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A. FRANKLIN SHULL, Consulting Editor

LABORATORY DIRECTIONS

IN

PRINCIPLES OF ANIMAL BIOLOGY

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Laboratory Directions

in

Principles of Animal Biology

BY

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FIFTH EDITION
SIXTH IMPRESSION

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LABORATORY DIRECTIONS IN PRINCIPLES OF ANIMAL BIOLOGY

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TO OUR

BEGINNING STUDENTS FOR WHOM

A KNOWLEDGE OF PRINCIPLES AFFORDS, IN

OUR OPINION, THE BEST APPROACH

TO ANY SCIENCE

PREFACE TO THE FIFTH EDITION

Revision of the "Principles of Animal Biology" seems always to call for changes in the laboratory work which it is designed to accompany. In addition, the continued search for better illustrative material and improved methods of presentation brings an accumulation of alterations which, if not incorporated in the formal printed instructions, results in growing confusion. Changes have been made in the order in which observations are made, and in terminology where current practice among zoologists appears to demand. Some new material is introduced, but of a sort not entailing necessary large expense. No change has been made for the sake of mere change. The fundamental aim of the course here outlined remains the same as was described in the preface to the first edition, which is reprinted in part on the following page.

The authors are much indebted to their colleague, Professor Harry W. Hann, for numerous suggestions for betterment and simplification of procedure.

A. FRANKLIN SHULL.

ANN ARBOR, MICH.,
January, 1942.

EXTRACT FROM
PREFACE TO THE FIRST EDITION

. . . No longer a purely morphological subject, zoology is not, in the opinion of the authors, properly treated in a purely morphological course. Good teachers have long recognized that dissection and classification alone would not make a zoologist, and have striven in lectures and recitations to provide the larger outlook which the science has come to possess. But this recognition seems hardly adequate. If in the lectures and recitations due attention is paid to the type dissections in the laboratory, morphology can scarcely avoid receiving an emphasis it does not deserve. If to avoid this overemphasis the recitations and lectures are devoted exclusively to evolution, distribution, ecology, genetics, etc., the laboratory exercises and recitations must seem unrelated to one another. Recitations and laboratory work thus become two courses which the student pursues simultaneously.

The only solution has appeared to be to make the laboratory work itself bear on the large questions of biology. The laboratory work may thus have a balance of its own, it does not need to be averaged with the recitations. This book contains directions for firsthand exercises which we believe have the emphasis properly placed. Morphology still receives more attention than any other division of the subject, but it is nearly everywhere directed to some end which is not merely structure. . . .

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LABORATORY DIRECTIONS

IN

PRINCIPLES OF ANIMAL BIOLOGY

Students electing the course in Principles of Animal Biology should furnish themselves with the following:

1 "Principles of Animal Biology" (by Shull, LaRue, and Ruthven).
1 "Laboratory Directions in Principles of Animal Biology."
2 teasing needles.
½ pound note paper, 2 dozen drawing sheets, 1 piece press board, all in strong manila envelope.
1 millimeter rule.
2 medicine droppers.
½ dozen slides.
2 dozen ¾-inch cover glasses No. 2, square or circular.
1 piece absorbent cloth for cleaning slides and covers.
1 5H Venus drawing pencil.
1 eraser with beveled end.
1 case for instruments is desirable.
8 maps of North America.
14 manila envelopes printed for zoology.
1 lock of approved style.

These instruments and supplies may be obtained in sets at the various dealers.

SCHEDULE

An attempt will be made to maintain a definite schedule in the work of the laboratory. Fill the blanks on page 2, from the notice posted on the bulletin board showing the date for beginning each subject and the number of periods allowed for it. Plan your work so that you may finish it in the time allotted.

Report number	Subject	Date of beginning study	Number of periods allowed
1.	Use of microscope		
2.	The cell		
3.	Cell division		
4.	From one cell to many cells		
5.	Activities of protoplasm		
6.	Reproduction		
7.	Breeding habits of vertebrate animals		
8.	Embryology of typical animals		
9.	Genetics		
10.	Homology		
11.	Taxonomy		
12.	Ecology and adaptation		
13.	Zoogeography		
14.	Paleontology		

LABORATORY ARRANGEMENTS AND REGULATIONS

Each student on leaving the laboratory should leave his place and instruments in good condition. Return the microscope, dissecting microscope, trays, etc., in good condition to their proper places. Clean your place at the table. Push the stool under the table. Do your part to leave the laboratory in good order for the next section.

1. Laboratory Notes Must Not Be Removed from the Laboratory without Permission.—At the conclusion of each laboratory period put *all your work, complete or incomplete,* in an envelope, properly labeled, and deposit it in the place indicated by the instructor.

2. Put your name, section, and laboratory seat number on your textbook, laboratory manual, and envelopes, so that they may be easily identified.

3. Report immediately to the instructor or his assistant any part of the laboratory equipment that is missing or out of repair.

LABORATORY RECORDS

The laboratory records consist of notes and drawings which should supplement each other. If laboratory records are to be accurate, they must be recorded at the time the observations are made, not hours or days afterward.

1. Notes.—The laboratory work of the course consists of 14 exercises. With the exception of the first, which is a preliminary drill in the use of the microscope, each exercise is designed to illustrate certain generalizations. The facts upon which these generalizations are based can, in many cases, best be recorded in the form of notes. Such notes, recorded while the observations are being made, will usually be isolated statements, often without connection with those that precede or follow. How frequently such notes should be made is left to the judgment of the student. They are intended solely as an aid to the memory. Obviously, therefore, these disconnected notes need not repeat statements made in the laboratory directions. Likewise, it is superfluous to write in the notes what the drawings show equally well. Unless called for by the instructor, these notes need not be handed in for inspection.

2. Summary.—When an exercise is completed, with his notes and drawings before him, the student should be able to draw certain conclusions from them or to state the principles which they illustrate. In most of the exercises, those conclusions or principles will be capable of clear expression in the form of a summary. If the student is in doubt as to what this summary should contain, it probably means that he has not grasped the significance of the exercise, and he should ask help. However, not all the exercises lend themselves equally well to recapitulation, and the instructor may indicate, in connection with each one, whether a summary is expected. When a summary is written, it is to be handed in with the drawings for inspection.

In lieu of a summary, an examination or a recitation may be given in certain exercises.

3. Drawings.—Drawings form a very essential part of the laboratory records. They should, therefore, accurately fulfill the purpose for which they are made. Many of them must be *detailed*, not caricatures of the general appearance of the object; when detail is desired, frequent comparisons of drawing and object must be made *during the process of drawing.* Drawings should in all cases be analytical, that is, should represent the student's analysis of the structures seen. They should, therefore, be made directly from the specimens themselves. Laboratory drawings should be considered not works of art but representations of faithful analysis. Sometimes brief *sketches* will suffice to illustrate a specific point; but even these must not be careless.

Special training in drawing is not presupposed. Any student can attend to certain features. Always use a sharp, hard pencil. Very lightly mark in the outlines and general features of the object to be drawn, erasing and redrawing any parts which are out of proportion or incorrect. Then carefully retrace the corrected outline, leaving a clean, sharp, single line. Leave no thick lines, or double lines, or loose ends, or gaps between the ends of lines where they do not belong. Draw even small granules with complete outlines, and of the proper shape and relative size. If granules are actually irregular, make them so. Remember that even minor errors offend the eye.

Make drawings large enough to show the required details.

Shade sparingly, and always with a definite purpose in view. Shading is rarely needed. An excellent method of shading for scientific pur-

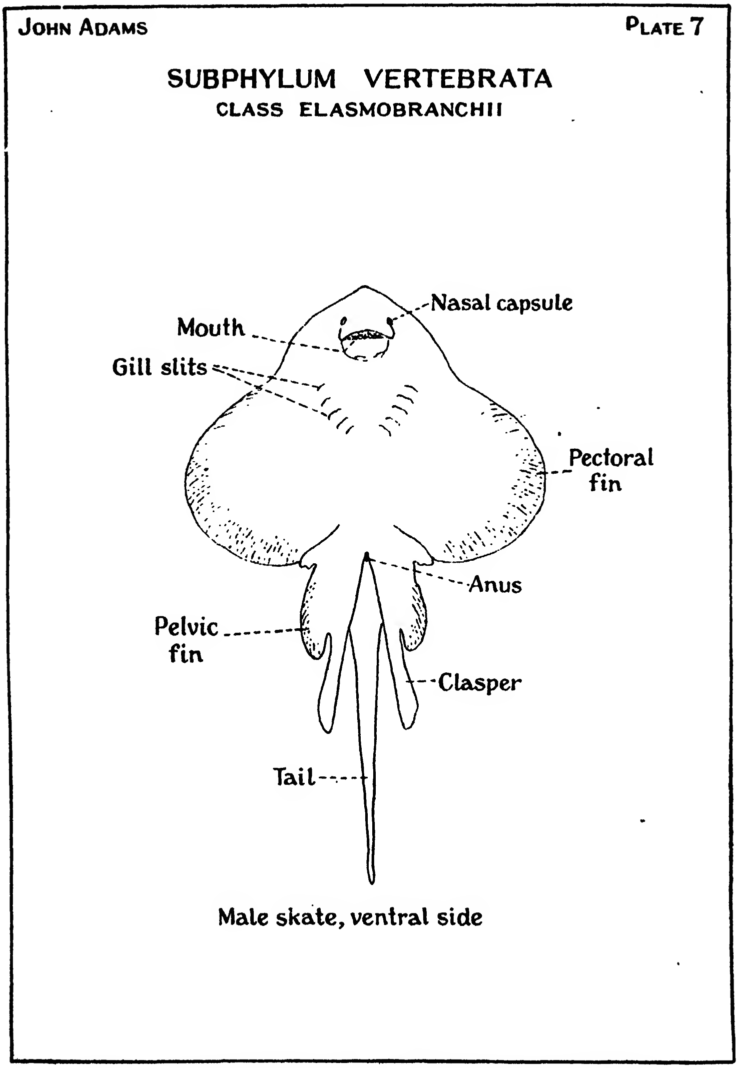

FIG. 1.—Sample plate showing suitable method of labeling.

poses is by the use of fine dots, placed irregularly but so as not to overlap or run together in blotches, a method known as stippling. In pencil work, an artist's tool called a blender may be used to secure an even gradation of shading.

4. Drawings and Legend.—The pages or plates of drawings should be numbered consecutively through the course in the upper right-hand corner (Plate 1, Plate 2, etc.). A sample plate is shown in Fig. 1. The student's name should appear in the upper left-hand corner. The individual structures in a drawing may be labeled with the entire names written beside the figure. Labeling must be neat and so inconspicuous as not to overshadow the drawing. If the drawing is done with a pencil, the labeling should be done with a pencil. A neat style of lettering for

A B C D E F G H I J K L M

N O P Q R S T U V W X Y Z

a b c d e f g h i j k l m

n o p q r s t u v w x y z

FIG. 2.—Recommended style of letters for labeling figures.

freehand work is shown in Fig. 2. Refer to it, if in doubt as to the correct form of letters.

Do not crowd the drawings on the plate but plan the plate so that there will be ample room for the drawings and the labels. Always take a fresh sheet of drawing paper when beginning a new exercise, so that drawings of two different exercises will not be found on one sheet.

Plates may be made to help clarify the purpose of laboratory work by bearing a heading indicating the exercise or project, and a subheading referring to a division of that exercise. Figure 1 was part of a projected lengthy survey of the vertebrate animals, and, as belonging to that survey, one of the drawings relating to the class Elasmobranchii. The drawing of yolk granules called for on page 12 of this book may well be placed on a plate headed The Cell, with a subheading Occasional Features.

FILING AND CORRECTION OF THE REPORT

At the conclusion of each laboratory period place all notes and drawings, finished or unfinished, in an envelope, fill in the blanks on the face of the envelope, and file the report in the place designated by the instructor. Notes and drawings must not be removed from the laboratory except by permission. They must be available for inspection at all times, except when the student is actually working on them. At the time indicated by the instructor for the completion of the study on each exercise, the report will be taken up, graded, and returned. The student, while in the laboratory, should make the corrections indicated and then place the work in an envelope, where it is to be kept until the end of the semester. At that time the notes and drawings, arranged in proper order, must be returned to the laboratory for inspection.

EXERCISE I

USE OF THE MICROSCOPE

Anyone who is not familiar with a modern laboratory microscope[1] should acquaint himself fully with its parts and their uses and care before proceeding to the biological exercises. After a study of Fig. 3 and identification of the parts and equipment of such an instrument, the student should learn how to illuminate the object, how to focus, how to change from "low power" to "high power," and the reverse; also which directions to turn the coarse adjustment and the fine adjustment to raise or to lower the body tube.

In using the microscope, note especially the following points:

1. When the substage condenser is used, the best illumination of the object is secured by the use of the plane mirror.

2. To eliminate the image of the window, trees, etc., in the field of view, first focus on the object, then lower the condenser a little. In some instruments this is accomplished by giving the condenser a short turn in a clockwise direction. In instruments like that illustrated in Fig. 3, it is done by turning the quick screw for focusing condenser.

3. Use only the lower iris diaphragm when the substage condenser is in position. The upper iris diaphragm is employed only when the condenser is removed.

4. In focusing, first use the low power, placing the front of the objective about one-fourth of an inch from the object. Then, with the eye at the ocular, focus *upward. Never focus downward while looking into the microscope,* as there is great danger thus of driving the objective against the object examined, to the great injury of both.

5. To focus with high power, first find the object with low power, and move the object *into the center* of the illuminated field. Then swing the high-power objective into place without raising the body tube. Focus carefully with fine adjustment.

6. Always use the lowest combination which will show the details which are to be seen. Never use high power until the object has first been carefully studied with low power.

7. When changing slides on the microscope, always turn the low-power objective into position before the new slide is placed on the stage.

[1] If students have had some experience with the microscope, it may be desirable to modify this exercise.

To do otherwise may result in injury to the cover glass and to the high-power objective.

8. Never wipe off the ocular or objective with handkerchief, cloth, or anything except *lens paper*, which will be furnished as needed.

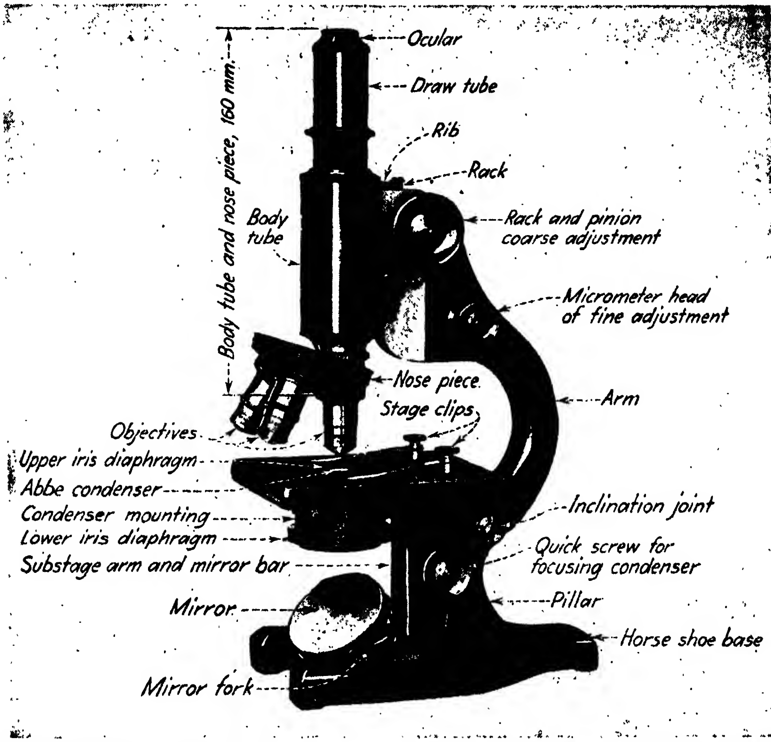

Fig. 3.—Modern microscope with its parts indicated. (*Courtesy of Spencer Lens Co.*)

9. In case the ocular or objective cannot be readily cleaned or is injured in any way, take it at once to one of the instructors. Do not try to clean it yourself.

10. Carry a microscope by its arm.

11. Report *at once* to the instructor any missing parts or injuries to the microscope.

PREPARED SLIDE OF A PRINTED LETTER

Use some familiar object, like a printed letter mounted on a slide, to ascertain the salient features of the microscope and the methods of employing it.

1. Focusing.—Place the 4x ocular and 16-millimeter objective in position and adjust the mirror so that the light from the window passes up through the tube of the microscope. Now holding the slide so that the printed letter on it is in reading position (that is, right side up) put it on the stage of the microscope with the mounted letter in the center.

Lower the tube of the microscope by means of the coarse adjustment until the objective almost touches the cover glass; then with the eye at the ocular slowly move the tube *upward* until the letter on the slide appears distinct.

2. Relation of Object and Image.—Make a *drawing* of the letter as seen by the unaided eye (right side up), and another *drawing* of the image made by the microscope. Before making your drawing, refer again to the instructions for labeling drawings and plates.

These drawings should be made of the same size as the object and image, respectively, and in the same position as seen when drawn. The image may be measured by laying a millimeter scale across the stage of the microscope at one side and looking into the microscope with one eye and at the scale with the other. After a little practice the scale will appear to lie over the image.

This sheet of drawings is your Plate 1. Always follow this style in making up your plates.

Now, using note paper and ink, state how the image differs from the object.

3. Illumination.—Note carefully the brightness of the field of vision and the appearance of the letter; it is illuminated by transmitted light. Tilt the mirror and observe the change in the intensity and character of the light. The object is now viewed by reflected light, which must be employed for all opaque objects.

4. Magnification.—Determine what combination of ocular and objective gives the lowest magnification, what combination the highest, etc. This can be done by examining an object, the printed letter used above, or something smaller, with each combination and noting the size of the image.

After having determined the order of magnification, determine the amount of magnification, using that combination of ocular and objective which gives the least magnification. Mount on the microscope an object of known size (the printed letter will serve if it has been measured). Lay across the stage a millimeter rule. By looking into the microscope with one eye and at the millimeter rule outside with the other, as directed above, both the object and the rule can be seen at once and a measurement obtained. From the known actual size of the object compute the magnification. As a check, calculate the magnifying power of the same combination by multiplying the initial magnifying power of the objective (usually stamped on the objective by the manufacturer) by the magnifying power of the ocular (which is likewise marked on it). Compare this computed magnification with the power determined by measurement.

Compute the magnifications obtained by the other three combinations of oculars and objectives, by means of the powers stamped on them, and then write in your notes a completed table of magnifications like the following, beginning with the lowest and ending with the highest.

Ocular	Objective		Magnification
	Focal length	Magnifying power	
x	millimeters	x	diameters
x	millimeters	x	diameters
x	millimeters	x	diameters
x	millimeters	x	diameters

Conclusion of First Laboratory Period.—The record of the work of this laboratory period constitutes report 1. Place all the notes and the plate (see that each sheet of your work has your name on it) in an envelope, fill in the blanks, giving as the subject of this report "Use of the Microscope," and put the envelope in the place indicated by the instructor. Put everything away in good order. Put wastepaper in the wastebasket; push the chair or stool under the table; leave the table in as good condition as you would like to find it. Take this book of laboratory directions home with you and study carefully the general laboratory regulations, the statements concerning the laboratory records, and the use of the microscope.

Be ready for a quiz at any time on the work completed.

EXERCISE II

THE CELL

One of the first great generalizations in biology concerned the part played by cells in the structure of living things. That generalization is fundamental to all branches of the science. Although a great principle cannot be rediscovered in a few hours time, its chief features can be illustrated. This exercise is devoted to such illustration. At its close, if it be assumed that the objects here used are representative, it should be possible to draw inferences that are comparable to the cell doctrine in nature, if not in extent. Follow the general problem and its subdivisions, as stated below.

THE GENERAL PROBLEM

To determine the structure of the cell and the extent of its occurrence as a unit of structure in living things.

1. Structural Features Common to Most Cells.—A complete catalogue of common features of cells cannot be made with limited materials; but if cells in general agree in possessing the structures shown by all the cells used in 1*a*, 1*b*, 1*c*, 1*d*, and 1*e* below, a provisional list of such common structures is thereby provided.

1*a*. Place a drop of water on a clean slide and mount in it a small piece of stratum corneum of frog skin (the outermost layer that is repeatedly shed). Spread the specimen flat, and cover with a cover glass. Examine with the microscope, trying out different light intensities.

Note the units of which the tissue is composed. These are the *cells*. Each contains a dense mass, the *nucleus* (plural, *nuclei*), which is usually visible. If the nucleus is not readily distinguishable, remove the cover glass or mount a fresh piece of stratum corneum. Draw off the excess water with filter paper or a blotter, and add a drop of erythrosin, which is a staining solution. After half a minute remove the surplus stain, add a drop of distilled water, and put on a cover glass. The nucleus should now be clearly differentiated from the surrounding material. The remainder of the cell, besides the nucleus, is nearly structureless and is known as the *cytosome*. Both nucleus and cytosome are composed of protoplasm. The surface layer of each of these cells is the *cell membrane*.

Draw a group of three or four cells, each one about half an inch in diameter. An outline of the cells and their nuclei will suffice, but it should be neat. Label nucleus, cytosome, and cell membrane.

1*b*. As an alternative to 1*a*, or in addition to it, examine the cells which line your own cheek. They may be removed by rubbing the inside of the cheek gently with the blunt end of a pair of ordinary forceps. Mount in water on a slide, adding a little of a dilute solution of the stain methylene blue. Look for the nucleus, as distinguished from the cytosome, and for the cell membrane. *Draw* one or more cells.

1*c*. Examine a section of the liver of a frog or salamander, which you will find in a tray on the table. Is the liver made up of cells? What part of the cell is most intensely stained? Is a nucleus found in each cell? If not, explain its apparent absence.

1*d*. Examine a slide of stained turtle, snake, bird, or salamander blood. *Draw* an oval corpuscle in outline, showing the nucleus. This is one of the corpuscles which gives the blood its red color, but if it is plainly red in your preparation it has been stained.

1*e*. From a culture containing protozoa (one-celled organisms) mount a drop of water. Before putting on the cover glass, examine the slide with low power to see that the organisms are present. Then add a drop of acetic methyl green, mixing the stain with the drop containing protozoa, and put on the cover glass.

