well-marked *lateral lip*. Observe that the mouth is bounded above by a denticulate horny *beak*, lying within the circular lip.

β. The *pedal gland;* opening by a wide aperture, at the base of a depression between the head segment and the foot. Insert a seeker into it—it can be readily introduced for a distance of more than an inch.

γ. With the animal still in this position, note that the shell (and visceral sac which underlies it) is carried altogether to the creature's right side.

Place the snail right side uppermost, and examine:

δ. The *genital orifice*, situated a short distance behind the bases of the tentacles of the right side.

ε. Gently raise the shell, and note, underlying its free edge, the thickened glandular border of the pulmonary sac. Enclosed by valve-like folds of this there lies the large *respiratory orifice*. Situated side by side with, and a little to the right of, this, is the *anus*. (The excretory orifice also opens at this point, but within the lip of the respiratory one. It is not visible without dissection. Cf. Sect. C. 1 *d*.)

ζ. The *genital furrow*, a feebly defined integumental groove, extending from the base of the pulmonary sac to the genital orifice.

B. **The shell or exoskeleton.**

a. It forms a continuous investment. Its free edge is produced into a whitish porcellanous reflected

border or *peristome*. It is thrown into a spiral of four whorls.

b. Note its texture and colour (cf. p. 272). As the apex (first formed part) is approached, a smooth friable texture and a pearly lustre are assumed.

c. The *columella*. If, with the peritreme directed towards you, the shell be carefully opened up with scissors, the columella will be seen as a central pillar or axis. Cut into this with care; it will be found to be hollow, and closed in below by an overgrowth of the peritreme—the shell is thus a spirally coiled tube.

In young shells the columellar cavity opens freely below, by an aperture or *umbilicus*.

d. The *columellar muscles*. Remnants of the glistening white tendons of these are often to be found, attached to the upper end of the shell axis. (Cf. Sect. E. 1 *a*.)

e. The *hybernaculum* or *epiphragm*. Examine a dormant specimen, and note that the mouth of the shell is completely closed by this. Remove it and examine under a low power; note the perforation of its central area.

C. **The pulmonary sac and its associated structures.**

1. Examine the *pulmonary sac* from above. It consists of a membranous expansion of the body-wall, which overlies the entire antero-dorsal region of the visceral hump. Large blood-vessels are developed within its walls; and there is visible through it, on the right side, the yellow *excretory organ*. Insert a scissors

into the respiratory orifice and make an incision along the right-hand border of the sac, cutting clear of the excretory organ. Carry a second incision along the thickened base of the sac towards its left side, and reflect the whole. Note:

a. The floor of the pulmonary chamber; thin and transparent, there being seen through it the dead-white reproductive organs.

b. The *rectum*, a thin walled tube coursing along the floor of the pulmonary chamber at its extreme right hand border.

Follow it to the *anus*. It opens, at the base of a groove-like depression in the lip of the respiratory sac, below and to the right of the respiratory orifice.

c. The *excretory gland*, a considerable yellowish mass, lying in the posterior right hand end of the pulmonary sac. Make an incision into it and wash out its contents; note the thickened spongy texture of its glandular lining, which is thrown into a series of folds by the underlying blood-vessels.

d. The *excretory duct*, a yellow thin-walled tube, running to the right of and parallel with the rectum. Its orifice lies a little above and to the right of the anus. Insert a blowpipe into this and inflate the whole; note that the duct is continued along the right hand border of the gland to its summit.

If examined minutely, it will be seen that the excretory duct is continued on beyond the above-named orifice as a well marked *excretory groove*, whose walls are contractile. This courses over the base of the rectum dorsally to the anus, and, passing

backwards and downwards, terminates to the left of the respiratory orifice.

c. The *pericardium*, a small sac lying in a recess of the left hand border of the excretory gland. Remove its front wall and examine the *heart;* it is subdivided into a single auricle and ventricle.

2. Cut away the upper part of both the excretory organ and pericardium, and remove so much of them as remains, together with a portion of the adjacent visceral sac. Wash carefully until clear of sediment, and examine in water under a low power.

A short ciliated duct will be found, passing from the base of the pericardium to the excretory gland, into which it opens by a *reno-pericardial aperture.*

D. **The alimentary organs.**

1. Place the animal on its left side, and pin it down through the muscular foot. Remove the lung-sac and liberate the rectum from its surroundings. Next dissect away the right half of the body wall and visceral sac.

There will be seen filling up the greater part of the body cavity the dead-white generative organs; remove these *en masse*, whereupon there will be clearly visible—

a. The *crop;* a large sac-like organ, filling a considerable portion of the "body cavity". It is generally rendered the more conspicuous on account of the yellow colour of its contents. Its lining membrane is thrown into a series of longitudinal folds; these are visible through its thin walls, giving it an apparent longitudinal striation.

b. Follow the crop forwards, it will be found to arise from the roof of the thick-walled muscular *buccal mass*, as a simple œsophageal tube.

c. The *sac of the radula*, a small backwardly directed diverticulum of the floor of the buccal mass.

d. The *salivary glands*, two irregular whitish masses closely applied to the sides of the crop. They are confluent postero-dorsally, and each is connected with the roof of the mouth by an elongated *salivary duct*. Follow one of these forwards, its base becomes enlarged as it enters the mouth, at the re-entering angle between the œsophagus and buccal mass.

e. Follow the crop backwards, it will be found to pass into the visceral sac under cover of the right lobe of the digestive gland (a yellowish brown mass, filling the apical whorls of the visceral hump). Turn this gland downwards and forwards, and note, lying beneath it, the small sac-like *stomach*, into the hinder end of which the œsophagus is seen to pass.

f. The *digestive gland*. Buried up in its small right lobe will be found the white *ovotestis* (remove this with care). Its left lobe is much the larger of the two; it is subdivided into three *lobules* which adapt themselves to the coils of the intestine. Arising from these are seen well-defined ducts; follow them and note that they unite to form a single short *digestive duct*.

g. The *stomach*. Open this from the side, being careful not to injure the right lobe of the digestive gland, and wash out its contents. It is a simple

sac, slightly subdivided into two by a longitudinal fold. Four apertures will be found in its walls; they are—

α. The œsophageal orifice, opening into its hinder end.

β. The origin of the intestine, lying below and to the right of α.

γ. The apertures of the digestive ducts; opening—that of the left side just above the intestinal orifice—that of the right into its apex (this duct skirts the free edge of its gland).

h. The *intestine*. After leaving the stomach it passes upwards and forwards, altogether to the left side. It then makes two turns upon itself, approaching the right side as it does so, and finally courses along the right border of the pulmonary sac. (Cf. Sect. C. § 1 *b*.)

E. **The buccal mass and odontophore.**

1. Examine the *buccal mass* from the side and make out its muscles. The *constrictor* fibres have already been referred to, as constituting the muscular wall of the whole structure. Of those which remain the more important are—

a. The *retractor;* a large sheet, arising from its floor and side walls below the sac of the radula, and passing back, side by side with the immense *retractor pedis* fibres arising from the foot, to be inserted into the columella or shell axis. (Cf. Sect. B. *d*.)

b. The *protractors;* delicate muscles arising from its side walls, and passing downwards and forwards, to be inserted into the cephalic integument.

c. The *levators;* delicate muscles arising just above the protractors, and passing upwards to be inserted into the cephalic wall, near the bases of the smaller tentacles.

d. The *depressors*, small muscles underlying the protractors, and passing obliquely backwards.

2. Carefully remove one half of the buccal mass, cutting to the near side of the middle line. Examine under water and note—

a. The *odontophore*, a cushion-shaped elevation of the floor of the mouth, completely covered in mucous membrane. There overlies it a yellowish ribbon-shaped band—the *lingual ribbon* or *radula;* follow this back into its sac (see Sect. D. 1. *c*) and note that as that is approached it assumes a whitish colour and membranous texture.

b. The *radula.* Remove this bodily, with a pair of forceps; transfer it to a glass slide, cover in water and examine under a low power. Note the presence of an immense number (between twelve and thirteen thousand) of chitinous teeth. Those which are functional can be readily distinguished by their yellow colour and sharp pointed cusps. Examine that portion of the radula which lay within the sac. Note the transparent immature teeth, becoming simpler and more papilla-like as the hindermost border is reached. Examine in like manner the front end. It is beset by teeth whose cusps are worn down, and reduced, in many cases, to the condition of mere functionless rudiments.

c. Examine the functional region under a $\frac{1}{4}$ or $\frac{1}{6}$ objective. Note that the bases of the two sets of lateral teeth (*uncini*), by which the radula is for the most part beset, are concave externally. Follow those of one side inwardly—a single median longitudinal row (*rachis*) will be reached, the bases of whose teeth are concave on either side.

The rasping surface of each median tooth is produced into a pointed cusp. In the lateral teeth there appears, on the inner side of this, a second smaller one which increases in size relatively, as the free edge of the radula is approached. There appears, at the same time, a smaller external accessory cusp, which is not represented in the median teeth.

d. The *odontophoral cartilages;* two gristly masses to whose presence the elevation of the floor of the mouth is due. There are attached to their bases a series of small *intrinsic muscles*, arising from the side walls of the buccal mass.

e. The *horny beak;* seen to be formed in relation with a special fold of the lining membrane of the roof of the mouth. It lies wholly within the *circular lip*, seen in section to be thick and fleshy.

f. The *salivary duct;* opening into the roof of the mouth by a minute orifice situated just above the odontophore.

F. **The Pedal Gland.**

This is best examined at this stage, by removing the foot to one side of the middle line. It has the appearance of a white fluffy-looking mass, lying immediately above the pedal disc, and extending back for two-thirds the length of the same. Open it up with care—it consists of a cœcal

diverticulum of the integument, opening below the mouth by an expanded orifice." (Cf. Sect. A. 1. *c.* *β*.)

Compare the same, as seen in a transverse section across the foot. Note its thick glandular walls and central lumen. It is accompanied on either side by a well-defined *lateral pedal blood sinus*.

G. **The Reproductive Organs.**

1. Pin the animal down as directed in Sect. D., and remove the right half of the body-wall posterior to the genital orifice, together with the rectum. Dissect off the visceral sac and examine in order—

   a. The *ovotestis* or *hermaphrodite gland;* a small white mass, buried up in a fossa of the right lobe of the digestive gland. Remove sufficient of the latter to fully expose it.

   b. The duct of the ovotestis or *hermaphrodite duct;* a short highly convoluted glistening white duct, passing upwards from the ovotestis towards the main mass of the reproductive apparatus.

2. Remove sufficient of the right lobe of the digestive gland to liberate the ovotestis and hermaphrodite duct; note—

   a. The *albumen gland;* a large greyish white structure lying, apex downwards, to the immediate left of the duct of the ovotestis.

   ' This gland swells up very rapidly under water, in the manner of the glandular segment of the Frog's oviduct already described (p. 53). Dissection may conveniently be carried on under weak alcohol.

In the vicinity of the base of this gland, the genital organs come into close apposition with the intestine and aortic vessels. Carefully liberate them *en masse* from their surroundings and pin down under water—the albumen gland with its flattened face uppermost. Now follow in order—

b. The further course of the *hermaphrodite duct.* On reaching the albumen gland it becomes suddenly constricted, to form an exceedingly delicate thread-like tube. As this is usually of a dead-white colour it can be seen through the more transparent albumen gland, in the superficial portion of which it lies buried. It passes for a short distance towards the apex of this gland, and then, turning sharply upon itself, is continued straight back towards the base of the same; it here becomes suddenly enlarged to form a convoluted duct with sacculated walls, which is continued on towards the genital orifice.

The transition between the two segments of the hemaphrodite duct here described, is so sudden as to make it appear that they are distinct structures; hence it is that the sacculated segment is sometimes spoken of as an *oviduct.*

c. Its walls are beset externally by a continuously straight series of glandular follicles, making up an *accessory gland* (so-called *prostate*).

d. Open up the albumen gland and note its spacious *central duct.* It will be found to enter the enlarged "head" of the sacculated portion of the hermaphrodite duct. Follow the hermaphrodite duct onwards —its sacculations gradually disappear, and it suddenly divides into two—*oviduct* and *vas deferens.*

e. The *oviduct;* a very short thick-walled tube, entering into immediate relationship with a large white *sac* —the *dart sac* (§ 3 *e*).

f. The *vas deferens;* a thin-walled tube, arising side by side with *g*, but of lesser calibre than it. Its inner end is tied down to the female duct; leaving this it passes under the retractor muscle of the right tentacles and becomes enlarged to form

g. the *penis*, apparent now as the elongated swollen end of the *vas deferens*. It passes to the exterior side by side with the female duct.

It is attached to the body-wall of the left side by a ribbon-shaped *retractor muscle.* Cut this across and displace the whole organ.

h. The *spermatophoral gland* or *flagellum;* an elongated thread-like diverticulum of the base of the penis. It is much coiled.

3. Remove the entire reproductive system from the body, together with a portion of the body-wall immediately adjacent to the sexual orifice. Pin the whole down carefully under water, and open up the larger ducts and their appendages, as they stand related to the exterior. Make out in order:

a. The *genital cloaca* or *vestibule;* a shallow integumental pit, opening on to the exterior by the single genital orifice.

b. The *penis.* Remove a portion of its enlarged eversible base. It opens into the front wall of the vestibule; its lining membrane is produced into a fold or *prepuce*, above which there is usually an eminence.

c. The *oviduct.* Follow this down; its first segment already described (§ 2 *c*) is sometimes termed the *vagina.* This opens by a tumid lip into a thin-walled terminal segment, which enters the vestibule side by side with the penis.

d. The *spermatheca* or *receptaculum seminis* (receptacle of the spermatophores); an elongated diverticulum of the above-named terminal segment of the oviduct, bound down during life to the hermaphrodite duct (§ 2 *b*). Liberate it from this, and note that it arises by a short neck which subdivides into two cœcal diverticula—a longer and stouter coiled one, and a shorter one which terminates in a globular enlargement (this lies, during life, tucked away in the bend of the first coil of the intestine. Cf. § 2 *a*).

e. The *dart-sac;* an immense melon-shaped diverticulum of the base of the oviduct, in life backwardly directed. Open it up and note its powerful muscular walls; lying within it will be seen

f. the *dart* or *spiculum amoris*, a four-bladed calcareous projectile. Its head overlies a papillate ingrowth of the lining membrane of the sac.

g. The accessory mucus or *digitate glands;* two branching masses opening side by side into the basal segment of the oviduct, immediately above *e.*

H. The circulatory system.

Examine an animal freshly removed from its shell, before it is in any way dissected. The *pulmonary vein* will be seen running in the wall of the lung-sac, in front of and in a line with the excretory organ. Make an incision in this and

introduce a small syringe or medicine-dropper filled with injecting material. Seize the vessel and the inserted nozzle between the finger and thumb of the left hand, and inject. If this simple operation be carefully performed, all the leading vessels which carry aerated blood will be injected.

1. Lay open the pulmonary-sac, and note:

   a. The *efferent pulmonary vessels;* a uniformly arranged series of trunks bringing in the blood from the lung-sac; they unite to form a large *pulmonary vein* which enters the auricle.

   b. The *efferent renal vessels;* two or three small trunks conveying the blood from the excretory organ to the pulmonary vein.

   During the process of injecting these become very rapidly filled, and in the exercise of the pressure necessary to inject the whole arterial system their walls are not unfrequently ruptured.

2. Open the heart (cf. Sect. C. 1 *e*). Note the thick muscular wall of the ventricle, and the *auriculo-ventricular valves*—so disposed as only to admit of a passage of the blood from the auricle to the ventricle.

3. The *arterial system.* Remove the right half of the body-wall and visceral sac, and together with them the rectum and greater portion of the genital apparatus—cutting the sacculated hermaphrodite-duct across at about its middle.

   There will thus be exposed the great arteries. Work them over in the following order:

   a. The *aortic trunk*, a very short vessel, arising from the base of the ventricle. It lies to the left of the head of the first coil of the intestine, immediately

below which it suddenly subdivides into an anterior and a posterior branch.

b. The *anterior aorta;* a well-defined vessel running parallel with the sacculated portion of the hermaphrodite-duct. On entering the body-cavity it passes beneath the first coil of the intestine and the adjacent globular head of the spermatheca (G. § 3 *d*); it then travels to the right side of the body and runs on, beneath the crop, to the anterior extremity. Branches are given off along its course to the genital apparatus, foot, crop, salivary glands and tentacles, with the adjacent integument; it finally perforates the sheath of the nervous system, ending in relation with the floor of the buccal mass. Special branches accompany the rectum and pedal gland.

c. The *posterior aorta;* a considerable vessel which accompanies, at its outset, the first limb of the intestine. It descends into the visceral sac, and is mainly distributed to those portions of the alimentary canal and its appended glands contained within the same.

4. The *venous system.* With the exception of certain well-defined sinuses and vessels detailed below, the venous channels consist mainly of a series of lacunar spaces, in free communication with the "body-cavity." Reference has already been made (Sect. F.) to the *lateral pedal sinus*, and if, in an uninjured Snail, the point of the injecting apparatus be carefully inserted into this, no difficulty will be found in demonstrating the existence of the vessels here described.

Having injected the animal, reflect the lung-sac,

cutting close alongside the rectum, in order that that portion of it bearing the smaller pulmonary vessels may be reflected intact.

Note in order:

a. The *circulus venosus pulmonis;* a great sinus surrounding the base of the pulmonary sac.

b. The *marginal sinus* of the visceral sac; a spacious channel continuous with *a.* posteriorly and running within the thickened edge of the visceral sac.

> On exposing the interior of the pedal sinus in an uninjected specimen, there will be seen opening into it the orifices of adjacent lacunae. During the process of injection from the above sinus the fluid introduced passes through these—partly into the body-wall, partly into the "body-cavity"—in either case finally reaching the pulmonary circulus, towards which all those channels in which venous blood circulates eventually converge.

c. The *afferent pulmonary vessels;* arising from the pulmonary circulus on all sides. They alternate with striking regularity with the efferent pulmonary vessels, the two sets of trunks being connected by a well-differentiated respiratory plexus.

The afferent pulmonary vessels of the left side and front wall of the lung-sac are elongated. Those of its right side are very short, but in so far as their relations to the lung-sac are concerned they differ from their fellows in no other respect; after leaving that to reach the heart however, they enter the excretory organ and break up within it into a *renal plexus,* from which the *efferent renal vessels* previously described (Sec. H. 1 *b*) arise.

The walls of the vessels concerned in respiration are frequently pigmented. The entire pulmonary system can therefore be generally followed in an uninjured specimen, without the aid of injection, by examining with care the wall of the lung-sac from the outside.

I. **The nervous system.**

1. Pin the animal down right side uppermost and open up the lung-sac, cutting along the edge of the rectum; divide the latter, together with the adjacent excretory-duct and lung-sac, across its middle transversely, and reflect the left hand half. Next remove the right half of the wall of the body and visceral-sac, together with the tentacles of the same side and the greater portion of the genital apparatus. Remove the foot to the level of the pedal gland, and with it the right half of the retractor pedis muscle. Follow in the order given :

a. The *central nervous system.* It consists of a circum-œsophageal mass, buried up in a loose connective-tissue *sheath.* Carefully dissect away the superficial portion of the sheath, until the nerve ganglia are exposed (looked at thus from the side, but one set will be seen); they are—

b. The *supra-œsophageal ganglia;* two considerable masses lying above the gullet, connected together by a short band-like *commissure.*

c. The *pedal ganglia;* two large masses lying immediately above the pedal-disc. They distribute a considerable series of fibres ventrally to the foot and pedal gland, and a smaller set laterally to the adjacent body-wall. Each pedal ganglion is united

to the supra-œsophageal of its own side by a *cerebro-pedal commissure.*

d. The *parieto-splanchnic ganglia;* a series of ganglionic masses lying in close apposition, immediately behind and a little above *c.* They give off fibres (on the right side three sets and on the left two), which enter the body wall at the base of the visceral sac.

The anterior fibres (*pallial nerves*) enter the thickened border of the lung sac, within which they anastomose.

The ganglia of either side are connected with the supra-œsophageal by a *cerebro-splanchnic commissure,* which runs parallel with but is somewhat shorter than the cerebro-pedal one.

e. The *posterior-visceral nerve;* a single nerve arising from *d.* altogether to the left side. It passes back immediately beneath the anterior aorta (which it accompanies) to enter the visceral sac. On doing so, it gives off branches as under—

α. A main one, running on to reach the left side of the visceral sac.

β. A right one, whose fibres can be traced to the albumen-gland and duct of the ovotestis.

γ. A dorsal one, arising from the main trunk, in the immediate vicinity of the enlarged head of the spermatheca. It is distributed to the excretory-organ and heart.

2. Remove sufficient of the cephalic wall of the right side to expose the whole buccal mass. Look for—

a. The *buccal nerves.* That of the right side will alone be seen; it arises from the supra-œsophageal ganglion, and passes downwards and forwards towards the buccal mass. Trace it with care—it passes beneath the dorsal protractor muscle (see Sect. E. 1 *b*), and terminates in a small *buccal ganglion*, lying just behind the enlarged base of the salivary-duct.

Just before it reaches the muscle referred to, it usually gives off two or three branches to the antero-lateral wall of the buccal-mass.

b. The *anterior visceral nerves;* a system of fibres arising from the above ganglion. The following can be seen with ease.

α. A ventral branch, distributed to the œsophagus.

β. A dorsal one, to the salivary gland, the duct of which it accompanies.

γ. An anterior one, to the roof of the buccal-mass.

3. Remove the whole nervous system, together with the buccal-mass and tentacle-bearing portion of the cephalic integument. Pin down under water with its posterior end uppermost, and dissect off as much as possible of the sheath of the nerve-collar. Note, in addition to the nerves already dissected—

a. The *buccal commissure;* a delicate tract of nerve-fibres, seen, on raising the cut end of the œsophagus, to connect the buccal ganglia of opposite sides. It passes beneath the gullet.

b. The *anterior aorta.* Its cut end is clearly visible at this stage. (Cf. Sect. H. 3 *b*.) It perforates the nerve-collar ventrally, running beneath the parieto-splanchnic and above the pedal ganglia.

c. The *tentacular nerves;* arising, together with the *labial nerves*, from the sides of the supra-œsophageal ganglia. The nerve to the optic tentacle is connected with a special ganglionic lobe.

J. **The Sense-Organs.**

1. Obtain a specimen in which the tentacles are fully extended. Remove the cephalic integument and tentacles, together with the nervous system, *en masse;* pin down very carefully under water and dissect under a lens. Open up the tentacles and note—

a. The *retractor muscles;* two ribbon-shaped bands arising from the extreme anterior ends of the tentacles; they pass back to be inserted into the shell axis, side by side with the larger retractors already described (Sect. E. 1 *a*).

The muscles of the smaller pair of tentacles receive slips from the adjacent cephalic integument.

b. The *labial tentacle.* Note that the upper end of its retractor muscle is darkly pigmented. Trace up the *tentacular nerve* which accompanies the muscle; it ends in a large pear-shaped *ganglion*, from which fibres are distributed to the integument investing the free end of the tentacle.

The nerve sends a branch to the adjacent cephalic integument.

c. The *ocular tentacle* and *eye.* Its retractor muscle and nerve repeat the conditions described for *b*; a

large terminal ganglion is present, in relation to the integument investing its free end.

The *visual organ* is perceptible as a minute black spot, lying to the outer side and a little in front of the ganglion. A delicate *optic nerve* can be traced back from it to the tentacular nerve, which it enters a short distance behind the above-named ganglion.

2. Remove the eye together with a small portion of its related integument, and examine in water under a low power. Note—

   a. The *choroid;* a pigmented investment for the eye, under cover of which the optic nerve passes to reach the retina.

   b. The *crystalline lens;* seen to project freely beyond *a.* in front. It lies under cover of a delicate *cornea.*

3. The *auditory organ.* Pin one half of the nerve-collar down under water, and, having dissected off the superficial stratum of its sheath, examine with a lens. Note—

   a. The *auditory nerve;* a delicate undulating trunk, lying in the interspace between the cerebro-pedal and cerebro-splanchnic commissures. Follow it up—it arises from the supra-œsophageal ganglion. Below, it enters the *otic vesicle,* now seen as a minute white speck, overlying the point of apposition of the pedal and parieto-splanchnic ganglia.

   b. The *otic vesicle.* Remove this, together with a small portion of its surrounding tissues, and

transfer *in toto* to a glass slide. Cover and examine in water under a low power. It appears as a conspicuous round vesicle, lodging a refractive black-looking granular contents.

Examine under a high power. It is seen to consist of a small sac (*otocyst*) closed on all sides and in no way connected with the exterior. It is filled with a fluid contents, in which are suspended a large number of small oval calcareous corpuscles or *otoliths*. Trace the passage of the auditory nerve into the vesicle.

K. **The reproductive elements.**

Remove a small piece of the ovotestis, and mount in aqueous solution of Eosin. Examine under a low power and note—

a. The *hermaphrodite follicles;* small cœcal diverticula of the ovotestis, from the epithelial lining of which the sexual products are derived. They are arranged in grape like aggregates.

The appearances met with will be found to vary with the season of the year. If spermatogenesis is going on there will be found, floating in the central cavity of each follicle, spermatozoa in all stages of development. If the ova are undergoing maturation, they will be found, in all stages, still adherent to the germinal epithelium.

Crush under the weight of a cover-glass and examine under a high power.

a. The ripe *spermatozoa*. Each is an elongated filiform body, bearing a small head-like enlargement at one end. They are commonly met with in aggregates.

Immature spermatozoa are at times present in abundance. They can be recognised by the relatively large size of the 'head', which is oval and can be distinctly seen to lodge a small nucleus. (Cf. *Lumbricus* and *Rana.*)

b. The *ripe ovum.* Examine under a high power.

α. Its *shape;* usually round, more rarely oval.

β. The absence of a distinct vitelline membrane.

γ. The *protoplasm;* consisting of a central uniformly granular *vitellus*, which graduates peripherally into a superficial clear layer.

δ. The *germinal vesicle;* a large round structure, usually containing one immense *germinal spot.*

L. **Development.** (The Pond Snail, *Lymnæus stagnalis.*) See p. 278.

Examine the developing eggs from time to time, and look especially for—

a. The *fertilized ovum* (*oosperm*) *during segmentation.* It divides up into two sets of cells—a smaller rapidly dividing clearer set, and a larger slowly dividing yolk-laden set (the presence of the yolk gives these an opaque coarsely granular appearance). Look out for *polar bodies* (see p. 278).

b. The same at the *close of segmentation.* Looked at from the outside the larger cells appear as a dark mass, lying within the smaller more transparent ones, which have now completely overgrown them.

c. The *gastrula* phase, immediately following upon *b.* Examine from beneath and note

α. the *blastopore;* a small slit-like orifice on the flattened under surface.

Examine from the side in optical section.

β. The *archenteron;* a sac-like pit opening externally by the blastopore, its walls are formed of the invaginate yolk-bearing *endoderm* cells.

γ. The *ectoderm;* a single layer of investing cells, the product of the more transparent rapidly dividing ones referred to above.

δ. The *cleavage-cavity* or *blastocœle;* a spacious cavity, enclosed between the investing and invaginate layers.

d. The *Trochosphere* larva; recognizable by its egg-shaped contour and rotatory movements.

α. The *mouth;* a small median orifice situated at the enlarged end.

β. The *trochal ridge;* a saddle-like band encircling the dorso-lateral area pre-orally. Look for its cilia.

Examine in optical section and note—

γ. The *foot* (it first appears at this stage); a median ventral outgrowth of the body wall just behind the mouth.

δ. The *stomodæum;* a blind sac-like involution of the integument, its aperture giving rise to the mouth.

ε. The *archenteron;* now partially surrounded by a conspicuous large-celled granular mass — the *digestive gland*, arising as an outgrowth of its wall.

Note the *bilateral symmetry* of the larva at this period. The blastopore appears shifted back, as the result of elongation of the embryo and

displacement, upon the development of the foot. An integumental pit known as the *shell-gland*, appears at the hind end of the body during this stage.

e. The *Veliger* larva. A head-segment is now becoming differentiated, and the rotatory movements of the animal are less marked. Bilateral symmetry is becoming disturbed. Note especially—

α. The *velum;* a ciliated *pre-oral* fold, occupying the position of the original trochal-ridge.

β. The *foot;* now greatly increased in size and utilized for purposes of locomotion.

γ. The *eye-spots;* two black masses at the sides of the head segment.

f. The *advanced larva.* Tentacles are now appearing, as paired outgrowths of the cephalic wall (the eyes are not carried up by them as in Helix). The original bilateral symmetry no longer obtains. Note the beat of the heart; the presence of the visceral sac and of its related shell—at this stage a transparent chitinous cap. The velum is undergoing a marked reduction.

If any difficulty is experienced in examining the embryos in the living state, they should be liberated from the surrounding albuminous investment by means of a couple of needles, and submitted, for 20 minutes, to the action of ½ p.c. Osmic acid solution. They may then be transferred to alcohol of increasing strength, and preserved for future examination.

V.

THE FRESH-WATER MUSSEL

(*Anodonta Cygnea*).

UNDER the name of 'Fresh-water Mussel' two distinct kinds of animals, which are not unfrequently abundant in our ponds and rivers, are included; namely, the *Anodonta* and two or three kinds of *Unio*. The *Anodonta* is chosen for special study here, but what is said about it applies very well to nearly all parts of *Unio* except the shell.

The animal is enclosed in a shell composed of two pieces or *valves*, which are lateral, or right and left, in relation to the median plane of the body. The more rounded and broader end is anterior, the more tapering, posterior. If placed in a vessel of water, at the bottom of which there is a tolerably thick layer of soft mud or sand, and left quite undisturbed, the *Anodonta* will partially bury itself with its anterior end directed obliquely downwards; and the valves will separate at their ventral edges for a short distance. At the edges of this 'gape' of the shell the thickened margins of a part of the contained body which is called the *mantle*, become visible, and between them a large, whitish, fleshy, tongue-shaped structure—the *foot*—not unfrequently protrudes, and is used to perform the sluggish movements of which the *Anodonta* is capable. Mud '*tracks*' are left behind the animal when in motion, as can be seen on observing its natural habit in slow running or still shallow

waters. If some finely dividing colouring matter, such as indigo, is dropped into the water, so as to fall towards the gape, it will be seen to be sucked in; while, after a short time, a current of the same substance will flow out from an opening between the two edges of the mantle on the dorsal side of the posterior end of the body; and these 'inhalent' and 'exhalent' currents go on, so long as the animal is alive and the valves are open. Any disturbance, however, causes the foot, if it was previously protruded, to be retracted, while the edges of the mantle are drawn in and the two valves shut with great force. This *adduction* results from the contraction of two thick bundles of muscular fibres, which pass from the inner face of one valve to that of the other, one at the anterior and the other at the posterior end of the body, and are called the *anterior* and *posterior adductors*. The valves of the dead *Anodonta* always gape, and if they are forcibly shut they spring open again. The reason of this is the presence of an elastic band or *ligament*, which unites the dorsal margins of the two valves, for some distance, and is put upon the stretch when the valves are forcibly brought together. The natural tendency to *divarication* of the valves resulting from this, is held in check during life by the contractions of the aforenamed adductor muscles. These respond to a nervous stimulus, and, on the death of the animal, that being withdrawn, the full force of the elasticity comes into play, the valves becoming divaricated to their utmost extent.

The animal can be extracted from the shell without damage, only by cutting through these muscles close to their attachments. It is bilaterally symmetrical, the foot proceeding from the middle of its ventral surface. There is a total absence of any distinct head segment such as is seen in the Snail. The mouth is median and situate between

a projection, which answers to the under surface of the anterior adductor muscle, and the superior attachment of the foot. On each side of the mouth are two triangular flaps with free pointed ends—the *labial palpi*—and behind these, on each side, two broad plate-like organs, with vertically striated outer surfaces, are visible. These are the *gills* or *branchiæ*. In the dorsal region, the integument is soft and smooth; on each side, it is produced into large folds, the lobes of the mantle or *pallium*, which closely adhere to the inner surface of the valves of the shell, and end, ventrally, in the thickened muscular and glandular margins already mentioned. They pass into one another in front of the mouth; at the sides, they are united with the dorsal edges of the outer gill-plates; and, behind, they extend upwards and on to the dorsal face of the body, finally passing into one another above and in front of the anus, which is small, tubular, prominent and median. Thus the anus is inclosed in a part of the cavity bounded by the two mantle-lobes which lies above the gills, it is relatively small and shallow, and is termed the *supra-branchial* or *cloacal chamber;* while the gills, the foot, and the palps, hang down into the relatively large *infra-branchial* chamber which occupies the space between the mantle-lobes for the rest of their extent. During life the posterior margins of the mantle lobes are prolonged for a short distance behind the free edge of the valves, and they come into apposition at the point of attachment of the gills in such a way as to give rise to two tubes or *siphons*—a dorsal *supra-branchial* one which receives the anus, and a ventral one communicating with the *infra-branchial* chamber alone. The dorsal siphon is the channel through which the exhalent currents pass; the ventral, that for the inhalent currents.

The currents are produced and kept up by the action of the cilia, which abound upon the gills, labial palps and inner face of the mantle. The gills are perforated by innumerable small apertures, and the cavities contained between the two lamellæ of which each is formed, are in communication, above, with the cloacal chamber. The cilia work in such a way as to drive the water in which the animal lives from the outer surface of each gill towards its interior. Hence, to a large extent, the current which sets from the infra-branchial to the cloacal chamber.

The current of water which is thus continually drawn into the infra-branchial chamber carries with it minute organisms, *Infusoria*, *Diatoms* and the like, and many of these are swept to the fore part of the chamber, where they enter the mouth, and are propelled by the cilia which line its cavity into the alimentary canal. The latter presents a short and wide gullet, a stomach surrounded by a digestive gland, a long intestine coiled upon itself, in a somewhat complicated manner, and, finally, a rectum, which lies in the middle line of the dorsal aspect of the body, traverses the pericardium and the heart which lies therein, and ends in the anus.

The subdivision of the mantle-cavity into supra and infra branchial chambers is, in the long run, the result of confluence of adjacent gill-laminæ with each other and with the pallial lobes. The gill-perforations, the groove enclosed by the labial palps and the aperture of the mouth towards which it leads, all open into the lower or inhalent chamber; while the upper or exhalent one receives the anus, and both genital and excretory orifices. During life the thickened margins of the pallial lobes are in close apposition; consequently, the infra-branchial chamber is closed below and in front, both it and the supra-branchial

one communicating with the surrounding medium through the agency of the siphonal prolongations of the mantle border alone. The walls of the two chambers and the surfaces of all the organs contained within them, together with the whole lining membrane of the alimentary canal, are ciliated, and the currents thereby induced set in, as before stated, by the lower and out by the upper siphon. One of three courses is open to the inhalent current with its suspended food material—it may pass either through the gills, through an interspace between the gills and the body wall, or into the mouth. Take whichever course it may, it finally reaches the supra-branchial chamber. It follows that the insetting current is both a nutritive and a respiratory one, while the outsetting one serves to carry away the waste products of respiration and digestion, together with the products of the excretory and genital organs.

The clue to the real meaning of the more important structural features in the organization of this animal, is to be sought in an understanding of the above facts.

Digestion, that is solution of the proteinaceous and other nutritive matters contained in food, is effected in the stomach and intestine; and the nutritious fluid, thus formed, transudes through the walls of the alimentary cavity and passes into the blood contained in the blood-vessels which surround it. This blood is thence carried into a large sinus, which occupies the middle line of the body under the pericardium and between the *organs of Bojanus* (see Laboratory Work E), and receives the greater part of the blood returning from all parts of the body. From this median *vena cava*, branches are given off to the gills and open into the extensive vascular network which those organs contain. From this, again, trunks lead towards the pericardium and open into one or other of the two auricles of the heart, which

communicate by valvular apertures with the ventricle. The ventricle gives off two aortic trunks; from these branches are given off which divide into smaller ramifications for the different regions of the body, and for the viscera, and finally terminate in channels which answer to the capillaries of the higher animals.

The pericardial cavity, in which the heart is lodged, is situated in the posterior half of the dorsal region of the body. Through its thin dorsal wall, and, still better, when it is carefully laid open, the heart can be seen beating. The auricles contract, and, after them, the ventricle; the wave-like contraction of the latter being much the more easily visible. The lips of the auriculo-ventricular apertures are so disposed that the blood is impeded from flowing back into the auricles, when the ventricle contracts, and is forced out, either forwards or backwards, through the two aortæ. From these it finds its way to the capillaries, and returns from them to the *vena cava;* whence it is carried, through the organs of Bojanus, to the branchiæ. Here it becomes purified of carbonic anhydride, and receives oxygen from the water in which the branchiæ are plunged; and it is finally brought back in an arterialized condition to the heart. There is reason to believe that the mantle, which is highly vascular, may perform an accessory respiratory function, in a manner similar to that of the inner wall of the Crayfish's carapace (cf. p. 182).

The heart is therefore systemic and propels aerated blood. As the heart, in most animals, first appears either in close proximity to, or in the substance of, the wall of the embryonic alimentary canal, there is no real anomaly in its position in the *Anodonta*.

The blood of the *Anodonta* is colourless, and contains colourless amœboid corpuscles.

The majority of the vessels which convey the blood from

the vena cava to the branchiæ, traverse the walls of the dark-coloured organs—the organs of Bojanus—which have already been mentioned; and they here part with their nitrogenous waste matters—the organ of Bojanus playing the part of a kidney. The cavity of the organ of Bojanus communicates, on the one hand, with the pericardium; and, on the other, with the exterior, by an aperture to which reference has already been made. Thus the cavity of the pericardium communicates directly with the exterior, though by a roundabout way.

The organ of Bojanus consists of a pair of modified tubes, each of which, like the nephridium of the worm, can be resolved into three segments—an internal non-glandular, a middle glandular, and a terminal muscular one. An outsetting current is induced by the ciliary action of the lining epithelium of each of these organs, and their relations to the pericardium of this animal, are as those of any one pair of segmental organs to the body-cavity in the worm. If so be that the pericardium of *Anodonta* is, like that of the Frog, a direct derivative of the body-cavity, the excretory organ is in no way anomalous in its relationships.

The digestive gland of this animal is mainly concerned in the elaboration of a digestive fluid. The production of combustible carbo-hydrate material—a function by no means confined to the liver in other animals—goes on to a considerable extent in other far removed parts of the body, *glycogen* being formed. The connective-tissues and especially the mantle lobes are remarkable for its presence, it being elaborated in the form of large intra-cellular vesicles, as the product of activity of certain of the connective-tissue corpuscles[1].

