LABORATORY DIRECTIONS IN

GENERAL BIOLOGY

By Edwin Grant Conklin
PROFESSOR OF BIOLOGY
PRINCETON UNIVERSITY

Third Edition

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INTRODUCTION

THE BIOLOGICAL SCIENCES

Read: Calkins, Biology, pp. 1-5; *or*
Sedgwick and Wilson; General Biology, pp. 6-8; *or*
Shull, Animal Biology, pp. 1-6; *or*
Woodruff, Foundations of Biology, pp. 1-5.

GENERAL BIOLOGY is the science which deals with the most general and fundamental characteristics of living things, whether plants or animals. The study of plants alone is known as Botany, of animals alone as Zoology. A thorough study of any plant or animal includes a knowledge of its (1) Physiology, which deals with its physics and chemistry, its functions and activities; (2) Morphology, which deals with its structure, whether gross (Anatomy), microscopic (Histology), or developmental (Embryology) and also with its classification (Taxonomy); (3) Ecology, which treats of its relations to its environment; (4) Biogeny, which deals with the origin of the individual plant or animal (Ontogeny, Heredity, Development, Genetics, etc.) or with the origin of races, species and larger groups of individuals (Phylogeny, Evolution).

In studying any plant or animal it is desirable that it should be considered from all of these aspects, but some organisms are better suited than others for the study of one or another of these subjects. Accordingly this course is divided unequally into three parts, the first of which deals chiefly with the Physiology and Morphology of the organisms studied, the second with their Ecology and the third with Biogeny.

THE AIMS AND METHODS OF LABORATORY WORK IN BIOLOGY

The purpose of all laboratory work is to study nature at first hand; but in order to save time it is necesary to utilize knowledge slowly acquired by many generations of previous students. Therefore we do not follow Agassiz's motto: "Study Nature, not Books," but, rather, "Study Nature and Books." The educational value of laboratory work lies chiefly in the cultivation of accuracy and independence both of observation and of judgment, and in the deeper and more lasting impression which is made of that which we have actually seen and handled. Each student is expected to make for himself the observations and experiments hereafter indicated. The assigned readings which are given at the beginning of each topic should be carefully read *before coming to the laboratory. In the laboratory these Directions must be studied and followed.* Only in this way can a great waste of time, effort and material be prevented.

A record of every observation or experiment must be entered in the prescribed note book, under numbers corresponding to those in these Directions. This record should consist of drawings and descriptive notes, and every page should bear the name of its author and date. The record for each topic must be inspected and passed by an instructor before any new topic may be undertaken.

To each student in the laboratory is assigned a locker containing a microscope, reagents, glassware, etc., for the safe keeping of which he is held responsible. The microscope is the most complex and delicate instrument in this outfit, and work with it should be preceded by a study of the following description of its parts and directions as to its use.

I. THE MICROSCOPE.

Read: "How to Use and Care for the Microscope." Spencer Lens Co.; *or*
"Use and Care of the Microscope," Bausch and Lomb Optical Co.

A. DESCRIPTION. The body or *tube* bears the lenses and is supported upon a *stand* which also carries a *mirror* to cast light

upon the object examined through a hole in the flat *stage* upon which the object is placed. This hole can be made of various diameters by means of the *iris diaphragm.* A lens for concentrating light, and known as a *condenser,* is placed between the mirror and the stage. The lens at the upper end of the tube is the *ocular* or eye-piece; there are two oculars, of different magnifying power. The combination of lenses at the lower end of the tube is the *objective;* in this microscope there are two objectives of different magnifying power, one marked 3, the other 6; the former (low power objective) is in focus, i. e., gives a clear image of the object examined when its lower end is about ⅔ in. above the object, the latter (high power objective) is in focus when it is about ⅛ in. above the object. The objectives are carried upon a *nose-piece,* by revolving which either one of them may be brought to lie at the lower end of the tube. The tube is really double, one tube being telescoped within another. By holding the body firmly in the left hand and taking hold of the projecting brass ring just below the eye-piece with the right, the inner tube may be drawn out some distance, thus lengthening the body and increasing the magnification. The length of the tube without lenses and nose-piece is 150 mm. and it can be drawn out to 195 mm.; with the nose-piece the tube is 10 mm. longer. A table of magnifications of the different lenses with a given tube length is found on the inside of each microscope case.

B. Use. 1. Reflect light, from white clouds if possible, upon the object. Where all the light is needed, use *concave mirror;* where light is intense and a low magnification is required, use *plane mirror.*

2. Use smaller diaphragms with higher powers.

3. To focus lenses upon an object, use the *coarse,* then the *fine adjustment;* the former movement is by means of a *rack and pinion.* The rack is the toothed plate along the back of the tube, the pinion is a smal cog wheel which fits into the rack and is turned by the two *milled wheels* on each side of the tube. The *fine adjustment* is by means of a *milled screw head* back of the pinion. If the fine adjustment screw is turned in the direction in which a clock's hands move the tube is lowered, turned in the reverse direction it is raised. In using high power turn the tube down nearly to the object, and then, while looking through the microscope, bring the object into focus by slowly turning the tube upward.

Never focus *down* upon the object, since by this method there is danger of crushing the lens into the object. Keep one hand on the fine adjustment when looking at an object and vary the focus constantly to bring all the fine details of structure into view.

4. Do not use higher power objective without *cover glass* over object examined.

5. Always use the lower power before the higher one; and always use the lowest possible power sufficient for distinct vision.

6. Do not touch lenses with fingers. If the field is blurred or the object dim either the cover glass or the lenses are at fault. If the cover glass is dirty remove it and clean it; if the fault is in the eye-piece the particles of dirt revolve when the eye-piece is rotated. If the field is still dim the objective is dirty and must be removed and cleaned. In cleaning the lenses never use anything but clean tissue paper supplied for that purpose. If necessary, the lenses may be moistened by breathing upon them; if this is not sufficient, consult the instructor.

7. Keep both eyes open, using either the right or the left. The strain of microscopic work on the eyes is usually due to the fatigue of constantly closing one eye. If you cannot see the object with both eyes open use an eye-shade provided for that purpose.

8. *Never leave the laboratory without first placing the microscope in its case and locking it and all your apparatus in your locker.*

II. PREPARING OBJECTS.

The preparation of objects for examination under the microscope is termed *mounting.* Objects are usually mounted on pieces of glass 3 x 1 in., known as *slides.* Observe the following directions:

1. If the object to be studied is a mass of cells, separate it into very small pieces by means of *teasing needles;* if it is a fluid use only a very small drop. If too much fluid has been used it will run out from under the cover glass and the excess must then be soaked up with filter paper. Temporary preparations are usually mounted in water, permanent ones in balsam.

2. The lenses of the microscope, the upper side of the cover glass and the lower surface of the slide must be perfectly clean and dry.

3. Having placed the object in a small drop of mounting fluid

take a cover glass in your left hand, rest one edge of the cover on the slide near the drop, and support the opposite edge on a teasing needle; lower the cover glass gradually over the drop, being careful to inclose no air bubbles. Do not press upon the cover glass.

4. Before putting a permanent preparation away label it carefully with the name of the object and the method of preparation.

5. Never use reagents haphazard, but only when you have a definite purpose in view. Reagents are used for fixing, hardening, preserving, staining, dehydrating, clearing, embedding and mounting. *Fixing* is the process of killing and hardening the living thing so that it preserves as nearly as possible its natural form. *Staining* is the dyeing of the object so that some parts are more deeply colored than others. *Dehydrating* is the process of removing the water from the object, usually by alcohol. *Clearing* usually consists in substituting some oil for the alcohol which is in the object. *Embedding* is the process of permeating and surounding the object with some substance such as paraffin, preparatory to cutting sections of it.

6. Miscroscopical slides which have been prepared in this way are valuable, sometimes very valuable, and when such preparations are given out for use they must be handled carefully. Do not crush the cover glass or slide by focusing down on it. Do not pick up your microscope with a slide on the stage, as it is very likely to fall on the floor and be broken. Do not leave slides on the table when you have finished with them but return them to the desk or to the box from which you received them. *A charge will be made for every slide that is broken.*

III. NOTES AND DRAWINGS.

1. Drawings should be made of every object studied; this is necessary not only as a record of what has been seen, but also as an aid to accurate observation. Make your drawings a record of what you actually see and if you cannot see what the directions call for consult an instructor. Do not attempt to make drawings without the object before you and do not make rough sketches and then finish them from memory. In general make outline drawings without shading. Where certain structures occur in large numbers it is sufficient to represent them in only a part of the drawing. Label all important Structures by means of reference lines and marginal words. Use hard pencils (4H), with very sharp points,

and make the drawings large enough so that all details can be represented without confusion.

2. To draw to scale:—Place paper at base of microscope and endeavor to trace outlines as seen with left eye while seeing point of pencil at same time with right eye. The pencil point must appear to coincide with the part of the object being drawn. Do not move the eyes.

IV. EXAMINATION OF COMMON OBJECTS.

1. Mount a few fibres of wool, cotton, linen and silk in water on different slides, cover with cover glasses and examine first under a low power, then under a high one. How do the fibres differ? *Sketch and label one of each under the lower power and then under the high power of the microscope, drawing to scale.*

2. Examine a drop of emulsion (oil suspended in water) and notice peculiar effects of refraction when lenses are focused upon different portions of a drop.

3. Examine bubbles of air in water. These may be obtained by running water under a cover glass supported at one side by a bit of paper and then tapping on the cover glass with a needle. What differences can you see between these and oil drops?

PART I

GENERAL PHYSIOLOGY AND MORPHOLOGY

A. CHEMICAL AND PHYSICAL CHARACTERISTICS OF LIVING THINGS

Read: Sherman, Chemistry of Food and Nutrition, pp. 1-102; *or*
Nutrition, pp. 1-102; *or*
Mathews, Physiological Chemistry, pp. 1-187; *or*
Hawk, Physiological Chemistry, pp. 1-147.

The bodies of all living things are composed of about 15 chemical elements and a great number of chemical compounds: 97 per cent of the human body consists of carbon, hydrogen, oxygen and nitrogen, and 3 percent of 11 other elements. Three-fourths of all the hydrogen and nine-tenths of all the oxygen are combined to form water. In addition to water and mineral salts living things contain carbon compounds, or "organic compounds." Compounds of carbon, hydrogen and oxygen form *Carbohydrates* and *Fats;* compounds of carbon, hydrogen, oxygen and nitrogen form *Proteins.*

I. CARBOHYDRATES (Starches, Sugars, etc.).

Carbohydrates of physiological importance are:—
Monosaccharids ($C_6 H_{12} O_6$)—dextrose, levulose, glucose.
Disaccharids ($C_{12} H_{22} O_{11}$)—cane sugar, malt sugar, milk sugar.
Polysaccharids $(C_6 H_{10} O_5)^n$—starch, dextrin, glycogen, cellulose.

1. MONOSACCHARIDS (glucose, fructose, etc.). Cannot be split into simpler sugars.

a. Dextrose. Take a one per cent solution and test solution as follows:

Boil 5cc. of Benedict's[1] or Fehling's[2] solutions in a test tube. Result?

Then add 8 drops of dextrose solution and boil again. Result? This is the "dextrose test." Copper sulphate ($Cu\ So_4$ in an alkaline solution is "reduced" to yellow cuprous hydroxide ($Cu\ OH$) or to red cuprous oxide (Cu_2O) when boiled with a reducing sugar.

Test your urine for sugar in this way.

2. DISACCHARIDS (Sucrose, maltose, lactose) can be split into monosaccharids by hydrolysis.

a. CANE SUGAR (Sucrose). Take a few drops of 1% solution and test with Benedict's or Fehling's Fluid. Is it a reducing sugar?

b. HYDROLYSIS OF CANE SUGAR. Boil some of the 1% solution with a few drops of hydrochloric acid. Cool, neutralize, and apply Benedict's test. Is a reducing sugar present?

3. POLYSACCHARIDS. Native starch. a. Mount a scraping of potato in water and examine under microscope. Study and draw structure of starch grains. Run a drop of iodine solution under cover. What happens?

b. Grind a little commercial starch in a mortar and shake with cold water. Filter and test filtrate with iodine. Explain result.

c. Test solubility in boiling water. Note character of resulting solution. Dilute and add a drop or two of iodine solution. What results, and why?

d. Cellulose (plant cell-walls). Cotton fiber is almost pure cellulose. Note insolubility in water and alcohol. Is it insoluble in acids? Alkalies? Does it react with iodine? Treat with 40 per cent sulphuric acid and then add iodine. What results? Treat with Schultze's chlor-zinc-iodide.[3] Explain results. This is known as the "cellulose test."

[1] Benedict Fluid: Copper sulphate, 17.3 grams; Sodium or potassium citrate, 173.0 grams; Sodium carbonate, 200 grams; Distilled water to make 1000 cc.

[2] Fehling's Fluid:—(1) Copper sulphate, 34.65 grams; Distilled water to make 500 cc.; (2) Potassium hydroxide, 125.0 grams; Rochelle salt, 173.0 grams; Distilled water to make 500 cc. (3) Mix equal parts of (1) and (2) when needed for use.

[3] Schultze's Chlor-zinc-iodide is made as follows:—(1) Dissolve 110 grams of zinc in 300 cc. hydrochloric acid and evaporate to 150 cc.; (2) Dissolve 12 grams of potassium iodide in as little water as possible and add 0.15 grams iodine; (3) Mix (1) and (2), and filter if necessary.

4. FERMENT ACTION: ENZYMES.

a. Salivary diastase (ptyalin). Collect a few cc. of saliva in a test tube; dilute with about five volumes of water and filter into two test tubes; boil one of the tubes and leave the other unboiled. Add to each tube an equal volume of dilute starch paste and place tubes in incubator warmed to 40 C. for fifteen minutes. Then divide the substance in each tube into two portions and test one of each of these for starch (iodine) and the other for sugar (Benedict's). What effect does ptyalin have on starch? What effect does boiling have on ptyalin?

II. LIPINS (Oils, Fats, Yolk. etc.).

1. OILS AND FATS. (1) Note physical properties, differences in melting point, etc., of three fats—olive oil, butter, and tallow. Test solubilities of these fats in water, alcohol, chloroform, ether. (2) Shake a few drops of olive oil with water in a test tube. What happens? Set tube aside for a few minutes. What happens? Shake up a few drops of the oil with one percent sodium carbonate instead of water, examine with microscope, and note difference in results. (This is an emulsion.) (3) Rub a thin film of butter on a slide, and put on a drop of the dye known as Sudan III. Observe under microscope what occurs.

Stain thin sections of Castor bean and of Lima bean with Sudan III. Is oil present in both?

2. CHEMICAL TESTS FOR FATS. The reaction with ether and with fat stains are two well-known tests for fats.

III. PROTEINS (Albumins, Peptones, Albuminoids, etc.).

Use white of egg as type of protein.

1. Carefully pour white of egg into a dish. This is approximately a 12 per cent solution of a protein (albumin) in water. Notice its consistency. Test its reaction with litmus paper; is it acid, alkaline, or neutral? A 10 per cent solution of this white of egg has been made by shaking it up with 9 times its volume of distilled water and filtering.

2. COAGULATION. (1) Coagulation by heat. Have a water bath with water at the boiling temperature. Put some of the undiluted albumin in a test tube and place in the water bath. Does it coagulate? Try a little of the 10 per cent solution in the same way. Does it coagulate? What is the effect of dilution on coagula-

tion by heat? (2) Coagulation by chemicals. To 5 cc. of the 10 per cent albumin add a few drops of 3 per cent copper sulphate. Try also strong nitric acid and sulphuric acid, allowing a drop or two to run down the side of the test tube. Try also 95 per cent alcohol. (3) Test urine for albumin as follows: a. Boil 10 cc. of urine in a test tube; if turbidity appears add a drop or two of strong acetic acid; if turbidity disappears it was due to phosphates, if not to albumin. b. Put 2 or 3 cc. of strong nitric acid in a test tube, then add urine, allowing it to run gently down the side of the slanted test tube; if albumin is present a ring of coagulum will form between the acid and the urine.

3. CHEMICAL TESTS FOR PROTEINS: *Xanthoproteic Reaction.* Dilute some of the 10 per cent albumin till it is about 2 per cent; place a small quantity in a test tube. Add a few drops of nitric acid. What occurs? Boil. What occurs as to color and other changes? Cool the solution and add ammonia until the acid is neutralized. Note color produced. (This is the essential feature of this reaction.) Try in the same way a weak solution of gelatin (albuminoid); does it coagulate? Does it give the xanthoproteic color?

4. ACTION OF ENYZMES ON PROTEINS. Thin pieces of boiled white of egg were placed in artificial gastric juice, made by adding pepsin to a 0.2 per cent solution of hydrochloric acid and allowed to stand 24 hrs. Some of the digested albumin was placed in one dialyzer and some fresh, undiluted albumin in another. Test the water below each membrane for albumin.