Any organism in the water will be killed and its nucleus stained by the methyl green. Find several kinds of nucleated organisms.

In the cells studied thus far the nucleus, cytosome, and cell membrane have been demonstrated. During the remainder of this study note carefully whether these structures are present in the cells studied. The protoplasm in the cytosome of living cells is largely of the nature of a very fine emulsion or suspension. A somewhat similar emulsion may be obtained by shaking oil in water. Examine a drop of such an emulsion under the microscope. A very common type of cell membrane is only differentiated protoplasm; the structure of the nucleus is studied below.

2. Other Structures Found in Some Cells.—Besides the foregoing structures, which are found in most cells, there are others that occur frequently but are absent from many cells.

2*a*. Examine a leaf from the growing tip of Elodea. Focus carefully upon the upper surface of the leaf. Note the cell *wall* which limits the protoplasm of the cell. Is this cell wall relatively thick or thin in comparison with a cell membrane? Does it appear to be more rigid or less rigid than a cell membrane?

Observe in the remainder of the exercise whether all cells have cell walls or cell membranes.

2*b*. Using the same green leaf of Elodea examine the cells for colored bodies. These are *plastids*. Are they distributed throughout the cell or restricted to some portion of it? If the latter, where? Color of fruits and of many flowers may be due wholly or in part to the presence of colored plastids. *Chromoplast* is a general name for all colored plastids, whereas the word *chloroplast* is used to designate only green plastids.

Draw one cell of Elodea, showing the wall with its connections with the walls of neighboring cells, and including its plastids. This figure should be 2 inches or more in length. Plastids that appear double or are otherwise peculiarly shaped should be included if found. Be on the lookout for plastids in other unstained cells to be studied later.

2*c*. In mounted sections of Hydra, study the cells of the innermost layer. Hydra is a small, many-celled animal related to jellyfishes, corals, sea anemones, and hydroids. Its body is composed of two distinct layers of cells separated by a noncellular layer. Note particularly the large clear spaces within the inner cells. These spaces are *vacuoles*. *Draw* in detail a group of three cells of this layer, showing structures present, selecting for this purpose a place where the cells are cut lengthwise.

2*d*. Remove a frog's egg from its jellylike covering, then tease out (tear up finely with needles) in water the substance of the egg upon a slide, separating the particles until they form a very thin layer on the slide, and mount under a cover glass. Examine with the microscope. The fine granules are yolk material (stored food). *Sketch* a group of them, showing shapes and sizes carefully.

Make a list of the structures studed in section 2 which are found in many but not all cells.

3. Some Features of the Nucleus.—The shape of the nucleus should be noted in the cells of the stratum corneum, sections of liver of a frog or salamander, in muscle, in the silk glands of the larva of the hawk moth, and in Spirostomum. See demonstrations for the last two.

3*a*. Study a longitudinal section of a dorsal root ganglion (a small mass of nervous tissue occurring near the junction of spinal nerves with the spinal cord) of a cat. Look for a single rounded body near the center of many of the nuclei. This is the *nucleolus*. Note its color. Observe the *chromatin* which occurs as granules in the nucleus. Compare with the nucleolus in color. *Draw* in detail a cell of the ganglion to show nucleolus or nucleoli and chromatin granules. The sections on these slides were stained with two stains. All parts of each cell were subjected to the same processes. How do you account for the differences in color?

4. Structure of a Moderately Simple Living Cell.—*Amoeba* furnishes an example of such a cell.

4*a*. Mount some ooze from a culture containing Amoeba and endeavor to find a specimen. If an amoeba cannot be found, ask help, but *do not discard the slide.* It may have amoebas on it, even if they are not at once discovered.

4b. Describe the general appearance of Amoeba, its color or lack of color. Be specific.

4c. The blunt processes thrust out from the body are *pseudopodia* (singular, *pseudopodium*). Do they change shape or size? If so, make three *sketches*, in outline only, of the entire amoeba at intervals to show these changes. What relation exists between the pseudopodia and the movement of the body as a whole? In some species there is only one pseudopodium.

4d. On using high magnification note the outer clear layer of protoplasm, often quite thin; this is the *ectosarc*. Within the *ectosarc* is the granular *endosarc*. Note the movements within these two layers, especially in the formation of a pseudopodium. Which layer moves more rapidly when free to move? What conclusion may be drawn regarding the relative fluidity of ectosarc and endosarc? Give reasons for the answer to this question.

4e. In large specimens vacuoles containing particles of food may be seen. The larger food vacuoles may be recognized by their contents. In which layer are they? What are their contents? These contents are cell inclusions, not part of the cell.

4f. Look for one or more pulsating vacuoles. These are not always visible; but if the specimen be watched for a few minutes, small vacuoles may be seen which increase in size and finally move to the surface, where they collapse. The disappearance of the vacuole is one feature that distinguishes the contractile vacuole from the more persistent food vacuoles. The latter, moreover, contain food particles.

4g. Find the nucleus, a rounded, highly refractive, somewhat grayish body, occurring in some species in a vacuolelike structure. Is it in the ectosarc or endosarc? If you do not see the nucleus clearly in your specimen, consult the demonstration of a stained specimen.

4h. Make an accurate *drawing* of Amoeba not less than 2 inches in diameter, showing all the structures noted in the foregoing study. Ask for instructions if in doubt on any point. Label all parts.

5. Divergence of Cells from Typical Form.—Cells sometimes depart widely from the form or structure that is most common. What is the chief modification of each of the following cells?

5a. Cartilage Cells.—The ends of all long bones are covered with a glistening white cap of cartilage. In young animals many bones are first laid down in cartilage, which is later replaced by bone by a complex process. Secure a thin piece of cartilage from a dish on the supply table, or with a very sharp scalpel or safety razor blade shave off a very small, thin piece of cartilage from the surface of the end of a bone provided for the purpose, and place it in a drop of water on a clean slide. Cover, and examine the thin edge of the cartilage with the microscope.

How are the cells arranged? Do the cells touch one another? The intervening substance is the *matrix*. What inference may be made concerning its origin? *Draw* a few groups of cells, showing the structure of at least two of them, and representing also the matrix.

5*b*. *Bone Cells.*—Examine prepared sections of dry bone. The dark spots are the spaces or *lacunae* (little lakes) formerly occupied by the bone cells. Projecting from the lacunae are minute wavy channels, the *canaliculi* (little canals), into which, in life, extend slender processes

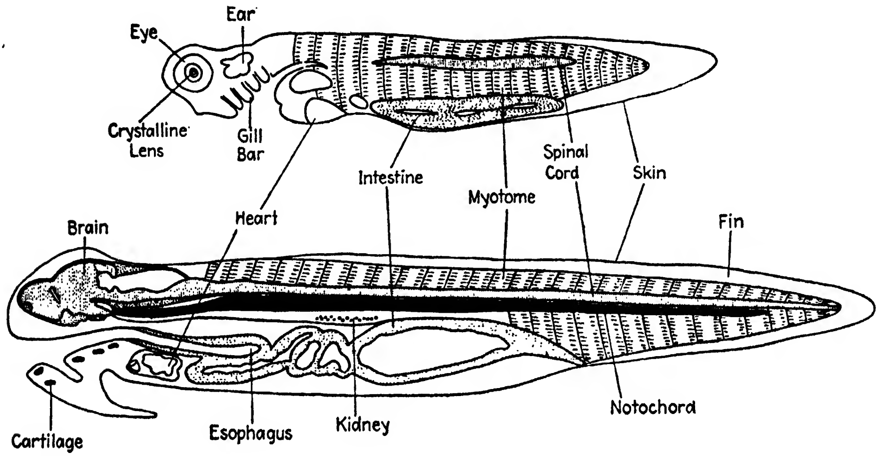

FIG. 4.—Longitudinal vertical sections through a larval salamander, representing organs and tissues shown in such sections. Below, through the median plane; above, to one side of the median plane.

(like pseudopodia) of the bone cells. Larger openings, for blood vessels, may occur in the preparations. The remainder of the specimen consists of the hard parts, mostly calcium salts, deposited in the matrix. The fleshy parts of the bone are dried and shriveled in these preparations. What is the origin of the matrix?

Draw carefully a lacuna with its canaliculi as representing the form of a bone cell.

5*c*. *Hair.*—Remove a hair from your eyebrow, mount it in water and examine at different points along its length both on the margin and on the upper surface. Can you detect anything that would indicate cells? *Sketch* a segment of the hair to show them. In the sketch the diameter of the hair should be ½ inch.

5*d*. *Wool.*—Wool is similar to hair in its composition. Examine the minute fibers from a woolen blanket or undyed woolen yarn. Are there any indications of cellular structure?

5*e*. *Human Blood.*—Examine a demonstration of stained human blood. Anyone who desires to examine his own blood will be shown how to do so with comparative safety and with the minimum of pain. Mount the blood, and examine the red corpuscles. What is their color when seen singly? Is there a nucleus?

6. Is the Whole Animal Body Made Up of Cells?—This may be answered partly from objects previously studied, partly from new material.

6a. Examine a longitudinal section of a small salamander. Identify the parts by consulting Fig. 4, and make a list of all the organs which you think are made up of cells. Do you find any organs not made up of cells? If so, make a list of them also. If you are in doubt as to the interpretation of any of the observations, consult the instructor. (The presence of nuclei may be taken as evidence of the existence of cells if the cell outlines cannot be determined.)

6b. Recall also in connection with this problem the parts of the animal body studied in 1*a*, 1*b*, 1*c*, 2*c*, 3*a*, 4, 5*a*, 5*b*, 5*c*, 5*d*, 5*e*.

SUMMARY

Describe the cell in generalized terms, emphasizing the structures that are nearly always present, but indicating also the occasional components. Point out some of the more extreme modifications exhibited by cells. Discuss also whether and to what extent cells or cell products enter into the composition of all living things. Use as largely as possible the evidence afforded by this exercise, and state the possible inferences with due regard to the incompleteness of the information obtained.

EXERCISE III

CELL DIVISION

In the common type of cell division[1] characteristic changes occur in the nucleus, the cytosome, and the centriole. The most important changes take place in the *chromatin*, the deeply staining portion of the nucleus. The nuclear membrane disappears, and the chromatin, which was arranged in very diffuse manner, appearing as fine particles, gradually rearranges itself into fine coiled threads (*fine spireme*) which shorten and thicken into coarser, more loosely coiled threads (*coarse spireme*). From the coarser threads are developed, by further shortening and thickening, definite bodies called *chromosomes*. The number of chromosomes is different for different species and is in general constant for any given species.

In the cytosome a spindle-shaped figure composed of threadlike structures is formed. At the ends of the spindle, where the threads converge, are two deeply staining bodies, the *centrioles*,[2] from which other threads radiate in all directions. These latter radiating threads are the *astral rays*. Upon the middle of this spindle the chromosomes take their place in a flattened group, the *equatorial plate*. Each chromosome either splits longitudinally and equally or is duplicated, and the two new chromosomes go to opposite ends of the spindle. Thus two new groups of chromosomes are formed, each of the same number as was present in the group from which they came. A cell membrane forms between the groups of chromosomes, dividing the cell into two cells. The chromosomes of each group now undergo a series of changes approximately the reverse of those in the early stages of division; that is, they become diffuse again, thereby forming two new nuclei like the original one.

[1] This exercise, which logically belongs here, may be postponed until just before Exercise VIII (Embryology) if it is thought that the students' inexperience with the microscope renders such delay desirable.

[2] In some animal cells there is only a minute dot at the end of the spindle, in others a minute dot enclosed in a larger object. It is this smaller particle which has been called a *centriole*. When surrounded by a very small amount of other staining material, the whole structure is sometimes called a *centrosome*, but there is much confusion of the two terms. For elementary purposes they are often used interchangeably, though the word centriole seems preferable.

For convenience, the process of mitosis may be divided into four intergrading stages: (1) the *prophase* which includes the early stages prior to the equatorial plate; (2) the *metaphase*, in which the chromosomes constitute the equatorial plate across the middle of the spindle; (3) the *anaphase*, in which the new chromosomes are distributed to opposite ends of the spindle; and (4) the *telophase*, which includes the division of the body of the mother cell and the reconstruction of the daughter nuclei. A cell that is not in any stage of division may be said to be in *interphase*.

MITOSIS IN THE SEGMENTING EGG OF ASCARIS

Mitosis may be readily studied in the segmenting egg of *Ascaris megalocephala* (a roundworm parasitic in the intestine of the horse), in the skin of young salamander larvae, or in the segmenting egg of the whitefish. The description below applies directly to Ascaris but may be modified to apply to the others.

A knowledge of the nature of the specimens of Ascaris in which mitosis is studied will obviate some confusion. The salient features follow.

a. The segmenting eggs are in the uterus, a tubular organ, which is cut in thin sections. If the sections are cut longitudinally, each ribbon shows the walls of the uterus at the edges, with the eggs between.

b. The eggs have been fertilized, so that in the earliest stages two nuclei (egg nucleus and spermatozoon nucleus) are present.

c. The eggs are turned in all possible positions, so that sometimes only one, sometimes both, of the nuclei may show; also the later division figures may be observed in various positions.

d. Each section includes only fractions of the eggs, so that only portions of the nuclei or spindles may be present, or these structures may be wholly lacking.

e. After the first division of the egg, only certain of the cells divide in the same manner as the first segmentation. The directions below apply to the first division and later ones of the same kind.

1. Interphase Nucleus.—Study a cell not undergoing division. Note the *nuclear membrane*, the diffuse arrangement of the chromatin in the nucleus, and one or more *net knots* (the larger granules of chromatin). How is the chromatin distributed through the nucleus? The protoplasm of which the cytosome is composed is called the *cytoplasm*. What is the appearance of the cytoplasm in these cells?

Draw a cell with interphase nucleus, showing also the nature of the cytoplasm in at least part of the figure. The thick membrane around the egg, and at some distance from the egg, should be drawn in this figure but may be omitted in those that follow.

2. Prophase.—Under this term falls almost half the process of mitosis, including a wide variety of changes.

2*a*. Find a cell in which the chromatin is arranged in distinct but still slender threads (*fine spireme*). Where are these found in the nucleus? Observe the nature of the cytoplasm. Look for a darker mass, the *centrosphere,* near the nucleus. A dark central granule may or may not be visible in this mass. *Draw.*

2*b*. Select a cell in which the chromatin is in thick wormlike strands (*coarse spireme*). Is the nuclear membrane still present? If so, where in the nucleus are these strands? Observe any objects in the cytosome. The centrosphere may be divided into two parts near together, each part with lines radiating in all directions into the cytoplasm. Each part is an *aster.* The two asters may be connected by other lines, the double structure being the *amphiaster.* If only one aster is present, how do you account for the absence of the other? *Draw.*

2*c*. Find cells in which the nuclear membrane has disappeared, and in which the chromosomes, now quite thick and distinct, have no definite arrangement. Count the chromosomes. Look in the cytosome for an amphiaster. Each aster should contain a central granule, the *centriole.* If only one aster is seen, where is the other?

3. Metaphase.—In a later stage the chromosomes are arranged in a flat group. Seen on edge, they form a nearly straight line; viewed from the flat side of the group, the chromosomes are readily distinguishable. How many are there? This group of chromosomes is the *equatorial plate.* In a cell in which the equatorial plate is seen on edge, note the two *centrioles* (that is, observe the amphiaster). The lines connecting the asters with each other are called *spindle fibers.* Those connecting the aster with the chromosomes are *mantle fibers.*

Draw a cell with the spindle in side view, that is, with the equatorial plate seen on edge. *Draw* another cell to show the equatorial plate as viewed from one of the centrioles.

While in the equatorial plate, or earlier, the chromosomes become double, either by splitting or through production of a new chromosome by each one. If you do not find this stage readily, ask for a demonstration either in Ascaris, or in the skin of a salamander, or in some other cell. *Draw.*

4. Anaphase.—Find cells in which the chromosomes have begun to separate into two groups. If possible, count the chromosomes in each group. Note the form of the spindle. Are there fibers between the groups of chromosomes? *Draw* either an early or a late stage; that is, one in which the groups of chromosomes are still near together or are widely separated.

5. Telophase.—Search for a later stage than 4, showing the two groups of chromosomes separated by a cell membrane which has divided the original cell into two cells. Is the nuclear membrane present around the groups of chromosomes? Are the centrioles visible? Do any signs of the spindle remain? *Draw.*

SUMMARY OR REVIEW

At the close of this exercise the student should either (1) write a connected account of cell division in Ascaris or (2) participate in a recitation or take an examination covering the same subject.

EXERCISE IV

FROM ONE CELL TO MANY CELLS

Increase of complexity among living things is manifested in two ways: (1) in their evolutionary history and (2) in their individual development. Many biologists believe there is a significant connection between the two. The former is illustrated at length in this exercise by means of various organisms, roughly in accordance with one theory of the way in which it has been brought about. The latter is touched only lightly, in advance of its fuller treatment in Exercise VIII, in order that a comparison may be made.

When a unicellular animal divides, two daughter animals are formed, which usually separate from one another. Thus one-celled organisms are always of small size, invisible or barely visible to the unaided eye. In some unicellular animals, after division, the daughter cells adhere. This results in the formation of a *colony*, whose cells are all alike. Such colonies are strictly limited in size. Animals that are unicellular, whether separate or colonial, and in which all cells are alike are called *protozoa*.

When the fertilized *ovum* (egg) of a higher animal divides, the daughter cells adhere. Certain of the cell descendants (the *germ cells*) retain their power of giving rise to new individuals, while the others lose this power and may be called *somatic* or *body* cells. The somatic cells of an animal may be variously modified (*differentiated*) and differently arranged, and they may become so numerous as to produce a body of considerable size. Animals that are made up of two kinds of cells, germ and somatic, are called *metazoa*, or many-celled animals.

1. A Colony.—Examine prepared slides of Gonium, Pandorina, and Eudorina. What is the form of the colony? Do the cells touch one another? If not, what holds them together? If living specimens are available, look for *flagella*, two whiplike processes projecting from each cell; they are not likely to be seen in preserved material.

Are all the cells in any one group alike, or are some distinctly different from others? If reproducing specimens are available, note particularly whether all cells are dividing. Are these organisms protozoa or metazoa?

Draw Gonium, Pandorina, and Eudorina. Only one or two cells in each drawing need be finished in detail, the others merely in outline.

2. The Most Primitive Differentiation.—Study prepared specimens of *Pleodorina illinoisensis* (which is perhaps only a variety of *Eudorina* *elegans*) and *P. californica*. What is the shape of these organisms? About how many cells are present in each one? Are the cells near the surface, or in the center, or both?

Examine a specimen of *P. illinoisensis* in which none of the cells is reproducing. Are the cells all alike? If not, what difference exists? How many cells of each kind are there? Examine another specimen in reproduction. Are all the cells dividing? Connect this observation with the preceding one regarding different kinds of cells. Referring to the introductory paragraphs of this exercise, apply suitable names to the kinds of cells found. Compare with Gonium, Pandorina, Eudorina.

Observe *P. californica*. Are the cells all alike? If not, about how many cells of the different kinds are there?

Draw Pleodorina illinoisensis and *P. californica*, labeling the kinds of cells. Only one or two cells of each kind need be finished in detail, the others in outline.

3. Volvox.—Volvox, like Pleodorina, is a free-swimming organism found in fresh-water ponds. Study living specimens, if available, in a salt cellar with the dissecting microscope. Otherwise stained specimens on slides will be furnished. Compare with Pleodorina as to size, shape, and number of cells.

Are the cells all alike? Note the numerous small cells of nearly uniform size. These are the *somatic cells*, held together by a gelatinous substance. In what part of Volvox are these cells located? Connecting the somatic cells are slender strands of protoplasm. By counting in several instances, determine how many of these connecting strands project from each cell. If living Volvox is available, focus on the edge of a specimen, and find *flagella* projecting from each cell. Does the beating of these flagella result in movement of the individual cells or of the whole organism? From the structure of Volvox, would you say the cells are independent of one another?

Besides the small somatic cells, observe the larger bodies in Volvox. These are either *reproductive cells* or *daughter individuals* derived from reproductive cells. Determine where they are located. The reproductive cells are of three kinds: (*a*) *parthenogonidia*, which by cell division give rise asexually to daughter individuals; (*b*) *ova* or *eggs*; and (*c*) *spermatozoa* (male reproductive cells).

3*a*. *Parthenogonidia*.—Look for these in very small (young) individuals. They are somewhat larger than the somatic cells but never grow very large without dividing. Look for cells that have divided into two, four, eight, or more cells, forming small *daughter individuals*. In older individuals look for daughters of various sizes. Eventually they break out of the parent.

3*b*. *Ova* are not at first distinguishable from parthenogonidia. They later become opaque with stored food and grow considerably in size without dividing. Find ova with *shells* (spiny in some species) covering them. The shell indicates that the egg has been fertilized by a spermatozoon and has gone into a resting condition. The fertilized ova later give rise to new Volvox. How many ova in the specimens studied?

3*c*. *Spermatozoa* occur nearly as frequently as ova but are more difficult to find, since they are less conspicuous. When present, they usually occur in groups and may have the appearance of cigars tied in wide circular bundles. In some species an individual may produce only one kind of cell. Look for bundles of spermatozoa in such a specimen.

If the species studied is one in which the same individual produces both ova and spermatozoa, the spermatozoa mature before the ova. In such species, look for spermatozoa in individuals having unfertilized eggs (without shells). When an individual with unfertilized ova is found, search its surface for small areas from which a few somatic cells seem to be lacking. Focus on such areas with high power and at a deeper level than the layer of somatic cells. Several bundles of spermatozoa may sometimes be found together. If you fail to find them, ask to have them pointed out to you.

Draw a specimen having parthenogonidia or daughter Volvox, representing the whole organism in outline, and the parthenogonidia or daughter Volvox more in detail. The outline should be at least 3 inches in diameter. Show the somatic cells in a portion of the figure. *Draw* a fertilized ovum and a sperm bundle in detail, not in a drawing of Volvox. May any advance in complexity of Volvox over Pleodorina be observed? If so, in what respects?