The nervous system of the *Anodonta* consists mainly of

[1] Similar glycogenous cells are met with in the walls of the lacunar spaces and on the 'mesenteries' of the Snail.

three pairs of yellow ganglia; the *cephalic*, situated at the sides of the mouth; the *pedal*, placed in the foot; and the *parieto-splanchnic*, on the under face of the posterior adductor muscle. They are united by commissural cords which connect the cephalic ganglia with one another, and with the pedal and parieto-splanchnic ganglia, respectively. Traces of a small fourth pair of ganglia may be obvious on the cerebro-splanchnic commissures, near the anterior end of the pericardium. As the parieto-splanchnic ganglia are immediately connected with a patch of sensiferous epithelium in the roof of the inhalent siphon, they are sometimes regarded as *olfactory*. The only other sense organs which have been discovered, are a pair of auditory vesicles, connected by nervous cords with the pedal ganglia.

The sexes are distinct. The *testes* and *ovaria* are similar in character, being racemose glands, which, in the breeding season, occupy a great part of the interior of the body. There is one gland on each side, opening by a minute aperture close to that of the organ of Bojanus.

The spermatozoa have minute, short, rod-like bodies, to which a long, filamentous, active *cilium* is attached; they are thrown off in enormous numbers, and make their way out with the exhalent currents.

The ova are spherical, and the vitelline membrane is deficient at one point, leaving a terminal aperture or *micropyle*, through which, in all probability, the spermatozoon makes its entrance. When fully formed, multitudes of these ova pass out of the oviducal apertures and become lodged in the chambers of the gills, particularly the external ones, which during autumn and winter are completely distended by them and the embryos to which they give rise.

Segmentation is holoblastic, and the early developmental phases resemble, in their main features, those already de-

scribed for the Snail—the original bilateral symmetry is however never disturbed.

The embryos when hatched, are so wholly unlike the parent *Anodonta*, that they were formerly thought to be parasites, and received the name of *Glochidium*. Each is provided with a bivalve shell, and each valve has the form of an equilateral triangle united by its base with its fellow, by means of an elastic hinge, which tends to keep the two wide open. The apex of the triangle is sharply incurved, and is produced into a strong serrated tooth, so that when the valves approach, these teeth are directed towards one another. The mantle is very thin, and the inner surface of each of its lobes presents three papillæ, terminated by fine pencils of hair-like filaments. The oral aperture is wide, and its margins are richly ciliated. There is a single adductor muscle and a rudimentary foot, from which one or two long structureless filaments, representing the *byssus* of the sea-mussel, proceed. These byssal filaments become entangled with one another and tend to keep the 'Glochidia' in their places.

The gill-laminæ of *Anodonta* will accommodate some three millions or more of these *Glochidia*. If the animal is living in company with fish it will eject them, whereupon they attach themselves to floating bodies—very commonly to the tails, fins or gills, of fishes—by digging the incurved points of their valves into the integument in the latter case, and holding on by them as if they were pincers. In this situation they become encysted in an epidermal overgrowth of the host's body, within which they undergo a final metamorphosis.

The alimentary canal and foot become more marked with the increased development of the whole body; the gills appear, in the form of filamentous outgrowths of the body-

wall, which become plumose and subsequently unite to form the adult gill-lamellæ; the byssus is thrown off, and the larval shell and adductor muscle are replaced by newly-formed valves provided with two adductors. The young *Anodonta* at length drops off and falls into its ordinary habitation in the mud, a bilaterally symmetrical animal, differing from its parent only in the absence of functional reproductive organs.

LABORATORY WORK.

The contraction of the foot and consequent displacement of the viscera, resultant upon death under chloroform, may be overcome by killing the animal in water sufficient to cover it—heat slowly to about 40° C.

In an animal freshly removed from the water only the shell or *exoskelcton* is visible, but if killed as above directed this will be slightly open, the foot will be protruded as in life and the edge of the membrane lining it (the *mantle*) will be visible. Raise one valve of the shell, by separating the mantle from it with the handle of a scalpel, and then cut through two strong bodies (*the adductor muscles*), one at each end of the animal, which pass from valve to valve and prevent their separation. The two valves will now be united only by their *ligament*, as the result of whose elasticity they gape; cut through this, thus liberating one valve and work over—

A. **General external characters.**

1. In the animal now laid bare may be distinguished—

   a. A *dorsal border* turned towards the hinge of the

shell, and nearly straight. A curved *ventral border*, opposite the dorsal.

b. A wider *anterior end.* A narrower *posterior end.*

c. A *right and left side.*

2. Pin the animal down under water without removing its remaining valve (insert the pins obliquely, so that they embrace, and exercise a downward pressure upon, the edges of the valve). Examine in order—

a. The *mantle* or *pallium;* a bilobed semitransparent membrane, one lobe lining each valve of the shell. Examine its ventral border, it is yellowish in colour and thickened to form a *pallial muscle.*

b. The *adductor muscles* of the valves; two immense masses whose cut edges are visible at opposite ends of the body.

c. The *retractor pedis* muscles; smaller masses, one at the upper end of each adductor. The anterior one is posterior, and the posterior one anterior, to its corresponding adductor.

d. The *protractor pedis* muscle; uniform in diameter with *c.* It lies a little below the anterior retractor.

e. The *lesser retractors;* insignificant bundles of fibres arising from the extreme dorsal surface of the body wall.

3. Turn back the parallel lobe, and note underlying it—

a. The ventral portion of the *body.* It projects forwards and downwards, immediately behind the anterior adductor muscle: it is thickened ventrally

to form the locomotor *foot;* a large, yellowish, somewhat ploughshare-shaped mass, whose apex projects freely between the mantle-lobes.

b. *The gills or branchiæ;* two lamellar organs on each side of the body, extending to its posterior end.

c. *The labial palps;* a pair of membranous folds on each side, in front of the gills and immediately below the protractor pedis muscle. Note the structural similarity between them and the gills.

B. **The pallial-lobes, in relation to adjacent structures and the exterior. The branchial chambers and siphons.**

Remove the animal completely from its shell, by detaching the other mantle-lobe from the valve to which it is fixed and cutting through the attachments of the muscles to the same.

Pin down under water (the pins should preferably be thrust through the adductor muscles). Raise the pallial lobe nearest you with forceps and remove it, cutting along its line of confluence with the underlying organs and the body wall. Examine carefully—

a. The *infra-branchial chamber;* bounded above and externally by the pallial lobe. *a*—*c* of Section A. 3 lie within it.

Follow the cut edge of the mantle, working from behind forwards. It courses along the dorsal border of the external gill lamella, whence it passes downwards and backwards to reach the labial palps; it skirts these in a similar manner, and is continued on beneath the anterior adductor muscle.

b. The *mouth;* a wide aperture, lying immediately behind and below the anterior adductor muscle. It is encircled by the labial palps.

c. Examine the thickened posterior border of the pallial lobe. It is prolonged backwards for a short distance as a pigmented tentacle-bearing lip —the wall of the *ventral siphon.* Compare the living animal, as it lies with its anterior end buried in the sand, and note—

α. The projection of the ventral siphon beyond the free edges of the valves.

β. The close apposition of the thickened ventral margins of the pallial lobes.

γ. The *siphonal tentacles.* A series of small contractile finger-like lobes, the larger of which project freely across the siphonal passage in the path of the insetting current.

Touch one of them with a bristle and note the immediate closure of the siphonal aperture —the tentacles are highly sensitive. Examine one of the longer ones under a lens; its base is swollen and it terminates in a slightly expanded and flattened extremity.

δ. Examine the relations of the gills. The outer one is confluent with the pallial lobe externally and with the inner gill internally, for its whole length: the inner one is confluent with the body wall for a short distance in front and with its fellow of the opposite side behind; there is a spacious cleft between it and the body wall

d. Insert the point of a scissors into the pallial lobe immediately above the gill laminæ, and make an incision which shall pass under the posterior adductor muscle and immediately over the line of attachment of the outer gill-lamina. A large opening will be found in the dorsal middle line, in the region of the posterior adductor muscle; insert the scissors blade into this and cut obliquely backwards. There will now be laid bare the *supra-branchial or cloacal chamber.*

α. Follow its boundaries. It ends blindly in front, immediately above the most anterior point of attachment of the gills. It opens posteriorly by two orifices; a dorsal median one referred to above, and a posterior one, whose lips are produced into a short smooth-walled *dorsal siphon.* It is walled in dorsally and externally by the pallial lobes and ventrally by the gill-laminæ.

β. The *suspensory ligament* of the gills can now be seen; it is a membranous fold arising immediately beneath the posterior adductor muscle. Insert a seeker from below into the supra-branchial chamber, through the cleft between the body wall and inner gill. It passes internally to this ligament, which therefore subdivides the greater part of the supra-branchial chamber into two portions, each of which is in open communication with one gill-cavity.

γ. Remove the anterior portion of *β* and note, opening into the inner subdivision of the supra-branchial chamber, the *genital and excretory*

orifices; two small apertures lying one above the other and embraced postero-ventrally by a common lip. (For further details see Sects. E. and L. *a.* ζ.).

δ. The *rectum;* a yellowish thin-walled tube coursing over the hind face of the posterior adductor muscle. It opens by a terminal wide-mouthed anus.

Introduce into the immediate vicinity of the ventral siphon of the living animal some finely divided colouring matter. It will be drawn into this with the inhalent current induced by the ciliary action at work, and ejected, a little later, through the dorsal siphon with the exhalent one. (Cf. pp. 308—9).

ε. Note the position and general relations of the *pericardial cavity*. It lies in the middle line immediately above the supra-branchial chamber, wholly in front of the posterior adductor muscle. Examine in relation to it

α. the *organ of Keber* (*pericardial gland*); a red-brown thickening of the adjacent body-wall and pallial-lobe; it is most conspicuous anteriorly, above the gills.

β. Lay open the cavity of the pericardium and note the *rectum;* a yellowish tube, passing through it on its way to the supra-branchial chamber. It is surrounded at its middle by the yellow thick-walled ventricle. (For further details see Sects. F. and L.)

Keber's organ can be advantageously seen at this stage, if looked at from within

C. **The alimentary organs.**

1. Remove the valve, mantle-lobe, gills and labial-palps of the left side, and open up the pericardium.

   Pin down under water (preferably without removing the right valve) and dissect away the body-wall; the coils of the intestine will come into view, two which lie parallel to one another at the posterior end of the body, being probably those first seen. Note, lying within the body—

   a. The *digestive gland;* a large greenish-brown mass, filling the interspace between the anterior adductor muscle and the pericardium.

   b. The *reproductive gland;* a yellowish-white mass lying around the coils of the intestine, and filling the greater portion of the body-cavity.

2. Remove one half of the digestive gland piecemeal and pick away the reproductive gland, until as much as possible of the course of the intestine is exposed. Work out the whole alimentary tube in detail, commencing at the mouth; examine in order—

   a. The *gullet;* a spacious tube lying immediately behind the anterior adductor muscle; it runs upwards and backwards to enter

   b. the *stomach*, a large irregular sac lying immediately in front of the pericardium.

      Both *a.* and *b.* are buried up in the substance of the digestive gland; the ducts of this will be seen opening into *b.* by a series of very large orifices. Examine the relationship of one or more of these to the lobes of the gland itself. Cf. Sec. L. *d.*

   c. The *intestine.* It arises by a wide aperture from the floor of the stomach; insert a scissors blade

into it and lay bare its entire lumen—its course is as follows. It first passes to the left side downwards and backwards, and, on nearing the hinder end of the body, turns upwards towards the pericardium; it then bends sharply upon itself and passes downwards and backwards, in a line with the free posterior edge of the body-wall. On reaching the middle ventral region it is again bent sharply upon itself, passing upwards and backwards altogether to the right side; at about the middle of the reproductive gland it becomes suddenly greatly enlarged, and passes (parallel with the first segment) upwards and forwards towards the anterior end of the pericardium. It enters this at its base, and, running upwards and backwards, leaves it postero-dorsally, finally passing over the posterior adductor muscle. (Cf. Sect. B. *d.* δ.)

d. Having laid bare the interior of the intestine, reduce its walls to the condition of median longitudinal section and examine the intestinal valve or *typhlosole;* a thick rich yellow ingrowth of its lining membrane. It is very conspicuous in the first and the enlarged terminal segments.

e The *labial-palps, in relation to the mouth.* Obtain an undissected specimen, and, after removal from the shell, cut away the anterior end of the body, to the level of the labial-palps; pin down and examine from beneath. The aperture of the mouth is oval. The labial-palps diminish in size as the mouth is reached and they embrace it in a lip-like fashion; they are confluent on either side and their free

edges enclose a *ciliated groove* which leads directly to the oral aperture.

f. Scrape off a little of the *epithelium* of the intestine; mount in eosin and examine under a high power. Note the presence of cilia.

> Should difficulty be experienced in following the coils of the intestine, recourse may be had to injection. Mix equal parts, by bulk, of plaster of Paris and water, stir well and strain through fine muslin; inject with a small syringe *per anum*.

D. **The gills or branchiæ.**

a. Cut out one of the gills and examine it under water; it will be found to consist of two lamellæ united by their ventral edges and enclosing a central cavity, which opens into the epibranchial chamber above. The gill-cavity is subdivided by partitions, which pass from one lamella to the other, and carry the larger blood-vessels of the respiratory plexus.

b. Carefully isolate a small piece of one lamina; mount in water and examine with 1 inch obj. It will be seen to be traversed by a great number of small perforations, whose walls are supported laterally by short chitinous rods; the substance of the gill is permeated by a meshwork of large vessels.

c. Examine under a higher power: the margins of each cleft are lined by a ciliated epithelium.

E. **The excretory organ (organ of Bojanus).**

Remove the animal from its shell and pin down through the adductor muscles. Dissect off the pallial

lobe as directed for Sect. B.; lay open the pericardial chamber and remove the auricle. The excretory organ will now be visible as a blackish mass underlying the floor of the pericardium; it becomes more marked posteriorly, and extends behind the pericardium, terminating immediately in front of the posterior adductor muscle.

Remove the anterior two-thirds of the outer gill-lamina leaving its cut edge, and then dissect away carefully a portion of the anterior third of the outer wall of the inner lamina; there will thus be laid bare

a. The *excretory orifice* (renal aperture); a small pore situated immediately below and behind the anterior end of the pericardium; it opens into the supra-branchial chamber, immediately above the generative orifice. (Cf. Sect. B. *d*. γ.) Insert a seeker into it and wash the whole under a gentle current until quite clean; the seeker will be found to project into

b. the *excretory vestibule* (non-glandular portion of Bojanus); a thin-walled *muscular* sac, extending back to the hinder end of the pericardium. Open it up and gently raise its cut edge; at its extreme anterior end it will be seen to be in communication with its fellow of the opposite side, by an immense oval *inter-renal aperture*.

c. Underlying *b*. and visible through its thin wall, there will now be seen the *glandular segment* of the organ. Open up the posterior portion of this, it is tubular and its walls are plicated.

Examine the cut edges of the whole with care; note the transition from the glandular to the mus-

cular segments, as seen in the cut edge of the roof of the hinder portion of *b*.

d. Follow the glandular segment forwards; it becomes constricted anteriorly (in the region of the inter-renal aperture) to give rise to the *thin-walled segment;* a short whitish tube, which opens into the extreme anterior end of the pericardial chamber by a small valvular *reno-pericardial aperture*. (Cf. Sect. F. 2. *a*.)

F. **The heart.**

1. Dissect an *Anodonta* from its shell, and remove the whole ventral portion of the body, well below the pericardium. Pin the upper portion down under water dorsal surface uppermost, and carefully lay bare the pericardial cavity.

The heart will now be exposed; it is a yellowish transparent sac, probably exhibiting regular contractions, composed of a median and two lateral chambers, these are

a. the *ventricle;* a median pear-shaped body, its thick end directed backwards; it embraces the hind segment of the alimentary canal. All parts of the wall of the ventricle do not contract together; but a wave of contraction passes, from one end of it to the other, like the peristaltic contraction of the intestine in one of the higher animals.

b. *The auricles;* one of these will be seen on each side, if the ventricle be gently pushed out of the way: each is a somewhat pyramidal sac, continuous with the ventricle at the apex of the pyramid.

c. Remove the dorsal wall of the ventricle and of a portion of one auricle. Note the thick spongy

nature of the former: at its point of communication with the auricle will be seen the *auriculo-ventricular valves;* long pocket-shaped flaps, so disposed as to admit only of a flow of the blood from the auricle to the ventricle.

2. Remove all but the bases of the auricles, and cut the rectum across at its extreme anterior end; turn it and the heart backwards, so as to lay bare the floor of the pericardium. Running along the middle line of this will be seen a large blood-sinus, the *vena cava:* it lies between the two *excretory-organs.*

a. At the extreme front end of the pericardiac floor, immediately under the point at which the intestine enters the cavity, will be found the *reno-pericardial apertures;* pass a bristle, or seeker, into one of them and open up the excretory vestibule of the same side. The glandular segment will now be seen—the seeker projecting into it. Examine the general relations of the excretory organs, and their apertures of communication with the supra-branchial chamber. (Cf. Sect. E.)

b. Examine the floor of one of the auricles under a lens, first having washed the specimen quite clean. Note the large orifices of the *efferent branchial vessels* which open into it.

c. The *organ of Keber.* Follow its cut edge; it embraces the antero-lateral region of the pericardium. (Cf. Sect. B. *c. a.*)

G. **The circulatory system.**

1. The *arterial system.* Lay bare the pericardial cavity and insert the point of a medicine-dropper, filled with

injecting material, into the ventricle. Inject slowly, and dissect under water, right side uppermost. Remove the gills of the exposed side, together with the hinder three-fourths of the pallial-lobe; leave the labial-palps, but remove the entire body-wall. Work from the ventricle and follow in order—

a. The *anterior aorta;* a spacious trunk embracing the rectum dorso-laterally. It runs across the digestive-gland to the right side, distributing branches to it as it does so, and enters the body immediately behind the anterior retractor muscle. Pick away the digestive and reproductive glands, and follow its course; it subdivides into visceral and pedal branches, asunder.

α. The *visceral artery.* Its main branch accompanies the first coil of the intestine; it distributes blood to the alimentary and reproductive organs.

β. The *pedal artery.* Follow its further course; its main trunk skirts the upper surface of the foot, and distributes a number of branches to the substance of the same. Soon after leaving the anterior aorta, it gives off a *labial* branch to the palps, and a *pallial* one to the anterior adductor muscle and pallial-lobe.

b. The *posterior aorta;* a much shorter trunk than *a.* It passes beneath the rectum and is mainly distributed to the posterior adductor muscle, the pallial lobes and body-wall.

The two pallial arteries of either side anastomose within the substance of the thickened border of the mantle-lobe.

2. Make a perforation in the roof of the ventricle, and pass the injecting apparatus through it into one of the auriculo-ventricular apertures; inject under a steady pressure, wash and examine the undissected animal under water. There will be seen—

a. The *pallial sinuses;* a series of irregular channels permeating the substance of the pallial lobe.

b. The *efferent pallial vessel;* a circular trunk, running along the upper surface of the thickened mantle-edge; it receives branches from the pallium at all points.

Trace it upwards—it follows the line of attachment of the pallial-lobe to the underlying organs (see Sect. B.); it is coincident, along the line of attachment of the gills, with the efferent-branchial sinus (*d.*).

c. Remove the greater portion of the pallial-lobe, and examine the *efferent-branchial vessels*—a series of short parallel trunks, lying in the walls of the gill-lamellæ; each is formed by the confluence of lesser vessels, coming in from the gill substance. They open into

d. the *efferent branchial sinus*, a spacious chamber overlying the gills; in the pericardial region it becomes expanded to form the auricle (Cf. Sect. F. 2. *b.*).

3. Remove the rest of the mantle-lobe and open up the underlying external gill-lamina, along its line of junction with the same. There will be seen running along the top of the gill, at the base of the suspensory ligament (Sect. B. *d.* β.), the *afferent-branchial sinus*—a well-defined longitudinal blood-space; make a perforation in this and inject,

backwardly and forwardly. Wash carefully, and remove a good portion of the outer wall of the external lamina; there will be seen

a. the *afferent branchial vessels*, a series of short parallel trunks, conveying the blood from the above-named sinus to the gills.

Examine the afferent branchial sinus and note that the injection has passed from it into the ventro-external portion of the excretory organ, having filled the *efferent renal vessels*.

b. Open up the pericardium, and remove the auricle, thereupon exposed. Make a small hole in the middle of the *vena cava* (Sect. F. 2) and inject both backwardly and forwardly, exercising a gentle pressure. Wash carefully, and note that the excretory organ is now completely injected, the colouring matter having passed from the vena cava into the *afferent renal vessels*.

c. Remove the anterior half of the gill-laminæ, and dissect to the level of the *vena cava*. Follow this into the body; it emerges immediately behind the rectum, and is seen to be formed by the confluence of a number of venous channels—some of the more important of which accompany the intestinal coils. Follow it backwards; it can be traced to the under surface of the posterior adductor muscle.

The vena cava can be dissected from above, as directed in Sect. F, with comparative ease, in an uninjected specimen; its lumen will admit of the passage of a seeker.

H. **The pallial lobe.**

a. Tease up a portion of the substance of the pallial

lobe of an animal which has been killed under chloroform. Conspicuous among the cells thus isolated there will be found large oval or rounded ones, each lodging a central refractive globule.

b. Remove the mantle-lobe from a mussel which has been killed as above and subsequently preserved in alcohol, and split it into two. Transfer a portion of one half to a glass slide, torn surface upwards, and treat with iodine solution; the whole will be studded with small brown spots. Examine under a high power; the spots are seen to be identical with the globules observed in *a.* (their chemical reactions are those of *glycogen*).

I. The nervous system.

1. Pin the animal down so as to get the body absolutely rigid; remove the mantle-lobe and gills of one side and slit open the organ of Bojanus.

Wash until quite clean and examine under water.

a. Find the *cerebro-splanchnic commissures;* two parallel white cords traversing the excretory organ. Trace the near one backwards; it passes round the posterior retractor tendon towards the under side of the posterior adductor muscle. Turn this latter over, so as the better to display its under surface, and note

b. *the parieto-splanchnic ganglia;* two elongated yellow masses confluent in the middle line, seen on removing the membranous investment from the ventral surface of the above-named muscle. They distribute branches to the same, to the gills and pallial lobe.

c. Carefully snip away the membranous tissue at the base of the labial palps; there will thus be laid bare the *cerebral ganglia*.

Each is about the size of a pin's head, and somewhat triangular in form; it lies immediately in front of the protractor pedis tendon, and distributes fibres to the anterior adductor muscle, labial-palps and pallial lobe. Examine, in connection with the one exposed

α. the *inter-cerebral commissure*.

A short cord uniting the two cerebral ganglia across the middle-line, above the gullet.

β. The further course of the *cerebro-splanchnic commissure*.

Dissect off the fibrous tissue between the anterior retractor and protractor tendons, and remove the body-wall, between these and the anterior end of the pericardium. The commissure will be exposed, on carefully picking away the superficial portions of the digestive and reproductive glands along the same line.

d. Remove a small portion of the body-wall along the point of origin of the muscular foot, and pick away the genital gland until the *pedal ganglia* are reached; they are a pair of deep-orange-coloured oval bodies, each rather larger than a big pin's head, applied to one another in the middle line, near the point of junction between the body and the muscular foot.

Branches are given off to the muscles of the foot and to the auditory organ. See *infra*.

e. The *cerebro-pedal commissure;* a cord which runs

upwards and forwards, from the pedal to the cerebral ganglion of either side.

(Cf. Sect. I. *f*.)

The *cerebro-splanchnic commissures* run close together and parallel with each other, in their course from behind forwards; on leaving the pericardial area, they suddenly diverge and pass downwards and forwards to meet the cerebral ganglia. If one of the commissures be removed and examined under a low power, there may occasionally be found, at the point of divergence, an enlargement (*visceral ganglion*).

J. **The auditory organ.**

a. This is rather difficult to dissect out in *Anodon:* it is a small sac which may be found by tracing back the posterior cords given off from the pedal ganglion, to a branch of one of which it is attached. There is a vesicle connected with each pedal ganglion.

Failing the above, the *otocyst* can best be found by removing a portion of the reproductive gland lying immediately behind the pedal ganglion, and examining under a low power.

b. If a fresh *Cyclas*[1] be obtained, and its foot removed, mounted in water, and examined with 1 inch obj., the auditory sac can readily be seen with a constantly-trembling particle, the *otolith*, in it.

K. **The shell or exoskeleton.**

a. Its two hardened lateral pieces or *valves;* each with a straight dorsal and a curved ventral edge,

[1] *Cyclas cornea*.—a small fresh-water lamellibranchiate mollusk.

and an anterior larger and posterior smaller end, the latter compressed dorso-laterally—note the soft chitinous ventral edge of each valve.

b. The *umbo;* a small blunt eminence on the dorsal border of each valve near its anterior end; its apex is directed forwards.

Note the delicate texture of this area.

c. The *ligament;* an elastic uncalcified part of the exoskeleton behind the umbones, uniting the two valves and tending to divaricate their ventral edges.

d. *External markings.* The outside of the shell is greenish brown, and on it are seen a number of concentric *lines of growth*, running parallel to the margin of the shell and more numerous towards its ventral edge.

e. Detach one valve by cutting through the ligament with a scissors; before removing the animal examine under water, and note the relations of the *chitinous ventral edge* (*a.*). It extends along the straight dorsal border of the valve in front of the ligament; and from that point in front, as from the posterior edge of the ligament behind, it is sharply inflected—passing inwardly to be reflected on to the free border of the pallium. (Cf. Sect. L. *a.* γ.)

f. *Internal markings.* The interior of the valve is white and iridescent: on it are seen, near the dorsal border, two oval scars, the *anterior* and *posterior adductor impressions.*

Joining the two adductor impressions is a double curved line, the *pallial impression*, which marks the point of attachment of the pallial muscle.

In front of the posterior adductor impression, is seen a small scar of attachment of the *posterior retractor* muscle.

Behind the anterior adductor impression are two others, one opposite its upper, the other opposite its lower end: the former indicates the point of attachment of *anterior retractor*, the latter of the *protractor pedis* muscle.

The adductor and retractor scars are not unfrequently confluent, and there extends from each into the umbo a fainter tapering impression, indicative of the growth of the muscles themselves as the animal has increased in size.

g. Prepare (as directed for the Crayfish on p. 203) a dried section across the middle of one valve, cut at right angles to its long axis. Mount in Canada balsam and examine under a low power, note—

α. The *epiostracum;* a thin uncalcified superficial layer; it is greenish-yellow in colour, and very frequently torn away in the process of grinding.

β. The *prismatic layer;* composed of elongated columns of calcified substance running parallel with each other.

γ. The *nacreous or pearly layer;* immediately internal to *β*. Its relative thickness is proportionate to the age of the animal from which the valve was taken.

Examine under a high power—it is finely granulated and traversed by delicate longitudinal striæ.

δ. Make a tangential section across the prismatic layer and examine under a low power. The

prisms are mostly hexagonal or pentagonal in contour; transverse diameter variable, often in proportion as the section passes through their inner ends.

ϵ. Dissolve out the earthy matter from a piece of a valve, by treatment with weak hydrochloric acid. An *organic basis* remains behind, conformable in shape to the fully formed structure.

I. **The study of transverse sections.**

Remove one valve from an animal which has been dead some 6—8 hours, and make sections as directed below—cutting through the soft parts with a razor or sharp scalpel and through the remaining valve with a bone-forceps. Examine under water, and if some of the more minute parts to which attention is directed do not fall in the plane of section, dissect until they are reached.

Sections *a*, *b*, *c*, *e*, and *f* to be transverse to the long axis of the body, *d* to be oblique.

a. Through the anterior pericardial region, immediately behind the excretory and genital orifices. Work over—

α. The *body;* median, laterally compressed below. Note the muscular nature of the *body-wall;* it is thickened ventrally to form the locomotor foot—above the gills it is expanded dorso-laterally and modified to form the *organ of Keber.*

β. The *pallial lobes;* membranous outgrowths of the thickened dorsal region of the body-wall, they are in close apposition with the valves. Note the thickening of their free borders to form the *pallial muscles.*

γ. The *exoskeleton.* A continuous investment for the exposed surfaces of the mantle. It consists of a pair of *calcareous valves*, which pass—dorsally into a *cornified ligament*—ventrally into a *chitinous free-border*, which is inflected on to the edges of the pallial muscles.

δ. The *branchiæ.* Their *descending laminæ;* that of the outer gill confluent with the pallium, that of the inner gill in part confluent with the body wall and with its fellow of the opposite side: their *reflected laminæ:* confluent along their line of mutual apposition. Note, in relation to the latter and to the supra-branchial chamber, the *suspensory ligament* of the gills. (Cf. Sect. B. *c*, δ, and *d*, *β*.)

ε. The *body-cavity.* This is almost obliterated; note the large *pericardial chamber*, situated in the dorsal middle line.

ζ. The *viscera.* The cut edges of the *intestine* will be seen, lying within the body and traversing the pericardial chamber.

The *excretory organ;* median and paired, lying immediately beneath the pericardial chamber. The glandular segments of opposite sides are seen to be approximated in the middle line; note the *reno-pericardial apertures* lying immediately above and internal to the attachments of the inner gill lamellæ. The muscular segments are here confluent in the middle line, enclosing a spacious cavity; dissect to the level of the *excretory orifice* of one side, and note that it is a perforation of the floor of this segment, opening

into the supra-branchial chamber, internally to the suspensory ligament of the gills.

The *cerebro-splanchnic nerve commissures* will be found, closely applied to the ventro-internal walls of the glandular segments of the above. The *vena cava* here lies in the middle line immediately below them.

The *reproductive gland;* a yellowish mass, filling up all the available space in the infra-branchial portion of the body-cavity.

Dissect it away on one side to near the level of the excretory orifice; there will thus be laid bare the *genital duct.* Insert a bristle into this and open it up; it communicates with the supra-branchial chamber immediately beneath and internal to the excretory orifice (Cf. Sect. B. *d*, γ). Follow the duct down and note the orifices of the smaller ducts which open into it, and by whose confluence it is formed.

A good notion of the ramifying nature of this duct may be obtained by means of injection.

η. Pick away the reproductive gland and note the *transverse muscles;* delicate tracts of tissue passing between the body-wall of opposite sides.

Examination of the above section will render it clear that the pallial lobes are paired outgrowths of the dorso-lateral portion of the body-wall. The cavity which they bound during life, is sub-divided—as the result of the confluence and attachments of the gills—into infra and supra branchial chambers; the latter being partitioned off into two portions, the inner of which receives the excretory and genital ducts.

b. Through the middle pericardial region, across the heart and auriculo-ventricular valves. Compare generally with *a*, and work over, in addition.

α. The *gill-lamina; the inner one is free internally, the supra and infra-branchial chambers being thus in open communication.*

β. The *excretory organ.* Note the increase in calibre of the glandular segment, as its posterior end is neared; the *vena cava* is here interposed between the muscular segments of opposite sides.

The *cerebro-splanchnic commissures* lie close together beneath the vena cava. Cf. *a. ζ.*

γ. The *pericardial chamber and heart.* The former is here at its maximum of development. Observe the fleshy wall of the ventricle and the thin auricles; the *auriculo-ventricular valves* are pocket-shaped in section, their mouths being directed towards the ventricle.

Note the course of the rectum; it traverses the ventricle, but is in no way bound down to it.

c. Through the middle of the posterior-adductor muscle.

α. The attachments of the *adductor muscle;* it traverses the body and pallial lobes, and is inserted directly into the substance of the valves of opposite sides.

β. The *rectum;* lying in the dorsal middle line immediately above the adductor muscle.

γ. The *supra-branchial chamber;* completely shut off, by the confluence and attachments of the gills, from communication with the infra-bran-

chial one, except through the gill-slits. Its dorsal prolongation (cf. Sect. B. *d*), seen, in section, as a small cavity overlying the rectum.

δ. The *parieto-splanchnic ganglia;* two yellow bodies confluent in the midddle line, buried up in a connective tissue sheath, immediately beneath the posterior adductor muscle.

d Obliquely backwards, through the stomach and first coil of the intestine. Examine from behind.

α. The *stomach;* a spacious sac, passing into the intestine below. The orifice of the gullet is seen as a wide transverse aperture, situated near its roof. Immediately below this there is a corresponding depression, which receives the *main ducts of the digestive gland*—two enormous tubes admitting the seeker with ease; note the presence of a series of lesser ducts.

β. The *intestine;* passing straight down in the middle line. Note the *typhlosole;* a median ingrowth of the lining membrane of its front wall.

γ. The *labial palps*. These, like the gills, are confluent—externally with the pallial-lobes, internally with each other and the body wall; their free edges enclose a ventral *ciliated groove*.

e. Through the anterior adductor muscle, immediately in front of the mouth.

α. The *adductor muscle*. Cf. *c*. *α*.

β. The *mouth;* median, transversely elongated. It lies immediately beneath the adductor muscle, and is embraced on all sides by the labial palps, which are confluent round it. Note that they

are so disposed that the *ciliated groove* (cf. *supra*) leads directly to the mouth aperture.

γ. The *foot;* a fleshy mass, projecting forwardly, immediately below the mouth.

f. Dissect away the adductor muscle and labial palps on one side, and note the relations of the *retractor and protractor pedis muscles.* Their fibres are largely derived from the muscular layer of the body-wall; follow them to their attachments. (Cf. Sect. A.)

Find the *cerebral ganglion* of the same side (see Sect. I. *c*) and trace the course of the *inter-cerebral commissure;* it runs over the gullet, in the substance of the labial-palps. Follow in like manner the *cerebro-pedal commissures,* until the *pedal ganglia* are reached; note that these—like the parieto-splanchnic ones—are confluent in the middle line.

M. **The reproductive elements and the larva.**

a. The animals are diœcious, but the reproductive organs are similarly constructed in both sexes: they vary much in size with the season, being large in winter and spring, but small at other times.

b. Tease up a small portion of the ovary in eosin solution, and examine under a high power. The ripe ova are large, rounded, rarely ovoidal bodies, and their special features are—

α. The *vitelline membrane;* usually separated from the ovum by a cavity filled with a coagulate albuminous fluid.

β. The *micropyle;* a perforation, or frequently a short neck-shaped prolongation of *a.*

Look for specimens in which the ovum is prolonged into a stalk passing through the above —the remnant of an original pedicle of attachment to the germinal epithelium.

γ. The *germinal vesicle;* very large and transparent; it is rounded and usually contains two *germinal spots*—a larger and a smaller one in close apposition.

c. The *Glochidium larva.* If the outer gill appear to be thick and distended, it will be found full of the above. Note, while living, the characters of their shells, the entangled *byssus* filaments, with which they are provided, and the spasmodic contractions of the adductor muscle.

Preserve a portion of the larva-laden gill (taken from an animal killed under chloroform) in spirit; when well hardened stain with magenta and examine under a low power. Look for individuals whose valves gape, and note—

α. The *larval exoskeleton;* composed of two transparent valves, united dorsally by an elastic hinge.

β. The *shell-teeth;* formed on either side as a spur-like inflection of *a.* Each terminates in a sharp upwardly directed spike, and its exposed surface is produced into a number of parallel serrated ridges.

γ. The *adductor muscle;* a powerful transverse band passing between the upper parts of the two valves.

δ. The *mantle.* This consists, at this stage, of very large cells which project freely into the enclosed

cavity. Three sets of bristle-like appendages are developed in connection therewith; an upper one—passing obliquely downwards immediately beneath the adductor muscle, and two shorter ones passing upwards, immediately above the shell-teeth.

c. The *byssus;* an elongated, transparent, much tangled filament, projecting beyond the free edges of the valves.

d. Examine a similar larva from the side. The valves are quite transparent, and there can be seen through them

α. the *adductor muscle;* appearing as a median dark mass.

β. The *visceral mass;* a somewhat less conspicuous mass lying in one of the top corners, altogether to one side.

γ. The *byssus organ;* a conspicuous transparent tube, arising from β. and coiled upon itself. It is unpaired, being present on one side of the body only.

δ. Note the shape of the shelly *valve;* it tapers off below to form the shell-tooth. Focus to the level of this, and examine its serrations. The valve is seen to be dotted all over by minute perforations—*pore-canals.*

VI.

THE FRESH-WATER POLYPES (*Hydra viridis and H. fusca*).

If a waterweed, such as duckweed, from a pond, is placed in a glass and allowed to remain undisturbed for a short time, minute gelatinous-looking bodies of a brownish or green colour may frequently be found attached to it, or to the sides of the glass. They have a length of from $\frac{1}{4}$ to $\frac{1}{2}$ of an inch, rarely more, and are cylindrical or slightly conical in form. From the free end delicate filaments, which are often much longer than the body, proceed and spread out with a more or less downward curve, in the water. These threads, which are the *tentacles*, may vary in number[1]; if touched they rapidly shorten and together with the body shrink into a rounded mass. After a while, the contracted body and the tentacles elongate and resume their previous form. These are *Polypes*, the brown ones belonging to the species usually termed *Hydra fusca*, the green to that called *H. viridis*. The polypes generally remain attached to one spot for a long time, but they are capable of crawling about by a motion similar to that of the looping caterpillar; and, sometimes, they detach themselves and float passively in the water.

[1] In *H. Hexactinella*, an Australian species, their number is reported to be invariably six.

When any small animal, such as a water-flea, swimming through the water comes in contact with the tentacles, it is grasped, and conveyed by their contraction to the aperture of the wide mouth, which is situated on the summit of a cone (*hypostome*) in the middle of the circle formed by the bases of the tentacles. It is then taken into a cavity which occupies the whole interior of the body; the nutritive matters which it contains are dissolved out and absorbed by the substance of the *Hydra;* and the innutritious residuum is eventually cast out by the way it entered. Small pieces of meat, brought within reach of the tentacles, are seized, swallowed and digested in the same manner.

If a *Hydra* is well fed, bud-like projections make their appearance upon the outer surface of the body. These gradually elongate and become pear-shaped. At the free end a mouth appears; and around it minute processes are developed and grow into tentacles; and thus a young *Hydra* is formed by gemmation from the parent. This young *Hydra* becomes detached sooner or later, and leads an independent existence; but, not unfrequently, new buds are developed from other parts of the parent before the first is detached, and the progeny may themselves begin to bud before they attain independence. In this manner, temporarily compound organisms may be formed. Experiments have shewn that these animals may be cut into halves or quarters and that each portion will repair its losses, and grow up into a perfect *Hydra;* and there is reason to believe that this process of fission sometimes occurs naturally.

The *Hydra* multiplies by budding through the greater part of the year; but in the summer sexual organs appear in the form of projections of the surface of the body. These, when ripe, may be resolved into a larger and a smaller set. The latter may appear on any part of the

body, but they are not unfrequently restricted to the free end at or near the bases of the tentacles. Within them (*testes*) great numbers of minute *spermatozoa*, each moved by a vibratile cilium, are developed and eventually set free.

The former are large globular bodies, from one to eight in number, usually formed near the attached end of the polype. Each becomes much larger than the testis, and is the *ovary*. Within it is developed a single large egg-cell, or *ovum*. This ovum, which is a huge nucleated cell, is impregnated by the spermatozoa and undergoes division into two parts. Each of these again divides into two; and so on, until the ovum is broken up into a number of small embryo-cells. The mass of embryo-cells thus formed becomes surrounded with a thick, usually tuberculated or spinous, case; and, detaching itself from the body, forms the 'egg,' from which a new Hydra is developed.

