

# LABORATORY DIRECTIONS IN GENERAL ZOÖLOGY

BY

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AND

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*Professor of Zoology, University of Missouri*

*THIRD EDITION, REVISED*

NEW YORK

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LABORATORY DIRECTIONS  
IN GENERAL ZOÖLOGY



## TEXTBOOK OF GENERAL ZOÖLOGY

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By WINTERTON C. CURTIS, Professor of Zoölogy, and MARY J. GUTHRIE, Professor of Zoölogy, University of Missouri. Third Edition, rewritten and reset. 682 pages, 6 by 9. 486 figures. Cloth.

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By WINTERTON C. CURTIS

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## PREFACE TO THE THIRD EDITION

THIS edition conforms in the main to the preceding one, although it has been fully revised in the light of the authors' experience in the University of Missouri and that of teachers in other institutions whose suggestions have been incorporated. Among other additions is an extension of the work in vertebrate embryology and one outlining such experiments in the genetics of *Drosophila* as we think feasible for an introductory course. The list of figures suitable for laboratory reference has been substantially extended, since we have found that such figures, available at the convenience of the student, are much more useful than the chart figures commonly used for a similar purpose in most laboratories. As in the earlier editions the work outlined is designed to accompany the authors' "Textbook of General Zoölogy," the third edition of which has recently appeared.

We are again indebted to colleagues in the department of Zoölogy at the University of Missouri and others for valued suggestions.

UNIVERSITY OF MISSOURI  
COLUMBIA, MISSOURI  
April 1, 1939





## PREFACE TO THE SECOND EDITION

THIS new edition of the "Laboratory Directions in General Zoölogy" has been revised to conform to the second, revised edition of the "Text-book in General Zoölogy" by Curtis and Guthrie. No extensive alteration of directions that have been used for so many years has seemed desirable, although changes in sequence and procedure have been introduced where our experience has shown them to be effective. The course in General Zoölogy at the University of Missouri now consists of three one-hour lecture periods and two two-hour laboratory periods for one semester. The omission of certain exercises and the substitution of demonstration of dissections by students, or frequent laboratory quizzes, for the more time-consuming required drawings for some exercises have made it possible to study the same amount of material as before. However, the requirement of a drawing record remains in most of the exercises, to be utilized or not as the instructor desires.

Since the appearance of the First Edition many users of the Directions in our own laboratory, as well as in other institutions, have made suggestions which are gratefully acknowledged. We are particularly indebted to our colleagues, Dr. Rudolph Bennitt, Dr. Katharine R. Jeffers, Mr. John A. Cameron, and Mr. C. Kenneth Collings, for suggestions with reference to the preparation of this edition. Such illustrations as are new have been drawn by Miss Coral Fleenor, biological artist of the University of Missouri.

UNIVERSITY OF MISSOURI  
COLUMBIA, MISSOURI  
May 30, 1933



## PREFACE TO THE FIRST EDITION

THESE directions are a revision and extension of publications by the senior author in 1912 and 1913, and by Curtis and Dodds in 1919. Beginning as printed outlines intended only for use in the University of Missouri, they have come into use in a number of other institutions, and it now seems desirable to provide for more general distribution. The present edition is, therefore, an outcome of some twenty years' development of the introductory course in Zoölogy at the University of Missouri. It is the result of progressive experimentation in both form and content. The authors know it "works" with underclass students in such an institution, because it is the product of so much experience, not only their own but also that of their colleagues and assistants, who have contributed to each revision.

The course, as given at the University of Missouri, occupies one semester and consists of three two-hour laboratory periods and two one-hour lecture periods with time for quizzes taken from both. This is, of course, woefully inadequate for a well-rounded introduction to Zoölogy, but is perhaps as much as can be expected where students in the College of Arts and Science are expected to meet a similar requirement in physical science. Obviously, the present volume includes more than can be undertaken within these limits. It has been made more comprehensive, because the authors have often found it desirable to vary the content in their own laboratory work and because a publication for general use may properly include more than the minimum needs of a single institution.

The course at Missouri is required, as an alternate to Botany, in the Arts College, and also for premedical and agricultural students, since it has been the opinion of the faculties concerned that the course as given satisfied both the cultural and the professional requirements. Because the authors' own conviction is thus supported by that of their colleagues, they feel that the work outlined is suitable for these three groups of students to whom Zoölogy is most commonly offered. Specialization is obviously desirable in later courses, but the first course should be sufficiently general to meet all needs. Because General Zoölogy is given as an underclass subject at Missouri, these directions are adapted to underclassmen, but they are believed to be

quite usable with older students who are beginning the subject. The nature of the course of which this laboratory work forms a part and the authors' purpose in introducing a much greater number of illustrations than is common in laboratory manuals are set forth under the "Remarks to Instructors" which follow this Preface.

It is impossible to acknowledge the services of all who have contributed to the several revisions and thus to the present work. The authors' colleague, Dr. G. W. Tamreuther, and particularly Dr. G. S. Dodds, formerly of the University of Missouri, have been especially helpful. The late Dr. George Lefevre, although not participating actively in any of the revisions, was always keenly interested in the success of the laboratory work. Among many other assistants, E. A. Martin, F. L. Hisaw, E. E. Nelson, F. O. Coe, Hope Hibbard, Harriet Johnston, and Agnes Orbison have made valuable suggestions in present and past editions. The acknowledgments of figures and other matter taken directly from other authors appear in connection with each item throughout the volume. The authors desire to thank the publishers and others concerned for all such courtesies. To Mr. George T. Kline, biological artist of the University of Missouri, the authors are indebted for his careful work in preparing most of the illustrations from preliminary drafts and selections made by themselves. C. E. Wilson and Wiley Crawford, students at the University of Missouri, have drawn the figures on pages 72 and 77 from nature and other sources.

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## REMARKS TO INSTRUCTORS

THE course in general zoölogy, of which the work outlined by these directions is a portion, is not a course of the "phylum" variety, although representatives of all the more important phyla are here included. In the phylum course, each animal is considered, primarily, as a representative of the group to which it belongs, its more general biological aspects being regarded as incidental. Such a course may be necessary in the later curriculum of students specializing in the subject. But in our opinion it fulfills neither the professional nor the cultural requirements of an introductory course in zoölogy. What is needed is the teaching of zoölogical facts and principles by such methods as are most effective, and not a survey of animal types. We agree with the advocates of the "principles" course in their main contention. The question at issue is, how may these principles be taught most effectively.

The principles course is not a new concept, although it has been exploited as such in recent years. There seem to have been two main forms of organization for courses in general zoölogy as given in American institutions during the past fifty years. On the one hand, there have been courses of the phylum type; and on the other, those which have aimed at the illustration of fundamental principles by the study of a limited series of forms. An extreme development of the latter was the "General Biology" of Sedgwick and Wilson (1886) which originally included but a single animal and a single plant, the earthworm and the fern. In a later edition (1895) this text was extended to include unicellular animals and plants, and further elaborated. A more typical example of the form of organization which has used the animals studied as representative of biological principles appears in the courses which have been built upon such books as Parker and Parker's "Practical Zoölogy," Borradaile's "Manual of Zoölogy," and similar works. Although largely morphological with some physiological additions, these texts, supplemented by lectures, have served the purpose where instructors have taught principles rather than phyla; and despite their chapter headings they seem to have been conceived as principles courses by their authors.

The course in general zoölogy at the University of Missouri has been gradually developed from such a beginning. Years ago when

we began using Parker and Parker's text, the frog, the Protozoa, the hydra, and the other animals included were studied as representatives of biological principles and not of animal phyla. With the developments in zoölogy of recent years, increasing attention has been given in the lecture room to the physiological, developmental, and genetic aspects of the science. Various textbooks were used, but the program of the course was always based upon the conviction, first, that we should teach principles, not phyla, and second, that the best way to teach principles is by the intensive study of a limited number of animals suited to illustrate them, followed by demonstrations of the application of these principles in other cases. In the history of zoölogical investigation it appears that the best understanding of principles has usually been gained not by the initial study of many cases, but by the intensive study of a single favorable example with subsequent extension and verification in other instances. Boveri with *Ascaris*, Morgan with *Drosophila*, and Woodruff with *Paramecium* are illustrations. We believe it is so in the teaching of students. Have them study the one case as thoroughly as time allows, and then show them how to apply the principles over a wide range of cases. If too many cases are presented and the comparisons made at the outset, they cannot see the forest for the trees. When they have studied one tree intensively they can understand that different kinds of trees present various modifications and that a forest is nothing but a collection of trees.

To illustrate specifically, the course in general zoölogy as conducted at the University of Missouri and now outlined by the "Text-book of General Zoölogy," which these Directions are designed to accompany, includes the following topics:

### **I. The Structure and Activities of Vertebrate Animals.**

1. Vertebrate organ-systems are studied by dissection and a limited number of physiological experiments. The complex multicellular animal is analyzed as a physiologically balanced unit made up of specialized, coördinated systems. Function is explained in terms of the requirements for protoplasmic maintenance.
2. Cell specialization is illustrated by the histology of vertebrates.
3. Special activities of cells during cell division and gametogenesis are presented with special reference to the behavior of the chromosomes in preparation for the study of heredity and variation.



4. Reproduction and development in vertebrates are discussed with emphasis on the cell movements and early localizations of organ-system primordia. The frog as a laboratory form is compared with other chordates in order to bring out the fundamental sequence of events and its various modifications.

II. **Heredity and Variation**, with emphasis on the experimental and theoretical aspects of modern genetics. No laboratory work is given in this topic.

III. **The Structure and Activities of Representative Invertebrate Animals.**

1. The Protozoa, as physiologically balanced cells or unicellular organisms, are compared with the vertebrate as a unit, as well as with its individual specialized cells. The requirements for the maintenance of protoplasm are further illustrated as in all the forms studied.
2. Origin of somatic cells and their increasing specialization and organization into the typical body-plans are traced through protozoan colonies, the hydra, earthworm, and other invertebrates. Increasing specialization of the sensory-neuro-muscular mechanism in multicellular, free-living animals is pointed out. Variations in organ-systems and similarities in fundamental functions are emphasized in comparison with the vertebrates. Dissection and microscopic study of selected forms constitute the laboratory program.
3. Parasitism, symbiosis, colony formation, and society organization, as well as other animal interrelations, are considered in connection with the various groups.
4. General ecological principles are illustrated wherever possible.
5. The economic importance of other animals to man is discussed particularly in connection with the Mollusca and Arthropoda.

IV. **The Classification and History of Animals.**

1. The principles of classification are considered in general and illustrated for a phylum by the animal studied in the laboratory, as well as by related demonstration specimens.
2. The evidence for organic evolution as the manner of origin of the numerous types of present-day animals is presented,

and the theories of the factors conditioning this evolution are analyzed.

**V. The History of Zoölogy and Its Unsolved Problems** are indicated in connection with the various other topics, not segregated in the course.

In a course of this nature we believe that the principles of zoölogy can be taught more effectively than by any other method of approach. There is no getting away from the fact that the study of an animal in its entirety, rather than a piece at a time, is the most convenient form of laboratory organization. It is also, as we believe, the most effective method of teaching principles, because it makes possible the intensive study of the single case and subsequent extension of the principle to other cases, which is more effective than smattering studies of many forms with the resulting confusion in the mind of the student.

Contrary to the arrangement of many courses, the vertebrate animal, as represented by the frog, is used as the introductory type. We justify this procedure on the following grounds. In studying the frog, the student is examining vertebrate structure and function in general and is able to utilize the biological knowledge he already has concerning his own body and the bodies of familiar animals. Experience indicates that the great majority of students know more biological facts in the vertebrate field than anywhere else. They are at least upon familiar ground, even though most of their supposed information concerning human anatomy and physiology may prove to be inaccurate. Moreover, it is not necessary to have the class learn the use of the microscope at the same time that they are becoming accustomed to the special procedure of the laboratory, which is new and difficult for many individuals. However, we recognize the fact that the use of a complex animal for introductory purposes is contrary to the practice of many excellent teachers. *If it is desired to begin with the Protozoa, these directions can, we think, be used without serious inconvenience, provided attention is given to the few places where references are made to previous work.* A word of general explanation to the class and specific explanations here and there will suffice. Even if the frog is studied at the end instead of at the beginning of a course, there should be no difficulty beyond the fact that the earlier directions are, of necessity, somewhat more explicit than the later ones.

A word may be said regarding our concept of the relationship between laboratory, textbook, and lectures in a course of this nature. It is possible to organize the instruction in a variety of ways. Lectures may be made the primary factor around which everything else

is grouped, or the textbook may be so used, or the laboratory work. Teaching is such a personal matter that each instructor must develop his own methods. Nevertheless, there are some fundamentals of good teaching in any subject. If laboratory work is to mean what we assume that it does, and not merely a routine of mechanical activity and memorization, it must be used in training the student to appreciate the original sources of biological knowledge and the inductive method of science. In a first course of zoölogy this may seem hopeless, but the attempt should be made, even though so many students seem constitutionally unable to learn in terms of anything but memorizing from secondary sources. If laboratory study is to fulfill its assumed function as a modicum of first-hand observation that enables the student to understand what he reads in his textbook and hears in lectures, there should be a close correlation between these three phases of the instruction. If the method pursued be an inductive one, the lectures and text will not be continually telling the student in advance what he may expect to see next day in the laboratory, nor discussing what was studied with scant explanation weeks ago and has, therefore, lost all vividness. A truly inductive approach to any topic gives only the minimum of explanation and text assignments before the student sees for himself. When such first-hand knowledge has been secured, even though the amount be limited, the instructor can build securely the superstructure of his explanations and discussions of principles. These directions have been developed in conformity with such a scheme. They are in most places so written that the student may proceed with the initial laboratory work in any topic without much preliminary discussion by the instructor. To illustrate concretely, if students first examine the *amœba* in the laboratory, being guided by such directions as these, a subsequent lecture may omit much that would have been necessary had the lecture preceded any laboratory work. Likewise, text assignments can be read more understandingly if they have been preceded by some actual observation. The ideal scheme, according to this method, would be to have the student return to the laboratory for the completion of his study after such lectures and assignments; and finally to have the whole gathered together in summary and quiz by the teacher. Of course, the exigencies of scheduled periods often prevent such a procedure. The details must rest with the teacher, if he follows the inductive method. And, in any case, he must do the job in the way his mind works. What can be insisted upon is that the course organization that results in a series of lectures given with little or no reference to the current matter of the laboratory defeats the fundamental purpose of laboratory study, which is to give the greatest

possible basis in first-hand knowledge. Personally, we incline to an inductive organization, because it follows the same course as the deductive method of scientific reasoning.

With this procedure in mind, these directions have been written and revised. They aim to give the student adequate explanations for dissection, but wherever possible to make him see for himself and not to tell him what he can tell himself. The method of verification has been used extensively, but only so far as we have found it necessary in practice. If the directions seem too full in places, it should be remembered that we have aimed to place in the student's hands what is essentially a textbook to guide his laboratory study and enable him to understand for himself. The better students in our laboratories find it possible to get most of the facts with the aid of these directions. The instructor's time is thus used in making sure that each individual has made suitable dissections or preparations and in explaining facts beyond the simpler observations possible in the laboratory. We recognize the difficulty in training students to read such directions and acknowledge that reading directions is not an end in itself, nor is it an intellectual process of the first order. But where many have never been trained to any close reading of exact subject matter such training is an indispensable preliminary to more independent work. In advanced courses, the directions should become more and more general, until the student can be thrown largely upon his own resources.

Pursuant to this intention of making the laboratory directions an aid to thoughtful study as well as to technical procedure, we have included a considerable number of figures. It is the experience of most teachers that charts and models are useful in the laboratory in so far as they do not show what the student may be expected to see in his own material. Many charts are objectionable in the laboratory because they tend to take the place of first-hand observation. When, however, the chart supplements what the student sees for himself, the combination is highly satisfactory. With few exceptions the drawings here included have been so chosen. They relate directly to what the student is supposed to find in his individual material, but cannot be used as substitutes for his own observations. In our own laboratory, where there are several sections working in different rooms at the same time, we must either carry charts from room to room or duplicate them; and in addition, many charts are objectionable for the reason above mentioned. In smaller institutions, chart collections are often inadequate. We therefore believe that these drawings add much to the effectiveness of laboratory study. The instructor can use them as charts, with the advantage that in talking from them in informal

explanations or comments each student has the chart before him. As we have found unlabeled mimeographed figures to be very effective when given out for study and completion by the student, it is suggested that supplementary labeling, shading, and coloring will prove an effective means of enforcing thorough study of many of the figures here included.

In summary and conclusion, it may be said that we believe in the "principles" course and that the introductory course in our own institution has developed along these lines for the past forty years. We think, however, that the best way to teach principles is to let the student study the single animal intensively and then extend to other forms the principle recognized in the single case. Although many courses in zoölogy begin with the cell, we think that the more effective procedure is to begin with a familiar animal and thus utilize any information the student may already have. The vertebrates in general and the frog in particular seem well suited for this purpose. The insertion of the figures in these directions will, we believe, tend to make the laboratory work more like real study and less mechanical. There is no general formula for good teaching, although we believe in the inductive method of presentation wherever possible, because this is the distinctive method of science. A humanistic outlook is also desirable in one who teaches an introductory course, because for the most part we are teaching future citizens of many sorts and not future zoölogists. An inductive approach, which tends to make the concrete facts observed by the student the point of departure in any discussion, a humanistic attitude toward zoölogy and human life, and a sense for what is interesting and essential seem to us the most important elements of good teaching for any course in general zoölogy.



## REMARKS TO THE STUDENT

**The Meaning of Laboratory Study in Zoölogy.**—As the laboratory work undertaken in a course in zoölogy may be new to the student, a word may be said regarding its nature and significance. The basis for all scientific knowledge is first-hand examination of objects in nature or in the laboratory. Intelligent men no longer dispute over the number of teeth the horse has and cite as authority the references to horses in the Bible, as was done in the Middle Ages, or appeal to the writings of Aristotle. They look in a horse's mouth and see for themselves; or if a horse is not available, they consult the works of men who have examined horses and who, it is thought, have truthfully recorded their observations. The strength of all scientific knowledge rests upon the fact that men have seen for themselves, provided, of course, that they have taken the time and trouble to see correctly. Where it is impossible to observe the facts at first hand we take the recorded observations of others, on the assumption that we could observe what they say they observed had we the opportunity. But all such knowledge rests upon second-hand authority and can never be so convincing as that of our own senses, provided, of course, that we are trained for such observation.

To illustrate from other fields, when we study English literature the best method is to read the writings of English authors and not merely to read what someone else says about what these authors have said and how they have written. It is desirable to be guided in such study by a competent teacher and, after reading an author, to know what critics have said regarding his work; but there can be no substitute for the study of the original sources, which in this instance are the writings themselves. Again, in the study of history the original source of information is not the textbook, as so many people suppose. Elementary textbooks in history are sometimes based entirely upon second-hand and even third- or fourth-hand knowledge of the facts, with resulting distortions. The original sources in history are the records of past events, made at the time of their occurrence, and other historic documents; even these are often incomplete and may be inaccurate. If we want to understand the Constitution of the United States, we will do well to begin by reading the document itself and

studying the records of the conditions under which it was written, instead of taking what someone else says about it, even though we will be aided by knowing the interpretations that have been placed upon its phraseology by careful students of law and society.