IV. ENZYMES (Organic Ferments).

1. Chemical substances probably allied to proteins though they have never been completely isolated. They are formed by animal or plant protoplasm and act as catalyzers in many chemical reactions within living things.

2. They are classified, according to what they do, as:

Amylo-lytic or Starch Splitting (Diastase, Ptyalian, etc.).
Lipo-lytic or Fat Splitting (Lipase, Steapsin, etc.).
Proteo-lytic or Protein Splitting (Pepsin, Tripsin, etc.).
Sugar Splitting (Maltase, Invertase, Lactase).
Alcohol forming (Zymase).
Coagulating (Thrombin, Rennin).
Oxidizing (Oxidase, etc.)

3. You have already observed the action of ptyalin on starch, and of pepsin on albumen. Write the chemical formula for the former of these reactions.

V. HORMONES (Chemical Messengers).

Chemical substances, possibly enzymes, usually formed by ductless glands and poured into the blood. They stimulate or inhibit many vital processes (Thyroidin, Adrenin, Secretin, etc.)

VI. VITAMINS.

Accessory food substances of unknown chemical nature, produced by animals and plants. May act as enzymes or hormones. Essential to life, but minute quantities sufficient. ("Water-soluble B," "Fat-soluble A," etc.).

VII. PROTOPLASM. (Living substance, material basis of life).

Composed of all the preceding classes of substances together with water and various salts. Protoplasm is not a single chemical compound but is an organized mixture of many compounds, especially proteins. There are innumerable kinds of protoplasm.

B. MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF LIVING THINGS

Protoplasm exists only in the form of cells, which are individual masses of protoplasm, each containing a denser body, the nucleus.

I. CELL STRUCTURES.

Read: Calkins, Biology, pp. 6-29; *or*
Sedgwick and Wilson, General Biology, pp. 20-40; *or*
Parker, Elementary Biology, pp. 56-79; *or*
Shull, Animal Biology, pp. 70-83; *or*
Woodruff, Foundations of Biology, pp. 6-29.

A. PLANT CELLS.

1. Carefully tear in two a leaf and from the torn edge pick off a small piece of the transparent covering (*epidermis*). Mount in water and examine under the microscope. Draw about 10 adjacent epidermal cells, making each cell about half an inch in diameter.

2. In prepared sections through the root-tip of an onion observe the shape and size of the cells. Draw about 10 adjacent cells. Having found one or more complete cells with round nucleus observe and make a *drawing* about 2 inches in diameter of a single cell showing the following parts: 1. Cell Membrane. 2. Nucleus. 3. Cytoplasm (protoplasm surrounding nucleus). In the nucleus observe: 4. Nuclear Membrane. 5. Nucleolus. 6. Chromatin (stained granular part of nucleus). 7. Achromatin (unstained part of nucleus).

B. Animal Cells.

1. With the handle of a scalpel gently scrape the inside of your lip or cheek and mount the scrapings in a drop of water. Draw several cells. Run a drop of aceto-carmine under the cover glass by placing the drop on one side of the cover and applying a bit of filter paper to the other side. The nucleus stains deeply owing to the fact that it contains chromatin. Draw one cell showing nucleus, cytoplasm, and cell-membrane; making the drawing about 2 inches in diameter.

2. In similar manner observe the cells and cell structures in prepared slides of the skin of a frog.

2. Mount a drop of frog's blood and examine under the low power and then under the high. Draw several of the red, also of the white, corpuscles.

II. CELL FUNCTIONS.

Each cell performs all the fundamental functions of life—it nourishes and reproduces itself, is contractile and sensitive,—though some cells are devoted more exclusively to one of these functions than to the others (Specialization). In this place we study only the functions of reproduction and movement.

1. Cell Reproduction. All cells reproduce by division; the nucleus first divides, in one of two ways, after which the cell body constricts into two. Nuclear division occurs by the indirect process (Mitosis) or by the direct process (Amitosis).

(1). Indirect Nuclear Division (Mitosis).

In prepared slides of the growing root-tip of the onion observe nuclei in the following stages of division:

(a) Early Phophase, in which the nucleolus has disappeared and the chromatin granules have united to form threads; (b) Late

Phophase, showing disappearance of nuclear membrane and formation of chromosomes (chromatic rods) from the threads; (c) Metaphase or Equatorial Plate, showing the chromosomes in the equator of the cell, each dividing by a longitudinal split; (d) Early Anaphase, showing the daughter chromosomes separating toward the poles of the cells; (e) Late Anaphase, showing union of daughter chromosomes to form daughter nuclei; (f) Teleophase, in which the cell-body divides and the nuclei; return to the "resting condition."

Draw a cell in each of these stages of division.

(2). Direct Nuclear Division (Amitosis). In prepared slides of the follicile cells surrounding the egg of the cricket observe various stages in the direct division of the nucleus. *Draw cells in which* (a) *the nucleolus is dividing, but the nucleus is still spherical,* (b) *the nucleus is dumb-bell shaped,* (c) *the nucleus is divided into two.*

2. PROTOPLASMIC MOVEMENT. With a pair of fine forceps pull off some of the hairs which grow on the stamens of the flower of the spiderwort (Tradescantia) and mount them in water under a cover glass. Observe: (1) The hair is made up of a succession of cells, with corrugated walls. (2) Just within the cell wall is the granular protoplasm, strands of which may be seen moving or circulating. (3) Within this protoplasm is a clear spherical or ovoid body, the nucleus. (4) Most of the center of the cell is occupied by a purple, homogeneous fluid, the cell sap.

If the flowers of Tradescantia are not available use one of the leaflets of the water weed Elodea canadensis. Observe the green bodies (chloroplasts) within the cells. Do they circulate?

Make a drawing showing these structures, and indicate by arrows the direction of the protoplasmic movement.

C. CLASSIFICATION OF PLANTS AND ANIMALS

Read: Calkins, Biology, pp. 162-166; *or*
Parker, Elementary Biology, pp. 137-147; *or*
Parker and Parker, Practical Zoology, pp. 215-228; *or*
Shull, Animal Biology, pp. 260-274; *or*
Woodruff, Foundations of Biology, pp. 348-351.

All living things are classified as plants or animals depending upon certain peculiarities of structure and function. In general

plants have rigid cell walls of cellulose (C_{12} H_{20} O_{10}), which is lacking in animals; the food of plants consists of relatively simple chemical compounds, whereas that of animals is much more complex; also plants are usually less active than animals.

The minor subdivisions of both animal and plant kingdoms are very numerous and are known as Orders, Families, Genera, Species and Varieties. The scientific name of any animal or plant consists merely of the name of the genus and species to which it belongs. This method of naming animals and plants is due to Linnæus (1707-1778) and is known as *binomial nomenclature.*

Inspect the specimens in the Museum, Herbarium and Vivarium, and become familiar with as many as possible of the subdivisions and classes named above. Enter in your laboratory notes in the following manner the scientific name (copied from the labels of specimens on exhibition) of some one member of each phylum and class of the animal kingdom, so far as represented in the exhibits:

PHYLUM	CLASS	GENUS	SPECIES
Cnidaria	Hydrozoa	Hydra	fusca

The animals and plants which will be studied in this course are common forms which are found in the vicinity of the laboratory. The particular forms to be studied are chosen because they illustrate especially well certain general principles or characteristics.

Locate in the tables of classification the position and relation to other animals or plants of each organism studied in the laboratory, museum or field.

D. METAPHYTA

Metaphyta are many-celled plants, with more or less differentiation of the cells and tissues for particular functions. The lower Metaphyta belong to the Cryptogamia or flowerless plants, the higher ones to the Phanerogamia or flowering plants. Owing to limitations of time, it is not possible in this course to study more than one representative of the Metaphyta, and for this study one of the higher flowering plants is chosen. *viz.*, the common bean.

PHASEOLUS VULGARIS, String Bean

(Subkingdom Phanerogamia, Division Spermatophyta, Subdivision Angiospermae, Class Dicotyledonae.)

Read: Bigelow, Applied Biology, pp. 66-121; *or*
Coulter, Barnes and Cowles, Text Book of Botany, Vol. 1, pp. 295-484; *or*
Curtis, Nature and Development of Plants, pp. 1-129; *or*
Duggar, Plant Physiology, pp. ; *or*
Ganong, Text Book of Botany, pp. 1-178; *or*
Physiology of Plants, pp. *or*
Huxley and Martin, Practical Biology, pp. 460-481; *or*
MacDougall, Plant Physiology, *or*
Vines, Text Book of Botany, pp. 666-783, *or*
Woodruff, Foundations of Biology, pp. 61-114.

A. MORPHOLOGY.

I. SEED.

In beans which have been soaked for 24 hours in water observe:—

1. *Shape, Size, Color.*

2. The *Seed Coat,* a tough outer membrane.

3. The *Hilum,* or scar, where it was attached to the parent plant.

4. Dry the surface of the seed and squeeze it gently; water will exude from a small hole near the hilum, the *Micropyle.*

Draw a bean to show all of these features, in profile and also in face view.

II. EMBRYO.

Remove the seed coat and observe the *Embryo,* which fills the whole space within the seed coat; note the following parts of the Embroyo:—

a. Two *Cotyledons* or seed leaves, which constitute most of the bean seed; they are attached to one another by their bases. Mount in water scrapings from one of the cotyledons and examine under microscope; also test scrapings with iodine and others with Benedict's Solution. What is the chief constituent of a Cotyledon?

Separate the two Cotyledons and observe:—

b. The *Hypocotyl* (stem and root) on the margin of the Cotyledons with its apex toward the micropyle.

c. The *Plummule* (bud) between the Cotyledons, composed of two small primary leaves and a minute bud between them.

Draw an embryo to show the inner face of a cotyledon with hypocotyl and plumule attached.

CHIEF SUBDIVISIONS OF THE ANIMAL KINGDOM

SUBKINGDOM			PHYLUM (=TYPE)	SUBPHYLUM	CLASS
A. PROTOZOA Without gastric cavity, germ layers or tissues.			I. PROTOZOA Entire body consists of a single cell which may be independent or joined with others to form a colony		1. Flagellata (Euglena) 2. Infusoria (Paramecium) 3. Sarcodina (Ameba) 4. Sporozoa (Gregarina)
B. METAZOA With gastric cavity, germ layers and tissues; animals develop from eggs through cleavage, blastura and gastrula stages.	(a) *Protaxonia* (= Coelenterata) With persistent gastrular axis, and radial, biradial, or bilateral symmetry.	(a) *Acoelomata* Without true coelom or mesothelium, but with blastocoel and mesenchyme.	II. SPONGIARIA (Sponges) Fixed forms, chiefly marine, Gastrula attaches by oral pole. Colonies formed by budding.		1. Calcarea 2. Hexactinelida 3. Demospongia (Commercial Sponge)
			III. CNIDARIA Attached polyps, or free medusae with Stinging Cells, Gastrula attached by apical pole. Colonies formed by budding.		1. Hydrozoa (Hydra) 2. Scyphozoa (Jellyfish) 3. Anthozoa (Coral)
			IV. CTENOPHORA. Jelly spheres, entirely marine; 8 meriodional rows of swimming plates.		
	(b) *Heteraxonia* (=Bilaterata) Chief axis not that of gastrula. Bilateral symmetry.		V. PLATYHELMINTHES. Worms flattened dorso-ventrally. Oral pole of gastrula usually on ventral side; apical pole (with brain and sense organs) near anterior end.	(a) Platoda	1. Turbellaria (Planaria) 2. Trematoda (Flukes) 3. Cestoda (Tapeworms)
				(b) Nemertinea	(Proboscis Worms)
			VI. ROTIFERA. Microscopic animals with head (trochal disk) trunk and tail (foot).		
			VII. NEMATHELMINTHES Thread Worms. Mostly parasitic, with unsegmented bodies covered by dense cuticle.	(a) Nematoda	(Round Worms)
				(b) Gordiacea	(Hair Worms)
				(s) Acanthocephala	(Anchor Worms)
		(b) *Coelomata* With coelom which may be limited to pericardial cavity.	VIII. CHAETOGNATHA. Small marine Arrow Worms, body divided into head, trunk and tail; setae on sides of mouth.		(Sagitta)
			IX. ANNELIDA. Ringed worms with segmented bodies, each somite inclosing		1. Chaetopoda (Bristle Worms) 2. Gephyrea (Siphon Worms)

* ARTHROPODA. Animals (a) Branchiate

X. ARTHROPODA. Animals with jointed bodies and legs; covered by dense coat of chitin; without cilia.	(a) Branchiata (With gills)	1. Crustacea (Crayfish)
	(b) Tracheata (With tracheae)	1. Onychophora (Peripatus) 2. Hyriapoda (Thousand Legs) 3. Insecta (Insects) 4. Arachnida (Spiders)
XI. MOLLUSCOIDEA. Unsegmented animals usually attached by stalk; crown of tentacles (Lophophore) around mouth.		1. Phoronida 2. Brachipoda 3. Polyzoa (Colonial forms)
XII. MOLLUSCA. Unsegmented animals with reduced coelom; usually with rasping organ (Radula) in mouth and with free fold of body wall (Mantle) which secretes shell.	(a) Aglossa (Without Radula)	1. Pelecypoda (Oyster, Clam)
	(b) Glossophora (With Radula)	2. Amphineura (Chiton) 3. Gasteropoda (Snail) 4. Scaphopoda 5. Cephalopoda (Squid)
XIII. ECHINODERMATA. Marine animals, 5-rayed and apparently radially symmetrical, but really bilateral; with ambulacral system of water vesels and tube feet.		1. Holothuroidea (Cucumber) 2. Echionoidea (Sea Urchin) 3. Asteroidea (Starfish) 4. Ophiuroidea (Brittle Star) 5. Crinoidea (Stone Lilies. Usually attached)
XIV. CHORDATA. Segmented animals with axial skeleton (Notochord), with tubular nervous system on dorsal side and alimentary canal on ventral side, with gill slits opening through lateral walls of pharynx.	(a) Adelochorda	Enteropneusta (Balanoglossus)
	(b) Urochorda	Tunicata (Ascidian, Sea Squirt
	(c) Cephalochorda	Acrania (Amphioxus)
	(d) Vertebrata	1. Cyclostomata (Lamprey) 2. Pisces a. Elasmobranchii (Sharks) a. Ganoidei (Armored fish) c. Teleostei (Bony fish) d. Dipnoi (Lung fish) 3. Amphibia (Frogs, Toads) 4. Reptilia (Reptiles) 4. Aves (Birds) 6. Mammalia (Mammals)

CHIEF SUBDIVISIONS OF THE PLANT KINGDOM

Subkingdom	Division	Subdivision	Class
	I. Thallophyta (Plants without true stems, roots or leaves)	Algae (Sea weeds and simpler fresh-water weeds.)	1. Cyanophyceae, Blue-gr Algae 2. Chlorophyceae, Gre Algae 3. Phaeophyceae, Bro Algae 4. Rhodophyceae, Red Al
		Fungi (Plants without chlorophyll and with saprophytic nutrition)	5. Myxomycetes, Sli Moulds 6. Schizomycetes, Bacteri 7. Phycomycetes, Moulds 8. Ascomycetes, Sac fung 9. Basidiomycetes, Sm Rusts, Muskrooms 10. Fungi imperfe ti (life tories imperfectly kno
A. *Cryptogamia* (Flowerless plants)	II. Bryophyta (Mosses and Liverworts)		11. Hepaticae, Liverwort 12. Musci, Mosses
	III. Pteridophyta—(Vascular cryptograms)		13. Eusporangiatae, Op glossum, Marattia, etes 14. Leptosporangiatae, Fe Marsilia 15. Spenophyllineae, Sp ophyllum 16. Equisetineae, Horse 17. Calamarineae, Calan 18. Lycopodineae, Cl mosses 19. Lepidodendrineae, L dodendren
B. *Phanerogamia* (Flowering plants)	IV. Spermatophyta (Seed Plants)	Gymnospermae (Plants with naked seeds)	20. Cycadolfilicineae, Cyc filices 21. Cycadineae, Cycads 22. Bennettitineae, Benn tales 23. Cordaitineae, Corda Ginko, Gnetum 24. Pinoideae, Conifers, Yews
		Angiospermae (Plants with covered seeds)	25. Monocotyledoneae, M ocots 26. Dicotyledoneae. D

III. GERMINATION.

In young seedlings grown in moist blotting paper observe:—

1. The expanding *Cotyledons.* Color?

2. The branching *Root* which has grown out from the apex of the hypocotyl.

a Mount a small branch of a root in water and examine under the low power. Observe the delicate *root hairs* standing at right angles with the rootlet. On what portion of the rootlet are they most abundant?

3. The smooth round stem (*hypocotyl*) lying between the Cotyledons and the root.

4. Between the Cotyledons is the expanding plumule, showing the *primary leaves* and the bud between them.

Draw a germinating plant to show all of these parts.