4. Hydra.—Hydra is more complex in its organization than Volvox but much more simple than the earthworm, which is to be studied later. It is a fresh-water animal found in lakes, ponds, and streams, attached to the surface of dead leaves, aquatic plants, and other objects. Two species are commonly found, the brown species (*Hydra oligactis*) and the green species (*Hydra viridissima*). Study a living specimen in a salt cellar containing a small amount of water. Examine with the unaided eye, with the dissecting microscope, and with the low power of the compound microscope. Identify *body, foot, tentacles, hypostome* (the conical eminence at the center of the circle of tentacles), and the *mouth* at the tip of the hypostome.

4*a*. *Somatic Cells.*—Focus on the margin of the body, and note a clear outer layer of cells, the *ectoderm*. The serrations found there indicate roughly the extent of the principal cells of the ectoderm. Among these, find numerous round bodies smaller than the ectoderm cells, the *nematocysts*, or *stinging bodies*. The nematocysts are lodged in cells called *cnidoblasts*, which may not be separately visible in the living animal. In what part of the animal are the nematocysts most abundant? Do you find groups of them anywhere? The structure of the nematocysts should be studied from specimens prepared for this purpose.

The darker part within is another layer of cells, the *endoderm*. The more or less central clear space bounded by the endoderm is the *digestive cavity*, also called *coelenteron* or *gastrovascular cavity*.

Examine mounted cross sections of Hydra. Note the two layers of cells, the ectoderm and the endoderm, surrounding the digestive or gastrovascular cavity. The bulk of the ectoderm is made up of the cells previously observed as serrations at the surface, approximately rectangular in section and not very deeply stained. These are called *epithelial cells*. Among the epithelial cells are pear-shaped or oval bodies, the nematocysts. Look for the cnidoblasts in which the nematocysts are contained. Numerous small, deeply stained cells among the bases of the epithelial cells are called *subepithelial cells*. From the subepithelial cells the cnidoblasts are derived.

4*b*. *Germ Cells*.—If available, examine a living specimen bearing one or more *spermaries* or *testes*. What is the shape of this organ? Do you note any movement within the spermary? The moving bodies are the *spermatozoa*. *Sketch* an entire specimen showing the spermaries. (Use a prepared slide if a live specimen is not at hand.) Examine a cross section of Hydra through a spermary. The spermatoza are deeply stained cells in a dense mass. What is their relation to the ectoderm and endoderm?

Examine either a living or a stained specimen bearing an *ovary*. *Sketch* to show this organ. What is the relation of the ovary to the ectoderm and endoderm? (When an ovum is fertilized by a spermatozoon, it develops into an embryo. See demonstration.)

Are the somatic cells of Hydra all alike? If not, how many kinds may be observed? Are the cells of one kind grouped together or scattered over the body? If the answer to the last question is different for different kinds of cells, specify the difference in your notes. Are the germ cells all alike?

5. The Earthworm.—As an example of more complex organization, study the earthworm. The aim is to illustrate tissues, organs, and systems, and the study should be made with this object always in mind.

5*a*. *External Features*.—Note that the body is divided into segments known as *somites* or *metameres*. A segmented animal is said to be *metameric*, or to exhibit *metamerism*.

Observe that the animal has a *dorsal* or upper surface and a *ventral* or lower surface. It has also an *anterior* end and a *posterior* end. Consequently it has also a right and left side. Since the earthworm can be divided by only one plane into two corresponding halves, it is said to be bilaterally symmetrical. Where does this one plane pass?

The following external features are referred to in the dissection:

Setae, minute horny bristles arranged in rows on each side of the body. Pass a preserved worm through your fingers in both directions. What does the result indicate?

Clitellum, a swelling of the body in the region of metamere 32. On its ventral side is a pair of thickened ridges, the tubercula pubertatis.

Prostomium, a small rounded projection at the anterior end, overhanging the mouth.

Mouth, an opening at the anterior end leading to the *buccal cavity.*

5b. *General Internal Structure.*—The worm furnished you is cut down the dorsal side. Separate the cut edges a little, just behind the clitellum, and note the transverse partitions or *septa* (singular, *septum*) which divide the body cavity or *coelom* into compartments. The coelom surrounds the digestive tract. Note the relation between the septa and the intersegmental furrows on the surface of the worm.

Now cut or tear the septa carefully on each side for about an inch. This may be done with the point of a sharp dissecting needle. Lay the worm ventral side down in the dissecting pan and pin the body wall out flat as far as the septa have been cut. Slant the pins outward so as to leave room to work between them. Then with the point of the needle tear the septa forward and backward, putting in pins as the operation proceeds. When this dissection is completed the septa should have been cut to the same depth on each side. Be careful not to injure any of the internal organs. Remember the general rule in dissection, to cut nothing unless you know what it is and why you cut it.

Readjust the pins in the anterior region so that they pass through the walls of the fifth, tenth, and fifteenth somites. This will facilitate counting them in locating the organs. Now study the following systems of organs.

5c. *Reproductive System. Male Organs.*—In somites 9, 11, and 12, notice the three pairs of whitish bodies partly covering the alimentary tract. These bodies are the *seminal vesicles.* In them are located the *testes* which produce the spermatozoa.

None of the remaining male organs is visible without careful dissection. They may be omitted from the study of the dissection but should be studied from a chart and from figures in the textbook ("Principles of Animal Biology").

Female Organs.—These consist of the paired *ovaries* in the thirteenth somite and a pair of oviducts in the fourteenth. Both organs are small, are concealed by the overlying organs, and need not be found. See wall chart.

Close to the septa separating somite 9 from 10 and 10 from 11 are two pairs of small whitish bodies, the *seminal receptacles.* Mature spermatozoa received from another worm are stored in these. Be careful in the course of the dissection not to remove or injure the reproductive organs.

5d. *Blood System.*—In the dissected worm the dorsal blood vessel is found imbedded on the dorsal side of the digestive tract. Follow the dorsal vessel forward. In somites 7 to 11, inclusive, will be found paired, tubelike red bodies (variously colored in preserved worms), the *hearts,* which are connected with the dorsal blood vessel. The hearts extend ventrad, forming semicircular loops on each side of the digestive tract. They unite below with a *ventral blood vessel,* which extends backward along the ventral side of the digestive tract. If the hearts cannot be seen, carefully dissect away the remaining portion of the very heavy septa which obscure the hearts and other organs of somites 7 to 12. The ventral vessel will be seen later in cross sections. In life the hearts propel the blood from the dorsal to the ventral vessel. Smaller vessels are found throughout the body. Some of the more prominent of these may be found in each segment back of the hearts connecting the dorsal blood vessel with the body wall and the intestine. What is the function of the blood system? How is this function served in Hydra?

5e. *Digestive System.*—The digestive system consists of a tube extending through the whole length of the body. It is modified into various parts. Beginning at the anterior end, these are as follows.

The *mouth* has already been found. It leads into the *buccal cavity* in the first three somites. Be careful not to injure the *brain,* a whitish bilobed structure situated on the dorsal side of the buccal cavity in somite 3.

The *pharynx* is the thick-walled portion following the buccal cavity. It extends to about the seventh somite. The walls are firm and muscular. Test the consistency of this structure by pressing with your dissecting needle.

The *esophagus* is a long, slender portion behind the pharynx. It is partly covered by the hearts and reproductive organs, and in the anterior part by heavy septa. The hearts and reproductive organs must not be removed or injured, but the reproductive organs may be carefully turned aside in order to reveal the esophagus.

The *crop* is an enlargement following the esophagus. It is situated directly behind the last pair of seminal vesicles in somites 15 to 16 (usually). Press it with a needle to determine whether it is thick- or thin-walled.

The crop is followed by the whitish *gizzard.* Is it thick- or thin-walled? Behind the gizzard, the *intestine* extends to the posterior end of the worm where it opens to the exterior by means of the *anus.*

Compare the digestive system of the earthworm with that of Hydra.

5f. Excretory System.—This consists of *nephridia*, fluffy organs located on each side of the intestine in most of the somites. They are readily seen with the unaided eye, but for their structure consult a demonstration. What is their general form?

5g. Nervous System.—In the third somite is the small, whitish bilobed structure, the *brain*, already noted above, resting on the buccal pouch. In the posterior part of the worm push the intestine aside, and note the white *nerve cord.* How far does it extend forward and backward? The slight thickenings of the nerve cord are the *ganglia.* Note the small nerves running out from the ganglia. Find the connection between the brain and the nerve cord in the anterior portion. The connecting cords are called the *circumpharyngeal connectives.*

Make a *drawing* of the dissection showing the structures that have been studied. Or, at the option of the instructor, a *drawing* may be furnished for labeling. Ask for instructions.

5*h.* Examine prepared slides of cross sections of the earthworm under the dissecting microscope. The intestine is seen in the middle of the section. Determine the dorsal and the ventral sides of the section. This may be done by using some of the following organs as landmarks.

In the intestine note the *typhlosole* which is an infolding of the dorsal wall of the intestine. On the dorsal side of the intestine is the dorsal blood vessel. Beneath the intestine is the *ventral blood vessel,* supported by a thin membrane or *mesentery* seen in the cross section as a wavy line. Near the ventral blood vessel is the *nerve cord.* The intestine consists of four layers. On the inside is a single layer of slender *epithelial cells.* Outside of this is a *circular muscle layer;* then a *longitudinal muscle layer* reduced to a few fibers; and covering the intestine is a thick layer of *peritoneal cells.*

Observe the *coelom* or body cavity directly between the intestine and the body wall. In it may be found portions of *nephridia* and sometimes portions of *septa.*

The body wall consists of four distinct layers. Lining the coelom is a very thin layer of cells, the *peritoneum.* Outside this membrane is a layer of more or less featherlike structures, the *longitudinal muscles.* Outside these is a layer of *circular muscles.* External to these is the *hypodermis;* how many layers of cells in it?

Draw an outline figure showing the form and position of the various layers of tissue and other organs in outline, but do not fill in details. The boundaries of the layers and organs are sufficient. Be careful to make this drawing with the dorsal side toward the top of the page. (At the option of the instructor a drawing may be furnished for labeling. Ask for instructions.)

Does the earthworm possess germ cells and somatic cells? If so, where are the germ cells? Are they of more than one kind? How many kinds of somatic cells are there? Compare their distribution over the body with their distribution in Hydra. Which arrangement appears to you the more complex? The more specialized? What are organs? Systems? Does Hydra have any approach to organs? If so, where?

6. Development of the Individual.—The successive degrees of complexity of the organisms studied in this exercise are roughly paralleled by the stages in the development of a multicellular animal. This correspondence led to a generalization known as the *biogenetic law*, according to which the evolutionary history of a species is epitomized or recapitulated during the embryonic development of each individual. With this principle in mind, study the early stages in the embryonic development of the starfish. These stages are similar to the developmental stages of many other metazoa.

On a single slide all the desired stages may be found. Find and *sketch* in order each of the following stages: 1 cell, 2 cells, 4 cells, 8 cells, 16 cells, *blastula*, and *gastrula*. The blastula is composed of many cells arranged in the form of a hollow sphere. Are the cells of the blastula uniform in size? The gastrula is produced by the invagination (pushing in) of the cells of one side. Are the invaginated cells larger or smaller than those remaining outside? The cavity of the blastula is the *blastocoele*, the new cavity in the gastrula is the *archenteron* (meaning primitive gut), and the opening of the archenteron to the exterior is the *blastopore*. The outer cellular layer is the *ectoderm*, and the inner layer is the *endoderm*.

Compare the undivided egg with a single-celled protozoon, the blastula with Volvox, and the gastrula with Hydra. In the last comparison, what parts of the gastrula and of Hydra correspond to one another?

SUMMARY

If the organisms and developmental stages used in this exercise indicate in any way the evolutionary changes of animals, state concisely what would seem to be the nature of the steps leading to the formation of the metazoa. Look for generalized conditions, not specific structures. Each organism studied should represent an important step in this change.

EXERCISE V

ACTIVITIES OF PROTOPLASM

All types of animals from the simplest to the most complex have the same physiological problems to face. All of them grow and reproduce their kind. In order to grow, they must utilize material known as food. This food is taken in by the living animal and either provides energy for the activities of the animal or is built up into living substance. When it is used for energy it is literally burned. As a result of this burning process water and carbon dioxide are formed as end products. These and some other substances are of no further use to the animal and are passed out, that is, excreted. In order to accomplish the combustion of food, it is essential that oxygen be taken in. The absorption of gaseous oxygen and the giving off of gaseous carbon dioxide are well-nigh universal among animals and are together known as respiration.

In this exercise an attempt is made to demonstrate some of the vital activities in both protozoa and metazoa. Activity is more difficult to observe than structure; hence only those phases of the subject which lend themselves to easy demonstration without the aid of complicated apparatus can be included.

1. Protoplasmic Activities in Protozoa.—The physiological processes of one-celled animals may be fairly illustrated by those of Paramecium.

1*a*. Mount some living paramecia on a slide. Study the general form of the animal. Are the two ends alike? Is the same end always directed forward as it moves? If so, which end? Note that there is a wide depression in the front half of the animal. This is called the *oral groove*. It leads back into a funnel-shaped tube which enters the interior of the cell. This tube is the *gullet*. Examine a clay model of Paramecium and observe the relations of oral groove and gullet.

Is there any difference in the appearance of the outer and inner layers of the animal? The outer is the *ectosarc*, the inner the *endosarc*. The endosarc contains granules as well as larger masses which are *food vacuoles*.

Find the *pulsating vacuoles*, one near each end of the body. These appear as circular clear spots or as a group of *radiating canals*, or both.

Make a *drawing* three or four inches long to show the various structures you have observed in Paramecium.

Prepare a fresh mount and stain with acetic methyl green or aceto-carmine. This will reveal the presence of a large nucleus. Paramecium really has two nuclei, a large *macronucleus* and a small *micronucleus*. The latter can be observed only when more pronounced stains are used. Search for it in a demonstration slide. Include the macronucleus and micronucleus in the drawing you have already made.

1*b*. *Ingestion*.—Mount some fresh paramecia, adding a very small quantity of ultramarine blue or Chinese ink suspended in water before putting on the cover glass. Are any of the particles taken in? If so, where? How are they arranged inside? Minute organisms are taken in in the same manner, as food, and the enclosing droplets form the *food vacuoles*. Where are the food vacuoles most abundant?

1*c*. *Digestion*.—Mount again some fresh paramecia and add a drop of a 0.001 per cent solution of the dye neutral red before putting on the cover glass. Examine demonstration test tubes containing respectively acid, basic, and neutral solutions of the dye neutral red. Now return to the slide of paramecia and examine the specimens. Do you find the food vacuoles colored? How do you interpret the result? Observe whether there is any movement of food vacuoles in the interior of Paramecium. What is the general direction of their movement?

1*d*. *Respiration*.—Air is bubbled through a series of three flasks, the first containing barium hydroxide, the second a paramecium culture, and the third barium hydroxide. Barium hydroxide and carbon dioxide, when together in a solution, recombine to produce, in part, barium carbonate, which is very insoluble in water and is at once precipitated. What happens in the two bottles of barium hydroxide? Explain the result.

1*e*. *Excretion*.—Study the behavior of the pulsating vacuoles. Do they change in size? Disappear? When the radiating canals are most conspicuous, in what condition is the central vacuole? What becomes of the contents of the vacuole? What waste material is probably being excreted? What other means has Paramecium of excreting wastes?

1*f*. *Osomotic Behavior*.—Mount some fresh paramecia in a moderately concentrated salt solution. How do you explain the change of shape?

1*g*. *Movement*.—Mount some paramecia in water on lens paper, whose fibers serve to trap them. Observe minute hairlike projections from the surfaces of the cells. These are the *cilia*, and they are responsible for the movement of the animal. Does the body rotate while swimming? What arrangement of the cilia could account for this motion? Paramecium also tends to swerve toward one side. Could this be due to stronger cilia on any portion of the surface? If so, where? Fill in the cilia in a portion of your *drawing* of Paramecium.

1*h*. *Responses to Stimuli.*—Paramecium responds to various environmental differences. Observe the position of paramecia in a long vertical tube. Where in the culture dish are the animals mostly found? What, therefore, is their response to gravity? Observe what happens when a paramecium in swimming meets an obstacle. The reaction that follows is called the *avoiding reaction.* Make a *diagram* to illustrate the reaction, showing the position of the animal before and its several positions afterward. Its responses to temperature differences and chemical substances are similar to the response to mechanical obstacles.

1*i*. The reproductive activities of Paramecium are considered in the next exercise.

2. Protoplastic Activities of Metazoa.—Many-celled animals possess, in general, the same activities as single-celled animals. In the metazoa, however, not all the cells take a conspicuous share in any one activity. There is a division of labor, and some cells perform chiefly one function, others another.

2*a*. *Ingestion.*—Ingestion of food in the higher animals is too familiar a process to require demonstration. In the earthworm, recently studied, the pharynx is connected to the body wall by a large number of muscular strands. When these contract, a suction is produced, the pharynx behaves like a vacuum cleaner, and the earth which the worm uses as food is sucked into the mouth.

2*b*. *Digestion.*—All food must be made soluble before it can be utilized by the body. The process of making food soluble is known as digestion. Various digestive juices play a part in this change.

To a drop of dilute boiled-starch suspension add a drop of iodine solution. Note the color that results. Iodine may thus be used as a test for the presence of starch. To a few cubic centimeters of starch suspension in a test tube add a small quantity of saliva. After half an hour, test again with iodine. What does the color indicate? Add a little iodine to a solution of glucose. What has become of the starch in the original suspension?

In the stomach, digestion is due to the presence of a secretion known as the *gastric juice.* Examine sections cut across the stomach of a frog. Note the cavity of the stomach and the thick wall surrounding it. On the inner surface of the wall are numerous pits or *glands.* These glands produce the gastric juice, which contains the enzyme *pepsin* and a little hydrochloric acid. *Draw* a single gland.

The action of the gastric juice may be illustrated by the following experiment, which should if possible be started at the beginning of a laboratory period. Place a small piece (half as large as a pea) of hard-boiled white of egg into each of three test tubes or dishes. To one tube add 10 cc. of a 0.4 per cent solution of hydrochloric acid (about 9 cc. of the concentrated acid to a liter of water); to another, 10 cc. of a solution of pepsin in water (2 grams of pepsin to a liter of water); to a third, 10 cc. of a 0.2 per cent solution of pepsin in 0.4 per cent hydrochloric acid. Put all the tubes into a water bath or incubator, and keep at a temperature of 40°C. Observe the three tubes at the end of the laboratory period and again at the next laboratory period. What conclusion do you draw from the experiment?

2c. *Absorption.*—Digested food passes through the lining of the digestive tract by diffusion or by osmosis.

Diffusion may be demonstrated by means of a faintly acid agar solution containing a little neutral red. It will be remembered (see 1c) that neutral red is yellow in alkaline solution and red in acid solution. Some of the agar is poured while hot into a test tube. When it is cool and solid, add a small quantity of dilute sodium hydroxide. The progress of diffusion of the sodium hydroxide can be followed by watching the color change of the agar. Observe the speed of diffusion during one laboratory period, then notice how far diffusion has progressed by the next period. Is the diffusion rate constant, or does the rate at first differ from its later speed?

Osmosis.—Tie a piece of pig's bladder, which has been immersed for some time in water, over the end of a thistle tube containing a concentrated sugar solution, and support the tube (large end down) in a beaker of water. Mark the level in the tube and examine it at intervals to record changes in the level. What is the explanation of the changes? Make a *diagram* of the apparatus.

Obtain a drop of frog blood (or human blood) and add several drops of a concentrated salt solution. Note changes in the appearance of the cells. How can you explain these changes? To another drop of blood, add several drops of distilled water. Explain what happens.

2d. *Circulation.*—In higher animals the size of the body is so great that a transportation system is required to carry oxygen and the digested food to cells in all parts of the animal. This is accomplished by the blood vessels, which, with the heart or hearts, form the circulatory system. The blood vessels carry not only digested food and oxygen; they are also instrumental in carrying the waste products away from the cells.

Examine a demonstration of the beating hearts and the main blood vessels of an earthworm, closely held in a glass chamber under a dissecting microscope.

Examine the web of a frog's foot, the tail of a tadpole, or the tail fin of a small fish, under a microscope. Observe the network of blood vessels. How large are the capillaries as compared with the blood cells that pass through them?

2*e*. *Respiration.*—As a class demonstration breathe through an apparatus consisting of two flasks of barium hydroxide solution with tubes so arranged that the inspired air bubbles through the solution in one flask, while the expired air bubbles through the other. Refer to 1*d*, and explain the result.

Place a mouse in a small closed chamber to which air is admitted by bubbling it through a flask of barium hydroxide solution, and from which air is withdrawn by bubbling it through another flask of a similar solution. Attach the apparatus to a suction pump or aspirator or to the compressed air apparatus to produce a current of air through the chamber containing the mouse. Note the difference between the two solutions. What does it indicate?

2*f*. *Excretion.*—Examine a section of the kidney of a frog. In the ventral half of the section note the rounded bodies, the *renal corpuscles*. The interior mass in each of these is composed of blood capillaries, around which are two layers of thin cells. *Draw* a renal corpuscle. Study the figures of kidney structure in "Principles of Animal Biology" to determine the relation between these capillaries and the adjoining tissues. Note, in the dorsal half of the section, numerous tubes cut at various angles. What is the relation between these tubules and the renal corpuscles? (See "Principles of Animal Biology.") Among the tubules observe minute blood vessels, recognizable by the reddish elliptical blood cells in them.

What is the essential feature of the structure of the kidney which makes it a suitable excretory organ? Ascertain from "Principles of Animal Biology" the course of the waste matter in leaving the kidney.

2*g*. *Responses to Stimuli.*—The responses of higher animals are more complicated than those of the single-celled animals. In an animal like the frog most of the responses are the result of *reflex actions*. In a reflex action a sensory structure or receptor receives a stimulus and sends an impulse to the nervous system, which in turn sends it to a muscle or a gland and the latter, which is called the effector, makes a definite response.

In a pithed frog provoke a reflex action by pinching one of the toes on the lower leg. Touch the flank of the frog with a piece of filter paper moistened with acid. Observe what follows. Consult "Principles of Animal Biology." What is the probable course of reflex arcs involved in the foregoing experiments?