In zoölogy, the animals themselves and not the men who write the texts and reference books are the primary sources of information. It may be presumed, of course, that the author of a textbook has a wide range of first-hand knowledge concerning the matters of which he writes. But in many instances he must take what other zoölogists have written, because no one man can have more than a limited amount of knowledge based upon his own observations. He will, however, be able to make a critical estimate of the work of others, if his general training as a zoölogist and his first-hand knowledge are sufficient. Nevertheless, the specimen or the experiment in the laboratory, the animal in the field, the conditions in nature are the primary sources of information in zoölogical science. Although it is easier to have someone tell you about such matters than to see for yourself, when you see objects for yourself you may understand them in a manner that is not possible by any other means.

This, then, is the meaning of laboratory work in zoölogy. You may, so far as time allows, see for yourself something of the basis for the statements that are made regarding animal life. What you can see within the limits of such a course as this, or even what you could see in a lifetime of study, would be insignificant as compared with the whole of living nature. But in such study one is dealing with the real objects and not with pictures or descriptions of them. Although such a means of obtaining information is laborious, it is the most certain one available to the human understanding. The laboratory is so called because its work is laborious for both the mind and the hands, in contrast to the ease with which supposed knowledge is obtained by the imagination.

The features of animals that may be conveniently subjected to examination will appear as the work proceeds. The anatomy, or structure, of the animal body is extensively studied; but function, or how the structures act, can never be neglected. Just as the structure of automobiles is interesting principally because it explains how automobiles "function," so the anatomy of animals should be studied in the light of their functions. In many instances, however, it is so much more difficult to observe the parts in action that laboratory observation becomes principally anatomical, with study of functions only so far as is feasible under the conditions of student work. In the present course, structure, as seen in the laboratory and further described in lectures



and texts, is made the basis for an understanding of the functions of parts and of the activities of each animal as a whole. The structure of a series of representative animals and the manner in which they react externally and internally are examined. Upon this basis of first-hand information, the discussion of more general zoölogical principles is developed. Since it is found unsatisfactory to have students examine animals in the laboratory and take no records of their observations, we have recourse to note taking; and since the study of structure consumes so large a proportion of the time, note taking is principally in the form of drawings. The writing of descriptions or the tabulation of experiments is also used wherever it is advisable. In most cases, however, a simple figure properly labeled constitutes a better record of what you have seen than any description you could write. Hence drawings are the common accompaniment of laboratory work in general zoölogy. Drawing is irksome for those who do not draw with facility. Nevertheless, it is the experience of teachers that such graphic representation is an effective means by which observations can be recorded in work of this nature.

There is further justification for note taking by drawings in the fact that when you draw an object you are obliged to study it more carefully than you can usually force yourself to do without drawing. The drawing enforces more careful observation, as anyone will find if he watches himself at work. If there were no other justification, this alone would be sufficient reason for what is commonly required of students in zoölogy. If you draw well, such work will be a pleasure; and if you draw poorly, you will find yourself seeing more than you could in any other way.

**The Technique of Laboratory Drawing.**—Since the recording of observations by means of figures is so extensively used as a means of note taking, it will be helpful if we explain at the outset the simpler principles of such representation. This may be accomplished by showing first how lines may be drawn in the two dimensions of a sheet of paper to suggest the third dimension or depth of objects, and then proceeding to applications in the drawing of structures such as are seen in the laboratory. Referring to the accompanying Fig. 1, if we draw two parallel lines, as in A, they suggest nothing more; when the lines are inclined, as in B, they suggest the convergence of the parallel lines of a railroad track or similar object stretching into the distance. A rectangle, C and D, may be similarly treated. A cylinder seen directly from the side, as in E, looks like a rectangle; when we think of it as slightly tipped it looks like F and may be so drawn. Again the rectangle drawn in G may be shown as though tipped backward, as in H;

or a solid object may be indicated by addition of lines to the right, as in I.

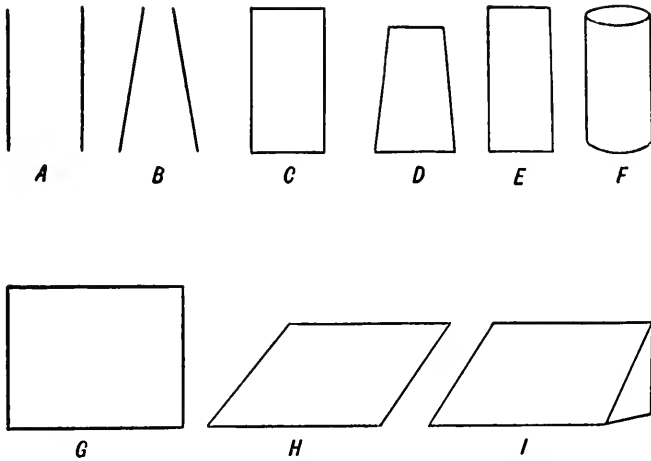


FIG. 1.—Use of lines in mechanical drawings.

If we deal with one object lying above another, as shown in Fig. 2, the principle is to have the lower object hidden by the upper one or

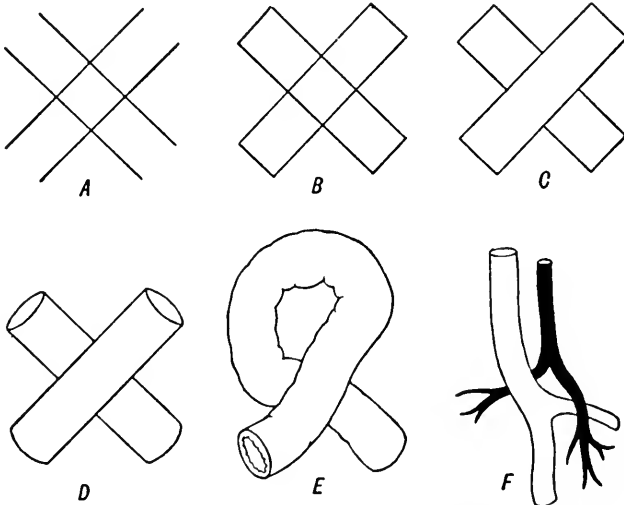


FIG. 2.—Use of lines in representation of various objects.

shown by lines that are broken. Thus, the two pairs of parallel lines in Fig. 2 A suggest nothing of depth; they are like the crossing of two street-car tracks. The rectangles in B are no better, but in C it is

clear that we are showing one object that is above another. How necessary it is to have the lower object disappear sharply behind the upper one will be seen if the reader will take a pencil and prolong one or more lines of the lower rectangle slightly within the border of the upper one in Fig. 2 C. As in Fig. 1 F, we may modify Fig. 2 C to present the appearance of the two cylinders shown in Fig. 2 D.

The necessity of showing one object over another occurs in every biological drawing of any complexity that attempts to represent a third dimension. The principle of such representation is simple, and its application can be easily mastered. For example, a loop of an intestine would be represented as in Fig. 2 E. The relationships of two neighboring blood vessels might be shown as in 2 F. Again, part of a sphere appears as in Fig. 3 A when seen from a straight side view;

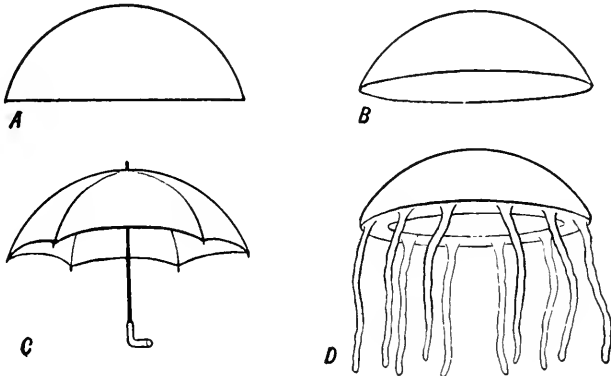


FIG. 3.—Use of lines, as in Fig. 2, to show dome-shaped bodies.

but 3 B looks more like such an object. The representation of an umbrella in 3 C, and that of a jellyfish in 3 D, apply this principle in the representation of familiar objects. In these last two cases the distance of the parts on the far side is suggested further by fainter lines. By these simple methods, drawings on a flat surface are made to express the third dimension, that is, to give the impression of depth seen in the real object.

What has been said above applies to line drawings or those in which no shadows are indicated. It is surprising how much can be shown by lines alone, as in the accompanying Fig. 4, in which the careful drawing of the posterior end of the body gives a remarkable suggestion of distance. The line drawing is the foundation for all biological figures of the sort under consideration. You should learn first to make good line drawings. In some laboratories these alone

are permitted. A further device to show the parts in contrast is the use of black and white, as in Fig. 2 F. The simplest way to do this is by using the pencil to darken limited parts of the figure. If too many parts are thus darkened the contrasts will be lost. Or one may use the common red and blue crayons, but this should be done with great care and the color kept within the boundaries of the blood ves-



FIG. 4.—Drawing of a nematode, in which the perspective is shown by lines at the posterior end of the body and by shading in the head region.

(From N. A. Cobb, 1914, *Yearbook, U. S. Dept. Agriculture.*)

sel or other parts. Experience shows that few students will do crayon work with sufficient care not to merely smudge; hence it is not favored by most teachers. In ink drawings where this technique of darkening is used, the special parts are merely painted in, with care not to overlap the outline and to fill the area completely, as in Fig. 2 F.

An even more effective means of indicating the third dimension is by what is called shading. This is not mere darkening, as explained in the previous paragraph, but rather the distribution of pencil or ink

marks and color to indicate shadows. If you will place yourself so that there is but one source of light, as when you sit at a table with a window or desk lamp to the left, you will see that a shadow borders each finger where your hand is outspread upon a sheet of paper. Elevating individual fingers will show how the shadows are changed by different positions of the parts. In a room with windows on more than one side or with many lights, the shadows are confused, and out of doors the conditions are often complicated. Their representation is one of the great problems of the artist. In our understanding of familiar objects seen at a distance, shadows, perhaps even more than lines, are the indicators of the third dimension. Look at the next building you approach when the sun is shining, and see how the lines change as you shift the angle of view and how the shadows bring out

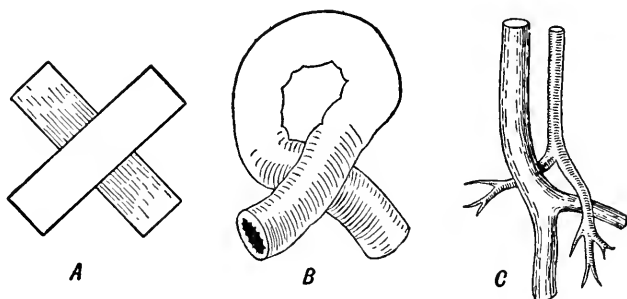


FIG. 5.—Principles of shading applied to different objects.

various parts. Perspective, or the way lines are projected on a plane surface, and shading, or the way shadows fall, are constantly seen by human beings and unconsciously interpreted with precision. It is an interesting study to analyze the impressions that make us conclude that one object is here and another beyond it as we walk out of doors or look from a window.

As is the case with black and white contrast, many teachers disapprove of shading, because it is so commonly misused by unskillful students. Unless carefully done, it is likely to result only in concealing inaccuracies of outline. We believe, however, that a minimum of shading gives very satisfactory results and that it may be recommended after the student has learned the principles of the line drawing, which should always be completed before shading is begun. How simple shading may be used is indicated by Fig. 5 and by the head parts of the worm shown in Fig. 4. When we look at most objects, the light, as has been said, comes from more than one source. To obviate this difficulty, architects and engineers, in making mechanical draw-

ings, limit the source of light to the upper left-hand corner at an angle of  $45^\circ$  and draw the shadows accordingly. Such mechanical drawings may be very elaborate. We shall not attempt too much but merely show how the suggestion of shadows in suitable places will add to the line figure. Thus in Fig. 5, as compared with Fig. 2, the addition of the shadow that would be thrown by the upper objects upon the lower ones makes the appearance much more realistic. In Fig. 4 the tentacles below the mouth are left white and thus contrast with the shadowed background of the body in this region. The mouth is darkened for the same reason that one would darken the opening to a cave. The way in which a touch of such shading adds to the line drawing is shown in Fig. 8 by the stippling of the limbs where they join the body. Other examples will be found in the drawings throughout the present

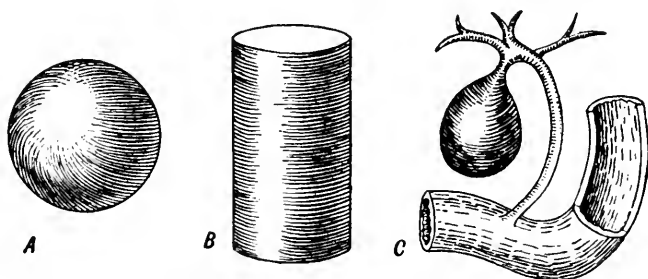


FIG. 6.—Principles of shading applied in representation of a sphere, a cylinder, and in the organs of an animal's body.

volume. Having completed a good line drawing, you can try adding a bit of shading here and there until you learn how to handle the same, making it a rule to add less rather than more, since there is always danger of smudging. The three principles of shading that are important for our purposes are the shadows on a sphere, those on a cylinder, and those cast upon an object by one above it, as shown in Fig. 6, and in Fig. 5 as previously explained. Since application of these principles and those of line drawing appears in the figures throughout this book, it may be helpful to examine these drawings. Even though you are unfamiliar with the objects represented, you can appreciate the means employed to suggest the third dimension by lines and shadows. It is easier to add such shadows to a line drawing made with pencil than to one made with ink, but the principles in distribution of light and shade are the same in the two cases. Delicate effects may be obtained in pencil drawings by rolling a piece of filter paper into a close cylinder about 5 mm. in diameter and twisting it into two pieces.

The rough ends thus formed make an excellent "stump" for rubbing shaded areas. However, practice is necessary to avoid smudging.

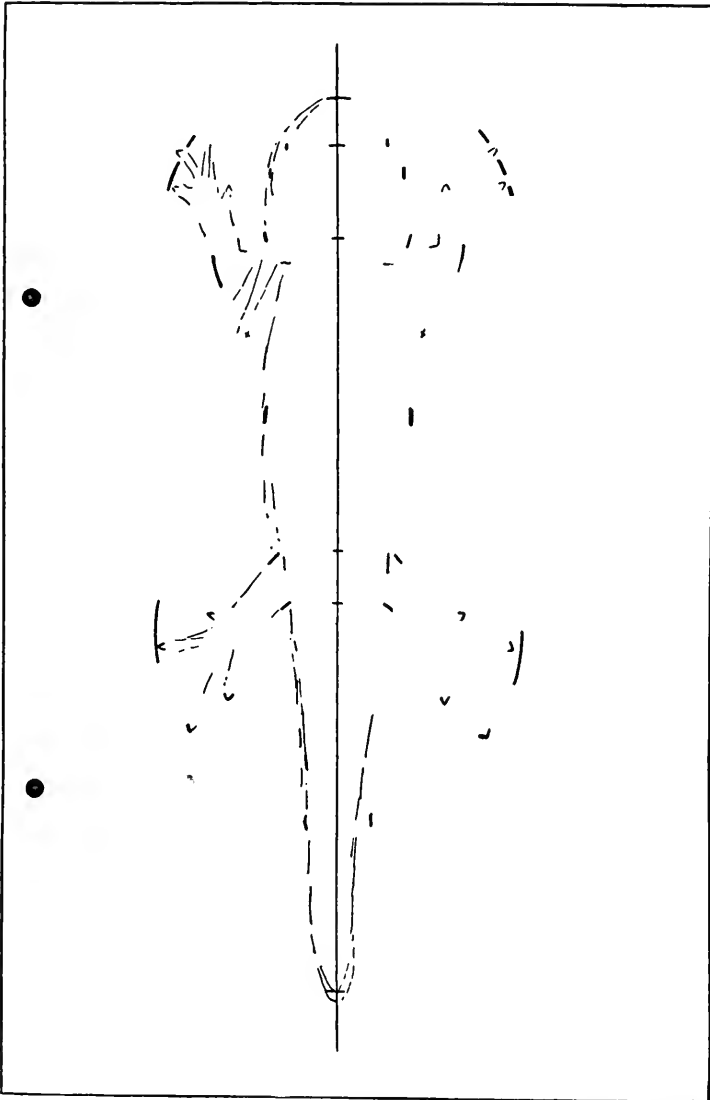


FIG. 7.—Preliminary stage in the making of the drawing shown in Fig. 8.

The procedure in constructing a finished laboratory drawing may now be explained. Suppose you are studying the external structure of a young salamander and wish to represent what you have observed

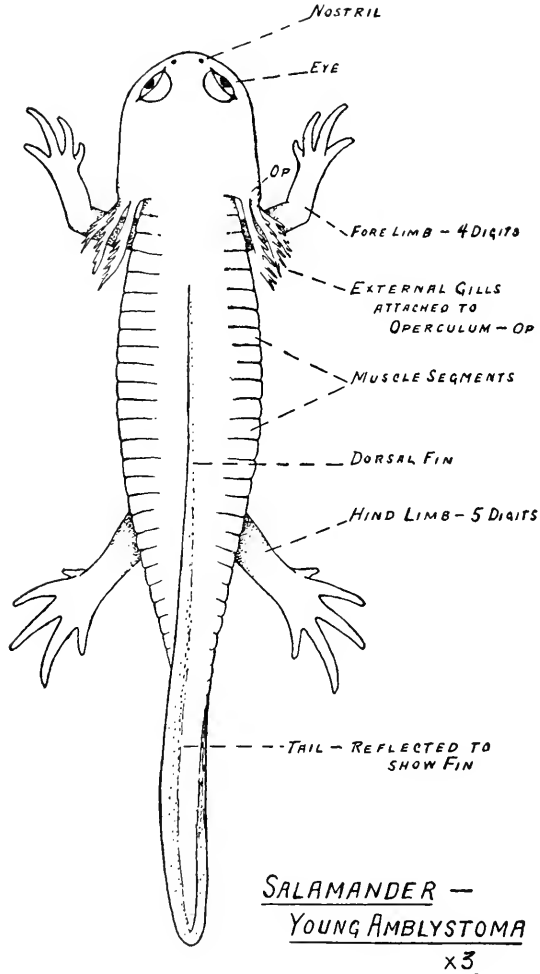
in the form of a drawing rather than by written notes. Where the object is bilaterally symmetrical, it is helpful to use a median line drawn temporarily as a guide and later erased. Such a line may be laid down faintly as in Fig. 7, care being taken to have it parallel with the edges of the page. After deciding the scale on which the figure is to be made, the principal dimensions may be laid off, with a compass or otherwise, along this line; and a number of right and left measurements be indicated, as in Fig. 7. With these as a guide, faint sketch lines may be made with the pencil, until the correct outlines have been hit upon, and then replaced by the final outlines, which should be firm and of uniform thickness, like the ink lines of the drawing reproduced in Fig. 8. It is well not to attempt too much preliminary measuring at the stage shown by Fig. 7, but to train yourself to see all the lesser proportions with the eye after a few principal ones have been measured. In drawing under the microscope, you must use this latter method entirely in the absence of special devices not feasible for use in elementary courses. When the drawing is completed (Fig. 8) the parts should be shown correctly and clearly by an outline figure, which can then be touched up with a little shading in the right places. Such a drawing is not photographic. It is a record of relationships observed and hence is always somewhat diagrammatic. But this does not mean that it is inaccurate.