IV. SEEDLINGS. (1-2 weeks old.)

Carefully dig up a seedling 1-2 weeks old and observe:—

1. The cotyledons lifted above the soil by the growth of the hypocotyl. Test portions of a cotyledon for starch and dextrose. What is the significance of their withering during the growth of the plant?

2. The branching *roots* which anchor the plant in the soil. Observe particles of soil attached to the root hairs. Do roots ever grow from any portions of the stem except the extreme lower end?

3. The heart-shaped *primary leaves* with long stalk (*petiole*) attached to opposite sides of the stem. At its distal end the petiole expands into the *blade* with three main *veins,* each of which branches repeatedly, thus giving rise to a *net-veined* leaf characteristic of Dicotyledons (Exogens). On each side of the petiole, near the base of the blade, is a rudimentary leaflet, the *stipel,* and on each side of the base of the petiole is a small leaf, the *stipulei.*

4. At the apex of the stem is the bud from which the central axis of the plant will develop, and a similar bud occurs in the *axil* (upper angle between leaf and stem) of every leaf.

Draw a seedling showing all of these parts.

V. MATURE PLANT (8-10 weeks old).

(A). *Vegetative Organs. Gross Anatomy.*

In a mature plant observe:—

1. The further development of the *root system. Root tubercles*

about ⅛ inch or less in diameter, and containing nitrogen-fixing bacteria, may occur on some of the rootlets.

2. The disappearance of the cotyledons and the scars on the stem where they were attached.

3. The primary leaves as in the younger plant.

4. The *stem* growing up between the primary leaves, and consisting of *nodes,* from which leaves and branches arise, and *internodes.*

5. The *secondary leaves* are compound, each consisting of three or more ovate leaflets, with rudimentary leaflets (stipels) at the base of each. Are stipules present at the base of the petioles? Do the secondary leaves occur in opposite pairs, as in the primary ones?

6. In the *axils* of the leaves are buds which may give rise to *branches and flowers.*

(B) *Histology.*

(I.) The Stem

1. Study a thin transverse section through an internode, first under the low power and then under the high power. Note:—

a. The central *pith* often with a cavity and with *pith-cells* around cavity.

b. The *fibro-vascular* bundles arranged in a ring outside the pith. Commencing at the side nearest the pith in each bundle: (1) Small openings which are cross sections of the *spiral vessels;* (2) Larger openings, the *pitted vessels;* (3) Small thick-walled *wood cells* between the vessels. These three constitute the wood or *xylem* of the bundle; (4) The *cambium zone,* composed of thin-walled cells in radial rows; (5) The *bast* or *phloem,* composed internally of *bast-cells* and *sieve tubes* and externally of rounded *bast-fibers* with thickened walls.

c. The *cortex,* consisting of several layers of large, rounded cells containing chlorophyll.

d. The *medullary rays,* radiating rows of cells passing between the bundles.

e. The *epidermis,* composed of a single layer of squarish-looking cells containing no chlorophyll and some bearing hairs. Between some of the epidermal cells are openings, the *stomata,* each bounded by two small *guard cells.*

Draw and label the section.

2. Study a longitudinal section through part of an internode and mount in water and study under microscope. Working from the central cavity note the following:—

a. *Pith cells.*

b. *Fibro-vascular bundles,* each containing (1) *Spiral vessels,* (2) *Wood cells,* (3) *Pitted vessels,* (4) *Cambium zone,* (5) *Bast-cells,* (6) *Bast-vessels,* large elongated cells with oblique perforated septa (*sieve tubes*), (7) *Bast fibers.*

c. *Cortex cells..*

d. *Epidermis* with occasional *stomata.*

Draw section.

3. Study longitudinal section through a node and compare it with that through the internode. Observe the bundles passing out from the stem into the leaf. Draw.

(II.) The Leaves.

1. In prepared sections of a leaf observe the following parts:

a. *Colorless epidermis,* with occasional *stomata* on upper and lower surfaces.

b. Mesophyll consisting of (1) *Palisade cells,* perpendicular to surface and containing chlorophyll; (2) *Spongy parenchyma,* composed of irregular branched cells containing chlorophyll, and forming the lower half of the leaf substance.

c. *Intercellular spaces* through the whole mesophyll communicating with the exterior through the stomata.

d. Here and there sections of *veins.* Make out in them the same parts as in the fibro-vascular bundles.

Draw the section.

2. Peel off a strip of epidermis from a leaf, mount in water and examine under microscope. Observe:—

a. The large *epidermal cells* with wavy margins and no chlorophyll and occasional hairs.

b. Here and there the *stomata* with two curved *guard cells,* containing chlorophyll, bounding each opening.

Draw.

3. Gently pull a mid rib (vein) in two across its long axis; note the fine threads uniting the two broken ends; cut them off with sharp scissors, mount in water, and observe under microscope that they are composed of partially unrolled *spiral vessels.*

Draw one of these.

(C.) *Reproductive Organs or Flowers.*

1. *Position.* Where do flowers occur? Judging from their positions, to what vegetative parts are they homologous?

2. Observe *shape* and *color.*

3. *Parts of flower;* observe:—

a. The green *Calyx,* composed of five *sepals* which are fused to form a cup. What vegetative parts do they resemble?

b. The showy *Corolla,* composed of five *petals,* one on the upper (dorsal) side, the *standard,* two on the two sides, the *wings,* attached by narrow stalks, two on the lower (ventral) side, united by their median borders to form the *keel,* which is much folded and twisted over the inner parts of the flower. What vegetative parts do the petals represent?

c. The *Stamens* (male parts of flower) ten in number, with broad bases, narrow filaments, and enlarged yellow ends, the *anthers;* the nine ventral ones united by their bases to form the *stamen tube,* and one dorsal one free (not fused). Tease out the contents of anther in water and examine under high power; it contains numerous *pollen grains* which produce the *sperms,* or male elements.

d. The *Pistil* (female part of flower), a long greenish, tapering body within the stamen tube, consisting of an enlarged basal portion, the *ovary* or pod, a narrow filamentous portion, the *style,* and an enlarged terminal portion bearing a tuft of delicate white papillae, the *stigma.* Slit open the ovary and observe the ovules attached along its ventral side; each contains the *embryo sac* inclosing an *egg;* the latter develops into the *embryo* found within the seed.

Draw a flower split open along the dorsal mid-line so as to show all of these parts; also make separate figures of anthers and pollen, of ovary and ovules.

B. PHYSIOLOGY.

I. METABOLISM.

1. Photosynthesis of Carbohydrates.

a. *Chlorophyll.*

Place about 100 sq. cm. of young leaves in 60 cc. of 95 per cent alcohol, cover dish and place in a darkened water bath at 50°-55°

C for 5-10 minutes. Pour solution into clean test tubes and (1) examine color in transmitted and reflected light. (2) Focus light into interior of tube with a lens and observe fluorescence. (3) Wrap one tube in black paper, leave another unwrapped, and expose both to bright light to observe effect of light. (4) Note the color of the leaves which were in alcohol. (5) Observe also color of etiolated and variegated leaves of Coleus. (6) Class demonstration of spectroscopic lines of chlorophyll (Ganong's Physiology, pp. 82-84).

Record in your note book results of these experiments.

b. *Demonstration of effects of light on living leaves.*

1. Keep a potted bean plant in a dark room for a day or two, and then expose to sunlight for 2 hours, having previously screened a portion of one leaf with (a) a strip of lead foil.

2. Remove screened leaves from plant, immerse in hot water, and place in a flat dish of alcohol until white; pour off alcohol and cover leaves with a solution of iodine.

Sketch leaves and explain results.

(The leaves may be preserved afterwards in alcohol and developed again in iodine solution.)

c. *Demonstration of Source of CO_2 in Photosynthesis.*

Remove two leaves with petioles, place cut ends of latter in small bottles of water and put one leaf in sealed jar containing soda lime to absorb CO_2, the other in sealed jar without soda lime; expose both jars to sunlight for 2 hours and then treat both leaves as in b (2).

Record and explain results in your notes.

d. *Demonstration of Formation of O_2 in Photosynthesis.*

1. Observe bubbles of gas escaping from a submerged water plant (Cabomba, Elodea).

2. Place supported funnel over plant and conduct bubbles into test tube filled with water and held in inverted position in jar of water.

3. Test collected gas with phosphorus match or with glowing match stick.

Describe and explain results.

2. SYNTHESIS OF PROTEINS.

a. *Demonstration of Source of Nitrogen.*

1. Compare the relative growth of seedling beans in (a)

Distilled water, (b) Pasteur's[6] solution without sugar, (c) Detmer's[7] solution.

2. Compare growth of peas in (a) Soil sterilized by steam, (b) Soil rich in bacteria, (c) Soil inoculated with Nitrogen-fixing bacteria.

Describe and explain results.

3. Demonstration of Respiration.

a. Place germinating seedlings of bean on moist filter paper supported in a closed jar over baryta water. Observe the latter after a day or two. What does the precipitation in the latter indicate?

b. Place other germinating seeds in a sealed jar from which oxygen has been removed by any of the following methods: (1) By exhausting air with an air pump, (2) by absorbing oxygen over pyrogallate of potash, (3) by replacing air by hydrogen. Compare growth of such seeds with others growing in jars containing atmospheric oxygen.

What conclusions do you draw from these experiments?

c. Fill two thermos bottles with (1) Germinating seeds; (2) Similar seeds killed by 5 per cent formalin. Place the bulb of a delicate thermometer in the midst of the seeds and compare the temperature in the two for the two or three days.

Explain results.

4. Water Absorption, Movement, Transpiration.

a. *Demonstration of Osmosis.*

Fill a parchment bag with molasses; in the open end tie a glass tube, and immerse the bag in a jar of warm water. Observe the rise of molasses in the tube.

Explain.

b. Plasmolysis.

Mount filaments of Spirogyra on three slides from the following solutions: (1) 5 per cent, (2) 10 per cent, (3) 20 per cent cane-sugar solutions. Observe under microscope effects on cell contents. As soon as contents of (2) or (3) begin to shrink replace solution by tap water and observe results.

Explain.

[6] See p. 32.

[7] Detmer's solution:—Calcium Nitrate, 1 gram; Potassium Chloride, .25 gram; Potassium Phosphate, .25 gram; Magnesium Sulphate, .25 gram; Distilled water, 1000 cc.

c. Plant Turgor.

Cut off three leaves and place the cut end of one in a saturated solution of sodium chlorate, of another in tap water, and leave the third in the air. At the end of one hour *compare and explain results.*

d. *Demonstration of Root Pressure.*

Cut off the stem of a vigorous plant 1-2 inches above the ground; attach to the stump by a tight-fitting rubber tube an S-shaped glass tube with one limb drawn out into a long capillary tube, and with oil in the loop of the S to prevent evaporation; water the plant and observe the rise of sap (or oil) in the capillary tube.

e. *Demonstration of Water Movement.*

Rate and Path of Ascent. Cut under water a colorless shoot and transfer the cut end to a strong aqueous solution of Eosin; observe and time the rise of color in the fibro-vascular bundles to the leaf. Cut sections of the shoot and observe where the color occurs.

Describe results.

f. *Demonstration of Transpiration.*

1. Take a vigorous potted plant and cover the pot and soil with waterproof coverings, so that all loss of water must be through the stem and leaves. Weigh the plant on a good balance at intervals and *tabulate the loss through two or three days.*

2. Apply to the upper and under surfaces of a leaf discs of filter paper which has been treated with cobalt chloride (Ganong, p. 190). In the presence of moisture the blue discs turn red. *Where is transpiration most active?* Where are stomata most numerous.

II. IRRITABILITY.

1. *Demonstration of Geotropism.*

a. Place well-soaked seeds of bean and corn in different positions on a sheet of cork covered with cotton flannel and fasten them in place by pins stuck around them. Set the sheet of cork on edge in a glass jar containing 1-2 inches of water; cover jar tightly and set in a warm place. Observe from day to day the direction of growth of roots and stems. After this direction is well estab-

lished turn cork sheet through 90° or 180° and observe subsequent directions of growth of roots and stems.

Sketch experiment and explain results.

2. *Demonstration of Phototropism.*

a. Stem and leaves. Place growing seedlings of beans, corn or oats in a dark box open on one side toward the light and observe the leaf and stem positions after a few days.

b. Roots. Place seedlings of radish grown in a jar of water in a dark box illuminated from one side and observe direction of growth of roots.

What is the influence of light on the direction of growth in shoots and roots?

3. *Hydrotropism.*

Plant various seeds in an inclined trough of wire netting filled with wet sawdust. *Observe and explain the direction of growth of the roots.*

SPIROGYRA.

Read: Huxley and Martin, Practical Biology, pp. 397-407; *or* Parker, Elementary Biology, pp. 192-198; *or* Woodruff, Foundations of Biology, p. 61.

(Subkingdom Cryptogamia, Division Thallophyta, Subdivision Algae, Class Chlorophyceae.)

1. Place a few filaments of the living plant in water on a slide, cover and examine with low power. Draw a small portion of one of the filaments showing its division into cells.

2. Examine with high power. Note the cell walls and the connection between adjacent cells. Note also in each cell the long, band-like, green *chloroplastids*. Count them and make out their arrangement. Make a drawing about two inches in length of a single cell, showing the chloroplastids and the connection with adjacent cells.

3. Treat the preparation with iodine solution. Note the changes of color in the rounded bodies imbedded in the chloroplastids. These are reserve food bodies (*pyrenoids*), and their blue color after treatment with iodine indicates the presence of starch.

4. Examine the cell wall carefully and note the thin layer of *cytoplasm* which lines it internally, and the large *vacuole* filled with cell sap, which occupies the greater part of the cell. Focus

carefully near the center of the cell and find the *nucleus,* surrounded by a thin layer of the cytoplasm.

5. Add all of these parts to your drawing.

6. *Asexual reproduction*:—Examine a number of filaments carefully to see whether there is evidence of recent multiplication by transverse division of some of the cells. Draw.

7. *Sexual reproduction*:—Examine, with low power, a stained and mounted preparation which shows two filaments in process of *conjugation.* Study with high power and draw as many stages in the process of conjugation as can be found, including the fully formed zygotes.

PROTOPHYTA

One-celled plants in which the entire body consists of a single cell, which may be independent or may be joined with others to form a colony.

SPHAERELLA (HAEMATOCOCCUS)

(Protophyta, Flagellatae, Division Thallophyta, Subdivision Algae, Class-Chlorophyceae.)

Read: Parker, Elementary Biology, pp. 23-35; *or*
Parker, Practical Zoology, pp. 240-250; *or*
Woodruff, Foundations of Biology, pp. 30-38.

A. RESTING STAGE.

(1). Spread out in water some sediment containing Sphaerella, put on a cover glass, and look with low power for red or green spheres. Having found one examine with high power and note:

(a). Size, variable; draw several to scale.

(b). Form; spheriodal.

(c). Structure; a sac surrounding the contents, which latter consist of protoplasm, chromatophores, a nucleus and sometimes a vacuole.

(d). Color; red, green or partly one and partly the other.

Where is the coloring matter always situated?

(2). Place a drop of iodine solution on the slide at the edge of the cover glass, apply a bit of blotting paper at the other side, thus drawing the iodine solution under the cover. What parts stain? How does it affect the nucleus and the chromatophores?

(3). Look for individuals in the process of division, some elongated with transverse lines of division, others divided into two or more smaller portions often lying within the sac of the parent. *Draw various stages in this reproduction by fission.*

B. MOTILE STAGE.

(1). After dried, resting forms have been in water for twelve hours examine for motile forms and note their movements.

(a). An active transition from place to place.

(b). A rotary motion around the long axis.

(2). Note the following kinds of motile forms:

(a). Large individuals, the *macro-zoospores,* of the same size as the resting forms, each surrounded by a thin colorless cell, wall, separated from the protoplasmic body by a clear space, which is bridged by protoplasmic strands ("bridles").

(b). Much smaller motile forms, each surrounded by no separate cell wall, but with two flagella at the pointed end of "beak." These are the *micro-zoospores.*

(3). In the macro-zoospores, note: Color, structure, contents, sac (cell wall), flagella, protoplasmic bridles. Which end goes ahead in swimming? How are the contents held in place within the sac? In an individual which has nearly ceased movements study the concessive positions assumed by the flagella and their mode of bending to and fro. Treat with iodine: The protoplasm is killed and the flagella are rendered conspicuous.

Draw individuals in motile stages to show all of the above mentioned points.

EUGLENA VIRIDIS.

(Flagellata, Plant or Animal?)

Read: Parker, Elementary Biology, pp. 44-48; *or*
Parker and Parker, Practical Zoology, pp. 251-258.