2*h*. *Movement.*—Observe a preparation of the *gastrocnemius* muscle from the calf of a frog's leg. The upper end of the muscle is supported from the thigh bone, held in a clamp. A weight may be suspended from the lower end. Apply an electrical stimulus to the muscle and note the result.

Examine a longitudinal section of gastrocnemius muscle. Note the dark and light bands or striations. Observe also the *myofibrils*, which extend the length of the muscle. Find the nuclei. Is there more than one in a muscle cell? *Draw.*

Not all muscles have striations. Thus the muscle cells surrounding the digestive tract or the blood vessels are unstriated. In the section of frog stomach, note the wide layers of muscular tissue in the outer layers of the stomach wall. The muscle cells are cut both lengthwise and across. *Draw* a few of those cut lengthwise.

2*i*. *Reproduction.*—The reproduction of metazoa will be considered in the next exercise.

SUMMARY

Review briefly the nature of the several physiological processes studied in this exercise and the methods of carrying them on. If any of these processes has an especially close relation to the others, state the relation.

EXERCISE VI

REPRODUCTION

Living organisms come into existence only from other organisms through some form of reproduction. It was once supposed that living things were sometimes produced directly out of nonliving matter, a phenomenon now referred to as *abiogenesis.*

ABIOGENESIS

One of the principal reasons for the old belief in abiogenesis is shown to be invalid by the following experiment, which is to be performed as a class demonstration. Into each of several clean, sterilized petri dishes or test tubes place a small amount of nutrient agar solution; put covers on the petri dishes and cotton plugs in the test tubes and sterilize under 15 to 18 pounds steam pressure. Allow to cool. One half of the preparations are opened in the presence of the class, exposing the agar to the air of the laboratory for about 15 minutes. The unexposed preparations are kept as controls.

Examine the dishes at frequent intervals for signs of growing organisms (molds, bacteria colonies, and yeasts). On which cultures do they appear first? Source of the growths? Discuss possible sources of contamination in the controls[1] if growths occur in them. Why would naturalists formerly have supposed these growths to arise by abiogenesis?

TYPES OF REPRODUCTION

Living organisms give rise to other organisms like themselves; that is, they possess the power of reproduction. Since the life of the individual is in every case limited, it is this reproductive capacity that prevents any race from dying out.

Reproduction may be of two general kinds: (1) *sexual* (either *bisexual* or *parthenogenetic*) and (2) *asexual.* It is the purpose of this exercise to determine, as far as possible, from a limited number of examples, the essential features of each of these types of reproduction.

A. Bisexual Reproduction

Look in the cultures of Paramecium for individuals swimming about in pairs side by side. Such specimens are conjugating.

[1] Note that air is not entirely excluded by the covers of the petri dishes. A more careful experiment is not needed, however, to illustrate the method of attacking the theory that abiogenesis occurs.

1. Conjugation in Paramecium.—The nuclei of conjugating specimens can be studied only with the aid of prepared slides. The essential part of the process is the exchange of portions of the micronuclei. Several demonstrations of this stage will be provided. *Draw* carefully, representing the body in outline, and the nuclei in detail. Consult "Principles of Animal Biology" for an account of conjugation.

2. Sexual Reproduction in Metazoa.—Sexual reproduction may be illustrated in Volvox, Hydra, and Ascaris.

2*a*. How does sexual reproduction occur in Volvox? If this is not clear in your mind from a previous exercise, examine slides of Volvox. Compare the sizes of the *gametes* (sexual cells) in Volvox. What is the name given to each of these kinds of cells?

2*b*. Recall sexual reproduction in Hydra. What structure contains the male sexual cells? The female sexual cells? What is the name of the cells contained in each of these structures? If your recollection of these facts is not clear, examine mounted slides of Hydra to show these features.

2*c*. In dissections of Ascaris, a roundworm parasitic in the pig, examine the long coiled reproductive organs of the male and of the female. In sections through the uterus of Ascaris find the large round *ova*. Among the ova and penetrating some of them, find small, somewhat triangular bodies. These are the *spermatozoa*. They were delivered by the male into the uterus of the female. In the great majority of species of metazoan animals the sexes are separate as in Ascaris. Such species are said to be *dioecious*. Species like the earthworm and the tapeworm, which have both male and female organs in the same individual, are said to be *monoecious*, and the individuals of these species are *hermaphroditic*. The anatomy of the sexual organs of a dioecious species will be studied later, in the exercise on breeding behavior.

B. Unisexual Reproduction, or Parthenogenesis

The eggs of certain species of rotifers, crustaceans, insects, and others normally develop without fertilization.

3. An Aphid.—A laboratory experiment will be conducted using the aphid, or plant louse, Macrosiphum, and the chrysanthemum[1] as a host plant. Several chrysanthemum plants should be carefully examined to discover if they are free from plant lice. If they are free, then a single immature plant louse should be placed on each plant, and the plant should be covered with a lantern globe closed at the top with cheesecloth or muslin. The plants will now be placed on a shelf and cared for by an assistant. Make a record of the date and just what was done.

[1] Another species of Macrosiphum, and certain other aphids, may be raised on potato plants.

After a time interval of a number of days count and record the number of individuals on each plant, the date, and the number of days elapsed since the lice were put on the plants. If the interval has been long enough, some of the progeny may also have borne young.

How many parents were concerned in the act of reproduction in Macrosiphum? How can you be sure? Why was an immature aphid used? To prove that this reproduction is sexual rather than some form of budding (asexual), it would be necessary to show that development of the new individual started from an egg (a cell that has undergone maturation, see Exercise VIII). This proof is obtainable, but not suitable for elementary study.

C. Asexual Reproduction

The more common cases of asexual reproduction fall into two groups: fission and budding. The latter type (budding) may be either external or internal.

4. Fission.—This implies approximately equal division of the parent's body, and neither fraction can properly be called the parent of the other.

4a. Fission in a protozoon (Paramecium). Try to find living paramecia that are dividing by means of transverse constriction about the middle, but do not spend much time in search. If living animals undergoing fission are found, note the position and depth of the constriction. Look for pulsating vacuoles. How many and where? If dividing animals are found, watch them at intervals until the process of division is completed.

In specimens stained and mounted on slides, observe carefully the condition of the nuclei. Note that each paramecium has two nuclei, a large *macronucleus* and a minute *micronucleus*. The micronucleus in paramecia that are not undergoing division occurs in or near a little hollow on the side or surface of the macronucleus. Look for it carefully. In fission each of the two nuclei divides, a half going into each of the daughter cells. Each daughter gets one of the old contractile vacuoles and produces a new one.

Make *three drawings* each 2 inches long showing an individual not dividing and an early and a late stage of fission. Represent the body by an outline and make the nuclei dark.

5. External Budding.—External budding consists in the protrusion of an externally visible portion (less than half) of the body to form a new individual, or a separation of a minor part of the body without such protrusion.

5a. Budding in the metazoon Hydra. Select hydras that bear buds of various sizes, representing stages in growth of these buds. Note that the cavity of the bud is directly continuous with the cavity of the parent.

The bud is formed by the simple outpushing of both layers of cells of the parent's body and the subsequent development of tentacles and mouth. Tentacles are produced by a process similar to budding.

Make an outline *drawing* of parent and bud.

6. External Budding and Colony Formation in a Hydroid.—Obelia is an animal closely resembling Hydra, being called a hydroid and belonging to the same phylum, Coelenterata. Obelia reproduces by budding, the buds remaining attached to the parent, and a colony is thereby produced. It grows on wharves and rocks in sea water. Under the dissecting microscope note the treelike form of a single stem. Specimens in watch glasses or mounted permanently on slides may be used for this purpose and for the identification of the kinds of individuals and their parts indicated below. Use the compound microscope for parts of this study.

6*a*. *Hydranths*, bearing tentacles, are located at the ends of the branches. Each hydranth is enclosed in a cuplike sheath or *hydrotheca*, which is a continuation of the tough membranous covering of the whole colony (the *perisarc*).

In an expanded hydranth note the body with the *hypostome*, an elongated projection in the midst of the tentacles. The hydranth is morphologically the equivalent of a hydra. Note that the fleshy part of the hydranth extends into the stalk and is continuous with the fleshy part of the entire colony. This fleshy part of the colony is called the *coenosarc*. The cavity in the body of the hydranth continues through the coenosarc.

6*b*. *Gonangia* (singular, *gonangium*) are club-shaped bodies usually found in the angles between the hydranths and the main stalk. They consist of a central fleshy core, or *blastostyle*, usually bearing buds, the whole being surrounded by a transparent sheath, the *gonotheca*. The blastostyle with its sheath is comparable to a hydranth and is to be regarded as a different type of individual. Note that the blastostyles do not have tentacles; hence thay can capture no food. How can they be nourished?

6*c*. *Medusae* may be found on the sides of the blastostyle as rounded projections. On large old blastostyles some of the medusa buds show the beginning of tentacles at the margin. If these are not visible in your specimens, see the demonstrations. The medusae detach themselves later from the blastostyle, emerge from the sheath through an opening at its distal end, which in younger gonangia is closed by the broad end of the blastostyle, and live a free-swimming existence. Examine one of the free medusae in a demonstration. Note the *manubrium* in the center of the under surface. It is homologous with the hypostome and contains the *mouth*. Four *radial canals* extend out from the manubrium as far as the *circular canal* along the margin. The *reproductive organs* are usually borne on the radial canals. The medusae reproduce by eggs and spermatozoa. The structure of a medusa may be better appreciated from an examination of larger medusae belonging to other species such as Gonionemus or Polyorchis.

The treelike branch you have examined is not an entire colony. In a demonstration, note that numerous such branches may be connected by a horizontal creeping portion, the *hydrorhiza*, from which the branches arise.

Obelia illustrates a simple form of *polymorphism*, in that it comprises three kinds of individuals differing in form: (1) the hydranth or nutritive individual, (2) the blastostyle with its sheath, and (3) the medusa, the dispersing member of the species.

The life cycle of Obelia is a form of *alternation of generations* in that asexual individuals of one kind give rise to sexual individuals, which in turn give rise to asexual individuals, the sexual and asexual individuals being unlike in structure. A life cycle that includes this sort of alternation is termed *metagenesis*.

Draw a branch showing a hydranth and a well developed gonangium in detail. *Draw* also a medusa.

7. External Budding and Colony Formation in a Tapeworm.—In a tapeworm there is at one end an enlargement called a *scolex*, bearing suckers, and in some species hooks. Near the scolex is a region of great proliferation of cells, where transverse constrictions appear, which mark off the individual buds from one another. This region may be called a budding zone. The buds remain attached for some time and continue to grow. In this way an elongated chainlike colony consisting of a number of links is formed. From time to time one or more of the oldest links of the chain are released. Those released are farthest from the scolex.

Make a *sketch* of a tapeworm showing the position of the budding zone.

8. External Budding in a Worm.—Use Nais, Aeolosoma, Chaetogaster, Dero, or Microstomum, indicating in your notes or drawings which was used. When reproduction by budding occurs in any of these forms, the elongated body becomes constricted transversely and later separates into two parts. The budding zone is marked by considerable proliferation of cells and by other forms of cellular activity. In some cases the worm may show several budding zones, producing several young individuals simultaneously. Such chains of individuals resemble colonies, but the union lasts for only a short time.

Examine a demonstration of a budding worm (a living one if possible). *Sketch.*

9. External and Internal Budding in Bryozoa.—The common bryozoan Plumatella has a general resemblance to hydroid colonies, such as Obelia, and may be found in most fresh-water bodies, attached to submerged vegetation, logs, or stones. They are preferably studied alive, but permanent preparations on slides may be used.

9*a*. Examine the tips of several branches of a colony of Plumatella. Rounded projections there are early stages of *external* buds, from which new tentacle-bearing members of the colony are produced. *Sketch* the end of a branch, including such a bud and the nearest full-grown individual.

9*b*. Note the U-shaped digestive tract of a full-grown individual. From the bottom of the U observe the slender strand of tissue, the *funiculus*, leading obliquely to the body wall. In the funiculus find one or more enlargements; these are *internal* buds, or *statoblasts*. Toward which end of the funiculus are they largest? What does this indicate? Mature statoblasts are released into the body cavity and reach the outside by decomposition of the parent's body; they may be collected with a very fine net at the surface of the water.

Sketch an individual with several statoblasts in its funiculus, or add the funiculus and statoblasts to the figure drawn in 9*a*.

9*c*. Examine a statoblast. Note that it is composed of two saucer-shaped valves enclosing a germinal mass of cells. Observe at the margin of each valve a ring of air chambers which cause the statoblast to float. The structure can best be observed if the statoblast has begun to germinate, so that the valves are slightly separated from one another.

Sketch a germinating statoblast if one is available, otherwise one that has not started to develop. Label the valves, the ring of air chambers, and the germinal mass.

10. Internal Budding in Sponges.—Fresh-water sponges exhibit both external and internal budding. The external buds are so intimately united with the parent that their limits cannot be determined, and their production looks merely like growth. The internal buds are very definitely recognizable. As autumn approaches, certain cells in the body wall aggregate into spherical groups and become surrounded by a protecting shell. These spherical bodies are called *gemmules*. Examine a specimen containing them. In some species several gemmules may be enclosed in a common envelope. The adult sponges die in the autumn, but the gemmules live through the winter and develop into new sponges in the spring. Crush a gemmule to demonstrate that it contains cells; that it is not an egg is shown by the fact that there are many cells in it. Examine gemmules that have been boiled in *caustic soda* to destroy the cells inside and to make the shells more transparent. Find a small *foraminal aperture*, with or without a projecting tube, through which the small sponge emerges in the spring.

Using the compound microscope, make a *drawing* of a gemmule an inch in diameter, or show a group of gemmules in a common envelope. Show the foraminal aperture in one of the gemmules.

SUMMARY

The summary should consist of a discussion of abiogenesis, and of sexual and asexual reproduction, or a comparison of the two modes of reproduction, pointing out distinguishing or essential features of each. Discuss also the results of budding in those species of metazoa in which the bud does not separate from the parent.

EXERCISE VII

BREEDING HABITS OF VERTEBRATE ANIMALS

A knowledge of the anatomy of the reproductive organs is essential to an understanding of the breeding habits of vertebrates. To gain this knowledge, the student should work out the structure of the male and female reproductive system in the frog, using for this purpose dissections which are placed on the table. Consult the wall charts and the diagram in the textbook showing the relative location of the organs and their connections. Examine also a model of the frog showing organs and specimens partly dissected.

1. Male Reproductive Organs.—In the dissection furnished you, observe the following:

1*a*. The *kidneys*, two flattened oval structures side by side, near the anterior ends of which are:

1*b*. The *testes* (singular, *testis*), two yellowish bodies of ovoid shape. Push one of them aside and observe:

1*c*. The *vasa efferentia* (singular, *vas efferens*), delicate white tubes passing between the testis and the median edge of the kidney, best seen in fresh preparations under low magnification.

1*d*. The *ureters*, tubes, one passing backward from the lateral margin of each kidney. They connect the kidneys with:

1*e*. The *cloaca*, a short passage which is a continuation of the large intestine. (The large intestine and part of the small intestine are included in your specimen.) The cloaca discharges to the exterior through the anal aperture.

1*f*. The *Muellerian ducts*, two irregular white tubes extending from the cloaca forward to a point in front of the kidneys, present in certain of the smaller species but not in the bullfrog. They correspond to the oviducts of the female but are functionless in the male.

Carefully *draw* the male reproductive system. Discover if possible how the spermatozoa reach the water. Examine wall chart.

(Note.—The *postcava*, a large vein lying between the kidneys and formed by branches coming from those organs, and the large yellow branching *fat bodies* can hardly fail to attract attention during the foregoing study, but they are not reproductive organs.)

2. Female Reproductive Organs.—In the demonstration dissection furnished find:

2a. The *ovaries,* two large lobed masses containing black and white eggs (or the ovaries may be much smaller and white).

2b. The *oviducts,* two thick convoluted tubes extending longitudinally beside the ovaries.

2c. The *uterus,* a thin-walled portion of the posterior end of each oviduct. Each uterus connects with:

2d. The *cloaca,* a continuation of the large intestine, as in the male. Try to discover how the eggs escape into the water.

Draw the female reproductive system. Indicate the path of the eggs by means of arrows.

Compare your drawings of the reproductive systems of the frog with the charts showing similar diagrams for the other vertebrates. Be sure that you understand the function of each organ in the frog and in a mammal. Bring the textbook "Principles of Animal Biology" to class for the following work.

TYPES OF EGGS IN RELATION TO METHODS OF REPRODUCTION

Full notes on this exercise are desired, and particular attention must be given to the conclusions or summaries called for.

3. Types of Eggs of Oviparous Forms.—Eggs whose development occurs outside the body of the female possess characteristics illustrated by the following examples.

3a. Examine the eggs of two fishes (perch and whitefish) and three amphibians (a frog, a toad, and the salamander *Ambystoma maculatum*), which are deposited in water and fertilized as laid. Describe the covering and the differences in the way in which the eggs are held in a mass. In what respects are the eggs of all these forms alike?

3b. Examine the egg of a turtle, a crocodilian, an egg-laying snake (such as a racer, king snake, or gopher snake), and a bird, which in each case is fertilized within the body of the mother and subsequently laid in places exposed to air. Describe the difference in the texture of the covering of the two types *3a* and *3b*. Read the description of shell structure given in "Principles of Animal Biology."[1]

3c. Read the account of fertilization in the textbook and explain the relation between the habits of the animals in *3a* and *3b* and the nature of the egg covering.

4. Types of Eggs of Ovoviviparous and Viviparous Forms.—Eggs that develop within the body of the female have characteristics illustrated by those of the following animals.

4a. Examine the demonstrations of the developing eggs in position in the body of an ovoviviparous reptile (the garter snake). In what

[1] For convenience this book will in later paragraphs be frequently referred to simply as the textbook.

part of the reproductive system do these eggs or embryos lie? Are they attached in any way to the walls of this tube? In a series of garter-snake embryos of different stages of development, note the gradual decrease in quantity of yolk with increase in size of the embryo.

4*b*. On slides prepared for the purpose, locate the eggs in the ova·y of a viviparous species (such as the cat). How do these eggs compare in size with those you have previously examined? Are these eggs supplied with any appreciable quantity of yolk?

4*c*. Examine a demonstration of mammalian embryos (mouse, pig, cat) in position in the uterus and describe the relation of the developing young to the uterus (and, therefore, to the body) of the mother.

4*d*. In comparing oviparous, ovoviviparous, and viviparous forms, do you observe any apparent relation (1) between size of egg and source of nutrition and (2) between size of egg and number of young produced?

BROODING HABITS

The care bestowed by oviparous animals upon their eggs usually consists either of simply guarding them or of incubating them, that is, raising the temperature of the eggs so as to facilitate development.

5. Guarding the Eggs without Incubation.—When the parents are cold-blooded, they can do little more for their eggs than to protect them. They are unable to hasten their development in any very appreciable degree.

5*a*. Read the paragraph on care of the fertilized eggs in the textbook.

5*b*. Examine the demonstration specimens or, in the absence of these, the figures of the marsupial frog (Gastrotheca) and a fish which carries the young (Hippocampus), noting the position of the brood pouch.

5*c*. Study an exhibit showing the miller's thumb (a fish) guarding its nest; also a similar exhibit of the four-toed salamander. Examine the photograph of a blue-tailed skink guarding its eggs.

6. Incubation of Eggs and Brooding the Young.—Incubation and brooding are prevalent among warm-blooded animals (birds and mammals).

6*a*. Refer again to the section of the textbook on the care of the eggs cited above and read also the section on care of the young.

6*b*. Examine the series of bird and mammal nests in the laboratory, and describe at least three nests representing different types of construction.

6*c*. Note the position of the brood pouch in a marsupial (the opossum), or in the illustration of a kangaroo (see textbook).

BIRTH STAGES

By birth stage is meant the stage of development attained at the time of birth or hatching.

7. Oviparous and Ovoviviparous Species with a Larval Period.—Compare the young and adult of the common lamprey, a frog, and the spotted salamander *Ambystoma maculatum.* Describe the differences in the mouth, eyes, form of body, and appendages (legs and fins).

8. Species without a Larval Period.—Compare the newly born young of a shark, a garter snake (or turtle, or lizard), an English sparrow, a chick, a mouse (or rat), and a guinea pig. Describe the differences in the stage of development at time of birth as shown by the relative size, strength, the covering of scales, hair, or feathers, and the eyes. Comparing the two birds just examined with one another and the two mammals with one another, and drawing on your knowledge of the habits of these animals, do you see any evident relation between the degree of development at birth and the amount of care bestowed on the young?

SUMMARY

Summarize the generalizations you have discovered in your study of reproductive habits and the types of eggs in vertebrate animals.

EXERCISE VIII

EMBRYOLOGY OF TYPICAL ANIMALS

Embryology treats of the changes undergone by the germ cells in preparation for reproduction, the union of the two kinds of germ cells in fertilization, the cleavage of the fertilized egg, the arrangement of the cells into definite germ layers, and the folding and differential growth of parts of these layers, and in some instances migration of the cells contained in them, to form organs. In species passing through a larval stage, the metamorphosis of the larva into the adult is likewise part of the subject matter of embryology.

GAMETOGENESIS AND FERTILIZATION

The series of changes undergone by the male and female germ cells before fertilization is known as *gametogenesis*. With respect to germ cells of the male it is called *spermatogenesis;* of the female, *oögenesis*.

1. Spermatogenesis.—The undifferentiated male germ cells are known as *spermatogonia*. These multiply by ordinary mitosis. When ordinary mitosis stops, each cell increases in size and is known as a *primary spermatocyte*. Each primary spermatocyte divides into two *secondary spermatocytes*. Each of these, in turn, divides into two *spermatids* which metamorphose into *spermatozoa*. Thus out of each primary spermatocyte four spermatozoa are formed. During this process the number of chromosomes is reduced one half, a change referred to as *meiosis*. A wall chart should be studied for the outline of the process.

1*a*. Examine sections of the testis of a grasshopper or katydid (Amblycorypha is excellent). At one end of each section spermatogonia will probably be found, at the other end mature spermatozoa, and between the two ends spermatocytes in various stages.