Drawings should be labeled as shown in Fig. 8. The label words should always be written horizontally on the page and be so spaced and aligned as to present a neat appearance. The label lines drawn to each word are preferably broken rather than continuous, because too heavy lines in the labeling may detract from the drawing itself. In the ideal figure of this sort, the drawing, not the labeling, should be the part that attracts most attention; but when one looks at the labeling this should be distinct and unquestionable. Label words should be printed or neatly written. Below the figure, or elsewhere on the page and uniformly throughout your laboratory book, there should be an appropriate title with scale indicated, and your name in accordance with the procedure in a particular laboratory. Labeling is not so easy as it seems. The appearance of a good drawing is sometimes ruined by poorly executed label words and lines. You must learn by experience, but suggestions may be obtained by examining the different figures in these directions, in all of which care has been exercised to make the labeling clear but at the same time subordinate to the actual drawing.

As explained at the outset, the drawing is a means to an end, namely, the recording of facts and the enforcement of careful observa-



tion. As a requirement it seems justified by the experience of teachers, even when expected of students who have little aptitude for this combination of mechanical and artistic work. Accuracy is all-impor-



John Smith, SEAT 22

FIG. 8.—Page from a student's laboratory notebook, showing a line drawing that is finished and labeled in appropriate fashion (cf. Fig. 7).

tant in the scientist. Neatness is desirable because it is so often the necessary accompaniment of accurate work. If you feel you "cannot draw" you need not be discouraged, since the test of laboratory work is understanding. A figure drawn by an unskilled draftsman may

show more real knowledge of relationships than one drawn by a person who is merely facile with hand and eye. It is necessary in science to be clever with the head, even more than with the hands. In every large class there are those who are painfully handicapped in a mechanical way, but whose intelligence is of a high order. These may never make remarkable drawings, but they can learn zoölogy. We have dealt at length with the technique of such drawing because it is a very practical difficulty with many students. This should not lead to the impression that we regard the drawing as an end in itself, nor that good laboratory work can be done only by those who are skilled in this particular. Nor should it appear that there is no other technique in the study of zoölogy.

In conclusion, we may speak of the equipment necessary for such work. Good paper is a prime necessity. For pencil work, Whiting's "Linen Ledger" is very satisfactory for ordinary uses and not expensive for a drawing paper. Byron and Weston's "Linen Record" is a much better paper and suitable for pen as well as pencil. Standard drawing pencils should always be used, 4 H for most work, and 3 H or 2 H for special purposes as found by experience. E. Faber's "Ruby" eraser is good for general purposes, and E. Faber's "Van Dyke" for sponging out dark lines. Pencil sharpeners may be used, but the good draftsman puts the final touches on his own pencil with a pencil file. A simple compass is convenient in the drawing of larger organisms. In advanced courses, ink drawings are required in many laboratories. Water-proof drawing ink is used, and special drawing pens such as the "crow-quill." For beginners the pencil is best, because it can be corrected by erasure unless too sharp a point or too much pressure has been used.

**The Laboratory as a Place of Study.**—In addition to its function as an opportunity for obtaining first-hand knowledge, the laboratory gives opportunity for training in observation and interpretation. As in all real education, you must train yourself. The teacher can insist upon a high standard of excellence and furnish opportunity and inspiration, but education is within you. Carefulness, accuracy, independence, and, above all, honesty are requisites of all good work in science, whether of student or investigator. The requirement of laboratory drawing often tempts students to substitute for their own original figures copies of what other students have done, or to draw upon figures in textbooks. This, of course, is cheating pure and simple, and when detected must be dealt with on that basis. A more common shortcoming is a form of self-deception and dependence familiar to instructors. One often finds that the same mistake is repeated by all

the students at a table. Upon examining such cases, it appears that someone got it wrong and the rest have followed like sheep, really thinking they have seen what they have drawn or described. If you find yourself doing this, try to have more confidence in your own eyes and hands. You can see what is called for in such a course as this as well as the next man, and perhaps better if you put your mind on it; and you should determine that even though others may follow a wrong lead you will not. Moreover, do not add items that you have not seen to notes or drawings, thinking you must always show everything. An instructor would rather have you explain in labeling that the nucleus or any other part "was not seen" than to have you draw it from your imagination. He knows that there are often details that the best of students may not find within a reasonable time and that the material does not always prove satisfactory even when care is used. He has more respect for the individual who does not claim to find everything, but finds enough to make it clear that he is getting all that can be fairly expected. It is well to be honest with your instructor, but even more important to guard against habits of self-deception. These general remarks apply to all parts of your work.

There is sometimes a tendency to treat laboratory work as a mechanical process and to regard the real studying in a course as something done from books. The latter are, of course, important; but the motto of Agassiz, "Study nature, not books," recognizes the importance of the laboratory and the field. Proper understanding of a topic considered in the laboratory often involves knowledge of facts previously ascertained in lectures and textbook. Hence it is important that you keep yourself well prepared on all assignments and on reviews of past work. The understanding of what is seen today often depends upon what was seen in the laboratory last week. It is easy to let the work degenerate with an unthinking manipulation of hands and instruments. To be successful it must be a process of thoughtful study. Read your directions with care. If you follow them explicitly and the instructor has furnished you with suitable material, you will see what is called for with a minimum of assistance. To a considerable extent, these directions are your instructor. If you do your part well, the individual who is your laboratory teacher can spend most of his time seeing that you have the best of opportunities and explaining to you the general conclusions that can be drawn from such detailed facts as you are observing. Instead of mere drudgery over details, you will then be getting something of the great principles of the science, and understanding them because you know the facts upon which they are founded.

If the laboratory is to be the place of study above indicated, it must be quiet and orderly. To this end you should not only avoid confusion by following the directions but also consider others. Bring to your seat at the beginning of the period all articles likely to be needed, thus avoiding unnecessary moving about the room. When demonstrations are put on exhibition, be careful not to injure them, and be sure to leave all material not intended for your exclusive use in good condition for your fellow students. Your coöperation is asked in making the laboratory a quiet and pleasant place of work and in so handling its equipment that everyone may derive the greatest benefit.

You will probably not appreciate all that is here said when you read these remarks at the beginning of your course. Read them again at the end of a week and also a month later. You will then know what your difficulties are and may be helped to solve them.

# LABORATORY DIRECTIONS IN GENERAL ZOÖLOGY

## THE FROG

PHYLUM CHORDATA

SUBPHYLUM VERTEBRATA

CLASS AMPHIBIA

### I. ANATOMY AND PHYSIOLOGY

#### A. ACTIVITIES AND EXTERNAL FEATURES

##### **Exercise 1.**—Activities.

(a) Observe living frogs in water and on a table. How is each pair of limbs used for locomotion in and out of water? Which of these modes of locomotion seems to be the primary one for the frog? Give your reasons. Observe the position of the frog as it floats in the water. Is it a good position for a quick retreat to the bottom? Observe just how the frog makes this retreat when frightened. Compare the structure and uses of the limbs in the frog and other animals with which you are familiar, such as the dog, ape, duck, and chicken. What is the position of the eyes and nostrils when the frog is floating? Of what use do you think these structural features are to the frog? Is the resting position of the frog when out of water such as to enable it to make a quick escape from danger? On what does the frog depend for protection from other animals? Compare the frog in this respect with the deer, horse, bear, turtle, rattlesnake, and man. Is the frog well or poorly protected? Be prepared to list some of the more obvious means of protection which familiar animals possess.

##### **Exercise 2.**—External Features.

(b) The following directions apply particularly to the leopard frog, *Rana pipiens*. They may be used, however, for any of the common species. Examine a preserved frog or one recently killed. Recognize **anterior** and **posterior ends**, **dorsal** and **ventral surfaces**, **right** and **left sides**; be sure you understand the meaning of and can define the terms used here and later. The animal is **bilaterally symmetrical**, that is, it can be divided into right and left halves each of which is

the mirrored image of the other. Notice the soft, moist skin and the absence of hair, feathers, and scales. What is the relation of such a skin to the habitat of the frog? Do you think the frog can live in dry places? Observe the distribution of color on the skin. Are the right and left sides colored alike? Are the dorsal and ventral surfaces colored alike? Do you know of any other animals that have a comparable distribution of pigment? The body of the frog, like that of many other animals, has three divisions: the **head**, the **trunk**, and the **appendages**. Is the head of the frog sharply separated from the trunk? For what is the long, flexible neck of birds and mammals used? What structure has the elephant which compensates for its short neck? Find the **mouth**, the **anus**, or posterior opening of the digestive tract, the **external nares**, or **nostrils**, and the **eyes**. Compare the **eyelids** with those of the human eye. Just posterior to the eye is the **tympanic membrane**, or **eardrum**. Is the ear of the frog similar to your own? The **fore** and **hind limbs**, or appendages, have the same general structure; **upper arm**, **forearm**, **wrist**, and **hand** correspond to **thigh**, **shin**, **ankle**, and **foot**. Consider how this similar fundamental structure is modified for quite different purposes in the two pairs of limbs. Compare with the limbs of a human being and other familiar vertebrates.

(c) Place the frog, dorsal surface up, in a dissecting pan, pinning out the fore and hind limbs with their digits separated. Draw on a scale of 1 as thus seen from the dorsal aspect, applying the principles of scientific drawing explained on p. xxix. Show all the parts to which your attention has been called in the foregoing section, if they appear in this view. Finish the drawing in simple outlines, without shading, and label carefully.

## B. THE BODY WALL AND CÆLOM

### Exercise 3.—The Body Wall.

(a) Fasten the frog, ventral surface up, in a dissecting pan by means of a pin thrust obliquely through each limb. Lift the **skin** with the forceps and cut through it, but not through the underlying **muscles**, with the scissors along the mid-ventral line from the posterior end of the trunk to the tip of the jaw. The skin is separated from the muscles except at certain places, and the spaces between the skin and the muscles contain a colorless fluid called **lymph**. Cut the skin outward, at right angles to the first cut, in the region of the arms and again at the posterior end of the trunk. Pin back the flaps. Notice the **blood vessels** of the skin and examine with a handlens.

The **veins** are conspicuous and dark-colored while the **arteries** are usually colorless and inconspicuous. Can you explain this difference? Arteries and veins are distributed throughout the body in a similar manner.

(b) Cut through the muscle layer a little to one side of the median line and open the **body cavity**, or **cœlom**. Cut and pin back the muscle layer as you did the skin in order that the **viscera**, as the organs of the body cavity are called, can be seen. In the region of the arms it will be necessary to cut through certain bones, the arrangement of which should be observed in a prepared skeleton. Do not cut too deep and injure large blood vessels in this region.

#### Exercise 4.—Identification of the Organs in the Cœlom.

(c) In the following identification of organs they may be displaced and pushed aside as necessary but must not be cut or removed

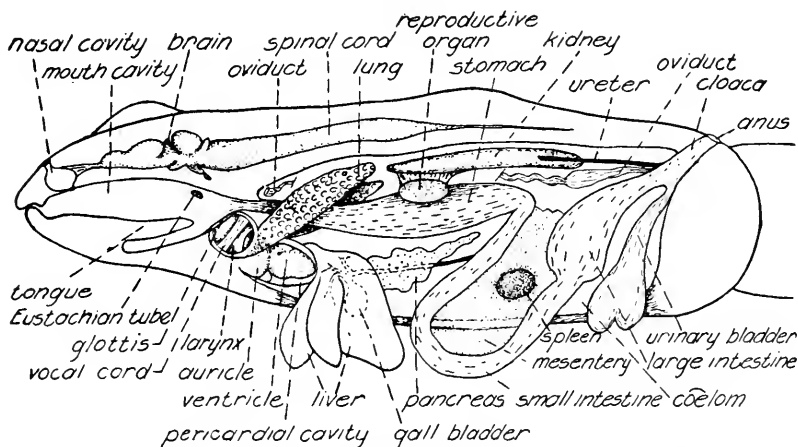


FIG. 9.—The frog, shown as if cut in the median, longitudinal plane; semidiagrammatic.

until specific directions are given. Work with care, and do not injure structures that will be studied in detail later. Some familiarity with the position of the organs can be gained from Fig. 9.

(d) At the anterior end of the cœlom the **heart** will be seen surrounded by a thin membrane, the **pericardium**. In a recently killed frog the heart will be beating; observe how it contracts. Immediately posterior to and at the sides of the heart are the three lobes of the **liver**. Between the right and median lobes of the liver is the round, greenish **gall bladder**. Lift up the left lobe of the liver and find the **stomach**, which is continuous anteriorly with a short **esophagus** lead-

ing from the mouth cavity. Turn the median lobe of the liver toward the head and note that the stomach is continuous posteriorly with the **small intestine** which leads to the **large intestine** at the extreme posterior end of the coelom.

(e) If your specimen is a mature female the **ovaries** will appear as conspicuous paired structures filled with black-and-white eggs. Find the places of attachment of the ovaries and note their general shape. Remove both ovaries by picking them away with your forceps, but be careful not to injure other organs. The large black-and-white eggs will be laid at the next breeding season. Observe the smaller eggs by using the handlens. Estimate the total number of eggs. With so many eggs in each female why has not the earth become crowded with frogs? Removal of the ovaries will reveal a pair of long, coiled, white **oviducts** stretching from the anterior to the posterior end of the coelom. Leave these in place.

(f) If the specimen is a young or immature female the **ovaries** will appear as paired, ruffled structures lying on each side of the mid-line and dorsal to the intestine. Small eggs can be identified in these organs with the aid of a handlens. Remove one of the ovaries by picking it away with forceps, but do not injure other organs.

(g) If you have a male frog the **testes**, a pair of small, light-colored, elongated bodies, will be found by pushing the small intestine aside.

(h) The ovaries and testes are attached to the ventral surfaces of the paired **kidneys**, which are behind the dorsal lining of the coelom (Fig. 10). From the anterior end of the kidneys the finger-like processes of the yellow **fat-bodies** extend. On the ventral surface of each kidney is a narrow, yellow band, the **adrenal** or **suprarenal gland**. In immature females the oviducts will be found as slender, somewhat coiled tubes running near the lateral margins of the kidneys and stretching the entire length of the coelom. In males the **rudimentary oviducts** will be found in the same position. The **spleen** is a dark red, round organ located in the membranous **mesentery** that holds the small intestine in place. At the extreme anterior end of the body cavity find the paired **lungs** covered by the right and left liver lobes. At the extreme posterior end of the coelom a thin-walled sac, the **urinary bladder**, will be seen partially covering the large intestine. If the specimen has been recently killed the lungs and urinary bladder can be inflated by means of a blowpipe.

(i) The organs in the posterior end of the coelom should be exposed more completely. Examine a skeleton and note the form and position of the bone at the base of the hind legs. Insert the end of



a pipette or handle of a needle into the anus. Then, with a scalpel, cut through the skin and thin sheet of muscle in the mid-line between the bases of the hind legs. A somewhat translucent region will then be seen separating the muscles of the legs in the mid-line. Carefully controlling your scalpel, cut directly down through this region, without injuring the urinary bladder or the posterior end of the digestive tract. Now separate the legs widely and pin them down. Find where the urinary bladder is attached at its anterior end and free it from its membranes. Turn the bladder to one side and observe that the large intestine narrows posteriorly and is continuous with a slender tube, the **cloaca**, which communicates with the outside by way of the **anus**. The neck of the bladder is attached to the ventral surface of the cloaca and should not be injured.

### C. THE DIGESTIVE SYSTEM

#### Exercise 5.—The Mouth Cavity.

(a) The digestive system consists of the **digestive tract** and the attached **digestive glands**. The **mouth cavity** is the most anterior region of the digestive tract and, in addition, is a part of the respiratory system. Only the structures which are directly related to the digestive system of the frog will be described now. Open the mouth wide, cutting a little at the angles of the jaws if necessary. Locate the **teeth** by rubbing with a finger. Are they found on both jaws? Examine with a handlens. Which way do the teeth slant? How can the frog use its teeth? Observe two patches of teeth on the roof of the mouth; these are the **vomerine teeth**, so called because they are attached to a bone known as the vomer. Posteriorly, the mouth cavity narrows and is continuous with the esophagus, which leads to the stomach. How does the place of attachment of the **tongue** differ from that in man? How does the frog use its tongue?

#### Exercise 6.—The Cœlomic Organs of the Digestive System.

(b) Identify again the remaining parts of the digestive tract, namely, the **esophagus**, **stomach**, **small intestine**, **large intestine**, and **cloaca** (Fig. 9). Notice the transparent, sheetlike **mesentery** which is attached to the stomach and intestine. Understand from charts or diagrams the relation between the mesentery and the **parietal peritoneum**, which is the innermost layer of the body wall and lines the cœlom, and the **visceral peritoneum**, which covers all organs that are suspended in the cœlom (Fig. 10). Observe the blood vessels in the mesentery. What are the functions of the mesentery? The visible

glands of the digestive system are the **liver** and the **pancreas**. Notice again the three lobes of the liver, and, also, the **gall bladder** in which the **bile**, a digestive juice secreted by the liver, is stored. Bile passes from the gall bladder to the intestine through the **bile duct**, a very delicate tube that lies in the mesentery and can be seen only in favorable specimens. The pancreas, a cream-colored gland of irregular shape, is in that part of the mesentery which connects the stomach with the first loop of the small intestine. **Pancreatic juice** is a digestive juice secreted by the pancreas and discharged through pancreatic ducts into the bile duct where the latter passes through the pancreas on its way to the intestine. What are the functions of the

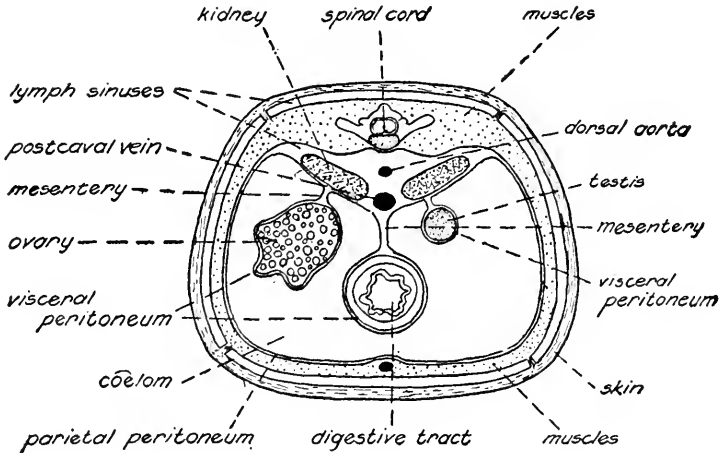


FIG. 10.—The frog; a transverse section in the region of the kidneys and reproductive organs. The specimen is shown as a male on the one side and as a female on the other; diagrammatic.

bile and pancreatic juice? Other digestive glands occur in the walls of the stomach and small intestine but are invisible without magnification.

(c) In order to see as much as possible of the digestive system at one time for the purpose of drawing, it is necessary to snip the mesentery, *except for that part which stretches between the stomach and the first part of the small intestine and encloses the pancreas*, and spread the small intestine out on the animal's right side. Do not cut the tract itself. The lobes of the liver may be turned toward the head so that the stomach and pancreas are revealed. Push the urinary bladder to one side to uncover the large intestine and cloaca. Draw

( $\times 2$  or  $3$ ) the digestive system as thus seen, surrounded by an outline of the trunk and head, and show the position of the mouth cavity and esophagus.