Place a drop of water containing Euglena on a slide, and after covering look with the low power for green spindle-shaped organisms which swim swiftly. Having found them study with the high power and note: (1) Size. (2) Color—due to chlorophyll. The anterior end is colorless. Near the anterior end is a red pigment spot, the stigma. (3) Shape, fusiform; the anterior end is blunter

than the posterior and bears a long flagellum which may be lost in some specimens. The flagellum arises from the bottom of a pit, the "gullet," or "mouth opening." Observe the contractile vacuole and "reservoir" near the anterior end and the nucleus and the paramylum bodies near the center of the body. What color do the paramylum bodies take when stained with iodine? Is there any cell sac?

Look for animals in the encysted condition, showing stages in division. Determine by the use of Schultze's solution whether or not there is cellulose in the cyst.

Movements are of two kinds: (a) Rapid swimming movements, in which the flagellum is carried forwards. (b) Worm-like movements, contractions and expansions by which the anima crawls about. The latter movements are characteristic of Euglena and are called "euglenoid" movements. Draw at intervals to show changes in shape.

Record the points in which Euglena resembles a plant; also those in which it resembles an animal. Which do you conclude that it is?

Make drawings and notes to show all that you have observed.

SACCHAROMYCES CEREVISIAE, Yeast.

(Division-Thallophyta, Subdivision Fungi, Class-Ascomycetes.)

Read: Calkins, Biology, pp. 29-34; *or*
Parker, Elementary Biology, pp. 71-81; *or*
Sedgwick and Wilson, General Biology, pp. 184-191; *also*
Woodruff, Foundations of Biology, pp. 213, 310.

A. MORPHOLOGY.

Place some growing yeast in a drop of water on a slide and examine under a low power, then under the high one. Observe the small oval bodies or yeast cells. Note: Size; is it constant? Measure several. Shape; does it change? Nature of surface. Mode of union. Is there any regular number or arrangement of cells in the various groups? How many cells in a complete yeast plant? Structure: Observe the cell wall; contents. Is a vacuole present? Where is it found? Do you ever find more than one? Is it contractile? A nucleus is present but it can be demonstrated only by the most careful staining. Place a piece of blotting paper over the cover glass and press firmly upon it; in this way some of the cells will be bursted and the sac and contents can be studied separately. (a). What is the nature of the sac? Its color? Is the

color of the cell due to the cell wall or contents? Is there any opening in the sac through which food can be ingested? Are there any organs of locomotion? Is the wall elastic? (b). What is the physical nature of the contents? Its color?

Draw several cells to show size, mode of union and structure.

B. CHEMICAL REACTION.

1. Run a drop of aceto-carmine under the cover glass and observe which individuals stain soonest and most deeply. Do the crushed cells stain as readily as the entire ones? Does the sac stain?

2. Treat another drop of yeast with dilute caustic potash. What happens to the cells?

3. Kill some yeast cells by boiling them with water in a test tube. Mount some of this dead yeast and stain with aceto-carmine. Does it stain differently from the living yeast? What inferences may be drawn?

4. Mount a fresh drop of yeast on a slide and treat with a drop of iodine. What is the effect on the cells? Is starch present in the fluid? Is there any starch in the cells themselves?

Make drawings of the yeast cells showing the effect of the reagents.

C. PHYSIOLOGY.

In the following experiments the amount of growth which has taken place may be roughly measured by the increase of the turbidity in the liquid. It may be tested microscopically by the number of buds to which the cell has given rise.

1. Effect of food supply upon growth. Take five test tubes, each one-third full of the solution named: (a) distilled water; (b) 10 per cent solution of sugar in water; (c) Pasteur's solution without sugar; (d) Pasteur's solution with sugar;[4] (e) Mayer's pepsin solution.[5]

Carefully label each tube and put a drop of yeast into each; shake the tubes thoroughly and tightly plug the mouth of each with a wad of clean absorbent cotton and allow them to remain

[4]Pasteur's Solution:—

Potassium phosphate	2.0	grams
Calcium "	.2	"
Magnesium sulphate	.2	"
Ammonium tartrate	10.0	"
(Cane sugar	150.0	")
Distilled water	837.6	"

[5]Mayer's Solution:—

Cane sugar 15% sol.	...20	cc.
Dihydropotassic phosphate	1	grams
Calcic phosphate	1	"
Magnesium sulphate	...1	"
Pepsin	23	"

for two or three days. Examine the tubes from day to day and judge, from microscopic examination and the turbidity, in which fluid the yeast grows best. In which are the most bubbles of gas formed? Does the formation of gas bear any relation to the growth? This is saprophytic nutrition.

2. Reproduction. (a) Budding. With the microscope examine cells from each of the test tubes. In which have the cells the largest number of buds? In which the smallest? How many buds may a cell have? Show by drawings the steps in the formation of a mature bud. What is the difference between budding and fission? (b) Endogenous Spore Formation sometimes occurs in yeast, but is difficult to observe and may be omitted from your notes.

3. The effect of growth of yeast upon food supply. (a) Taste of the Pasteur's solution with sugar in which yeast has been acting for a day or two. Compare with a solution in which there is no yeast. How do you explain the difference? (b) Examine the distillate of a solution containing sugar in which yeast has been growing for a day or two. Notice that it has the taste and odor, and burns with a pale blue flame, characteristic of alcohol. (c) Nature of the gas given off. Take two test tubes, fill the first ⅓ full of clear baryta water, fill the second about ½ full of yeast which is actively giving off bubbles of gas. Insert a cork in this second tube and connect the two by a bent glass tube, one end of which passes through the cork into the air space above the yeast, the other end of which dips below the surface of the baryta water. What changes take place in the baryta water? This is a test for carbon dioxide. (d) Chemical reaction of fluid yeast. Determine by the use of litmus paper whether fluid yeast is acid or alkaline in its nature. What do you suppose the cause of this to be?

Prepare a written statement giving as far as possible an explanation of all the facts you have observed in the experiments in this section.

BACTERIA.

(Division-Thallophyta, Subdivision Fungi, Class Schizomycetes.)

Read: Calkins, Biology, pp. 34-43 *or*
Parker, Elementary Biology, pp. 82-94; *or*
Sedgwick and Wilson, General Biology pp. 192-204; *or*
Woodruff, Foundations of Biology, pp. 44-53.

A. PREPARATION OF CULTURES FOR FUTURE STUDY

1. BACTERIA OF HAY INFUSION.

Fill three test-tubes ½ full of a fresh infusion of hay: (1) carefully close one tube with cotton wool and boil a few minutes; (2) do the same with a second tube and then boil it again after 24 hours and repeat the boiling for several days if convenient; (3) leave a third tube open and do not boil it; set all three in a warm place where they can be observed from time to time.

2. BACTERIA OF THE AIR.

Clean a smooth potato with a stiff brush and water, removing with a knife all injured portions as well as the buds ("eyes"). Sterilize the potato in boiling water for thirty minutes, then cut in slices by means of a knife sterilized in a Bunsen flame. Place the slices of the potato on a sterilized glass plate and leave the clean cut surface exposed to the air in the room for one hour. Cover with a sterilized bell jar, under which some distilled water is placed to maintain a moist atmosphere, and set aside for several days. If any organisms develop on the potato they must have come from the air of the room.

3. BACTERIA OF HYDRANT WATER.

Take a sterilized gelatin culture plate in a Petrie dish, open the dish and quickly allow a few drops of hydrant water to run across the gelatin. Close the dish at once and set it aside for several days. If bacteria were present in the water one or more colonies of them will be found along the path of the drop.

4. BACTERIA OF MILK.

Dilute 1 cc. of milk with 100 cc. of sterilized water. Add 1 cc. of this dilution to a Petrie dish of "litmus agar," cover and place in the incubator for 36 hours.

B. STUDY OF DIFFERENT STAGES, TYPES AND CULTURES OF BACTERIA

I. Active Stages.

Study bacteria from various media, viz.: Pasteur's Solution, Beef Tea, Infusions of Hay and Peas, Potato and Gelatin Cultures, Sewage, etc., and observe and *draw* the following forms:

1. *Cocci;* rounded forms occurring singly or in bead-like rows; without flagella.
2. *Bacilli;* rod-like or thread-like forms.
3. *Spirilla;* spiral forms which may consist of many turns (Spirillum Spirochaeta) or of only a fraction of one turn (Vibrio "Comma bacillus").

In these various forms observe the following points:

First; size, measure.

Second; structure. Can you notice any change of shape in an individual? Any difference between the external and internal portions? Any peculiarity of the ends in the longer forms?

Third; movements. Some vital, others purely physical (*Brownian movements*). The former progressive, the latter vibratory and irregular. Study the Brownian movements in particles of Chinese ink in water. Put a few drops of fluid containing bacteria on a slide, hold the slide over a Bunsen flame and kill the bacteria by boiling, cover and examine with high power. Can you notice any movement of the dead bacteria? Compare with movements of living ones.

II. Resting Stages.

Examine the scum ("*Zoogloea*") from the surface of various liquids, especially the hay infusion; it consists of myriads of bacteria in a resting condition imbedded in a gelatinous substance, the "bacterial jelly."

III. Staining and Mounting.

Spread a small drop of liquid containing active bacteria on a clean cover glass and let it dry slowly; then pass the glass through a Bunsen flame two or three times to coagulate and fix the bacteria upon the glass. Put a drop of Methylen Blue or Gentian Violet upon the glass. After five minutes rinse with distilled water and mount in a drop of water upon the slide. If a permanent mount is

desired thoroughly dry the glass after rinsing and mount in Canada Balsam.

Treat some of the Zoogloea in the same way and observe that the bacteria stain more deeply than the substance in which they are imbedded.

IV. Bacteria from the Mouth.

Take some scrapings from the teeth, dilute with water, mount and study the various forms under a high power. How many kinds of bacteria can you find?

V. Study of Cultures in Hay Infusion.

Study the tubes of hay infusion which were prepared and set aside on a previous day (see (A. 1). Note the changes which take place in each of the tubes. Does the infusion in any of the tubes become turbid and in which one is this most marked? Determine by microscopic examination what the cause of the turbidity is. How do you account for the differences between the tubes? Where did these organisms come from and how did they get into the tubes? Keep the tubes under examination for several days or weeks and observe in what tubes a scum forms on the top of the liquid. Does the formation of this scum have any influence on the turbidity of the fluid? Study the scum under the microscope and determine what it consists of and whether it differs in the different tubes. What ultimately becomes of the scum? What changes, if any, are there in the odor of the fluid during the period of observation, and how do you account for them? Are different kinds of bacteria found in the tubes? If so, make sketches to show them. Do the bacteria in the same tube differ in form from day to day? If so, sketch them in the order in which they appear. After one week what is the condition of the fluid and the bacteria found in each of the tubes? *Write up an account of the phenomena you have observed and give your explanation of them.*

VI. Study of Cultures on Potato. (See A. 2.)

Observe on the potato variously colored spots or "colonies." Are all of these colonies bacteria? Are all the organisms in a colony alike? What is the significance of this fact? What kinds of organisms are most abundant in the air of the room? Can they undergo drying without being killed? Can they grow and multiply without food and moisture?

VII. Gelatine Plate Culture from Hydrant Water.

Are colonies present on the plate? If so, how many? Are they all alike in shape and color? What kinds of organisms are found in the different colonies? Are they more or less numerous than in air or milk?

VIII. Agar Plate Culture from Milk.

By means of a "counting plate" determine the approximate number of colonies present in the agar, and calculate the number of bacteria present in 1 cc. of undiluted milk (the 1 cc. of milk used in the culture was diluted 100 to 1). Colonies which have the form of minute footballs belong to the group of "colon bacilli" and come from the intestinal tract of some mammal, in this case probably from a cow. If colon bacilli are numerous it indicates that the milk has not been taken under sanitary conditions. What does the changed color of the litmus agar indicate?

C. DEMONSTRATIONS.

I. Living Bacteria Seen With Dark-Field Illumination.
II. Prepared Slides of Pathogenic Bacteria.

F. PROTOZOA

One-celled animals in which the entire body consists of a single cell, which may be independent or may be joined with others to form a colony.

1. PARAMECIUM CAUDATUM, Slipper Animalcule

(Phylum Protozoa, Class Infusoria, Order Ciliata.)

Read: Calkins, Biology, pp. 60-75; *or*
Parker, Elementary Biology, pp. 106-120; *or*
Parker and Parker, Practical Zoology, pp. 261-286; *or*
Sedgwick and Wilson, General Biology, pp. 168-172; *or*
Woodruff, Foundations of Biology, pp. 39-43, 244-248, 340-342.

A. MORPHOLOGY.

Put a small drop of water containing Paramecia on a slide; surround it with cotton wool to limit the movement of the animals and cover with a glass; examine with the low power of the microscope and then with the high power. Note:

1. SIZE: measure.

2. SHAPE: fusiform, rounded at the anterior end, bluntly pointed at the posterior end.

3. LOCOMOTION: due to cilia uniformly distributed over the whole surface. Note also movements of flexion (bending).

4. STRUCTURE. The two protoplasmic layers: (ectoplasm and endoplasm).

a. Ectoplasm (Cortex): the firm elastic outer layer; its deeper part marked by oblique *myophan striations.*

(1). The cuticle, a delicate superficial layer differentiated from the underlying protoplasm.

(2). Cilia, delicate vibratile filaments arising from the ectoplasm and protuding through openings in the cuticle. These openings can be seen on a specimen from which the water is allowed to evaporate.

(3). Trichocysts: minute oval sacs in deeper part of the ectoplasm arranged perpendicular to the surface; when the animal is irritated, e. g. by iodine, a stiff thread can be shot out and projected beyond the cilia. They are probably defensive organs.

(4). Two contractile vacuoles in the ectoplasm of the dorsal side about ⅓ of the animal's length from each end. While dilating they are nearly spherical, but at the moment of contraction separate canals can be seen radiating from them.

(5). The oral groove begins at the anterior end of the left side and runs back to the mouth near the middle of the ventral side. The cilia of the groove drive food particles into the mouth. Run some Chinese ink in water under the cover glass and note that some of it is carried into the groove.

(6). The mouth is an aperture in the ectoplasm at the posterior end of the groove through which food passes into a narrow tube, the gullet. Watch the ink collect at the inner end of the gullet into a ball which is suddenly passed into the endoplasm. Watch the course of the food ball within the endoplasm until it is finally ejected through the anus.

(7). The anus is a temporary aperture between the mouth and the hinder end of the body—visible only at the moment of ejection of fecal matter.

b. The Endoplasm (Medulla) is the more fluid protoplasm filling the central portion of the body. In it observe:—

(1). The food vacuoles, which are spherical spaces in the endoplasm filled with water containing food particles.

(2). The circulation of the endoplasm is rendered obvious by the food vacuoles and the granules, which are carried round in a definite direction.

(3) The nucleus is an elongated oval body near the center of the body of the animal. It is best seen after the death of the animal, or in stained specimens.

(4). The micronucleus is a much smaller body applied to one side of the nucleus and resembling it in staining reactions.

Make a full-page drawing of an animal to show the above-mentioned structures.

B. PHYSIOLOGY.

I. Metabolism.

1. Ingestion of food. Place some Paramecia on a slide with powdered Chinese ink and watch the formation of food balls in the gullet and their ingestion. Study the formation of a food vacuole.

2. Circulation of Endoplasm. Observe and sketch the changes of position of the food vacuoles in the body and show by arrows the course of circulation. Time the circulation by noting the time at which the ink is added and that at which the first ink ball completes the circuit.

3. Egestion. Observe, if possible, the egestion of ink from the body. *Show by a drawing where and how this takes place.*

4. Digestion. Observe and draw the changes in color, etc., of food as the vacuoles circulate through the body, also the changes in the size of vacuoles and their fluid contents. What do these changes indicate?

5. Nature of Food. Study the nature of the contents of the food vacuoles of normal Paramecia and find, if possible, what they feed upon. Is it animal or vegetable matter? Does Paramecium choose its food? Stain with iodine and see if any starch is used as food.

6. Excretion. The contractile vacuoles are excretory organs for getting rid of water and nitrogenous waste (urea). Study and *sketch* a vacuole in various stages of contraction and expansion. Time the contractions and expansions and record the results in your notes. Place Paramecia in a thick solution of Chinese ink

and observe the extrusion of a clear drop of fluid at the moment of contraction of the contractile vacuole.

7. Respiration. Place a number of the animals in a drop of phenolpthalein (which loses its rose-color in the presence of carbon dioxide) and note the result; also note the manner in which the animals collect at the surface of a dish in which the water is very foul. What do these observations teach? Are there any organs of respiration?

II. Reproduction.

1. Fission. Observe a Paramecium in the process of division. In a stained preparation note what changes take place in the nucleus and micronucleus during this process. Can you detect any difference between the two daughter individuals? How do the contractile vacuoles, the buccal grooves and the gullets arise in the two? *Draw three stages in fission to show all of these points.*

2. Conjugation. Study and *draw* living individuals in the act of conjugation. What portions of the body are in contact? Is there any distinction of size or sex in the two individuals? In stained preparations study the nuclear changes which takes place during conjugation. *Draw three specimens to show different stages in the nuclear changes.*

III. Irritability.

1. Automaticity. Does the animal appear to act of its own accord or only through the influence of external stimuli?

2. Movement. How many kinds of movement does the animal exhibit? What are the organs of locomotion? With a dissecting lens observe the movements of an animal in a drop of water. Does it move in straight lines? Does it keep one side uppermost? How does it alter its course? Can it move backwards? By means of lines and arrows plot the movements of an animal during one minute. By means of powdered ink observe the direction of currents over the body. What is the direction of the currents in the buccal groove?