Microscopic examination shews that the body of the *Hydra* is a sac, the wall of which is composed of two membranes, a transparent outer (*ectoderm*), and a coloured inner (*endoderm*). The tentacles are tubular processes of the sac, and therefore are formed externally by the ectoderm and lined internally by the endoderm. Both the endoderm and the ectoderm are made up of nucleated cells; the inner ends of certain of these are prolonged into delicate fibres, those of the ectoderm, which are most marked, running parallel with the long axis of the body. The green colour of the *Hydra viridis*, long thought to result from the presence of chlorophyll grains imbedded in the protoplasm of the endoderm cells of the animal itself, is now satisfactorily proved to be due to the presence of microscopic vegetable organisms (algæ) capable of being cultivated and propagated after removal from the animal. They are referable to the genus *Chlorella* (? *Zoochlorella*); and during life a condition

of mutual inter-relationship between the plant and animal known as *symbiosis*, in which each utilizes certain waste products of the other, is probably maintained. There is some reason for thinking that the brown or orange-coloured particles predominant in the endoderm of the other species, and less rarely present in *H. viridis*, may be perhaps disintegrating Chlorelloids.

The cells of the ectoderm, and especially of that of the tentacles, contain very singular bodies,—the so-called *urticating capsules*, *thread cells*, or *nematocysts*. These are oval bags, with thick and elastic walls, containing a spirally coiled or looped filament which can be unrolled, presenting the appearance of a long filament attached to the capsule, and often provided with recurved spines near its base. When the nematocyst is ejected these spines or barbs lie in close apposition, with their pointed ends directed forwards and their bases under tension; on reaching the attacked prey they undergo a forcible displacement, fixing the body of the nematocyst and clearing the way for the introduction of the more delicate thread. These thread-cells appear to exert a noxious influence upon the animal's prey. Very rarely, nematocysts are to be found in individual cells of the endoderm; there is reason to believe that they are introduced with the captured prey, but argument from analogy to allied hydroids renders it probable that they may be developed *in situ*.

The larger endoderm cells of *Hydra* are throughout life amœboid, and the like is partly true of the ectoderm in at least the young state of one variety (*H. viridis var. Baker*). The *Hydra*, then, may be compared to an aggregate of *Amœbæ*, arranged in the form of a double walled sac with accompanying structural and functional differentiation.

The cavity of the body alone represents a stomach and

intestine; there are no organs of circulation, respiration or urinary secretion; the products of digestion are doubtless transmitted, by imbibition, from cell to cell, and those of the waste of the cells exuded directly into the surrounding water. While the *Hydra* has none of the special apparatuses which are termed glands, definite secretory cells are nevertheless present. Among the more important of these are those developed in the ectoderm of the foot which are utilized for purposes of adhesion, and the secretory cells of the endoderm, most numerous in the hypostome, which is eversible.

Nematocysts are generally, but not invariably, ejected, if any portion of the body which bears them be touched. Certain of the ectoderm cells, usually, if not always, lodging nematocysts, bear each a stiff filament or *cnidocil* such as can readily be seen in life projecting beyond the free surface of the tentacles. Continuity has been traced between these cells or *cnidoblasts* and certain small *nerve-cells* sparsely diffused in the deeper layer of the ectoderm; the whole constituting an elementary neuro-sensiferous apparatus, through the agency of which control of at least the cnidoblasts and their contained urticating capsules is exercised.

The fully formed *Hydra* may further be compared to those animals previously dealt with, at that stage in their development when the body consists of a double-walled sac (cf. especially the gastrula stage of the Snail and its representative in the Crayfish). The inner layer gives origin, in the latter, to the digestive epithelium and its appended glands; in *Hydra* it forms the digestive layer. The ectoderm is, in all, protective, and from it such neuro-sensiferous organs as are formed, exclusively arise. Between the applied surfaces of the ectoderm and endoderm there is interposed a gelatinous middle layer, which, under the

action of reagents, appears as a structureless membrane. This furnishes attachment for the cells themselves and support for the body generally; its optical characters in the prepared state are expressed in the term *supporting lamella*, its real nature more nearly in that of *mesogloea*.

It is possible that the longitudinal fibres connected with the cells of the ectoderm may be specially contractile, and represent muscles; but, however this may be, each cell has its own independent contractility. Similar fibres, disposed transversely, arise from the bases of the endoderm cells. These have been met with only in the body, and they appear to be in connection with the fibres of the ectoderm cells, by means of delicate fibrils which perforate the above-named mesogloea.

LABORATORY WORK.

1. Put into a beaker some water containing bodies to which Hydræ are attached, and place the beaker in a window not exposed to direct sunlight: in the course of some hours many Hydræ will be found attached to that side of the glass which is turned towards the light. Note their size, form, colour, mode of attachment and movements.

2. Transfer a Hydra, by means of a pipette, on to a slide with plenty of water. In order to avoid crushing, place the animal between two coverslips, so arranged as to support the edges of a third one which shall overlie it. Examine with a low power.

a. **Form.**

α. *The body proper;* cylindrical, varying much in length and diameter with the state of extension of the animal; its conical free end (*hypostome*) with an opening (*mouth*) at its summit.

It is often difficult to observe the mouth, especially in the green species. It may be readily seen however if a Hydra, placed in a drop of water without a coverslip, be watched under an inch objective, until it turns its anterior end up towards the observer. Occasionally, under these circumstances, the hypostome may be everted.

β. *The tentacles:* ranged round the mouth; their number and shape; their varying length and diameter; the knob-like eminences on them.

Look for developing tentacles, shorter and more rigid than the rest. Very rarely, one or more may branch.

γ. *The base* (so called *foot*): a flattened disc; narrower or wider than the body, according to the state of extension of the latter. It is functional as an organ of adhesion. If the animal be attached, its secretory product can often be seen, as a transparent laminated mass, interposed between the body and the surface of attachment; if the animal be free, the disintegrating remains of the same are often to be found adherent to the 'foot'; if it be forcibly detached, rupture of the base of the polype may ensue, the animal appearing to possess that which has been erroneously termed an 'anus'.

δ. *The buds;* young Hydræ, of various sizes and stages in development, attached to the sides of the parent. There may be one or more of them.

ε. The *sex-organs*, if present; colourless prominences, variable in number and position (cf. Sect. 8).

δ, ε may be one or more absent, or all may coexist, in individual specimens.

b. **Structure.**

α. The animal evidently composed of two layers, an outer transparent, *ectoderm*, and inner, *endoderm;* the latter alone containing chlorophyll in the green species, or such colouring matter as is present in the brown one. The ectoderm is marked out into areas, and may with care be seen to be composed of distinct cells, though this is a little difficult to make out in fresh specimens.

β. The *body-cavity;* least obvious in the green species, frequently visible in the brown ones as a darker central area with which the mouth-opening is continuous. Note that it extends into the tentacles; corpuscles can, with care, be seen floating within these, when extended.

c. **Movements.**

α. The general *contractility* of the animal; it is constantly either extending or shortening its body and tentacles, and so altering its form and place.

β. Its *irritability;* slight pressure or other stimulus immediately causes it to contract.

3. **The tentacles and nematocysts.** Attention has already been called to the knob-like eminences of the former. Note that these are widely separated in extension; closely applied in contraction. Examine one of them under a high power, with the tentacle fully extended.

a. The *cnidocils;* well defined stiff processes, each pointed and standing out from the free surface.

b. The *nematocysts* or *thread-cells;* highly refractive bodies embedded in the common mass. They can be resolved into

α. Smaller and more numerous ones; ovoidal, and situated almost invariably at the bases of the longer cnidocils.

β. Larger and less numerous ones (very often one only) lying near the middle of the mass; globular when seen *en face*, flask-shaped when looked at from the side.

c. Run in a drop of acetic acid magenta solution and watch, under a low power, the ejection of the threads of the nematocysts. Note from what parts of the body they are thrown out. Examine, under a high power, a portion of a tentacle beset by them. There will be found:

α. Short, deeply staining, stout threads, usually thrown into a spiral. Note that these are related to the smaller nematocysts.

β. Delicate whip-lash filaments, from 8 to 10 times the length of α. Trace them to their origin from the larger nematocysts.

d. Should opportunity offer, examine any small organism or other prey that may have been seized by the tentacles. When about to be swallowed it will be found to be studded by nematocysts, especially of the smaller kind.

4. **The asexual buds.** (Cf. Sect. 3 *a.* δ) Examine in relation to the parent, under a high power.

a. The *body-cavity;* in young buds continuous with that of the parent; in buds ripe for dehiscence completely constricted off therefrom. Note that the *mouth* is formed as a secondary perforation.

b. The *tentacles;* variable in number. A solitary one may appear first, the second and third being symmetrical with respect to each other. Compare the fully-formed animal.

> The rate of development of the tentacles, individually or collectively, varies under change of temperature, and their order of appearance is somewhat inconstant. There are usually in the fully-formed animal (8—12 hrs.), 6 for *H. fusca*, 8 for *H. viridis*.

5. **The study of prepared sections.** Place one or more Hydras, preferably in full diet, in Kleinenberg's picric acid solution; after two hours' immersion therein, transfer to alcohol of increasing strengths. When fully hardened, stain with borax-carmine, imbed in paraffin and cut into transverse sections. Mount in Canada balsam and examine under a low power.

a. The *ectoderm;* of uniform thickness and composed, for the most part, of squarish-looking cells which stain lightly.

b. The *endoderm;* composing the inner two-thirds or more of the body-wall, extremely variable in thickness. Its component cells are highly vacuolated.

c. The *body-cavity* (*enteron*); enclosed by *b*. Variable in capacity in different sections, in accordance with the state of contraction of the body-wall.

d. *a* and *b* will be seen to be separated by a hard line,

which stains deeply. Examine this under a high power and look for

α. the *mesoglœa* or *supporting lamella;* visible as the above-named line.

β. *Kleinenberg's fibres* (contractile-processes of the ectoderm-cells); obvious, if the section be a good one, as a parallel series of deeply-stained dots immediately external to *α*.

e. Examine the ectoderm under a high power, selecting your thinnest sections. Look for

α. The *larger ectoderm cells* (*epitheloid layer*); conical, their bases being external, and fairly uniform in contour. Each is composed of a lightly staining protoplasm, nucleated and at times vacuolated. Note the strong granulation (*cuticle*) of their free outer borders.

β. The *interstitial tissue* (*sub-epitheloid layer*); composed of aggregates of smaller cells, at the bases of *α*; rendered conspicuous by its affinity for the stain and by the great development of nematocysts.

Cf. sections across a tentacle. The knob-like protuberances previously seen (Sect. 2 *a*. *β*) will be found to consist of aggregates of these cells which have reached the surface. Similar eminences, less regularly disposed, will be found in the body-wall.

f. Examine, in like manner, the *endoderm*.

α. The *larger cells* (*epitheloid layer*). *Shape*, irregular; *size* and *contour*, variable.

The *cell-protoplasm;* largely replaced in one or more clear vacuoles. It forms peripherally a cell membrane, and is generally densest at the base, where it is aggregated to form the so-called 'foot.'

β. The *secretory cells* of the endoderm; deeply-staining pear-shaped cells, interposed between the bases of the larger ones. Numerous in sections across the hypostome, fewer in those across the body. They may be recognized by their tapering inner ends, the highly-granular nature of their cell-protoplasm, and by their small nucleus.

γ. The *sub-epitheloid layer;* composed of small undifferentiated cells among the bases of α and β.

g. Place a Hydra in 1 per cent. osmic acid solution for 24 hours, and then tease up a portion of the endoderm in weak glycerine, under a low power. Examine, under your highest objective, and look for the *flagella;* long whip-lash filaments, one to six being borne upon a single cell.

As these structures are capable of withdrawal, and usually retracted during digestion[1], cells will be present which do not bear them. Look for specimens showing stages in their elongation.

6. **Structural analysis of the individual cells.** Preserve some Hydras in Müller's fluid (2—3 days). Transfer to alcohol of increasing strengths, and finally tease up under a low power in eosin or hæmatoxylin solution. Alternatively, preserve for a similar period in 1 per cent. solution of ammonia bichromate, transfer to alcohol and tease up in carmine solution. Select the best preserved cells and examine, under your highest power, in order—

a. The *larger ectoderm cells;* (cf. Sect. 5. *e*). Their *nuclei*. Their protoplasm; differentiated superficially into a finely granulated *cuticle*. Look especially for

[1] As would appear to be the case with the cilia of the intestinal epithelium of the earthworm (cf. p. 267).

Kleinenberg's fibres (Sect. 5. *d.* β); one or more of these may be connected with an individual cell. They arise as outgrowths of the constricted cell-base, the whole frequently having the appearance of an inverted T with a thick stem (cell-body) and greatly elongated arms (the fibres).

b. The *cells of the interstitial tissue* (cf. Sect. 5. *e.* β) much smaller than *a*, and often separable from one another only with difficulty. Numbers of them will be found to contain two nuclei (evidence of active division).

c. The *nematocysts;* highly refractive bodies to be found in both *a* and *b*—in the latter in abundance and in all stages of development. Look for the undermentioned (cf. Sect. 3).

α. The *larger nematocysts;* each consists of a *body*, ovoidal and truncated, with a strongly-marked double contour due to the thickness of its wall; a *neck*, inverted and beset by three or four powerful spines; a *filament*, lying within the base of the body, coiled into a spiral the edges of which may occasionally be seen (cf. Sect. 3).

Compare a large nematocyst in the everted state; the summit of the neck is beset by a series of excessively delicate spines.

β. The *smaller nematocysts;* rarely present in the larger cells. Each is ovoidal and much smaller than *a*, having a similar double contour. The *filament;* but $\frac{1}{8}$ to $\frac{1}{12}$ the length of, and very much stouter than, that of *a*; at rest looped, in eversion frequently spiral (cf. Sect. 3).

γ. Examine, in detail, a nematocyst in relation to its *parent-cell.* The cell-protoplasm will be seen to form a delicate envelope, thickened only around the nucleus, which stains deeply and is to be found in close apposition with the nematocyst.

δ. The *cnidoblasts;* recognizable by the presence of a stiff spinous process or *cnidocil,* usually projecting from one corner of the cell-body.

Note the variable size of the cnidocils (cf. Sect. 3). Nematocysts may or may not be present; where they are it will generally be found that the longer cnidocils are associated with the smaller thread-cells and *vice-versâ.*

ε. Look for *nerve-cells;* small and stellate with greatly attenuated offshoots (nerve fibres) and deeply-staining and relatively large oval or rounded nucleus. They are rare and to be found only with difficulty.

ζ. *Immature nematocysts;* occasionally to be met with in the isolated state. Bottle-shaped and bearing a short blunted filament, which subsequently becomes invaginated (be careful not to confound this with the cnidocil).

d. The larger *cells of the endoderm.* Examine cells freshly isolated from specimens killed with osmic acid vapour. Note, in addition to the characters already observed (Sect. 5).

α. The *cell-base;* not unfrequently expanded and prolonged out to form *contractile processes,* identical with, but shorter and less marked than, those of the ectoderm cells.

β. The *nucleus;* variable in position with the state of distension of the cell; generally marginal.

γ. Superadded to the cell-protoplasm. In *H. viridis* the *Chlorelloids;* small round vegetable organisms fairly uniform in size, for the most part aggregated at the cell-base. They stain very feebly, and may or may not contain chlorophyll.

In *H. fusca.* The *sooty corpuscles;* irregular and transparent, rarely rounded; sooty particles may or may not be present. Look for the presence of chlorophyll; occasionally to be found in individual cells. Cf. pp. 344—345.

δ. Compare the living cells, as obtained by crushing a *Hydra.* They are highly amœboid. Watch the nature of their movements.

e. The *mesoglœa.* Search under a low power for stray pieces of this; obvious as transparent shreds of membrane with parallel striæ. There will be found in connection therewith—

α. Nuclei and fragments of cells, mostly of the interstitial tissue.

β. Contractile processes; firmly adherent to the mesoglœa, the above-named striation being due to their presence.

Look for fragments in which the two sets of striæ cross each other (cf. p. 347).

γ. Compare a piece of the same, obtained from the freshly-killed animal. If forthcoming, press gently upon the cover-glass with the point of a needle; it will be found to be gelatinous and compressible.

7. **Food material and digestion.** Work through all your sections under a low power, and look for ingested prey.

a. *Small organisms;* especially *Entomostraca* and other small crustacea. When present one such will generally be found, tightly embraced by the endoderm.

Examine under a high power and look for evidences of digestion (*intra-enteric*) and assimilation.

b. *Smaller organisms;* especially *Infusoria*, *Diatoms*, and the like. Frequently to be met with in the central cavity; they may however be found, unchanged or in process of digestion (*intra-cellular*), within the individual cells of the endoderm.

8. **The reproductive organs.** (Cf. Sect. 2. *a.* ε).

a. *The testes;* small conical colourless eminences below the point of attachment of the tentacles, more rarely irregularly scattered over the whole body.

Gently flatten out a testis in eosin or magenta by pressure on the coverslip, and examine with a high power. According to its state of maturity the following contents will be found in it—

α. A collection of the smaller ectoderm (interstitial) cells of variable shape.

β. The same, but having become ovoidal and hyaline.

γ. Cells otherwise like β, but with a long filament proceeding from them.

δ. *Ripe spermatozoa;* bodies consisting of a very small oval nucleated head to which a very delicate flagellum is attached, by the movements of which they swim about in the water. They may frequently be seen in motion within the unruptured testis.

b. *The ovaries;* one or more in number, larger than *a*, and situated near the base of the polype.

When young, each appears as a hill-shaped enlargement of the ectoderm; when ripe, it becomes rounded and very prominent.

c. Press out an ovary: according to its stage of development there will be found in it—

α. Ectoderm cells with a marked preponderance of the smaller form (interstitial tissue).

β. Imbedded among α, one (*ovicell*) which has become larger and clearer than the rest, and possesses a distinct central *germinal vesicle*.

d. The ripe ovum. Conspicuous in *H. viridis* by its green colour. It consists of a great irregularly branched (amœboid) mass of protoplasm (*vitellus*), in which is a clear space (*germinal vesicle*) containing one larger and a number of smaller *germinal spots*.

Examine under a high power, and note—

α. The absence of a vitelline membrane.

β. The *yolk granules;* exceedingly large and modified to form the so-called 'pseudo-cells'. Each is rounded or oval, thickened on one side to form a plug-shaped ingrowth and filled with a fluid contents.

γ. The *Chlorelloids;* present in *H. viridis;* identical with those found in the endoderm (Sect. 6. *d.* γ).

e. The *segmented ovum* (*oosperm*): composed of a large number of small cells. Its thick horny capsule, rough on its external surface.

VII.

THE BELL-ANIMALCULE (*Vorticella*).

THE bell-animalcule is one of a very large group of animals called the *Infusoria*, on account of the fact that many members thereof make their appearance in infusions of certain animal and vegetable substances.

The higher multicellular animals begin their existence as simple nucleated cells, and the single nucleated cell which constitutes the whole animal in its primitive condition divides and subdivides until an aggregation of similar cells is formed. And it is by the differentiation and metamorphosis of these primitively similar histological elements that the organs and tissues of the body are built up. In the Infusoria, the protoplasmic mass which constitutes the germ does not undergo this process of preliminary subdivision, but such structure as the adult animal possesses is the result of the direct metamorphosis of parts of its protoplasmic substance. Hence, morphologically, the bodies of these animals are the equivalents of a single cell; while, physiologically, they may attain a considerable amount of complexity.

The Infusoria abound in fresh and salt waters, and many make their appearance, as before stated, in organic infusions, their germs either being contained in the substances infused, or being wafted through the air. Their diffusion is greatly facilitated by the fact that many of them retain their

vitality when dried, and reduced to the condition of an excessively light dust; while their rapid propagation is, in the main, due to their power of multiplying by division, with extraordinary rapidity, when duly supplied with nourishment. The majority are free and provided with numerous cilia by which they are incessantly and actively propelled through the medium in which they live; but some attach themselves to stones, plants, or even the bodies of other animals. A few are parasitic, and the bladder and intestines of the Frog are usually inhabited by several species of large size.

The Bell-animalcules are Infusoria which are fixed, usually by long stalks, to water plants, or, not unfrequently, to the limbs of aquatic Crustacea. They are barely visible to the unaided vision. The body has the shape of a wine-glass with a very long and slender stem, provided with a flattened disc-like cover. What answers to the rim of the wine-glass is thickened, somewhat everted, and richly ciliated, and the edges of the disc are similarly thickened and ciliated. Between the thickened edge of the cover, or *peristome*, and the edge of the disc, is a groove, which, at one point, deepens and passes into a wide depression, the *vestibulum*. From this a narrow tube, the *œsophagus*, leads into the central substance of the body, and terminates abruptly therein; and when fæcal matters are discharged, they make their way out by an aperture which is temporarily formed in the floor of this vestibule. The outermost layer of the substance of the body is denser and more transparent than the rest, forming a *cuticula*. Immediately beneath the cuticle it is tolerably firm and slightly granular, and this part is distinguished as the *cortical* layer or *ectosarc;* it passes into the central substance or *endosarc*, which is still softer and more fluid.

In the undisturbed condition of the Bell-animalcule, the stem is completely straightened out; the peristome is everted, and the edges of the disc separated from the peristome; the vestibule gaping widely and the cilia working vigorously. But the least shock causes the disc to be retracted, and the edge of the peristome to be curved in and shut against it, so as to give the body a more globular form. At the same time, the stem is thrown into a spiral, and the body is thus drawn back towards the point of attachment. If the disturbing influence be continued, this state of retraction persists; but if it be withdrawn, the spirally coiled stem slowly straightens, the peristome expands, and the cilia resume their activity.

In the interior of the body, immediately below the disc, a space, occupied by a clear watery fluid, is seen to make its appearance at regular intervals—slowly enlarging until it attains its full size and then suddenly and rapidly disappearing by the approximation of its walls. This is the *contractile vesicle* or *vacuole*. It communicates with the exterior at the moment of contraction, and in all probability performs an excretory function. If the Bell-animalcule is well fed, one or more watery vesicles of a spheroidal form, each containing a certain portion of the ingested food, will be seen in the soft central mass of the body. And by mixing a small quantity of finely divided carmine or indigo with the water in which the *Vorticellæ* live, the manner in which these food-vesicles are formed may be observed. The coloured particles are driven into the vestibule by the action of the cilia of the peristome and the adjacent parts, and gradually accumulate at the inner end of the gullet. After a time the mass here heaped together projects into the central substance of the body, surrounded by an envelope of the accompanying water; and then suddenly breaks off,

as a spheroidal drop, henceforward free in the soft central substance. Such being the mode of formation of the vesicles, they have been termed *vesicles of ingestion;* in some Bell-animalcules, they are carried round with the deeper layer of protoplasm or endosarc in a movement of circulation, passing up one side of the body, then crossing over below the disc and descending on the other side. Sooner or later the contents of these vesicles are digested, and the refuse is thrown out of the body, surrounded as a rule by a watery vacuole or *vesicle of egestion.* This process takes place by an aperture leading into the vestibule, which exists only at the moment of extrusion of the fæces, and is indistinguishable at any other time.

A portion of the substance of the body, which is slightly different in transparency and in its reactions to colouring substances from the rest, is called the *nucleus* or *endoplast.* It is elongated and bent upon itself into a crescentic or horseshoe shape.

The numerous species and varieties of bell animalcules are, for the most part, colourless. Green varieties are however occasionally to be met with, the green colour being due to a deposit of chlorophyll within the endosarc, comparable to that seen within the endoderm of the green-hydra. This colouring matter may be restricted to small bodies or *chloroplastids* identical with those of the polype referred to, or diffused throughout the cell-protoplasm (endosarc) in a manner such as, there is good reason to believe, is never the case in the vegetable organism.

The Bell-animalcules multiply in two ways; partly by *longitudinal fission*, when a bell becomes cloven down the middle, each half acquiring the structure previously possessed by the whole; and partly by *gemmation* from the *endoplast*, in which latter case the endoplast divides into a

number of rounded spore-like masses, which are ultimately set free as locomotive germs.

Sometimes a rounded body, encircled by a basal circlet of cilia but having otherwise the characters of a *Vorticella* bell, is seen to be attached to the base of the bell of an ordinary *Vorticella*. It was formerly supposed that these were buds, but it is now known that they are independent individuals, formed as the result of repeated longitudinal fission, which have attached themselves to that to which they adhere and are gradually becoming fused with it, so that the two will form one indistinguishable whole. There is here a process of "conjugation" sexual in nature, and it is, moreover, preliminary to the budding of the endoplast and the subsequent formation of germs.

Under certain circumstances a *Vorticella* may become *encysted*. The peristome closes and the bell becomes converted into a spheroidal body, in which only the nucleus and the contractile vesicle remain distinguishable. This surrounds itself with a structureless envelope or *cyst*, the whole process sometimes following that of conjugation and preceding that of germ formation. It does not appear however that this encystment is invariably associated with reproduction; for the encysted animal after remaining for a longer or shorter time in a temporary condition of rest, may emerge and resume its former state of existence.

The two genera of Infusoria which most commonly occur in the Frog are *Nyctotherus* and *Balantidium*. Both are free and actively locomotive, and the former is particularly remarkable for its relatively large size and semilunar contour, and for the length and distinctness of its curved œsophagus. *Balantidium* is pyriform, and has a very short œsophageal depression.

LABORATORY WORK.

A. Examine duckweed roots, confervæ, &c, with $\frac{1}{4}$ inch objective avoiding pressure; having found a group of *Vorticellæ* note the following points with a higher power.

1. **In the extended state of the animal.**

a. *The body.*

a. Its size (measure).

b. Form; broadly speaking, that of an inverted bell: note—

α. The prominent everted rim (*peristome*).

β. The flattened central *disc* projecting above the peristome.

γ. The *cilia* fringing the disc.

δ. The depression between the peristome and disc.

ε. The *vestibulum;* a chamber in the hollow between the peristome and disc.

c. Structure.

α. The thin, transparent, homogeneous external layer (*cuticle*).

β. The granular layer (*ectosare*) inside the cuticle, longitudinally striate at the base of the bell (*myophan layer*).

γ. The central more fluid part (*endosarc*) not sharply marked off from β.

The various clear spaces (*food vacuoles*) in it, containing foreign (ingested) bodies (Diatoms, Protococcus, &c.).

δ. The *contractile vesicle;* its position, in the cortical layer just beneath the disc; its systole and diastole.

ε. The *nucleus;* an elongated curved body in the deeper layer; sometimes nearly homogeneous, sometimes more distinctly granular. The nucleus is usually indistinguishable until after treatment with iodine, or acetic acid and magenta (cf. § 5).

ζ. The *gullet;* sometimes seen in optical transverse section as a clear round space; sometimes seen sideways as a canal opening above into the vestibulum, and ending abruptly below in the body-substance.

η. The *anus;* look for the egestion of solid particles.

b. *The stalk.*

α. Its length and diameter (measure).

β. Its structure; the external homogeneous layer (*sheath*) continuous with the cuticle; the highly refractive centre (*axis* or *contractile filament*) continuous with the cortical layer of the bell.

2. **In the retracted state.**

a. *The body.*

α. Its form; pear-shaped; rounded off above; neither disc nor peristome visible.

β. The clear transverse space near the top, indicating the interval between the retracted disc

and the rolled-in peristome. In this space the cilia can frequently be seen moving.

γ. Structure; as in 1. **a.** *c.*

b. The stalk; thrown into corkscrew-like folds.

3. **The movements of Vorticella.**

a. The ciliary movement.

α. Examine the cilia carefully; delicate homogeneous processes; their length, diameter and form; their position.

β. The continuity of the cilia with the cortical layer.

γ. The function of the cilia; their rapid movements, alternately bending and straightening: the *co-ordination* of these movements; they work in a definite order; note the currents produced in the neighbouring water (if necessary introduce a few particles of carmine under the coverslip); the sweeping of small bodies down the gullet.

b. The movements of the contractile vesicle. Tolerably regular rhythmic distension and collapse (diastole and systole).

c. The currents in the endosarc carrying round the ingested bodies.

d. The movements of the animal as a whole. (Examine under $\frac{1}{2}$ inch or $\frac{1}{4}$ inch obj.)

α. Its extreme *irritability;* it contracts on the slightest stimulation, often without any apparent cause.

β. The movements which occur in contraction;

the coiling up of the stalk; the rolling in of the disc. The rapidity of these movements.

γ. The mode of re-expansion; the stalk straightens first; then the peristome is everted; finally the disc and its cilia are protruded.

4. Stain with iodine or magenta; the cuticle uncoloured —the rest stained; the nucleus especially becomes deeply coloured.

5. Treat with acetic acid; the contents soon disappear (except perhaps some swallowed bodies)—the cuticle later or not at all.

6. Look for the following in various specimens—

α. *Multiplication by fission, early stage;* a bell partially divided into two by a vertical fissure starting from the disc.

β. *The same, late stage;* two complete bells on one stalk; the result of completion of the fission. The development of a basal circlet of cilia by one or both of these bells.

γ. Free swimming unstalked bells (detached bells from β).

δ. *Conjugation;* the attachment of a small free swimming bell to the side of a stalked one.

ε. *Encystation;* the body contracted into a ball and surrounded by a thickened structureless layer, the contractile vesicle being persistently dilated.

B. Other forms closely allied to *Vorticella* which may be met with, and which will do nearly as well for examination, are;—

a. **Epistylis.** Bell-shaped animals growing on a *branched non-contractile* stalk.

b. **Carchesium.** A form very like *Vorticella* but borne on a *branched* contractile stalk.

c. **Cothurnia.** An almost sessile form, provided with a cup or envelope into which the bell can be retracted.

[The activity of the movements of the free Infusoria interferes with the complete examination of the living animal. It is well therefore to add a little osmic acid solution to the drop of water under examination. This kills such Infusoria as *Paramæcium*, *Nyctotherus* and *Balantidium* instantly, without destroying the essential features of their organization.]

VIII.

THE PROTEUS ANIMALCULE (*Amœba*) AND THE COLOURLESS BLOOD CORPUSCLE.

Amœbæ are minute organisms of very variable size which occur in stagnant water, in mud, in damp earth and elsewhere, and are frequently to be obtained by infusing any animal matter in water and allowing it to evaporate while exposed to direct sunlight. Parasitic *Amœbæ* are known to exist.

The *Amœba* has the appearance of a particle of jelly, which is more or less granular and fluid in its central parts, but usually becomes clear and transparent, and of a firmer consistency, towards its periphery. Sometimes *Amœbæ* are found having a spherical form and encased in a structureless sac, and in this encysted state they exhibit no movements. More commonly, they present incessant and frequently rapid changes of form, whence the name of "*Proteus Animalcule*" given to them by the older observers; and these changes of form are usually accompanied by a shifting of position, the *Amœba* creeping about with considerable activity and, in most cases, with no constancy of direction.

The changes of form, and the movements, are effected

by the thrusting out of lobe-like prolongations of the peripheral part of the body, which are termed *pseudopodia*, sometimes from one region and sometimes from another. Occasionally, a particular region of the body is constantly free from pseudopodia, and therefore forms its hindmost part when it moves. Each pseudopodium is evidently, at first, an extension of the denser clear substance (*ectosarc*) only; but as it enlarges, the central, granular, more fluid *endosarc* flows into its interior, often with a sudden rush.

The protoplasm is in some *Amœbæ* crowded with small watery vacuoles. In most there is present a clear space which makes its appearance at intervals, in a particular region of the ectosarc, and then disappears by the rapid approximation of its walls. After a while, a small clear speck or a line appears at the same spot and slowly dilates until it attains its full size, when it again rapidly disappears as before. Sometimes two or three small clear spots arise close together, and run into one another to form the single large cavity. The structure thus described is termed the *contractile vesicle* or *vacuole*, and its rhythmical systole and diastole often succeed one another with great regularity. There is reason to think that it may be excretory like the corresponding structure in Vorticella.

One part of the *Amœba* generally exhibits a rounded or oval *nucleus*, while a still smaller body of about the $\frac{1}{10}$ part of the diameter of the nucleus, which may possibly be compared to the para- or micro-nucleus of certain other unicellular organisms, may be present (cf. p. 374).

The gelatinous body of the *Amœba* is not as a rule bounded by anything that can be properly termed a cuticular membrane; all that can be said is, that its external or limitary layer is, in most cases, of a somewhat

different constitution from the rest, so that it acquires a certain appearance of distinctness when it is acted upon by such reagents as acetic acid or iodine, or when the animal is killed by raising the temperature to 45° C. Physically, the ectosarc might be compared to the wall of a soap-bubble, which, though fluid, has a certain viscosity, which not only enables its particles to hold together and form a continuous sheet, but permits a rod to be passed into or through the bubble without bursting it; the walls closing together, and recovering their continuity, as soon as the rod is drawn away.

It is this property of the ectosarc of the *Amœba* which enables us to understand the way in which these animals take in and throw out again solid matter, though they have neither mouth, anus, nor alimentary canal. The solid body passes through the ectosarc, which immediately closes up and repairs the rent formed by its passage. In this manner, the *Amœbæ* take in the small, usually vegetable, organisms, which serve them for food, and subsequently get rid of the undigested solid parts.

Ingested matter is invariably taken in, in conjunction with a watery *vacuole of ingestion* such as has been described for *Vorticella* (pp. 361—2). If the matters thus introduced be nutritive and digestible the vacuole persists, during at any rate the earlier stages of digestion; later, the unassimilable innutritious portions of the same are cast out surrounded by a *vacuole of egestion*. Experiment has led to the belief that *Amœbæ* will not digest fat globules or starch grains In the case of the latter the grains have been seen to be thrown out 6—7 days after ingestion, unchanged in their optical characters and in their behaviour towards certain reagents and unaccompanied by the vesicle of egestion.

It is of interest to note that in the case of small vegetable organisms the cellulose coats have been at times observed to be thrown out in a distorted, if not a digested and disintegrating condition.

The chemical composition of the bodies of the *Amœbæ* has not been accurately ascertained, but they undoubtedly consist, in great measure, of water containing a protein compound, and are similar to other forms of protoplasm. They absorb oxygen and give out carbonic acid, and the presence of free oxygen is necessary to their existence. When the medium in which they live is cooled down to the freezing point their movements are arrested, but they recover when the temperature is raised. At a temperature of about 35° C. their movements are arrested, and they pass into a condition of "heat-stiffening," from which they recover if that temperature is not continued too long; at 40° to 45° C. they are killed.

Crystals are generally to be met with in the cell protoplasm, but of their origin and significance nothing is at present known.

Electric shocks of moderate strength cause *Amœbæ* at once to assume a spherical still form, but they recover after a while. Strong shocks kill them and, at the same time, bring the nucleus distinctly into view.

If *Amœbæ* are not to be found, their nature may be understood by the examination of the *colourless corpuscles* of the blood. (Cf. ch. I. pp. 121–123.)

The colourless corpuscles of the blood of some of the cold-blooded vertebrates, such as Frogs and Newts, may be kept alive for many weeks in serum properly protected from evaporation; and if finely divided colouring matter, such as indigo, is supplied to them, either in the body or out of it, they take it into their interior in the same way as true

Amœbæ would. In the earliest condition of the embryo, the whole body is largely composed of such nucleated cells as the colourless corpuscles of the blood; and the colourless corpuscles must be regarded as simply the progeny of such cells, which have not become metamorphosed, and have retained the characteristics of the lowest and most rudimentary forms of animal life.

The *Amœba* is an animal, not because of its contractility or power of locomotion, but chiefly because it is devoid of the power of manufacturing protein from bodies of a comparatively simple chemical composition. The *Amœba* has to obtain its protein ready made, in which respect it resembles all true animals, and therefore is, like them, in the long run, dependent for its existence upon some form or other of vegetable life.

Amœbæ multiply by fission in a manner similar to that described for the bell-animalcule; the nucleus first divides, the cell becoming subsequently cleft in two. Occasionally an *Amœba* has been seen to engulph another of smaller size than itself; and there is reason to believe that this process, originally thought to have been one of cannibalism, may probably be one of conjugation of dissimilar individuals, for reproduction, akin to that seen in the bell-animalcule.

LABORATORY WORK.

A. Place a drop of water containing *Amœbæ* on a slide, cover with a cover glass, avoiding pressure, and search over with $\frac{1}{4}$ inch obj.: having found an *Amœba*, examine with a higher power.

1. *Size;* differing considerably in different specimens. Measure.

2. *Outline;* irregular, produced into a number of thick rounded eminences (*pseudopodia*) which are constantly undergoing changes: sketch it at intervals of five seconds.

3. *Structure.*

   a. Outer hyaline layer (*ectosarc*), tolerably sharply marked off: inner granular layer (*endosarc*) becoming more fluid centrally.

   b. *Nucleus;* a roundish more solid-looking body, often highly granular.

   c. Look for the *paranucleus*, a small granular body lying (if present) close to the nucleus, towards which it always remains constant in position.

   d. *Contractile vesicle;* (if present) a roundish clear space in the ectosarc which disappears periodically, and after a short time reappears; its slow diastole—rapid systole.

   e. *Ingested foreign bodies;* Diatom cases, *Desmidiæ*, &c.

4. *Movements.*

   a. Watch the process of formation of a *pseudopodium.* A hyaline ectosarcal lobe first appears; then, as it increases in size, the granular endosarc flows into it.

   b. Locomotion; watch the process,—a pseudopodium is thrown out, then the rest of the body is drawn up to it, and the process is repeated.

   c. If the opportunity presents itself, watch the processes of the ingestion and egestion of solid

d. Observe the movements on the hot stage; warmth at first accelerates the movements, but as the temperature approaches 40° C. they cease, and the whole mass remains as a motionless sphere.

e. Effects of electrical shocks on the movements.

5. *Mechanical Analysis;* crush. The whole collapses, except sometimes the nucleus, and even that after a time disappears: there is no trace of a distinct resisting outer sac.

6. *Chemical Analysis;* Treat with magenta and iodine. The whole stains, and there is no unstained enveloping sac. Iodine produces no blue colouration unless starch has been swallowed; if so, blue specks become visible.

7. Look for encysted specimens; and for specimens which are undergoing fission.

8. Another form of *Amœba* is not unfrequently found which differs from that just described in being much less coarsely granular, and in having ill-defined ectosarc and endosarc, together with much longer, more slender and pointed pseudopodia. Another common form progresses rapidly with a slug-like movement, only throwing out pseudopodia at its anterior end.

B. **White Blood-Corpuscles** (human).

Prick your finger and press out a drop of blood: spread out on a slide under a coverslip, avoiding pressure, and surround the margin of the coverglass with vaseline or oil. Neglect the red corpuscles, and examine the larger and much less numerous colourless ones.