**Exercise 7.**—The Digestive Tract in Cross Section.

(d) The digestive organs must now be removed. Leave the heart, lungs, large intestine, oviducts, testes, and related structures in place and uninjured. Cut across the stomach at its anterior end, and sever the small intestine near its union with the large intestine. Take out the stomach, small intestine, spleen, pancreas, liver, and gall bladder after cutting the mesentery that holds them in place.

(e) With a sharp scalpel, a razor blade, or with scissors, cut cross sections of the stomach toward its posterior end. Place these under water and study the cut surfaces with a handlens. Identify the **mucous membrane**, a conspicuous layer lining the cavity of the stomach, the **submucosa**, a thin, somewhat brownish layer of varying thickness, the **muscular layer**, conspicuous and of uniform thickness, and the **visceral peritoneum**, which is very thin and visible only where it is continuous with the mesentery. What are the functions of the different layers? The mucous membrane and submucosa will be folded if the stomach is not expanded with food. What are the functions of such folds? Draw a section ( $\times 5$  to  $8$ ).

**Exercise 8.**—Gastric Digestion.

(f) The conditions under which digestion occurs in the living animal can be duplicated to a considerable degree in experiments with non-living material. Digestion of food will occur in test-tubes if the proper chemicals are employed. The process is more rapid at the temperature of the body but goes on quite actively at room temperatures.

(g) The way in which gastric digestion occurs can be found out by means of a simple experiment. Label four test-tubes as follows: (1) fibrin and distilled water; (2) fibrin, distilled water, and pepsin; (3) fibrin, distilled water, and hydrochloric acid; (4) fibrin, distilled water, pepsin, and hydrochloric acid. Place one small piece of fibrin, a protein, in each tube and fill it half full of distilled water. Add one-half pipette full of pepsin to tubes 2 and 4, and two drops of hydrochloric acid to tubes 3 and 4. Mix the contents of each tube thoroughly. Use care not to spill acid on the tables or on your clothing. Notice the changes that occur in the fibrin during the laboratory

period. Record your observations and make a general concluding statement concerning the conditions under which digestion occurs in the tubes. This is to be done in the form of a table such as the one shown in Fig. 11.

Gastric Digestion				
Tube No.	1	2	3	4
Materials put in	Fibrin Water	Fibrin Water Pepsin	Fibrin Water Hydrochloric Acid	Fibrin Water Pepsin Hydrochloric Acid
Observations on condition of fibrin				
Conclusion				

FIG. 11.—Form of table for record of Exercise 8.

### Exercise 9.—Salivary Digestion.

(h) In order to find out how salivary digestion occurs fill each of two test-tubes to a depth of one inch with starch paste. Test the contents of the first tube for starch as follows: Transfer with your pipette two drops of the starch paste to a glass slide. Add to this one drop of iodine solution. A blue color reaction with iodine indicates the presence of starch. Now test the remaining contents of tube 1 for sugar, as follows: Add to the contents of the tube half as much Benedict's or Fehling's solution, mix, and heat. Keep the solution in the tube agitated in order to avoid burning yourself. A yellow or red precipitate indicates the presence of sugar. To the starch paste in the second tube add half as much saliva from your mouth. Mix thoroughly and let stand for ten minutes. Test the contents of tube 2 for starch and sugar as described above. If digestion is not complete a purplish color with iodine will indicate the presence of erythro-dextrin. Collect some saliva in a third test-tube and test for starch and sugar as described above. Keep all tests until the experiment is completed. Record the results of the tests and make a general concluding statement concerning the changes which occur in salivary

digestion. This is to be done in the form of a table such as the one shown in Fig. 12.

Salivary Digestion			
Tube No.	1	2	3
Contents of tube	Starch	Starch Saliva	Saliva
Result of test for starch			
Result of test for sugar			
Conclusion			

FIG. 12.—Form of table for record of Exercise 9.

#### Exercise 10.—The Absorption of Food.

(i) Food in the cavity of the digestive tract is surrounded by the mucous membrane which lines the digestive tract. The thin-walled capillaries and lymphatics which carry blood and lymph, respectively, run in the submucosa (Fig. 13). The food must pass through the mucous membrane and into the capillaries and lymphatics before it can be distributed by the circulatory fluids to all parts of the body. The mucous membrane and the walls of the blood and lymph vessels are semipermeable membranes, that is, some substances can pass through them but others cannot. Some foods are in a condition to be absorbed when they enter the body; others must be changed by digestion into substances which can pass through the mucous membrane and the walls of the capillaries and lymphatics. Parchment and similar animal membranes which are no longer alive act as semipermeable membranes and can be used in the construction of a simple apparatus to demonstrate the passage of diffusible food substances through such membranes. A piece of the parchment is tied over the end of a good-sized tube. Into this tube is put a solution of the food to be tested, and the tube is then lowered into a larger vessel containing water. After some time, tests are made of the water in the outer vessel to determine whether any of the substance in the inner tube has passed through the parchment membrane.

(j) Such an apparatus will be constructed and demonstrated before the class, and the tests to determine the presence of the substances

used will be explained. In one apparatus, starch paste is in the inner tube; in another, a sugar solution is in the inner tube. Do tests show that these substances pass through the membranes? If both starch paste and saliva are placed in the inner tube what happens? Record the results and conclusions of the experiment in the form of a table

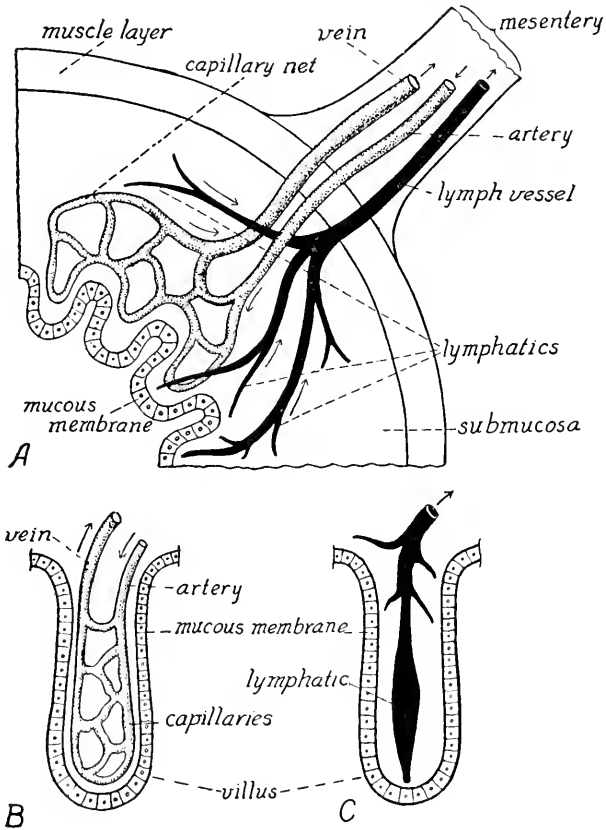


FIG. 13.—Blood and lymph vessels in the wall of the digestive tract; diagrammatic. *A*, a portion of the entire wall. *B*, capillaries in a villus of the intestine. *C*, lymphatic in a villus of the intestine. Arrows indicate direction of circulation.

such as the one shown in Fig. 14. Make diagrams to show the parallel between the apparatus used and the structure of the digestive tract. What facts can be cited against the physical process of osmosis as a complete explanation of the absorption of food in the living animal?

Absorption of Food						
Apparatus	Apparatus 1		Apparatus 2		Apparatus 3	
	Tube A	Tube B	Tube A	Tube B	Tube A	Tube B
Materials put in	Water Sugar	Water	Water Starch	Water	Water Starch Saliva	Water
Materials found after two days						
Conclusions						

FIG. 14.—Form of table for record of Exercise 10.

#### D. THE EXCRETORY AND REPRODUCTIVE SYSTEMS (URINO-GENITAL SYSTEM)

##### Exercise 11.—The Female Urino-genital System.

(a) The **ovaries**, which are the reproductive organs or gonads of the female, have been recognized and probably removed. The mesentery, or **mesovarium**, of each can possibly still be seen. The two **oviducts** are very long and coiled. They do not connect with the ovaries, but the funnel-shaped anterior end of each opens into the body cavity. To find the anterior ends of the oviducts it is necessary to lift the lungs and examine the extreme anterior wall of the coelom. Observe the ovaries and anterior end of the oviducts in a demonstration of a freshly killed specimen. The **eggs**, or female germ cells, break from the surfaces of the ovaries and are free within the body cavity until they pass into the open ends of the oviducts. Within the oviducts each egg is covered with a gelatinous substance which swells upon contact with the water and forms the conspicuous jelly envelopes after the eggs have reached the outside by way of the cloaca and anus. At its posterior end each oviduct expands to form a thin-walled portion in which the eggs accumulate just before being laid. This expanded portion of each oviduct is connected to the body wall, the large intestine, and the other oviduct by mesenteries which must be dissected away to show the real size and shape of this part of the oviduct. The oviducts open separately on the dorsal wall of the cloaca, opposite the opening of the urinary bladder.

(b) The **kidneys**, or excretory organs, have been identified behind the peritoneum of the dorsal wall of the cœlom. Carefully remove the right oviduct and completely expose the kidneys. The **ureter** is a slender, light-colored duct, arising from the outer edge of each kidney near its posterior end and extending backward to the cloaca. It conveys the urine from the kidney to the cloaca. Do not confuse the ureter with the several large, white nerves on the body wall dorsal to the ureters, or with the large, dark-colored blood vessel which enters each kidney close to its ureter. Difficulty in locating and tracing the ureter arises from the fact that it is attached to the dorsal surface of the expanded portion of the oviduct. The ureter is, however, entirely distinct from the oviduct; each oviduct and each ureter opens separately on the dorsal wall of the cloaca. Be prepared to demonstrate your dissection to the instructor and to explain the dorso-ventral relationships of the parts. Make a drawing ( $\times 3$  or  $4$ ) of the female urino-genital organs, including the cloaca and the bladder, as seen from the ventral aspect. It may be well to show only one of the oviducts. Indicate in this drawing the size and position of one ovary by a dotted outline. Be very careful to show accurately the relationship of ureters, oviducts, bladder, and cloaca. Examine a dissection of the urino-genital system of a male frog. Now take up the work of Exercise 13.

**Exercise 12.**—The Male Urino-genital System.

(c) The **kidneys**, or excretory organs, have been identified behind the peritoneum of the dorsal wall of the cœlom. The **testes**, which are the reproductive organs or gonads of the male, are a pair of small, elongated, light-colored bodies suspended from the anterior end of the kidneys. The mesentery that supports each testis is called a **mesorchium**.

(d) Running along the outer margin of each kidney is a slender, coiled structure, the **rudimentary oviduct**, which corresponds to the oviduct of the female, but is not functional in the male. These rudimentary oviducts expand at their posterior ends and connect with the dorsal wall of the cloaca opposite the place of attachment of the bladder, in the same manner as the functional oviducts of the female.

(e) The **ureter** is a slender, light-colored duct, arising from the outer edge of each kidney near its posterior end and extending backward to the cloaca. It conveys the urine from the kidney to the cloaca. Do not confuse the ureter with several large, white nerve cords on the body wall dorsal to the ureters, or with a large, dark-colored blood vessel which enters each kidney close to its ureter.



Difficulty in locating and tracing the ureter arises from the fact that it is attached to the dorsal surface of the expanded portion of the rudimentary oviduct. The ureter is, however, entirely distinct from the oviduct; each oviduct and each ureter opens separately on the dorsal wall of the cloaca. Each testis discharges its **seminal fluid**, containing the male germ cells, through the **ductus efferentes** (vasa efferentia), several very slender ducts which can perhaps be seen running through the mesorehium from testis to kidney. Inside the kidney these ducts connect with the ureter, which functions as a **ductus deferens** (vas deferens) through which the seminal fluid reaches the cloaca. Make a drawing ( $\times 3$  or  $4$ ) of the male urino-genital organs, including the cloaca and the bladder, as seen from the ventral aspect. Be very careful to show accurately the relationship of ureters, oviducts, bladder, and cloaca. Examine a dissection of the urino-genital system of a female frog.

#### E. THE CIRCULATORY SYSTEM

##### Exercise 13.—The Heart and Larger Arteries.

(a) Remove the large intestine, cloaca, bladder, oviducts, gonads, and fat-bodies, but be careful not to injure the kidneys, blood vessels,

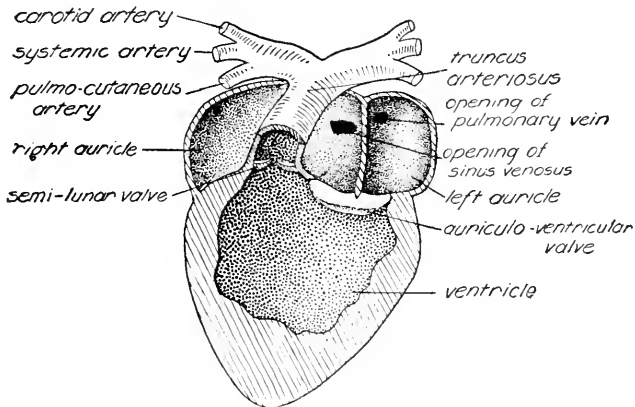


FIG. 15.—Heart of the frog, with the ventral walls of the ventricle and auricles removed.

and nerves. Cut away any remaining portions of the **pericardium**, the thin membrane surrounding the cavity in which the heart lies. The heart consists of three chambers: the **ventricle**, a thick-walled, conical portion, and in front of this the **right** and **left auricles** which are thin-walled and lie on each side of a single large vessel, the **truncus arteriosus**, which passes diagonally forward from the ventricle (Fig. 15).

Recall the action of the heart if observed in Exercise 4. What is its function?

(b) Blood leaves the heart through the **truncus arteriosus** which arises from the right side of the ventricle, runs obliquely forward over the ventral surface of the auricles, and then divides into right and left branches, each of which subdivides in the same manner. Using your forceps and a dissecting needle, follow the right branch as it divides into three arteries. The most anterior branch is the **carotid artery** which goes to the head. The most posterior branch of the **truncus arteriosus** is the **pulmo-cutaneous artery** which runs to the lungs, after sending a branch to the skin. The middle branch is the **systemic artery** which runs first in a dorsal and then in a posterior direction. A **subclavian artery** arises from the systemic artery and passes to the fore leg by the side of a conspicuous white nerve. Follow the right systemic artery posteriorly and find its place of union with the left systemic artery to form the **dorsal aorta**. At the place of union a large branch, the **celiaco-mesenteric artery**, is given off and runs through the mesentery to the digestive tract; this artery will have been partly removed. The dorsal aorta runs posteriorly in the mid-dorsal line. Carefully free the right margin of the right kidney and lift it enough to see the delicate **renal arteries** that pass from the dorsal aorta to the kidneys. Determine how the renal arteries arise and trace their distribution on the ventral surface of each kidney; use your handlens. Posterior to the kidneys the dorsal aorta divides to form the two **iliac arteries** which carry blood to the hind legs. Make a drawing ( $\times 5$ ) of the heart and principal arteries as thus dissected, from the ventral view; the kidneys are not to be shown. Use arrows to indicate the direction of blood flow. Be sure you know to what organs these vessels are carrying blood; the general relationships are shown in Fig. 16 A. Larger frogs in which a colored mass has been injected into the arteries should be examined if available.

#### Exercise 14.—The Veins.

(c) Some of the principal veins, especially those making up the hepatic portal system, were removed from your specimen with the digestive and reproductive organs. In the region of the kidneys the veins should be in place. Find the **renal portal veins** that extend from the hind legs to the lateral margins of the kidneys, just anterior to where the ureters emerge. Lift the outer edge of the right kidney and trace the right renal portal vein as it branches on the dorsal surface of the kidney. What is a portal vein? The **dorso-lumbar vein**, which has been cut on the right side in freeing the kidney, can

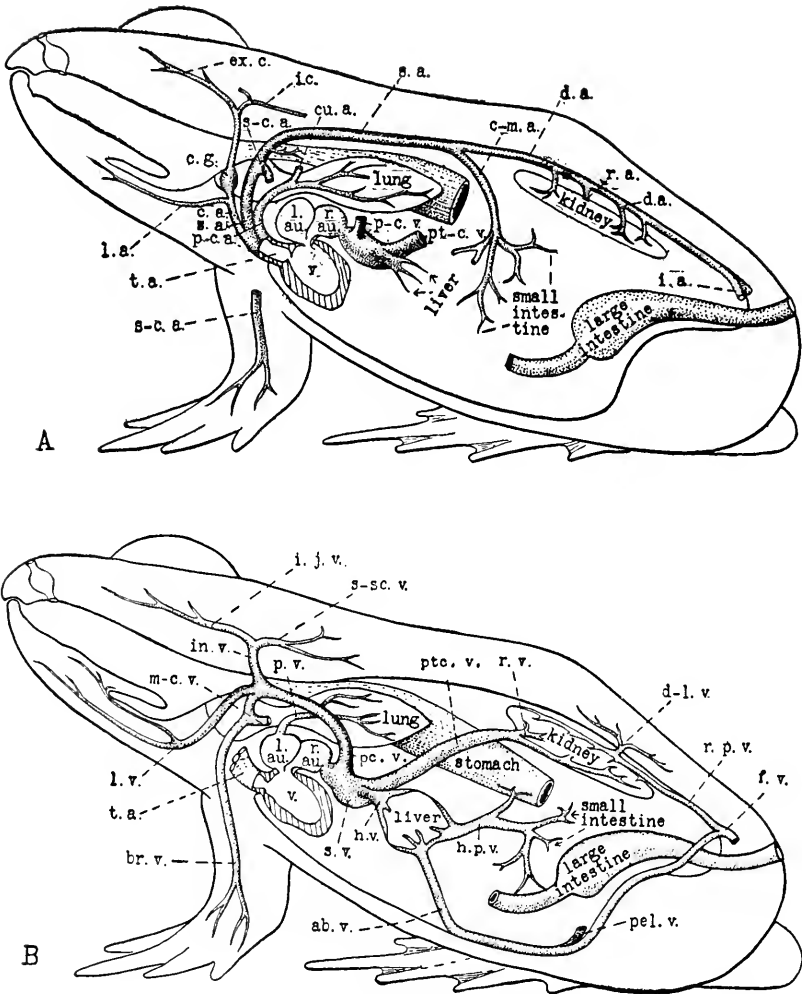


FIG. 16.—The circulatory system of the frog; from lateral view. *A*, arteries. *B*, veins. *ab.v.*, abdominal vein; *br.v.*, brachial vein; *c.a.*, carotid artery; *c.g.*, carotid gland; *c-m.a.*, coeliaco-mesenteric artery; *cu.a.*, cutaneous artery; *d.a.*, dorsal aorta; *d-l.v.*, dorso-lumbar vein; *ex.c.*, external carotid artery; *f.v.*, femoral vein; *h.p.v.*, hepatic portal vein; *h.v.*, hepatic vein; *i.a.*, iliac artery; *i.c.*, internal carotid artery; *i.j.v.*, internal jugular vein; *in.v.*, innominate vein; *l.a.*, lingual artery; *l.a.u.*, left auricle; *l.v.*, lingual vein; *m-c.v.*, musculo-cutaneous vein; *p-c.a.*, pulmo-cutaneous artery; *p-c.v.*, left precaval vein; *pel.v.*, pelvic vein; *pt-c.v.*, postcaval vein; *p.v.*, pulmonary vein; *r.a.*, renal artery; *r.a.u.*, right auricle; *r.p.v.*, renal portal vein; *r.v.*, renal vein; *s.a.*, systemic artery; *s-c.a.*, subclavian artery; *s-sc.v.*, subscapular vein; *s.v.*, sinus venosus; *t.a.*, truncus arteriosus; *v.*, ventricle.

be seen on the left side, passing from the muscles of the back to the dorsal surface of the kidney. On the ventral surface of each kidney you will see the conspicuous **renal veins** which pass toward the mid-line to unite and form a single median vessel, the **postcaval vein**. This vein passes through the liver and part has been removed with the liver; find the cut end anterior to the kidneys. Understand by what vessels blood enters and leaves the kidneys and what the blood loses and gains in these organs.