3. Sensitivity. Is the animal sensitive to touch or pressure? How does it behave when in contact with a solid body? Place a small drop of salt solution colored with Chinese ink on a slide and note whether the animals are sensitive to this substance. In similar manner test them with 1/50 per cent and 1/10 per cent acetic

acid, and also with a bubble of carbon dioxide. Place animals in a tube heated at one end and cooled at the other. What results? In a similar manner test them with the electric current. Also test their sensitivity to light and gravity. *Record all of your results.*

IV. AMOEBA PROTEUS.

(Phylum Protozoa, Class Sarcodina, Order Rhizopoda.)

Read: Calkins, Biology, pp. 44-59; *or*
Parker, Elementary Biology, pp. 1-22; *or*
Parker and Parker, Practical Zoology, pp. 229-238; *or*
Sedgwick and Wilson, General Biology, pp. 158-167.

Place a small drop of sediment from a vessel containing Amoeba on a slide with a drop of water; cover with cover glass and search for Amoeba with low power. If not easily found, prepare several such slides and examine them after they have been standing for some minutes, so that the Amoebae may crawl out of the sediment. When an Amoeba is found examine with a high power and note:

A. MORPHOLOGY.

1. SIZE; is it visible to the naked eye?

2. SHAPE; is it regular? Constant? Are the pseudopodia of the same size and shape? Do they ever branch? How many do you find? *Sketch at intervals of one minute for five minutes.*

3. STRUCTURE: An outer clear layer, the ectosarc (ectoplasm), and an inner granular more opaque substance, the endosarc (endoplasm). Is the boundary between the two layers a sharp one? Where is the ectosarc thinnest? Is there a membrane outside this layer? Are all the granules of the endosarc of the same size? Which layer is the more fluid? In the ectosarc a clear vesicle may be found which appears and disappears; this is the contractile vacuole. How long does it take to contract, how long to expand? Are there any visible contents? Is there more than one contractile vacuole?

In the endosarc a round, clear body may be found, which does not change shape; this is the nucleus. Is it more solid than the surrounding protoplasm? What is its shape? Size? Is it always in the same place? There are often also in the endosarc various foreign bodies which serve as food, such as diatoms, desmids, green cells, etc. *Draw to show structure.*

4. Stained Specimens: In a prepared specimen, stained and mounted, observe nucleus, ectosarc, indosarc, etc. *Draw.*

B. PHYSIOLOGY.

1. Movements: Is Motion continuous? Regular? How is it produced? Watch process of formation of a pseudopodium. What part does the ectosarc play in the process? The endosarc? Watch an active Amoeba and *trace on paper its path of motion.* Are there permanent anterior and posterior ends? Does there seem to be any difference in surface tension between the anterior and posterior ends? Are the currents in the endosarc constant? *Indicate in a drawing the course of the currents by arrows.* Where are the currents swiftest? Where slowest? Are cilia present on any portion of the body?

2. Nutrition: If possible watch the process of taking in food and of its egestion. What does the animal eat? How and where does it take in food? Are food vacuoles formed? Is there a definite course of circulation of food within the body? Where is the food digested? How distributed? How are gaseous, liquid and nitrogeneous waste substances expelled from the body?

3. Reproduction is difficult to observe and may be omitted from notes.

4. Irritability: Are there any indications that Amoeba is sensitive to stimuli?

Does Amoeba show any reflex movement? Is its behavior more or less varied than that of Parmecium?

Enter answers to all these questions in your notes or drawings.

G. METAZOA

Metazoa are many-celled animals in which there is differentiation into at least two body layers, the *Ectoderm* and the *Endoderm;* the former is the organ of relation, the latter the organ of nutrition; in addition all have ova and spermatozoa. In all metazoa the fertilized ovum undergoes repeated divisions (Cleavage) which lead up to the formation of a hollow sphere of cells (Blastula) and from the latter arises a two-layered condition (Gastrula), the outer layer being the *Ectoderm,* the inner the *Endoderm;* between these two a third layer, the *Mesoderm,* is usually formed.

Ectoderm and Endoderm consist of cells closely packed together into a layer, such a grouping of cells being called *Epithelium.* Mesoderm, at its first appearance, usually consists of scattered cells with large spaces between them, such loosely connected

cells being called *Mesenchyme;* later closely packed layers of mesoderm cells may be formed that are known as *Mesothelium.*

The cells of the different layers of the gastrula differ from one another, and in the course of further development differentiations appear among the cells of the same layer. In this way Tissues, i. e., differentiated groups of like cells and their products. From the two primitive tissues, epithelium and mesenchyme present in the blastula and gastrula, all other tissues are derived, as shown herewith.

1. EPITHELIUM gives rise to:	2. MESENCHYME gives rise to:
a. Epithelial tissue	a. Connective tissue
b. Muscular tissue	b. Skeletal tissue
c. Nervous tissue	c. Vascular tissue
d. Germinal tissue	d. Storage (reserve) tissue

These different tissues will be studied in the laboratory in connection with each animal considered.

The various functions of animal life, which in the Protozoa are all performed by a single cell, are performed in the Metazoa not only by many cells and tissues but also by groups of different tissues united to form Organs, each with a specific function, and by groups of organs united to form Systems, each having some one general function, as shown in the following table:

FUNCTIONS		ORGANS	SYSTEMS
I. METABOLISM	Ingestion, Digestion, Egestion	Mouth, Teeth; Stomach, Intestine; Anus	Alimentary
	Respiration	Trachea, Lungs, etc.	Respiratory
	Excretion	Kidneys, Bladder, etc.	Excretory
	Circulation	Heart, Arteries, Veins, etc.	Circulatory
	Assimilation, Growth, Dissimilation	Have no special organs or systems	
II. REPRODUCTION	Asexual	No special organs or systems	
	Sexual	Ovaries, Testes, Uterus	Genital
III. IRRITABILITY	Reception of Stimuli	Sense Organs	
	Transmission of Stimuli	Nerves	Nervous
	Coördination	Ganglia, Brain	
	Movement	Muscles	Muscular

These various organs and systems will be considered in detail in connection with each of the animals studied.

DEVELOPMENT OF OVUM

In prepared slides of Echinoderm eggs observe the following stages:—1. *Cleavage;* 1-cell, 2-cells, 4-cells, 8-cells, 16-cells, 32- or 64-cells. Observe the appearance of a cleavage cavity after the 8-cell stage.

2. *Blastula*:—Observe the hollow sphere composed of a single layer of cells (Epithelium). Are there any indications that scattered cells (Mesenchyme) migrate into the cavity of the blastula (Blastocoel)?

3. *Gastrula*:—Note the flattening and ultimate infolding of the blastula at one pole. Do the cells at this pole differ in appearance from the others? The infolded cells constitute the endoderm, the outer layer the ectoderm. The infolded cavity is the *Gastrocoel,* or digestive cavity; the opening to the exterior is the *Blastopore.*

Draw and label the stages and structures named above.

HYDRA VIRIDIS OR FUSCA, Freshwater Hydra.
(Phylum Cnidaria, Class Hydrozoa.)

Read: Calkins, Biology, pp. 76-102; *or*
Parker, Elementary Biology, pp. 221-236; *or*
Parker and Parker, Practical Zoology, pp. 289-314; *or*
Woodruff, Foundations of Biology pp. 118-121.

A Metazoan which throughout life remains in a two layered condition, like a gastrula. Observe with naked eye, or with pocket lens, the hydras in a jar of water where they have been undisturbed for some time. Notice the general habitus of body, method of obtaining food, etc. Transfer a hydra to a slide with plenty of water, and observe with the dissecting microscope; afterward cover, supporting the cover glass so as not to crush the animal, and examine wtih the low power of the compound microscope.

I. Form.

The body: What is the general shape? Do its length and breadth vary? It is usually attached at one end, the foot, by a kind of sucking disk and terminates at the other in a conical projection, the hypostome, with the mouth at its summit. The mouth is a small aperture, but it can be greatly dilated to take in food. It opens into a central digestive cavity, the enteron. The tentacles

are hollow processes of the body wall. How many are there? Compare the number of tentacles in brown and in green hydras. Is there more than one circle of tentacles? Observe the knob-like swellings on the tentacles. Measure the length of the tentacles when expanded; when fully contracted. For what purpose are the tentacles used? Buds:—Young hydras of various sizes and stages of development may be attached to the sides of the parent. Are colonies formed by budding? Why?

Draw an entire animal, with all the parts named above.

II. Structure.

1. The body wall of the animal is composed of two layers of cells, one within the other. (a) The *Ectoderm* is the outer layer. What is its color? How much of the thickness of the body wall does this layer form? (b) The *Endoderm* is the inner lining of the body cavity (digestive cavity). In the green species (Hydra viridis) it contains chlorophyll bodies; in the brown species, H. fusca, it contains "sooty corpuscles." Which layer is the thicker? (c) The supporting layer or *Mesoglea* is a thin gelatinous layer between the ectoderm and the endoderm.

2. The tentacles. Examine a tentacle with the high power. Of how many layers is it composed? Focus up and down so as to obtain views (optical sections) at various levels. Is the tentacle hollow or solid? The elements of the two layers can be most easily seen in the tentacles. Observe the following:

(a) The ectoderm cells are large and conical with their apices directed inward. The boundaries of the outer ends form a mosaic, their inner ends rest directly on the supporting lamella. Do these cells vary in shape when the tentacle is extended or contracted?

(b) The interstitial cells are small rounded cells placed between the inner ends of the large ectodermal cells.

(c) The cnidoblasts or "thread cells" are modified interstitial cells prolonged at the outer end into a cnidocil or "trigger" and containing an oval, highly refractive capsule, the nematocyst. The capsule is filled with fluid and contains a spirally wound filament formed by the doubling in of the wall of the capsule at one pole. The nematocysts form knob-like swellings on the tentacles. They are of two kinds: (1) smaller, more numerous ones situated at the bases of the longer cnidocils and containing short stout threads; (2) larger ones lying near the middle of each knob-like

swelling, globular in shape when seen from the face, flask-shaped when seen from the side; they contain long, slender filaments armed with barbs at the basal end. Run in a little iodine and observe the ejection of the threads of the nematocysts. Note that the threads are turned inside out in the process of discharge, the basal portion being discharged first. What is the use of the barbs? The hollow thread? The fluid in the cysts? Do nematocysts occur anywhere else than on the tentacles?

(d) The endoderm cells line the cavity of the tentacles. They are large and some of them bear flagella by which currents are caused. Focus on the middle of the thickness of a tentacle and observe the flagella on the endoderm cells and the nutrient particles streaming up and down the cavity of the tentacle. What difference can you detect in the relative numbers of these elements (cells) in the various parts of the body?

Make drawings of a tentacle to show the characteristic layers, and cellular elements.

III. Reproduction.

1. Asexual reproduction occurs by the formation of hollow out-growths from the sides of the body wall. Each of these acquires a mouth and tentacles at the distal end of its body and finally, constricting at the base, separates from the parent animal. Look for such buds in various stages of development.

2. Sexual reproduction: hydra is monoecious (hermaphroditic), the same animal producing eggs and spermatozoa.

(a) The spermaries are swellings of the body wall produced by the local multiplication of interstitial cells, and covered on the outside by a cap formed of large ectodermal cells. The spermaries are situated just below the tentacles. How many do you find? Is the number constant? Find a ripe spermary and observe the movement of the spermatozoa within the capsule. By gentle pressure upon the cover glass break open the capsule and observe the swimming of the spermatozoa and their size and shape.

(b) The ovaries usually develop later than the spermaries and are formed near to the base of the animal. How many do you find? Is the number the same in the brown and green species? Single cells of each ovary enlarge to form the ovum, while the other cells nourish it and form a capsule about it.

Make drawings of buds and of the sexual organs.

IV. Study of Prepared Sections.

Examine series of transverse and longitudinal sections of hydra prepared by the paraffin method, and note the large central enteron surrounded by a body wall of two layers of cells.

1. The ectoderm. Is it of uniform thickness? In it observe: (a) Large squarish or conical cells. Do they contain nuclei and vacuoles? Their basal ends are continued into muscle fibres (Kleinenberg's Fibres) which are mainly longitudinal in direction, and in cross-section appear as a row of refractive dots on the surface of the supporting lamella. Over the outer surface of these cells is a thin cuticle. At the foot the ectoderm cells are more columnar and contain granules. (b) Interstitial cells are present over the body and tentacles but absent in the foot; they stain deeply. (c) Nematocysts, abundant in the tentacles, less numerous on the body and absent on the foot. Are they found in the endoderm?

2. The supporting lamella. A thin, deeply staining layer between the ectoderm and the endoderm. Is it composed of cells?

3. The endoderm cells; variable in shape and size. They are of two kinds: (a) Larger cells, irregular in shape and size, containing vacuoles, and with the nucleus flattened and near the basal end. In H. viridis the basal part of each cell contains rounded bodies, chloroplastids, coated with chlorophyll. In H. fusca similar bodies are present, "sooty corpuscles," devoid of chlorophyll. The basal ends of these cells are often prolonged into muscular processes like those of the ectoderm cells, but transverse in direction. (b) The smaller secretory cells, pear-shaped and lying between the bases of the larger ones. These last mentioned cells are numerous in the walls of the hypostome but fewer elsewhere. Their protoplasm is granular and they stain deeper than the larger cells.

Make a drawing of each section.

V. Study of Isolated Cells.

Place living hydra on a slide, draw off the water and cover for a few minutes with a drop of Haller's Fluid. Cover and tap gently upon the cover glass to separate the cells.

Select and draw good examples of the varieties of cells mentioned.

LUMBRICUS TERRESTRIS, The Earthworm

(Phylum Annelida, Class Chaetopoda, Order Oligochaeta.)

Read: Darwin, The Formation of Vegetable Mould through the Action of Worms; *also*
Sedgwick and Wilson, General Biology, pp. 41-104, *or*
Calkins, Biology pp. 131-161 *or*
Parker and Parker, Practical Zoology, pp. 318-341; *or*
Woodruff, Foundations of Biology, pp. 121-129.

A. EXTERNAL CHARACTERS.

Place a preserved worm in a dissecting dish, cover with water, and observe:—

1. General form, color, iridescence.

2. Anterior and posterior ends? How do they differ? Dorsal and ventral sides; how distinguished? Right and left sides; are they symmetrical?

3. Body divided into metameres, or somites by grooves around it. Count the somites.

4. Between the 29th and 35th somites, a swollen light-colored region, the *clitellum*. How many somites does it cover?

5. The setae, stiff light-colored spines projecting from the surface of each somite, and easily felt with the fingers. How many are there on each somite, and how are they arranged? Do they all point in the same direction? Remove a seta, mount it in a drop of water, and examine it under the compound microscope. What is its general shape? Do its ends differ?

6. The cuticle. Soak an alcoholic specimen in water for a few minutes, and then strip off some of the cuticle. What is its color? Texture? Examine some from the ventral surface and note the cuticular sacs in which the setae are imbedded. What is their shape? Arrangement? Examine the cuticle under the high power and observe the striae crossing one another at right angles. At some of the intersections are pores to allow the escape of secretions of the epidermis.

7. Apertures. (a) The mouth, in front of the first somite, and below a protuberant lobe, the prostomium, which runs across the first somite on its dorsal surface. (b) The anus, a vertical slit at the end of the last somite. The following apertures are not easily seen, and must be looked for with a hand-lens, or a dissecting microscope. They can often be seen by drying the surface of the

worm, and then gently squeezing it, when a small drop will come out of the openings. (c) Sexual apertures. (1) Openings of spermaducts, or vasa deferentia; two openings surrounded by swollen areas on the ventral surface of the 15th somite. From these openings, grooves are often found passing back to the clitellum. (2) Openings of oviducts; two small pores on ventral surface of the 14th somite. (3) Openings of the seminal receptacles or spermatheca, two openings on each side between the 9th and 10th, and 10th and 11th somites, in line with the outer row of setae and posterior to them. (d) Nephropores; openings of the segmental organs or nephridia; two openings in each somite, one on each side, just dorsal to the ventral pair of setae.

Draw the anterior and posterior portions of the body to illustrate all that you have observed.

B. INTERNAL ANATOMY.

Extend the worm, ventral side down, in a dissecting pan, and fasten firmly by a pin at each end (the anterior one through the prostomium only); cover with water, and cut open carefully from behind forward with fine scissors making the incision along the dorsal side a little to one side of the dorsal median line. Do not cut deep, but merely through the body wall. Carefully cut through the partitions or septa along each side, stretch out the body wall to right and left, and fasten with pins.