Note

1. *Size;* (measure).
2. *Form;* changing much like that of the *Amœba*, but less actively. Draw at intervals of ten seconds.
3. *Structure;* Some more and some less granular; ectosarc and endosarc less distinct than in the *Amœba*. Nucleus rarely visible in the fresh state. No contractile vesicle.
4. Treat with dilute acetic acid: the whole is clarified and a nucleus is brought into view in a more or less central position. Occasionally two nuclei may be seen.
5. Stain with magenta; the whole becomes coloured, the nucleus most intensely.
6. Place a fresh preparation on the hot stage, and gradually warm up to 50° C. The movements are at first rendered more active, but ultimately cease, the pseudopodia-like processes being all retracted and the whole forming a motionless sphere.

IX.

YEAST (*Torula* or *Saccharomyces Cerevisiæ*).

YEAST is a substance which has been long known on account of the power which it possesses of exciting the process termed *fermentation* in substances which contain sugar.

If strained through a coarse filter, it appears to the naked eye as a brownish fluid in which no solid particles can be discerned. When some of this fluid is added to a solution of sugar and kept warm, the mixture soon begins to disengage bubbles of gas and become frothy; its sweetness gradually disappears; it acquires a spirituous flavour and intoxicating qualities; and it yields by distillation a light fluid—*alcohol* (or spirits of wine) which readily burns.

When dried slowly and at a low temperature, yeast is reduced to a powdery mass, which retains its power of exciting fermentation in a saccharine fluid for a considerable period. If yeast is heated to the temperature of boiling water, before it is added to the saccharine fluid, no fermentation takes place; and fermentation which has commenced is stopped by boiling the saccharine liquid.

A saccharine solution will not ferment spontaneously. If it begins to ferment, yeast has undoubtedly got into it in some way or other.

If the yeast is not added directly to the saccharine fluid, but is separated from it by a very fine filter, such as porous earthenware, the saccharine fluid will not ferment, although

the filter allows the fluid part of the yeast to pass through into the solution of sugar.

If the saccharine fluid is boiled, so as to destroy the efficiency of any yeast it may accidentally contain, and then allowed to come in contact only with such air as has been passed through cotton wool, it will never ferment. But if it is exposed freely to the air, it is almost sure to ferment sooner or later, and the probability of its so doing is greatly increased if there is yeast anywhere in the vicinity.

These experiments afford evidence (1) that there is something in yeast which provokes fermentation, (2) that this something may have its efficiency destroyed by a high temperature, (3) that this something consists of particles which may be separated from the fluid which contains them by a fine filter, (4) that these particles may be contained in the air; and that they may be strained off from the air by causing it to pass through cotton wool.

Microscopic examination of a drop of yeast shews what the particles in question are.

Even with a hand-glass, the drop no longer appears homogeneous, as it does to the naked eye, but looks as if fine grains of sand were scattered through it; but a considerable magnifying power (5—600 diameters) is necessary to shew the form and structure of the little granules which are thus made visible. Under this power, each granule (which is termed a *Torula*) is seen to be a round, or oval, transparent body, varying in diameter from $\frac{1}{2500}$th to $\frac{1}{7000}$th of an inch (on the average about $\frac{1}{3000}$th).

The *Torulæ* are either single, or associated in heaps or strings. Each consists of a thin-walled sac, or bag, containing a semi-fluid matter, in the centre of which there is often a space full of a more clear and watery fluid than the rest, which is termed a 'vacuole.' The sac is comparatively

tough, but it may be easily burst, when it gives exit to its contents, which readily diffuse themselves through the surrounding fluid. The whole structure is called a 'cell;' the sac being the 'cell-wall,' and the more solid portion of the contents the 'protoplasm.' It appears that a nucleus is also present, but on this point there is still some difference of opinion.

When yeast is dried and burned in the open air it gives rise to the same kind of smell as burning animal matter, and a certain quantity of mineral ash is left behind. Analysed into its chemical elements, yeast is found to contain Carbon, Hydrogen, Oxygen, Nitrogen, Sulphur, Phosphorus, Potassium, Magnesium and Calcium; the last four in very small quantities.

These elements are combined in different ways, so as to form the chief proximate constituents of the *Torula*, which are (1) a Protein compound, analogous to Casein, (2) Cellulose, (3) Fat, and (4) Water. The cell-wall contains all the Cellulose and a small proportion of the mineral matters. The protoplasm contains the Protein compound and the Fat with the larger proportion of the mineral salts.

These *Torulæ* are the 'particles' in the yeast which have the power of provoking fermentation in sugar; it is they which are filtered off from the yeast when it loses its efficiency by being strained through porous earthenware; it is they which form the fine powder to which yeast is reduced by drying, and which, from their extreme minuteness, are readily diffused through the air in the form of invisible dust.

That the *Torulæ* are living bodies is proved by the manner in which they grow and multiply. If a small quantity of yeast is added to a large quantity of clear saccharine fluid so as hardly to disturb its transparency, and the whole is

kept in a warm place, it will gradually become more and more turbid, and, after a time, a scum of yeast will collect, which may be many thousand, or million, times greater in weight than that which was originally added. If the *Torulæ* are examined as this process of multiplication is going on, it will be found that they are giving rise to minute buds, which rapidly grow, assume the size of the parent *Torula*, and eventually become detached; though, generally, not until they have developed other buds, and these yet others. The *Torulæ* thus produced by gemmation, one from the other, are apt long to adhere together, and thus the heaps and strings mentioned, as ordinarily occurring in yeast, are produced. No *Torula* arises except as the progeny of another; but, under certain circumstances, multiplication may take place in another way. The *Torula* does not throw out a bud, but its protoplasm divides into (usually) four masses, each of which surrounds itself with a cell-wall, and the whole are set free by the dissolution of the cell-wall of the parent. These *endogenous* reproductive bodies are termed *spores*, and retain their power of germination for a long time.

As each of the many millions of *Torulæ* which may thus be produced from one *Torula* has the same composition as the original progenitor, it follows that a quantity of Protein, Cellulose and Fat proportional to the number of *Torulæ* thus generated, must have been produced in the course of the operation. Now these products have been manufactured by the *Torulæ* out of the substances contained in the fluid in which they float and which constitutes their food.

To prove this it is necessary that this fluid should have a definite composition. Several fluids will answer the purpose, but one of the simplest (Pasteur's solution) is the following:

Water	(H_2O).
Sugar	($C_{12}H_{22}O_{11}$).
Ammonium Tartrate	($C_4H_4(NH_4)_2O_6$).
Potassium Phosphate	(KH_2PO_4).
Calcium Phosphate	($Ca_3P_2O_8$).
Magnesium Sulphate	($MgSO_4$).

In this fluid the *Torulæ* will grow and multiply, But it will be observed that the fluid contains neither Protein nor Cellulose, nor Fat, though it does contain the elements of these bodies arranged in a different manner. It follows that the *Torula* must absorb the various substances contained in the water and arrange their elements anew, building them up into the complex molecules of its own body. This is a property peculiar to living things.

If, on the other hand, some *Torula* cells be added to a quantity of pure distilled water, it will be found not only that no growth or multiplication of the cells takes place, but that the amount of their protoplasm actually undergoes a perceptible diminution. Hence we see that there are processes going on in the cells which tend to diminish their substance.

The chemical changes going on in a living cell, such as that of yeast, may conveniently be spoken of collectively as its *metabolism*. Among these changes those which result in the building up of more complex chemical bodies, and thus in an increase of the organic substance of the cell, constitute its *constructive* metabolism or *anabolism*, while to those which bring about a diminution of organic substance, with evolution of simpler compounds, such as carbonic anhydride, the term *destructive* metabolism or *catabolism* has been applied.

It must be pointed out that the anabolic processes of the

organism not only result in the building up of its organic substances, but also involve an accumulation of potential energy. If a quantity of dried yeast is burned a definite amount of heat is given off, due to the conversion of this potential energy into the kinetic form. Conversely, the catabolic processes involve a change of potential into kinetic energy. The evolution of heat by actively growing yeast is due to changes connected with its destructive metabolism.

The *Torula* being alive, the question arises whether it is an animal or a plant. Although no sharp line of demarcation can be drawn between the lowest form of animal and of vegetable life, yet *Torula* is an indubitable plant, for two reasons. In the first place, its protoplasm is invested by a cellulose coat, and thus has the distinctive character of a vegetable cell. Secondly, it possesses the power of constructing Protein out of such a compound as Ammonium Tartrate, and this power of manufacturing Protein is distinctively a vegetable peculiarity. *Torula* then is a plant, but it contains neither starch nor chlorophyll, and it cannot obtain the whole of its food from inorganic compounds, thus differing widely from the green plants. On the other hand, it is, in these respects, at one with the great group of *Fungi*. Like many of the latter, its life is wholly independent of light, and in this respect, again, it differs from the green plants.

Whether *Torula* is connected with any other form of *Fungi* is a question which must be left open for the present. It is sufficient to mention the fact that under certain circumstances some Fungi (e.g. *Mucor*) may give rise to a kind of *Torula* different from common yeast.

The fermentation of the sugar is in some way connected with the living condition of the *Torula*, and is arrested by all those conditions which destroy its life. At the same time

it appears that the conditions which are most favourable to the growth and multiplication of the *Torula* are unfavourable to the process of fermentation. Thus the latter goes on most vigorously in the absence of free oxygen, while its presence is favourable to the increase of the yeast-cells. The greater part of the sugar is resolved into Carbonic anhydride and Alcohol, the elements of which, taken together, equal in weight those of the sugar. A small part (4—5 per cent.) breaks up into Glycerine and Succinic acid, and one or two per cent. is not yet accounted for, but is perhaps assimilated by the *Torulæ*.

This is the more probable as *Torulæ* will grow and multiply actively in a solution in which sugar and Ammonium Nitrate replace the Ammonium Tartrate of the former solution, in which case the carbon of the Protein, Cellulose and Fat manufactured must be obtained from the sugar. Moreover, though oxygen is essential to the life of the *Torula*, it can, as already mentioned, live in saccharine solutions which contain no free oxygen, appearing, under these circumstances, to carry on its life by the substitution of the abnormal catabolic process of fermentation for the normal respiration.

It has further been ascertained that *Torulæ* flourish remarkably in solutions in which sugar and pepsin replace the Ammonium Tartrate. In this case, the nitrogen of their protein compounds must be derived from the pepsin; and it would seem that the mode of nutrition of such *Torulæ* approaches that of animals.

LABORATORY WORK.

Sow some fresh baker's yeast in Pasteur's fluid[1] with sugar and keep it in a warm place: as soon as the mixture begins to froth up, and the yeast is manifestly increasing in quantity, it is ready for examination.

A. **Morphology.**

1. Spread a little out, on a slide, in a drop of the fluid, and examine it with a low power ($\frac{1}{2}$ inch objective) *without a cover-glass.* Note the varying size of the cells, and their union into groups.

2. Cover a similar specimen with a thin glass and examine it under a high power ($\frac{1}{8}$ objective).

   a. Note the size (measure), shape, surface and mode of union of the cells.

   b. Their structure: cell-wall, protoplasm, vacuole.

      α. *Cell-wall;* homogeneous, transparent.

      β. *Protoplasm;* less transparent; often with a few clear shining dots in it.

      γ. *Vacuole;* sometimes absent; size, position.

[1] See Appendix E, p. 494.

δ. The relative proportion of cell-wall, protoplasm, and vacuole in various cells.

Draw a few cells carefully to scale.

3. Run in magenta solution under the cover-glass. (This is readily done by placing a drop of magenta solution in contact with one side of the cover-glass, and a small strip of blotting-paper at the opposite side.)

a. Note what cells stain soonest and most deeply, and what part of each cell it is that stains: the cell-wall is unaffected; the protoplasm stained; the vacuole unstained, though it frequently appears pinkish, being seen through a coloured layer of protoplasm.

4. Burst the stained cells by placing a few folds of blotting-paper on the surface of the cover-glass and pressing smartly with the handle of a mounted needle: note the torn empty and colourless, but solid and uncrushed transparent cell-walls; the soft crushed stained protoplasm.

5. Repeat observation 3, running in iodine solution instead of magenta. The protoplasm stains brown; the rest of the cell remains unstained. Note the absence of any blue coloration; *starch is therefore not present.*

6. Treat another specimen with potash solution, running it in as before: this reagent dissolves out the protoplasm, leaving the cell-wall unaltered.

7. After treatment with ether and alcohol, and staining with hæmatoxylin solution, a single deeply-stained body, probably of a nuclear nature, may be distinguished in each cell.

8. Sow a few yeast-cells in Pasteur's solution in a moist chamber and keep them under observation from day to day; watch their growth and multiplication.

9. Spore-formation: take some dry German yeast; suspend it in water and shake so as to wash it. Let the mixture stand for half-an-hour: pour off the supernatant fluid, and, with a camel's hair pencil, spread out the creamy deposit in a thin layer on fresh cut potato slices or on a plate of plaster of Paris, and place with wet blotting-paper under a bell-jar: examine from day to day with a very high power (800 diam.) for *spores*, which will probably be found on the eighth or ninth day.

B. PHYSIOLOGY.

(Conditions and results of the vital activity of *Torula*.)

1. Sow a fair-sized drop of yeast in—

   a. Distilled water.

   b. 10 per cent. solution of sugar in water.

   c. Pasteur's fluid without the sugar.

   d. Pasteur's fluid with sugar.

   e. Mayer's pepsin solution[1].

Keep all at about 35° C., and compare the growth of the yeast, as measured by the increase of the turbidity of the fluid, in each case. "*a*" will hardly grow at all, "*b*" better, "*c*" better still, "*d*" well, and "*e*" best of all. Note that bubbles of gas are plentifully evolved from both the solutions which contain sugar.

[1] See Appendix E, p. 494.

That any growth at all takes place, in the case of experiments *a* and *b*, is due to the fact that the drop of yeast added contains nutritious material sufficient to provide for that amount of growth.

2. Prepare two more specimens of "*d*" and keep one in a cold—the other in a warm (35° C.) place, but otherwise under like conditions. Compare the growth of the yeast in the two cases; it is much greater in the specimen kept warm.

3. Prepare two more specimens of "*d*"; keep both warm, but one in darkness, the other exposed to the light: that in the dark will grow as well as the other; sunlight is therefore not essential to the growth of *Torula*.

4. Sow some yeast-cells in Pasteur's solution in a flask, the neck of which is closed by a plug of cotton wool, and boil for five minutes; then set it aside; no signs of vitality will afterwards be manifested by the yeast in the flask; it is *killed* by exposure to this temperature.

5. [Take two test tubes; in one place some yeast, with Pasteur's solution containing sugar; in the other place baryta water, and then connect the two test tubes by tightly fitting perforated corks and a bent tube passing from above the surface of the fluid in the first tube to the bottom of the baryta water in the second; pass a narrow bent tube, open at both ends, through the cork of the baryta water tube, so that its outer end dips just below the surface of some solution of potash[1]. All gas formed in the first tube will now bubble through the baryta water in the second, and, from thence, any that

[1] The object of the potash is to shield the baryta water from any carbonic anhydride that may be in the atmosphere.

is not absorbed will pass out through the potash into the air. An abundant precipitate of barytic carbonate will be formed which can be collected and tested. The fermenting fluid, therefore, evolves carbonic anhydride.]

6. [Grow some yeast in Pasteur's solution (with sugar), in a nearly closed vessel (say a bottle with a cork through which a long narrow open tube passes): as soon as the evolution of gas seems to have ceased, distil the fluid in a water bath and condense and collect the first fifth that comes over: redistill this after saturation with potassic carbonate, and test the distillate for alcohol by its odour and inflammability.]

7. [Determine that heat is evolved by a fluid in which active alcoholic fermentation is going on. Place 200 cc. of fresh yeast in a flask, and add 1 litre of Pasteur's fluid with sugar: put another litre of the fluid alone in a similar flask, cover each flask with a cloth and place the two side by side in a place protected from draughts. When gas begins to be actively evolved from the yeast-containing solution, take the temperature of the fluid in each flask with a good thermometer; the temperature of the one in which fermentation is going on will be found the higher.]

X.

PROTOCOCCUS (*Protococcus pluvialis*[1]).

If the mud which accumulates in roof-gutters, water-butts, and shallow pools be collected, it will be found to contain, among many other organisms, specimens of *Protococcus*. In its vegetative condition *Protococcus* is a spheroidal body $\frac{1}{350}$ to $\frac{1}{4000}$ of an inch in diameter, composed, like *Torula*, of a structureless tough transparent wall, inclosing viscid and granular protoplasm. The chief solid constituent of the cell-wall is cellulose. The protoplasm contains a nitrogenous substance, doubtless of a proteinaceous nature, though its exact composition has not been determined, and some small starch-grains are sometimes to be found in it. Certain definite portions of the protoplasm, termed *chromatophores*, contain a red or green colouring matter. The latter is called *chlorophyll*. These chromatophores occupy a large part of the cavity of the cell, so that under a low power the whole contents appear to be coloured.

Individual *Protococci* may be either green or red; or half green and half red; or the red and green colours may co-exist in any other proportion.

In the middle of the cell is a distinct *nucleus*, containing a nucleolus.

[1] The names Hæmatococcus, Chlamydococcus and Sphærella pluvialis have also been applied to this form, but it must not be confused with the more abundant *Pleurococcus* which everywhere forms a green powdery layer on trees, palings, &c.

The influence of sunlight is an essential condition of the growth and multiplication of *Protococcus;* under that influence, it decomposes carbonic anhydride, appropriates the carbon, and sets oxygen free. The energy thus derived from the sun's rays is stored up in the potential form, to be again partly expended in the growth of the plant, and in the movements of its spores. It is this power of obtaining the carbon which it needs from carbonic anhydride, which is the most important distinction of *Protococcus*, as of all plants which contain chlorophyll, from *Torula* and the other *Fungi.*

As *Protococcus* flourishes in rain-water, and rain-water contains nothing but carbonic anhydride, which it absorbs along with other constituents of the atmosphere, ammonium salts (usually ammonium nitrate, also derived from the air) and minute portions of earthy salts which drift into it as dust, it follows that it must possess the power of constructing protein by rearrangement of the elements supplied to it by their compounds. *Torula*, on the other hand, is unable to construct protein matter out of such materials as these.

Another difference between *Torula* and *Protococcus* is only apparent: *Torula* absorbs oxygen and gives out carbonic anhydride; while *Protococcus*, on the contrary, absorbs carbonic anhydride and gives out oxygen. But this is true only so long as the *Protococcus* is exposed to sunlight. In the dark, *Protococcus*, like all other living things, undergoes oxidation and gives off carbonic anhydride; and there is every reason to believe that the same process of oxidation and evolution of carbonic anhydride goes on in the light, but that the loss of oxygen is far more than covered by the quantity set free by the carbon-fixing apparatus, of which the chlorophyll forms an essential part.

The multiplication of *Protococcus* takes place by means of actively locomotive spores, termed *zoospores*. These are of two kinds. The larger, or *macrozoospores*, are produced by the division of the contents of one of the ordinary cells into two or four portions. These portions assume a pear-like shape and become free by the resorption of the wall of the mother-cell. When they first escape the zoospores are naked protoplasmic bodies (*primordial* cells) destitute of any cell-wall. They swim actively through the water by means of two delicate processes of the protoplasm, termed *cilia*, which are inserted at the pointed end of the zoospore. The movement is of a double kind, the *progressive* motion, in which the pointed ciliated end goes foremost, being accompanied by a *rotation* of the zoospore about its long axis. The macrozoospore soon acquires a thin cell-wall, through which the cilia protrude. The wall does not remain in immediate contact with the protoplasm of the primordial cell, but becomes separated from it by a space containing clear cell-sap. This space is however traversed by fine radiating protoplasmic threads. The internal structure is similar to that of the vegetative cell, each zoospore containing a nucleus and chromatophores. The ciliated end consists of colourless protoplasm only. The movement of the cilia is so rapid, and their substance is so transparent and delicate, that they are invisible until they begin to move slowly, or are treated with reagents, such as iodine, which colour them, and arrest their movements.

The smaller, or *microzoospores*, are produced by the division of the vegetative mother-cell into a larger number of portions, the number being always some power of two. They differ from the macrozoospores, apart from their size, in never acquiring a cell-wall so long as their movement continues. In other respects the two kinds of zoospores agree.

Both eventually come to rest, assume a globular shape, lose their cilia and form a thick cell-wall around their protoplasm, thus reproducing the vegetative condition of the plant.

Both kinds of zoospores are sensitive to light, swimming towards the source of light, when its intensity is moderate, and away from it when its intensity is increased.

For reasons similar to those which prove the vegetable nature of *Torula*, *Protococcus* is a plant, although its zoospores are curiously similar to the Monads among the lowest forms of animal life. But it is now known that many of the lower plants, especially in the group of *Algæ*, to which *Protococcus* belongs, also give rise to locomotive spores propelled by cilia, like those of *Protococcus*, so that there is nothing anomalous in the case of the latter.

Like the yeast-plant, *Protococcus* retains its vitality after it has been dried. It has been preserved for as long as two years in the dry condition, and at the end of that time has resumed its full activity when placed in water. The wide distribution of *Protococcus* on the tops of houses and elsewhere, is thus readily accounted for by the transport of the dry *Protococci* by winds.

LABORATORY WORK.

A. **Morphology.**

a. *Vegetative stage.*

1. Spread out in water some mud from a gutter or similar locality, and put on a cover-glass. Look for the red or green Protococcus cells with a low power. Having found some, put on a high power and make out the following points.

Size; (measure)—very variable.

Form; more or less spheroidal, with individual variations.

Structure; cell-wall, protoplasm, chromatophores, sometimes a *vacuole,* always a *nucleus* containing a nucleolus.

Colour; generally green—sometimes red—sometimes half and half—sometimes centre red, periphery green—the colouring matter always in the protoplasm of the chromatophores only.

Draw carefully to scale.

2. Apply the methods of mechanical and chemical analysis detailed for *Torula.* (I. A. 3. 4. 5. 6.) The application of iodine is especially useful for bringing out the limits of the chromatophores, and also the nucleus and nucleolus. The same reagent will also often show the presence of some small starch-grains. Treat a specimen with strong iodine solution and then with sulphuric acid (75 per cent.): the cell-wall will become stained blue.

3. Look out for cells the contents of which are dividing up to form zoospores.

b. *Zoospores.*

a. Mount a drop of water containing zoospores of Protococcus, and examine with a high power. Note the actively locomotive green bodies, of which two varieties can be distinguished.

α. Cells of relatively large size derived from the vegetative cells by division of their contents into 2 or 4 portions. These are the *macrozoospores.* Observe the thin colour-

less cell-wall, surrounding the protoplasm, but separated from it by a clear space. Note in various specimens—The two cilia prolonged from the protoplasm through apertures in the cell-wall; their motionless part within the wall; their vibratile portion outside it. The colourless thin external layer of the protoplasm collected into a little heap at the point from whence the cilia arise. The delicate colourless processes radiating from the outer protoplasmic layer to the interior of the cell-wall. The colour—usually green, but frequently one bright red spot is present.

Here, as in the vegetative stage, the colouring matter is limited to the relatively large chromatophores, and here also a nucleus is present.

β. Cells of smaller size but much like the above if the cellulose sac were removed, and the radiating processes extending to it from the protoplasm withdrawn. These are the *micro-zoospores.*

b. Try to find specimens in which the movements are becoming sluggish, and see the cilia in motion.

c. Stain with iodine; this kills the cells, and stops their movements, and frequently renders the cilia very distinct.

d. Try to find zoospores which have come to rest, and are beginning to form a new cell-wall.

[B. PHYSIOLOGY.

1. Get some water that is quite green from containing a large quantity of Protococcus; introduce some of it into two tubes filled with and inverted over mercury, and pass a small quantity of carbonic anhydride into each: keep one tube in the dark and place the other in bright sunlight for some hours. Then measure the gas in each tube and afterwards introduce a fragment of caustic potash into each; the gas from the specimen kept in the dark will be more or less completely absorbed (= carbonic anhydride), that from the other will not be absorbed by the potash alone, but will be absorbed on the further introduction of a few drops of solution of pyrogallic acid (= oxygen). Protococcus, therefore, in the sunlight, takes up carbonic anhydride and evolves oxygen. A comparative experiment may be made with a third tube containing water but no Protococcus.

2. Place some water containing numerous zoospores of Protococcus near a window, exposed to bright sunlight. Observe that the majority of the zoospores accumulate on the side *away from* the source of light.

Next remove them to some distance from the window, or otherwise diminish the intensity of the light acting on them. Observe that now the zoospores accumulate on the side *towards* the light.

The zoospores of Protococcus are thus seen to be *photometric*, i.e. they seek or avoid the light according to the degree of its intensity.]

XI.

SPIROGYRA.

In ponds and tanks, the water of which is tolerably pure, we constantly find in summer floating masses, of a light green colour, which feel slippery when handled. A very superficial examination is sufficient to show that these masses consist of a vast number of very long and fine green threads. The organisms in question may belong to various genera of a group of plants called the *Confervoid Algae:* one of the genera, representatives of which are most commonly found, is *Spirogyra.*

On microscopical examination we find that each thread of Spirogyra consists of a single, unbranched, row of cylindrical cells, of very variable length, but of constant diameter throughout the filament. There is no distinction of apex and base; both ends of the filament are quite alike, and neither is attached to any substratum. We have to do here with a plant which is *morphologically* multicellular, for each thread consists of many cells. All these cells, however, are uniform in structure, and each performs for itself, so far as can be ascertained, all the functions of the plant. Hence Spirogyra may be spoken of as *physiologically* a unicellular plant. In each of the cells there are one or more (sometimes as many as 10) bright green spiral bands, and by these the genus may at once be recognized. Their number varies according to the species, which are very numerous, and in many cases hard to

distinguish. The most favourable for investigation are of course the larger ones, and among these the most convenient forms are those in which the spiral bands have somewhat lax windings, so that they do not interfere with the view into the interior of the cell.

Each cell of Spirogyra is bounded on the outside by a cellulose wall. This consists of a number of layers, which however can only be made out distinctly by the use of reagents such as potash. In many species the wall is surrounded by a thick gelatinous sheath. Immediately inside the cell-wall and closely applied to it is a layer of protoplasm, to which the name *primordial utricle* is applied. In Spirogyra this is so thin that it is difficult to make out under natural conditions except with a very high power. It can however be easily demonstrated by the use of what are termed *plasmolysing* re-agents, such as solution of common salt, which withdraw water from the interior of the cell, and thus cause its contents to contract. The contracted mass will then be seen to be bounded on all sides by a thin membrane, which is the primordial utricle. The latter is of precisely the same nature as the protoplasm in the cells of Yeast or Protococcus. Its occurrence in the form of a relatively thin parietal layer, while the greater part of the cavity of the cell is occupied by a large vacuole, is characteristic of most vegetable cells in their mature condition.

The green spiral bands correspond to the chromatophores in Protococcus, and bear the same name, each band being spoken of as a single chromatophore. Each cell may therefore contain one chromatophore, or several, according to the species. In these bodies all the chlorophyll of the plant is contained. They themselves consist of protoplasm and retain their form unaltered when the chlorophyll is extracted by alcohol or other solvents, and they are imbedded in the primordial utricle throughout their

whole course. At intervals along each chromatophore round bodies will be observed which appear green like the rest of the band, but on careful examination are each found to contain a small colourless mass of proteid substance, termed the *pyrenoid.* This pyrenoid is of a crystalline form, usually appearing hexagonal in optical section, and can be brought out more clearly by the use of staining reagents. Bodies of this nature are of very general occurrence in the chromatophores of the Algæ. It is only around the pyrenoids that starch is formed, and if the plant has been exposed to the light before examination, the pyrenoid will be found to be surrounded by a layer of small starch-grains often present in so great a mass that the band is distinctly swollen at the places where they occur.

As already mentioned the greater part of the interior of the cell is occupied by a single large vacuole, containing cell-sap, i.e. water in which certain inorganic salts, and certain organic substances, such as sugar and acids, are dissolved.

In addition to the structures already described each cell of Spirogyra contains a conspicuous *nucleus.* Its position varies somewhat in different species, for in some cases it is placed in the middle of the cell, while in others it is nearer to the external wall. Forms with a central nucleus are more convenient for observation. The shape of the nucleus also shows certain specific differences. A very common form is that of a biconvex lens, the edge of the lens being turned towards the lateral walls of the cell, and the circular faces towards its ends. The nucleus is surrounded by a layer of protoplasm, from which long processes radiate which traverse the vacuole and are attached at their further ends to those parts of the chromatophores in which the pyrenoids are situated. Both in these processes and in the primordial utricle delicate protoplasmic currents, can be detected, flowing in all directions (cf. Tradescantia p. 441).

The nucleus contains a relatively large *nucleolus*, the substance of which seems to be homogeneous, and somewhat resembles that of the pyrenoids in its reactions. The nucleus itself usually appears to be finely granular. Its real structure is however fibrous, but for the study of these details the nucleus of Spirogyra is not favourable.

The growth of Spirogyra in length is accompanied by the increase in number of its cells. This increase takes place by the repeated division of each cell into two equal parts, by means of a transverse wall; the process is not localized in any special region of the filament; all its cells are alike capable of division. In this fact Spirogyra differs from all the more perfect plants to be subsequently considered, and here also we have an indication of the physiological equivalence of the constituent cells of Spirogyra.

The process of cell-division takes place, under normal conditions, in the night, the products of assimilation which have been accumulated in the daytime being used up during the darkness to form new cell-wall and protoplasm. The division is however dependent on temperature, and by keeping the plants cold during the night it is possible to make them postpone their cell-division till the morning, when it can be conveniently observed. The nucleus divides into two, the daughter nuclei being at first connected by fine protoplasmic threads. In the meantime a transverse ring of cellulose is formed by the protoplasm around the cell, midway between its two ends. This ring is continuous, at its outer edge, with the lateral wall on which it abuts. By further additions from the protoplasm it gradually grows inwards until its inner edges meet, so that a complete disc of cellulose is formed across the cavity of the cell. The contents necessarily become constricted by the inward growth of the new cell-wall, and are eventually severed into two

distinct halves, each containing one of the daughter nuclei. The whole process, especially as regards the division of the nucleus, is a complicated one, but it is not necessary to enter into the details here. Cell-division, of which this may be taken as a type, is the general mode of formation of cells throughout the vegetable kingdom. In the higher plants however it is usual for the new wall to be formed simultaneously over its whole area, instead of by progressive growth from without inwards.

The *Reproduction* of Spirogyra takes place in quite a different way from that of the plants hitherto considered, and affords one of the simplest examples of a sexual process. In the form in which it here occurs this process is termed *conjugation*, which may be defined as the union of two externally similar cells to form a single reproductive cell. Conjugating specimens may be distinguished from vegetative ones by their more tangled filaments, and duller colour, and by feeling rougher when handled. When under cultivation Spirogyra can usually be induced to conjugate by allowing the water in which it is growing to evaporate very gradually. In the ordinary form of conjugation two filaments lying side by side send out short lateral protrusions, one from each cell. The protrusions from the one filament grow to meet those from the other, their contiguous walls become absorbed, and in this way the two filaments become connected by a number of transverse tubes, arranged like the rungs of a ladder. Changes now begin to take place in the contents of the cells, and it is at this stage that a difference first shows itself in the behaviour of the two filaments. In the one filament the contents of each cell contract away from the wall, so as to lie freely in the cavity of the cell, as a distinct sac bounded by the primordial utricle. In the other filament these changes take place somewhat later.

After a time the contents of the cells which first underwent contraction begin to move into the connecting tube. The entire contents pass over and fuse completely with those of the cell which receives them. Recent researches have shown that the corresponding constituents of the contents of each cell unite one with another, primordial utricle fusing with primordial utricle, chromatophore with chromatophore, and, which is the most important point, nucleus with nucleus.

The cell formed by conjugation is termed the *zygospore*. It is at first spherical in shape and destitute of any cell-wall of its own. It soon increases in size, by taking up water, assumes an elliptical outline, and becomes surrounded by a cell-wall. The latter consists, in its mature condition, of three layers, the middle one of which is of a brown colour, while those on either side of it are colourless. The wall is *cuticularized*, that is to say, it has undergone a chemical change which renders it almost wholly impermeable to water. Meanwhile changes go on in the contents of the zygospore, the most important being the appearance of oil in place of starch. In this condition the zygospore passes through a long stage of rest, during which it can withstand great extremes of temperature, and is not injured by periods of drought. On germination each zygospore bursts its cuticularized wall and grows out directly into a new plant.

We have seen that in the normal case of conjugation just described a distinct differentiation of the filaments into male and female individuals is shown, as all the cells of each of the conjugating filaments behave in the same way, either giving up their own contents or receiving those of the other thread. This sexual difference however has not yet become completely fixed, for in certain cases adjoining cells of one and the same filament conjugate with each other

This process, to which the name of *monœcious* conjugation may be applied, occurs in the same species which are usually reproduced by the normal ladder-like or dicœcious conjugation.

Some allies of Spirogyra show no demonstrable difference of sex between the conjugating cells, for the zygospore is formed in the connecting passage, each cell thus appearing to take an exactly equal part in the process.

The mode of reproduction in Spirogyra and its allies, simple as it is, may serve as the type of all sexual reproduction. Among the innumerable modifications under which the sexual process takes place, the one point which is found to be constant is the fusion of the nuclei of two distinct cells.

A few words on the *Physiology* of Spirogyra may be added. Its nutrition takes place in all important respects in the same way as that of Protococcus. The carbonaceous food of the plant is obtained by decomposition of the carbon dioxide dissolved in the water, and this of course only goes on under the influence of light. Starch is the first easily demonstrable product of this process, though it is certainly not the first product actually formed. It is easy to show that the formation of the starch-grains is dependent on the action of light. If the plant be kept for some hours in the dark all the starch will be found to have disappeared. A very short exposure to daylight is sufficient to induce its renewed formation. Under the action of direct sunlight a demonstrable amount of starch may be developed within so short a time as five minutes. It will be remembered that the starch-grains are formed around the pyrenoids. As regards the part played by the latter in the process we have no definite knowledge at present, but similar proteid crystalloids are found elsewhere in connection with starch-forming corpuscles.

Spirogyra will not of course grow in distilled water any more than will Yeast or Protococcus. If the water be supplied with the following salts the Spirogyra will be able to obtain all the food it requires: Potassium Nitrate, Calcium Sulphate, Magnesium Sulphate, Calcium Phosphate, Sodium Chloride, and Ferric Chloride. The sodium chloride is only of indirect service as neither of its elements is essential as a food-constituent. As regards the other elements required by the plant, we already know that Carbon, Hydrogen, and Oxygen are the elements of starch and cellulose, while the protoplasm and nucleus are built up of these elements with the addition of Nitrogen, Sulphur and Phosphorus. Iron is necessary for the formation of chlorophyll, while potassium plays an essential, though obscure, part in the process of assimilation. Calcium and Magnesium have been shown by experiment to be necessary for the nutrition of green plants, but their exact function is still doubtful.

LABORATORY WORK.

A. Morphology.

I. **Vegetative condition.**

1. Naked-eye characters.

   Observe that the masses of Spirogyra consist of long, delicate, bright green, unbranched filaments, which in the vegetative condition are quite smooth and glossy.

2. Microscopical characters.

   Mount one or two filaments of Spirogyra in water and observe first with the low, and then with the high power.

a. Note that each filament is composed of a series of cylindrical cells, that it is unbranched, and that all its cells are alike (apart from slight differences of length). Observe especially that there is no difference of structure between the two ends of the filaments.

b. In each cell observe

α. The cell-wall, completely surrounding the cell. It consists chiefly of cellulose and gives the same reactions as the cell-wall of Protococcus (p. 393). On treatment with potash solution, the *stratification* of the cell-wall can be made out with a high power.

β. The protoplasmic *primordial utricle*, completely lining the cell-wall on the inside. This is colourless and appears finely granular under a very high power. In order to see the primordial utricle better, *plasmolyse* the cell by running in 10 p. c. salt solution. The primordial utricle will now contract away from the cell-wall and be easily seen.

γ. The bright green spiral *chromatophores*. These are from 1 to 10 in number, according to the species. Observe the roundish bodies occurring at intervals in each chromatophore. These are the *pyrenoids*, each of which is surrounded by a layer of starch-grains if the plant has been exposed to light. Run in Iodine solution to demonstrate the presence of starch.

δ. The large vacuole occupying the greater part of the interior of the cell.

ε. The *nucleus* in the middle of the cell. In most

species its form is that of a biconvex lens, as described in the text. Inside it is the *nucleolus*. The nucleus is surrounded by a layer of protoplasm. Observe that strands of protoplasm radiate from the latter, crossing the cavity of the cell, and attached at their outer ends to those parts of the chromatophores where the pyrenoids occur.

ζ. The minute structure will be better made out by means of staining. Place some Spirogyra in saturated solution of Picric acid[1] for not less than 12 hours. Then wash thoroughly in distilled water. The chlorophyll will now have entirely disappeared. Stain the filaments thus prepared with Borax carmine solution[2] for an hour or more. Then mount in dilute glycerine[3] and examine with the highest power available. The cell-wall will be unstained. The protoplasm of the primordial utricle, chromatophores, and radial strands will have taken the stain very lightly. On the other hand the nucleus will be decidedly, and the nucleolus very deeply, coloured. The pyrenoids stain deeply, and their crystalline form can now be clearly made out. The layer of starch-grains surrounding each pyrenoid remains quite colourless. It is especially in material prepared in this way that the relation of the radial protoplasmic strands to the pyrenoids can be traced.

η. The process of *cell-division*, as described in the text, can be best observed by keeping the Spirogyra at a low temperature during the night, and

[1] Appendix, E.

[2] Appendix, E.

[3] The best plan is to mount in water and gradually run in glycerine under the cover-slip.

examining in a warm room the following morning. In cold weather it is sufficient to put the vessel containing the Spirogyra out-of-doors; in summer it may be surrounded with ice.

In mounting the filaments to observe the division it is necessary to support the cover-slip by means of two bristles or fragments of thin glass, so that it may not press on the object. The higher the power used the better.

II. **Reproduction.**

1. Look out for masses of Spirogyra in which the filaments are tangled, and of a dull-green colour, as described in the text. These will generally be found late in the summer, or at any time when the water in which the plant is growing is getting low.

2. Examine such filaments first with a low power, in order to find the various stages of conjugation. The details at each stage are then to be made out with the high power. Observe

   a. Pairs of parallel filaments which have begun to produce the lateral protrusion. Note that the protrusions in the filaments are formed opposite one another.

   b. Similar pairs in which the protrusions have already become continuous by absorption of their walls at the point of contact, so that they now form transverse tubes connecting the filaments. Note that at this stage the cells of the one filament show a contraction of their contents, while those of the other are unaltered.

   c. Stages in which the cell-contents of the one filament are passing over through the connecting tubes into the cells of the other.

The details of the fusion of the contents are best made out in material prepared and stained as described above (I. 2. *b.* ζ.). Without some such treatment the nuclei cannot be seen at this stage at all.

d. Stages of development of the *Zygospore*, formed from the united contents of the two cells. Observe its *form*, at first spherical, then oval, and the gradual thickening of its wall, which ultimately consists of 3 layers. Treat a ripe zygospore with Sulphuric acid. Observe that its wall is not dissolved, showing that it has become *cuticularized*. Observe the abundant oil-drops in the contents of the Zygospore.