1*b*. Note the small size of the spermatogonia. Find some undergoing mitosis. In a polar view of an equatorial plate determine as nearly as possible the number of chromosomes. Record the number. Note the large *X chromosome*. *Draw* a spermatogonium in some stage of division showing as nearly as possible the number of chromosomes.

1*c*. The spermatocytes are larger than the spermatogonia. Among them find cells undergoing mitosis. From an anaphase of the division of a secondary spermatocyte determine as nearly as possible the number of chromosomes. If in doubt whether you are observing the correct stage, ask to have one shown to you. How does the number of chromosomes in each anaphase group in the secondary spermatocyte compare with the number in the spermatogonia? The number in these anaphase groups is the number that goes into the spermatozoa. *Draw* a spermatocyte in anaphase to show as nearly as possible the number of chromosomes.

1*d*. *Draw* a mature spermatozoon, from either the grasshopper or katydid or from a mammal, of which a demonstration may be provided.

2. Oögenesis and Fertilization.—Since, in the animal selected for the study of the female germ cells, the processes of oögenesis and fertilization occur in large part simultaneously, they are studied together. The chronological order of events is followed.

2*a*. Examine sections of the uterus of *Ascaris megalocephala*. For the form of the uterus see a specimen of Ascaris. The large rounded bodies in the uterus are *oöcytes* or later stages. The nature of these sections has been explained in Exercise III. While in the ovary, before growth began, the female cells were *oögonia*.

2*b*. In the uppermost row of sections, which is from the inner part of the uterus, find *primary oöcytes*, each containing a triangular dark body with a distinct black nucleus. These triangular bodies are *spermatozoa* which have already penetrated the primary oöcytes. Some spermatozoa may also be found among, but not yet in, the oöcytes.

2*c*. Note the nuclei of the primary oöcytes. Some will have formed spindles preparatory to the first division.

2*d*. In the second row of sections, from a point a little lower down in the uterus, observe oöcytes undergoing their first division. The chromosomes are arranged in two quadruple bodies or *tetrads*. Each tetrad is composed of two chromosomes brought together in a process known as *synapsis*, the chromosomes of the pair having doubled so as to form four parts. The nucleus of the spermatozoon, surrounded by more darkly stained protoplasm, may also be seen in some specimens. Select a clear specimen, and *draw*.

2*e*. In the third row of sections are *secondary oöcytes* undergoing the second division. The secondary oöcyte is surrounded by a thick membrane. Within this membrane, usually adhering to its inner surface, is found in some sections a small dark object, the *first polar body*. This and the secondary oöcyte constitute the two daughter cells formed by the first division described in 2*d*. If the polar body is not seen, explain its absence.

In the secondary oöcyte observe the spindle, bearing two double bodies, the *dyads*. Each dyad is half of one of the tetrads described in 2*d*. When this second division is completed, two single bodies (*chromosomes*) will have gone into the *second polar body* (a very small cell), and two remain in the *mature ovum.* The nucleus of the spermatozoon may be visible in some specimens.

Draw a specimen showing a spindle with clear dyads.

2*f.* In the fourth row of sections, the second division is already completed and the two polar bodies are visible at the surface of the *mature egg* in some of the specimens, one on the surface of the egg, the other usually adhering to the inner surface of the membrane.

The first polar body in some animals divides so that out of the original oöcyte four cells are formed, one of which is the mature egg and the others the polar bodies which are without function.

Observe in the interior of the mature egg the two large vesicular nuclei, containing scattered granules of chromatin. One of these is the *egg nucleus,* the other the *spermatozoon nucleus.* Both take part in the *first cleavage,* and their union constitutes the final step in *fertilization.*

Draw an egg with these two nuclei.

2*g.* In the fifth row of sections, the fertilized ovum is undergoing division or cleavage. Two-celled and four-celled *embryos* will be found. In a favorable specimen showing the chromosomes as long strands, count the chromosomes in one cell. This number is known as the *diploid* number. Recall the number of chromosomes in the mature ovum (see 2*e* above), which is known as the *reduced* or *haploid* number. The diploid number is restored at fertilization.

Compare gametogenesis in the male and female germ cells (see chart).

EMBRYONIC DEVELOPMENT OF THE FROG

The developmental processes of the various groups of vertebrates are quite similar. The development of one of them, therefore, serves to illustrate the process in all, just as the formation of spermatozoa and ova in the grasshopper and Ascaris is typical of the corresponding processes in other animals. The frog is used in this exercise to illustrate the more easily observed stages.

1. During the First Day.—Examine an unsegmented egg, using a dissecting microscope and reflected light, preferably with a black background. The middle of the black half of the egg is the *animal pole,* the middle of the white half the *vegetative pole.*

Using now transmitted light (from the mirror), examine the jelly surrounding the egg. How many layers? Their relative thickness?

Draw an unsegmented egg, with the animal pole toward the top of the plate. Make the egg itself ¾ inch in diameter and the jelly in proportion. Label.

Draw also, without the jelly, the 2-cell, 4-cell, 8-cell, and either the 12- or 16-cell stages. Compare the cells around the animal pole with those near the vegetative pole with respect to size.

2. During the Second Day.—By the beginning of the second day after fertilization, if the temperature is not too low, hundreds of cells have been produced, and the embryo is in the blastula stage (see Exercise IV).

Examine a bisected blastula and note the cavity, or *blastocoele,* within it. How large is the blastocoele? Is it in the center?

2*a*. *Early Gastrula.*—During the early *gastrula* stage the cells near the border between the black and white areas begin to be tucked into the hollow interior of the mass, along a crescent-shaped line. This crescentic opening is the *blastopore.* Note that the cells on one side of it are white, on the other side black. Why could not this invagination occur precisely at the vegetative pole? *Draw,* with the blastopore in the middle of the figure, convex side up. Label blastopore.

2*b*. *Late Gastrula.*—The invagination of the cells into the interior is now occurring along a circular line; that is, the blastopore is now a circle. The white cells within this circle constitute the *yolk plug.* The yolk plug is all that is left of the vegetative half of the egg that has not retreated into the interior. *A neural groove* may be present but is more likely not to be observable at this stage. *Draw,* turning the blastopore nearly to the right side. Label animal pole and yolk plug.

2*c*. *Neural Groove Stage.*—The *neural fold* is a ridge or elevation on the surface of the embryo. The fold is continuous and in the form of an elongated ring, wide at one end and narrow at the other. Later the wide part forms the *brain* and the narrow part the *spinal cord.* The groove between the neural folds is the *neural groove.* At a somewhat later stage the neural folds of the two sides come together above the neural groove and fuse, forming a *neural tube,* which differentiates into the brain and spinal cord. *Draw,* with the dorsal side toward you, that is, showing the whole nervous system.

3. About the Fifth Day.—By this time the external form of the embryo is no longer even approximately spherical.

3*a*. *Early Larva.*—Note the prominent *tail;* the V-shaped *sucker* under the *head;* and the rounded body, its form due to the yolk still present. At each side of the neck there may be a prominence, the *gill plate. Draw,* in side view, but tilt the ventral side up enough to show the sucker. Omit shading.

4. About the Eighth Day.—At ordinary temperatures the embryo hatches out of the jelly about the eighth day, though it is seldom active for some time thereafter unless it is disturbed.

4*a*. *Tadpole.*—Note the *external gills* developed from the gill plate of an earlier stage; the *operculum,* a fold of skin partially covering the gills and extending entirely across the ventral side of the body; the broad *tail* with its thin margin or *fin;* the V-shaped *segments* or *myotomes* into which the muscles in the axis of the tail are divided; the angular *mouth* beneath the head; the two *suckers* formed by the division of the original one sucker; and the *eye*, a whitish spot surrounded by a darker ring on each side of the head above. The *nasal pits*, minute depressions at the anterior end of the head, will be visible in clean specimens.

Draw a tadpole, tilting up the ventral side enough to show the mouth and suckers. Omit shading.

5. Older Tadpole.—After several months or a year, depending on which species is used, the young is a well-developed tadpole, of some size and possessing prominent hind legs.

5a. Note the following external features observed in the eighth-day tadpole: tail, fin, myotomes, mouth, eyes, and nasal pits or nostrils. In addition find:

The *hind legs*. The fore legs are present but concealed beneath the operculum.

The *spiracle*, an opening on the left side. Its front edge is the edge of the operculum (see stage 4), which has fused with the body everywhere except at this point. Water passes out of the gill chamber through the spiracle.

The horny *jaws* with which the tadpole scrapes off little particles of food from objects in the water.

5b. Internal Features.—Slit open the body wall on the ventral side and turn the flaps back. Observe:

The much coiled *intestine*, and the *mesenteries* supporting its coils. Compare the relative length of the intestine of the tadpole with that of the adult frog (see demonstration of the latter).

The *liver*, a brownish body to the right of the intestine (the observer's left), and the *gall bladder* on the posterior side of the liver.

The *pancreas*, an irregular whitish organ along the anterior part of the intestine.

The *heart* with its whitish *ventricle* anterior to the intestine. Push the intestine to one side and beneath it find:

The *fat bodies*, branching yellow organs.

The two *kidneys*, elongated reddish brown bodies lying against the dorsal wall in the posterior part of the body cavity.

The small *reproductive organs* lying near the anterior ends of the kidneys. It is difficult to distinguish the sexes at this stage.

The *lungs*, two flattened black or grayish structures attached at the anterior end of the body cavity toward the sides, and free at their posterior ends. They are rudimentary and still functionless at this stage.

Open the gill chamber by slitting through the operculum and observe:

The brownish fluffy *gills*. Probe between them into the mouth. The openings from the gill chamber into the mouth and pharynx are the *gill slits*.

The *fore legs* within the opercular cavity behind the gills.

Draw the dissected tadpole, displacing the organs slightly if necessary to show the foregoing features.

Compare the structure of a tadpole with that of an adult frog, dissections of which will be furnished as demonstrations, and make a list of the differences you discover.

SUMMARY

Outline briefly the essentials of the process of embryonic development from gametogenesis to the adult, basing your account on your laboratory work.

EXERCISE IX

GENETICS

Fundamental principles of heredity may be quickly illustrated by experiments with the vinegar fly *Drosophila melanogaster*. This fly may be obtained in its wild form by exposing near a fruit stand in warm weather a bottle containing some of the food described below. The mutant varieties to cross with the wild type or with one another are furnished by certain biological supply houses, or they may be had from other teachers who use them in classwork. Once a group of suitable mutants has been collected, it may be maintained the year round by the culture methods which follow.

1. Preparation of Food.—The following ingredients will make about 100 food cultures:

3750 cc. water.

400 grams corn meal (coarse meal works better than fine).

700 cc. molasses (cheap brands will do).

75 grams agar (dissolves best if chipped).

1 cake yeast mixed with 100 cc. of water.

Dissolve the agar in about three fourths of the water, by boiling. Soak the corn meal in the remaining one fourth of the water. When the agar is dissolved, add to it the molasses and bring to a boil. While it boils vigorously, add the moistened corn meal, stirring to prevent lumps. Boil about 7 minutes. Sterilize 100 half-pint milk bottles, either in an autoclave or by inverting them in a closed can and subjecting them to hot steam. Pour into each bottle, to a depth of ¾ inch, some of the boiled agar-molasses-corn-meal gruel. Close the bottle with a sterile stopper of cotton covered with gauze or cheesecloth, and set aside to cool. When cool add four or five drops of the yeast emulsion and shake it over the surface of the agar mass. The cultures are now ready for the flies and should be used within 24 hours. If any are not to be used at once, they may be stored without the yeast in a refrigerator above freezing; add yeast shortly before using. Cultures in which molds or bacteria grow must be discarded.

If fewer than 100 bottles are needed, reduce the ingredients in the same proportion except the water. Proportionately more water is needed in small batches—10 per cent more for 15 to 20 bottles—to compensate for evaporation during preparation.

2. Rearing the Flies.—The flies must usually be etherized for ease of handling. Turn a culture bottle with its bottom toward the window; the flies will move toward the light. Remove the cotton plug and place over the mouth of the culture bottle the mouth of a similar, but empty and dry, bottle. Holding the two bottles together mouth to mouth, turn the empty bottle toward the light. When the flies have gone into the empty bottle, close the latter with a cork stopper, in the bottom of which is a short wire bearing at its lower end a wad of cotton containing a few drops of ether. The flies gradually become quiet, and most of them fall to the bottom of the bottle. A minute after all of them have ceased motion, remove the stopper and pour the flies out on a white card, where they may be sorted out with a camel's hair brush or a needle, under a lens if necessary.

Place the etherized flies to be used as parents in an experiment in a small cup or horn of paper, and set this cup in one of the culture bottles. Replace the cotton plug. Remove the flies after 7 or 8 days, for their offspring may emerge as adults soon thereafter. If it is desired to obtain as many offspring as possible from a given group of parents, transfer the parents to a fresh culture bottle on removing them from an old one, and leave them there for another 7- or 8-day period. Continue this as long as the flies live.

Remove the offspring at intervals, examining them and making whatever records are desired, over a period of a week from the time when the first offspring emerge. It is not safe to continue much more than a week, since the next generation may begin to appear soon thereafter and so confuse the experiment.

3. Recognition of the Sexes.—Females are usually stouter than the males. More certain recognition marks are found, however, on the ventral side of the abdomen. The white area on the under side of a female extends backward to an acute angle almost at the tip of the abdomen, where it ends in a minute black speck on the ovipositor. The light area under the male does not extend to the tip of the abdomen but is reduced by a crescent-shaped black area on the under side of the tip. The concave edge of this crescent is toward the anterior end, and near this anterior edge is a small brown elevation consisting of the male genitalia.

4. Virgin Females.—Females used for experiments must be obtained before they have had an opportunity for an uncontrolled mating. In stock bottles of flies, the pupae are found attached to the sides of the bottle or to the paper towel, if any of the latter projects above the agar. The pupae turn dark in patches shortly before the adult flies are to emerge from them. From a culture bottle in which there are many dark pupae, remove all adult flies. Replace the cotton plug and wait several hours. Adults that emerge in that time will mostly still be pale and unable to fly. The females among them may safely be regarded as virgin and should be isolated. It is not necessary, of course, to take any such precautions with males.

5. Maintenance of Stocks during Idle Seasons.—During seasons when crosses are not being made, the growth of the flies may be retarded and the labor of keeping up the stocks greatly reduced by rearing them at low temperature. A constant temperature of 17°C. about doubles the time required to reach maturity, as compared with the optimum temperature for growth, which is 24 or 25°C. Or the adult flies, at least of the more vigorous types, may be kept for as long as 30 days in a food bottle in a refrigerator whose temperature may fall as low as 2 or 3°C., then brought to room conditions, and, in a fresh food bottle, allowed to produce offspring. The adult offspring may in turn be kept in the refrigerator for a long time.

6. Simple Cross.[1]—For this cross the wild type and the mutant brown[2] eye color may be used.

6*a*. Obtain virgin females from one of these stocks and males from the other. Put four or five of each sex in a culture bottle. Label carefully, naming the females and males separately, and date. Keep in a moderately warm place (24°C. is very favorable). If larvae are not found 4 days later, repeat the cross.

[1] The experiments here outlined are arranged in the order of their desirability for elementary exposition of principles, based partly on the ease with which the experiments may be performed, partly on the simplicity or the fundamental importance of the phenomena which they illustrate. It is likely that most elementary classes will not wish to perform all of them. It is suggested that they be done in the order in which they are given, omitting linkage if there is to be any curtailment and sex-linked characters if further restriction is necessary.

If the exercise is to be done in very limited time, the operations of the experiments may all be performed by the teacher and the bottles of offspring handed to the students for examination and record. To do this, the experiments must be started several weeks in advance. Ten days to two weeks must be allowed for each generation, depending on temperature. Thus, in an experiment extending to the F_2 generation, the original mating should be made nearly four weeks in advance and must be repeated two weeks later when the F_1 flies are mated, so that both F_1 and F_2 generations will be available simultaneously when students are ready for them. Still further reduction of time may be effected by using only demonstrations of preserved material, but much of the value of the exercise is thereby lost. The whole exercise may even be omitted without destroying the continuity of the course.

[2] As a substitute for brown eye in this experiment, curled wing or vestigial wing or scarlet eye or claret eye or sooty body may be used. There are many other mutations, but it is important that the first example be a character that is easily and certainly recognized. Vestigial wing is especially useful for this reason, but its low viability disturbs the ratios of the different kinds of individuals.

6*b*. Remove the parents a week after the cross is made, and either transfer to a fresh bottle or destroy them.

6*c*. When adult offspring (F_1) appear in the culture, examine them, etherized, for their eye color. Which color is dominant?

6*d*. Put a few of the F_1 flies into a fresh culture bottle, taking care that both sexes are well represented. Is it necessary that the females be virgin in this mating? Remove the F_1 flies a week later.

6*e*. When adult offspring from 6*d* appear, etherize and examine all of them for eye color. What is this generation called? Record the number of flies having each color.

6*f*. Prepare a chart like the following, using the symbol *b* for brown eye, *B* for the wild type, to show the facts and explanation of the foregoing experiment:

Appearance of parents		......		......
Genetic formulas of parents		....		....
Formulas of germ cells of parents		..		..
Formula of F_1 flies			....	
Appearance of F_1 flies			......	
Formulas of eggs of F_1 females		..	..	
Formulas of spermatozoa of F_1 males		..	..	
Formulas of F_2	....	....	....	....
Appearance of F_2	......	......	......	......
Expected ratio in F_2	.. ..	:	.. ..	
Observed numbers in F_2	... .	:	... .	

6*g*. What feature of an F_2 generation indicates that the original parents differed in only one pair of characters?

4. Backcross.—The backcross may be made with F_1 flies from the wild × brown cross above but is here described for another mutation—curled wing.

7*a*. Cross the wild type with the curled-wing mutant by placing a few virgin females of one of these stocks in a culture bottle with several males of the other. Remove the adult flies after a week.

7*b*. When adult F_1 offspring appear, record their wing character. Which type is dominant?

7*c*. Mate some of the F_1 flies of one sex with some curled-winged flies of the opposite sex taken from the stock culture. Does it make any difference from which of these cultures the females are taken? Must they be virgin in either instance? Remove the adult flies after a week.

7*d*. When the adult offspring from the foregoing backcross emerge, classify the flies with respect to wing shape, and count the different kinds.

7*e*. Prepare a chart comparable to that in 6*f* but modified to show the facts and the explanation of the backcross experiment.

7*f*. If you knew nothing of the matings made in a certain experiment but obtained from a given cross 83 offspring of one kind and 79 of another, what would you conclude regarding the genetic formulas of their parents?

8. Two Independent Characters.—The simultaneous use of brown eye and scarlet eye in Drosophilia illustrates not only the behavior of two pairs of genes independent of one another in their distribution to the germ cells but also a striking interaction of two genes to produce a character unlike that produced by either one alone.

8*a*. Cross a few brown-eyed flies with scarlet-eyed ones, obtaining the virgin females from either stock. Remove the adults after a week.

8*b*. When adult F_1 offspring emerge, note their eye color. Would it be possible for F_1 to have this eye color if brown and scarlet eye color were produced by contrasting genes of the same pair?

8*c*. Remove some of the F_1 flies of both sexes to a fresh food bottle and destroy them a week later. When F_2 individuals emerge, classify them with respect to eye color and count the number in each class. What new eye color is present? How do you account for it?

8*d*. Prepare a chart arranged to show appearances, genetic formulas of individuals and germ cells, expected proportions in F_2, and actual numbers of flies. Follow the plan of 6*f* in general, but note that appearances now involve two characters, genetic formulas of flies consist of four symbols, and formulas of germ cells of two symbols.

9. Sex-linked Characters.—Because a female Drosophilia has two X chromosomes which she receives one from each parent, whereas a male has only one X received from his mother, any character whose gene is in the X chromosome is unequally inherited by the two sexes.

9*a*. Mate a few yellow-bodied virgin female flies to wild-type males.[1] Remove the adults after a week.

9*b*. When F_1 adults emerge, classify them, taking note of the sex as well as of the body color, and count the individuals of each kind. How does the F_1 generation differ from the F_1 in previous crosses?

9*c*. Remove a few F_1 flies of each sex to a fresh culture bottle, and take them out after a week.

9*d*. When the F_2 adults appear, classify them as to both sex and body color, and count individuals. How does the F_2 generation differ from the F_2 from a cross involving a nonsex-linked character?

9*e*. Complete in your notes a chart like the following to explain the results just obtained, using y to symbolize yellow, Y the wild-type gray body.

[1] The cross may be made also by taking the virgin females from the wild stock and the males from the yellow, but the difference between sex-linked and other characters is most strikingly shown in the way here described.

Appearance of parents	......		......	
Genetic formulas of parents	....		....[1]	
Germ cells of parents	..		..	..[1]
Formulas of F_1 flies	....		....	
Appearance and sex of F_1 flies	......		......	
Germ cells of F_1 flies	..	..	..	..
Formulas of F_2 flies	....	....	....	....
Sex and color of F_2 flies	......	......	......	......
Expected proportions of F_2 flies	..	..	..	..
Observed numbers of F_2 flies	....	....	....	....

10. Linkage.—When two genes are in the same chromosome, they go to the same germ cell unless the chromosome breaks between them and exchanges pieces (and genes) with its homologous mate. Discoverable breakage of the chromosomes may be assumed to occur only in a minority of the cells. As a consequence, ratios of different types of individuals may be considerably modified.

For the simplest illustration of linkage, it is desirable to have a stock exhibiting the recessive character of each of the linked pairs, so that, when the cross is made, the two recessive genes come from one parent, the two dominant genes from the other.

10*a*. Mate some wild-type flies with others having both brown eyes and vestigial wings. The virgin females may be taken from either of these stocks. After a week remove the adults.

10*b*. When F_1 flies emerge, note the color of their eyes and the character of their wings. Which gene of each pair is dominant?

10*c*. Obtain virgin F_1 females, and backcross them with males of the brown-eyed vestigial-winged stock. It is important that the cross be made this way, but it may profitably be made both ways. Remove the parents after a week.

10*d*. When the adult offspring from the foregoing backcross appear, sort them into the four classes: (1) brown vestigial, (2) brown long, (3) red vestigial, and (4) red long (wild type). Count the flies in each group. Are they equally numerous? Would they be equally numerous if the two pairs of genes were not linked? A deficiency is to be expected in the classes having vestigial wings, because of the lower vigor and higher mortality of these flies.