Observe the following structures, dissecting as little as possible to make them out:

I. General Features.

1. Body wall, thick and firm and composed of three layers: (a) A thin cuticle on the outside; (b) a more or less colored layer, the epidermis; (c) a light-colored, and much thicker layer internal to the epidermis, the muscular layer.

2. Body cavity or coelum, with the digestive tract passing through it from mouth to anus, and septa or transverse partitions dividing it into as many chambers as there are somites. Each septum passes from the digestive tract to the body wall. What is the relation of the septa to the external grooves?

3. Seminal vesicles, large lobed bodies between the 10th and 15th somites, partly covering the digestive tract.

4. Dorsal or supra-intestinal blood vessel, generally full of

blood, and seen on top of the digestive tract, along the dorsal median line. In the 7th to 11th somites it gives off laterally 5 large pulsatile vessels, or "hearts," which pass around to the ventral side of the digestive tract.

5. Nephridia; or segmental organs, light-colored fluffy bodies attached to the posterior side of the septum, right and left, in each somite.

Make a sketch to show the above organs in place.

II. Digestive Tract.

Make out the following parts in the order named.

1. Pharynx, thick and muscular, extending back into the 6th somite and attached to the body wall by many radiating muscles.

2. Oesophagus, the narrow portion from the 6th to the 14th somites. On its sides in the 11th and 12th somites are 3 pairs of light colored swellings, the calciferous glands. Place one of the glands in a watch glass of dilute hydrochloric acid; explain results.

3. Crop. A large thin walled expansion in the 15th and 16th somites.

4. Gizzard, immediately posterior to the crop in the 17th and 18th somites, and with thick muscular walls.

5. Stomach-intestine, extending from the gizzard to the anus. It expands in each somite, and is contracted by each septum. Along its dorsal surface is a dark colored body, the liver, or pancreas. Cut open the intestine along one side and note the large ridge on its dorsal internal surface, the typhlosole. Cut open the gizzard and the crop, and note the lining of these structures and the character of the food contained.

Make a sketch of the digestive tract, showing the above mentioned structures.

III. Vascular System.

The dorsal blood vessel and the "hearts" have been mentioned. To observe the other principal vessels, remove the crop, gizzard, and oesophagus. Cut the oesophagus away from the pharynx, pull it gently back while cutting the septa which hold it in position, and leaving all the other organs in place. This will lay bare the white nerve cord on the median ventral line of the body cavity. Upon it the supra-neural blood vessel may be seen. In removing these parts of the digestive tract, the sub-intestinal vessel may be seen on its ventral side.

IV. Reproductive System.

1. Seminal vesicles (for storage of own sperm); composed of 3 pairs of white sacs arising from a median portion below the oesophagus. This median portion is subdivided into an anterior and a posterior part.

2. Seminal receptacles (for receiving sperm from another worm); 2 light colored sacs on the ventral surface of the body-wall, on each side of the median line and attached to septa between the 9th and 10th and 11th somites.

3. Ovaries; very small light colored bodies with pointed tips and rounded bases on the anterior wall of the 13th somite, not very far from the middle of the ventral surface, one on each side, right and left.

4. Oviducts; these are also not easily seen, but form what appear as thickenings of the wall between 13th and 14th somites.

5. Cut off the lateral lobe of a seminal vesicle, cut open its median part and carefully wash out its soft contents to show the following structures; great care in dissection and observation is necessary. (a) Vasa Efferentia; large folded or convoluted masses which form the funnel-like openings, one on each side of the median line in the 10th and 11th somites. From these, delicate thread-like ducts pass back on each side to unite in somite 12 to form the Vas Deferens, which passes along the body wall one on each side of the median line, as far back as somite 15 where it opens to the exterior. (b) Testes; four small white bodies, a pair in each somite, inside the seminal vesicles in part concealed by the funnels of the vasa efferentia, and attached to the posterior surfaces of the septa between somites 9 and 10, and 10 and 11, two on the right and two on the left of the median line.

Make a sketch of the reproductive system and explain the function of each part.

V. Nervous System.

1. The nerve cord; extending the whole length of body on the median ventral line, lying in the body cavity but near the body wall. In each somite it expands to form a ganglion and gives off three pairs of nerves. (a) Two large pairs arise from the ganglion. (b) One smaller pair arises from the slender part of the cord (connective) near the anterior end of the somite.

2. Circum-oesophageal nerve ring. Raise the oesophagus and

trace the nerve cord anteriorly to its division into right and left halves which pass around the digestive tract to form a ring which unites with the brain on the dorsal side of the pharynx.

3. Brain; connected as above shown with the ventral nerve cord, but lying dorsal to the pharynx. Note the nerves given off from the brain and also from the connectives on the side of the pharynx.

Make a drawing of the nervous system.

VI. Body Wall.

Pin out part of the body wall quite flat and note that the muscular layer of the wall is interrupted along four longitudinal lines in which are the setae in sacs or setigerous glands; four of these occur in each somite. Between somites 12 and 13 some of these glands are conspicuously large; tease out one and note under the microscope the color, shape and hardness of the setae.

C. ANATOMY AND PHYSIOLOGY OF LIVING SPECIMENS.

I. Movements.

1. Place a worm upon moist filter paper and observe the direction and method of movements.

2. In small light-colored worms note the contraction of the dorsal blood vessel and the movements of the blood toward the anterior.

3. Gently touch different parts of the body and note which are the most sensitive.

4. Place the worm under a glass vessel with some cotton saturated with chloroform, the vapor of which will render the animal insensible; when motion has ceased remove the worm and cut it open as in the specimen previously dissected, but only in the anterior region and a little to one side of the median line. Keep the specimen wet with physiological salt solution.

II. Vascular System.

If the specimen is not quite dead observe:

a. The contraction of the hearts, dorsal vessel, and sub-neural vessel; in the latter the wave of contraction passes backward.

b. Small blood vessels passing from the dorsal vessel to the digestive tract, and from the ventral vessel to the body wall and to the septa.

c. Fine vessels seen upon the septa, body walls and the nephridia.

III. Coelomic Fluid.

Puncture the body where not yet opened and take out in a fine pipette some of the fluid of the body cavity, examine under a high power and note:—

1. White amoeboid corpuscles.
2. Yellow granules, from the chlorogogue cells (See D II. 1).
3. Bacteria or other foreign bodies, especially Gregarina, which are often present.

D. HISTOLOGY.

I. Body Wall.

Examine prepared transverse sections of the body; observe body wall, now seen to be made up of five layers:

1. Cuticle, a thin non-cellular layer (membrane) often torn off.
2. Deric epithelium or epidermis, a single layer of cells many of which are swollen (gland cells).
3. A thin outer layer of circular muscle fibres with blood vessels and connective tissue nuclei among them.
4. A thick layer of longitudinal muscle fibres or plates, arranged in elongated groups of elliptical form.
5. Peritoneum or coelomic epithelium, a thin layer of granular protoplasm containing nuclei, lining the body cavity.

II. Digestive Tract.

In its wall four layers are to be seen.

1. Chlorogogue cells, large and more or less elongated and irregular.
2. An outer layer of longitudinal muscle fibres cut across.
3. A layer of circular muscle fibres and of blood vessels (not easily made out).
4. Enteric epithelium; a single layer of elongated cells with stained nuclei and a thin cuticle over their central ends through which fine cilia project into the lumen of the gut.

III. Nervous System.

In a transverse section of the ventral cord, note:

1. An outer muscular sheath or coat.
2. Large ganglion cells in groups or clusters.

3. A mesh work of fine fibres.
4. Very large clear "giant fibres" each in a definite sheath.
5. In some of the sections the nerves are to be seen as they pass from ganglion cells to the body wall.

IV. Body Cavity.

Some of the sections will show the following structures:

1. The corpuscles of the coelomic fluid.
2. Blood vessels cut across and filled with coagulated blood.
2. Mesenteries or dorsal and ventral membranes connecting the digestive tract with the body wall on the median line.
4. Septa and nephridia cut at various angles.

Make a full-page outline of a cross section to show all of these organs and in the ventral sector fill in histological details of (a) *Body Wall,* (b) *Body Cavity and Nervous System,* (c) *Digestive Tract.*

CAMBARUS, The Crayfish.

(Phylum Arthropoda, Class Crustacea.)

Read: Huxley, The Crayfish, pp. 1-226; *also*
Parker and Parker, Practical Zoology, pp. 346-37-; *or*
Calkins Biology, pp. 166-186.

A. GENERAL CHARACTERS.

I. Body.

Note that the animal has a body proper and a series of paired appendages. The body is bilaterally symmetrical and divided into a posterior jointed *abdomen* and an anterior portion the *cephalothorax*. The entire body is covered by a hard calcarious shell the *exoskeleton* which is flexible at the joints where movement may take place.

II. Appendages.

Note that all of the *appendages* are jointed that they are attached in pairs to the ventral surface of the body and that they vary much in size and form.

Make a drawing of the crayfish as seen from the dorsal side.

III. APERTURES.

Make out the following *apertures* in the body wall:

1. The mouth seen under the anterior part of the cephalothorax after separating from one another the crowded appendages.

2. Anus, a much elongated slit upon the lower side of the terminal piece of the abdomen, the telson.

3. Genital openings on the basal joints of the legs: (a) In the male on the delicate papilla on the last appendage of the cephalothorax (one on the right and one on the left); (b) in the female an opening with a valve-like edge on the antepenultimate appendage of the cephalothorax (one on the right and one on the left).

4. Auditory organs: A small opening on the appendage (antennule) just under each eye stalk.

5. Green glands: A large opening on the ventral side of the first joint of the next following appendage (antenna) on each side.

B. ABDOMEN.

This is made up of six segments or somites bearing appendages and a terminal, seventh piece, the *telson,* which is subdivided by a transverse hinge and bears the anus. Carefully examine the third abdominal somite. The following surfaces are found upon it: (a) *Tergum,* the dorsal arched portion overlapped anteriorly by the preceding tergum. (b) *Sternum,* the ventral portion between the appendages, composed of a transverse bar and a more calcified cuticle where movements take place in bending the abdomen. (c) *Pleuron,* the downward projecting portion on each side, overlapped in front by the pleuron of the preceding segment. The appendages are attached to the body by soft flexible cuticular parts of the exoskeleton.

Each abdominal appendage consists of the following parts:

a. *Protopodite*: This is the proximal part of the appendage and is divided into a long joint, and a small ring-like piece by which it is attached. It bears distally two parts,

b. *Endopodite*: This is the part nearer the middle line.

c. *Exopodite*: The portion farther from the middle line.

C. CEPHALOTHORAX.

a. The large shield-like part of the exoskeleton covering the cephalothorax above and on the sides is the *carapace,* which is prolonged in front into the frontal spine or *rostrum.*

b. A groove, the *cervical suture* runs across the carapace and marks off the head from the thorax.

c. On the ventral side, the region between the appendages is very narrow; the anterior appendages project forward and not downward as do the more posterior ones.

d. The *locomotor appendages* are attached to the thorax; the posterior pair are upon a movable somite while all the others arise from a fused single mass continuous with the head.

e. The free lateral part of the carapace, above the appendages, is the *branchiostegite.* Raise its edge and see that it covers the gills.

f. Respiratory organs: Remove one of the branchiostegites, study the gills under water and observe: Six of them are attached to the appendages, *podobranchiae;* eleven of them are attached to the soft cuticle joining the appendages to the body *arthrobranchiae.* At the anterior end of the branchial cavity a canal leads forward toward the mouth and in this lies the flat part of the second maxilla called the *scaphognathite.*

D. APPENDAGES.

Starting at the posterior end carefully remove all of the appendages from one side *with all the basal parts of each,* see which are alike and then *draw one of each kind or set* (*13 figures for the female, 15 for the male*) keeping all of the small ones in water in watch glasses.

I. Abdominal Appendages or Swimmerets.

Composed of a two jointed *protopodite,* and an *exopodite* and *endopodite* each with many joints; found on all but the first and sixth somites (and the second also in the male) where the appendages are more or less modified.

II. Thoracic Appendages.

There are five pairs of ambulatory, and three pairs of masticatory appendages (the *maxillipedes.*)

a. The posterior pairs of ambulatory appendages have the following seven joints: (1) *Coxopodite,* the short and very thick basal joint. (2) *Basipodite,* a very small and conical joint. (3) *Ischiopodite,* cylindrical and with a groove around it. (4) *Meropodite,* very much longer than the last. (5) *Carpopodite,* about half as long as the last. (6) *Propodite,* slender and long. (7) *Dactylopodite,* the short, pointed terminal piece. Of these (1) and

(2) probably correspond to the protopodite of the abdominal appendages, and the other five to the endopodite, as may be seen by comparing all of the other appendages with the third maxilliped.

b. The next pair of appendages have in addition a *branchia* and *epipodite* upon the coxopodite extending up into the branchial chamber.

c. The third and fourth appendages (counting forward) differ in having the propodite produced opposite the dactylopodite to form a pair of forceps.

d. In the large anterior pair of ambulatory appendages, the *chelae,* the forceps is greatly enlarged and the basipodite and ischiopodite are united into one piece.

e. The third or posterior maxilliped should be carefully studied. Note: (1) The large basal part, *protopodite,* bears a long five jointed *endopodite* and a slender many jointed external *expodite,* besides a curved lamella, *epipodite,* lying in the branchial chamber and bearing a branchia. (2) The protopodites and endopodites make up together a seven jointed organ like the ambulatory appendages.

f. The second maxilliped differs from the last mentioned, chiefly in the size of the endopodite.

g. In the first maxilliped the endopodite is short and flat, the protopodite two jointed and foliaceous, the epipodite has no gill.

III. Cephalic Appendages.

These are the maxillæ, mandibles and antennæ.

(a) Second or post maxillae; the endopodite is not jointed, while the two parts of the protopodite are subdivided or cleft; the large oval plate, *scaphognathite,* acting to bail water out of the branchial chamber, represents the epipodite and probably also the exopodite. (b) First maxilla; this is very small and lies close to the mandible. It is divided into three parts representing the coxopodite, basipodite and endopodite. (c) Mandibles; each has a strong basal part bearing a two jointed palp or endopodite. (d) Post antenna (antenna proper); each has a two jointed protopodite with the opening of the green glands on a tubercle on the proximal joint, the scale-like plate is the exopodite and the long, filiform, many jointed part is the endopodite. (e) First antenna (antennula); here the protopodite has three joints and bears a long many jointed endopodite, and a similar exopodite, while upon its large proximal joint is the opening of the auditory organ, surrounded by hairs.

Compare your drawings of the different kinds of appendages, labelling homologous parts with the same name; the 19 pairs may be regarded as modifications of such a one as the third maxillipede.

E. INTERNAL ORGANS.

Pin the crayfish down under water, dorsal side up, and carefully remove the carapace bit by bit with strong forceps, commencing at the free posterior border.

I. Circulatory System.

1. *Heart.*

Posterior to the cervical suture, a median chamber is laid bare, the pericardial sinus, within which lies the polygonal, flat heart which has six openings into the pericardinal sinus, two on the dorsal surface, two on the lateral surfaces, and two on the ventral surface.

2. *Arteries.*

Running anteriorly from the heart are: (a) the *opthalmic* artery in the mid-line and lateral to this, (b) a pair of *antennary* arteries, (c) a pair of *hepatic* arteries; posterior to the heart are: (d) the median *abdominal* artery from which (e) the *sternal* artery runs to the ventral side just posterior to the heart.

Draw the heart and arteries.

II. Reproductive Organs.

Carefully remove the heart to expose the reproductive organs. (a) Testes. In the male; these form a Y-shaped mass with the smallest of the three lobes passing back along the median line. (b) Vas deferens. Cut away the thorac wall on one side and trace the much convoluted tube from the union of the posterior and anterior lobes of the testes down to the external genital opening on the posterior ambulatory appendage on that side. (c) Ovary. In a female specimen the larger reddish ovaries have the same general form and position as the testes in the male. (d) Oviducts. These are short and go directly down from the ovary to the openings on the third, or middle, ambulatory appendages.

Make a drawing of your dissection, showing all these organs in place.

III. Digestive Tract.

(a) Carefully remove the anterior part of the carapace and notice the very large sac-like stomach anterior to the heart. Pass a probe into it through the mouth and short oesophagus. (b) Dissect away the exoskeleton and muscles and follow the intestine from the stomach to the anus. Immediately posterior to the stomach is the "mid gut" having a short dorsal diverticulum on it. The remainder of the intestine is the "hind gut." (c) The digestive gland (the so-called "liver") forms a yellow mass opening by a duct on each side of the mid gut. Wash away its contents if the duct cannot otherwise be found. (d) Remove the stomach and cut it open along one side (under water) and note a large round (cardiac), and a narrow posterior (pyloric) portion. The chitinous lining forms in the cardiac portion three conspicuous tooth-like thickenings, the so-called "gastric mill." In the pyloric region ridges, set with hairs, reduce the lumen of the stomach to a narrow slit.