B. PHYSIOLOGY.

1. Keep some filaments of Spirogyra in distilled water. Observe that although they may remain alive for some time, growth soon ceases entirely.

Place other filaments in a food-solution[1] containing the salts enumerated in the text. Under proper conditions of light and temperature active growth of the filaments and division of their cells will now take place.

2. Place a vessel containing Spirogyra in the dark for 12 hours. Then mount some filaments, test with Iodine, and observe that the starch has entirely disappeared.

Expose some of the filaments to as bright a light as possible. Observe again after a short interval (varying from 5 minutes to an hour or more according to the intensity of the illumination). Test with Iodine as before, and observe that starch-grains have again made their appearance around the pyrenoids.

[1] Appendix, E. For Spirogyra the solution should be considerably diluted.

XII.

BACTERIA (*Schizomycetes*).

UNDER these names a considerable variety of organisms, for the most part of extreme minuteness, are included.

They may be defined as globular, oblong, rod-like or spirally coiled masses of protoplasmic matter enclosed in a more or less distinct structureless membrane, in most cases devoid of chlorophyll and multiplying by transverse division. The smallest are not more than $\frac{1}{30000}$th of an inch in diameter, so that under the best microscopes they appear as little more than mere specks, and even the largest have a thickness of little more than $\frac{1}{10000}$th of an inch, though they may be very long in proportion. Many of them have two conditions—a still and an active state. In their still condition, however, they very generally exhibit that *Brownian* movement which is common to almost all very finely divided solids suspended in a fluid. But this motion is merely oscillatory, and is readily distinguishable from the rapid translation from place to place which is effected by the really active *Bacteria.*

In a large number of forms the presence of *cilia* has been detected. In *Spirillum volutans*, for example, one of the largest species, there is a cilium at each end of the spirally coiled body. In some cases it is probable that these cilia consist of protoplasm, and are really the organs by which

the movement is effected. In other Bacteria however, there is reason to believe that the so-called cilia are simply prolongations of the cell-membrane, and destitute of any power of independent movement. In the latter case the movement of the organism must be due to some other cause, such as the contractility of the protoplasmic body as a whole. Many forms, such as the *Vibriones*, so common in putrefying matters, appear obviously to have a wriggling or serpentiform motion, but this is an optical illusion. In this Bacterium the body does not rapidly change its form; but its joints are bent zigzag-wise, and the rotation of the zig-zag upon its axis, as it moves, gives rise to the appearance of undulatory contraction. A cork-screw turned round, while its point rests against the finger, gives rise to just the same appearance.

In other forms, however, such as Spirillum, true contractile curvatures have been observed.

There is still some doubt as to the presence of a nucleus. The delicate membrane of the Bacteria is in all cases surrounded on the outside by a gelatinous layer, often difficult of detection. In the still state, however, this gelatinous substance is often developed to a very large extent, forming a continuous mass of considerable size, in which the individual cells appear embedded. This is termed the *Zoogloea* form of the Bacteria. The membrane of the Bacteria, with its gelatinous layers, corresponds to the cell-wall of Protococcus or yeast. Its reactions, however, vary in different cases, sometimes agreeing with those of cellulose, and sometimes indicating an albuminous composition.

All forms of Bacteria, of which the life-history has been fully investigated, are found to produce resting *spores*. These bodies make their appearance when the supply of food has become exhausted or the conditions have otherwise

grown unfavourable. Either the spore may arise as a small cell in the interior of certain of the vegetative cells, or the latter may themselves become directly converted into spores. In either case the spore is characterized by its more granular protoplasm and firmer cell-wall. On germination, which may be delayed until after a long period of rest, the hard membrane is thrown off, and the protoplasm of the spore resumes the ordinary vegetative development.

Bacteria grow and multiply in Pasteur's solution (without sugar) with extreme rapidity, and, as they increase in number, they render the fluid milky and opaque. Their vital actions are arrested at the freezing point, but no temperature has yet been reached low enough to kill them. They thrive best in a temperature of about 30° C. but, in most fluids, they are killed by a temperature of 60° C. (140° F.). This, however, only applies to the vegetative condition, for the spores can in many cases resist a temperature very considerably over the boiling point of water.

In many points of their physiology Bacteria closely resemble *Torulæ;* and a further resemblance lies in the circumstance that many of them excite specific fermentative changes in substances contained in the fluid in which they live, just as yeast excites such changes in sugar.

All the forms of putrefaction which are undergone by animal and vegetable matters are fermentations set up by *Bacteria* of different kinds. Organic matters freely exposed to the air are, in themselves, nowise unstable bodies, and if due precautions have been taken to exclude *Bacteria* they do not putrefy, so that, as has been well remarked, "putrefaction is a concomitant not of death, but of life."

Bacteria, like *Torulæ* and *Protococci*, are not killed by drying up, and from their excessive minuteness they must

be carried about still more easily than *Torulæ* are. In fact there is reason to believe that they are very widely diffused through the air, and that they exist in abundance in all ordinary water and on the surface of all vessels that are not chemically clean. They may be readily filtered off from the air, however, by causing it to pass through cotton-wool.

The Bacteria have no near relationship to any of the other plants described in this book, and are introduced chiefly on account of the great importance of the various fermentative changes which they produce.

The life-history of the Bacteria is only completely known in comparatively few species. Some of them have been found to be highly *pleomorphic*, that is to say, the same species may appear under very different forms at different periods of its development. In other cases the life-history is comparatively simple. Hence the various forms mentioned in the Laboratory work must not be regarded as necessarily representing so many distinct species.

LABORATORY WORK.

1. Infuse some hay in warm water for half-an-hour—filter, and set aside the filtrate: note the changes which go on in it—at first clear, in 24 or 36 hours it becomes turbid; later on, a scum forms on the surface and the infusion acquires a putrefactive odour.
2. Rub some gamboge down in water and examine a drop of the mixture with a high power: avoid all currents in the fluid and watch the *Brownian movements;* note that they are simply oscillatory—not translative.

3. Take a drop of fluid from a turbid hay infusion—and examine it, using the highest power you have; in it will be found multitudes of

Moving Bacteria. Note their—

a. *Form;* elliptic or rodlike—sometimes forming short (2—8) jointed rows.

b. *Size;* breadth, very small but pretty constant; length, varying, but several times greater than their breadth: measure.

c. *Structure;* an outer more transparent layer enveloping less transparent matter: in the compound forms the envelope appears only where two joints come in contact, so that the rod looks as if made up of alternating transparent and more opaque substances.

d. *Movements;* some vital, and some purely physical (*Brownian*). The former various but progressive: the latter a rotatory movement round a stationary centre; study it in a drop of boiled infusion in which the Bacteria are all dead.

4. Treat with iodine—only the more opaque parts stain; probably then we have to do with protoplasm, enveloped in nonprotoplasmic matter.

5. **Resting Bacteria.** (*Zooglœa-stage.*)

a. Examine the scum from the surface of a hay infusion; it exhibits myriads of motionless Bacteria, embedded in gelatinous material.

b. Treat with iodine; the Bacteria stain as before: the gelatinous uniting material remains unstained.

6. Besides the form hitherto described, which would be known as *Arthrobacterium*, many other Schizomycetes will certainly be found, both in the pellicle and the fluid beneath. The following may be especially mentioned:

   a. **Micrococcus.** Bodies much like Arthrobacterium but short and rounded, and occurring singly, or in bead-like rows. They may be found free or in a Zoogloœa stage.

   b. **Bacillus.** Threads composed of straight cylindrical joints much longer than those of Arthrobacterium, but of similar structure: they are always free-swimming.

   c. **Vibrio.** Like Bacillus, but with bent joints.

   d. **Spirillum.** Elongated unjointed threads rolled up into a more or less perfect spiral: frequently two spirals interwine. In some of the largest forms a vibratile cilium can be made out on each end of the thread.

   e. **Spirochæte.** Much like Spirillum, but longer and with a much more closely rolled spiral. A very actively motile but not common form.

7. Examine various putrefying substances for different forms of Bacteria. Successful cultures may be made on hard-boiled white of egg, or slices of potato, kept moist under a bell-glass. Various brilliantly coloured micrococci including the blood-red "Micrococcus prodigiosus" will make their appearance on such cultures. An infusion of pea-flour, filtered, is especially favourable for Spirochæte.

8. Place some fresh-made hay infusion in three flasks; boil two of them for three or four minutes, and while one is boiling briskly stop its neck with a plug of cotton-wool and continue to boil for a minute or two: leave the necks of the other two flasks unclosed, and put all three away in a warm place.

a. In a day or two abundant Bacteria will be found in the unboiled flask.

b. In the boiled but unclosed flask Bacteria will also appear, but perhaps not quite so soon as in *a.*

c. In the flask which has been boiled and kept closed Bacteria will not appear, if the experiment has been properly performed, even if it be kept for many months.

XIII.

MOULDS (*Penicillium, Eurotium and Mucor*).

Torula, *Protococcus* and *Amœba* are extremely simple conditions of the two great kinds of living matter which are known as Plants and Animals. No plants, except perhaps the *Bacteria*, are simpler in structure than *Torula* and *Protococcus*, and the only animals which are simpler than *Amœbæ*, are essentially *Amœbæ* devoid of a nucleus and contractile vesicle. Moreover, however complicated in structure one of the higher plants may be in its adult state, when it commences its existence it is as simple as *Torula* or *Protococcus;* and the whole plant is built up by the fissive multiplication of the simple cell in which it takes its origin, and by the subsequent growth and metamorphosis of the cells thus produced. We have already seen that the like is true of all the higher animals. They commence as nucleated cells, essentially similar to *Amœbæ* and colourless blood-corpuscles, and their bodies are constructed by aggregations of metamorphosed cells, produced by division from the primary cell. It has been seen that *Torula* and *Protococcus*, similar as they are in structure, are distinguished by certain important physiological peculiarities; and the more complicated plants are divisible into two series, one produced by the growth and modification of cells which have the physiological peculiarities of *Torula* and contain no chlorophyll, while the other, and far larger, series contains chlorophyll,

and has the physiological peculiarities of *Protococcus*. The former series comprises the *Fungi*, the latter all other plants, only a few parasitic forms among these being devoid of chlorophyll.

The *Fungi* take their origin in *spores*, a kind of cells, which, however much they may vary in the details of their structure, are essentially similar to *Torulæ*. Indirectly or directly, the spore gives rise to a long tubular filament, which is termed a *hypha*, and out of these hyphæ the Fungus is built up.

One of the commonest Moulds, the *Penicillium glaucum*, which is familiar to every one from its forming sage-green crusts upon bread, jam, old boots, &c. affords an excellent and easily studied example of a Fungus. When examined with a magnifying glass, the green appearance is seen to be due, in great measure, to a very fine powder which is detached from the surface of the mould by the slightest touch. Beneath this lies a felt-work of delicate tubular filaments, the hyphæ, forming a crust like so much blotting-paper, which is the *mycelium*. From the free surface of the crust innumerable hyphæ project into the air and bear the green powder. These are the *aerial hyphæ*. On the other hand, the attached surface gives rise to a like multitude of longer branched hyphæ, which project into the fluid in which the crust is growing, like so many roots, and may be called the *submerged hyphæ*. If the patch of *Penicillium* has but a small extent relatively to the surface on which it lies, multitudes of silvery hyphæ will be seen radiating from its periphery and giving off many submerged, but few or no vertical, or subaërial branches. Submitted to microscopic examination, a hypha is seen to be composed of a transparent wall (which has the same characters as the cell-wall of *Torula*) and protoplasmic contents, which fill the tube

formed by the wall, and present large central clear spaces, or vacuoles. At intervals, transverse partitions, continuous with the walls of the tube, divide it into elongated cells, each of which contains a correspondingly elongated protoplasmic sac, or *primordial utricle*. Each cell contains a number of small nuclei, the presence of which can only be detected by the use of staining reagents. Thus in *Penicillium* we have our first example in the vegetable kingdom of *multinucleate cells*. The hyphæ frequently branch, and, in the crust, they are inextricably entangled with one another; but every hypha, with its branches, is quite distinct from every other. Those aerial hyphæ which are nearest the periphery of the crust end in simple rounded extremities; but the others terminate in brushes of short branches, and each of these branches, as it grows and elongates, becomes divided by transverse constrictions into a series of rounded spores arranged like a row of beads. The spores formed in this manner are termed *conidia*, and the hyphæ bearing them are termed *conidiophores*. At the free end of each filament of the brush the conidia become very loosely adherent, and constitute the green powdery matter to which reference has been made. Examined separately, a *conidium* is seen to be a spherical body, composed of a transparent sac, enclosing a minute mass of protoplasm and a nucleus, in all essential respects similar to a *Torula*. If sown in an appropriate medium, as for example Pasteur's solution, with or without sugar, the *conidium* germinates. Upon from one to four points of its surface an elevation or bulging of the cell-wall and of its contained protoplasm appears. This rapidly increases in length, and, continually growing at its free end, gives rise to a hypha, so that the young *Penicillium* assumes the form of a star, each ray being a hypha. The hyphæ elongate, while side branches

are developed from them by outgrowths of their walls; and this process is repeated by the branches, until the hyphæ proceeding from a single conidium may cover a wide circular area, as a patch of mycelium. When, as is usually the case, many conidia germinate close together, their hyphæ cross one another, interlace, and give rise to a papyraceous crust. After the hyphæ have attained a certain length, the protoplasm divides at intervals, and transverse septa are formed between the masses thus divided off from one another. But neither in this, nor in any other Fungus, are septa formed in the direction of the length of the hypha.

Very early in the course of the development of the mycelium, branches of the hyphæ extend downwards into the medium on which the mycelium grows; while, as soon as the patch has attained a certain size, the hyphæ in its centre give off vertical aërial branches, and the development of these goes on, extending from the centre to the periphery. The outgrowth of pencil-like bunches of branches at the end of these takes place in the same order; and these branches, becoming transversely constricted as fast as they are formed, break up into conidia, which are ready to go through the same course of development.

The conidia may be kept for a very long time in the dry state, without their readiness to germinate being in any way impaired, and their extreme minuteness and levity enable them to be dispersed and carried about by the slightest currents of air. The persistence of their vitality is subject to nearly the same conditions of temperature as that of yeast. Pullulating cells, resembling Torulæ, are not unfrequently derived from the conidia of Penicillium and many other of the lower Fungi, but they must not be confounded with true Yeast.

In Penicillium we have for the first time a differentiation

of the body of the plant into distinct organs. On the one hand we have the hyphæ, constituting the *vegetative* part of the organism, on the other the *conidia*, the function of which is purely *reproductive*.

The hyphæ again, are either *submerged* or *aerial*. The former may be described from a purely physiological point of view as the *root*, being characterized by the two functions of attaching the plant to the substratum, and of taking up food-material from it. The aërial hyphæ, on the other hand, in so far as they absorb oxygen from the air, and bear the reproductive organs, are analogous to the *shoot*, or leafy stem of the higher plants.

Besides the conidial organs of reproduction above described, Penicillium also occasionally produces reproductive structures of another kind, the development of which is far less simple. A complex truffle-like fruit (*cleistocarp*) is produced from them, but these phenomena will be more easily studied in another mould, the *Eurotium Aspergillus glaucus*. Though less abundant than Penicillium, this plant is of very common occurrence, especially on such substances as preserved fruits. Apricot jam, for example, may be used for growing the Eurotium upon. In the vegetative structure and the asexual reproduction there is little difference from Penicillium. All the hyphæ are stouter, and the conidiophores are unbranched. Each head of conidia can be distinguished separately with the naked eye, which is not the case with the more minute Penicillium. After the fungus has been growing for some weeks the cleistocarps will begin to make their appearance. They may be recognized as small round yellow bodies, easily distinguished from the heads of conidia by their colour and their sessile position. The development of these fruits is somewhat complicated. A branch grows out from one of the hyphæ,

and becomes spirally coiled, like a cork-screw. This branch is called the *ascogonium*. A second branch (sometimes termed the *pollinodium*) is formed in the immediate neighbourhood of the first. This applies itself closely to the ascogonium. Subsequently the ascogonium increases greatly in size, and produces a number of clavate branches called *asci*, in the interior of each of which eight spores are formed. In the meantime other branches, developing into closely interwoven hyphæ, arise from below the ascogonium, and form the dense envelope of the fruit. The production of spores in asci is characteristic of a very large group of Fungi. It is not probable that any act of fertilization occurs in this group, though the ascogonium and "pollinodium" of such Fungi as Eurotium have been regarded as sexual organs. The spores formed in the asci are termed *ascospores*, and reproduce the ordinary form of the Fungus.

If some bread be placed in a jar and kept very wet and moderately warm, its surface will, in two or three days, be covered with white cottony filaments, many of which rise vertically into the air, and end in rounded heads, so that they somewhat resemble long pins. The organism thus produced is another of the Fungi—the mould termed *Mucor stolonifer*.

Each rounded head is a *sporangium;* the stalk on which it is supported rises from one of the filaments which ramify in the substance of the bread, and are the *hyphæ*. In this species the hyphæ send out branches (the so-called "stolons") which grow out from the bread, and on reaching any substratum, such as the plate on which the bread rests, may produce a new crop of sporangia. Each hypha is, as in *Penicillium*, a tube provided with a tough thickish structureless wall, which is composed of a form of cellulose,

and is filled by a vacuolated protoplasm. In old specimens, transverse partitions, continuous with the walls of the hyphæ, may divide them into chambers or cells, but the plant is as a rule unicellular, or rather non-cellular, as regards its vegetative organs. The hyphæ contain very numerous nuclei. The stalk of the sporangium is a hypha of the same structure as the others. The wall of the sporangium is beset with minute asperities composed of oxalate of lime, and it contains a great number of minute oval bodies, the *spores*, held together by a transparent intermediate substance. When the sporangium is ripe, the slightest pressure causes its thin and brittle coat to give way, and the spores are separated by the expansion of the intermediate substance, which readily swells up and finally dissolves, in water. The greater part of the wall of the sporangium then disappears, but a little collar, representing the remains of its basal part, frequently adheres to the stalk. The cavity of the stalk does not communicate with that of the sporangium, but is separated from it by a partition, which bulges into the cavity of the sporangium, forming a central pillar or projection. This is termed the *columella* and stands conspicuously above the collar, when the sporangium has burst and the spores are evacuated.

The spores are oval and consist of a sac, having nearly the same composition as the wall of the hypha, which encloses a mass of protoplasm and a nucleus. When they are sown in an appropriate medium, as for example in Pasteur's solution, they enlarge, become spheroidal, and then send out several thick prolongations. Each of these elongates, by constant growth at its free end, and becomes a hypha, from which branches are given off, which grow and ramify in the same way. As all the ramifying hyphæ

proceed from the spore as a centre, their development gives rise, as in *Penicillium*, to a delicate stellate *mycelium*. At first, no septa are developed in the hyphæ, so that the whole mycelium may be regarded as a single cell with long and ramified processes, or as an organism which does not show a cellular structure at all. The distinction between this condition and the multicellular structure of Penicillium is not so sharp as it appears at first sight, for it has been shown that even in plants which consist of many cells the protoplasm is very generally continuous, through minute perforations in their walls. From near the centre of the mycelium a branch is given off from a hypha, rises vertically, and after attaining a certain length ceases to elongate. Its free end dilates into a rounded head, which gradually increases in size, until it attains the dimensions of a full-grown sporangium; and, at the same time, the protoplasm contained in this head becomes separated from that in the stalk by a septum, which is curved towards the cavity of the sporangium, and constitutes the columella. The wall of the sporangium, thus formed, becomes covered externally with a coat of oxalate of lime spines. As the sporangium increases in size, its protoplasmic contents become marked out into a large number of small oval masses, which are close together, but not in actual contact. Each of these masses next becomes completely separate from the rest, surrounds itself with a cellulose coat, and becomes a spore; while the protoplasm not thus used up in the formation of spores, appears to give rise to the gelatinous intermediate substance, which swells up in water, referred to above.' The walls of the spores become coloured, and that of the sporangium gradually thins, until it is reduced to little more than the outer crust of oxalate of lime. The sporangium

now readily bursts, and the spores are separated by the swelling and eventual dissolution of the gelatinous intermediate matter.

There appears to be no limit to the extent to which the *Mucor* may be reproduced by this process of *asexual* development of spores, by the fission of the contents of the sporangium; nor does any other mode of multiplication become apparent, if the mould be grown in a fluid medium and abundantly supplied with nourishment.

But when growing in nature, a method of reproduction is set up which represents the sexual process in its simplest form, such as we have already seen in Spirogyra. Adjacent hyphæ, or parts of the same hypha, give off short branches, which become dilated at their free ends, and approach one another, until these ends are applied together. The protoplasm in each of the dilated ends becomes separated by a septum from that of the rest of the branch; the two cells thus formed open into one another by their applied faces, and their protoplasmic contents becoming mixed together, form one spheroidal mass, to the shape of which the coalesced cell-membranes adapt themselves. Here, as in Spirogyra, the product of conjugation is termed a *zygospore*. Its cellulose coat becomes separated into an outer layer of a dark blackish hue and an inner colourless layer. The outer coat is raised into irregular elevations, to which corresponding elevations of the inner coat correspond.

Placed in suitable circumstances, the *zygospore* does not immediately germinate; but, after a longer or shorter period of rest, the walls burst, and a bud-like process is thrown out, which grows out into a mycelium. Under favourable conditions this may at once assume the normal development, but if the supply of food be scanty, the mycelium

remains rudimentary, and immediately gives rise to a sporangium, in which the asexual conidia are produced.

When *Mucor* is allowed to grow freely on such a substratum as bread, or at the surface of a saccharine liquid, it takes on no other form than that described; but, if it be submerged in the same liquid, the mode of development of the younger hyphæ becomes changed. They break up, by a process of constriction, into short lengths, which separate, acquire rounded forms, and at the same time multiply by budding after the manner of *Torulæ*. While these changes are in progress, an active fermentation is excited in the fluid, so that this "*Mucor-Torula*," functionally as well as morphologically, bears a resemblance to the yeast-plant, from which however its life-history shows it to be quite distinct.

If the *Mucor-Torula* is filtered off from the saccharine solution, washed, and left to itself in moist air, the "*Torulæ*" give off very short aërial hyphæ, which terminate in minute sporangia. In these a very small number of ordinary Mucor spores is developed, but, in essential structure, both the sporangia and the spores resemble those of normal *Mucor*.

LABORATORY WORK.

A. Penicillium.

Prepare some Pasteur's fluid, and leave it exposed to the air in saucers in a warm place; if *Penicillium* spores are at hand add a few to the fluid in each saucer: if spores cannot be obtained, the fluid, if simply left to itself, will probably be covered with *Penicillium* in ten days or a

fortnight. Sometimes, however, the fluid will overrun with *Bacteria*, to the exclusion of everything else. And very frequently other moulds, such as *Eurotium*, or *Mucor*, may appear instead of or along with *Penicillium*.

1. NAKED-EYE CHARACTERS. Note the powdery-looking upper surface, white in young specimens, pale greenish in older, and later still becoming dark sage-green: the smooth pale under surface: the dense tough character of the mycelium.

2. HISTOLOGICAL STRUCTURE.

a. The mycelium.

a. Tease a bit out in water, and examine first with low, and then with a high power: it is chiefly made up of interlaced threads or tubes—the

α. *Hyphæ.* Note their diameter (measure)—form—subdivisions (*cells*)—mode of branching—and structure: the external homogeneous cell-wall; the granular less transparent protoplasm; the small round vacuoles, the nuclei (only to be seen on treatment with alcohol, and staining with hæmatoxylin). Draw.

β. *The intermixed "Torulæ."* Note their size and number.

b. Hold a bit of the mycelium between two pieces of carrot, and cut a thin vertical section with a sharp razor: mount in water and examine with low and high power.

b. The submerged hyphæ.

Small branched threads hanging down from the under surface of the mycelium: repeat the observations

c. The aërial hyphæ and conidiophores.

Tease out in water a bit from the surface of one of the greenish patches; observe the difficulty with which water wets it. Examine with low and high power.

Note;—

α. The primary erect hypha.

β. Its division into a number of branches.

γ. The division of the terminal branches by constrictions into a chain of conidia. Draw.

d. The conidia.

a. Their *Size* (measure).

Form; spherical.

Structure; cell-wall, protoplasm, vacuole, and nucleus.

b. Stain different specimens with magenta, hæmatoxylin, and iodine, to bring out the above points of structure.

c. Treat another specimen with potash. The cell-contents gradually disappear.

e. The germination of the Conidia, and building up of the Mycelium.

a. Sow some conidia in Pasteur's fluid in a watch-glass; protect from evaporation, and watch the development of the mycelium (examine the surface with a low power); then the formation of aërial hyphæ; finally the production of new conidia.

b. Sow Conidia in Pasteur's fluid in a moist chamber, and watch from day to day; note the formation of eminences at one or more points on a conidium; the elongation of these eminences to form hyphæ; the branching and interlacement of the hyphæ.

B. EUROTIUM ASPERGILLUS-GLAUCUS.

1. Place some apricot jam under a bell-jar and keep moist and warm. In a few days the Eurotium will probably make its appearance. A crop of conidia will first be produced. Neglect these, and look out, after some weeks, for the *cleistocarps*, as described in the text.

2. Mount some of the mycelium and fruits in water, and observe first with a low and then with a high power. Examine

a. The mature fruits. These are approximately spherical in form. Observe the outside of the fruit formed of dense cellular tissue. *Burst* some of the fruits by pressure on the coverslip. Observe the asci in the interior, each containing eight ascospores.

b. The development of the fruit. Make out the spirally coiled branch forming the ascogonium, and the "pollinodium" in contact with it. Observe the outgrowth of hyphæ from below the ascogonium, to form the envelope of the fruit.

C. MUCOR STOLONIFER.

1. Place some bread under a bell-jar and keep very moist and warm; in from 24 to 48 hours its surface will nearly always be covered by a crop of erect aërial mucor-hyphæ, each ending in a minute enlargement (*sporangium*) just visible with the unassisted eye.

2. Snip off a few of the hyphæ with a pair of scissors, mount in water, and examine with 1 inch obj.

a. Large unbranched hyphæ, each ending in a spherical enlargement (*sporangium*).

3. Examine with $\frac{1}{8}$ obj.

a. The hyphæ.

α. *Their size;* they greatly exceed the hyphæ of *Penicillium* both in length and diameter.

β. *Their structure;* homogeneous cell-wall, granular protoplasm, vacuoles; septa absent except close to the sporangium.

γ. Treat with iodine and magenta or hæmatoxylin; the protoplasm is stained and nuclei may be made out.

δ. Treat another specimen with Schulze's solution; the cell-wall is stained violet.

b. The sporangia.

Examine with $\frac{1}{8}$ obj.

a. *Their size* and *form.*

b. *Their structure.*

α. The homogeneous enveloping cell-wall, covered by irregular masses of calcic oxalate.

β. The granular protoplasmic contents: unsegmented in some; divided into a great number of distinct oval masses (*spores*) in others.

γ. The projection into the sporangial cavity of the convex septum (*columella*) which separates the hypha from the sporangium.

δ. The *collar* projecting around the base of the columella of burst sporangia.

c. Stain some with iodine; others with Schulze's solution.

c. The spores or conidia.

a. Crush some ripe sporangia by gentle pressure upon the cover-glass. Examine with $\frac{1}{8}$ obj.

α. The size of the spores (*measure*).

β. Their form; cylindrical and elongated.

γ. Their structure.

δ. Stain with iodine and magenta or hæmatoxylin.

XIV.

STONEWORTS (*Chara and Nitella*).

THESE water-weeds are not uncommonly found in ponds and rivers, growing in tangled masses of a dull green colour. Each plant is hardly thicker than a stout needle, but may attain a length of three or four feet. One end of the *stem* is fixed in the mud at the bottom, by slender thread-like roots, the other floats at the surface. At intervals, *appendages*, consisting of *leaves*, *branches*, *root-filaments*, and *reproductive organs*, are disposed in circles, or *whorls*. In the middle and lower parts of the plant these whorls are disposed at considerable and nearly equal distances; but, towards the free upper end, the intervals between the whorls diminish, and the whorled appendages themselves become shorter, until, at the very summit, they are all crowded together into a terminal bud, which requires the aid of the microscope for its analysis.

The parts of the stem, or *axis*, from which the appendages spring are termed *nodes;* the intervening parts being *internodes*. When viewed with a hand-magnifier the internodes exhibit a spiral striation.

In *Chara*, each internode consists of a single, much-elongated cell, which extends throughout its whole length, invested by a *cortical layer*, composed of many cells, the spiral arrangement of which gives rise to the superficial marking which has been noted. And this multicellular

structure is continued from the cortical layer, across the stem, at each node. The stem therefore consists of a series of long, axial cells, contained in as many closed chambers formed by the small cortical cells. The nodes are the multicellular partitions between these chambers. The branches are altogether similar in structure to the main stem. The leaves are also similar to the stem, so far as they consist of axial and cortical cells, but they differ in the form and proportions of these cells, as well as in the fact that the summit, or free end, of the leaf is always a much-elongated pointed cell. The most important distinction, however, consists in the fact that the growth of the leaves is *limited* while that of the axis is unlimited. The branches spring from the acute angle between the stem and the leaf, which is termed the *axil* of the leaf; and, in the same position, at the fruiting season of the plant are found the reproductive organs. These are of two kinds, the one large and oval, the *oogonia*, the other smaller and globular, the *antheridia*. Both, when ripe, have an orange-red colour, and are seated upon a short stalk

If a growing plant be watched, it will be found that it constantly increases in length in two ways. New nodes, internodes, and whorls of appendages are constantly becoming obvious at the base of the terminal bud; and these appendages increase in size and become more and more widely separated, until they are as large and as far apart as in the oldest parts of the plant. The appendages at first consist exclusively of leaves and root-filaments (*rhizoids*), and it is only when these have attained their full size, that branches, oogonia and antheridia are developed in their axils. Sometimes rounded cellular masses appear in the axils of the leaves, and, becoming detached, grow into new plants. These are comparable to the *bulbs* of higher plants.

If the innermost part of the terminal bud, which constitutes the free end of the axis, or stem, be examined, it will be found to be formed by a single nucleated cell, separated by a transverse septum from another. The former of these is the *apical cell*, from the divisions of which all the other cells are derived; the latter is the last segment cut off from it. Beneath this last follows another cell, which has already undergone division into several smaller cells by the development of longitudinal septa. This is the most newly-formed node. Below this again is a single cell, which is both longer and broader than those at the apex, and is an internodal cell. Each nodal cell is the sister-cell of the internodal cell next below it, both having arisen by the transverse division of a single segment. Next follows another node, composed of more numerous small cells than the first. Some of the peripheral cells of this node are undergoing growth and division, and thus give rise to cellular prominences, which are rudiments of the first whorl of leaves. In the still lower parts of the stem the internodal cells get longer and longer, but they never divide. The nodal cells, on the other hand, multiply by division, but do not greatly elongate. From the first, the nodal cells overlap the internodal cell, so as to meet round its equator, and thus completely invest it externally. And, as the internodal cell grows and elongates, the overlapping parts of the nodes increase in length and become divided into internodal and nodal cells, which take on a spiral arrangement, and thus give rise to the cortical layer.

Thus the whole plant is composed of an aggregation of simple cells, just as is the case with *Hydra* among animals; and, while it lives, new nodes and internodes are continually being added at its summit, or *growing point*. The internodal cells which give rise to the middle of the stem undergo no important change, except great increase of size, after they are once formed. The nodal cells, on the con-

trary, undergo division with comparatively little increase in size. And out of them, the nodes, the cortical layer, and all the appendages, are developed.

In all the young cells of *Chara* a *nucleus* of relatively large size is to be seen imbedded in the centre of the protoplasm, and the latter is enclosed in a structureless cell-wall, composed of cellulose. As the cell grows larger, the centre of the protoplasm becomes occupied by a watery fluid, and its thick periphery, which remains applied against the cell-wall, constitutes a sac, or *primordial utricle*, in which the nucleus is imbedded. In the larger cells the primordial utricle is readily detached and made to shrivel up into the middle of the cell by treatment with strong alcohol. In the older cells the nucleus divides up into a number of portions.

Numerous small green bodies—*chlorophyll grains*—are imbedded in the outer, or superficial, part of the primordial utricle, and they increase in number by division, as the cell enlarges. These chlorophyll grains are composed of protoplasmic matter, which frequently contains starch granules, and is impregnated with the green colouring substance.

During life, the layer of the primordial utricle which lies next to the watery contents of all the larger cells is in a state of incessant rotatory motion, while the outermost layer which contains the chlorophyll grains is quite still. In the large cells, so long as the nucleus is discernible, it is carried round with the rotating stream.

The *antheridium* is a globular spheroidal body with a thick wall, made up of eight pieces, which are united by interlocking edges. The four pieces which make up the hemisphere to which the stalk of the antheridium is attached, are foursided, the other four are triangular. From the centre of the inner, concave face of each piece a short process, the handle or *manubrium*, projects into the cavity of the hollow sphere. At the free end of the manu-

brium is a rounded body, the *capitulum*, which bears six smaller *secondary capitula;* and each secondary capitulum gives attachment to four long filaments divided by transverse partitions into a multitude (100 to 200) of small chambers. Thus, there may be as many as 20,000 to 40,000 chambers in each *antheridium* ($8 \times 6 \times 4 \times 100$ or $\times 200$). The several pieces of which the wall of the antheridium is composed, the manubria, the capitula, the secondary capitula and the chambers of the filaments are all more or less modified cells, as may be proved by tracing the antheridia from their earliest condition, as small processes of the nodal region, to their complete form. The cells of the filaments are, at first, like any other cells; but, by degrees, the nucleus of each becomes changed into a thread-like body, thicker at one end than at the other, and coiled spirally like a corkscrew. From the thin end two long cilia proceed; and, when the cells have burst, and the spermatozoids are set free, they are propelled rapidly, with the small end forwards, by the vibration of the cilia. These spermatozoids answer to the spermatozoa of animals, and represent the male element of *Chara.*

The oogonia are borne upon short stalks, the end of which supports a large oval central cell or *ovum;* five spirally-disposed sets of cells invest this, an aperture being left between the investing cells at the apex of the oogonium. When the antheridia attain maturity they burst, the spermatozoids are set free, and swarm about in the water. Some of them enter the aperture of the oogonium, and, in all probability, pierce the free summit of the oval central cell, and enter its protoplasm; but all the steps of this process of impregnation have not been worked out. The result, however, is, that the contents of the impregnated central cell now called the *oospore* become full of starchy and oily

matter; the spiral cells forming its coat acquire a dark colour and hard texture, and the oogonium, detaching itself, falls into the mud.

After a time the oospore germinates; a tubular process, like a hypha, protrudes from it through the aperture between the investing cells and almost immediately gives off a branch, which is the first root. The tube elongates, and becomes divided transversely into cells, the protoplasm of which developes chlorophyll. Very soon, the further growth of this *pro-embryo* is arrested. But one of the cells, which lies at some distance below the free end of the pro-embryo, undergoes budding, and gives rise to a set of leaves (which are not arranged in a whorl), amidst which a bud appears, which has the structure of the terminal bud of the adult *Chara* stem, and grows up into a new *Chara*-plant.

We have then, in *Chara*, a plant which is *acrogenous* (or grows at its summit), and which becomes segmented by the development of appendages, at intervals, along an axis; which multiplies, asexually by bulb-like buds, and also multiplies sexually by means of the spermatozoids (male elements) and central cells of the oogonia (female elements); in which the first product of the germination of the oospore is a simple filament, from which the young *Chara* is developed by the germination and growth of one cell; so that there is a sort of *alternation of generations*, though the alternating forms are not absolutely distinct from one another.

Chara flourishes in pond-water under the influence of sunlight, and by the aid of its chlorophyll CO_2 is decomposed, so that its nutritive processes must be the same as those of *Protococcus*. From its complete immersion, and the absence of any duct-like, or vascular tissues, it is probable that all parts absorb and assimilate the nutriment contained

in the water; and that, except so far as the reproductive organs are concerned, there is a morphological differentiation of organs, unaccompanied by a corresponding physiological differentiation.

Nitella is a rarer plant than *Chara*, and is simpler in structure, its axis being devoid of the cortical layer. In other respects, however, it is very similar to *Chara*, and its structure is more easily made out.

[The *Characeæ*, or plants belonging to the genera *Chara* and *Nitella*, are found in all parts of the world, and belong to the class of *Algæ*, which also includes the sea-weeds.]

LABORATORY WORK.

A. NAKED-EYE CHARACTERS.

Note the slender elongated axis (*stem*); the whorled appendages (*leaves*); the *nodes* and *internodes;* the shortening of the latter towards the apex of the stem; the *rhizoids.*

a. *The roots;* small; serving chiefly for attachment, the plant getting most of its nutrition, through other parts, from matters dissolved in the water.

b. *The leaves;* their sub-divisions (*leaflets*); their form, size, &c.

c. *The oogonia* and *antheridia;* their position, size, form, colour.

Draw a portion including two or three internodes.

B. HISTOLOGICAL STRUCTURE.

a. The stem.

1. Examine the outside of a fresh internode with a low power, or pocket lens, to see the spirally-arranged cortical cells.

2. Hold a bit of fresh stem between two pieces of elder-pith or imbed it in paraffin, and, with a sharp razor, cut thin transverse and longitudinal slices through nodes and internodes. Note the cavity of the large central *internodal* cell; the *cortical* cells, round the internodal cell; the *nodal* cells, and the interruption of the central cavity at the nodes.

3. Examine similar sections in specimens treated with spirit, and also preparations made by teasing or pressing out in glycerine bits of stem from chromic acid (0·2 per cent.) preparations: make out in these,—

   α. The nodal, internodal, and cortical cells.

   β. The wall, protoplasmic layer (*primordial utricle*), nucleus, and vacuole of each cell. (The nucleus will have undergone fragmentation in the older cells.)

4. Examine sections from the fresh stem to make out the points detailed in B. **a.** 3. β. The protoplasm and nucleus are difficult to see. Note the chlorophyll-granules. (See B. **b.** γ.)

5. Stain sections of the fresh stem with iodine, and with magenta: note the results.

b. The leaves.

Examine fresh and chromic acid specimens.

α. The large uncovered terminal cell.

β. Then a series of internodal cells, separated from one another, and covered-in, by nodal cells: the cell-wall, protoplasm, nucleus, and vacuole of each.

γ. The oval *chlorophyll-granules*, arranged so as to leave an oblique uncoloured band round

each cell; the position of these granules, in the more superficial layer of the protoplasm.

δ. The protoplasmic movements (see C. *a*.).

c. The terminal bud.

Dissect out chromic acid specimens as far as possible with needles, and then press gently out in glycerine. Note in different specimens—

a. The terminal or apical cell:

α. Its *form:* hemispherical, the rounded surface free; the flat surface attached to the cell below it.