10*e*. Complete in your notes a chart like the one shown on page 57, in which the chromosomes in the flies and in their germ cells are indicated by straight lines. The formulas are to be indicated not in the usual way

[1] The absence of a second X chromosome and of a second gene for body color in the male Drosophila may be indicated by a zero (0). There is another type of chromosome there, but it contains no discoverable gene for this body color; hence the zero refers to the absence of a homologous gene, not the absence of a chromosome.

employed in preceding charts but by placing dots on these chromosome lines to indicate the locations of the genes, and writing the symbols of the genes beside the dots. Use *b* for brown, *B* for red eye, *v* for vestigial, and *V* for long wing.

ORIGINAL CROSS

Appearance of parents	Brown Vestigial	Red Long
Chromosomes and genes of parents		
Germ cells of parents		
Chromosomes and genes of F_1 flies		
Eyes and wings of F_1 flies	......, ...	

BACKCROSS

Chromosomes and genes of F_1 females

Chromosomes and genes of brown vestigial males

Germ cells of F_1 females without breakage of chromosomes

Germ cells of F_1 females with breakage of chromosomes

Germ cells of brown vestigial males

Chromosomes and genes of the backcross flies	Appearance of the backcross flies	Numbers of backcross flies	Numbers expected without linkage
	......, ...	....	....
	......, ...	....	....
	......, ...	....	....
	......, ...	....	....

SUMMARY

Point out the features of heredity which appear to you to be of fundamental importance. Using your knowledge of gametogenesis, explain these fundamental features by showing how they follow from the behavior of chromosomes. Include the answers to such questions as these: (1)

Why do body cells of animals contain two genes for each character? (2) Why do the mature germ cells have only one gene of each pair? (3) Under what circumstances are the genes of different pairs free to go either to the same germ cell, or to different germ cells, in gametogenesis?

If your experiments included sex-linked or other linked characters, extend the chromosome explanation, started above, to include these phenomena.

EXERCISE X

HOMOLOGY

Structures or organs having a similar evolutionary origin, no matter how different they may be now, are said to be homologous with one another. In the absence of *direct* evidence of the evolutionary origin of structures, inferences are drawn from the embryonic development and from adult anatomy. Animals whose organs arise in a similar manner in the embryo are believed to have descended from a common ancestor, on the ground that only inheritance from a common source could make them alike. Striking and widespread similarity of adult structure is also believed to be an inheritance from a common ancestry.

EMBRYONIC ORIGIN OF VERTEBRATE LIMBS

The general features of the origin of fore and hind limbs of vertebrate animals are of service as evidence of homology.

1. Limb Buds of Frog or Toad Tadpole.—Examine early stages of the tadpole showing limb buds.

1*a*. In a very early stage note the rounded prominences, the hind-limb buds, at the base of the tail. *Draw* an outline of the whole animal, 2 inches long, in side view, and make the limb buds dark.

1*b*. In an older embryo observe that each limb bud is now elongated, and that the distal end is broadened and shows signs of division into several *digits*. How many? *Draw* the limb bud, considerably enlarged, without the body.

1*c*. Determine the number of digits in the hind foot of an adult frog.

2. Limb Buds of Chick Embryos.—Examine chick embryos in the early stages of development.

2*a*. In a chick embryo, after 72 to 80 hours of incubation, note that the body is in the form of an inverted letter J (see wall chart). The bent part of the inverted letter is the neck of the embryo, and the broadened tip of the bent portion the head. The longer and more slender limb of the inverted letter is the trunk. On the head will be found large rounded prominences. These are the *eyes*.

The *limb buds* are two semicircular prominences on each side of the trunk. Are they alike? *Draw* an outline of the body 2 inches long, and represent the limb buds dark. The anterior portion of the embryo should be toward the top of the page.

2*b*. Examine a chick embryo after 100 to 120 hours of incubation. The general features may be recognized from the description above. In addition, observe that the limb buds are elongated, that their distal ends are flattened, and that the division into digits has begun. (The latter feature is best observed if the light falls obliquely on the flattened surface of the limbs, so as to throw shadows in the hollows.)

Draw the limb, either wing or leg, omitting the body. Compare the origin of the wings and legs of the chick with the origin of the hind legs of the tadpole.

DIVERGENCE OF ADULT VERTEBRATE LIMBS

From their similar beginnings in the limb buds of the embryo, the limbs have diverged to their characteristic forms in the adult.

3. Hypothetical Pentadactyl Limbs.—Before comparing the adult limbs of the frog, pigeon, and man, study a chart representing the skeleton of a hypothetical pentadactyl (five-toed or five-fingered) limb.

3*a*. *Fore Limb.*—Note the *shoulder girdle*, composed of *clavicle, scapula,* and *coracoid;* the upper arm or *humerus;* the *glenoid fossa*, the partial cavity formed by the girdle bones, into which the head of the humerus fits; the fore arm, composed of *radius* and *ulna;* the wrist with its 10 *carpal bones;* the body of the hand with its five *metacarpals;* and the *digits* or fingers, composed of *phalanges.* How many phalanges in each finger?

3*b*. *Hind Limbs.*—Note the *pelvic girdle* composed of *ilium, ischium,* and *pubis;* the leg bone or *femur;* the *acetabulum,* or socket which receives the head of the femur; the lower leg with its *tibia* and *fibula;* the *tarsals,* 10 in number, in the ankle; the five *metatarsals* forming the body of the foot; the digits or toes, composed of *phalanges.* How many phalanges in each toe?

4. The Limbs of Man.—The bones of the arms and legs of man are most useful for a first comparison with the hypothetical limbs described above.

4*a*. *The Arm.*—Study the *pectoral* or shoulder girdle, composed of the *scapula* or shoulder blade, the *clavicle* extending from the shoulder to the sternum or breastbone, and the *coracoid,* a hooklike process fused to the head of the scapula, which in youth starts as a separate center of ossification; the arm bone or *humerus;* the fore arm with its *radius* (on the thumb side) and the *ulna;* the *carpals* in the wrist (number?); the *metacarpals* in the body of the hand; and the *phalanges.* How many phalanges in each digit?

4*b*. *The Leg.*—Compare the human leg bones with those of the typical pentadactyl hind limb. Note similarities and differences.

The pelvic girdle is fused into a single bone, the *innominate*, on each side. The *ilium* is the broad expanded portion of the innominate above the hip socket or *acetabulum*. The *ischium* projects downward and somewhat backward from the acetabulum. The two *pubes* of the opposite sides meet in the middle line in front, from which point two branches project, one upward and outward to the acetabulum, the other backward and downward to the lower end of the ischium.

In the leg proper observe the *femur* or thigh bone; the *tibia* (larger) and the *fibula* in the lower leg; the *tarsals* in the ankle (number?); the *metatarsals* in the body of the foot; and the *phalanges*. How many phalanges in each digit?

5. The Limbs of a Frog.—The limb bones of the frog show comparatively small deviations from the human type.

5a. The Fore Limb.—Omit the pectoral girdle. Compare the bones of the arm with those of the typical pentadactyl arm. Note the *humerus* in the upper arm, and the *radio-ulna* in the fore arm. Which of the fused bones is the radius? Study also the irregular *carpal* bones of the wrist (number?); the *metacarpals* in the body of the hand, the one of the thumb being rudimentary; the *phalanges*, present in the second, third, fourth, and fifth digits, but wanting in the first. How many phalanges in each digit?

5b. The Hind Limb.—Compare with the typical pentadactyl hind limb. The pelvic girdle consists, on each side, of a long bone, the *ilium*, extending forward from the *acetabulum;* the *ischium*, a rounded flat bone behind the acetabulum; and the *pubis*, a triangular bone below the acetabulum. The last is more or less translucent in fresh preparations. Each bone forms a part of the acetabulum. They may be readily distinguished in the skeleton of a young frog. Observe the *femur* in the thigh, and the *tibiofibula* in the lower leg. Which edge of the latter represents the tibia? There are four *tarsals*. Two of them are much elongated; beyond these are the other two, small irregular bones. Study also the *metatarsals* in the body of the foot, and the *phalanges* in the toes. How many phalanges in each toe? A rudimentary sixth toe may be present on the inner side of the foot.

6. The Limbs of a Bird.—The bones of the wings and legs of birds are more greatly modified than in most other groups of vertebrates.

6a. The Fore Limb or Wing.—Compare the bones of the pigeon or chicken wing with the typical pentadactyl fore limb as well as with the other forms already studied.

The *pectoral girdle* consists of the *scapula*, a sword-shaped bone projecting back over the ribs; a *coracoid*, sloping downward and backward and joining with the sternum or breastbone; and the two clavicles fused to form the *furcula* or wishbone. Observe the *humerus* in the upper arm and the *radius* and *ulna* (larger) in the fore arm. Only two free *carpals* are present, and they may be hidden in the ligaments of the wrist. See a thoroughly cleaned skeleton to find them. The remaining carpals are fused with three metacarpals to form a large irregular bone, the *carpometacarpus*, consisting of two rods joined at the ends. The larger of the two rods represents in the main the third metacarpal. At its base, on the anterior edge, is a tubercle which represents the second metacarpal. The fourth metacarpal is the more slender one of the two rods. The first and fifth digits, present in the embryo, are wanting in the adult.

The *phalanges* of all three fingers are reduced. The second finger has a single spinelike or triangular phalanx. The third has two phalanges, and the fourth finger one, which may be closely applied to the first phalanx of the third finger.

6*b*. *The Hind Limb.*—Compare the leg of the pigeon with the typical pentadactyl limb and with those of the other forms studied.

The pelvic girdle is fused into a single bone except in young birds. The *ilium* is the broad flat part above. The pubis is the slender curved rod at the lower margin of the girdle, behind the acetabulum. It is partially separated from the rest of the girdle by a long cleft. The *ischium* lies above this cleft and below the large opening behind the acetabulum. Observe the *femur* in the thigh, and the *tibiotarsus* in the lower leg. The *fibula* is a slender bone fused to the tibiotarsus near its upper end. The *tarsals* are not present as distinct bones, some of them being fused with the tibia and some with the metatarsals. The latter tarsals, together with the fused metatarsals, form the *tarsometatarsus* of the foot. Note that it is a triple bone, the second, third, and fourth metatarsals being fused. At the proximal end they are fused with some of the tarsal bones. The first metatarsal is a separate bone applied to the inner edge of the tarsometatarsus at its distal end. There are four digits. The distal *phalanx* of each is modified for the support of the claw. The first digit points backward, the second, third, and fourth forward. How many phalanges in each digit?

Draw two of the limb skeletons studied and label fully.

Prepare a chart similar to the one partially filled on the following page, stating in the last three columns whether the bone in question is in any striking way modified from the hypothetical ancestral condition (3*a*, 3*b*), such as fusion with another bone, reduction in number, absence, unusual size, in the three animals named. If a bone is not significantly different from the corresponding one in the hypothetical ancestor, it may be recorded as "primitive." When the chart is finished, note the number of modified bones, and the extent of the modifications, in the last three columns. What do you infer from the contrast shown?

7. Analogy.—Two structures which serve the same function in different animals but which have not arisen in the same way in evolution are said to be analogous. They are not homologous.

7a. Compare the wing of an insect, preferably a clear one showing veins as in dragonflies or May flies, with the wing of a bird or a bat. Is there any structural similarity that would suggest a common evolutionary origin?

7b. Compare one of the walking legs of a crayfish or lobster with the human leg. Where are the muscles located in relation to the skeleton? Is the general plan of structure the same in both? What do you infer regarding their origins?

HOMOLOGY CHART

	Hypothetical limb	Man	Frog	Bird
Pectoral girdle	Clavicle Scapula Coracoid			
Fore limb	Humerus Radius Ulna 10 carpals 5 metacarpals 14 phalanges 5 digits			
Pelvic girdle	Ilium Ischium Pubis			
Hind limb	Femur Tibia Fibula 10 tarsals 5 metatarsals 14 phalanges 5 digits			

SUMMARY

Present in organized form the argument in favor of common descent that is afforded by the embryonic origin and the adult skeletal anatomy of vertebrate limbs. On the basis of the structure of the limbs, does man stand high or low in the animal series? What bearing has homology on the classification of animals?

EXERCISE XI

TAXONOMY

Taxonomy (Gr. taxis = arrangement + nomos = a law) is the arrangement of known facts according to law. As applied to animals, taxonomy has for its object the discovery of the pedigree of every animal from an evolutionary standpoint, that is, its kinship or blood relationship, and, consequently, its position in the animal series or genealogical tree. Related animals are put together into a taxonomic group. The characters used in determining such relationship are the form and structure of the adults, young, and embryos, since these are believed to indicate the degree of kinship among organisms. The discovery of homologous structures in two or more animals is regarded as a sure indication of kinship.

In the work on taxonomy, numerous sketches should be made, but it is left to the student to decide what forms shall be drawn. As aids to memory, these sketches should serve two purposes. First, they should recall those features of animals which place the animals in certain groups; these features are listed in the following exercises under the heading Characteristics. Second, many animals should be simply *remembered* as belonging to certain groups, without the necessity of recalling the characteristics which put them in those groups. For this second purpose, the drawings of one phylum or of one class should be grouped together on consecutive pages.

Drawings are to be made on notepaper, not on the drawing sheets. Make frequent reference to the wall charts illustrating members of the various groups.

At the end of the exercise on Taxonomy, the student should be able to place any animal studied in its proper group and should be prepared for a test of his ability to do so.

THE PHYLA OF ANIMALS

All animals have been arranged according to their supposed relationships into phyla (Gr. phulon = tribe, race, stock). All members of a single phylum possess certain characteristics in common and differ in certain of these respects from the members of every other phylum. The principal characteristics of each phylum are listed, with illustrative examples, in the following exercises.

1. Phylum PROTOZOA (Gr. protos = first + zoon = animal).

Characteristics:

1*a*. Unicellular. Examine stained specimens of Amoeba, Euglena, or other similar forms. The single nucleus, together with the absence of cell boundaries within the animal, demonstrates that it is a protozoon.

1*b*. If cells are attached to one another, all are alike. Examine stained preparations of Epistylis, Carchesium, and Synura.

2. Phylum PORIFERA (Lat. porus = pore + ferre = to bear).

Characteristics:

2*a*. Usually radially symmetrical. Examine Grantia or other sponge. How many planes can be passed through the longitudinal axis, each dividing the body into two parts that are mirrored images of each other?

2*b*. Multicellular. Examine a cross section of Grantia. Note that many cells are present.

2*c*. Numerous pores. Examine the surface of a dried specimen of Grantia with a dissecting microscope. Also the inner surface of a specimen split open (keep in alcohol or water).

2*d*. Collared flagellate cells line certain sections of Grantia, in which these cells will probably be much shrunken. Learn from illustrations their appearance when fresh.

2*e*. Skeleton composed of spicules or spongin. For spicules examine the surface of Grantia and a slide bearing isolated spicules of Grantia. Examine also the skeleton of a siliceous sponge. For spongin, tear off a minute portion of a bath sponge, place between two slides, and examine with a compound microscope.

2*f*. Aquatic, mostly marine. Spongilla is a fresh-water form.

3. Phylum COELENTERATA (Gr. koilos = hollow + enteron = intestine).

Characteristics:

3*a*. Diploblastic. Examine a cross section of Hydra. Note the two layers of cells.

3*b*. Radially symmetrical. How many planes can be passed through the longitudinal axis of Hydra, each dividing the body into halves which are approximately mirrored images of one another? How many such planes through a jellyfish? How many through a hydranth of Obelia? A sea anemone? A coral?

3*c*. Single gastrovascular cavity with only one opening, the mouth. Note the hollow interior of Hydra as shown in cross sections. Observe also the coenosarc of Obelia.

3d. Nematocysts. Examine these in preparations of Hydra and hydranths of Obelia. If living material is available, examine nematocysts of Hydra that have been discharged.

3e. Tentacles. Observe the sinuous projections from the hydranths of a hydroid, and from the margin of a jellyfish.

3f. Aquatic, mostly marine. Hydra and at least one of the colonial hydroids are fresh-water forms.

4. Phylum PLATYHELMINTHES (Gr. platus = broad + helmins = an intestinal worm).

A. Characteristics:

4a. Bilaterally symmetrical. Examine Planaria. How many planes may be passed through the body, each dividing it into two parts that are mirrored images of one another?

4b. Single gastrovascular cavity (may be wanting in parasitic forms). Examine an entire planaria; note the gastrovascular cavity and its branching form. It has but one opening, the mouth, as in the Coelenterata.

4c. Unsegmented. In Planaria note that the body is not divided into a series of segments.

4d. Body flattened above and below.

4e. May be (1) free-living in water or soil or (2) parasitic in or on other animals.

B. Special Features:

In the tapeworm observe:

4f. That the animal is not segmented but is colonial, the members of the colony being attached in a linear series. Each individual is called a *proglottis*.

4g. The enlarged *scolex* at one end of the chain. The individuals of the colony are successively budded off from the scolex. Note the *hooks* and the *suckers*. What is the use of these structures?

4h. The absence of a gastrovascular cavity. Why is it not necessary?

4i. The reproductive organs make up the greater part of the body.

5. Phylum NEMATHELMINTHES (Gr. nema = thread + helmins = an intestinal worm). A phylum rich in species, yet seldom attracting general attention.

A. Characteristics:

5a. Cylindrical in form. See Ascaris or any other species available.

5b. Bilaterally symmetrical. Meaning of this expression? Verify in Ascaris or any other species.

5c. Unsegmented. See any nematode, for example, Ascaris.

5d. Alimentary canal with both mouth and anus. See demonstrations.

5e. Body cavity, probably not a true coelom, surrounding the alimentary canal. Examine a dissection of Ascaris, and observe that the body wall may be cut through without opening the digestive tract. Was such a cavity present in any of the preceding phyla?

B. Economic Representatives:

Some of the most dangerous parasites of man and other animals as well as pests of plants are found among the Nemathelminthes. Among the demonstrations are:

5f. Ascaris, parasitic in the intestine of pigs, horses, and man.

5g. Trichinella, which causes trichinosis in pigs, rats, and man.

5h. Necator, the hookworm, the cause of "hookworm disease" in human beings.

6. Phylum ECHINODERMATA (Gr. echinos = a sea hedgehog + derma = skin).

Characteristics:

6a. Radially symmetrical. Meaning of this expression? Verify in a starfish; in a sea urchin. (There are exceptions to radial symmetry, especially in minor features.)

6b. Parts usually arranged in fives, but larger numbers are not uncommon. Verify in starfish; in sea urchin; in brittle star.

6c. Generally covered with spiny exoskeleton of calcareous matter. Observe in starfish; in sea urchin; in brittle star. Compare, however, with sea cucumber.

6d. Possess tube feet for locomotion. These are connected with a water-vascular system in the body and operate by means of suction.

6e. All marine.

7. Phylum ANNELIDA (Lat. annellus = a little ring).

A. Characteristics:

7a. Segmented. Examine the rings in the body of an earthworm. Note also the sandworm Nereis. Compare in this respect with the Platyhelminthes and Nemathelminthes, which are also called "worms."

7b. Setae. Examine an earthworm. Each segment except a few at the anterior end is provided with several pairs of stiff bristles or setae which aid the worm in locomotion. How are the setae arranged? Observe the sandworm, with the flattened projections at the sides of the body, upon which are bunches of setae.

7c. Body cylindrical, or only slightly flattened above and below.

7d. Paired, but unjointed, fleshy appendages frequently present. Examine Nereis.

7e. Mode of life is terrestrial (earthworm), fresh-water (many species), marine (the sandworm Nereis), or parasitic (some leeches).

B. Special Feature:

7f. Suckers in leeches. These attach the animals to the body of the host whose blood they suck. Examine specimens of leeches.

8. Phylum MOLLUSCA (Lat. mollis = soft).

Characteristics:

8*a.* Body soft and unsegmented. Observe a clam in which the shell is either open or partly removed.

8*b.* Body bilaterally symmetrical (see Chiton, any clam, and a squid), or in part asymmetrical (any snail).

8*c.* Locomotion usually by a fleshy, muscular foot. In the clams the foot is wedge-shaped. In snails it is flat and used for creeping along surfaces. In cephalopods the foot is composed in part of a series of armlike projections.

8*d.* Body usually protected by a calcareous shell which may consist of two valves (clams), of a spirally wound tube (snails), or be concealed by the fleshy parts (squid), or wanting (nudibranchs).

8*e.* Possess a mantle, a thin membranous sheet that secretes the shell. This may be a single piece (snail, squid), or in two flaps lining the two valves of the shell (clam).

9. Phylum ARTHROPODA (Gr. arthron = joint + pous = foot). The Arthropoda include hundreds of thousands of species, probably a greater number than any other phylum.

A. Characteristics:

9*a.* Segmented. Examine any insect; a crayfish; a centipede; a spider.

9*b.* Paired jointed appendages (legs, antennae, mouth parts, etc.). How many pairs of legs in an insect? In a crayfish? In a centipede? In a spider? In a millipede?

9*c.* An exoskeleton of chitin covering the body. Observe in all the forms mentioned in the preceding paragraphs.

B. Special Considerations:

9*d*. Although each arthropod has a definite number of segments in its body, these segments are often fused in characteristic ways so that the number is not easy to determine. The number of appendages, or the embryonic development, is relied on in such cases to establish the correct number.

In insects, a number of segments are fused to form a *head*, others are fused to form a *thorax*, while the segments of the *abdomen* remain more or less movable upon one another. Make out these regions in a wasp, a grasshopper, or other insect.

In the crayfish, lobster, and allied forms, the segments of both head and thorax are fused into one immovable group called the *cephalothorax*, while those of the abdomen are movable. Make out these regions in specimens.

In spiders, the cephalothorax is one group of fused segments and the abdomen is also a group of fused segments. Make out these regions in specimens.

In the centipedes and millipedes, on the contrary, all the segments are movable except a small number in the head. Examine a specimen and note that the region behind the head is flexible.

10. Phylum CHORDATA (Lat. chordatus = having a chord or cord). The most commonly known animals, because they are large and conspicuous and some of them are domesticated.

Characteristics:

10*a*. Skeleton internal, composed of cartilage (shark) or bone (fish, man, etc.).