(d) Lift the tip of the ventricle of the heart and observe the thin-walled **sinus venosus** on the dorsal surface of the heart. A circular

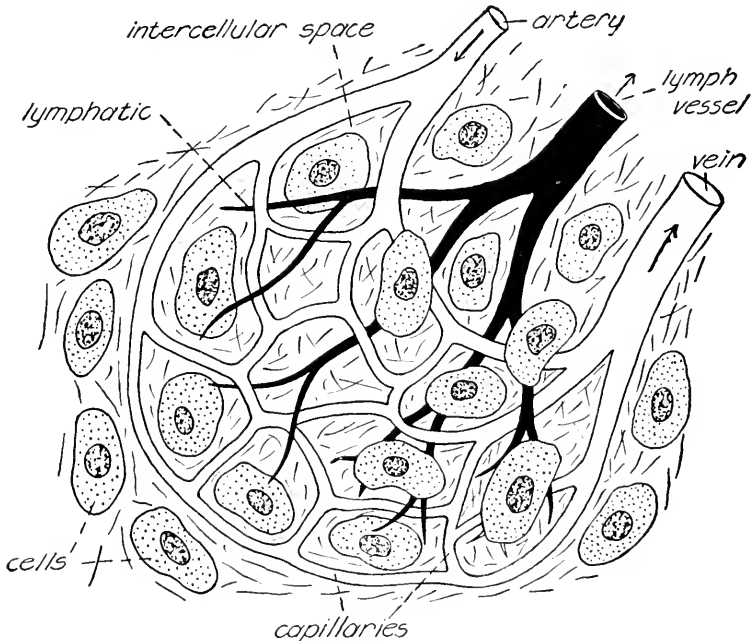


FIG. 17.—The capillaries and lymphatics in relation to cells throughout the body; diagrammatic.

opening in the sinus venosus will be seen; this is the place where the postcaval vein enters the heart. If your specimen has been dissected carefully, you will be able to find the two **precaval veins** which enter the sinus venosus anteriorly on each side. From what regions of the body do these veins return blood to the heart?

(e) Supplement what you have been able to see in your own specimen by observations on demonstration specimens. Study the diagrams of the circulatory system so that you become thoroughly acquainted with the names and distribution of the principal blood ves-

sels (Fig. 16). Be prepared to discuss the circulation of blood in terms of what is carried to and from the different organs of the body.

(f) Make a drawing ( $\times 5$ ) of the veins as dissected in your own specimen. Spread the kidneys apart in the mid-line and show them in outline. Pay especial attention to the representation of the origin of the postcaval vein. The branches of the renal portal veins are to be indicated by broken lines in the outline of the right kidney. Use arrows to indicate the direction of blood flow.

#### **Exercise 15.—The Capillaries.**

(g) Examine a demonstration of the circulation of the blood through the capillaries in the web of a frog's foot. How can you distinguish arteries, capillaries, and veins? Notice the pulse. Why does it occur? In what vessels is it seen? What is the relative size of the **blood cells**, or **corpuscles**, and the smallest blood vessels? Can you distinguish more than one kind of blood cell? Capillaries like these are present in all the organs of the body of a vertebrate animal (Fig. 17). What important relationships do capillaries have to metabolism? Understand the relationship between the smallest lymph vessels and the blood capillaries.

### **F. THE RESPIRATORY SYSTEM**

#### **Exercise 16.—The Lungs and Air Passages.**

(a) Remove the heart but do not injure the **lungs**. Carefully clean off the area between the anterior ends of the lungs and find the semi-transparent floor of the **larynx** with which the lungs are continuous.

(b) Study the mouth cavity again. Observe the two **internal nares** close to the vomerine teeth on the roof of the mouth cavity. Pass a bristle through the **nasal canal** on one side. Find the **glottis**, a vertical slit in a slightly raised area ventral to the esophagus on the floor of the mouth cavity. The posterior forked end of the tongue extends to the sides of the glottis in the living frog. Pass one end of your forceps through the glottis and observe the tip through the floor of the larynx. What is the course of air from the outside to the lungs? Compare with man (Fig. 18). Notice the openings of the **Eustachian tubes** on the roof of the mouth cavity near the angles of the jaws. These tubes connect the mouth cavity and the cavities of the middle ears (*cf.* Fig. 19). Understand their relationship to the respiratory system of aquatic vertebrates.

(c) Watch a living frog under a bell-jar. Time the respiratory movements of the nostrils, the floor of the mouth cavity, and the sides of the body. Understand the method of inhaling and exhaling air in

the frog and in man. Make comparisons with the drawing of air in and out of a pipette and with the pumping up of a tire. Why is it that a man can breathe through either his nose or mouth but the frog can breathe only with its mouth closed?

(d) Remove the esophagus, the lungs, the floor of the mouth cavity, and the lower jaw by lifting up the end of the esophagus where it was cut from the stomach and carefully cutting the attaching structures as you pull; cut at the corners of the mouth as necessary. With scissors, separate the removed piece into right and left halves by a cut passing through the glottis and exactly along the mid-line. Ob-

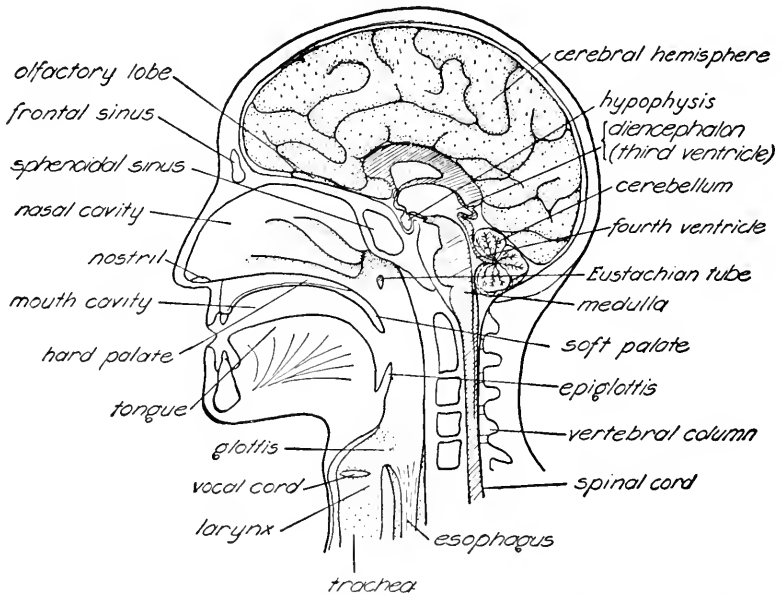


FIG. 18.—The human head, shown as if cut in the median, longitudinal plane; semidiagrammatic.

serve the size and shape of the larynx as seen from the inside, and find the openings into the lungs. The **vocal cords** are ribbonlike structures attached to the side walls of the larynx. How do they function? Compare this dissection with "half-frogs" prepared by the instructor. Make a drawing ( $\times 3$ ) of your dissection as seen from the cut surface. Indicate by arrows the course of the air from the outside to the lungs. Cut one lung lengthwise and observe its folded lining. What are the functions of the lungs?

## G. THE SENSE ORGANS AND THE NERVOUS SYSTEM

**Exercise 17.—The Ear.**

(a) In order to remove the skin from the ear make a V-shaped incision in the skin, with the point just in front of the ear and the sides of the V passing above and below it. Hold the skin at the point of the V with forceps, slowly pull it backwards, and expose a circular area still covered with the delicate **tympanic membrane** which is thickened at its center. Cut through this membrane, avoiding the thickened center, and thus lay open the cavity of the **middle ear** which connects with the mouth cavity by way of the **Eustachian tube** (Fig. 19). What is the function of the Eustachian tube? Demon-

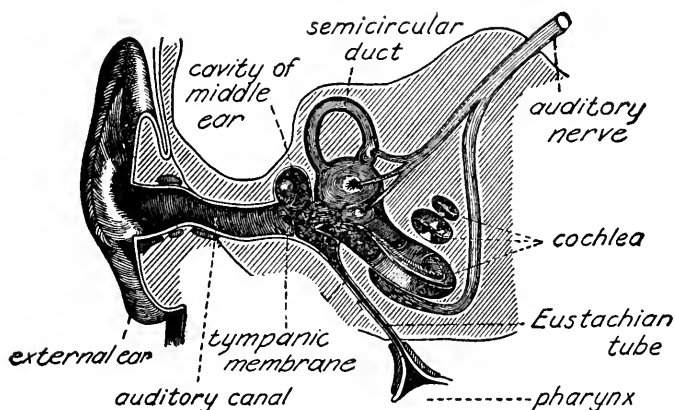


FIG. 19.—The human ear; diagrammatic. The cavity of the middle ear contains the ear-bones that transmit vibrations from the tympanic membrane to the inner ear and is connected by the Eustachian tube with the pharynx.

(From T. Hough and W. T. Sedgwick, "Human Mechanism," copyright, 1918, by Ginn and Co., reprinted by permission.)

strate its relation to the eardrum in the human body by holding your nose, closing your mouth, and exhaling. Notice the **columella** of the frog's ear, a slender bone extending upward and inward from the thickened center of the tympanic membrane to the skull. The function of the columella is to transmit sound waves across the cavity of the middle ear from the tympanic membrane to the nerve endings of the inner ear. The inner ear is embedded in the bone of the skull and is difficult to dissect. Understand its structure from demonstrations. A model of the human ear and dissection of a shark's inner ear should be examined if available.

**Exercise 18.**—The Eye.

(b) Remove the skin from the dorsal surface of the head. Take out the eyeball after cutting the **muscles** by which it is attached to its **socket**. What is their function? The **optic nerve** can be recognized where it enters the back of the eyeball (Fig. 20). The outermost layer on the back of the eyeball is the **sclera**. It is continuous with the transparent **cornea** in front. Notice the **iris** with its opening, the **pupil**. What is the function of the iris? Use your scissors to open the eyeball by a cut from front to back. Study the pieces under water. The **lens** is just behind the iris and, if not detached by the cutting, will be found attached to the iris. Internally, the eyeball is

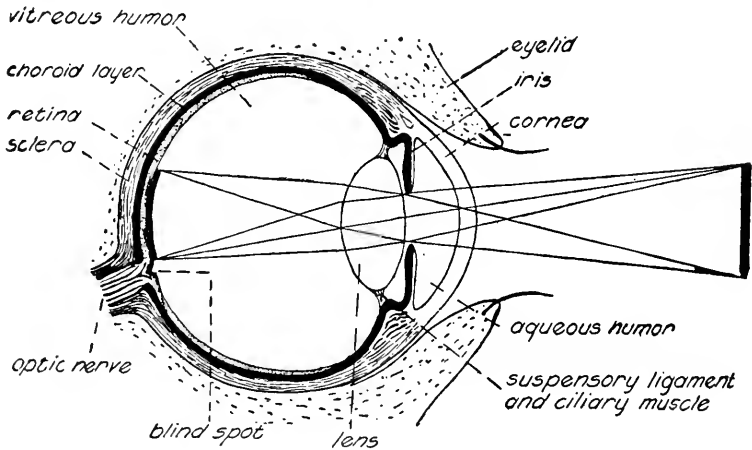


FIG. 20.—The eye, in section, showing details of structure and also the manner of formation of an image upon the retina; diagrammatic.

(Adapted from a figure in C. Hill, "Manual of Histology and Organology," copyright, 1923, by W. B. Saunders Co., printed by permission.)

separated into an outer cavity which lies between the lens and the cornea and contains a fluid called the **aqueous humor**, and an inner cavity which is filled with the **vitreous humor**. On the inner surface of the sclera, and closely applied to it, is the dark-colored **choroid**, a layer which is continuous with the iris. The innermost layer, which lines the cavity behind the iris, is the **retina**. In life the retina is closely applied to the choroid but may become separated from it during dissection. The retina is the layer with which the optic nerve is continuous. Other parts of the eye are devices to protect the retina and to transmit the light rays to it in the form of a sharp image. Make a drawing ( $\times 10$  or  $15$ ) of the eyeball as now opened. After completing this drawing, remove the lens, lay it on a printed



page, and observe the result. Examine a model of the human eye or dissection of a mammalian eye if available.

**Exercise 19.**—The Spinal Nerves and Sympathetic Trunks.

(c) The **spinal nerves** are visible on the dorsal wall of the body cavity as pairs of whitish cords running laterally from the region of the **spinal column**, or backbone. They arise from the spinal cord (Fig. 21) and emerge in pairs between the vertebræ which make up the spinal column. Surrounding the place of exit of each nerve is a light-colored mass known as the **periganglionic gland**. The **sympa-**

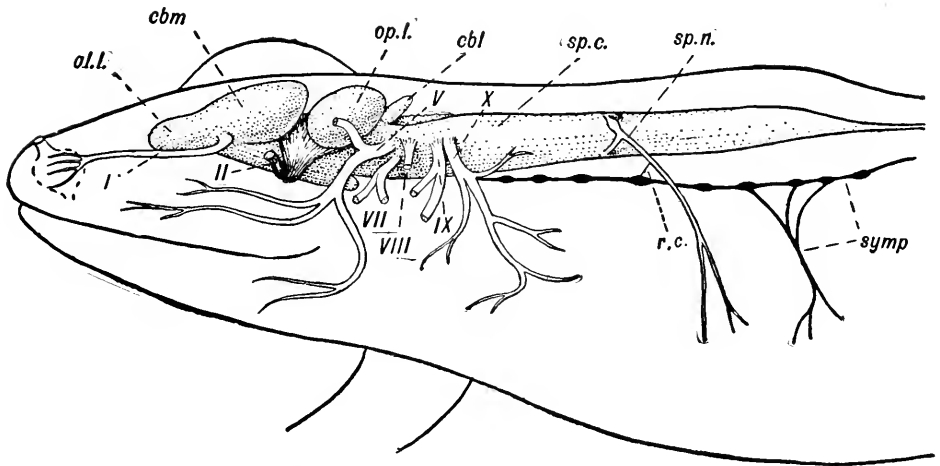


FIG. 21.—Nervous system of the frog, from lateral view. *cbl*, cerebellum; *cbm*, cerebral hemisphere or cerebrum; *ol.l.*, olfactory lobes; *op.l.*, optic lobes; *r.c.*, ramus communicans; *sp.c.*, spinal cord; *sp.n.*, spinal nerve; *symp.*, part of the sympathetic system; I, II, V, VII, VIII, IX, and X, cranial nerves. The third, fourth, and sixth cranial nerves are small and not shown in this figure.

**thetic trunks** consist of a delicate pair of nerves running parallel to the spinal column on each side and ventral to the spinal nerves. Each spinal nerve is attached to the neighboring sympathetic trunk by a **ramus communicans**. Nerves which have been removed extended ventrally from these main trunks of the sympathetic system to the organs of the body cavity. Parts of the sympathetic trunks will be found in your specimen and should be distinguished clearly from the spinal nerves. There are ten pairs of spinal nerves, designated by numbers beginning anteriorly. The large nerves which were seen in the examination of the subclavian artery are the second pair. Trace one of them as far as you can, separating the muscles of the fore leg

as necessary. The first nerve is a small one just in front of the second, to which it sends a branch. The third is a small nerve which comes close to the second and sends off a communicating branch. These connections between the first three nerves make up the **brachial plexus**. The fourth, fifth, and sixth nerves are very slender and run obliquely outward over the muscles of the back. What region do they seem to supply? The seventh, eighth, and ninth nerves are larger and run almost directly backward. They have various communicating branches which form the **sciatic plexus**, as a continuation of which the **sciatic nerve** will be seen. Follow it in one hind leg as far as you can, noticing the blood vessels, the **sciatic artery** and **sciatic vein**, which accompany the nerve. The tenth nerve is small and may be hard to find. It comes out through an opening in the **urostyle**, the elongated bone which terminates the spinal column, and runs almost directly backward to supply the bladder and cloaca. What is the function of nerves? Why are some of the spinal nerves larger than others? What is the significance of the brachial and sciatic plexuses? Make an outline ( $\times 3$ ) of the central portions of the spinal column. Add to this the spinal nerves, representing accurately their origin, courses, and relative sizes. Whatever part you have seen of the sympathetic system should be included in the drawing.

**Exercise 20.**—The Central Nervous System and the Origin of the Nerves.

(d) Cut off the fore legs close to the body. Remove the hind legs together by cutting across the posterior part of the trunk, and save them for a study of the muscles. The following dissection will be made more readily if the head and trunk are held in one hand with the axial skeleton supported by the forefinger. Remove the skin and muscles from the back with scissors and scalpel until the skull and vertebral column can be seen. Find the opening at the posterior end of the skull where the most anterior vertebra is attached. Insert the scissors into this opening in such a way as not to damage the soft brain, and make two short cuts in the form of a "V." The cut bone can be broken away with the forceps and the breaking continued forward until the entire **brain**, covered by its membrane, is exposed. Do not damage the membrane. Continue the dissection posteriorly by cutting off the dorsal part of each vertebra with a scalpel. To see the spinal cord more clearly break the vertebrae right and left with the fingers; it is also covered by a membrane. What is the importance of the large blood supply to the central nervous system as indicated by the numerous blood vessels in the mem-

branes? What happens when the blood supply of the human brain is reduced or shut off in limited areas?

(e) The brain and spinal cord are to be studied in place, but the membrane covering them should now be removed in order that the parts of the central nervous system can be seen more clearly. Beginning at the anterior end, the following parts of the brain can be identified as it lies in place: (1) The **cerebral hemispheres**, or **cerebrum**, a pair of elongated, nearly cylindrical structures, from the anterior ends of which the pair of **olfactory lobes** is indistinctly separated. (2) The **diencephalon**, a somewhat depressed region, the top of which may be covered by a very vascular, thin roof, the **anterior choroid plexus**; if this has been removed, the cavity of the **third ventricle** will be seen. (3) The **optic lobes**, a pair of prominent rounded bodies. (4) The **cerebellum**, a narrow transverse ridge just posterior to the optic lobes. (5) The **medulla oblongata**, part of the roof of which is thin, very vascular, and known as the **posterior choroid plexus**. This covers the **fourth ventricle**, which will be exposed by the removal of the choroid plexus. The medulla is the most posterior region of the brain and is continuous with the **spinal cord**. The spinal cord is not uniform in diameter throughout its length. Observe the slightly enlarged anterior **brachial region** and posterior **lumbar region**. What groups of spinal nerves arise in these regions? Beyond the lumbar region the spinal cord tapers rapidly and ends in the **filum terminale**.