β. *Structure:* cell-wall, protoplasm, nucleus; no vacuole present.

γ. Sometimes two nuclei, preliminary to division.

δ. Its mode of division; across the long axis of the stem, giving rise to two superimposed nucleated cells.

b. The further fate of the new cells derived from the segments of the terminal cell; work back in your specimens from the terminal cell.

α. The new cells are successively *nodal* and *internodal,* one nodal and one internodal cell being formed from each segment; the internodal cells enlarge, develope a large vacuole, and ultimately form the medullary cells of the internodes; they never divide.

β. The nodal cells divide freely, and do not increase much in size; they give origin to the nodes and the cortical cells.

c. The development of leaves: by the multiplication and outgrowth of nodal cells.

d. Their growth at the base, the terminal leaf-cell soon attaining its full size and not dividing.

e. *The development of branches;* from nodal cells in leaf-axils, which take on the character of terminal cells.

d. The oogonia.

Examine fresh, under a low power.

α. Made up externally of five twisted cells, bearing at their apices five smaller, not twisted cells.

β. Cut sections from embedded specimens, and examine with a high power: make out the large central nucleated cell or ovum; the fatty and starchy matters contained in it; stain with iodine.

γ. Press out chromic acid specimens in glycerine; make out the above points (**d.** *α*, *β*).

δ. Examine chromic acid specimens for young oogonia, and press them out in glycerine: make out in the youngest the five roundish cells surrounding a central one; then in older specimens the elongation, and twisting of the external cells, and the separation of their apices as five distinct cells.

e. The antheridia.

a. Examine, with a low power, a ripe (orange-coloured) one.

α. Make out its external dentated cells.

β. Tease out a ripe antheridium in water; and examine with a high power; note the flat, dentated, nucleated external cells; the cylindrical cell (*manubrium*) springing perpendicu-

larly from the inner surface of each; the roundish cell (*capitulum*) on the inner end of the manubrium; the six *secondary capitula* attached to the capitulum; the thread-like filaments (usually four) proceeding from each of the secondary capitula.

γ. The structure of these threads; each consists of a single row of cells, containing in unripe specimens nucleated protoplasm; in older specimens each contains a coiled-up *spermatozoid*.

b. *The spermatozoids* (or antherozoids).

α. Their form and structure; thickened at one end and granular; tapering off gradually towards the other end, which is hyaline and has two long cilia attached to it.

β. The movements in water of ripe spermatozoids.

[Sometimes Chara cannot be obtained, when Nitella, another genus of the same Natural Order, and of similar habit and structure, can. Nearly all the points above described for Chara can be made out in Nitella, with the following differences: the cortical cells of the stem and leaves are absent, and, in the commoner species, the plant is not hardened by calcareous deposit; the branches arise, not *one* from a whorl of leaves, but *two*; and the five twisted cells of the spore-fruit are each capped by two small cells, instead of one.]

C. PROTOPLASMIC MOVEMENTS IN VEGETABLE CELLS.

a. **Chara.** Take a vigorous-looking fresh Chara or Nitella cell (say the terminal cell of a leaf), and

examine it in water with a high power. Note the superficial layer of protoplasm in which the chlorophyll lies; it is stationary: focus through this layer and examine the deeper one; note the currents in it, marked by the granules they carry along: their *direction;* in the long axis of the cell, up one side and down the other, the boundary of the two currents being marked by the colourless band, in which no movements occur. Try to find the nucleus; it has usually undergone fragmentation in cells in which currents have commenced. The original nucleus or the products of its division are passively carried along by the currents. Sometimes it is very difficult, on account of the incrustation of the leaf-cells of Chara, to make out the protoplasmic movements in them; if this is found to be the case, the manubrial cells from an antheridium should be used instead.

b. **Tradescantia.** Examine in water, with a high power, the hairs which grow upon the stamens: they consist of a row of large roundish cells, each with cell-wall, protoplasm, nucleus, and vacuolar spaces. Note the protoplasm; partly forming a layer (*primordial utricle*) lining the wall and heaped up round the nucleus, and partly forming bridles running across the cell in various directions from the neighbourhood of the nucleus, and from one part of the protoplasm to another; observe the currents in these bridles; from the nucleus in some, towards it in others. Sometimes there are two currents in opposite directions in the same strand.

Observe also the various currents in the protoplasm of the primordial utricle.

c. **Vallisneria.** Take a leaf beginning to look old; split it into two layers with a sharp knife and mount a bit in water; examine with a high power. Note the larger rectangular cells, belonging to the deeper layers, with well-marked currents in them, which carry the chlorophyll granules round and round inside the cell-wall.

If no currents are seen at first, gently warm the leaf by immersing it for a short time in water heated to a temperature between 30° and 35° C.

d. **Anacharis*.** Take a yellowish-looking leaf: mount in water and examine with a high power; the phenomena observed are like those in Vallisneria. They are best observed in the single layer of cells at the margin of the leaf.

e. **Nettle-hair.** Mount an uninjured hair in water with the bit of leaf to which it is attached (it is essential that the terminal recurved part of the large cell forming the hair be not broken off); examine with the highest available power: currents carrying along very fine granules will be seen in the cell, their general direction being that of its long axis.

* Also called *Elodea*.

XV.

THE BRACKEN FERN (*Pteris aquilina*).

THE conspicuous parts of this plant are the large green leaves, or *fronds*, which rise above the ground, sometimes to the height of five or six feet, and consist of a stem-like axis or *rachis*, from which transversely disposed offshoots proceed, these ultimately subdividing into flattened leaflets, the *pinnules*. The rachis of each frond may be followed for some distance into the ground. Its imbedded portion acquires a brown colour, and eventually passes into an irregularly branched body, also of a dark-brown colour, which is commonly called the root of the fern, but is, in reality, a creeping underground stem, or *rhizome*. From the surface of this, numerous filamentous true roots are given off. Traced in one direction from the attachment of the frond, the rhizome exhibits the withered bases of fronds, developed in former years, which have died down; while, in the opposite direction, it ends, sooner or later, by a rounded extremity beset with numerous fine hairs, which is the apex, or growing extremity, of the stem. Between the free end and the fully formed frond one or more processes, the rudiments of fronds, which will attain their full development in following years, are usually found.

The stem branches in two ways; 1st by apparent dichotomy of the terminal growing point, and 2nd by the forma-

tion of adventitious buds. The latter are produced singly on the dorsal side of the leaf-stalks, near the base.

The attachments of the fronds are nodes, the spaces between two such successive attachments, internodes. It will be observed that the internodes do not become crowded towards the free end, and there is nothing comparable to the terminal bud of Chara with its numerous rudimentary appendages.

When the fronds have attained their full size, the edges of the pinnules will be observed to be turned in towards the underside, and to be bordered by a fringed membrane called the *indusium*, which roofs over the groove enclosed by the incurved edge. At the bottom of the groove brown granules are aggregated in large numbers, so as to form a streak along each side of the pinnule. The granules are the *sporangia*, and the streaks formed by their aggregation, the *sori*.

Examined with a magnifying glass, each *sporangium* is seen to be pouch-shaped, like two watch-glasses united by a thick rim. When ripe, it has a brown colour, readily bursts, and gives exit to a number of minute bodies which are the *spores*.

The plant now described is made up of a multitude of cells, having the same morphological value as those of *Chara*, and each consisting, at least when young, of a protoplasmic mass, a nucleus and a cellulose wall. These cells, however, become very much modified in form and structure in different regions of the body of the plant, and give rise to groups of structures called *tissues*, in each of which the cells have undergone special modifications. These tissues are, to a certain extent, recognizable by the naked eye. Thus, a transverse section of the rhizome shews a *circumferential zone* of the same dark-brown colour as the external

epidermis, enclosing a white *ground-substance*, interrupted by variously disposed *bands*, *patches*, and *dots*, some of which are of the same dark-brown hue as the external zone, while others are of a pale yellowish-brown.

The dark-brown dots are scattered irregularly, but the greater part of the dark-brown colour is gathered into two narrow bands, which lie midway between the centre and the circumference. Sometimes the ends of these bands are united. Enclosed between these narrow, dark-brown bands are, usually, two elongated, oval, yellowish-brown bands; and, outside them, lie a number of similarly coloured patches, one of which is usually considerably longer than the others.

A longitudinal section shews that each of these patches of colour answers to the transverse section of a band of similar substance, which extends throughout the whole length of the stem; sometimes remaining distinct, sometimes giving off branches which run into adjacent bands, and sometimes uniting altogether with them.

At a short distance below the apex of the stem, however, the colour of all the bands fades away, and they are traceable into mere streaks, which finally disappear altogether in the semi-transparent soft tissue which forms the growing end of the stem. Submitted to microscopic examination, the white ground-substance is seen to consist of large polygonal *cells*, containing numerous starch granules; this tissue is called the *ground-parenchyma*. The circumferential zone is formed of somewhat elongated cells, containing little or no starch, the thick walls of which have acquired a dark-brown colour. These cells constitute the *epidermis* and *hypoderma*. The dark-brown bands, on the other hand, consist of cells which are so much elongated as almost to deserve the name of *fibres*. Their walls are very thick and of a deep-brown

colour; but the thickening has taken place unequally, so as to leave short, obliquely directed, thin places, which look like clefts and are called *pits*. This tissue is termed *sclerenchyma*. The yellow bands, lastly, are *vascular bundles*[1] or *cylinders*. Each cylinder is surrounded on the outside by a layer of rather thick-walled, elongated, parallel-sided cells, constituting the *endodermis*. The cylinder itself consists of two main parts; a central portion, constituting the *xylem* or *wood*, and a peripheral portion which is the *phloem* or *bast*. The xylem consists chiefly of *vessels*, many of them of relatively large size. They are derived from cells, the transverse walls between which have been partially broken down. In the mature vessels the protoplasmic contents have entirely disappeared; they only contain water or air. Their walls are greatly thickened, the thickening having taken place along equidistant transverse lines, the thin spaces left between them being the pits. The vessels have become flattened against one another, by mutual pressure, so that they are five- or six-sided; and, as the markings of their flattened walls simulate the rounds of a ladder, they have been termed *scalariform vessels*. The cavities of these scalariform vessels are divided at intervals, in correspondence with the lengths of the cells of which they are made up, by oblique, often perforated partitions. In most Ferns there is no such perforation. Among the smaller vessels, a few will be found, in which the thickening forms a closely wound spiral. These are *spiral vessels*. They usually occur in two groups, and as they are the first elements of the xylem to be differentiated, they are said to constitute the *protoxylem*

[1] These "vascular bundles" in the Fern do not correspond to the structures of the same name, to be described below in the Broad-bean. Each "bundle" in the Fern-stem is equivalent to the whole "central-cylinder" of the Broad-bean, or of the Fern-*root*.

of the bundle. Among the vessels a few parenchymatous cells containing starch are scattered. The *phloem* is at once distinguished from the xylem by the smaller average size of its cells, and their thinner walls. The most conspicuous elements of the phloëm are the *sieve-tubes*, which consist of long cells, the lateral walls of which show groups of minute perforations. The outer layer of the phloëm is formed of long narrow cells, with comparatively thick walls. These cells are developed before the rest, and are hence termed the *protophloem*. Between the phloëm and the endodermis is a layer of parenchymatous cells containing starch, termed the *phloem-sheath*.

The rachis of a frond, so far as it projects above the surface of the ground, is of a bright green colour; and, in transverse section, it presents a green ground-substance, interrupted by irregular paler markings, which are the transverse sections of longitudinal bands of a similar colour. There are no brown spots or bands. Examined microscopically, the ground-substance is found to be composed of polygonal cells containing chlorophyll. These are invested superficially by an *epidermis*, composed of elongated cells. The pale bands are vascular bundles, of similar structure to those in the stem, with which they are continuous.

The vascular bundles, the green parenchyma, and the epidermis are continued into each pinnule of the frond. The epidermis retains its ordinary character on the upper side of the pinnule, except that the contours of its component cells become somewhat more irregular. On the under side, many hairs are developed from it, and the cells become singularly modified in form, their walls being thrown out into lobes, which interlock with those of adjacent cells.

Between certain epidermal cells on the under surface an oval space is left, forming a channel of communication between the interior of the frond and the exterior. The

opening of this space is surrounded by two kidney-shaped cells, the concavities of which are turned towards one another, while their ends are in contact. The opening left between the applied concave faces is a *stoma*, and the two cells are the *guard-cells*, and, as the *stomata* are present in immense numbers, there is a free communication between the outer air and the *intercellular passages* which exist in the substance of the frond. Those cells of the green parenchyma of the frond which form the inferior half of its thickness, in fact, are irregularly elongated, and frequently produced into several processes, or stellate. They come into contact with adjacent cells only by comparatively small parts of their surfaces, or by the ends of these processes. They thus bound passages between the cells, *intercellular passages*, which are full of air, and are in communication with similar, but narrower, passages, which extend throughout the substance of the plant.

The vascular bundles break up in the pinnules, and follow the course of the so-called *veins*, which are visible upon its surface; ducts being continued into their ultimate ramifications.

The growing point of the stem terminates in a single *apical cell*, by the divisions and subdivisions of which all the tissues of the stem and leaves are formed.

Each *root* presents an outer coat of epidermis, bearing a number of unicellular *root-hairs*, and enclosing parenchymatous and sclerenchymatous tissues traversed by a central vascular cylinder. The latter contains the same elements as are found in the bundles of the stem, but the xylem and phloëm have a different arrangement. The roots, like the stem, develop by means of an apical cell at the growing point, but this is not situated at the extreme end of the organ, as the growing point of the rhizome is, but is covered by a *root-cap* of protective cells.

The nutrition of the Fern takes place essentially as in the other chlorophyll-containing plants previously considered. The root-hairs constitute the organs for the absorption of water and dissolved salts. As regards the vascular bundles, the xylem has been found to be the tissue in which the water is conducted to the leaves, while the phloëm serves for the transference of assimilated food, especially proteids, from the leaves to all parts of the plant.

Passing on to the *reproductive* organs, the sporangia have first to be considered. The study of their development has shown that each sporangium arises from the growth and subdivision of a single epidermal cell of the leaf. The sporangium consists of the pouch-shaped head above described, which is borne on a short stalk. The interior of the former part is occupied by the spores, usually 64 in number, which are formed by the division into four of sixteen mother-cells. The thick rim of the sporangium (called the *annulus*) consists of a row of thick-walled cells extending from the stalk over the top of the sporangium. The annulus contracts in dry weather and thus tears the sporangium open, setting the spores free. Each of the latter is a single cell, and the outer layer of its wall is strongly cuticularized.

When the spores are sown upon damp earth, or a tile, or a slip of glass, and kept thoroughly moist and warm, they germinate. Each spore bursts its outer wall and gives rise to a tubular filamentous prolongation, the protoplasm of which contains chlorophyll-grains. At the base of this a similar but colourless process, the first *root-hair*, is developed. The green filament at first undergoes transverse division, so that it becomes converted into a series of cells. Then, the cells at its free end divide longitudinally, as well as transversely, and thus give rise to a flat expansion, which

gradually assumes a bilobed form, and becomes thickened in its middle part by division of its cells in a direction parallel to its surface. The thickened portion is termed the *cushion*. Numerous colourless, unicellular root-hairs are given off from the under surface of the little plant, which is called a *prothallus* or *prothallium*, and attach it to the surface on which it grows.

The prothallus attains no higher development than this, and does not directly grow into a fern such as that in which the spores took their origin; but, after a time, rounded or ovoidal elevations are developed, by the outgrowth and division of the cells which form its under surface. These bodies are of two kinds, the *antheridia* and *archegonia*. In the former, which may be developed on any part of the under side of the prothallus, the nucleus of each of the cells contained in their interior is converted into a spermatozoid somewhat similar to that of *Chara*, but provided with many more cilia. The antheridium bursts, and the spermatozoids set free from their containing cells are propelled through the moisture on the under surface of the prothallus by their cilia.

The processes of the second kind, the *archegonia*, acquire a more cylindrical form. The outside of the organ is formed of a single layer of cells, which are persistent, but those which are situated in the axis of the archegonium all disappear with the exception of that which lies at the bottom of its cavity. This is the *ovum* or *oosphere*, and when the archegonium is fully formed, a canal leads from its summit to this cell. The spermatozoids enter by this canal, and impregnate the ovum.

The ovum now begins to divide, and becomes converted into eight cells, which give rise to the stem, leaf, root and *foot*, of the fern-plant, the foot being the organ by which

the embryo is attached to the prothallus and derives food-supplies from it.

As the rhizome grows, and developes its fronds, it rapidly attains a size vastly superior to that of the prothallus, which at length ceases to have any functional importance, and disappears.

Thus *Pteris* presents a remarkable case of the alternation of generations. The large and complicated organism commonly known as the 'Fern' is the product of the impregnation of the ovum by the spermatozoid. This 'Fern,' when it attains its adult condition, developes sporangia; and the inner cells of these sporangia give rise, by a perfectly asexual fissive process, to the spores. The spores when set free germinate; the product of that germination is the inconspicuous and simply cellular prothallus; an independent organism, which nourishes itself and grows, and on which, eventually, the essential organs of the sexual process—the archegonia and antheridia—are developed.

Each impregnated ovum produces only a single 'fern,' but each 'fern' may give rise to innumerable prothalli, seeing that every one of the numerous spores developed in the immense multitude of sporangia to which the frond gives rise, may germinate.

LABORATORY WORK.

A. THE FERN-PLANT; ASEXUAL GENERATION.

a. External characters.

a. The brown underground stem or *rhizome*, with a lighter band (the *lateral line*) running along each side of it: its *nodes* and *internodes*.

b. The *roots* springing from the rhizome.

c. The *leaves* or *fronds* arising from the rhizome at intervals, along the lateral lines.

α. The great amount of subdivision of the frond: its main axis (*rachis*); the primary divisions or *pinnæ;* the ultimate divisions or *pinnules.*

β. The *sori;* small brown patches along the margin of the under surface of some of the pinnules.

γ. The *indusium* or membrane covering the sorus.

d. The *nodes* and *internodes* of the rhizome. The naked growing point at its extremity.

b. The rhizome.

1. Cut it across and draw the section as seen with the naked eye.

a. The outer brownish layer (*epidermis* and *hypoderma*); the latter thins aways somewhat opposite the lateral lines.

b. The yellowish-white substance (*ground-substance* or *parenchyma*) forming most of the thickness of the section.

c. The internal incomplete brown ring (*sclerenchyma*) imbedded in the parenchyma.

d. The small patches of sclerenchyma scattered about in the parenchyma outside the main sclerenchymatous ring.

e. The yellowish tissue (*vascular bundles* or *cylinders*) lying inside and outside the ring of sclerenchyma.

2. Cut a longitudinal section of the rhizome; make out on the cut surface b. 1. *a*, *b*, *c*, *d*, *e*.

3. Cut a thin transverse section of the rhizome, mount in water and examine with 1 inch obj.

   a. The single layer of much thickened epidermal cells.

   b. The small opaque angular contours of the hypodermal cells (*external sclerenchyma*).

   c. The large polyhedral more transparent parenchymatous cells.

   d. The small opaque angular contours of the cells of the internal sclerenchyma.

   e. The great openings of the vessels in the fibro-vascular bundles.

   Draw the section.

4. Examine with $\frac{1}{8}$ obj.

   a. The *epidermis:* its thick-walled cells.

   b. The *parenchyma:* its large thin-walled cells: their wall, protoplasm and nucleus: the great number of starch granules in them.

   c. The various patches of *sclerenchyma*, made up of thick-walled angular cells.

   d. The *vascular bundles.* Note in each :—

      α. Outside, a single layer of cells with brownish walls, containing no starch granules (*endodermis* or *bundle-sheath*).

      β. Within the bundle sheath a layer of small parenchymatous cells containing starch (*inner* or *phloèm sheath*).

      γ. Within the last layer comes the *bast* of the bundle (*phloèm*) consisting of—externally, two or more layers of small rectangular cells with

thickened walls (*protophloem*) and then a single row of large thin-walled cells (*sieve-tubes* or *bast vessels*) between which lie smaller thin-walled cells containing starch granules (*bast parenchyma*).

δ. The whole of the space within the phloëm is occupied by the *xylem* or *wood*. Note the greatly thickened walls of its large vessels, and their central cavity containing no protoplasm; the groups (usually two in number) of much smaller vessels (*protoxylem*); scattered here and there, in the spaces between the angles of the vessels, note the small parenchymatous cells (*wood parenchyma*) containing starch granules.

ε. Treat with iodine: observe the protoplasm stained brown; the starch granules deep blue, rendering some of the cells quite opaque and almost black-looking; the cell-walls of the xylem light brown.

5. Cut a thin longitudinal section of the stem and examine with 1 inch and then with $\frac{1}{8}$ obj. Make out the various tissues described in 3 and 4.

a. The *epidermis*, *hypoderma* and *parenchyma*, much as in the transverse section, except that the hypodermal cells are longer.

b. The *sclerenchyma* is seen to be made up of greatly elongated cells, tapering towards each end.

c. The vascular bundles; note in them—

α. The cells of the bundle sheath and of the phloëm sheath much as in the transverse

section but longer; the protophloëm cells, elongated, with thickened walls; the cells of the bast parenchyma somewhat elongated; the relatively large *sieve tubes*, of great length with oblique ends; their lateral walls marked by irregular groups of minute pores.

β. The *vessels* of the xylem: elongated tubes presenting oblique perforated partitions, at long intervals. Two forms of vessels will be seen, viz. *scalariform vessels*, with regular transverse thickenings on their walls and smaller *spiral vessels* (protoxylem), less numerous than the last form, with a continuous spiral thickening on their walls. Among the vessels the square-ended cells of the *xylem-parenchyma* will be recognised.

6. By macerating in Schulze's mixture (see Appendix E) the various cells and vessels may be isolated one from another, and their form better observed.

7. By making a series of transverse sections from the growing end of a stem the gradual development of the various forms of tissue from the originally uniform parenchyma (*meristem*) of the growing point, may be traced. If a section through the actual apex be obtained it will be possible to make out the relatively large, wedge-shaped *apical cell*.

c. The leaf.

1. Hold a small piece of a barren leaflet in pith or imbed it in paraffin and cut a thin vertical section at right angles to one of the veins.
Observe with low and high power

a. The epidermis of the upper and lower surface.

b. The *mesophyll*, the cells of which contain chlorophyll-grains in large numbers.

c. The transverse sections of the vascular bundles.

2. Slice off the epidermis from the under side of a leaflet. Mount in water and observe with a high power

a. the ordinary *epidermal cells* of irregular form,

b. the *hairs*,

c. the *stomata*, each stoma with its two kidney-shaped guard-cells.

d. The root.

1. Examine the tip of an uninjured root with a lens; note the relatively large *root-cap* covering the end of the root.

2. Make a transverse section of a mature root held in elder-pith, or imbedded in paraffin. Note, under a low power,

a. the *epidermis* with its *root-hairs*,

b. the cortex consisting of *parenchyma* towards the outside, and of very thick-walled *sclerenchyma* nearer the centre,

c. the central *vascular cylinder*.

e. The reproductive organs.

1. Examine a *sorus* with a low power without a cover-glass. It is composed of a great number of minute oval bodies, the *sporangia*, roofed in by the *indusium*.

2. Scrape off some sporangia and mount in water: examine with 1 inch obj.

a. *Their form:* they are oval biconvex bodies borne on a short stalk.

b. *Their structure:* composed of brownish cells, one row of which has very thick walls, and forms a

marked ring (*annulus*) round the edge of the sporangium.

c. Their mode of *dehiscence* (look out for one that has opened): by a cleft running transversely across the sporangium.

3. Burst open some sporangia by pressing on the cover-glass: examine, with $\frac{1}{8}$ obj., the *spores* which are set free.

a. *Their size:* measure.

b. *Their form:* somewhat triangular.

[*c.* *Their structure:* a thick outer coat, a thin inner coat, protoplasm, and a nucleus: crush some by pressure on the cover-glass.]

B. THE PROTHALLUS; SEXUAL GENERATION.

Prothalli may be obtained by sowing some spores on peat and keeping them warm and very moist for about three months. They are small deep green leaf-like bodies.

a. The Prothallus.

1. Transfer a prothallus to a slide, and mount it in water with its under surface uppermost. Examine with 1 inch obj.

a. *Its form:* a thin kidney-shaped expansion from which, especially towards its convex border, a number of slender filaments (*root-hairs*) arise.

b. *Its structure.*

a. *The leafy expansion:* it consists throughout most of its extent of a single layer of polyhedral chlorophyll-containing cells, but at a part (the *cushion*) a little behind the depression marking the growing point it is several cells thick.

β. *The root-hairs:* each composed of a single cell which contains no chlorophyll.

The *antheridia* and *archegonia:* the former can just be seen with an inch objective as minute eminences on the under surface of the older parts of the prothallus especially among the root-hairs; the latter are partly imbedded in the cushion.

b. The reproductive organs.

These are to be found by examining the under surface of the prothallus with $\frac{1}{8}$ obj.

1. The *antheridia.* Most numerous near and among the root-hairs.

   a. *Their form:* small hemispherical eminences.

   b. *Their structure:* made up of an outer layer of cells containing a few chlorophyll-granules, through which can be seen, according to the stage of development, either a single *central cell,* or a number of smaller cells (*mother-cells of spermatozoids*) resulting from its division: in the latter cells, in ripe antheridia, spirally coiled bodies (*spermatozoids*) can be indistinctly seen.

2. *The spermatozoids.*

   Some of these are sure to be found swimming about in the water if a number of ripe prothalli are examined.

   a. Small bodies, coiled like a corkscrew, thick at one end, and tapering towards the other, which has a number of cilia attached to it. To the thicker end of the spermatozoid is often attached a rounded mass containing colourless granules.

b. Treat with iodine; this stains them and stops their movements, so that their form can be more distinctly seen.

3. *The archegonia.* Make vertical sections of the prothallus passing through the cushion; this is best done while holding it between two pieces of pith. Note in the archegonia—

a. *Their form:* chimney-shaped eminences with a small aperture at the apex.

b. *Their structure.* Each is composed of a layer of transparent cells containing no chlorophyll, arranged in four rows, and surrounding a central cavity which extends into the cushion formed by the thickened part of the prothallus (**a.** 1. b. *a*). In this cavity lies, in young specimens, a large nucleated granular central cell, with two or three smaller granular cells (*neck canal-cells*) above it in the narrow upper part of the cavity; in older specimens this upper part is empty, forming a canal leading down to the central cell. The latter has now divided into two; the upper smaller cell resulting from its division forming the *ventral canal-cell*, which becomes disorganized, while the larger lower cell is the *ovum* or *oosphere.*

4. Examine a young fern-plant in connection with its prothallus, to the lower side of which it is attached by means of the *foot.*

XVI.

THE BEAN-PLANT (*Vicia Faba*).

In this, which is selected as a convenient example of a Flowering Plant, the same parts are to be distinguished as in the Fern; but the axis is erect and consists of a *root* imbedded in the earth and a *stem* which rises into the air. The appendages of the stem are *leaves*, developed from the opposite sides of successive nodes; and the internodes become shorter and shorter towards the summit of the stem, which ends in a terminal *bud*. Buds are also developed in the axils of the leaves, and some of them grow into branches, which repeat the characters of the stem; but others, when the plant attains its full development, grow into stalks which support the *flowers;* each of which consists of a *calyx*, a *corolla*, ten *stamens* and a central *pistil;* the latter is terminated by a *style*, the free end of which is the *stigma*.

The flower-stalks are modified branches, and the flower itself consists of several whorls of modified leaves.

The stamens form a tube which ends in ten filaments, four of which are rather shorter than the rest; and the filaments bear oval bodies, the *anthers*, which, when ripe, give exit to a fine powder, made up of minute *pollen* grains, each of which is a single cell. The pistil is hollow; and, attached by short stalks along the ventral side of it, or that turned towards the axis, is a longitudinal series of minute bodies, the *ovules*. Each ovule consists of a central conical *nucellus*,

invested by two coats, an *outer* and an *inner*. Opposite the summit of the nucellus, these coats are perforated by a canal, the *micropyle*, which leads down to the nucellus. One of the cells of the nucellus is very much larger than the rest; this is called the *embryo-sac*. In its interior several smaller cells are developed, the most important of which is the *ovum* or *oosphere*, which lies at the end of the embryo-sac towards the micropyle. When the pollen is deposited on the stigma the grains germinate. Each sends out a long filament, the *pollen-tube*, which elongates, passes down the style, and eventually reaches the micropyle of an ovule. Traversing the micropyle, the end of the pollen tube penetrates the nucellus, and comes into close contact with the embryo sac. The original nucleus of the pollen-grain has in the meantime divided into two, and one of the daughter nuclei divides again. All three pass down the tube, one nucleus reaches the embryo-sac, and then fuses with the nucleus of the ovum. This is the process of impregnation, and the result of it is that the ovum divides and gives rise to a cellular *embryo*. This becomes a minute Bean-plant, consisting of a *radicle* or primary root; of two, relatively large, primary leaves, the *cotyledons;* and of a short stem, the *plumule*, on which rudimentary leaves soon appear. The cotyledons now increase in size, out of all proportion to the rest of the embryonic plant; and the cells of which they are composed become filled with starch and other nutritious matter. The nucellus and coats of the ovule grow to accommodate the enlarging embryo, but, at the same time, become merged into an envelope which constitutes the coat of the seed. The pistil enlarges and becomes the pod; this, when it has attained its full size, dries and readily bursts along its edges, or decays, setting the seeds free. Each seed, when placed in proper conditions of

warmth and moisture, then germinates. The cotyledons of the contained embryo swell, and burst the seed coat, but in this species remain underground, and do not become green. The nutritious matters which they contain are absorbed by the plumule and radicle, the latter of which descends into the earth and becomes the root, while the former ascends and becomes the stem of the young Bean-plant. The apex of the stem retains, throughout life, the simply cellular structure which is, at first, characteristic of the whole embryo; and the growth in length of the stem, so far as it depends on the addition of new cells, takes place chiefly, if not exclusively, in this part. The growing point does not terminate in a single apical cell, as in the Fern, but consists of a number of small, actively dividing cells, termed collectively the *apical meristem*. The root likewise develops its tissues from an apical meristem, but this, as in the Fern, is protected by a root-cap.

The leaves cease to grow by cell multiplication at their apices, when these are once formed, the addition of new cells taking place at their bases. Each leaf is compound, the common petiole bearing from four to six *leaflets*.

The tissues which compose the body of the Bean-plant are similar, in their general characters, to those found in the Fern, but they differ in the manner of their arrangement. The surface is bounded by a layer of epidermal cells, among which are stomata similar to those described in the Fern. Within the epidermis is a broad zone of tissue, termed the *cortex*. The greater part of this zone consists of parenchymatous cells of the usual structure. At the four projecting corners of the stem however, the cortical tissue has a somewhat different structure, consisting of cells which have their walls much thickened at the points of junction. Within the cortex comes the *large central cylinder*, consisting

of a ring of vascular bundles surrounding the parenchymatous *pith* which extends to the very centre in the younger parts of the stem, while, in the older parts, the middle is occupied by a more or less considerable cavity, full of air. This cavity results from the central parenchyma becoming torn asunder, after it has ceased to grow, by the enlargement of the peripheral parts of the stem.

The arrangement of the vascular bundles in the Bean is not quite regular. Most of them, as already stated, form part of the central cylinder, but besides these there are two bundles, which are situated outside the cylinder. Each of these occurs opposite one of the projecting corners of the stem. Each vascular bundle consists of two halves; the *xylem* or wood, which is turned towards the centre of the stem, and the *phloem* or bast, which is turned towards its periphery. Bundles with this arrangement of xylem and phloëm are termed *collateral*. The wedge-shaped bundles of the ring are separated from one another by narrow bands of parenchymatous tissue, which extend from the parenchyma within the circle of vascular tissue (*medulla* or pith) to the outer limit of the cylinder. These are the *medullary rays*. In each of the bundles the xylem and phloëm are separated by a thin layer of small, and very thin-walled cells, termed the *cambium* layer. In the older parts of the stem this layer extends across the medullary rays between the bundles so as to form a continuous ring all round the stem. The tissues inside this layer are the wood and pith, while those outside it are the bast, cortex, and epidermis.

The great morphological distinction between the axis of the Bean and that of the Fern lies in the presence of this cambium layer. The cells composing it, in fact, retain their power of multiplication, and divide by septa parallel with the length of the stem, or root. Thus new cells are

continually being added, on the inner side of the cambium layer, to the thickness of the wood, and on the outer side of it, to the thickness of the bast; and the axis of the plant continually increases in diameter, so long as this process goes on. This constant addition to the outer face of the wood and the inner face of the bast is characteristic of the Dicotyledons and Gymnosperms, to which two groups all our forest trees belong. In the Bean this process of secondary thickening only goes on to a comparatively small extent.

At the apex of the stem, and at that of the root, the cambium layer is continuous with the cells of the apical meristem which retain the capacity of dividing in these localities. As the plant is thickest at the junction of the stem and root, and diminishes thence to the free ends, or apices, of these two structures, the layer of cambium and meristem may be said to have the form of a double cone. And it is the special peculiarity of the groups of plants above-mentioned to possess this doubly conical layer of constantly dividing cells, the upper end of which is free, at the growing point of the terminal bud of the stem, while its lower end is covered by the root-cap of the ultimate termination of the principal root.

The most characteristic tissues of the wood are pitted and spiral vessels, the spiral vessels being particularly abundant close to the pith. They are the first elements of the xylem to be formed. The outer part of the bast consists of elongated bast fibres, while the inner, or soft bast, contains sieve-tubes, the transverse walls of which are perforated.

Stomata are absent in the epidermis of the root: they are to be found, here and there, in the epidermis of all the green parts of the stem and its appendages, but, as in the Fern, they are most abundant in the epidermis of the under side of the leaves. As in the Fern, they communicate with

intercellular passages, which are widest in the leaves, but extend thence throughout the whole plant.

The blade of the leaf is traversed by the branched vascular bundles, the xylem being turned towards the upper, and the phloëm towards the lower surface. The parenchyma of the leaf, or mesophyll, is of two kinds; towards the upper surface the cells are closely packed, and elongated at right angles to the surface, forming the *palisade* parenchyma. Towards the lower surface the cells are of more irregular shape and very loosely arranged, and are termed the *spongy* parenchyma. Both kinds of cells contain chlorophyll-grains, but they are most abundant in the palisade cells.

The *root* has an epidermis, bearing unicellular root-hairs. Within this is a wide cortex of parenchyma, while the centre of the root is traversed by a central vascular cylinder, of *radial* structure, usually containing four bundles of xylem, and four of phloëm, which alternate one with another[1]. The lateral roots arise *endogenously*, from the outside layer of the vascular cylinder, opposite the xylem groups. They thus have to make their way through the whole of the cortex before reaching the surface. There are in typical cases four rows of lateral roots, corresponding to the four xylem-groups opposite which they originate.

The difference between a flowering plant, such as the Bean, and a flowerless plant, such as the Fern, at first sight appears very striking, but it has been proved that the two are but the extreme terms of one series of modifications. The *anther*, for example, is strictly comparable to a leaf bearing sporangia, the sacs in which the pollen is contained answering to the sporangia themselves. The *pollen grains* exactly resemble spores in their mode of development and answer to the *small spores* of those flowerless plants in which the spores are of two kinds—some spores giving rise to

[1] The central cylinder of the main root is directly continuous with that of the stem.

prothallia which develope only antheridia, and others to prothallia which develope only archegonia; instead of the same prothallia producing the organs of both sexes, as in *Pteris*. And the *pollen tube* may be compared to the first filamentous process of the spore. But, in the flowering plants, the protoplasm of the pollen tube does not undergo division and conversion into a prothallus, from which antheridia are developed, giving rise to detached fertilizing bodies or spermatozoids,—but exerts its fertilizing influence without any such previous differentiation, other than the divisions of its nucleus[1]. The connecting links between these two extreme modifications are furnished, on the one hand, by the Conifers, in which the protoplasm of the pollen tube becomes divided into cells, from which, however, no spermatozoids are developed; and by Selaginella, in which the protoplasm of the smaller spores (= pollen grains) divides into cells which form a unicellular prothallus, and a single antheridium in which the spermatozoids are produced.

On the other hand the *embryo-sac* is the equivalent of the large spore which gives rise to a prothallus bearing female organs. The ovum of the flowering plant corresponds to the ovum contained in the archegonium of the prothallus. There are other cells produced from the protoplasm of the embryo-sac, which probably answer to the cells of a prothallus. Here again the intermediate stages are presented by the Conifers and Selaginella. For, in the Conifers, the protoplasm of the embryo-sac gives rise to a solid prothallus-like endosperm, in which bodies called *corpuscula*, which answer to the archegonia, are formed, and in each of these an ovum is produced; while, in Selaginella the prothallus developed in the large spores does not leave the cavity of the spore, but remains in it like an endosperm.

[1] There is however an indication of cell-formation around two of the daughter-nuclei.

The physiological processes which go on in the higher green plants, such as the Fern and the Bean, resemble, in the gross, those which take place in *Protococcus* and *Chara*. For such plants grow and flourish if their roots are immersed in water containing a due proportion of certain saline matters, while their stem and leaves are exposed to the air, and receive the influence of the sun's rays.

A Bean-plant, for instance, may be grown, if supplied through its roots with a dilute watery solution of potassium and calcium nitrate, potassium phosphate and iron sulphate, and magnesium sulphate. While growing it absorbs the solution, the greater part of the water of which evaporates from the extensive surface of the plant. In sunshine, it rapidly decomposes carbonic anhydride, fixing the carbon, and setting free the oxygen; at night, it slowly absorbs oxygen, and gives off carbonic acid; and it manufactures a large quantity of protein compounds, cellulose, starch, sugar and the like, from the raw materials supplied to it.

It is further clear that, as the decomposition of carbonic anhydride can take place only under the combined influences of chlorophyll and sunlight, that operation must be confined, in all ordinary plants, to the tissue immediately beneath the epidermis in the stem, and to the leaves. And it can be proved, experimentally, that fresh green leaves possess this power to a remarkable extent. The decomposition of carbonic anhydride and of water appears to go on simultaneously, and as the result of the process, various carbohydrates, such as grape-sugar, make their appearance.

On the other hand, it is clear that, when a plant is grown under the conditions described, the nitrogenous and mineral constituents of its food can reach the leaves only by passing

from the roots, where they are absorbed, through the stem to the leaves. And, at whatever parts of the plant the nitrogenous and mineral constituents derived from the roots are combined with the carbohydrates produced in the leaves, the resulting compound must be diffused thence, in order to reach the deep-seated cells, such for instance as those of the cambium layer and those of the roots, which are growing and multiplying, and yet have no power of extracting carbon directly from carbonic anhydride. In fact, those cells which contain no chlorophyll, and are out of the reach of light, must live after the fashion of *Torulæ;* and manufacture their protein out of the nitrates and salts of ammonia taken up by the root, in combination with such bodies as grape-sugar, already formed in the leaves. Thus, the higher plant combines within itself the two, physiologically distinct, lower types of the Fungus and the Alga.