IV. Excretory Organs.

1. *Green Glands.*

In front of the stomach is a pair of sac-like structures, the *green glands,* or nephridia; each consists of a ventral glandular part and a dorsal saccular portion, the latter opens to the exterior by a duct.

2. *External Openings.*

On the basal joint of each antenna observe a papilla with the external opening of the green gland at its summit.

Draw a side view of the digestive tract and excretory organs.

IV. Nervous System.

Remove the muscles of the abdomen until the nerve cord is seen along the ventral wall of the body. (a) Note the relation of the ganglionic swellings to the somites. (b) Follow the cord into the thorax; here it enters a canal, the roof of which must be broken off bit by bit with forceps to show the nerve cord. Note the number of ganglia in the thorax. (c) The cord divides at the oesphagus into a right and left half which meet again at the brain. The brain, or supraoesophageal ganglion, lies just posterior to the eye stalks, close to the exoskeleton, and sends a large nerve into each of the eye stalks.

Make a drawing of the nervous system.

RANA, The Frog.

(Chordata, Vertebrata, Amphibia.)

Read: Parker and Parker, Practical Zoology, pp. 1-228; *or* Holmes, The Biology of the Frog, pp. 1-358.

A. GENERAL STRUCTURE.

I. External Characters.

Note the smooth moist skin over the entire animal; the absence of exoskeleton; the head, trunk, two pairs of limbs; the absence of a tail and of a neck.

a. *The head.* Observe:

1. The eyes are prominent and have lids; the ears are marked out by a modified part of the skin, *membrana tympani* posterior to the eyes; the two *anterior nares,* or nostrils; the position of the mouth opening; the soft flexible throat and hard parts of the endoskeleton felt on the dorsal side of the head. Observe the movements of the throat in respiration.

2. After the frog has been killed with chloroform (see below II, 1, a.) pass a bristle far into the anterior nares and one into the ear through a hole cut in the *membrana tympani;* on opening the mouth the bristles will indicate its communications with the nostrils and tympanic cavity. The second bristle appears in the *Eustachian recess* at the side of the posterior part of the mouth. In the male a small opening anterior to this recess leads into the *buccal sac* which can be distended, by means of a small blowpipe. Turn the fleshy tongue forward and notice its mode of attachment. Note the slit of the *glottis* and the posterior opening of the mouth into the oesophagus; pass a bristle into the former and a large probe into the latter. There are thus two median openings from the mouth cavity and six paired openings in the male frog; four in the female. Note the small teeth.

b. *The Trunk.*

This tapers towards the posterior end where the *cloacal aperture* is seen near the dorsal surface. Beneath the skin the hard endoskeleton can be felt on the dorsal side and on the anterior part of the ventral side.

c. *The limbs.*

1. The anterior pair divided each into three regions, *brachium*

(upper arm), *antebrachium* (fore arm), *manus* (hand); the latter with four *digits,* the innermost of which bears a swollen cushion in the male.

2. The much longer posterior pair each divided into three regions, *femur* (thigh), *crus* (shank), *pes* (foot), the latter with five long digits connected by a web. There is a large firm prominence on the inner side of the ankle; *callosites* are found under the joints of both *pes* and *manus.*

Draw entire frog and label parts named above.

II. Internal Characters.

1. General.

a. Place the frog under a bell-jar with a sponge saturated with chloroform; when dead pin out under water on its back.

b. Cut through the skin along the median ventral line from the posterior end to the jaw (raising the skin from the body and not cutting deep); cut transversely at each end of first cut and turn aside the two large flaps thus made.

c. On the flap of skin on each side is seen a large vein near the shoulder, the *musculo-cutaneous vein.* The muscular walls of the abdomen are covered by a thin, shining connective tissue, sheath, the *eponeurosis,* through which in the median region is seen the *rachis abdominis* passing from the pelvis to the sternum and somewhat divided by transverse lines into segments or *myotomes.* Through this muscle is seen the dark blood of the *anterior abdominal* vein in the median line.

d. With a pair of forceps raise the body wall and carefully cut it through by a slit to the right of the median line; continue this cut from pelvis to sternum and make transverse cuts as in the skin so as to throw back a flap of body wall on each side; the left one should show the anterior abdominal vein on its exposed surface.

e. With forceps raise the sternum and carefully cut off the fibrous bands seen passing to soft organs dorsal to it; with strong scissors cut through the sternum and other hard parts on the median line carefully holding it up away from the soft parts dorsal to it. Turn each half outward and pin firmly; pin the anterior limbs out at full length.

f. The *liver* is conspicuous, forming a large brown mass with the pericardial sac just anterior to it.

g. Anterior to the heart note the broad flat transverse *mylohyoid* muscle through which can be seen the long first-vertebral nerve or hypoglossal. Note also the hard protuberant *larynx* and on each side of this a small soft body, the *thyroid gland.*

2. Circulatory System.

Carefully cut away the membranous pericardium to expose the heart; then *with great care* clean off bit by bit the tissue covering the vessels at the anterior end of the heart.

a. *Heart.*

(1) Note the firm conical posterior portion of the heart, the *ventricle.* (2) The cylindrical *truncus arteriosus* arises from the right side of the base or anterior end of the ventricle and passes obliquely forward to divide into two large branches. (3) The *atrium* forms a thin walled sac dorsal to the truncus and anterior to the ventricle (it is divided *internally* into two auricles). (4) The *sinus venosus* can be seen by carefully raising the ventricle to one side; it forms a thin sac dorsal to the ventricle and atrium and receives three large veins (two anterior or *superior venae* and one large posterior or *inferior vena cava.*) Two *pulmonary* veins open into the left auricle by a single opening.

b. *Pulsations of the heart.* Observe:

(1) A regular sequence of contraction and dilation. (2) The atrium contracts, then the ventricle, and immediately after the truncus. (3) On raising the ventricle, the sinus venosus can be seen to contract before the atrium. The contraction proceeds in the same order as that followed by the blood in passing through the heart.

c. *Arteries* (the efferent vessels).

The blood is carried through the common truncus arteriosus which gives rise to a right and left subdivision. Each of these divides into three branches derived from three embryonic arches:

(1) The most anterior branch or arch, the *carotid,* bears near its point of origin a pinkish glandular enlargement, the *carotid gland.* The common carotid divides into (a) *the external carotid* —situated near the median line but distributed to the superficial tissues of the head, and (b) the *internal carotid* which passes into the cranial cavity to supply the brain and sense organs (eye, internal ear).

(2) The middle or *systematic arch unites* with its fellow on the

opposite side to form the *dorsal aorta.* Trace the systemic arches dorsally around the oesophagus to their point of union. The dorsal aorta extends to the posterior end of the body cavity where it divides into two branches, the right and left *iliac* arteries to the hind legs. From each systemic arch a *subclavian* artery passes to the front leg. Named in order, beginning with the most anterior, the following branches arise from the dorsal aorta: (a) *coeliaco-mesenteric* (to liver, stomach and intestine), (b) *ovarian* (or *spermatic*) to gonads, (c) *renal* (to kidneys).

(3) The posterior or *pulmonary* arch runs to the lungs giving off on its way a large *cutaneous* branch which carries blood to the skin for aeration when the animal is submerged.

d. *Veins* (the afferent vessels).

The blood is returned to the heart through five main trunks, right and left *anterior venae cavae,* a median *posterior vena cava,* and right and left *pulmonary veins.* The venae cavae are joined together at the heart to form the *sinus venosus,* which opens into the right auricle.

(1) Each anterior vena cava is formed by the confluence of three veins: (a) *external jugular,* from face and throat; (b) *internal jugular,* from cranial cavity; (c) *axillary* formed by union of the *subclavian* from the arm, and the *musculo-cutaneous* from the skin and abdominal muscles.

(2) The posterior vena cava arises by the union of several large branches coming from each kidney (*renal* vein); anteriorly it receives a large vein from each lobe of the liver (*hepatic* veins).

The *anterior-abdominal* vein (unpaired) arises from the right and left *iliac* veins from the legs and runs to the liver and heart.

(3) Portal veins (veins arising from and ending in capillaries): Blood from the hind limbs is carried to the kidneys by the *renal-portal* veins which enter the kidneys along their external borders. The *hepatic-portal* vein carries blood from the stomach and intestine to the liver.

(4) The right and left *pulmonary* veins open into the left auricle through a common opening.

Make a diagram of the heart and vascular trunks.

Before leaving the laboratory open the skull and spinal canal as follows: Cut the skin along the median dorsal line and reflect it. With forceps pick off the muscles from the vertebrae. Open the neural canal by cutting into the membrane just posterior to the

skull, and bit by bit pick off the roof of the brain cavity with strong forceps. Tag your specimen with your name and put it into a jar of preserving fluid until the next exercise.

3. DIGESTIVE SYSTEM. (Preserved Specimen.)

Posterior to the heart note: (1) *The liver* with its larger left lobe divded into two parts; on raising the posterior border, the *gall bladder* is seen as a greenish sac on the right side; also the *hepatic-portal* vein, which enters the left lobe of the liver. *The stomach,* an elongated white body on the left side under the posterior edge of the liver. (3) A convoluted tube, *the intestine* passing from the stomach to the right and then posteriorly to finally enter the pelvic cavity as an expanded rectum. It is slung by a delicate membranous fold of peritoneum, the *mesentery,* which is full of blood vessels. (4) The *fat masses,* long slender yellow masses on each side in the dorsal part of the body cavity anterior to the reproductive glands.

Cut off all the dorsal part of the liver with strong scissors, cut open the body wall in the pelvic region without injuring the rectum. (1) the *cloaca* is now exposed; a probe may be run through it into the rectum. (2) Uncoil the intestine and fasten to one side to expose the *spleen* (a small red body near dorsal part of mesentery). (3) The *pancreas* is also seen as a pale-colored compact mass in the mesentery between the stomach, liver and small intestine. The bile duct from the gall bladder passes through the pancreas to open into the small intestine. (4) The oesophagus is a short straight tube; pass a probe from the mouth into the stomach.

Make a drawing of your dissection to show all of these parts.

4. URINO-GENITAL SYSTEM. (Preserved Specimen.)

Remove the stomach, liver, mesentery and connected organs.

(1) Posterior to the fat masses lie the *reproductive glands,* in the male yellow oval *testes;* in the female, folded or lobed, yellow *ovaries* (when the eggs are nearly ready for laying, each is a large sphere, light on one side and dark on the other, and the lobes of the ovary are so distended by great masses of ova as to fill most of the body cavity). (2) Sexual ducts: in the male, each testis sends numerous small thread-like ducts, *vasa-efferentia,* into the kidney lying just posterior and dorsal to it. In the female the *oviduct* is a long convoluted tube opening into the cloaca posteriorly and passing forward on each side to open by a funnel into the body

cavity near the oesophagus. It has no connection with the ovary. (3) The kidneys are elongated, red masses close to the vertebral column; on the ventral surface of each is an elongated yellowish body the *adrenal body*. Entering the kidneys on their external side are the *renal-portal veins;* leaving the kidneys on their mesial side are the branches of the *inferior vena cava*. (4) *Ureter*, a whitish duct on each side passing from the outer side of the kidney into the cloaca. (In the male this serves also as a vas deferens.) (5) The *urinary bladder*, a large bi-lobed sac ventral to the rectum (it can be inflated through the cloaca by means of a blow-pipe).

Make a diagram of the urino-genital system of your specimen.

5. The Nervous System. (Preserved Specimen.)

a. Dissection.

Finish the exposure of the brain and spinal cord. With strong forceps pick off bit by bit the roof of the skull, and remove the dorsal part of the vertebral arches in the same way. A delicate pigmented membrane (*pia mater*) covers the brain and the spinal cord but may be concealed in the latter region by soft substance (coagulation products after death) that can be washed away with a stream of water from a pipette.

b. The Brain.

If this is not injured in exposing it, note:

1. *Rhinencephalon* or anterior part made up of two *olfactory lobes* extending anteriorly from a common median part as two cylindrical so-called *olfactory nerves* to branch inside the nasal chambers.

2. *Prosencephalon* or *cerebrum* composed of two large masses, the *cerebral hemispheres*, separated by a median groove.
pheres. Upon it is a very small *pineal* gland and below this a cen-

3. *Diencephalon*, a mass between and posterior to the hemistral cavity, the third ventricle, bounded on the sides by masses called *optic thalami*.

4. *Mesencephalon*, showing on the dorsal side a pair of large, rounded, hollow bodies, the *optic lobes*.

5. *Mentencephalon* or *cerebellum*, a small mass extending across the anterior edge of a large triangular cavity, the *fourth ventricle*.

6. *Myelencephalon* or *medulla oblongata*, forms the remainder

of the brain posteriorly and contains the fourth ventricle which is covered by a vascular part of the *pia mater*.

c. Cranial Nerves.

Ten pairs of nerves arise from the brain:

1. *Olfactory,* a direct anterior continuation of the olfactory lobe.

2. *Optic,* also part of the original neural tube, arises from the optic chiasma on the ventral side of the diencephalon.

3. *Oculomotor,* arises from the ventral surface of the mesencephalon just posterior to the optic chiasma; distributed to some of the eye muscles.

4. *Patheticus,* arises from the dorsal part of the brain just posterior to the optic lobes; distributed to one of the eye muscles.

5. *Trigeminal,* arises from the side of the anterior part of the medulla; distributed to the scalp, face and jaw.

6. *Abducens,* arises near ventral fissure from anterior portion of medulla; distributed to one eye muscle.

7. *Facial,* arises from the side of the medulla near the origin of the 5th with which it leaves the cranial cavity by a single orifice; distributed to the facial area.

8. *Auditory,* arises from the side of the medulla with the 7th; sensory nerve to the ear.

9. *Glossopharyngeal,* arises with the 10th, just posterior to the 7th and 8th; distributed to the tongue and pharynx.

10. *Vagus* (pneumogastric), arises in conjunction with the 9th; distributed to larynx, heart, stomach and lungs.

d. Spinal Cord or Myelon.

1. Forming the continuation of the medulla oblongata posteriorly, it rapidly tapers at about the fifth or sixth vertebra to form a slender filament. On its dorsal surface is a median line, the dorsal fissure.

2. On each side ten *spinal nerves* arise from the cord. By carefully raising the cord a little, towards the posterior end, and searching with a pocket lens, the nerves are seen to arise each by two roots, one dorsal, one ventral.

Make a sketch of the central nervous sytem and cranial nerves as thus exposed.

e. Removal of Central Nervous System.

1. Cut the olfactory nerves away from the skull, gently turn the brain back cutting all the nerves close to the skull and thus remove as entire as possible the brain and spinal cord. Place in a dish of water and study the ventral side with a pocket lens.

2. *Optic chiasm or commissure,* a transverse elevation at the posterior end of the cerebral hemispheres continued up on the sides of the brain towards the optic lobes as the *optic tracts,* and giving rise in the other direction to the optic nerves, (cut off in removing the brain).

3. *Tuber cinereum,* a rounded somewhat two-lobed elevation posterior to the chiasm, continued ventrally into the conical *infundibulum,* which bears at its lower end a small conical mass, the *pituitary body* or *hypophysis cerebri.*

4. *Crura cerebri,* the large nerve bundles, extending anteriorly on each side from the medulla toward the cerebral hemispheres.

5. *Ventral fissure,* a median longitudinal groove along the ventral side of the medulla and spinal cord.

Draw the ventral surface of the brain and spinal cord.

f. Peripheral Nerves.

a. *Spinal Trunks.* In the dorsal wall of the body cavity observe:

(1) *Sciatic plexus*: A number of large nerves, on each side of the dorsal aorta, connected by branches and ending posteriorly in the sciatic (leg) nerve, while anteriorly it is formed from the 7th, 8th, and 9th spinal nerves. (2) Anterior to the sciatic plexus, three pairs of small spinal nerves, the 6th, 5th, and 4th pass obliquely outward and posteriorly along the wall of the body cavity. (3) *Brachial plexus,* formed from the union of the 2nd and 3rd spinal nerves; it goes to the arm.

b. *Sympathetic System.*

Raise the dorsal aorta and notice the two slender longitudinal *sympathetic trunks* dorsal to it, one on each side. (1) Each trunk has numerous enlargements or ganglia giving off fine nerves. (2) Large lateral trunks connect these ganglia with the spinal nerves. (3) *Periganglionic glands:* white masses of unknown function, surrounding the spinal nerves where they issue from the spaces between the transverse processes of the vertebrae.

Draw these peripheral nerves.

III. Museum Specimens.

Observe in the Museum, south wing, numerous preparations of organ systems of different vertebrates, and compare them with corresponding organ systems of the frog.