(f) Ten pairs of **cranial nerves** arise from the brain, and ten pairs of **spinal nerves** arise from the spinal cord in the frog (Fig. 22). Many of these nerves are too small to be seen easily but certain ones can be identified. Four pairs of cranial nerves can usually be found. The **olfactory nerves**, or first pair of cranial nerves, can be exposed by cutting away the bone immediately in front of the olfactory lobes; they are short nerves running to the nasal cavities. If they were not destroyed during the removal of the eyes the **optic nerves**, or second pair, can be seen by gently pressing the brain away from the side of the skull; they arise from the ventral surface of the brain. The **trigeminal nerves**, or fifth pair, arise from the anterior end of the medulla on each side and run to the face. The **auditory nerves**, or eighth pair, can usually be found near the fifth and run to the inner ear. Carefully push the spinal cord away from the vertebræ and find the roots, or places of origin, of the spinal nerves studied in Exercise 19. Draw the brain and spinal cord ( $\times 3$ ) as seen from dorsal view. Indicate the positions of the nerves you have found.

(g) The brain and spinal cord can now be removed from the animal. Insert a scalpel under the spinal cord toward its posterior end and lift it carefully. Cut any membranes or nerve roots that tend

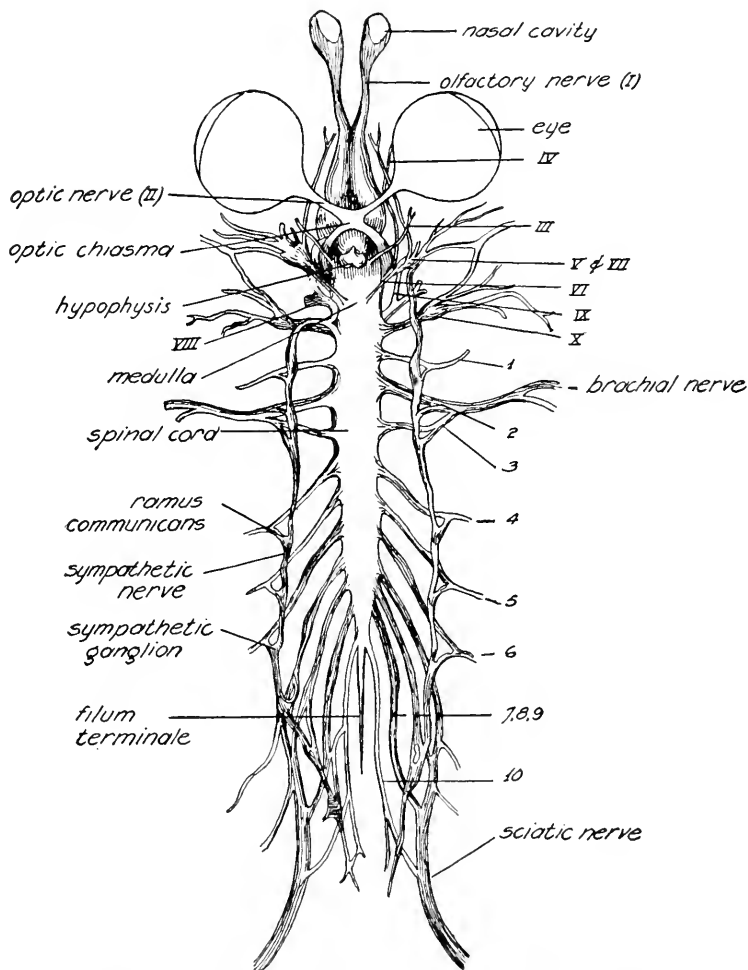


FIG. 22.—Nervous system of the frog, from ventral view. I-X, cranial nerves; 1-10, spinal nerves.

(Redrawn from R. Wiedersheim, "Comparative Anatomy of Vertebrates," copyright, 1907, by The Macmillan Co., printed by permission.)

to hold it in place, working toward the anterior end. In the region of the brain cut the optic nerves. Note the hypophysis, or pituitary gland, which is attached to the brain; this is a gland, not a part of the central nervous system. It can be lifted out with the brain.

After the olfactory nerves are cut the entire central nervous system can be placed in a watch glass and studied under water from the dorsal, ventral, and lateral aspects. Make any necessary corrections of your drawing. Identify again the **third** and **fourth ventricles**. Cut away the dorsal surface of the cerebral hemispheres and find the **first** and **second ventricles**, or **lateral ventricles** as they are called. The narrow canal connecting the third and fourth ventricles will probably not be seen. The fourth ventricle is continuous with the **central canal** which runs the length of the spinal cord. The cavity of the central nervous system contains the **cerebrospinal fluid**. It should be recalled that one of the distinguishing characteristics of the Phylum Chordata, of which the frog is a member, is the presence of a tubular central nervous system which is dorsal in position.

**Exercise 21.**—Localization of Functions in the Sensory-neuro-muscular System.

(h) It is possible to obtain some information concerning the localization of functions in the sensory-neuro-muscular system by successive observations on frogs from which parts of the nervous system have been removed. The reactions of a normal frog and of frogs in which the following parts of the nervous system have been destroyed will be demonstrated: (1) the cerebral hemispheres and diencephalon; (2) the entire brain; and (3) the entire central nervous system. In the frog in which the entire central nervous system has been destroyed, watch what happens when the sciatic nerve is cut, pinched, or otherwise stimulated. What happens when an electric current or other stimulus is applied directly to the gastrocnemius muscle? Observe a frog's heart which has been removed from the animal and placed in warm 0.7% salt (sodium chloride) or Ringer's solution. Your observations should be recorded in a table such as the one shown in Fig. 23.

Localization of Functions in the Sensory-neuro-muscular System

Frog	Parts Removed	Parts Present	Behavior
A			
B			
C			
D			

FIG. 23.—Form of table for record of Exercise 21.

Make a diagram of the central nervous system and indicate what parts have been present in each of the specimens used. Also make a diagram of a cross section of the spinal cord through the roots of a

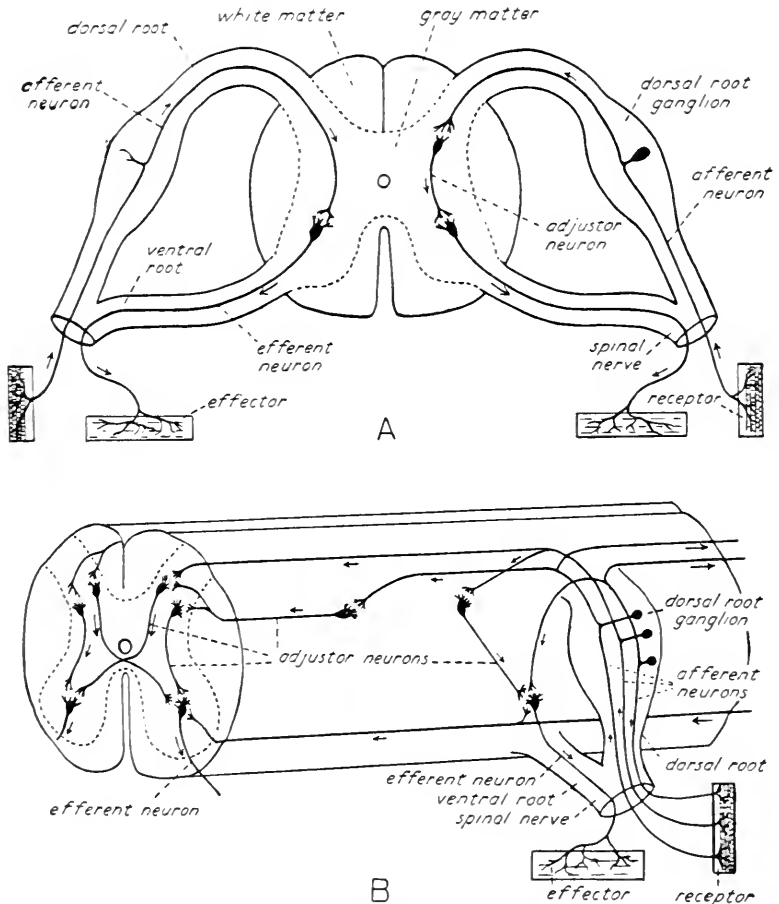


FIG. 24.—A, the spinal cord, in cross section, showing a pair of spinal nerves and the essential parts of a reflex arc; diagrammatic. The neurons necessary for the simplest type of reflex action are present on the left, while those of a typical reflex arc are represented on the right. B, the spinal cord showing some relations of neurons in reflex arcs; diagrammatic. The brain would lie to the right as the diagram is constructed. Arrows in both diagrams indicate the direction of transmission of the nervous impulses.

pair of spinal nerves and show the positions of the neurons of a typical reflex arc (cf. Fig. 24). On the basis of your observations, write out statements of the functions localized (1) in the sense organs, (2) in the peripheral nervous system, (3) in the spinal cord, (4) in the

medulla, cerebellum, and optic lobes, (5) in the diencephalon and cerebral hemispheres, and (6) in the muscles. In addition, on the basis of your observations and reading, state (7) the function of the entire central nervous system and (8) the function of the nervous system as a whole.

(i) When, in the course of the demonstrations mentioned in the preceding paragraph, did the frog die? Write a brief statement justifying your answer and explaining the meaning of life and death.

## H. THE MUSCULAR SYSTEM

### Exercise 22.—The Muscles.

(a) Remove the skin from an uninjured hind leg. The flesh is now seen to be made up of a considerable number of **muscles** which may be readily separated from each other. These muscles fall into two classes: the **flexors**, which bend a joint, and the **extensors**, which straighten it. Each muscle has a thickened middle portion and tapers toward the ends. Muscles are attached to bones by **tendons** which are continuous with the connective tissue sheath that surrounds and helps to bind together the numerous muscle cells that make up each muscle.

(b) Study the **gastrocnemius muscle**, the large muscle in the calf of the leg. To what bones is it attached? The large tendon at its posterior end is the **tendon of Achilles**. Compare this with the same tendon in man and other animals. Determine the relation of this muscle to movements of knee and ankle by moving the joints. Does it serve as flexor or as extensor for these joints? Look on the front of the leg for muscles which oppose the action of the gastrocnemius in the ankle region. Observe the two sets of muscles in the thigh. To what bones are they attached? What muscles are brought into play when the frog suddenly straightens the leg, as in jumping or swimming? Remove the muscles of the thigh, noticing their attachments and their mechanical possibilities. Make a diagrammatic drawing ( $\times 2$ ) showing the relation of the gastrocnemius muscle to the bones of the leg. Indicate, in the diagram or in writing, the motions produced by its contraction.

## I. THE SKELETAL SYSTEM

### Exercise 23.—The Skeleton.

(a) Study mounted skeletons. Identify the major parts and learn the names of the chief bones by reference to the list given here. Compare the skeleton of the frog with that of man and any other forms that are available. How do the several parts correspond and

how do they differ? Make a sketch of the skeleton of the frog from the dorsal view, showing as many of the bones as possible. The bones of the skeleton may be classified as follows:

I. The Axial Skeleton: the Skull and Vertebral Column.

The **skull** includes the **cranium**, or brain case, and the bones of the **face** and **jaws**.

The **vertebral column** is composed of nine **vertebræ**. Posterior to the last vertebra is the elongated **urostyle**. The first or most anterior vertebra is the **atlas** and has no **transverse processes** which are found on the other **vertebræ**. The ninth or most posterior vertebra is the **sacrum**, to which the hip girdle is attached.

II. The Appendicular Skeleton: Girdles and Appendages.

The shoulder girdle includes the **sternum**, or breastbone; the **clavicle**, or collar bone; the **coracoid**, a large bone just posterior to the clavicle; and the **scapula** and **suprascapula**, which together constitute the shoulder blade. The **hip girdle**, which is also called **pelvic girdle** and **innominate bone**, is represented in the frog by a V-shaped bone formed by the fusion of three paired bones, the **ilium**, the **ischium**, and the **pubis**. The hip girdle is attached to the sacrum by the elongated ilia which are seen parallel to the urostyle.

The **fore** and **hind limbs** are articulated with the shoulder and hip girdles respectively. The identity of the fundamental plan in each pair of limbs is shown by the following tabulation in which the bones are listed in order from the proximal to the distal end of the leg, that is, beginning with the bone which is articulated with the girdle.

BONES OF THE FORE LEG	BONES OF THE HIND LEG
<b>Humerus</b> (upper arm).	<b>Femur</b> (thigh).
<b>Radio-ulna</b> (forearm).	<b>Tibio-fibula</b> (shin).
<b>Carpals</b> (wrist), six small bones.	<b>Tarsals</b> (ankle), two long bones and several small ones.
<b>Metacarpals</b> (hand).	<b>Metatarsals</b> (foot).
<b>Phalanges</b> (fingers).	<b>Phalanges</b> (toes).

**Exercise 24.**—Joints.

(b) Remove the muscles from the **hip joint**, or place where the femur is articulated with the hip girdle. The joint is not at once exposed as it is covered by a thin, tough membrane known as the **capsular ligament**. This ligament attaches the femur to the hip girdle



and also encloses a cavity in the joint, the **synovial cavity**. In the living animal this cavity contains a small amount of fluid, the **synovial fluid**, which serves to lubricate the joint. Cut through this ligament and expose the **head** of the femur. Notice how it fits into its **socket**. In what directions can such a joint move? The hip joint is known as a ball-and-socket joint. Study the knee joint in like manner. How does it differ from the hip joint? The knee joint is known as a hinge joint.

## II. RELATED FORMS

**Exercise 25.**—The Frog's Relatives.

(a) It will be recalled from your reading that the Subphylum *Vertebrata* to which the Class *Amphibia* belongs is a division of the Phylum *Chordata*. Examine specimens of *Dolichoglossus* (*Balanoglossus*), representing the Subphylum *Hemichorda*; various tunicates or sea-squirts, representing the Subphylum *Urochorda*; and *Branchiostoma* (*Amphioxus*), representing the Subphylum *Cephalochorda*. Understand why these animals and the vertebrates are classified together as chordates.

(b) What are the distinguishing characteristics of the Subphylum *Vertebrata*? It is divided into seven classes. Understand the basis of this division, and examine specimens from the several classes. Recall any information concerning the internal structure of these vertebrates as compared with the frog.

## III. HISTOLOGY

### A. THE MICROSCOPE

**Exercise 26.**—The Parts of a Microscope and Their Use.

(a) Before beginning the study of histology, or microscopic anatomy, you must learn how to use the microscope. In this exercise it is important that you become familiar with the names of the parts of your instrument and the correct procedure for microscopic study. Since the microscope is an expensive instrument of precise construction it must be handled carefully and kept clean. The metal parts and the mirror can be cleaned with soft cloth, but the lenses must be cleaned only with lens paper or they may be scratched and permanently damaged. Do not unscrew any part of or attempt to repair your instrument; report any unusual condition immediately.

(b) The microscope is an instrument which magnifies objects. The **compound microscope** with which you are working consists essentially of two sets of **lenses** fixed in a **tube** by means of which they can be moved with reference to, or **focused** on, the object to be magnified,

which is placed upon the **stage** of the instrument (Fig. 25). The lenses that are attached to the **nosepiece** at the lower end of the tube, or near the object to be studied, are known as the **objectives**. There are two objectives, the shorter of which is a **low-power objective** and magnifies less than the longer or **high-power objective**. Since the nosepiece can be rotated either of these objectives can be brought into line with the tube. In addition to the objectives there are two other lenses, the **oculars**, or **eyepieces**, either of which can be inserted into the upper end of the tube or the end near the eye. The longer or **low-power ocular** magnifies less than the shorter or **high-power ocular**. The tube is raised or lowered with reference to the stage, and the object thereby brought into focus, by means of the adjustment screws, of which there are two. One of these moves the tube conspicuously and is called the **coarse adjustment**; it is used only when the low-power objective is in line with the tube. The other moves the tube very slightly and is called the **fine adjustment**; it is used with the high-power objective and to get a very sharp focus.

(c) In order to see a highly magnified object it is necessary to have it well illuminated. For most of your microscopic work the necessary illumination is obtained by transmitted light, that is, by light that passes through the object of study. The parts of the microscope that are used for illumination are located below the stage. The **mirror**, which is flat on one side and concave on the other, can be moved in any direction in order to reflect light from the sky or from a lamp through the hole in the stage. In many microscopes there is a set of lenses constituting the **condenser** between the mirror and the stage. This is a device for increasing the number of light rays that pass from the mirror through the object. If your microscope has no condenser and is near the source of light, use the flat surface of your mirror; otherwise, the concave surface will give better illumination. Sometimes, as with very transparent objects, it is desirable to reduce the amount of light, and it is always necessary to have brighter illumination when working with the high-power objective. The **iris diaphragm** is a shutter by means of which the degree of illumination can be varied. Find the lever by which it can be opened and closed.

(d) Objects to be studied with the compound microscope are **mounted**, either temporarily or permanently, on **glass slides**. Make a temporary mount of a small piece of paper ruled in millimeter squares. Clean a slide and a cover glass with a soft cloth; it must not be forgotten that success with microscopic work depends largely on cleanliness of lenses, slides, and cover glasses. Place the piece of paper near the middle of the slide and cover with a drop of water. Pick up

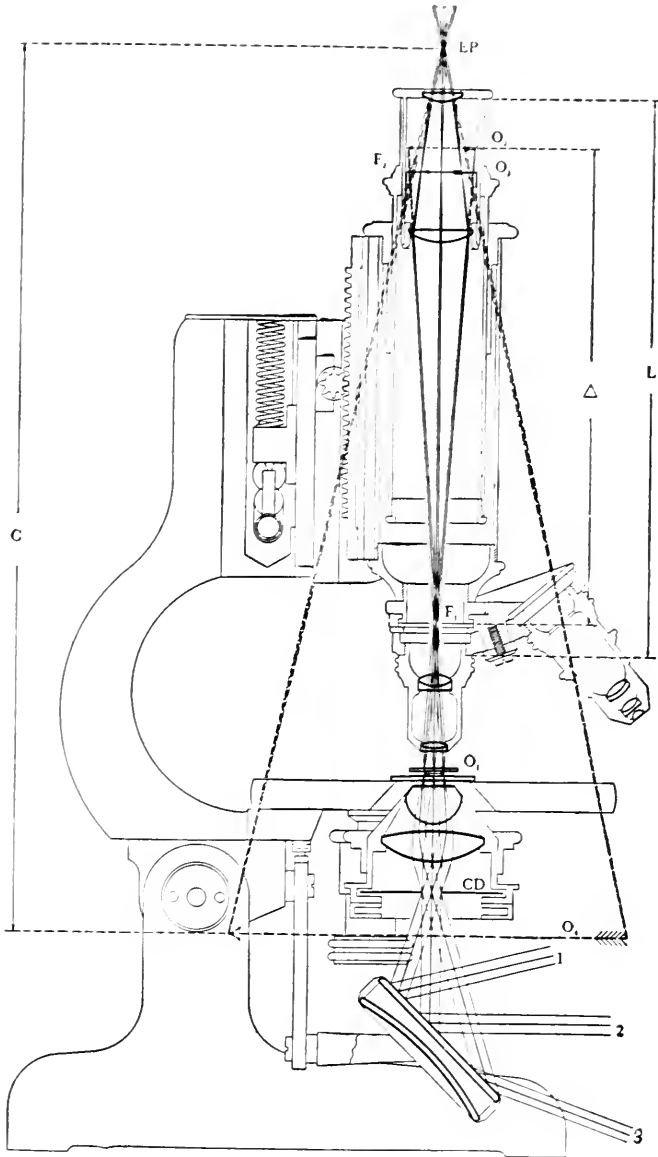


FIG. 25.—Diagram of a compound microscope, showing the passage of the light rays (1, 2, and 3) through the lenses to the eye (*EP*). The other letters refer to the manufacturer's description and need not be considered here.