10*b*. Dorsal skeletal axis of vertebrae (backbone) or a notochord. Examine cross sections and entire specimens of Amphioxus and Balanoglossus. Note the vertebral column in skeletons of bird, cat, frog, turtle, fish, snake, lizard.

10*c*. Typically two pairs of jointed appendages. Recall the modifications of these appendages found in the skeletons studied in Homology. Observe also the modifications of the limbs in the skeleton of a mole; of a seal; of a porpoise; of a snake.

10*d*. Central nervous system hollow, and dorsal in position. In these respects the Chordata differ from all other phyla. Examine a section of a salamander. Observe that its brain and spinal cord are hollow and dorsal to the alimentary canal.

10*e*. Gills or gill bars in adult, young, or embryo. See a fish, Amphioxus, and embryos of a mammal and a bird.

SUBDIVISION OF THE PHYLA

Phyla are divided into subgroups called *classes*. Classes are distinguished from one another in the same way as are phyla, but by means of characters less fundamental and less primitive than those used in separating phyla. Note that this is true in the analysis of one subphylum, the Vertebrata, in the following exercise.

The Classes of Vertebrates

1. Class PISCES (Lat. piscis = fish).

Characteristics:

1*a*. Body long and pointed, and provided with *dorsal fins*, *tail fin*, *ventral fin*, and two pairs of *lateral fins*. Verify in a specimen.

1*b*. Cold-blooded, aquatic, respiring by *gills*. Observe the gills in a fish.

1*c*. *Scales* cover the body; and a flap, the *operculum*, covers the gills. Verify.

2. Class AMPHIBIA (Gr. amphi = both + bios = life). Frogs, toads, salamanders, newts, etc.

Characteristics:

2*a*. Skin is without scales or other hard parts and is slimy, owing to a mucous secretion. Handle a living frog to observe these features.

2*b*. Usually possess two pairs of limbs with five digits each. Examine a toad, a frog, and a salamander for verification of, or exception to, this rule.

2*c*. A larval stage typically present. Observe tadpoles of several kinds.

2*d*. Young possess gills (observe a tadpole); adults usually breathe by lungs (see dissection of a frog, also the respiratory movements of a living frog).

2*e*. Cold-blooded animals usually spending part of their existence in water, part on land, capable of living either in water or on land.

3. Class REPTILIA (Lat. repere = to crawl). Lizards, snakes, turtles, alligators, etc.

Characteristics:

3*a*. Skin possesses scales or hard plates. Observe in a snake; in a turtle; in a lizard.

3*b*. Body not slimy.

3*c*. Breathe by means of lungs throughout life. Note the lungs in a dissection of a turtle or other reptile.

3*d*. Cold-blooded.

4. Class AVES (Lat. avis = bird).

Characteristics:

4*a*. Body covered with feathers. Examine one or more feathers under a lens. Compare with figures.

4*b*. Fore limbs modified as wings. Examine the skeleton of a wing and note its deviations from the typical vertebrate limb. Examine also the character of the feathers which add to the wing expanse.

4*c*. Absence of teeth in modern birds. Examine a bird skull.

4*d*. Warm-blooded. How does a fowl incubate her eggs?

4*e*. Terrestrial. Even wading and swimming birds spend the major portion of their time on land.

5. Class MAMMALIA (Lat. mamma = a breast). Man, monkeys, whales, bats, seals, and many common wild and domestic animals.

Characteristics:

5*a*. Skin covered with hair. Observe hair in squirrels, bats, or other quadrupeds; also spines in hedgehog or porcupine.

5*b*. Mostly quadrupeds. Some, however, progress on two feet (man), some by wings (bats).

5*c*. Young nourished after birth by secretion from mammary glands of mother.

5*d*. Warm-blooded. What is your own temperature?

SUBDIVISION OF THE CLASSES

To illustrate the subdivision of the *classes* of animals into smaller groups called *orders*, the Amphibia and Reptilia may be selected. There are but three orders of living Amphibia, and four orders of Reptilia. Note, in the following exercises, that the characters used to separate orders are less fundamental than those used to separate classes.

The Orders of Amphibia

1. Order CAUDATA. Salamanders, newts, etc.

Characteristics:

1*a*. Tailed. See specimens.

1*b*. External gills sometimes present throughout life (Siren, Necturus, Proteus), sometimes absent in the adult stage (Ambystoma, Triturus, Plethodon, Amphiuma, and others).

2. Order SALIENTIA. Frogs, toads.

Characteristics:

2*a*. Tailless. See specimens of frogs and toads.
2*b*. External gills absent in adult. See specimens of larvae and adults.
2*c*. Hind legs much longer than fore legs.

3. Order APODA. Caecilians.

Characteristics:

3*a*. Without limbs. See Siphonops.
3*b*. Eyes concealed. See Siphonops, and compare with any of the Salientia.

The Orders of Reptilia

1. Order TESTUDINATA. Turtles.

Characteristics:

1*a*. Body encased in a bony capsule composed of dermal plates. Observe any turtle. In a cleaned skeleton note how the shell is attached to the skeleton.
1*b*. Jaws without teeth. Examine a cleaned turtle skull.
1*c*. Quadrate bone immovable. Examine skull. The quadrate is at the angle of the upper jaw, and forms the articular surface for the attachment of the lower jaw.
1*d*. Vertebrae procoelous or opisthocoelous (concave in front or behind, respectively).
1*e*. Usually five digits in each fore foot, and four or five in each hind foot. Verify in as many specimens as possible.
1*f*. Only one nasal aperture in skull. Observe in any cleaned skull.

2. Order RHYNCHOCEPHALIA. This order is represented by only one species, which is found in the New Zealand region. Owing to the rarity of the material, the internal features listed below cannot be demonstrated.

Characteristics:

2*a*. Vertebrae biconcave (amphicoelous).
2*b*. Quadrate bone immovable. See figure of skull on chart.
2*c*. Pineal eye fairly well developed. Its position is marked externally by a rosette of small scales surrounding a small central scale on the top of the head behind the level of the eyes. Examine the dorsal side of a cleaned skull of Sphenodon, or a figure of one, and note the perforation of the bone in the region of the pineal body.
2*d*. Anus a transverse slit.

3. Order CROCODILINI. Crocodiles and alligators.

Characteristics:

3*a*. Vertebrae usually concave in front (procoelous).

3*b*. Fore limbs bear five digits, hind limbs four. Verify in specimens.

3*c*. Anal opening a longitudinal slit. Compare with a Rhynchocephalian and with a snake and a lizard in this respect.

3*d*. Quadrate immovable. See alligator skull. Note that the quadrate is fixed firmly in position by the surrounding skull bones.

4. Order SQUAMATA. Snakes, lizards, and chameleons.

Characteristics:

4*a*. Vertebrae usually concave in front. Verify on specimens.

4*b*. Quadrate freely movable. See skull of snake; also of a lizard. Advantage of this feature? What is the food of snakes?

4*c*. Anus a transverse slit. Examine several snakes and lizards.

SUBDIVISION OF THE ORDERS

Orders are divided into *families* on the basis of characters less fundamental than those which furnish the basis for the division of classes into orders. To illustrate the features that distinguish families, a few families of turtles[1] may be used. All the families listed below belong to one order, the Testudinata, and there are several families of this order that are not mentioned.

Family Differences among Testudinata.—Eight of the characters used by taxonomists to distinguish families of turtles from one another are listed below. Examine skeletons of turtles of the families Testudinidae, Chelydridae, Chelydidae, Trionychidae, Kinosternidae, and Cheloniidae. Prepare a chart of nine columns, and as many horizontal lines as families studied, putting the names of the families in the left-hand column, the characters studied at the heads of the other eight columns, and indicating in the appropriate spaces the characteristics of the several families.

1. Around the edge of the carapace (the convex dorsal shell) there is in some families of turtles a complete ring of marginal bones; in other families this ring is incomplete or wanting. Examine one specimen of each family, and state, in the first column of the table, after the respective

[1] Other orders may be equally good for division into families, but in any group substituted it is important that the characters studied be the true *family characters*, not those which merely happen to distinguish the families present in a given limited region.

family names, whether this ring of bones is complete, incomplete, or wanting.

2. Note the parietal bone, which adjoins the middle line of the top of the skull, and the squamosal bone, which overhangs the region of the ear. In some families of turtles these bones do not touch one another, and there is a depression between them; in other families these bones join one another by a suture and there is no depression. In the second column after each family name state whether these bones are separated or joined.

3. In some turtles the digits are short and somewhat separate from one another; in others the digits are long and are all joined in the flesh to form a paddle-shaped limb. Examine specimens of all available families and record your observations in the third column after the family names.

4. Study the plastron, the flat ventral plate of bones, from the inside. Count the bones and record the number in each family in your list.

5. In retracting the head, some families of turtles bend the neck sidewise, other families vertically. The vertebrae determine by their structure which direction the neck must bend. Make a record of the plane of bending in each family.

6. In some families the last cervical vertebra joins the first body vertebra by means of both the centra and the zygapophyses; in other families only the zygapophyses join. See "Principles of Animal Biology" for the meaning of these terms. Examine each family and record the method of articulation of these vertebrae.

7. The caudal (tail) vertebrae of some families of turtles are mostly opisthocoelous (centra concave behind); in other families they are mostly procoelous (centra concave in front). What is the nature of the caudal vertebrae in each family studied?

8. The nuchal plate is the middle bone of the carapace at the front margin. In some families it bears a long tapering projection to right and left; in others such a projection is wanting. Describe each family in your list in this respect.

When the foregoing study is complete and the observations are recorded, each family is defined by the characters listed after its name. These characters are the true family distinctions; that is, they mark off from one another not merely the families of turtles in a given limited region but the families of turtles anywhere in the world.

SUBDIVISION OF FAMILIES

Families are divided into genera, based on differences which are in general less fundamental than those distinguishing families from one another. This fact is illustrated by the genera of turtles in the following comparisons.

Generic Characters of Turtles.—There are many genera of turtles comprised in the family Testudinidae. To illustrate generic characters five or six genera of turtles of this family will be supplied for examination (for example, Emys, Chrysemys, Pseudemys, Terrapene, Graptemys).

Prepare a chart like the accompanying one, with the names of the genera in the left column, and the generic characteristics at the heads of the remaining columns. Select one of the turtles supplied, examine it for all the structural characters enumerated below, and tabulate in the spaces at the right of its name the characters you find it to possess. When you have completed this examination you will have a list of the external characters that distinguish this genus of turtles.

Examine other specimens of the same genus to see whether the characters you have listed are constant or variable, and record the situation as you find it.

Repeat this study for each genus supplied. At the close of the exercise your knowledge of these generic characters will be tested.

1. Are the feet, particularly the hind feet, (*a*) broad with webs between the toes and slender sharp nails on the toes or (*b*) stumplike without noticeable webs and with generally stronger and blunter nails?

2. Is the carapace (*a*) conspicuously high, narrow, and globular or (*b*) relatively low and broad?

3. Is a median ridge or keel on the carapace (*a*) present or (*b*) absent?

4. Does the nuchal plate have (*a*) a denticulate or (*b*) a smooth anterior margin?

5. Does the posterior lateral margin of the carapace (*a*) flare outward and upward or (*b*) only outward or (*c*) does it slope evenly downward? Is its margin here (*d*) smooth, (*e*) slightly irregular, or (*f*) denticulate?

6. Is the tail (*a*) of moderate size or (*b*) conspicuously small and short?

7. (*a*) Is the plastron firmly united to the carapace by a broad, bony ridge or (*b*) is there only a ligamentous connection between carapace and plastron?

8. (*a*) Is the plastron divided transversely a little posterior to the insertion of the fore legs by a ligamentous connection (hinge) or (*b*) is the plastron composed of one solid, unbending piece?

9. (*a*) Is there a sharp median notch in the anterior end of the upper jaw or (*b*) is there only a slight notch here or (*c*) is there none?

10. (*a*) Is there a prominent toothlike projection on each side of the notch in the tip of the upper jaw or (*b*) is there no such projection?

11. Is the lower jaw (*a*) sharp-pointed at its tip or (*b*) blunt-pointed or (*c*) is it smooth and spoonlike?

Place asterisks in the table beside those characters that seem to be constant and distinctive for each genus.

GENERA OF TESTUDINIDAE

Name of genus	Feet	Arch of carapace	Keel of carapace	Nuchal plate	Posterolateral margin of carapace		Tail	Union of plastron with carapace	Division of plastron	Median notch of upper jaw	Tooth-like projection of upper jaw	Tip of lower jaw
					Slope	Shape						
Terrapene												
Pseudemys												
Graptemys												
Emys												
Chrysemys												

SUBDIVISION OF GENERA

The division of genera into species rests usually upon characters that are quite superficial. Observe that this is true in frogs in the following examples.

Species Characters of Frogs of the Genus Rana.—Five species of the genus Rana which are common in southern Michigan and in other regions are selected for this comparison.

SPECIES OF RANA

Species		Lateral folds	Cheek patch	Free phalanges	Upper body markings	Under body markings	Under thigh markings	Size
Scientific name	Common name							
Rana catesbeiana	Bullfrog							
Rana clamitans	Green frog							
Rana pipiens	Leopard frog							
Rana cantabrigensis	Wood frog							
Rana palustris	Pickerel frog							

Prepare a chart like the accompanying one, in which each frog is given its species name and common name. In the remaining columns its characteristics are to be indicated as follows: whether lateral folds (ridges of skin along the back on either side) are present or not; whether there is a dark cheek patch or not; how many of the phalanges of the longest toe of the hind foot are free of (project beyond) the web; the nature of the markings on the upper surface of the body; the markings on the under surface of the body; the markings on the under surface of the thighs; and the general size, whether large, small, or medium.

The colors are altered somewhat in preserved specimens, but the markings are the same as in living frogs.

After the table has been completed, place asterisks beside those characters that will distinguish each species from all other species.

EXTENSION OF WORK IN TAXONOMY

If a longer time is to be used for taxonomy, keys may be introduced and students should learn the method of using them. As a rule, keys for such elementary work must be devised by the teacher, not taken directly from systematic works. Simplified keys may readily be made for a number of groups of animals. Material for keys may be found in the following works on large groups having general distribution: (1) Chapman, "Handbook of Birds of Eastern North America"; (2) Bailey, "Handbook of Birds of Western United States"; (3) Dickerson, "The Frog Book"; (4) Jordan, "A Manual of the Vertebrate Animals of the United States" (13th Ed.); (5) Walker, "A Synopsis of the Classification of the Fresh-water Mollusca of North America, North of Mexico"; and (6) Blanchard, "A Key to the Snakes of the United States, Canada and Lower California" (*Papers* of Michigan Academy of Science, Arts, and Letters, vol. 4).

Systematic works on smaller groups or with special reference to limited regions are the following: (1) Barrows, "Michigan Bird Life"; (2) Forbes and Richardson, "The Fishes of Illinois"; (3) Ortmann, "The Crawfishes of Pennsylvania"; (4) Ruthven, Thompson, and Thompson, "The Herpetology of Michigan"; and (5) Walker, "An Illustrated Catalogue of the Mollusca of Michigan": Part 1, Terrestrial Pulmonata.

SUMMARY

State the principles at the basis of classification. Discuss the nature of the characters that distinguish groups of different ranks, showing whether family distinctions are more fundamental or more superficial than the distinctions between genera, or between species, etc. That is, in general, are the differences between species less or greater than the differences between genera, and the differences between orders less or greater than the differences between families? Did small differences require less time, or more time, than large differences to arise by evolution? Which, therefore, is the older (in general), an order or a family? Which is older, a family or a genus? What are the relative ages of groups of the other ranks?

EXERCISE XII

ECOLOGY AND ADAPTATION

In this exercise will be studied a species of animal found in terrigenous bottoms of lakes, with special reference to the structures and habits which fit it for such habitat. Animal reactions will be studied in forms from other habitats. Drawings, answers to questions, and a summary should be handed in.

TERRIGENOUS BOTTOMS

Terrigenous bottoms are those formed by the washing in of material from the land. Such bottoms have certain well-defined characteristics.

Characters of Terrigenous Bottoms.—Examine photographs of portions of some lake showing (1) a considerable area of barren, sandy shoal and (2) a photograph of a limited portion of the bottom of such a shoal. If such habitat is easily available for actual observations, this part of the work could be done in the field with considerable profit. Observe the following features:

1. The almost complete absence of vegetation. How is this feature accounted for?

2. The waves, showing the beach to be wind-swept. What relation does this fact bear to (1) above? On which shore of a lake might such a beach be located?

3. The sand ripples. What causes them? Relation to (1) and (2) above?

4. Flecks of foam on the surface of the water. Cause?

5. In such an environment, what are the conditions with respect to (*a*) dissolved oxygen content of the water, (*b*) carbon dioxide, (*c*) decaying organic matter, (*d*) extremes of temperature as compared with the deeper water, (*e*) light, (*f*) molar agents, (*g*) materials for holdfasts, shelter, or abode?

6. By what methods can animals normally inhabiting such a situation maintain their positions there?

Mussels of Terrigenous Bottoms.—The relation of animals to the terrigenous bottom habitat may be represented as satisfactorily by freshwater mussels as by any other form. Use either Lampsilis or Anodonta.

1. Living Mussel.—Study living specimens in shallow dishes or in small aquaria containing water and provided with sand bottoms. Be careful *not* to *jar* them.

1*a*. Note the two *siphonal openings* with fringed borders at one end of the shell. With a pipette carefully and without touching the animal put some eosin or powdered carmine mixed with water just opposite the openings and demonstrate that water enters one (inhalent) and leaves the other (exhalent).

1*b*. Observe the large, fleshy, plowlike *foot* buried in the sand. It may be seen if the animal is near the sides of the glass dish or demonstrated by lifting the animal quickly before it contracts.

1*c*. Make a *sketch* or diagram of a mussel from the side, showing the position of the long axis of the shell, that of the surface of the sand, the siphonal openings (with direction of the current for each shown by an arrow), and the outline of the extended foot. The lower end is the anterior end, the upper is the posterior end. The dorsal surface is that bearing the *hinge* with its dark brown ligament. Represent the relative positions with care.

2. Anatomy of Mussel.—Study fresh or preserved material, including some females with young (glochidia) in the gills.

2*a*. Remove the right valve of the shell by cutting along its inner surface, with a stout knife, the strong *adductor muscles*, one near each end, and pushing the mantle from the valve to be removed. Place the half of the shell containing the animal in a dissecting dish and cover with water.

2*b*. Note the *mantle* lining the left shell valve, and the mantle cavity between the two lobes of the mantle. In the mantle cavity find:

2*c*. The *gills*, two leaflike structures on each side. Turn back the upper pair and find:

2*d*. The hard contracted *foot* near the anterior end on the ventral side. It continues backward into the *visceral mass* which contains alimentary canal, reproductive, circulatory, and excretory organs. These will not be dissected but may be seen in charts of typical mussels.

2*e*. The *labial palps*, triangular ridged flaps, two on each side just anterior to the gills.

2*f*. The *mouth* opening between the labial palps of the two sides. Probe it with the blunt end of a needle or other instrument. *Sketch* in natural position the foregoing parts in a second outline of the shell, making the drawing large.

2*g*. Tear apart the two lamellae of which each gill is composed, and note that these enclose vertical tubes which extend from the free edge of the gill to its attached dorsal edge. With scissors cut thick cross sections of the gill to show the lamellae and tubes. For finer details examine a stained cross section under the microscope. *Draw* a small portion of this section at a place showing some of the openings through which water enters the gills.

2*h*. Put a probe into the exhalent siphonal opening. It enters a channel above the attached edge of the gills. Cut through the gills by drawing a knife along the probe, and explore the gill chamber into which the vertical tubes from the gills open.

2*i*. Trace the course of the water from the inhalent siphon into the mantle cavity, thence through the gill tubes, and out at the exhalent opening.

2*j*. With dissecting microscope examine the surface of a living gill and note the numerous small openings leading into its interior. With compound microscope observe the cilia which cause water currents to pass in through the openings. These can best be seen in a portion of a single *lamella* (lateral half of one gill) mounted in common tap water between slide and cover glass and studied with high magnification. *Sketch* a little of the gill surface showing the cilia and their relation to the openings.

2*k*. How do you suppose the animal gets its food? Removes the waste products of respiration, digestion, and excretion? Breathes?

2*l*. Examine a specimen containing glochidia and note how the expanded spaces between the gill lamellae serve as brood pouches.

2*m*. Examine some of the glochidia of Anodonta in water under a low magnification. *Sketch* to show (1) the triangular valves of the shell, (2) the large tooth at the apex of each valve, (3) the strong *adductor muscle*, and (4) the threadlike *byssus* (of uncertain function).

2*n*. By reading one of the following references, or by consulting the instructor, learn how the glochidia attach themselves to fish and are distributed by them.

LEFEVRE, G., and W. C. CURTIS, Studies on the Reproduction and Artificial Propagation of Fresh-water Mussels, *Bulletin* 30, Bureau of Fisheries, pp. 107–201, 1912.

NEEDHAM, J. G., and J. T. LLOYD, "The Life of Inland Waters." Comstock Publishing Co., 1916. (See pp. 287–292.)

BAKER, F. C., The Relation of Mollusks to Fish in Oneida Lake, *Technical Publication* 4, N. Y. State College of Forestry at Syracuse University, 1916. (See pp. 219–223.)

2*o*. Examine the demonstration of towings made with a fine-mesh Birge cone net from the sandy shoal habitat where the mussels were collected. In general, what kinds of minute organisms occur there?

2*p*. Examine (demonstration) contents of the anterior part of the digestive tract. Can you recognize any of the organisms observed in 2*o*? Approximately what proportion of the contents is composed of organisms? If other materials are present, what are they and what is their source? By what means are the food particles brought to the mouth?

3. Relation to Environment.—The mussel must maintain an upright position by means of the foot so as to have its siphonal openings uncovered in order to feed and breathe. On what kind of a bottom would it thrive best? Why does it not occur on a solid, clean-swept rock bottom? What other organisms must live in the same body of water with it? In short, in what situations would you be most likely to find fresh-water mussels and why?

ANIMAL REACTIONS

Of importance in determining the habitat of animals is the manner in which they react to different factors in their environment. A few reactions will be observed here.