B. THE SKELETON.

In connection with a dried prepared skeleton, study a fresh skeleton, boiled for a *few* minutes after removing the skin and viscera.

I. General Arrangement of Parts of the Skeleton.

a. The main axis consists of the *vertebral* column continued anteriorly as the central part (brain case) of the skull.

b. Connected with the main axis are the supporting parts of the appendages, and the lateral parts of the skull.

1. The anterior appendages consist of a free limb (containing a *humerus, radio-ulna, carpus and digits,* supported by a *shoulder girdle* or *pectoral arch.*

2. The posterior appendages consist of a free limb (containing a *femur, tibio-fibula, tarsus* and *digits*) connected with the spinal column by the *pelvic girdle.*

C. HISTOLOGY.

I. Epithelium.

a. *Columnar epithelium*: Gently scrape the inner surface of a frog's intestine that has been preserved in Müller's fluid. The fragments removed, under a high power, are seen to be composed of elongated cells each with a nucleus and having one end more pointed than the other.

b. *Ciliated epithelium*: Cut off a bit of the mucous membrane from the tongue or roof of the mouth of a freshly killed frog, mount in physiological salt solution and examine under a high power. Note the appearance on the free edge due to the cilia; as the cilia become less active individual ones can be distinguished. Scrape off some of the epithelium and examine under a high power in physiological salt solution; note the shape of the individual cells with cilia at one end.

Draw both kinds of epithelium.

II. MUSCLE.

a. Tease out a bit of injected frog's muscle preserved in alcohol. (1) It is composed of elongated fibres, some of which may be split up somewhat into fibrilae. (2) Numerous blood capillaries are found among the fibres.

b. Examine with a high power: (1) Each fibre shows alternate darker and lighter bands, (2) A delicate sarcolemma or structureless membrane envelopes each fibre and can be easily seen at places where the fibres are broken or twisted.

e. Tease out fresh muscle in salt solution and examine with high power to note the above points; treat with acetic acid and observe the oval nuclei in the fibre. *Draw.*

III. NERVE.

a. Nerve fibres: Tease out a bit of fresh nerve in salt solution and examine with a high power. Note: (1) Well defined fibres, each with a double contour, together with white fibrous tissue make up the mass of the nerve. (2) Each fibre has a highly refractive border (*medullary sheath*) and a central homogeneous *axis cylinder,* well seen in torn specimens where also the very delicate, innermost membrane (*primitive sheath*), may be sometimes made out.

b. Ganglion cells: Examine prepared specimens of ganglion cells that have been stained to make out the structure of the cells.

Draw a nerve fibre and a ganglion cell.

IV. CARTILAGE.

Dissect out the tip of the delicate xiphisternal cartilage of a fresh frog, or slice a bit of the cartilage from the head of the femur with a razor; mount in salt solution and study under the high power. Note: (1) Large rounded cartilage cells scattered through a nearly invisible and structureless matrix which forms a refractive halo about each cell. (2) A distinct nucleus (or two) in each cell. (3) After some time the cells contract and thus a space is formed between the cell and the matrix. *Draw.*

V. BONE.

Examine with low power a section of bone (mammalian bone). Observe: (1) *Haversian canals,* rounded spaces often filled with air or dirt and then appearing black. (2) *Lamellae,* concentric layers about each haversian canal. (3) *Lacunae,* oval black spaces be-

tween the lamellae. (4) *Canaliculi,* minute dark lines radiating from the lacunae. (5) Other lamellae are to be found not arranged about canals, but filling in spaces not occupied by such systems. *Draw.*

VI. Connective Tissue.

a. White fibrous tissue: (1) Tease out a bit of fresh tendon in water; with a high power it is seen to be made up of fine wavy fibres in bundles; each fibre has faint outlines and does not branch. (2) Treat with acetic acid; most of the fibres swell and become invisible, but a few (yellow elastic fibres) and some elongated granular connective tissue cells remain visible.

b. Yellow elastic fibres: Tease out in acetic acid some of the tissue immediately under the frob's skin. Under a high power note: Branched fibres with well defined outlines; these may not be found until several specimens have been examined.

Draw both white and yellow fibres.

D. EMBRYOLOGY.

I. Early Cleavage Stages. Examine entire frog's eggs divided into two, four, eight and sixteen cells. Note in the two-cell stage that a grayish or slate colored area, crescentric in form, is present on one side of the egg, and that it is, as a rule, bisected by the first plane of cleavage. Note in the four-cell stage the relative distribution of the pigment on the anterior and posterior sides of the egg. Note in the eight-cell stage the relative sizes of the upper and lower cells, also the distribution of the pigment in the cells, and the location of the grey crescent. Note in the sixteen-cell stage the position of the planes of the fourth cleavage in the upper and lower cells. Examine a section of one of the early cleavage stages; note the nuclei surrounded by pigment. *Draw.*

II. Later Cleavage Stages. Examine two of the later cleavage stages (Blastula stages). Note the comparative sizes of the cells in the upper and in the lower hemispheres of the embryo. Examine a section of a late cleavage stage. Note the large cleavage cavity; the thinness of the roof, and the thickness of the floor. *Draw.*

III. Gastrula Stages. Study surface views of three gastrula stages showing: (1) The beginning of the dorsal lip of the blastopore, (2) the backward-growth of the dorsal lip, and the appearance of the lateral lips, and (3) the formation of the ventral lip.

In the latter the blastopore is circular in outline and the yolk plug fills up its opening. Study a longitudinal section of a gastrula stage. Compare with the section of the blastula and note all differences. Observe especially:

a. The lifting up of the floor of the segmentation cavity.

b. The slit-like archenteron, opening behind the dorsal lip of the blastopore.

c. The condition of the cells in the dorsal lip itself.

Draw to large scale showing ectodern, mesoderm and endoderm.

IV. Neural Plate. Study sections of three stages in the formation of the neural plate: (1) Stage with the neural plate widely open, (2) Sides of the neural plate rolling in, (3) Neural plate completely closed to form the neutral tube (brain and spinal cord).

Draw.

V. Tadpole. In cross sections of a young tadpole study and draw:

1. Origin of eye-vesicle from the fore brain, and development of the eye.

2. Cross section through the ear vesicles and hind brain, showing the gill region, with the heart beneath.

3. Cross section through the middle of the embryo to show neural tube and crest, notochord, aorta, pronephros, somites and gut.

Draw and label.

VI. Museum Specimens. Observe in the Museum, south wing, numerous preparations illustrating the development of vertebrates.

PART II

ECOLOGY

Read: Semper, K., Animal Life; *or all of the following*:
Gadow, The Wanderings of Animals;
Needham, General Biology, pp. 3-55, 368-433;
Phillips, Habits of the Honey Bee.

The following directions for laboratory work are general in character since they are intended to apply to various specimens collected in the field or brought from the Museum and Vivarium. Several specimens, illustrating different kinds of adaptations, etc., will be assigned, one after another, to each member of the class. Keep laboratory records for each specimen, and then the results of observations on the topics proposed.

A. RELATIONS TO INORGANIC ENVIRONMENT.

I. HABITAT.

Is the specimen a marine (Halobios), fresh-water (Limnobios) or terrestrial (Geobios) form? What are the evidences upon which your conclusion is based?

1. If aquatic, is it a bottom form (Benthos) or a top form (Plankton)? Give evidence for your conclusion.

2. If terrestrial, is it fitted for life in arid or swampy regions, or for subterranean, arboreal, or aerial life? Give reasons for your answer.

3. Are there any evidences that this species or its ancestors have ever changed habitat? If so, what are they?

4. Draw the specimen, devoting particular attention to those adaptations which have relation to the habitat.

II. CLIMATE.

1. Temperature. Does the organism show any particular adaptations to heat or cold? In what conditions does it pass the winter? The summer?

2. Moisture. Does it show adaptations for the prevention of the loss of moisture, or to protect it against too great moisture?

3. Winds. What adaptation, if any, does it show to winds?

4. Light. Is it a form which seeks or avoids strong light, and what adaptations does it show in this connection?

III. Movement.

1. Is the animal free-moving or sedentary? (a) If free moving is it passively carried by winds or currents, or does it move actively? Do the organs of locomotion indicate that it is fitted for swimming, walking, running, creeping, leaping, burrowing, or flying? Draw one or more of the locomotor organs. (b) If sedentary is it free or attached? Show by drawings the means of attachment. Are there any rudiments of locomotor organs? Are sedentary animals descended from free-moving ones, or *vice versa?*

2. Does this species undergo migrations? If so describe them. In the specimen assigned you by what means is the dispersal of the species assured, and what are the barriers to such dispersal?

IV. Geographical Distribution.

1. To what Zoogeographical Region of the earth is the animal native?

B. RELATIONS TO ORGANIC ENVIRONMENT.

I. Food.

Animals are monophagous or polyphagous, depending upon whether they live upon a single kind of food or on several kinds; they are carnivorous, herbivorous, or omnivorous depending upon whether they eat flesh, plants, or both.

1. Correlation of Food and Structures. Determine by means of the organs of prehension, the teeth, or the character of the mouth-parts of the specimen assigned you what is the nature of its food. Draw these characteristic structures.

2. Correlation of Food and Habits. Point out, if possible, the correlation between the food and the habits of the animal you are studying.

3. Storage of Food. Is this animal able to store up food in any form? If so describe the process.

II. Means of Defense and Offense.

1. Active. Is the animal you are studying able to defend itself actively or not? If so, draw and describe some of the organs used for this purpose.

2. Passive. If it defends itself passively describe the methods and structures by which this is done.

III. Interrelations Between Different Species.

If the species which you are studying is always associated with some other species, in which of the following groups does it belong?

1. Commensalism. The commensal alone benefits, but the host is not injured. In the case in hand, which is the commensal and which the host? Is the commensal permanently fixed to the host, or free to separate on occasions?

2. Symbiosis. The symbionts derive mutual benefit from association. These also may be free or fixed.

3. Parasitism. The parasite benefits at the expense of the host. Is the parasite an endoparasite or an ectoparasite? Is it temporary or constant?

IV. Coöperation Between Individuals of the Same Species.

Associations of individuals of the same species fall under one or another of the following heads:

a. Colonial forms without division of labor.

b. Colonial forms with division of labor.

c. Association of separate individuals without division of labor.

d. Association of separate individuals with division of labor.

1. Does the form which you are studying belong in any of these groups? What advantage, if any, is derived from association without division of labor? If physiological division of labor is present is it associated with structural diversity? If so, draw each of the types present, and determine their relations to one another.

2. Study a colony of bees, or of ants, and draw figures of the males, females and workers. Observe the varied activities of the members of the colony. If other members (castes, slaves) are present in the ant colony make a study of them also. Note the way in which the food is stored and the young are cared for. Study a section through honey comb, and if possible, observe the method in which it is formed. Observe and draw to scale worker cells, drone cells and queen cells, and if possible observe the kinds and relative quantities of food which are fed to the larval workers and queens.

V. Sexual Reproduction.

1. Sex. In many plants and lower animals the sexes are united in the same individual (Hermaphroditism); in higher animals the sexes are generally separate (Gonochorism). To which class does your specimen belong?

2. Primary and Secondary Sexual Characters. The ovaries and testes are primary sexual characters; all other sexual characters, which are dependent for their development upon the primary ones, are secondary sexual characters. Draw and compare the secondary characters which distinguish male and female, and, if possible, determine the significance of each.

3. Semination. Is semination internal or external? Draw the structures of the male and female which serve to bring the spermatozoa to the ova.

4. Types of Development. Where does the development of the embryo occur? Is the animal oviparous or viviparous? Do the embryos obtain their food by their own activities (larval developments), or from the mother (foetal development)?

5. Care of Eggs and Young. In the species you are studying are the eggs and young cared for? If so, how?

PART III

BIOGONY

A. ONTOGENY. Development of the Individual.

I. Asexual Reproduction.

Occurs by Fission, Budding, Segmentation, and has been studied in the Protozoa and Protophyta, Hydra, etc. If time permits study in detail prepared slides showing the process of fission in Stenostoma.

II. Sexual Reproduction.

a. Monogony; sexual reproduction with only one parent.

1. Parthenogenesis (virgin reproduction). Observe and draw water fleas (Daphnia) containing broods of young produced from unfertilized eggs. The same phenomenon may be seen in plant lice (Aphides.)

2. Paedogenesis (infant reproduction). Observe and draw stages in the development of unfertilized eggs in the larvae (sporocysts, rediae) of the tematode worm, Diplodiscus.

b. Amphigony; sexual reproduction with two parents.

1. Oogenesis and Spermatogenesis. Study prepared sections of; (1) Ovotests (hermaphrodite gland) of the snail, Planorbis, and draw a portion of the section to show the ova, the spermatozoa, and the method of development of each. (2) Ovary of the frog, showing eggs of very different sizes. Note the enormous size of the nucleus (germinal vesicle). It is filled with nuclear sap, in which are scattered nucleoli and fine threads of chromatin. Note the distribution of the yolk and pigment in the egg. *Draw.* (3) Testis of frog, showing mature spermatozoa, their heads attached in bundles to nurse cells and their tails extending into the lumen of the seminiferous tubule. Around the walls of the tubule are seen the following stages in the formation of spermatozoa: (a) Spermatogonia, cells with clear nuclei, at periphery, (b) Spermatocytes I, large cells with chromatin in clumps, (c) Spermatocytes II, smaller cells with densely staining nuclei, (e) Spermatozoa, with progressively elongating nucleus and cell body.

2. Maturation and Fertilization. The last two cell divisions in the oogenesis and spermatogenesis are known as the "maturation divisions" and lead to the reduction of the chromosomes in the mature egg and sperm to half the usual number. When the egg is fertilized the normal number is again restored.

Carefully study the maturation and fertilization of the egg of Ascaris megalocephala, with especial reference to the chromosomes. Observe that in the maturation of the egg (also of the sperm) the number of chromosomes is reduced to two, and in the union of the egg and sperm the number is increased to four, the normal number. *Draw eggs and sperm showing all of these points.*

3. Cleavage. Observe that in the cleavage of the egg of Ascaris each chromosome is split longitudinally, so that each daughter nucleus receives two chromosomes from the egg and two from the sperm. *Draw.*

Enumerate the evidences that the chromosomes contain the inheritance factors.

III. Combinations of Sexual and Asexual Reproduction.

1. Metagenesis. Alternation of asexual reproduction with sexual, as in hydromedusae.

2. Heterogeny. Alternation of monogonic reproduction with amphigonic, as in fluke worms.

IV. Heredity. Germinal likeness or variation as contrasted with environmental.

1. Mendelian (alternative) inheritance. Study and draw Museum exhibits illustrating this kind of inheritance.

2. Give Mendelian formulas and ratios to the third filial generation (F^3) for the offspring of (a) two homozygous parents, (b) two heterozygous parents, and (c) one homozygous and one heterozygous parent. Explain sex as a Mendelian character and show by formulas and ratios in which of these three groups it belongs.

3. Describe any cases of inheritance, known to you, which seem to be non-Mendelian, and show how they may be explained in accordance with Mendelian principles.

4. All members of the class are invited, but not required, to fill out a Family Record blank, giving details of their own heredity for the use of the Committee on Eugenics.

B. PHYLOGENY. Development of Races, Species and larger subdivisions.

I. VARIETIES AND SPECIES.

1. Varieties. In a large series of individuals of the same species pick out and draw individuals which represent the mean and the extremes of variation.

2. Species. In a genus containing a large number of species, pick out and draw species which represent the mean and the extremes of the series.

II. HOMOLOGIES.

a. *Comparative Anatomy.*

1. Draw and label corresponding parts of the limb skeletons of three vertebrates, having different modes of locomotion.

2. Draw and label corresponding teeth of three vertebrates, which eat different kinds of food.

3. Draw and label corresponding parts in the appendages of a lobster, or crayfish, and a crab.

4. Draw and label corresponding parts of the skeleton of a starfish and a sea-urchin.

How are such likenesses (homologies) to be explained?

b. *Comparative Embryology.*

1. Study and draw the adult and larva of an ascidian, and of a barnacle, and show how embryology throws light on phylogenetic relationships.

2. Compare the branchial clefts in an embroyo chick and shark, and indicate the phylogenetic significance of this resemblance.

c. *Paleontology.*

Study in the Museum the paleontological history of some family of animals, and trace its Evolution.

III. EXPERIMENT.

With the aid of books which will be assigned you, describe the principal races of some one domestic animal or cultivated plant and compare them with the original wild stock.

IV. FACTORS OF EVOLUTION.

Explain the origin of the peculiar structures of the specimen assigned you according to the following theories: (1) Lamarckism. (2) Darwinism. (3) Mutation.

Read on Ontogeny: Conklin, Heredity and Environment; *or*
Punnet, Mendelism; *or*
Walter, Genetics.

Read on Phylogeny: Darwin, Origin of Species; *or*
Morgan, A Critique of the Theory of Evolution; *or*
Scott, The Theory of Evolution.