That some sort of circulation of fluids must take place in the body of a plant, therefore, appears to be certain, but the details of the process are by no means clear. There is evidence to show that the ascent of fluid from the root to the leaves takes place, to a great extent, through the vessels of the wood, which in the higher plants have their transverse walls broken down so as to form very fine capillary tubes traversing both stem and root.

The mechanism by which this ascent is effected is of two kinds; there is a pull from above, and there is a push from below. The pull from above is the evaporation which takes place at the surface of the plant, and especially in the air-passages of the leaves, where the thin-walled cells of the parenchyma are surrounded, on almost all sides, with air, which communicates directly with the atmosphere through the stomata. The push from below is due in the first instance to the absorptive action of the root hairs. The

water they take up passes to the parenchymatous cells of the cortex of the root, and is thence pressed into the vessels. In a vine, for example, before its leaves have grown in the spring, this process, called "root-pressure," causes a rapid ascent of fluid (*sap*) absorbed from the soil. A certain portion of the fluid thus pumped up from the roots to the surface of the plant doubtless exudes, laterally, through the walls of the vessels (the thin places which give rise to the pits on the walls of these structures especially favouring this process), and passing from cell to cell, eventually reaches those which contain chlorophyll. The distribution of the carbohydrates formed in the chlorophyll-bearing cells, probably takes place by slow diffusion from cell to cell. The proteid compounds are in all probability conveyed through the sieve-tubes of the vascular bundles.

There is no doubt that all the living protoplasm of the plant undergoes slow oxidation, with evolution of carbonic anhydride. In the green parts, and in daylight, this process of respiration is disguised by the more conspicuous one of assimilation, in which carbonic anhydride is decomposed and oxygen given off. In the deeper seated cells, and in all parts of the plant when light is absent, respiration alone goes on. The supply of oxygen needful for this purpose is sufficiently provided for, by the air-passages which are to be found between the cells in all parenchymatous tissues. The replacement of the oxygen of the air thus absorbed, and the removal of the carbonic anhydride formed, will be sufficiently provided for by gaseous diffusion.

From what has been said, it results that, in an ordinary plant growing in damp earth and exposed to the sunshine, a current of fluid is setting from the root towards the surface exposed to the air, where its watery part is for the most part evaporated; while gaseous diffusion takes place, in the

contrary direction, from the surface exposed to the air, through the air-passages which extend from the stomata to the radicles; the balance of exchange being in favour of oxygen, in all the chlorophyll-bearing parts of the plant which are reached by the sunlight, and in favour of carbonic anhydride, in its colourless and hidden regions. At night, the evaporation diminishing with the lowering of the temperature, the ascent of liquid becomes very slow, or stops, and the balance of exchange in the air-passages is entirely in favour of carbonic anhydride; even the chlorophyll-bearing parts oxydizing, while no carbonic anhydride is decomposed.

LABORATORY WORK.

a. **General characters.**

- *a.* The erect central main axis (*root* and *stem*).
- *b.* The *branches* of the stem; some, mere repetitions of the main axis; others, modified and bearing flowers.
- *c.* The *nodes* and *internodes*.
- *d.* The *appendages*.
  - *α.* Foliage leaves.
  - *β.* Floral leaves.

b. **The root.**

- *a.* Its main central portion (*axis*).
- *b.* The *rootlets* attached to the axis in four rows.
- *c.* The *root-hairs*, only found on the younger parts of the root.

d. The *root-cap*, covering the tip of each rootlet: this is difficult to get whole out of the ground in the bean, but is readily seen by examining the roots of duckweed (*Lemna*) with 1 inch obj. In the latter plant it consists of several layers of cells forming a cap on the end of the root, and ending abruptly with a prominent rim some way up it. In the bean the root-cap can be well seen by making a longitudinal section of the radicle of the seed. See below, **f.** 1. *c.*

e. Make a transverse section of the main root of a seedling about an inch below its junction with the stem. Note that the whole root is destitute of chlorophyll. Observe with a low power

α. The epidermis.

β. The wide cortex.

γ. The central vascular cylinder. Note the four xylem-bundles alternating with the same number of phloëm-bundles.

δ. In a section passing through the insertion of a lateral root, observe that this arises *endogenously* and immediately outside one of the groups of xylem.

c. The stem.

1. Erect, green, four-cornered, with a ridge at each angle; not woody; the gradual shortening of the internodes towards its apex.

2. Cut a thin transverse section of the stem through an internode; note its central cavity, and the whitish ring of *fibro-vascular bundles* in it, which is harder to

cut than the rest: mount in water and examine with 1 inch obj.: note—

a. The *medullary* or *pith-cavity* in the centre of the section.

b. The *pith-cells*, around the central cavity: large and more or less rounded (*parenchyma*): sometimes with dotted walls from spots of local thinness on them (pits).

c. The *fibro-vascular bundles* arranged in a ring immediately outside the pith[1]. Two of them however will be found separate from the ring in two opposite corners of the stem. Commencing at the side nearest the pith, note in each bundle—

- *α.* The small openings formed by the transverse sections of the *spiral vessels* (protoxylem).
- *β.* The larger cavities of the *pitted vessels.*
- *γ.* The small thick-walled *wood-cells*, wedged in between the vessels. These three (*α*, *β* and *γ*) form the wood or xylem of the bundle.
- *δ.* The *cambium zone:* granular-looking, and composed of small angular thin-walled cells, ranged in regular radial rows.
- *ε.* The *bast* or *phloem.* It presents internally thin-walled cells of various sizes, the *bast parenchyma* and bast vessels or *sieve tubes.* Externally it appears in cross section to be composed of rounded cells with thickened walls; the *bast fibres* or *sclerenchyma.* Draw the section.

d. The *cortex*, consisting of several layers of large rounded cells containing chlorophyll. Note that at the four corners of the stem the walls of these

[1] *a*, *b*, *c*, and *e* together constitute the *central cylinder.*

cells are much thickened at the angles (*collenchymatous*).

e. The *medullary rays:* radiating rows of parenchymatous cells passing between the bundles and uniting *b* and *d:* not quite continuous, being interrupted by the *cambium zone* (*c.* δ.).

f. The *epidermis:* composed of a single layer of somewhat squarish looking cells, containing no chlorophyll. Note the *stomata*, their two small *guard-cells* being seen in section.

3. Cut a transverse section through a node, and compare it with that through the internode. Observe the bundles passing out from the stem into the leaf.

4. Cut a thin longitudinal section through part of an internode (if necessary the bit of stem may be imbedded in paraffin first), and mount it in water; working from the medullary cavity outwards, note the following layers, using at first a low power:—

a. *The pith-cells:* much as in the transverse section.

b. The fibro-vascular bundles presenting—

α. *The spiral vessels:* elongated tubes with a spiral thickening on their walls.

β. *The wood-cells:* elongated and with much thickened walls.

γ. *The pitted vessels:* much like α, but with their walls pitted instead of spirally thickened.

δ. *The cambium zone:* made up of small, prismatic, thin-walled cells, containing abundant protoplasm.

ε. The *bast parenchyma:* thin-walled elongated cells.

ζ. The *bast vessels:* larger elongated cells with oblique perforated septa (*sieve-tubes*).

η. The *bast fibres*[1], fusiform and thick-walled.

c. More parenchymatous cells, constituting the cortex.

d. *Epidermis:* composed apparently of cubical colourless cells: here and there the opening of a stoma (**d.** 2. *d.* β.) may be seen.

Draw the section.

5. Compare the transverse and longitudinal sections together, making out the corresponding parts in each.

6. Put on a high power, and examine each of the above-mentioned tissues carefully.

7. Stain with iodine: note the *cell-walls;* the *protoplasm* —its presence or absence, and relative quantity in the various tissues; the *nuclei* of the cells; *starch granules* in some, stained deep blue by the iodine.

d. The leaves.

1. *Their form and composition.*

a. Each leaf consists of a number of different parts, viz.:—

α. The stalk or *petiole.*

β. The four to six oval *leaflets* attached laterally to the stalk.

γ. The pair of small leaf-like expansions (*stipules*) at the base of the petiole.

δ. The rudimentary *tendril* terminating the petiole.

2. *The histological structure of a leaflet.*

a. Imbed a leaflet in paraffin or hold it between two bits of elder pith and cut a thin section from it,

[1] These fibres belong to the central cylinder, but do not, strictly speaking, form part of the bundle.

perpendicular to its surfaces. Let the section lie in alcohol[1] a few minutes to drive the air out of its intercellular spaces, and then mount it in water, and examine with 1 inch objective.

b. Begin at the upper surface (marked out by its more closely packed cells), and work through to the lower. Note—

α. The colourless *epidermal layer*—consisting of a single row of cells; the openings here and there in it (*stomata*).

β. Beneath the upper epidermis come elongated chlorophyll-containing cells, set on perpendicularly to the surface, forming the *palisade* parenchyma.

γ. Then come irregularly branched cells forming the lower half of the leaf-substance; these also contain chlorophyll. They constitute the *spongy* parenchyma.

δ. The epidermal layer of the lower surface; like α.

ε. The *intercellular spaces*, through the whole thickness of the leaf: the direct communication of some of them with stomata.

ζ. Here and there sections of *ribs* or *veins*: make out in them the same elements as in **c. 2.** *c.*

Draw.

c. Treat with iodine: make out the wall, protoplasm (*primordial utricle*), nucleus and vacuole of the cells: the chlorophyll grains, the starch granules.

d. Peel off a strip of epidermis from a leaf and examine with a low power: note—

[1] This will extract the colouring matter from the chlorophyll grains.

α. The large close-fitting cells, with irregularly wavy margins and no chlorophyll, which chiefly make up the epidermis.

β. The openings here and there in it (*stomata*); the two curved, chlorophyll-containing guard-cells bounding each stoma.

c. Gently pull a midrib in two across its long axis; note the fine threads uniting the two broken ends; cut them off with a sharp pair of scissors, mount in water and examine with $\frac{1}{4}$ or $\frac{1}{8}$ objective: they will be found to consist of partially unrolled spiral vessels.

e. The flower.

1. *Its general structure.*

a. Borne on a short stalk (*peduncle*).

b. Composed of four rows or *whorls* of organs.

α. The external green cup-like *calyx.*

β. Inside the calyx the *corolla:* the most conspicuous part of the flower.

γ. Inside the corolla the *stamens.*

δ. Within the stamens the *pistil.*

2. *The calyx.*

A cup terminated at its free edge by five prominent points, two dorsal, and three ventral: the five small midribs running along it (one to the end of each of the points) represent the free ends of five *sepals,* which are united below.

3. *The corolla.*

a. Composed of five pieces or *petals.*

α. On the dorsal side, a single large piece (*vexil-*

lum) expanded at its free end and folded over the rest.

β. On the sides, two oval pieces (*alæ*), each attached by a distinct narrowed stalk (*unguis*).

γ. The inferior part of the corolla (*carina*), composed of two oval pieces united along their lower edge but readily tearing apart.

4. *The stamens.*

a. Ten in number, each consisting of a stalk-like part, *the filament*, terminated by a small knob, *the anther.*

b. The union of nine of the filaments for three-fourths of their length to form the *staminal-tube*, the tenth being free: the sharp bend of the filaments towards the upper side at the point where they separate from one another.

c. Tease out an anther in water and examine with $\frac{1}{8}$ obj.: there will be found numerous—

a. *Pollen-grains:* small oval cells, with projections on the cell-wall in the equatorial region.

d. The anther of a bean is so small that sections cannot be made of it without considerable skill: the structure of an anther can however be easily made out by imbedding one from a tiger-lily in paraffin or holding it between two bits of elder-pith, cutting transverse sections, mounting in water and examining with 1 inch. obj.

a. It contains four chambers, *the pollen-sacs*, two on each side of the continuation of the filament, and in each chamber lie numerous pollen-grains.

β. By making careful transverse sections of young flower buds the stages of development of the anther and pollen may be made out. Observe especially the origin of the pollen-grains by the successive division of each of the numerous mother-cells, into two and then into four. Each of the four daughter-cells forms a wall of its own and becomes a pollen-grain.

5. *The pistil.*

a. It is found by tearing open the stamen-tube: it is a long green tapering body, somewhat flattened laterally and ending in a point (the *style*) which bears a tuft of strong hairs.

b. Slit it open carefully: its central cavity contains a number of small oval bodies, *the ovules*, attached along its ventral side by short pedicles.

c. It is difficult to get a section of a bean-ovule, but its essential structure may be readily made out by making thin transverse sections of the ovary of a large lily (where the ovules are closely surrounded by the tissue of the pistil) and examining with 1 inch obj.

α. The central cellular portion of the ovule (*nucellus*) made up of a large number of cells.

β. Its two coats, an inner (*primine*) and outer (*secundine*).

γ. The small passage (*micropyle*) leading through the coats down to the nucellus.

δ. In median sections of the ovule, a very large cell (the *embryo-sac*) will be seen in the nucellus just opposite the micropyle.

ε. The contents of the embryo-sac are best studied in material which has been preserved, when quite fresh, in absolute alcohol, and then transferred to a mixture of alcohol and glycerine. The alcohol is allowed to evaporate, and the sections are then made, and mounted in glycerine. With a high power the following structures may be made out in the embryo-sac :

αα. At the micropylar end three cells forming collectively the "*egg-apparatus*." The most deeply inserted of the three is the *ovum* itself, the other two are the *synergidae* and undergo no further development.

ββ. About the middle of the sac the large nucleus. After fertilization this divides, repeatedly, to form the nuclei of the *endosperm-cells*, which are soon absorbed in this plant.

γγ. At the end opposite the micropyle the three "antipodal cells."

f. The seeds.

1. Soak some dried beans in water for twenty-four hours; they will slightly swell up and be more readily examined than when dry.

a. Note the black patch (the *hilum*) on one end of the bean, marking where the stalk (*funiculus*) which fixed it in the pod was attached to it.

b. Having wiped all moisture off the bean gently press it while observing that part of the black patch which is next its broader end: close to the patch a minute drop of fluid will be observed to be pressed out through a small opening, the *micropyle*.

c. Carefully peel off the outer coat (*testa*) of the seed :

the two large fleshy *cotyledons* of the embryo will be laid bare. Joining the cotyledons together will be found the rest of the embryo: it consists of a conical part (the *radicle*) lying outside the cotyledons, with its apex directed towards the point where the micropyle was: and of the rudiments of the stem and leaves (*plumule*) lying between the cotyledons.

g. The process of fertilization.

This is difficult to follow in the bean; but by using different plants for the observation of its various stages it is fairly easy to observe all its more important steps.

1. A plant well adapted for seeing the penetration of the pollen tube into the stigma and style is the Evening Primrose (*Œnothera biennis*).

   Detach the style from the flower and hold the club-shaped stigma between the finger and thumb of the left hand. Moisten it with a drop of water and then make with a wetted razor several successive cuts through it. This will divide the stigma into several slices. Spread these out on a glass slide with a needle in water and examine the thinnest, after putting on a covering-glass.

   The triangular grains of pollen will be seen sending out from one angle a tube into the stigmatic tissue, which is easily seen from its slight difference in colour.

2. The entrance of the pollen-tube into the micropyle can be readily made out in some species of *Veronica*. The common *V. serpyllifolia*—often to be found in shady places on lawns—is well adapted for the purpose. A flower should be taken from which the

corolla has *just* dropped. Dissect out the minute ovary and, using the dissecting microscope, open with a needle one of its two cells in a drop of water; remove the mass of ovules and gently tease them apart. Then put on a covering-glass and examine with a low power till an ovule is found which shews the entry of the pollen-tube. The addition of dilute glycerine will make the ovule more transparent, so that after some time the embryo-sac can be seen, and the progress of the pollen-tube into the ovule followed.

3. In favourable median sections of the ovule of the lily prepared as directed in **e**. 5. *c*. *e*., the pollen-tube may be traced through the micropyle and between the cells of the nucellus to the embryo-sac.

4. Among other plants favourable for the study of the details of fertilization may be mentioned *Caltha* (marsh-marigold), *Helleborus*, and *Campanula* (Canterbury Bells). In each case thin transverse sections of the fruit are to be made, and the material is best prepared as above described.

APPENDIX.

A. GENERAL.

The animals and plants employed in this work should be obtained alive, and if possible by the student himself. Directions for killing and preserving them will be found under each head. Living specimens should be kept ready at hand, in order that their habits and movements may be studied side by side with their structure.

Apparatus required.

1. **Dissecting instruments**, as under

   Two or three **scalpels** of variable sizes, with straight blades.

   Two pairs of **scissors** with straight points (one large and one small).

   Two pairs of **forceps** (one large and one small) with straight points, the inner faces of which shall be ground or notched.

   A **seeker** with a tapering point.

   A German-silver **blow-pipe**.

   Two **razors** (for section-cutting).

   A **section-lifter**. This may most readily be made by beating out the terminal $\frac{1}{4}$th of a piece of copper-wire, 4 inches in length.

   3 or 4 **camel's hair brushes.**

   6 **needles** mounted in long wooden handles.

2. **Dissecting dishes.**

A small pie-dish 6 or 8 inches in length, and an ordinary salve-pot will meet all requirements. They should be half filled with a mixture of paraffin and lamp-black, put in hot, and weighted down with a piece of sheet lead.

3. **Injecting apparatus.**

Two or three $\frac{1}{2}$-**oz. medicine-droppers,** with their points drawn out to varying degrees of fineness.

A **1-oz. syringe,** preferably of metal, for histological work.

4. **A compound microscope**, preferably with a short body; fitted with low power (1 inch) and high power ($\frac{1}{6}$th inch) objectives. A shallow eye-piece of low magnifying power will suffice, but a higher one may conveniently be to hand.

5. **A hand-lens** : preferably of the pocket or watchmaker's type.

A simple dissecting microscope may conveniently be added.

6. **Sundries.**

Drawing material. Unlined paper; pencils H and HB, and a box of moist-colours. Paints may be employed in preference to chalks, and it is advisable to use corresponding colours in representing corresponding organs of different organisms, or corresponding parts of organs themselves.

In drawing, accurate representation in outline should be aimed at, and it will generally suffice to colour in light flat washes.

Microscopic slides and cover-slips.

Of the former, $\frac{1}{2}$ a gross, with rough or ground edges at will.

Of the latter, 2 oz., preferably $\frac{1}{8}$ square thin.

Glass **dipping-tubes** of various calibres and lengths; two or three to be drawn out to a point

Three or four small **thin glass beakers.**

Half a dozen **watch-glasses.**

Three or four **glass salve-pots** with lids.

B. ON DISSECTING.

Unless otherwise directed, all the dissections embraced by this work should be performed under water.

The undermentioned precautions are indispensable to success.

1. The animal or organ under dissection should be firmly pinned down, the pins being thrust through those more solid parts of the same, furthest removed from the point of operation.
2. Displacement of parts of organs, prior to removal or otherwise, should be effected by means of forceps, the larger ones being employed wherever possible (in order that the smaller ones may be the more fit for delicate work); the direct use of the fingers should be discouraged.
3. Never dissect under dirty water. Should the water become clouded, as it may do from numerous causes, change it at once and, in doing so, wash the dissection clean under a gentle stream.
4. In dissecting a given system remove nothing unnecessarily. Dissect away only such parts as may interfere with the immediate purpose of inquiry, and do this only after full deliberation.
5. Dissecting instruments should always be wiped perfectly clean and dry after use. The hinges should be occasionally oiled by means of a camel's hair-brush; and if put away for a lengthy period after use, all parts subject to immersion in water should be similarly treated.

C. ON INJECTING.

1. **Precautions**, indispensable to success under all circumstances.

   a. Injection should always be performed as soon after death as possible; and, in the case of the Frog, it is advisable to first remove the apex of the ventricle, in order to allow of the escape of as much blood as possible.

   b. In filling the syringe (or its equivalent) with injecting material care should be taken to first expel the contained air: when fully charged it should be held nozzle uppermost, while, by displacement of the piston, all trace of air is dispelled.

   c. In opening a blood-vessel prior to injection the exposed wall should be slit longitudinally with small scissors. Escape of as much blood as possible should be permitted. Before inserting the cannula or its equivalent, the whole should be well washed and the incision examined to make sure of the absence of a blood-clot or other obstructive agent.

   To this end the introduction of the cannula may be advantageously preceded by that of the seeker.

   d. If the cannula is to be tied in place (as must always be the case when injecting for histological purposes) the thread (preferably one of surgical silk) should be passed round the vessel and loosely knotted, prior to making the incision in its wall. The incision should be made a short distance from the thread, in a direction away from that in which it is intended to inject.

   Under such circumstances the knotted thread will be found to serve as a landmark, which experience will show to be occasionally necessitated, in the course of the work.

c. Upon removal of the injecting apparatus the cut end of the blood-vessel or other organ through which it was inserted should be immediately ligatured or clamped, in order to guard against reflux.

2. **Coarse injection, for anatomical purposes.**

Complete satisfaction may be obtained by using a mixture of French blue and water in the proportion of a teaspoonful to half a tumbler. The mixture should be well stirred immediately before use, and it may be introduced under water or otherwise as occasion demands. Most satisfactory results are to be obtained with this mixture by injecting piecemeal from such of the larger vessels as may be desirable.

For permanent anatomical preparations plaster of Paris may be preferably employed. Mix with two-thirds its bulk in water and colour with French blue or vermilion; stir thoroughly and strain through two thicknesses of fine muslin. The mixture thus prepared will remain for 8—10 minutes sufficiently fluid for all practical purposes.

3. **Fine injection, for histological purposes.**

Allow a given quantity of gelatine to stand for 3—4 hours in twice its bulk of cold water; heat slowly until quite fluid and colour to taste with Berlin blue or carmine. Inject when lukewarm, the animal being immersed in water at the same temperature.

In the above process the following precautions should be taken.

a. The point of the cannula should bear a slight contusion near its tip, round which the thread may bite when ligatured.

b. The cannula should be as short as possible, and there should be attached to its base an inch or so of india-rubber tubing to receive the nozzle of the syringe.

c. The cannula should not be inserted until all bleeding has ceased.

d. While injecting, a steady pressure should be maintained. Should resistance to this be offered, the operation must be interrupted until the cause of obstruction shall have been ascertained and, if possible, removed.

Obstruction generally arises from one or more of the undermentioned causes.

i. The presence of clotted blood in the smaller vessels. This source of difficulty is usually fatal to success.

ii. Resistance offered in the capillary systems, often resulting from the forcing back of the blood upon the great vessels. This may generally be overcome by puncturing a large vessel as far removed from the point of operation as possible (i. e. if injecting the arteries puncture one of the larger veins, or *vice versâ*).

iii. A too rapid cooling of the gelatine within the smaller vessels and capillary systems. To overcome this, increase the temperature of the water in which the animal is immersed.

e. Complete injection of a given capillary system ultimately results in visible distension of the organ concerned; should enlargement such as this become suddenly obvious the operation must be terminated, otherwise rupture and extravasation will ensue.

f. On withdrawal of the syringe the end of the india-rubber tube attached to the cannula should be either plugged with a glass-rod or other convenient stopper, or clamped; and the whole should be placed in cold water until the gelatine shall have set.

g. Animals or tissues thus injected should never be placed at once into strong alcohol, but into spirit of increasing strength, from 50 per cent. upwards as directed at Appendix E.

D. MICROSCOPE AND MICROSCOPIC EXAMINATION.

1. In using the microscope the first thing to be considered is the **illumination**. A position facing a window should be selected, and if the window be a north one there will be an advantage in the absence of direct sunlight, which should never be employed for microscopic work. The light from a south window is however equally good, if a white blind be used to exclude bright sunshine.

   The mirror must be so placed that the field of the microscope appears quite bright. The admission of light from the mirror to the object is regulated by the diaphragms. The size of the opening to be used should depend on the magnifying power. With a low power a large opening is necessary, or the whole field will not be illuminated; with a high power a smaller opening gives a better definition, though it diminishes the intensity of the illumination. Most modern microscopes are provided with a double mirror: the flat one should be used low down in illuminating with a low power; with a high power on the other hand, the concave one should be used obliquely and high up.

2. The microscope has two adjustments of **focus**. The coarse adjustment is effected by sliding the body of the microscope up and down in its tube, or in the larger instruments by a rack and pinion movement. The fine adjustment is worked by a screw. In all observations the low power should be used first, and then the high, if necessary. The focal distance of the low power is of course relatively long, usually either an inch or half an inch. Hence there is no difficulty in focussing and no danger of crushing the object. With the high power, which may have a focal distance of $\frac{1}{6}$ in., $\frac{1}{8}$ in., or less, great care is necessary, lest the objective should touch the cover-slip. It is best to slide the body of the

microscope very carefully down, until the object is just visible, and then to focus accurately with the fine adjustment.

3. Never observe with a high power until the object is covered with a cover-slip. If the object appears indistinct, this may be due to dirt or condensed vapour, on the cover-slip, the objective, or the eye-piece. If the first is in fault the distinctness will vary as the slide is moved. The presence of any foreign particles on the eye-piece can be easily detected by turning it round. A general indistinctness, which is not affected either by moving the slide, or by rotating the eye-piece, must be due to the objective having become dirty, or injured.

4. Should the objective be dirty, it must be cleaned with a soft linen rag, or with a piece of wash-leather, never with a coarse cloth. The greatest care must be taken that the objective does not become dirty, for even the most careful cleaning is likely to injure the lens. In order to keep the objective clean, it is essential that no more fluid should be used in mounting, whether temporarily or permanently, than will exactly fill the space under the cover-slip.

5. During observation the focussing should constantly be slightly varied, by means of the fine adjustment, as this greatly aids in getting a clear idea of the object.

6. It is best to cultivate a habit of using the microscope in the vertical position, so that the stage is horizontal. An inclined position of the instrument is only admissible in examining permanent preparations.

7. The body of the microscope should always fit accurately, but not tightly, in its tube. It should be oiled if it does not work up and down quite smoothly.

8. A **micrometer** of some kind is indispensable, and the purpose to be aimed at in its use is the knowledge of the size of objects under examination. An eye-piece mi-

crometer ruled into squares answers most purposes and the squares and objects delineated should be drawn to the proportions observed. Having thus a record of objects, drawn in proportion relative to the said squares, it remains but to ascertain the value of the latter. This is best done by using, in conjunction with the eye-piece micrometer above named, a stage micrometer ruled to intervals of known value; there will thus be superposed lines of absolute and relative measurement. The actual value of the squares of the eye-piece micrometer may by this means be once for all calculated, and a record of the same should be kept for each lens combination.

E. PREPARATION AND USE OF REAGENTS AND CULTURE SOLUTIONS.

The reagents employed in microscope work are best put up for use in ½ oz. bottles. Those marked thus * should be kept in glass bottles with ground necks and stoppers, their contents being removed by means of clean capillary tubes. The receptacles of the remaining ones should be corked, each cork to carry a thin glass rod long enough to reach near the bottom of the bottle.

1. **Acetic acid, Dilute.**

   Mix 1 cub. centimetre of glacial acetic acid with 99 cub. cent. of distilled water.

2. **Alcohol.**

   Methylated spirit should be kept ready to hand in stock bottles, diluted with water to various strengths, viz.—50 per cent., 75 per cent., 90 per cent. Upon placing any specimen, organ, or tissue in the same, at least 3—4 times its bulk in fluid should be employed. Immersion in the weaker solutions should not exceed 6—8 hours in the case of whole organs, or 2—3 hours in those of tissues in course of hardening for histological work.

The stronger solutions employed in the final stages of hardening or preservation should be replaced at intervals. In the case of preparations which have been transferred from acid solutions, the spirit must be repeatedly changed, until all excess of acid is removed.

Preparations of nervous tissues or sense-organs, if treated with alcohol, should be put at once, when quite fresh, into strong spirit.

3. **Ammonic bichromate, Solution of.**

Dissolve 10 grammes of crystallized ammonic bichromate in a litre of distilled water.

4. **Canada balsam*.**

A chloroform or turpentine solution of fairly fluid consistency should be employed. It must be kept in a well-stopped bottle with a wide neck.

5. **Carmine, Solution of.**

Carmine	2 grammes.
Strong solution of ammonia	4 cub. cent.
Distilled water	48 cub. cent.

Dissolve the carmine in the ammonia and water; leave in an unstoppered bottle until nearly all smell of ammonia has gone. Afterwards keep in a well-closed bottle. Dilute a small quantity with fifteen or twenty times its bulk of water, when required for use.

6. **Carmine, Borax, Solution of.**

Carmine	1 gramme.
Borax	4 grammes.
Distilled water	56 cub. cent.

To this solution add twice its volume of absolute alcohol. Filter.

7. **Chromic acid, Solution of.**

Dissolve 10 grammes of crystals of chromic acid in one litre of water. This gives a 1 per cent. solution, from

which weaker ones can readily be prepared when required.

Preparations should be placed in ·1 per cent. solution for the first 24 hours, that being ultimately replaced for a similar period by one of ·5 per cent. which should be changed if necessary. When well hardened they should be transferred to 75 per cent. alcohol.

When used for purposes of decalcification the maximum quantity should be employed, and to it a few drops of nitric acid may be added.

8. **Corrosive sublimate, Solution of.**

To a saturated aqueous solution add a few drops of acetic acid.

A relatively large quantity of the above must be employed, and the preparation, after at most an hour's immersion therein, should be well washed under running water before being transferred to alcohol.

9. **Eosin, Solution of.**

Aqueous and alcoholic solutions should be prepared;—the former for use with fresh material, the latter with that which has been previously hardened.

10. **Glycerine.**

By 'weak glycerine,' referred to in the text, is meant a solution composed of equal parts in bulk of glycerine and distilled water.

11. **Gold chloride, Solution of.***

A 1 per cent. solution is customarily employed.

Tissues submitted to the action of this reagent should be kept free of contact with metal.

12. **Hæmatoxylin, Solution of.**

a. Prepare a saturated solution of crystallized calcic chloride in 70 per cent. alcohol; then add alum to saturation.

b. Prepare a saturated solution of alum in 70 per cent. alcohol. Add 1 volume of *a* to 8 of *b*.

c. To the mixture of *a* and *b* add a few drops of a saturated solution of pure hæmatoxylin in absolute alcohol.

d. Filter.

This reagent stains with great intensity. Overstained preparations may be clarified by immersion for a longer or shorter period in acidulated alcohol (70 per cent. solution plus ·25 per cent. solution of nitric acid).

13. **Hæmatoxylin-ammonia, Solution of.**

Expose some crystals of Hæmatoxylin under a bell-glass to the action of the Ammonia gas given off from a strong solution. The crystals can then be dissolved in distilled water. This reagent must be prepared fresh when required.

14. **Iodine, Solution of.**

Prepare a saturated solution of potassic iodide in distilled water; saturate this solution with iodine. Filter. Dilute to a brown sherry colour.

It sometimes happens that the iodine and sulphuric acid tests for starch and cellulose fail, when dealing with fresh material. Under such circumstances a check experiment should be performed, as follows, viz. :—preserve in alcohol, transfer to weak solution of caustic potash for 6—8 hours, neutralize with dilute acetic acid and finally stain, as in ordinary, with iodine and strong sulphuric acid.

The presence of starch or cellulose should never be denied before both the above-named tests have failed.

Magenta, Solution of.

Dissolve 1 decigr. of crystallized magenta (roseine) in 160 cubic centimetres of distilled water: add 1 cub. cent. of absolute alcohol. Keep in a well-closed bottle.

16. **Mayer's Solution.**

15 per cent. solution of sugar-candy	20 cc.
Dihydropotassic phosphate	0·1 grm.
Calcic phosphate	0·1 grm.
Magnesic sulphate	0·1 grm.
Pepsin	0·23 grm.

17. **Muller's Fluid.**

Bichromate of potash	25 grammes.
Sodic sulphate	10 grammes.
Distilled water	1 litre.

18. **Osmic Acid, Solution of.**

Best bought ready made in the form of 1 per cent. solution.

The crystals are supplied in small tubes each containing 1 gramme. Such a tube should be broken up in 100 c. c. of distilled water, great care being taken to avoid contact with the resulting vapour.

This reagent should be kept in a black stoppered bottle, perfectly in the dark.

19. **Paraffin.**

See Appendix F.

20. **Pasteur's Solution.**

Potassium Phosph.	20	parts.
Calcium Phosph.	2	„
Magnesium Sulphate	2	„
Ammonium Tartrate	100	„
[Cane Sugar	1500	„]
Water	8376	„
	10,000	parts.

The sugar is to be omitted when Pasteur's fluid "without sugar" is ordered. Pasteur himself used actual yeast ash; the above constituents give an imitation ash, which, with the ammonium salt and sugar, answers all practical purposes.

21. **Picric Acid, Solution of.**
Make a saturated solution in distilled water.

22. **Picric Acid, Kleinenberg's solution of.**
To a cold saturated solution of the acid add 2 parts of nitric or concentrated sulphuric acid.

Filter, and add to the filtrate three times its bulk of water.

Immersion of from 3—5 hours will suffice for most preparations.

23. **Potash Solution.**
Dissolve 5 grammes of potassic hydrate in 100 cubic cent. of water.

24. **Sachs's Food-solution for green plants.**

Distilled water 1000 cub. cent.	
Potassium nitrate	1 gramme.
Sodium chloride	0·5 gramme.
Calcium sulphate.....	0·5 gramme.
Magnesium sulphate	0·5 gramme.
Calcium phosphate	0·5 gramme.

To this solution add a trace of a weak solution of ferric chloride. The calcium phosphate is only slightly soluble in water.

25. **Schulze's Macerating mixture.**
Dissolve 1 gramme of potassium chlorate in 50 cub. cent. of nitric acid. Immerse the tissue in this solution, and heat it. The tissue will then readily break up into its constituent cells, their middle lamellæ being dissolved.

Cold maceration often gives better results, but takes longer.

Take care that the gases given off from the mixture do not injure the microscope.

Schulze's Solution.
Dissolve some zinc in hydrochloric acid; permit the solution to evaporate, in contact with metallic zinc, until it has attained a syrupy consistence. Saturate the syrup

with potassic iodide, and then add enough iodine to make a dark sherry-coloured solution. The object to be stained must be placed in a little water, and then some of the above solution added.

27. **Silver Nitrate, Solution of.***
Dissolve 0·5 grammes of silver nitrate in 100 cubic cent. of distilled water. Keep in an opaque stoppered bottle.

28. **Sodic Chloride, Solution of.** (*Normal saline Solution. Salt solution.*)
Dissolve 7·5 grammes of sodic chloride in 1 litre of distilled water.

F. SECTIONS AND SECTION CUTTING.

Directions for preparing sections of the vegetable tissues will be found incorporated in the text; those which follow apply to the animal series alone.

1. **Imbedding.**
All tissues or embryos about to be imbedded, whether stained or unstained should have been first well hardened in 90 p.c. alcohol.

For imbedding, a mixture of hard and soft paraffins is most serviceable, such as shall melt at from 50° to 60°.

The preparation to be imbedded, if stained, should be first soaked in turpentine to saturation; if unstained, it may be transferred direct from the alcohol. In either case it must be placed in melted paraffin (the temperature of which must not exceed that of its melting point) until thoroughly permeated thereby. When ready for imbedding, take of the solid paraffin a piece of about the calibre of a candle and excavate at one end a pit, large enough to fully accommodate the preparation; then transfer the latter and fill the pit with melted paraffin. Put the whole aside, until quite cool and firmly set.

2. **Cutting.**
For this purpose an ordinary razor will suffice, so far as

the requirements of this volume are concerned. The edge should be kept permanently sharp.

Before cutting, pare away the imbedding material, so as to reduce that which surrounds the preparation to the minimum.

3. **Mounting.**

Transfer the sections as cut, paraffin and all, to microscopic slides previously prepared by one of the two undermentioned methods, and proceed as directed in either case.

a. White of egg method.

Smear the surface of the slide with a thin but uniform film of freshly drawn white of egg and deposit the sections in order of cutting. Gently heat the slide until the paraffin begins to melt and then put it aside. When set, put the whole bodily into turpentine and leave it until all the paraffin is dissolved out; upon examination the sections will be found to be firmly attached to the glass slide by means of the coagulated albumen.

Immersion in turpentine for an indefinite period will do no harm.

b. Kreasote and shellac method.

Smear the surface of the slide with a heated solution of white shellac in Kreasote. Submit the whole to the temperature of the melting point of the paraffin until the kreasote is evaporated, whereupon the sections will become firmly adherent to the glass by means of the shellac. Next immerse the whole in turpentine, and leave it at rest until the excess of imbedding material is dissolved out.

This method is best applicable to preparations which have been previously stained and clarified. The white of egg process is not only the simpler of the two, but the more advantageous, as the sections may be cut and

mounted unstained, that process and the subsequent clarifying being permissible after fixture to the slide.

4. **Final mounting.**

Allow sufficient of the canada balsam to drop upon the sections to fully cover them. When thoroughly diffused among them, smear the under face of a cover-slip with balsam and place one edge of it upon the slide supporting its body upon the point of a needle; by gradually withdrawing the latter the cover-slip will descend obliquely expelling the enclosed air.

Finished preparations should be examined from time to time, in order that loss of the mounting medium by evaporation may be made good.

5. **To prepare ground sections of shells, bones, or other hard parts.**

A small piece of the structure to be manipulated should be first isolated and then cemented in the desired position to a piece of plate glass, by means of canada balsam. When firmly set it should be ground down upon a hone or rough surface to the required thinness, and finally dislodged for mounting, by immersing the whole in benzole or chloroform. It may then be put up in canada balsam in the manner described above.

6. **Frozen sections.**

Material for this purpose should be preserved in weak glycerine. For the preparation of these sections an ether-spray freezing microtome is desirable; good preparations may however be made as follows.

Obtain a metal rod of the calibre of a candle and 2—3 inches in length: place the preparation (which, unless quite fresh, should be previously soaked in gum-water) on one end of it and add 6—8 times its bulk in ordinary fluid gum. Freeze with pounded ice and salt. Cut.

Mount in weak glycerine.

INDEX.

A.