1. Place a number of living planarians (Planaria sp.) in each of several finger bowls containing water and two or three pebbles about three-fourths of an inch in diameter. Allow these dishes to stand for some time absolutely undisturbed and add no food. Where are the animals? Why? What is the stimulus involved? As gently as possible, place a small, recently excised portion of the body of an earthworm or pieces of tubificid worms just below the surface of the water. Watch the dish intently for signs of activity on the part of the planarians. What kind of activity is manifested? What is the end result of this activity? To what kind of stimulus is it a response? Is it positive (going toward the source of the stimulus) or negative (going in the reverse direction)? Of the stimuli referred to above, which is the stronger? Evidences?

2. Observe land sowbugs (Porcellio sp.) in a petri dish half covered with a black paper envelope to exclude the light, but with the other half well lighted. Ten sowbugs have been placed in this dish and left undisturbed so that they might come to rest. What is the distribution of the animals and how do you account for it? What is the stimulus involved and how do they react to it?

3. Observe 10 land sowbugs in another petri dish half of which contains loosely laid thin sheets of mica, the other half being clear. In which half are most of the sowbugs? Which of the following factors, if any, are they reacting to: light, gravity, contact, moisture, temperature? Reactions to these are called respectively phototaxis, geotaxis, thigmotaxis, hydrotaxis, and thermotaxis.

4. In a pan, half of the bottom of which is covered with rather moist soil and half with dry soil, note the reactions to moisture in this species of land sowbug.

Make records of observations. In what sort of environment would you expect to find land sowbugs? Do you conceive the reactions of these animals to be advantageous to them?

5. Reactions with doubtful ecological significance may be observed in the vinegar fly Drosophila. Place some of these flies in a clean bottle, close with a stopper, and turn the top end of the bottle toward the window. Note the movement of the flies. Now turn the bottom of the bottle toward the window. How do you explain the result?

Place some of the flies in a glass tube closed at both ends. Hold the tube vertically, shading it so that no more light falls on it from above than from below. Note the distribution of the flies. Then turn the tube with lower end up. Is there any reaction to gravity?

SUMMARY

Point out for the mussel an interlocking of environmental factors, anatomical structures, physiological requirements, and life history.

From your study of animal reactions, what may you assume to be the probable relationship between these reactions and the particular habitat in which the species is found? Use a concrete example.

EXERCISE XIII

ZOOGEOGRAPHY

Zoogeography deals with the distribution of animals over the earth, and with the causes of their distribution. The object of this exercise is to show the correspondence between range, or distribution, and such environmental features as plant life, topography, latitude, and rainfall, and to show some of the reasons for changes in distribution through migration and extinction. The maps described below must be carefully prepared if they are to reveal the true relationship between distribution and environment.

GENERAL ENVIRONMENTAL CONDITIONS IN NORTH AMERICA

North America may be divided into several regions which have characteristic physical conditions. The flora of these areas is characteristic and thus provides an easy means of establishing their boundaries. It should be kept in mind that the boundaries of the different regions are sharply drawn only at the seashore; where the regions come together inland there is, in nearly every case, a zone of transition or interdigitation, owing to the fact that the environmental conditions change gradually and not suddenly.

1. Study carefully the vegetation map[1] of North America in "Principles of Animal Biology" (Shull, LaRue, and Ruthven) to ascertain the location of the following types of vegetation: coniferous forests, deciduous forests, tundras, prairies and plains, deserts, and tropical floras. Memorize the principal areas of each type.

2. Compare this vegetation map with a rainfall map of North America in the summer season, and with a relief map of North America or of the United States. What is the explanation of the treelessness of the tundras in northern North America? What sort of ocean current would explain the southeastern extension of the tundras to Newfoundland? Recalling that the prevailing winds in the northern United States are westerly, is there any relation between the desert areas of this country and the mountains along the Pacific Ocean? Other relations between vegetation areas and topographic or climatic features may occur to the student.

[1] A large-scale map of North America showing, in a general way, the distribution of the major types of vegetation, a summer rainfall map of North America, a relief map of North America or the United States, and a good atlas should be available for reference in the laboratory.

3. Compare this vegetation map with the map of the Natural Vegetation of the United States, prepared by the Department of Agriculture, and note the subregions into which the major regions may be divided. Note the transition areas. Read the pertinent sections in "Principles of Animal Biology."

DISTRIBUTION OF SOME TYPICAL NORTH AMERICAN ANIMALS

The following vertebrate animals of North America represent somewhat distinct types with respect to the factors determining their ranges. It should be understood that the range or distribution of a species of animal is that geographic area throughout which the species is to be found in most of the suitable habitats.

4. Terrestrial Animals.—Plot upon outline maps of North America the ranges of several exclusively terrestrial animals. Any of the following forms are suitable.

4*a*. The American bison and the moose upon one map (from Seton, "Life Histories of Northern Animals"). Compare their ranges with the vegetation areas. Examine a molar tooth of a bison and of a moose. What kind of food is each fitted for? Do you observe any relation between the teeth and the ranges of the animals?

4*b*. The willow ptarmigan. The summer or breeding range of the willow ptarmigan is enclosed roughly by a line extending from western and northern Alaska through northern Banks Island to the west coast of central Greenland, thence south to north central Quebec, west to James Bay, central Keewatin, central Mackenzie, south in the mountains as far as west central Alberta, and west to the eastern Aleutian Islands. After plotting this range, compare it with the vegetation areas. The willow ptarmigan feeds upon seeds, berries, insects, and buds and leaves of birch and willow. What factors of food supply or climate may determine its range?

4*c*. The prairie dog. The range of the black-tailed prairie dog, which may be plotted on the same map as the willow ptarmigan, is roughly enclosed by a line extending from southwestern North Dakota to east central South Dakota, thence nearly due south to central Texas (just west of Austin), west to southeastern Arizona, northeast to northeastern New Mexico, thence along the eastern border of the Rocky Mountains to north central Montana, and east to North Dakota. Compare this range with the map of the vegetation areas.

4*d*. The ground squirrel. The ground squirrel (*Citellus tridecimlineatus*) is found in a range bounded roughly by a line from the Strait of Mackinac southward along the eastern border of Michigan, thence through south central Illinois to eastern Kansas, south to southeastern Texas, thence to eastern New Mexico, north to central Montana, north-

west to central Alberta, thence to central Manitoba, through central Wisconsin to the Strait of Mackinac. It lives in the ground in open dry country, feeds on insects, seeds, and grains, and almost never climbs trees. Does its range bear any relation to topographic or vegetation features of the country?

4*e*. The porcupine. The range of this animal is given in Seton's "Life Histories of Northern Animals," vol. I, page 607, and may be copied on the same map with the ground squirrel. The porcupine leads an essentially arboreal life. Compare its range with the map of vegetation areas.

4*f*. The red-bellied snake (*Storeria occipitomacultata*) has been taken at the following localities: near the southern end of Lake Winnipegosis in Manitoba; northwestern Iowa; northeast central Kansas; St. Louis, Missouri; north central Florida; Washington D.C.; Mobile, Alabama; Ann Arbor, Michigan; Nova Scotia; Toronto, Ontario; Long Island; southwestern Missouri; Columbus, Ohio; Augusta, Georgia; Portland, Maine; Keewenaw Point on the south shore of Lake Superior, in Michigan. Plot these localities on a map.

This snake is partial to dry wooded places. Remembering this fact and knowing that the lower Mississippi Valley, including much of Mississippi and Louisiana and parts of Arkansas, Tennessee, Kentucky, and southeastern Missouri lying near the Mississippi River, is swampy, show on your map the probable range of this species.

5. Semiaquatic Animals.—Plot on separate maps the distributions of the following semiaquatic species.

5*a*. The cottonmouth moccasin (*Agkistrodon piscivorus*) has been found in the following localities: the Dismal Swamp in extreme southeastern Virginia; on the Wabash River in southeastern Illinois; Tuscaloosa, Alabama; extreme southern Florida; Fort Smith, Arkansas; San Antonio and Dallas, Texas; southeastern Missouri; Jacksonville, Florida; Charleston, South Carolina; Mobile, Alabama; New Orleans, Louisiana; and along the Rio Grande from its mouth to the mouth of the Pecos River. Plot these localities upon a map, and outline the probable extent of the range of this snake, remembering that it is an inhabitant of swamps and slow-flowing streams where it feeds largely on fish, and therefore is not to be expected in mountainous regions. Consult the topographic map of the United States in plotting this range.

5*b*. DeKay's snake (*Storeria dekayi*) has been found, among other places, near Portland, Maine; Boston, Massachusetts; Toronto, Ontario; Pensacola, Florida; Wilmington, North Carolina; south central Minnesota; throughout the southern peninsula of Michigan; St Louis, Missouri; Fort Smith, Arkansas; New Orleans, Louisiana; Guatemala; Brownsville, Texas; Mobile, Alabama; Little Rock, Arkansas; southwest central Oklahoma; Vera Cruz, Mexico; northeast central Kansas; Knoxville, Tennessee; Racine, Wisconsin; Pittsburgh, Pennsylvania; Washington, D. C.; and various localities in New York, Pennsylvania, Virginia, Ohio, and Indiana. Plot these localities on a map.

This snake is generally found near water of streams or lakes. It feeds upon earthworms, slugs, and other small animals. In the Florida peninsula it is replaced by a closely allied species. From these facts show on your map the probable range of this species. Does the range correspond to any vegetation area?

In none of the ranges plotted above under 4 and 5 do the limits coincide *exactly* with those of the vegetation areas. There are several reasons for the lack of precise correspondence, such as incomplete knowledge of the ranges, the gradual transition of one type of region into another or their dovetailing with one another, or different responses of different animals to the environment.

FACTORS OF DISTRIBUTION

The preceding work has shown that there is a more or less close correspondence between the ranges of animals and the environmental conditions. The following study deals with several well-known changes in distribution and the causes of these changes.

6. Factors of Distribution of the Potato Beetle.—The Colorado potato beetle has greatly altered its range almost within the memory of the present generation of men.

6*a*. On a map of North America plot the distribution of this beetle as it existed before 1850. Consult "Principles of Animal Biology." From the description of its feeding habits, what plant do you infer occupied the same range?

6*b*. On the same map plot the extensions of the range of the potato beetle since 1850. It occupies North America east of the Rocky Mountains, from southern Canada to Mexico and Central America, and east to the Atlantic seaboard from Nova Scotia to northern Florida. The species has crossed the Rocky Mountains in British Columbia, Washington, and Oregon, but not beyond the Cascade Range. It is also recorded from Arizona. What is the cause of this great extension of range?

6*c*. In the early part of the present century the potato beetle obtained a foothold in Germany but was exterminated. About 1920 it was found in three departments in France, near Bordeaux. How was it probably introduced into that country?

7. Factors of Distribution of Marsupials.—The marsupials are a large and diversified order of very primitive mammals. They are at present confined to Australia, Tasmania, and New Zealand, except that a few species are found in South America and one, the opossum, in southern North America. Familiarize yourself with this general range.

7a. Factors Now Modifying Range.—The coming of man into Australia, and the introduction of several of the higher mammals, is seriously threatening the extinction of the marsupials. The rabbit is assisting in the extinction of the herbivorous marsupials, and the fox and dingo are depleting both carnivorous and herbivorous types. Of these four inimical forms, including man, which are competitors, which enemies, of the marsupials?

7b. History of the Mammals.—In the Jurassic and Cretaceous periods (see geological time scale, "Principles of Animal Biology"), and even in the Eocene and lower (early) Oligocene, as determined from fossils, marsupials occurred in North America and Europe. They probably occurred in Asia at this time, although direct evidence is wanting. Since early Oligocene, marsupials have existed almost exclusively in South America and the Australian region and, since the Pleistocene epoch, also in North America (arboreal opossums only). Mammals of higher orders than the marsupials made their appearance in the northern continents, and first became numerous in the Eocene. In the succeeding epochs they attained a state of high development and differentiation.

7c. History of the Continents.—In Jurassic time Australia was connected with Asia across the archipelago which now separates them. Since Cretaceous time, if not earlier, Australia has been isolated from all other continents, except for a possible connection with South America through the antarctic region during at least part of the Tertiary. South America was separated from North America during most of the Tertiary, though there were connections between these continents in the lower Eocene and upper Miocene, and they were connected in the Pleistocene epoch.

7d. Assemble the facts given in the preceding paragraphs in a table arranged as follows:

Geological period	Connections of Australia		Connections of South America with North America	Distribution of marsupials	Distribution of higher mammals
	With South America	With Asia			
Recent...........					
Pleistocene........					
Pliocene..........					
Miocene..........					
Oligocene.........					
Eocene...........					
Cretaceous........					
Jurassic...........					

From a consideration of the facts shown in the table or recorded in 7*a*, write out an explanation of the past and present distribution of marsupials. How did the marsupials reach Australia? Why have they been able to maintain themselves there? Is there evidence that higher mammals are able to bring about the extinction of the marsupials? Why have not the higher mammals more generally reached Australia? How does South America compare with Australia as an asylum for the marsupials? Why? May the arboreal habit of opossums have any bearing on their ability to invade North America and to maintain themselves there?

SUMMARY

What relationship have you discovered between the general environmental conditions and the ranges of animals? Are ranges constant or variable? Explain.

Give instances, from the foregoing study, of the influence of food supply, competitors, enemies, man, time and place of origin of animal groups, and former distribution of land and sea upon the distribution of animals. Of what may barriers consist?

EXERCISE XIV

PALEONTOLOGY

In the exercise on Homology it was found that the limbs of different vertebrates begin their development in the same way, as a simple outpushing of the body wall, although the adult limbs may be quite unlike in the details of structure. These and other facts are believed to show that all vertebrate animals have descended from a common ancestor. If this belief is well founded, vertebrates have changed (evolved) greatly in the generations subsequent to the common ancestor.

In the following exercise it is shown in the case of two typical groups of animals, one vertebrate and one invertebrate, that such an evolution has actually taken place. Either or both of these exercises may be used at the option of the instructor. The change is demonstrated by the remains of animals preserved in the rocks as fossils. In general, the deeper rock strata contain the fossils of the more ancient animals, the more superficial rocks the more recent animals. Why?

In the study of the fossils used, reference should be made to the geological time scale in "Principles of Animal Biology." This time scale should be before the student throughout the exercise.

EVOLUTION OF THE TETRABRANCHIATE CEPHALOPODA

The tetrabranchiate cephalopods of the past lived within their shells, like the present-day Nautilus. Examine a bisected shell of Nautilus, also a shell of Nautilus containing the animal. Note that the shell is divided into a number of chambers by *septa*. These were successively produced from the center to the opening of the shell. The animal, as it grew, moved forward in its shell at intervals, and formed new septa behind it.

The ancient cephalopods lived in shell somewhat similar to that of Nautilus. The line of union of a septum with the outer wall of the shell is called a *suture*. The sutures of Nautilus are not visible externally because of a pearly layer, the *nacre*, on the outside. Fossil cephalopod shells, however, usually show these sutures. Examine a fossil Loxoceras, Orthoceras, or other orthocone.

Notes.—No preliminary notes on the cephalopods are required. The questions asked below are intended chiefly to direct attention.

The drawings and the summary (directions for which are given below) will answer most of them.

1. Study an orthocone (Loxoceras or Orthoceras, for example). This type of cephalopod was particularly common in the Ordovician and Silurian periods. What is the form of the shell? The form of the sutures? The specimen is usually only a fragment of the entire shell. Thus, in the Museum of Geology at the University of Michigan is a fragment of an orthocone 6½ inches in diameter at its larger end, 4½ inches in diameter at its smaller end. This fragment is 18 inches long. If the piece were completed at its smaller end, how long would it be? Since the animal lived only in the undivided chamber at the larger end of the shell, the shell was much larger than its occupant.

Modern cephalopods progress backward by means of the siphon. (Examine the siphon of a squid and understand its operation.) How would the long shell of an orthocone affect the animal's movements if it moved backward?

Draw an orthocone, giving its name, to show the form of the shell and of the sutures. A line drawing is sufficient but should be carefully made.

2. Examine a gomphoceran (Poterioceras or some other). These forms are recovered from the Ordovician to the Carboniferous periods. What is the shape of the shell? Form of the sutures? How much of the shell was occupied by the animal? Is this shell more cumbersome, or less so, than that of the orthocone?

Draw a gomphoceran carefully (line drawing).

3. In a nautiloid (Eutrephoceras is an example), what is the form of the shell? Of the sutures? Examine a bisected fossil nautiloid, if one is available, noting the form of the septa; compare it with the bisected shell of the modern Nautilus. The nautiloids were most abundant in Silurian and Devonian times, though some survived these periods, and one of them, the pearly Nautilus, is still living.

Draw a nautiloid, showing all the visible sutures.

4. Compare the shell of a goniatite (Aganides or some other) with those of the preceding forms, particularly the nautiloid. Note the form of the sutures. Goniatites were most abundant in Carboniferous times (see time scale).

Draw a goniatite, being careful to represent all the sutures in their correct form.

5. Study a ceratite (Ceratites or any other). What is the form of the suture? How do the sutures compare in complexity with those of a goniatite? The ceratites were largely Triassic.

6. Study an ammonite (Scaphites or any other). These reached their climax in the Jurassic to the Cretaceous periods. The sutures are very crooked fine lines on the surface. Do not confuse the coarse ridges

on the surface with them. Trace very carefully at least one suture completely around one coil of the shell before attempting a drawing. The chances for error are large, because adjoining sutures approach one another very closely at various points. Examine other ammonites if possible.

Draw an ammonite at least natural size, showing two of the sutures. The latter should be very accurate pictures of the specimen used, not merely a diagrammatic representation of the *kind* of sutures found in ammonites in general.

EVOLUTION OF THE HORSE

The development of the horse, as far as known from fossils, took place entirely in Tertiary time. The undiscovered ancestor was undoubtedly a small animal, with five toes on each foot, and nails instead of hoofs. In the following exercise the evolution of the horse will be traced with respect to (1) number of toes, (2) form of teeth, and (3) size of body and skull.

The Feet.—Many of the specimens used in the laboratory are casts of fossils and *must be handled with care.* The student should see some of the actual fossils also, if these are available.[1]

1. Examine casts of the bones of the fore and hind feet of Eohippus or Orohippus. They are of natural size. How many digits in each foot? Are any of the digits distinctly shorter than the rest?

From the wall chart note the geological age to which these forms belong. *Draw* both fore and hind foot, representing the proportions with care, and carefully distinguishing the individual bones. Indicate by Roman numerals which of the ancestral five digits are left (see "Principles of Animal Biology"). The figure may be less than natural size.

2. Study a cast of the foot of Mesohippus. How many digits? Which ones? What is the relative size of the various digits? Compare in size with Eohippus or Orohippus.

To what geological period does Mesohippus belong? *Draw* the foot of Mesohippus with care, indicating which digits are present.

3. Examine the fore foot of Hypohippus. Compare in size with the foot of Mesohippus. Note the size of the third digit as compared with the second and fourth. Is the third digit relatively larger, or relatively smaller, than in Mesohippus? Did the lateral digits of Hypohippus reach the ground? Observe the nodules at the back of the metacarpals at their proximal end. What do they represent? Which nodule is the larger? Does this relative size signify anything?

[1] Other genera of similar nature may be substituted for the ones named here. Hypohippus and Hipparion, which appear not to be in the direct line of evolution, may be omitted if desired.

In what geological time did Hypohippus exist?

4. Study the fore or hind foot of Merychippus. Compare in length with the fore foot of Hypohippus. Did the lateral toes reach the ground? Are there any indications of the first and fifth digits (compare Hypohippus)? How recent is Merychippus?

Draw a foot of either Hypohippus or Merychippus. If Hypohippus is selected for this figure, view it obliquely from the side, so as to include the vestige of one of the lateral metacarpals. Represent the individual bones carefully in their proper proportions.

5. Foot of Hipparion or Pliohippus. Compare in height with Merychippus. How well developed are the second and fourth digits? Compare with Hypohippus and Merychippus.

Geological period?

6. Equus, fore or hind foot, either fossil or modern. Compare in size with the earlier forms. Look for vestiges of the second and fourth digits.

Draw the fore or hind foot of Hipparion or Pliohippus or Equus with care. Turn in such a position as to show one splint bone.

The Teeth and Skull.—Casts must ordinarily be used for this work. Handle them with care.

1. Examine the skull of Eohippus. Note size of the jaws. Note position of orbit of eye relative to teeth. Ask for a specimen, photograph, or cast of a tooth of Eohippus. What is the relative length of the crown and the roots? (Note whether the roots are entire or not.) Observe the tuberculate surface of the tooth (that is, the cusps or prominences on it).

2. Study the skull of Mesohippus. Compare with Eohippus. Where is the orbit relative to the teeth? A fossil tooth, photograph, or cast will be furnished. What is the relative length of crown and root? What is the nature of the surface? *Draw* the tooth of Mesohippus, either from the original or from a cast or photograph, showing as accurately as possible (*a*) the length of crown and root and (*b*) the form of the upper surface. Shading is desirable to show the latter feature. View the tooth obliquely so as to include roots and upper surface in one figure.

3. In specimens, casts, or photographs of the tooth and skull of Merychippus, note (*a*) the size of jaw, (*b*) position of orbit, (*c*) the size of the crown of the tooth, (*d*) the character of the surface of the tooth. *Draw* the tooth of Merychippus or copy the photograph in a line drawing.

4. Compare the skull and teeth of Equus (either fossil or modern) with the preceding forms. Examine a bisected tooth and note the extent of the pulp cavity. *Draw* the tooth of Equus in oblique view to show roots and upper surface in one figure.

Size of Body.—From the casts or specimens of the feet, note the increase in size through successive geological periods. On a chart representing restorations of the entire animals, based on measurements of the fossil bones, note the increase in stature from Eohippus to Equus.

SUMMARY

State carefully the course of evolution of the tetrabranchiate cephalopods and of the horse with regard to the features studied in the foregoing exercise. Make reference to your figures. Note that some of the forms studied may not be in the direct line of descent but are probably offshoots. Which are these? In which continents did the early, middle, and late development of the horse chiefly take place? Make use of these points in your summary. The summary of the horse should include a table showing the continents where its development took place, the geological periods, and the changes in feet, teeth, skull, and stature.

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