(Courtesy of Bausch and Lomb Optical Company.)

the cover glass in such a way as not to soil it and, placing one edge of it at the edge of the drop of water, let it fall so that it covers the piece of paper. If this is done correctly there will be no air bubbles imprisoned between the slide and cover glass and no excess of water. Practice until you are successful, drying both slide and cover glass between attempts.

(e) Place the microscope squarely in front of you and adjust the height of your chair so that you look down the tube comfortably. Do not shift the microscope about after you get it ready for work. Have the low-power objective in line with the tube and 3 to 5 millimeters above the stage. Put the low-power ocular in position and open wide the iris diaphragm. Now look down the tube, keeping both eyes open, and move the mirror until you can see a circular area that is evenly and brightly illuminated; this is the **field** of the microscope. Place your mount of the piece of paper on the stage so that the paper is directly over the center of the opening. As you look down it raise the tube, by means of the coarse adjustment, until you see the lines clearly. How far away do they appear to be? How large do the millimeter squares seem to be? Move the slide to the right. Which way did the lines move as you watched them? Learn to move the object so that the image goes where you want it to go. Open and close the iris diaphragm and observe how the illumination is altered. Focus with the fine adjustment and observe the results. In which direction do you need to turn the adjustment screws to move the tube up; to move it down?

(f) Follow carefully the steps in changing to the high-power objective. Place a junction of two lines exactly in the center of the field and focus sharply. Without changing the focus or the position of the slide, rotate the nosepiece and thus bring the high-power objective in line with the tube. The lines will be seen indistinctly or not at all. Focus carefully up through one turn of the fine adjustment screw. If the lines do not come into focus reverse the turn and then focus down through one turn of the fine adjustment screw. Try the change from the low-power objective to the high-power objective several times and learn which way the fine adjustment screw must be turned, and how much, for your particular instrument. To master this operation now will save much time later. Do you find that you need to open the iris diaphragm to see clearly with the higher magnification? The low-power ocular will usually give you better results with the high-power objective than the high-power ocular will. Unless instructed to the contrary, use the low-power ocular with the high-power objective in subsequent exercises.

**Exercise 27.**—Determination of the Magnification Obtained with Different Combinations of Lenses.

(g) In order to determine the actual sizes of objects studied with the microscope it is necessary to know how many diameters the several combinations of lenses will magnify. This can be determined as follows, using first the low-power ocular and low-power objective. Focus sharply on the ruled lines of the preparation of millimeter paper, looking with your left eye. At the same time, looking with your right eye at your millimeter ruler on the table, measure one line and the space between two adjacent parallel lines as they appear when projected to the level of the table. This will seem difficult at first but can be done quite accurately. Since the lines are actually 1 mm. apart the measured distance will give you the degree of magnification obtained, at table level, with the low-power ocular and low-power objective of your microscope. Make several measurements and compare your results with those of your neighbors, keeping a record for check by the instructor. Repeat the process, using the high-power ocular instead of the low-power ocular but with the low-power objective still in position. Record your result for this second combination of lenses.

(h) Place the low-power ocular in the tube again and focus with the high-power objective. With this combination of lenses you cannot see a complete square millimeter; you see a smaller area at a greater magnification. In order to find out what the magnification actually is measure the diameter of the field. Then, move the slide so that one edge of the field coincides with the left edge of one of the ruled lines. Notice a fiber of the paper which is conspicuous and which coincides with the opposite side of the field. Move the slide until this fiber reaches the edge of the field first occupied by the line. Repeat this operation until you reach the left edge of the next ruled line; measure one line and one space. Multiply the measured diameter of the field as projected to table level by the number of times you shifted the slide in passing from one line to the next. This will give you the magnification with this combination of lenses. Insert the high-power ocular and observe the fibers of the paper. The magnification with this combination of lenses is very difficult to obtain by the method previously used. It can be calculated with reasonable accuracy on the basis of ratios if your determinations for other magnifications were carefully made. Record the magnifications for all combinations of lenses for use later.

(i) At the close of each laboratory period leave the low-power objective in line with the tube. Remove the slide from the stage;

clean and dry both slide and cover glass. Do not lift the microscope by the tube; if there is no handle, use the base.

### B. TISSUES

#### Exercise 28.—Squamous Epithelium.

(a) The outermost layer of the frog's skin which is composed of squamous epithelium is sloughed off when living frogs are confined in a small aquarium. It can, also, be obtained from the liquid in a jar of preserved specimens. The outer layer of your own skin is continually sloughing off in a similar manner except that it falls away in microscopic dry particles.

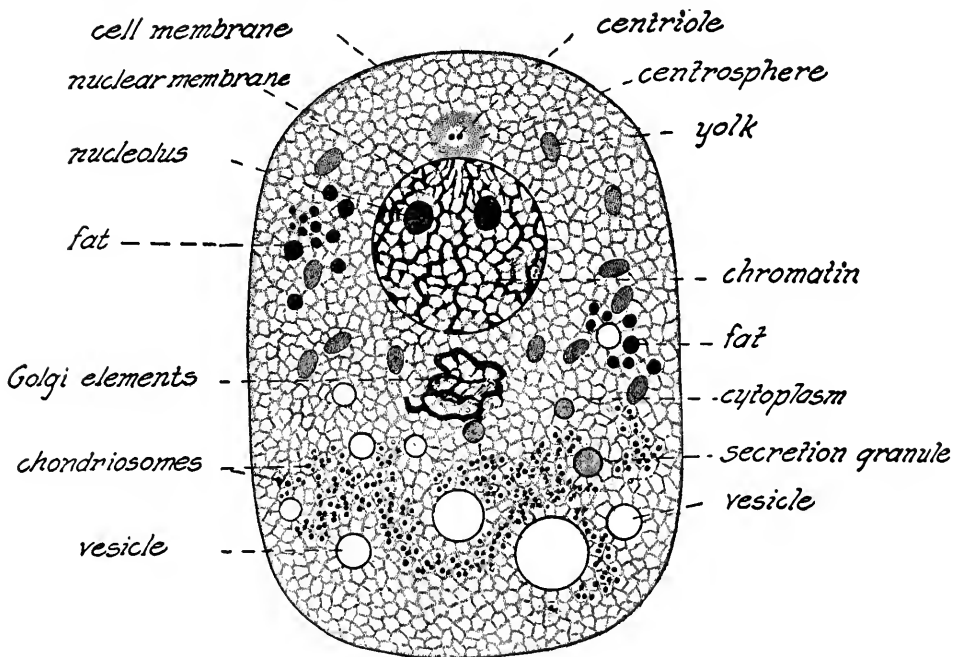


FIG. 26.—A composite animal cell in the vegetative phase; diagrammatic.

(b) Place a small piece of the outer layer of the frog's skin in a drop of water on a clean slide. Spread out the filmy mass without tearing, add a cover glass, and focus on the mass with the low-power objective in position. Adjust the iris diaphragm so that you can see the cell outlines clearly. How many sides do the cells have? How are they arranged? Observe with the high-power objective and note that each cell has a distinct **nucleus** surrounded by a **cytosome** (cf. Fig. 26). Two or more layers of cells may be seen in some places.

The cells thus observed are only the outer part of the skin of the frog which is much thicker than this and will be studied in a later exercise. What do you conclude regarding the relative thickness of these cells as compared with their length and breadth? Measure the diameter of a single cell as projected to table level. By dividing this measured distance by the magnification obtained with the combination of lenses in use, find the actual diameter of the cell. Make an accurate drawing of several adjacent cells, including the one measured. This drawing is to be  $3 \times$  the measured diameter, and the total magnification is to be recorded. Indicate, also, the source of the material studied.

**Exercise 29.**—Columnar Epithelium.

(c) Obtain, in a drop of the alcohol, a short piece of the frog's small intestine which has been cut from a fresh specimen and soaked for about twelve hours in 30% alcohol. This treatment is known as maceration and makes it easy to separate the cells. Pick up the piece of intestine with your forceps and shake one cut end in the drop of alcohol in such a way that some of the columnar epithelial cells of the mucous membrane become free in the liquid. Discard the piece of intestine and place a cover glass on the drop of alcohol. Using the low-power objective look for elongated cells, which will be seen singly and arranged in groups. Study a favorable group. Determine the exact shapes of typical cells and how the cells are fitted together. In some preparations **goblet cells** will be found; they are so called because the presence of a drop of mucus ready to be passed into the intestine makes the cell resemble a goblet. What is the position of the nucleus? Measure the length of a single cell as projected to table level. Make a drawing of this cell ( $3 \times$  its measured size) and of several cells grouped together. Record the total magnification and the source of the tissue.

**Exercise 30.**—Ciliated Columnar Epithelium.

(d) Obtain a small piece of the mucous membrane from the roof of the mouth cavity of a recently killed frog in 0.7% salt (sodium chloride) solution, or Ringer's solution. Scrape off the softer layer on the mouth side of the piece, discard the tough membranous material, tease apart the sticky mass that remains, and add a cover glass. With the low-power objective look for movement resembling the flickering of a flame in different parts of the mass which is made up of ciliated columnar epithelium. The vibration of small detached particles which are moving about may first attract attention. Examine, with

the high-power objective, a place where this movement appears. The cells are rather short columns which tend to be pointed at their inner ends and blunt at their outer, ciliated ends. However, when the living cells are torn apart they contract and appear as globular or bluntly cone-shaped bodies. The **cilia**, which are very delicate processes, cannot be seen individually but may be recognized as a flickering zone bordering the free ends of the cells. Observe the effect of the cilia upon small particles which may be in the preparation. Look for dying cells whose cilia are no longer in motion or have disappeared. In such a cell the relatively large nucleus can be distinguished. Measure the diameter of such a cell as projected at table level and draw it and several other cells ( $3 \times$  projected size). Record the total magnification and source of the tissue.

(e) Observe, in a demonstration of the roof of the mouth cavity of a frog or the gill of a mussel, the action of the cilia upon small objects and imagine their action upon microscopic particles. What is the function of these cilia? Examine, also, a demonstration of a permanent preparation of a section of the trachea, mussel gill, or other suitable material to see the cilia clearly.

#### **Exercise 31.**—Connective Tissue.

(f) Spread out a small piece of connective tissue on a slide in a drop of water, tease the edges, and add a cover glass. This tissue is most readily obtained from between the muscles of the leg in a preserved frog or from the inside of a cat's skin which has been preserved in formalin. Connective tissue has widely separated cells with a large amount of intercellular material in the form of fine fibers. These fibers are of two kinds: **white fibers**, very fine ones which run in wavy bundles, and **elastic fibers**, which are thicker, occur singly, and are straight. In some preparations one type of fiber will predominate and in some the other. In many preparations it may be hard to distinguish the two kinds. The cells of connective tissue are not easy to demonstrate but they may be seen by staining. Remove the cover glass and stain the piece of connective tissue with methyl violet for about a minute. Rinse in a drop of water and mount in a clean drop of water. If bundles of white fibers are seen, measure as projected at table level and draw ( $3 \times$  measured size). Record total magnification and source of material.

#### **Exercise 32.**—Cartilage.

(g) Examine sections of cartilage that have just been cut from the head of the femur of a recently killed frog and placed in 0.7%



salt (sodium chloride) solution, or Ringer's solution. After locating with the low-power objective the thinnest part of the section and distinguishing the cartilage proper from the regions in which bone is being formed, study with the high-power objective. Cartilage is a tissue having its cells widely separated by a **matrix** of a semitransparent, lifeless material. The cartilage cells lie in cavities, called **lacunæ**, surrounded by the matrix. In some lacunæ there may be two cells, or two lacunæ may be seen close together with flattened adjacent sides, indicating that cell division has recently taken place. Can you also find cells in fours? Measure the diameter of a cell as projected to table level. Make a drawing of this and several adjacent cells ( $3 \times$  the measured size). Record the total magnification and the source of the tissue.

### Exercise 33.—Bone.

(h) Pieces of dried bone, ground to thin sections, will be used. In these sections only the inorganic substance of the bone remains, but the extent and distribution of the **bone cells** are shown by the cavities which the cells once occupied. These **lacunæ** appear black because in the grinding of the section they become filled with dirt and air. Examination with the high-power objective will show the lacunæ as elongated, black areas which were formerly occupied by cells and, radiating from them, fine black lines, the **canaliculi**, which in life were occupied by delicate processes of the bone cells. Compare the structure here observed with that seen in cartilage. In some bones the cells are grouped about canals in which blood vessels run. These are called **Haversian canals**, and each one, together with its surrounding cells, constitutes a **Haversian system**. The bone cells and their nuclei can be seen in stained sections of decalcified bone. Examine, if available. Make a drawing ( $3 \times$  measured size) of a Haversian canal and some of its surrounding lacunæ. Record total magnification and source of material.

### Exercise 34.—Blood.

(i) Place on a slide a drop of frog's blood slightly diluted with 0.7% salt (sodium chloride) solution, or Ringer's solution. Cover at once, before it has time to dry, since drying changes the appearance of the cells. Examine with the low-power objective. Blood is a tissue composed of two kinds of **cells**, or **corpuscles**, suspended in a fluid, the **plasma**. What is the color of the **red blood cells**, or **erythrocytes**, as here observed? Determine their shape by observing them in different positions. The nucleus is readily seen. The **white blood cells**,

the **leucocytes** and **lymphocytes**, are much less numerous and very transparent; they are much smaller than the red cells. Some of them will be seen to be irregular in shape and you may possibly see that some of them change shape while you watch them. Their nuclei cannot be recognized. Make drawings of red blood cells in different positions, to show their shape. Make drawings of the white blood cells. These drawings are to be  $3 \times$  the measured size of cells as projected to table level. Record total magnification and source of tissue.

(j) Stain a fresh preparation with methyl violet by placing a small drop of the stain at the edge of the cover glass and allowing it to run under. Study especially the white blood cells in which the nuclei are now visible, and add to your drawing.

(k) Examine again the circulation of blood in the web of the frog's foot, looking for the two kinds of corpuscles and noting the relative size of the blood cells and the capillaries.

(l) Examine demonstrations of fresh and stained human blood. Compare with frog's blood with respect to size and shape of the red blood cells. Do you find nuclei? Do the white blood cells seem to differ in number or appearance from those of the frog? Observe, in a demonstration of undiluted blood, that the red blood cells may be arranged in rows like piles of coins. These are called **rouleaux**.

#### **Exercise 35.**—Non-striated Muscle.

(m) Obtain, in a drop of liquid, a piece of the digestive tract of the frog which has been macerated. Tease thoroughly and add a cover glass. Study first with the low-power objective and then with the high-power objective. The cells are elongated and taper toward their ends, and each has a prominent nucleus near the middle. Measure the length of a cell as projected to table level and draw ( $3 \times$  measured size). Record the total magnification and the source of the tissue.

#### **Exercise 36.**—Striated Muscle.

(n) Obtain a small bit of muscle from a freshly killed frog and place it at once in a clean watch glass half full of 0.7% salt (sodium chloride) solution, or Ringer's solution. Do not at any time give the material the least chance to dry. Fray out the piece with needles, separating but not injuring the individual fibers which you can see. Mount a few fibers on a slide in salt solution without staining. Also, stain a small mass of fibers as follows: put a drop of methyl violet on one end of a slide and a drop of water on the other; with forceps, put several fibers into the stain and leave them for about a minute;

transfer them to the water to wash off the surplus stain; mount in a clean drop of water. If the preparation is successful, the nuclei will be stained a deeper purple than the remainder of the fiber. Use these two slides in the following studies. For nuclei, the stained material should be used; for other features, the unstained fibers will be more satisfactory.

(o) Each muscle fiber is a single cell with a large number of nuclei. Compare the length of such a cell with other cells you have seen. Each fiber is composed of a large number of very minute **fibrillæ** which are indicated by rather indistinct longitudinal markings. There is a very much more distinct **transverse striation**, the feature from which this kind of muscle derives its name. Each fiber is enclosed in a very delicate cell membrane, the **sarcolemma**, which cannot be recognized except at places where the fiber is somewhat broken. Measure the width of a single cell as projected to table level. Make a drawing of this cell ( $3 \times$  its measured size). Record the total magnification and the source of the tissue. Review and correlate your observations on the examples of tissues by means of the table in Fig. 27.

### C. ORGANS

#### Exercise 37.—The Skin.

(a) The skin of the frog will be studied in permanently stained and mounted sections cut at right angles to the surface. These sections have been stained with two different dyes so that it is easy to distinguish the cytosome and nucleus of a cell. The skin is made up of two layers of which the outer is the **epidermis** and the inner is the **dermis**. The epidermis is composed of stratified squamous epithelium. Its flattened outermost cells have been studied from surface view in Exercise 28. What is the shape of the cells in the deeper layers? The dermis is composed of fibrous connective tissue in which numerous blood vessels are found. A layer of pigment cells will be observed immediately below the epidermis and another one near the base of the dermis. Pigment granules also occur in some of the epidermal cells. Notice the large, **simple alveolar glands** which open to the exterior by **ducts**. To which of the two layers of the skin do the cells lining the ducts and glands belong? What is the function of these glands? Have you anything comparable in your own skin? Can you identify non-striated muscle cells surrounding the expanded part of the gland? What is their function? Make a drawing ( $2 \times$  the measured size projected at table level) of a narrow vertical strip through the skin as seen with the high-power objective, showing in

Principal Types and Subdivisions			Examples of Occurrence
Epithelial tissue	Squamous epithelium (flattened cells)	Simple	Peritoneum and lining of blood vessels
		Stratified	Epidermis of skin
	Columnar epithelium (height of cells greater than width)	Simple	Mucous membrane
		Pseudo-stratified	Lining of trachea
Sustentative tissue	Connective tissue	Cells in mucous, reticular, or fibrous matrix	Dermis, submucosa, and tendons
	Cartilage	Cells in hyaline or fibrous matrix	Cartilaginous portions of skeleton
	Bone	Cells in calcified matrix	Bony skeleton
	Adipose tissue	Cells with stored fat drops	Subcutaneous fat deposits and fat bodies
Vascular tissue	Blood	White and red blood cells in plasma	
	Lymph	White blood cells in plasma	
Contractile tissue	Non-striated muscle	Sheets of mononuclear, spindle-shaped cells	Muscles of digestive tract
	Cardiac muscle	Syncytium of branching cells	Heart muscle
	Striated muscle	Bundles of multinuclear, cylindrical cells	Skeletal muscles
Nervous tissue	Nerve cells	Bipolar and multipolar neurons	Central and peripheral nervous system

FIG. 27.—Tissues and their occurrence in vertebrates.

detail the structure of the layers and including a gland and its duct. Label fully, giving the names of the layers, the tissues of which each is composed, and the functions of the tissues. Record the actual magnification.