Abdomen, 174, 190, 192, 238
Abdominal appendages, 195, 198; papilla, 188, 236
Acetabulum, 71
Adipose tissue, histology of, 131
Adrenal, 24, 52
Adventitious buds, 444.
Afferent branchial vessels, Crayfish, 226; Mussel, 328
Alæ, 477
Albumen gland, 289; duct of, 290
Alcoholic fermentation, 377, 383, 388
Algæ, 392, 396, 436
Alimentary Canal, Crayfish, 178, 207; Earthworm, 241, 253, 267; Frog, 10, 15, 45; Mussel, 308, 320; Snail, 274, 284
Alinasal process, 13, 64
Allolobophora, 240, 249
Alternation of generations, Chara, 435; Fern, 451
Ambulatory appendages, 175, 197
Amœba, 369—376
Amœboid movement, 123
Anabolism, 381
Anacharis, 442
Annulus, Fern, 449, 457; tympanicus, 118
Anodonta cygnea, 305—341
Antenna, 176, 200
Antennule, 176, 201
Anther, 460, 465, 477
Antheridium, Chara, 431, 433, 439; Fern, 450, 458, 466
Antherozoid (see Spermatozoid)
Antipodal cells, 479
Anus, Crayfish, 179; Earthworm, 241, 249; Mussel, 307; Snail, 281, 283
Aorta, Frog, 19, 20, 86; Snail, 293; anterior, Mussel, 326; Snail, 294, 299; posterior, Mussel, 326; Snail, 294
Aortic arches, 20, 85, 89, 92
Apical cell, Chara, 432, 438; Fern, 448, 455
Appendages, Chara, 430; Crayfish and Lobster, 174, 176, 177, 192, 195; development of, 237, 238
Aqueduct of Sylvius (see Iter)
Aqueous humour, 117
Arachnoid, 10
Archegonium, 450, 459, 466
Archenteron, Crayfish, 187, 236; Frog, 167, 170; Snail, 303
Artery, antennary, 225; antero-ventral, 224; carotid, 18, 20, 85; cœliac, 88; cœlicao-mesenteric, 87, 88; common iliac, 87; gastric, 225; genital, 87; hepatic, 180, 224; hypogastric, 87; inferior abdominal (see postero-ventral); inferior mesenteric, 87, 88; ophthalmic, 224; pedal, Mussel, 326; postero-ventral, 225; pulmo-cutaneous, 19, 20, 86; renal, Crayfish, 226; Frog, 87; rostro-antennary, 225; splenic, 88; sternal, 180, 224; subclavian, 86; superior ab-

dominal, 180, 224; superior mesenteric, 88; vertebral, 86; visceral, Mussel, 326
Arthrobacterium, 413
Arthrobranchiæ, 221
Articular, 62
Arytenoid, 21, 51
Ascent of sap, 468
Ascogonium, 420, 427
Ascospore, 420, 427
Ascus, 420, 427
Asexual generation: Fern, 451
Astacus fluviatilus, 173—239
Astragalus, 73
Atrium, 17, 81, 90
Auditory epithelium, Frog, 145; Lobster, 232
Auditory capsule, 59; organ: Crayfish, 186, 230; Cyclas, 331; Frog, 33, 118; development of, 161; Mussel, 331; Snail, 276, 300
Auricle, Frog, 17, 90; Mussel, 324; Snail, 275, 284
Auricular septum, 17, 91
Axil, Bean, 460; Chara, 431

B.

Bacillus, 413
BACTERIA, 408—414
Balantidium, 363
Basipodite, 195
Bast, Bean, 464, 472; Fern, 446, 453
Bast fibres, 464, 474
Bast parenchyma, 454, 473
Bast vessels, 454, 473
BEAN-PLANT, 460—481
BELL-ANIMALCULE, 359—368
Bidder's organ, 54
Bile ducts, 47
Bipolar nerve-cell, 139
Blastoderm, 236
Blastopore, Crayfish, 187, 236; Frog, 159, 160; Snail, 302
Blood, Crayfish, 226; Earthworm, 245, 262; Frog, 16, 121; Lobster, 182; Mussel, 310; Snail, 276
Blood glands, 245, 258—259
Body-cavity, Earthworm, 247 252; Frog, dev. of, 169; Hydra, 349, 351; Mussel, 335
Body-wall, Earthworm, 243, histology of, 264; Frog, 41, 43; Hydra, 344, 349
Bone, histology of, 129
Brachial plexus, 103
BRACKEN FERN, 443—459
Brain, 11, 24, 96, 170
Branch, 430, 443, 460
Branchiæ, Crayfish, 182, 220; Mussel, 307, 316, 317, 322, 335, 336; suspensory ligament of, 318, 335; Tadpole, 5, 6, 161, 162, 164, 171
Branchial clefts, 5
Branchio-cardiac canals, 181, 222
Branchiostegite, 174, 176, 193, 194
Bronchi, 50
Brownian movement, 408, 411
Brow-spot, 35, 36
Buccal-mass, 285, 286; sac, 241, 253, 255
Bud, Bean, 460; Chara, 432, 438; Hydra, 350
Bundle-sheath, 446, 453
Byssus, 313, 341; organ, 341

C.

CÆCUM, Crayfish, 208; Frog, 16; Lobster, 208
Calcaneum, 73
Calcar, 3, 73
Calciferous glands, 243, 254
Calcium, 379, 403
Caltha, 481
Calyx, 460, 476
Cambium, 463, 473
Campanula, 481
Canal cells, 459
Canalis centralis, 31
Capillaries, 93
Capitulum, 434
Capsulogenous glands, 247, 250
Carapace, 174, 190; development of, 237

Carbo-hydrate, 467
Carbon, 379, 390, 403
Carbonic anhydride, 381, 390, 402, 467
Carchesium, 368
Carina, 477
Carotid gland, 86
Carpopodite, 196
Carpus, 71
Cartilage, histology of, 129; replacement in bone, 14
Cartilage bones, 63
Catabolism, 381
Cell-division, hyaline cartilage, 128; Spirogyra, 399, 405; wall, 379
Cellulose, 379
Cement glands, 186, 235
Centrum, 56, 58
Cephalon, 174
Cephalothorax, 173, 190, 193, 237
Cerebellum, 25, 98
Cerebral hemisphere, 97
Cerebro-spinal axis, 10, 95; development of, 160, 168; nerves, 10
Cervical groove, 174, 175
Chara, 430—441
Chela, 175, 197, 237
Chlamydococcus, 389
Chloragogen cells, 242, 243, 267
Chlorelloids, 344, 345, 356, 358
Chlorophyll, Bean, 465, 467; Chara, 433; Fern, 447; Hydra, 344; Protococcus, 389; Spirogyra, 397; Vorticella, 362
Chondro-cranium, 12, 63
Choroid, coat, 33, 117, 300; plexus, 27, 100
Chromatophore, Protococcus, 389, 391, 393; Spirogyra, 397, 404
Cilia, Bacteria, 408; Chara, 434; Fern, 450; Protococcus, 391, 394.
Ciliary action, Frog, 124; Vorticella, 366
Ciliated groove, 322, 338
Circulation, Crayfish, 182; Frog, 19, 92
Circulatory system, Crayfish, 180, 222; Earthworm, 244, 259; Frog, 16, 80; Mussel, 309, 325; Snail, 275, 292
Circulus venosus pulmonis, 295
Circum-neural arcade, 265
Circum-œsophageal vessels, 245, 259, 260
Clavicle, 69
Clitellum, 245, 248
Cloaca, Frog, 2, 10, 47; orifice of in Tadpole, 163, 165
Cnidoblast, 346, 355
Cnidocil, 346, 355
Cocoon, 247
Cochlea, 33
Collateral bundle, 463
COLOURLESS BLOOD CORPUSCLE, 122, 369, 372, 375
Columella, Mucor, 421; Snail, 282; auris, 33, 34, 60, 65, 119
Commissures, ant, œsophageal, 229; buccal, 298; cerebro-pedal, Mussel, 330, 339; Snail, 297; cerebro-splanchnic, Mussel, 329, 330, 336; Snail, 297; circum-œsophageal, Crayfish, 227; Earthworm, 262; of brain, Frog, 26, 101; inter-cerebral, Mussel, 330, 339; lateral neural, 261; longitudinal ventral, Crayfish, 184, 227; Earthworm, 263; maxillo-palatine, 106; median ventral, 229; post-œsophageal, 229
Condylar facet, 56
Confervoid algæ, 396
Conidium, 417, 419, 426
Conidiophore, 417, 419, 426
Conifers, 466
Conjugation, Amœba, 373; Mucor, 423; Spirogyra, 400, 406; Vorticella, 363
Conjunctiva, 44
Connective tissue, histology of, 126
Coracoid, 69
Cornea, 117, 185, 300; facets of, 233, 234

Corolla, 460, 476
Corpora adiposa, 40, 54
Corpus callosum, 26, 101
Corpuscula, 466
Cortex, 462, 465, 472
Cortical layer, Chara, 430, 432
Cothurnia, 368
Cotyledon, 461, 480
Coxopodite, 195
Cranium, 63
CRAYFISH, 173—239
Cricoid, 21, 51
Crop, Earthworm, 241, 254; Snail, 274, 284
Crystalline cone, 185, 233, 234, lens, 117; Snail, 300, development of, in Frog, 172
Crystals, Amœba, 372, Hydra, 353
Cutaneous glands, 34, 146, 148; Earthworm, 243, 265
Cuticle, Crayfish, 189; Earthworm, 241, 251; Hydra, 352, 353; Vorticella, 360, 364
Cuticularized cell-wall, 401, 407
Cyclas, 331
Cylinder, central vascular, 446, 448, 456, 462, 465, 471, 472

D.

DACTYLOPODITE, 196
Dart, 277, 292; sac, 291, 292
Dentary, 62
Dermis, 146, 147
Development, Crayfish, 187, 235; Earthworm, 247; Frog, 5, 157; Hydra, 344; Lobster, 238; Mussel, 312, 340; Snail, 278, 302
Diaphragm, 43
Dicotyledons, 464
Digestion, Amœba, 371: Crayfish, 179; Earthworm, 242; Hydra, 343, 357
Digestive gland, Crayfish, 179, 207, 209, 215; Earthworm, 242; Mussel, 311, 320; Snail, 285; development of, 303
Digitate gland, 292
Disc, 364
Ductus arteriosus, 89; Botalli, 89
Duodenum, 45, 46
Dura Mater, 96

E.

EAR (see Auditory organ)
EARTHWORM, 240—271
Ecdysis, 189
Ectoderm, 236, 303; Hydra, 344; histology of, 351, 352, 353
Ectoplasm, 124
Ectosarc, Amœba, 370, 374; Vorticella, 360, 364
Efferent branchial vessels, Crayfish, 222; Mussel, 327
Egg-apparatus, 479
Egg-capsule, 235
Elodea, 442
Embryo, Bean, 461, 480; Fern, 451
Embryo-cells, 5, 158
Embryonic layers, 165
Embryo-sac, 461, 466, 478
Encephalon, (see Brain)
Encystment, Amœba, 369; Vorticella, 363
Endoderm, 236, 303; Hydra, 344 histology of, 351, 352, 353, 355
Endolymph, 33
Endophragmal system, 189, 201
Endoplasm, 124
Endoplast (see Nucleus)
Endopleurite, 194
Endopodite, 177, 193, 239
Endosarc, Amœba, 370, 374; Vorticella, 360, 364
Endoskeleton, Frog, 11, 55; Snail, 274
Endosperm, 466, 479
Endosternite, 194
Endotergite, 194
Epiblast, 166, 187
Epidermis, Bean, 462, 473; Earthworm, 245, 265; blood plexus of, 245; Fern, 445, 447, 453; Frog, 121, 146
Epiglottis, 49
Epimeron, 192, 194

Epiostracum, 203, 333
Epiphragm (see Hybernaculum)
Epipodite, 177, 183, 221
Epistylis, 368
Epithelium, 124
Eurotium, 419
Eustachian recess, 9, 48
Excretory organ, Crayfish, 183; Earthworm, 244; Frog (see Kidney); Mussel (see Organ of Bojanus); Snail, 275, 283; orifice, Mussel, 323, 335
Exoccipital, 60, 63, 65
Exopodite, 177, 193, 238
Exoskeleton, Crayfish, 173, 189, 191; Earthworm (see Cuticle); Frog, 11; Mussel, 331, 335; Snail, 274, 281; histology of, Crayfish, 202; Mussel, 333
External characters, Crayfish, 173, 190; Earthworm, 240, 248; Frog, 1, 6, 35; Hydra, 342, 348; Mussel, 305, 314; Snail, 272, 280
Eye, Crayfish, 184, 233; Frog, 2, 36, 44, 112; Snail, 273, 299
Eyelids, 2, 3, 36, 44, 112
Eyespot, 304
Eye-stalk (see Ophthalmite)

F.

FALCIFORM ligament, 43, 44
Fat, 379
Fat bodies (see Corpora adiposa)
Fenestra ovalis, 34, 65
Fermentation, Bacteria, 410; Mucor, 424; Yeast, 377, 382, 388
Fern, 443—459
Fertilization, Bean, 480; Chara, Fern (see Impregnation); Frog, 4; Mussel, 312; Snail, 277
Fibula, 72
Filament, 460, 477
Filum terminale, 30, 96
Flagellum (see Spermatophoral gland)
Flower, 460, 476
Flowering plant, 460—481.
Follicular epithelium, Crayfish, 220, Frog, 155
Fontanelles, 12—63
Food yolk, Crayfish and Frog, 187, 235
Foot, Fern, 450, 459; Hydra, 348; histology of, 346; Mussel, 334; Snail, 273, 280; development of, 304
Foramen magnum, 59; of Munro, 26, 100, 101
Foramina for exit of cranial nerves, 66
Fourth ventricle, 100
Fragmentation of nucleus, 437
FRESHWATER POLYPES, 342—358
FROG, 1—172
Fronto-parietal, 60
Fungi, 382, 416
Funiculus, 479

G.

GALL bladder, 45, 47
Ganglion, abdominal, 227; cerebral, Mussel, 330, 339; Gasserian, 28, 105; parieto-splanchnic, Mussel, 312, 329, 338; Snail, 276, 297; pedal, Mussel, 312, 339; Snail, 276, 296; spinal, 102; subœsophageal, Crayfish, 184, 228; supra œsophageal, Crayfish, 184, 227; Earthworm, 246, 262; Snail, 276, 296; sympathetic, 107; thoracic, 228; visceral, 331
Gastrolith, 210
Gastrula, 187, 236, 247, 278, 302
Gemmation, 362, 380
Genital cloaca, 276, 291
Genital duct, Crayfish, 206; 250; Frog, 40, 53; Mussel, 336; Snail, 276
Genital furrow, 281
Genital gland, Crayfish, 206; Frog, 40, 42, 53; Mussel, 336; Snail, 276, 301
Germinal epithelium, Frog, 155; Earthworm, 270; spots, Cray-

fish, 219; Mussel, 340; vesicle, Crayfish, 219; Earthworm, 270; Frog, 24, 155; Mussel, 340; Snail, 302
Germination, Bean, 461; Chara, 435; Fern, 449; Mucor, 423; Penicillium, 417, 426; Spirogyra, 401
Gills (see Branchiæ)
Girdle-bone (see Sphenethmoid)
Gizzard, 241, 255
Glenoid fossa, 68
Glochidium, 313, 340
Glottis, 9, 21, 49
Glycerine, 383
Glycogen, 151, 311, 329
Green gland, 183, 207, 216
Ground parenchyma, 445
Growing point, Bean, 462; Chara, 432; Fern, 448
Guard-cells, 448, 473
Gullet, Crayfish, 210; Mussel, 320
Gustatory organ, 110
Gymnosperms, 464

II.

Hæmatococcus, 389
Harderian gland, 33, 114
Head segment, 273
Heart, Crayfish, 180, 181, 206, 223; Frog, 17, 40, 43, 80, 89; Mussel, 310, 324, 337; Snail, 275, 284
Helix aspersa, *H. hortensis*, 272—304
Helleborus, 481
Hepato-pancreas, 180
Hermaphrodite duct, 276, 289; gland (see Ovotestis)
Hilum, 479
Hip-girdle, 71
Homarus vulgaris, 173—239
Humerus, 69
Hybernaculum, 272, 282
Hydra fusca, *viridis*, 342—358, *hexactinella*, 342
Hydrogen, 379, 403
Hyoid, 9, 14, 49, 67
Hyphæ, 416, 419, 420
Hypoblast, 167, 187
Hypoderma, 445
Hypodermis, Worm, (see Epidermis)
Hypostome, 343, 348; histology of, 346, 353

I.

Ileum, 45, 46
Ilium, 72
Impregnation, Bean, 461; Chara, 434; Crayfish, 235; Earthworm, 247; Fern, 450; Hydra, 344
Indusium, 444, 456
Infra-branchial chamber, 316
Infundibulum, 26, 99, 101
Infusoria, 359
Integument, Crayfish, 204; Frog, 34 (see also Skin)
Inter-articular membrane, 201
Intercellular passages, 448, 475
Internal ear, 119
Internodal cell, Chara, 432
Internode, Bean, 460; Chara, 430; Fern, 444
Inter-ocular gland (see Brow-spot)
Interstitial tissue, 352; histology of, 354
Inter-vertebral foramina, 57
Intestinal valve, Earthworm, 242, 255; Mussel, 321; Frog, 15, 46
Intestine, Crayfish, 179, 208; Earthworm, 242, 254; Frog, 39; Mussel, 320; Snail, 274, 286; Tadpole, 163; histology of, Earthworm, 264; Frog, 148
Intra-vertebral bodies, 12, 58
Iris, 113
Iron, 403
Ischiopodite, 196
Ischium, 72
Iter, 100

K.

KEBER'S organ, 319, 334
Kidney, 22, 40, 41; histology of, 151
Kleinenberg's fibres, 352, 353

L.

LABIAL cartilages, 64
Labial palpi, 307, 316, 321, 338
Labrum, 175, 176, 202; development of, 237
Lamina terminalis, 99, 100
Large intestine, 10, 15, 39, 41
Larynx, 21, 49, 51
Lateral intestinal vessels, 261
Lateral neural vessels, 259
Lateral œsophageal trunks, 245, 260
Lateral roots, 465, 471
Lateral ventricle, 100
Leaf, Bean, 460, 462, 465, 474; Chara, 430, 431, 437; Fern, 443, 455
Leaflets, Bean, 462, 474; Chara, 436; Fern, 443
Lemna, 471
Lesser omentum, 43
Ligament, of shell, 306, 332, 335
Ligamentum latum, 43
Lily, 477, 478, 481
Limb-girdle, 14, 55, 68
Limbs, Frog, 2, 36; development of 163, 164; Crayfish, 175
Lingual ribbon (see Radula)
Liver, 16, 39, 43, 47; histology of, 150
LOBSTER, 173—239
Lower layer cells, 158, 159, 166
Lumbo-sacral plexus, 103
Lumbricus herculeus, 240—271
Lungs, 21, 39, 43, 46, 50; development of, 164
Lymph, 16
Lymph-cavities, subcutaneous, 37, 44, 45
Lymph-hearts, 17, 94
Lymph sinus, circum-œsophageal, 94; sub-vertebral, 8, 16, 42
Lymph stomata, 126

M.

MACROZOOSPORE, 391, 393
Magnesium, 379, 403
Mandible, Frog, 9, 62; Crayfish, 200
Mantle, 305, 307, 316, 328, 334; development of, 340
Manubrium, 433
Manus, 2
Marginal sinus, 295
Maxilla, Crayfish, 175, 199, 213; Frog, 9, 61
Maxillipede, 175, 196, 197, 199
Mayer's solution, 386
Meckel's cartilage, 13, 67
Medulla oblongata, 98
Medullary rays, 463, 473
Membrana propria, 220
Membrane bones, 63
Membranous labyrinth, 33, 119, 120
Mento-Meckelian bone, 62, 67
Meristem, 455, 462, 464
Meropodite, 196
Mesencephalon, 98
Mesenteric septa, 243, 252, 265
Mesenteron (see Archenteron)
Mesentery, 8, 39, 42
Mesoarium, 42
Mesoblast, Earthworm, 247; Frog, 167, 169, 170
Mesoglœa, 347, 352, 356
Mesophyll, 456, 465
Mesorchium, 42
Metabolism, 381
Metacarpals, 70
Metastoma, 175, 176, 202, 213
Metatarsals, 73
Metencephalon, 98
Micrococcus, 413
Micropyle, Bean, 461, 478; Mussel, 312, 339
Microzoospore, 391, 394
Monœcious conjugation, 401

MOULDS, 415—429
Mouth, Crayfish, 175, 202; Earthworm, 249; Frog, 9, 44, 48; Hydra, 343, 348; Mussel, 306, 317, 321, 338; Snail, 274, 281; Tadpole, 161, 163.
Mucor, 420; Torula of, 424
Multinucleate cells, 417
Multipolar nerve-cell, 141
Muscles, abdominal, Crayfish, 205, 207; adductor, Mussel, 306, 315, 337; anal, 215; of body wall, Earthworm, 265; of buccal mass, 286: columellar, 282; constrictor laryngis, 50; of eye, 33, 113; of eyestalk, 233; of hind limb, Frog, 74—80; of hyoid, 50; limb, Crayfish, 204; mandibular, 205, 207; mylohyoid, 37; oblique of trunk, 38; pallial, 315; pectoral, 37; pedal, Mussel, 315, 339; of seta, Earthworm, 266; of stomach, Crayfish, 178, 213; transversalis, 38
Muscle, histology, striped, 133; unstriped, 132; physiology, 156
Muscular system, Crayfish, 204; Frog, 24
MUSSEL, 305—341
Mycelium, 416, 422, 425
Myelon (see Spinal cord)
Myophan layer, 364

N.

NARES, 2, 9, 48, 111
Nasal, 60
Nematocysts, 344, 346, 350; histology and development of, 354
Nephridium (see Segmental organ), blood plexus of, 258
Nephrostome, Earthworm, 244, 256, 257; Frog, 54
Nerve, histology of, Crayfish, 229; Earthworm, 264; Frog, 136; physiology, 156
Nerve-cells, Crayfish, 230; Earthworm, 246, 264, Frog, 139; Hydra, 346, 355
Nerves, 1st Cranial (olfactory) 27, 105; 2nd Cranial (optic) 27, 105; 3rd Cranial (oculomotor) 27, 105, 114, 116; 4th Cranial (pathetic), 28, 114, 116; 5th Cranial (trigeminal), 28, 105; 6th Cranial (abducent) 29, 115, 116; 7th Cranial (facial), 29, 106, 109; 8th Cranial (auditory), 29, 107; 9th Cranial (glossopharyngeal), 30, 104, 106; 10th Cranial (vagus or pneumogastric), 30, 106, 108; antennary, 228; antennulary, 228; buccal, 276, 298; ganglionic, Earthworm, 263, Crayfish, 227; hypoglossal, 31, 103; interganglionic, Earthworm, 263; Crayfish, 227; labial, Snail, 299; maxillo-mandibular, 105; ophthalmic, 105; optic, Crayfish, 228; Snail, 300; pallial, Mussel, 329; Snail, 297; palatine, 106; recurrent laryngeal, 110; spinal, 31, 96, 102; splanchnic, 107, 110; sympathetic, 32, 107; tentacular, 299; vaso-motor, 108; visceral, Crayfish, 229; Earthworm, 263; Snail, 297, 298; viscero-motor, 108
Nervous system, Crayfish, 184, 227; Earthworm, 246, 262; Frog, 10, 24, 95; Mussel, 312; Snail, 276, 296; sheath of, Earthworm, 263; Snail, 296
Nettle, 442
Neural arch, 56; canal, 56, 59; cavity, 10, 41; folds and plate, 160, 168
Neurochord, 246, 263
Neurosensiferous apparatus, Hydra, 346
Nitella, 436, 440
Nitrogen, 379, 403
Nodal cell, 432
Node, Bean, 460, 473; Chara, 430; Fern, 444

Notochord, 12, 168, 170
Nucellus, 460, 478
Nucleolus, 123, 389, 393, 399
Nucleus, 122; Amœba, 370; Bean, 461; Chara, 433, 434; Fern, 444; Mucor, 421; Penicillium, 417; Protococcus, 389, 391, 393; Spirogyra, 398, 404; Vorticella, 362, 365; Yeast, 379
Nyctotherus, 368

O.

OCCIPITAL condyle, 59
Odontoid process, 56
Odontophore, 274, 287, 288
Œnothera, 480
Œsophageal glands (see Calciferous glands)
Œsophagus, Earthworm, 254, 255; Frog, 43; Snail, 274, 285 (see also Gullet)
Oil, 407
Olfactory capsule, 64; epithelium, 144; lobe, 97; organ, Crayfish, 184, 232, Frog, 32, 111; ventricle, 100
Omosternum, 69
Oogonium, Chara, 431, 434, 439
Oosperm, 4, 158, 235, 302, 358
Oosphere, 450, 461 (see also Ovum)
Oospore, 434
Opercular membrane, 6, 162; development of, 163, 171
Ophthalmite, 176, 201
Optic bulb, 233, 234; chiasma, 99; cup, 172; lobes, 25, 98; pit, 237; stalk, 172; thalami, 26, 98; tract, 99; ventricle, 100
Organ of Bojanus, 311, 322, 335, 337
Os cruris, 72
Ossicles, Crayfish stomach, 178, 208, 210
Otocyst, 300, 331
Otolith, Crayfish, 231; Cyclas, 331; Frog, 120; Snail, 300
Ovary, Crayfish, 186, 218; Earthworm, 269; Flowering plant, 478; Frog, 23, 40, 52, 154; Hydra, 344, 357; Lobster, 219
Oviduct, Crayfish, 218; Earthworm, 269; Frog, 23, 40, 53, 54; Snail, 290, 292
Ovisac, 219
Ovotestis, 276, 285, 289
Ovule, 460, 478
Ovum, Bean, 461, 466, 479; Chara, 434; Crayfish, 219; Earthworm, 246, 270; Fern, 450, 459; Frog, 5, 24, 154; Hydra, 344, 357; Mussel, 312, 339; Snail, 277, 302
Oxygen, 379, 390, 403

P.

PALATINE, 61
Palato-quadrate cartilage, 63
Palisade parenchyma, 465, 475
Pallium (see Mantle)
Pancreas, 45; ducts of, 47; histology of, 149
Papillæ, of tongue, 34, 111
Paragnatha, 202
Parasphenoid, 61
Parenchyma, 453, 454
Paranucleus (Amœba), 370, 374
Pasteur's solution, 380, 384
Pectoral arch (see Shoulder girdle)
Pedal gland, 273, 281, 288; sinus, 289, 294
Peduncle, 476
Pelvic arch (see Hip girdle)
Penicillium, 416
Pericardium, Crayfish, 182, 222; Frog, 8, 40, 43; Mussel, 310, 319, 335, 337; Snail, 275, 284
Periganglionic glands, 32, 103
Perilymph, 34, 119
Periotic capsule (see Auditory capsule)
Peristome, 274, 282, 360, 364
Peristomium, 240, 249
Peritoneal pores, 244, 250
Peritoneum, Frog, 42; Earthworm, 243, 266
Perivisceral fluid, 244, 253
Pes, 3

Petal, 476
Petiole, 462, 474
Phalanges, manus, 70; pes, 73
Pharynx, Earthworm, 241, 253, 255; Frog, 5
Phloem, Bean, 463, 472; Fern, 446, 448, 449, 453
Phloem-sheath, 447, 453
Phosphorus, 379, 403
Photometric Zoospores, 395
Pia mater, 27, 96
Pineal gland, 26
Pinnule, 443, 447
Pistil, 460, 478
Pith, 462, 472
Pits, 446, 472
Pitted vessels, 464, 473
Pituitary body, 26, 99
Plasmolysis, 397
Pleomorphic Bacteria, 411
Pleurobranchiæ, 221
PLEUROCOCCUS, 389
Pleuron, 192
Pleuroperitoneal cavity, 7, 39, 41; membrane, 8, 42, 43
Plexus, brachial, 31; epidermal, 245; excretory, 261; lumbo-sacral, 31, 103
Plumule, 461, 480
Pod, 461
Podobranchiæ, 220
Polar bodies, 278, 302
Polar plate, 139
Pollen, 460, 465, 477
Pollen-sac, 465, 477; tube, 461, 480
Pollinodium, 420
Pore-canals, Crayfish, 203; Mussel, 341
Posterior commissure, 101
Potassium, 379
Pre-hallux (see Calcar)
Pre-maxilla, 9, 60
Pre-nasal process, 13, 64
Primine, 478
Primordial cell, 391
Primordial utricle, 397, 404, 417, 433
Procephalic process, 202
Processus spiralis, 140
Pro-embryo, 435
Pro-otic, 60
Propodite, 196
Pro-renal duct (see Segmenta duct)
Prosencephalon, 97
Prostate, 290
Prostomium, 240, 249
Protein, 379, 390, 467
Proteus animalcule, 369—376
Prothallus, 450, 457, 466
PROTOCOCCUS, 389—395
Protophloem, 447, 454
Protoplasm, 379
Protoplasmic movement, Anacharis, 442; Chara, 440; Nettle hair, 442; Tradescantia, 441 Vallisneria, 442
Protopodite, 177, 193, 239
Protoxylem, 446, 454, 472
Pseudopodia, 122, 370, 374
Pteris, 443—459
Pterygoid, 61
Pubis, 71
Pulmonary sac, 274, 282
Putrefaction, 410
Pylangium, 18
Pylorus, 15, 46
Pyrenoid, 398, 404

Q.

QUADRATE, 65
Quadrato-jugal, 61

R.

RACHIS, 288
Radicle, 461, 480
Radius, 70
Radula, 287; sac of, 285
Rana esculenta—temporaria, 161—172
Receptaculum ovorum, 269
Receptaculum seminis, 292
Rectum, Mussel, 337; Snail, 283

Renal organs (see Excretory organ and Kidney)
Renal vessels, Snail, 295
Reno-pericardial aperture; Mussel, 324, 335; duct, Snail, 275, 284
Reproduction, Amœba, 373; Crayfish, 187; Earthworm, 247; Frog, 5; Hydra, 343; Mucor, 423; Mussel, 312; Snail, 277; Spirogyra, 400, 406; Vorticella, 362
Reproductive organs, Bean, 465; Chara, 433; Crayfish, 186, 217; Earthworm, 246, 268; Eurotium, 419; Fern, 449, 456, 458; Frog, 23, 51; Hydra, 349, 357; Mucor, 421, 423; Mussel, 312, 320; Penicillium, 419; Snail, 276, 289
Respiration, Bean, 469; Crayfish, 183; Earthworm; 245; Frog, 3, 22; Mussel, 308; Snail, 275; Yeast, 383
Respiratory organs, Crayfish, 182, 207, 221; Frog, 21, 48; Mussel, 307, 322; Snail, 275, 282
Restiform body, 25
Retina, 33, 118; development of, 172; histology of, 141
Retinula, 234
Rhabdome, 185, 234
Rhinal process, 13, 64
Rhinencephalon, 97
Rhizoid, 431
Rhizome, 443
Root, Bean, 460, 465, 470; Fern, 448, 452, 456
Root-cap, 448, 462, 471
Root-filaments, Chara, 430
Root-hairs, 448, 449, 458, 465
Root-pressure, 469
Rostrum, 190, 238

S.

Sac (see Cell-wall)
Saccharomyces, 377—388
Sacculus, 33, 120
Sacrum, 57
Salivary duct, Snail, 285, 288; gland, Earthworm, 242; Snail, 285
Scalariform vessels, 446, 455
Scaphognathite, 174, 182
Scapula, 69
Schizomycetes, 408—414
Sclerenchyma, 446, 453, 454, 472
Sclerotic, 33, 117
Secondary thickening, 464
Secundine, 478
Seed, 479
Segmental duct, 169; organ, 244, 251, 252, 256
Segmentation, cavity, 166; of ovum, Crayfish, 187, 235; Earthworm, 247; Frog, 5, 158, 165; Mussel, 312; Snail, 278, 302
Selaginella, 466
Seminal receptacle (see Spermatheca); vesicle, 246, 268
Sense-organs, Earthworm, 246; Hydra (see Cnidocil)
Sepal, 476
Septum narium, 13, 66, 111
Seta, Crayfish, 203; Earthworm, 241, 249, 251, 266; sac of, 251, 266
Sex-organs (see Reproductive organs)
Sexual generation, Fern, 457
Sexual reproduction, 400, 402, 419, 423
Shell (see Exoskeleton)
Shell-teeth, 340
Shoulder-girdle, 68
Sieve-tubes, Bean, 464, 469, 473; Fern, 447, 454, 455
Sinus venosus, 17, 81, 90
Siphonal tentacles, 317
Siphons, 307, 317
Skeleton, Crayfish, 173, 189; Frog, 11, 55
Skin, Frog, histology of, 145
Skull, 12
Small intestine, 10, 15, 39, 45
Snail, 272—304
Somite, 174, 177, 240, 248
Sooty corpuscles, 356
Sorus, 444
Spermatheca, 250, 268, 292

Spermatophoral gland, 217, 291
Spermatophore, Crayfish, 235; Snail, 277
Spermatozoid, Chara, 434, 440; Crayfish, 218; Earthworm, 246, Fern, 450, 458; Frog, 23, 154; Hydra, 344, 357; Mussel, 312; Snail, 277, 301; development of, 270
Sperm-capsule, 235
Sphenethmoid, 61, 63, 66
Spiculum amoris (see Dart)
Spinal cord, 11, 25, 30, 96; histology of, 140
Spinous process, 56
Spiral vessel, 446, 455, 464, 473
Spirillum, 408, 413
Spirochæte, 413
SPIROGYRA, 396—407
Splanchnic cavity (see Pleuro-peritoneal cavity)
Spleen, 16, 40
Spongy parenchyma, 465, 475
Sporangium, Fern, 444, 449, 456; Mucor, 420, 427
Spore, Bacteria, 409; Fern, 444, 449, 457; Mucor, 421, 428; Penicillium, 417; Yeast, 380, 386
Squamosal, 60
Stamen, 460, 477
Starch, 389, 398, 402, 433, 445
Stem, Bean, 460, 471; Chara, 430, 436; Fern, 443
Sternal sinus, 226
Sternum, 192, 194
Stigma, 460
Stipule, 474
Stoma, Bean, 462, 464, 473; Fern, 448, 456
Stomach, Crayfish, 178, 208; Frog, 15, 39, 46; Mussel, 320, 338; Snail, 285
Stomodæum, 171, 303
STONEWORTS, 430—441
Stratification, 404
Striated spindle, 185, 233, 234
Style, 460, 478
Sub-neural vessel, 245, 260
Sub-ocular arch, 63, 64
Sub-tentacular lobe, 279
Succinic acid, 383
Suckers, 160, 162
Sulphur, 379, 403
Supporting lamella (see Mesogloea)
Supra-branchial chamber, 307, 318, 337
Supra-ilium, 72
Supra-intestinal vessel, 245, 259, 260
Supra-neural vessel, 245, 259
Supra-scapula, 69
Suspensorium, 13, 64, 65
Swimmerets, 192
Synangium, 18
Symbiosis, 344
Synangium, 18
Synergidæ, 479

T

TADPOLE, 5, 162
Tail, 162, 165
Tail fin, 162
Tarsus, 73
Teeth, Crayfish stomach, 178, 211, 212; Frog, 9, 15, 48
Telson, 174, 190, 194
Tendon, 74, 189
Tendril, 474
Tentacles, Hydra, 342, 348; development of, 351; histology of, 349, 352; Snail, 273, 280, 299
Tergum, 192, 194
Testa, 479
Testis, Crayfish, 217; Earthworm, 269; Frog, 23, 40, 53; histology, 153; Hydra, 357; Mussel, 312
Thalamencephalon, 25, 98
Thoraco-abdominal linkwork, 202
Thorax, 174
Thread cells (see Nematocysts)
Thymus, 24
Thyroid, 24
Tibia, 72
Tongue, 9, 34, 44, 49
Torula, 377—388
Tradescantia, 441

Transverse process, 50
Trochal ridge, 279, 303
Trochosphere, 279, 303
Truncus arteriosus, 17, 19, 81, 91
Tuber cinereum, 99
Tubular fibres (see Neurochord)
Tympanic cavity, 9, 33; membrane, 2, 9, 33
Typhlosole (see Intestinal valve)

U.

Ulna, 70
Umbilicus, 282
Umbo, 332
Uncini, 288
Unguis, 477
Unio, 305
Unipolar nerve-cell, 139
Upper layer cells, 158, 159, 166
Ureter, 48
Urinary bladder (see Urocyst)
Urino-genital duct, 48, 52, 54; organs, 51
Urocyst, 23, 39
Urostyle, 12, 55, 58, 59
Utriculus, 33, 120

V.

Vacuole, Chara, 437, 438; Mucor, 421, 428; Penicillium, 417, 425, 426; Protococcus, 393; Spirogyra, 397, 398, 404; Tradescantia, 441; Yeast, 378, 384
Vallisneria, 442
Valves, of heart, Crayfish, 223; Frog, 18; Mussel, 325, 337; Snail, 293
Valves, of stomach, Crayfish, 211, 212; of shell (see Exoskeleton)
Vasa efferentia, 23, 54
Vascular bundle, Bean, 463; Fern, 446, 453
Vas deferens, Crayfish, 217; Earthworm, 268; Frog, 23; Snail, 291
Vein, afferent hepatic, 82; afferent renal, 20, 83; anterior abdominal, 20, 37, 38, 44, 82; axillary, 85; cystic, 82; dorso-lumbar, 83; duodenal, 88; efferent hepatic, 20, 84; efferent renal, 20, 84; epigastric, 82; external jugular, 84; femoral, 83; gastric, 88; genital, 20, 84; great cutaneous, 20, 37, 85; hæmorrhoidal, 88; hepatic-portal, 20, 82, 87; inferior cava, 17, 20; innominate, 85; internal jugular, 85; lieno-intestinal, 88; lingual, 84; oviducal, 83; pelvic, 82; posterior cardinal, 169; pulmonary, Frog, 18, 21, 85; Snail, 292; renal portal, 20, 83, 89; sciatic, 83; subclavian, 85: superior cava, 17, 20; superior mesenteric, 88; vesical, 82
Veliger, 279, 304
Velum, 279, 304
Venous system, Crayfish, 181; Frog, 20; Mussel, 328; Snail, 294
Ventricle, Frog, 17, 81, 91; Mussel, 324; Snail, 275
Ventricles of brain, 26, 100
Veronica, 480
Vertebral body (see Centrum)
Vertebral column, 11, 55
Vesicle, contractile, Amœba, 370, 374; Vorticella, 361, 365; of egestion, Amœba, 371; Vorticella, 362; of ingestion, Amœba, 371; Vorticella, 362
Vesicula seminalis, 52
Vessel, Bean, 464; Fern, 446, 455
Vestibulum, 360, 364
Vexillum, 476
Vibrio, 409, 413
Vicia, 460—481
Visceral arch, 160, 161, 164, 170; cleft, 164, 171; pouch, 170, 171; sac, 273
Visual organ (see Eye)
Vitellus, 24, 270
Vitelline membrane, 155, 220, 270, 339
Vitreous humour, 117

Vocal sac, 9, 51; ligament, 21
Vorticella, 359, 368

W.

Wood, 446, 454, 463, 472
Wood-cells, 473

X.

Xiphisternum, 69
Xylem, Bean, 463, 464, 473; Fern, 446, 448, 449, 454
Xylem-parenchyma, 455

Y.

Yeast, 377—388
Yellow-brown tissue (vide Chloragogen cells)

Z.

Zonites, 241, 248
Zoochlorella (vide Chlorelloids)
Zooglœa, 409, 412
Zoospores, 391, 393
Zygapophyses, 56
Zygospore, Mucor, 423; Spirogyra, 401, 407

CAMBRIDGE: PRINTED BY J. & C. F. CLAY, AT THE UNIVERSITY PRESS.