**Exercise 38.**—The Wall of the Intestine.

(b) The intestine of the mud-puppy, *Necturus*, is better for this study than the intestine of the frog, but the structures to be observed are found in all vertebrates. On the slide are very thin sections cut across the intestine. Compare with the sections of the stomach that were previously studied (Exercise 7). As in the section of the skin, the nucleus and cytosome of each cell have been stained differently. Identify the gross features of the section with the handlens before studying with the microscope. There are six layers in the wall of the intestine. On the outside is the **peritoneum**, composed of a single layer of squamous epithelium which is seen from the edge. Beneath this is the **longitudinal muscle layer**, composed of non-striated muscle cells which are cut across in the section. Under this is the **circular muscle layer** which is similar to the longitudinal muscle layer, except that the non-striated muscle cells run around the intestine and are, therefore, cut lengthwise. Do you find any **blood vessels** in the muscle layers? Internal to the circular muscle layer is the **submucosa**, which is composed of fibrous connective tissue and contains many blood vessels. What is their function? What is the structure of their walls? Do any of them contain blood cells? The innermost layer, bounding the cavity of the intestine, is the **mucosa**, or **mucous membrane**, which is composed of a single layer of columnar epithelium. In this layer are seen many goblet cells, each with a drop of mucus. Can you find places where the mucus is passing into the intestine? Between the mucosa and the submucosa you may be able to see the **muscularis mucosæ**, a very thin layer of non-striated muscle cells. Understand the structure of each of the layers of the wall of the intestine and be able to explain the part each plays in the function of the intestine. Find the **mesentery**. With which layer of the intestine is it continuous? What kind of tissue forms the middle layer of the mesentery? Are there blood vessels in the mesentery? Make a drawing of a narrow strip across the wall of the intestine ( $1 \times$  measured size projected to table level), showing the cells exactly as they appear under the high-power objective. Label completely, giving the names of the layers, the tissues of which each is composed, and the function and full classification of each tissue. Record the actual magnification.

## IV. REPRODUCTION AND DEVELOPMENT

## A. RELATED CELL ACTIVITIES

**Exercise 39.**—Cell Division.

(a) The division of the cytosome of a cell is preceded by division of its nucleus. Although nuclear division occurs by two different methods, **amitosis** and **mitosis**, the latter method is almost universal. The life-cycle of a cell is divided into phases for purposes of discussion. Periods characterized by metabolism and growth, during which the cell is said to be in the metabolic or vegetative phase, alternate with periods of division during each of which the dividing nucleus of the cell passes through the **prophase**, **metaphase**, **anaphase**, and **telophase** and the cytosome is separated into two parts (Fig. 28). For the study outlined here, sections of growing onion, hyacinth, or may-apple root tips, or of the epithelium of salamander larvæ can be used. Examine the sections with the low-power objective and understand the relation of the parts. Then, with the high-power objective, observe the parts of the cells in the vegetative phase. Find cells in the different phases of division and note the changes which occur as the **mitotic spindle** and **chromosomes** are formed, as the half-chromosomes separate, and as the cytosome divides. Can you determine how many chromosomes are present in each cell?

(b) Draw (2 or 3  $\times$  the measured size as projected to table level) the outlines for six cells. Label these outlines vegetative phase, early prophase, middle prophase, metaphase, anaphase, and telophase. As you find and identify a cell in each of these stages add the details of its structure to the correct cell outline.

**Exercise 40.**—Differentiation of Germ Cells.

(c) Examine demonstrations showing **primary spermatocytes** or **primary oöcytes** and the two **meiotic divisions**. Understand the significance of the disjunction of the members of homologous pairs of chromosomes which occurs at this time (Fig. 29). The eggs of the nematode *Ascaris cquorum* (*A. megaloccephala bivalens*) are excellent material for this exercise, but many other forms can be used since the phenomena are universal among animals.

## B. THE GERM CELLS AND FERTILIZATION

**Exercise 41.**—The Germ Cells of the Female Frog.

(a) Examine in a watch glass of water a small mass of eggs from the ovary of a frog which has been preserved in formalin. Look with the handlens and the low-power objective of the compound microscope

for the smaller oöcytes among the larger ones. Explain this difference in size. A nucleus and a small amount of cytoplasm can be seen in

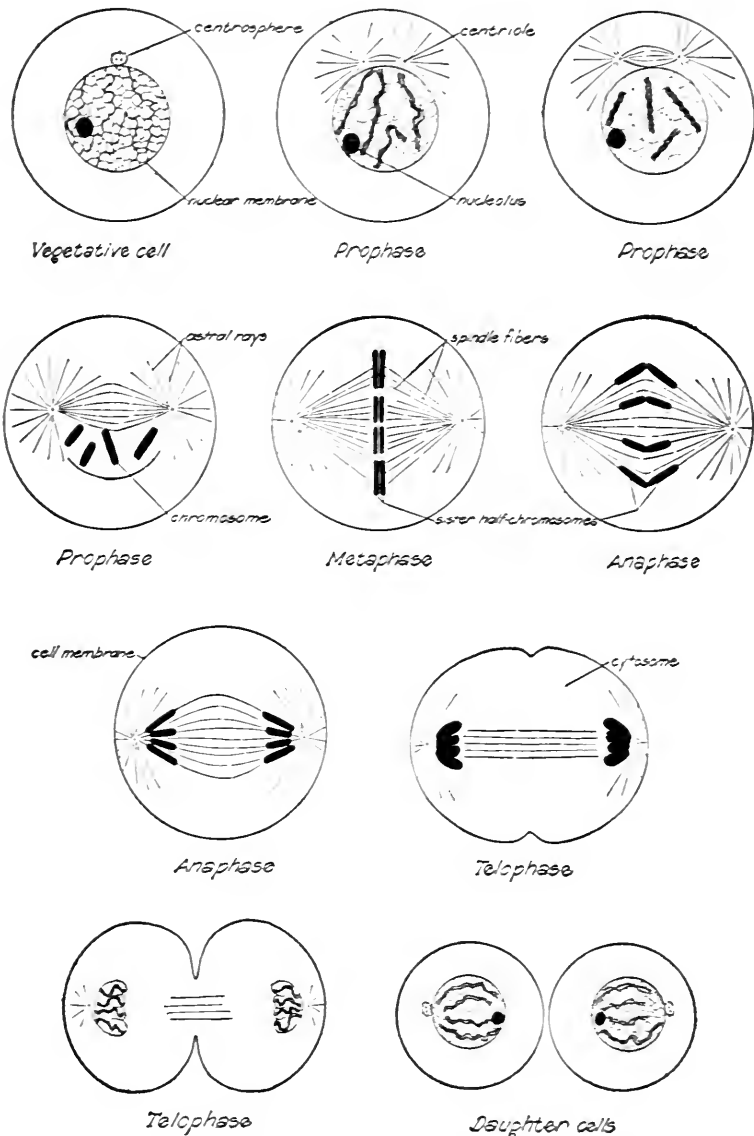


FIG. 25.—Mitosis and cell division in animal cells; diagrammatic.

the smaller oöcytes when examined under the low-power objective. The larger oöcytes are opaque, and their internal structure cannot be

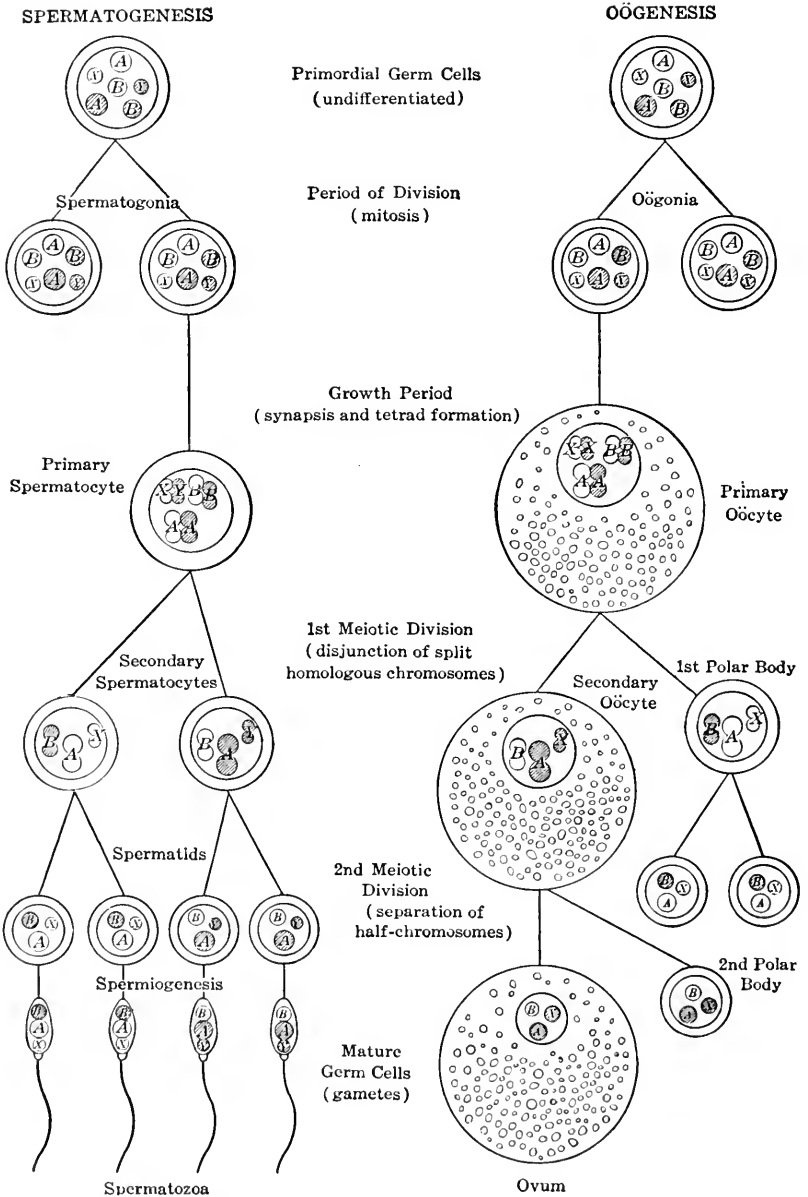


FIG. 29.—Gametogenesis in animals; diagrammatic. Homologous chromosomes are lettered alike; A and B are autosomes, X and Y are sex chromosomes. The chromosomes shown in black represent the paternal contribution, while the unshaded ones are from the mother. During the growth period food is stored in the cytosome of the primary oöcyte; this yolk remains in the ovum and is not distributed to the polar bodies.



seen in this material. They are single cells like the smaller ones but much yolk has been stored in the cytosome of each during the growth period of oögenesis which precedes the formation of the polar bodies. Draw (2 or 3  $\times$  measured size as projected to table level) one or more of the smaller oöcytes, showing the parts in outline only. Examine a permanently mounted and stained section of the ovary of a young frog. This will show the **follicle cells** surrounding each oöcyte; add these to your drawing.

(b) Remove a single large oöcyte from the ovary of a recently killed frog, crush on a slide under a cover glass, and examine with the high-power objective. What can you make out about the physical nature of the **protoplasm**? Observe the numerous **yolk bodies**. Is there any indication of the preformation of a new individual?

**Exercise 42.**—The Germ Cells of the Male Frog.

(c) The mature male germ cells, or **spermatozoa**, can be secured by cutting a testis from a freshly killed frog into small bits and teasing in water. Examine with the compound microscope and look for elongated bodies. Do these move about? Can you see how? Each spermatozoön consists of an enlarged portion or **head**, containing the **nucleus**, and the **mid-piece** from which extends the elongated thread-like **flagellum**, or **tail**. These parts are distinguished more easily after the addition of iodine. Like the female germ cell, the spermatozoön is a single cell (Fig. 30). Draw one or more spermatozoa (10  $\times$  measured size projected to table level).

**Exercise 43.**—Activation and Amphimixis.

(d) Examine demonstrations of favorable material showing the entrance of the spermatozoön into the female germ cell with its consequent **activation**. Activation is indicated by the lifting of the **fertilization membrane**, the initiation or completion of the meiotic divisions in many forms, and by the beginning of cell division, or **cleavage**. In normal fertilization **amphimixis** also follows the entrance of the sperm. During this process the two **haploid sets of chromosomes** that are present in the **male and female pronuclei**, or nuclei of the fully differentiated gametes, unite to give rise to the **diploid set of chromosomes** in the **zygote**.

**Exercise 44.**—The Fertilized Egg, or Zygote, of the Frog.

(e) In the frog the female germ cell is ovulated, or freed from the ovary, as a secondary oöcyte, that is, after the first meiotic division has occurred. How does it reach the oviduct? As the eggs pass along the oviduct, layers of the gelatinous secretion of the cells of the ovi-

duct accumulate around each one. During sexual union the eggs are shed from the anus of the female, and sperm from the anus of the male mingle with them. If spermatozoa enter the eggs before the jelly envelopes have swollen by absorption of water activation occurs. The second polar body is then formed and amphimixis follows. Since

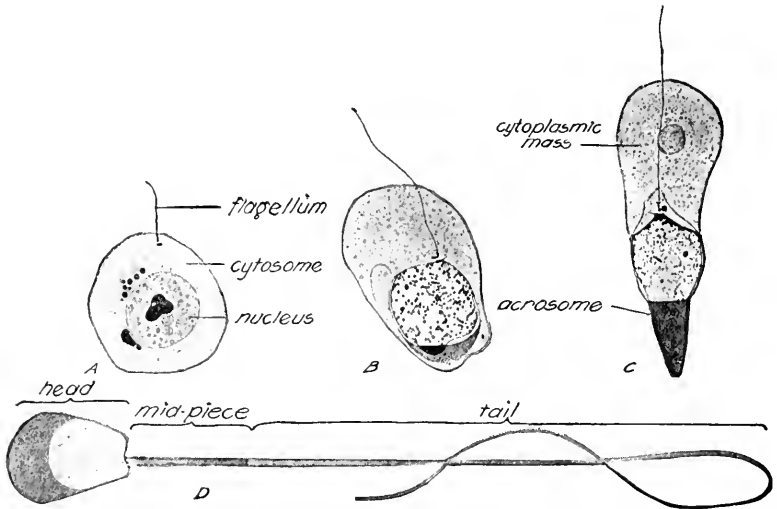


FIG. 30.—Stages in spermiogenesis in the guinea-pig. *A*, spermatid with early growth of flagellum from one centriole. *B* and *C*, showing the centrioles near the nucleus, the accumulation of cytoplasm along the flagellum, and the formation of the acrosome, which is the most anterior part of the spermatozoon. *D*, mature sperm, showing head, mid-piece, and tail; the cytoplasmic mass has been detached and lost.

(From F. Meves, 1899, *Archiv für mikroskopische Anatomie*, vol. 54.)

these changes can be seen only in sectioned eggs diagrams should be consulted.

(f) Examine several eggs which have the jelly well swollen. How is the jelly arranged? These and the subsequent stages should be studied in a watch glass containing enough water to cover the eggs. Use the handlens mainly, and in special cases the lowest magnification of the compound microscope with the mirror turned aside so that illumination is secured by the reflection of light from the surface of the egg. When it is desirable to remove the jelly from a preserved egg, it can be done by rolling the egg on a piece of filter paper. In the living egg the light-colored **vegetal hemisphere** is the heavier, and the darker surface is always uppermost since the egg turns readily within its envelopes. The center of the darker or pigmented **animal hemisphere** is called the **animal pole**; the opposite point on the sphere

is the **vegetal pole** of the egg. Draw several eggs ( $\times 3$ ), showing their envelopes of jelly as they lie together in the mass, and a single egg from a side view ( $\times 20$ ) to show the distribution of pigment and the jelly envelopes. In this and in all subsequent drawings from the side view, the animal pole should be toward the top of the page. Label the poles and indicate the polar axis by an arrow drawn as though thrust through the egg.

### C. CLEAVAGE STAGES

#### **Exercise 45.**—Collection and Study of Living Eggs.

(a) It is desirable that cleavage as well as later stages in development be studied in living eggs. The frogs and salamanders normally lay their eggs in spring and early summer, the period of egg-laying being as definite a characteristic of the species as any other form of behavior. If this study is made during the egg-laying season, go out and collect eggs for yourself, examining the breeding places and studying the activities of the animals under natural conditions. Are there any easily recognizable differences between males and females? Why do frogs croak more at this season?

(b) It is possible to induce ovulation in a female frog in the late fall and winter by inserting hypophyses from other frogs into her coelom. The hypophysis, or so-called pituitary gland, may have been seen lying in the cranial cavity and attached to the ventral surface of the brain. When the secretion of these extra glands is carried to the ovaries final growth of the egg cells occurs and ovulation follows. Eggs thus obtained can be fertilized by adding sperm from a frog's testis, and development will follow.

(c) Place a mass of living eggs in a shallow dish and keep in a well-lighted place but not exposed to direct sunlight for much of the day. Record the stage of the eggs when obtained and note the changes from day to day. Preserve your notes in the form of a written report to be handed in later. The influence of temperature upon the rate of development can be tested by placing part of the eggs out-of-doors on the cool north side of a building and comparing them each day with those having sunlight and the warmth of indoors. With proper care development can be followed until the tadpoles have completed their metamorphosis. At no time should the water in the dish be allowed to become too low from evaporation or to become foul from the growth of bacteria. A few green water plants will be beneficial. After hatching, the tadpoles can be fed upon bread or cracker crumbs, but too much food will foul the water and care must be used.

(d) Since egg-laying occurs only in the spring the following directions have been written with reference to preserved material. In studying this dead material, examine not only the exact stages specified but also look for intermediate conditions. Wherever possible arrange specimens in a series showing the transition from one stage to another, and thus obtain a concept of development which approaches that obtained by the study of living eggs and embryos. Reference can be made to Fig. 31 for the general sequence of early development.

**Exercise 46.**—The Two-cell Stage.

(e) After amphimixis has occurred, the nucleus of the zygote enters the prophase of mitosis. When the telophase is reached a constriction appears at the animal pole and rapidly passes toward the vegetal pole, dividing the zygote into two cells. This division is known as the first cleavage and the constriction is known as the **first cleavage furrow**. In this and subsequent stages the cleavage furrow indicates that a cell division following mitotic nuclear division is complete. During cleavage the vegetative phase is relatively short and divisions succeed one another at intervals that depend somewhat upon the temperature. In preserved material the two-cell stage is best studied in an egg with the cleavage furrow encircling about two-thirds of the circumference. Examine several specimens under water in a watch glass with the handlens and the low-power objective of the compound microscope. Draw one from side view to show the first furrow in process of formation or as it appears when completed. This and subsequent drawings should be made  $\times 20$ , in order that change in size as development continues can be shown. Indicate the polar axis by an arrow as in Exercise 44 and number the furrow. Do not represent the jelly or pigment in this or later drawings unless specified.

**Exercise 47.**—The Four-cell Stage.

(f) Examine several preserved specimens of eggs in which the **second cleavage furrow** is well advanced. What has happened internally before this furrow appears on the surface? The two nuclei divide at the same rate, and the cytosomal constrictions appear in both cells at the same time and together are known as a single cleavage furrow. Draw from the animal pole, numbering the furrows 1 and 2, and the poles as before.

**Exercise 48.**—The Eight-cell Stage.

(g) The **third cleavage furrow** is horizontal. How is it placed with reference to the equator of the sphere? With its completion the

eight-cell stage is formed. This stage has four smaller, deeply pigmented cells and four larger, lighter-colored cells; cleavage in the

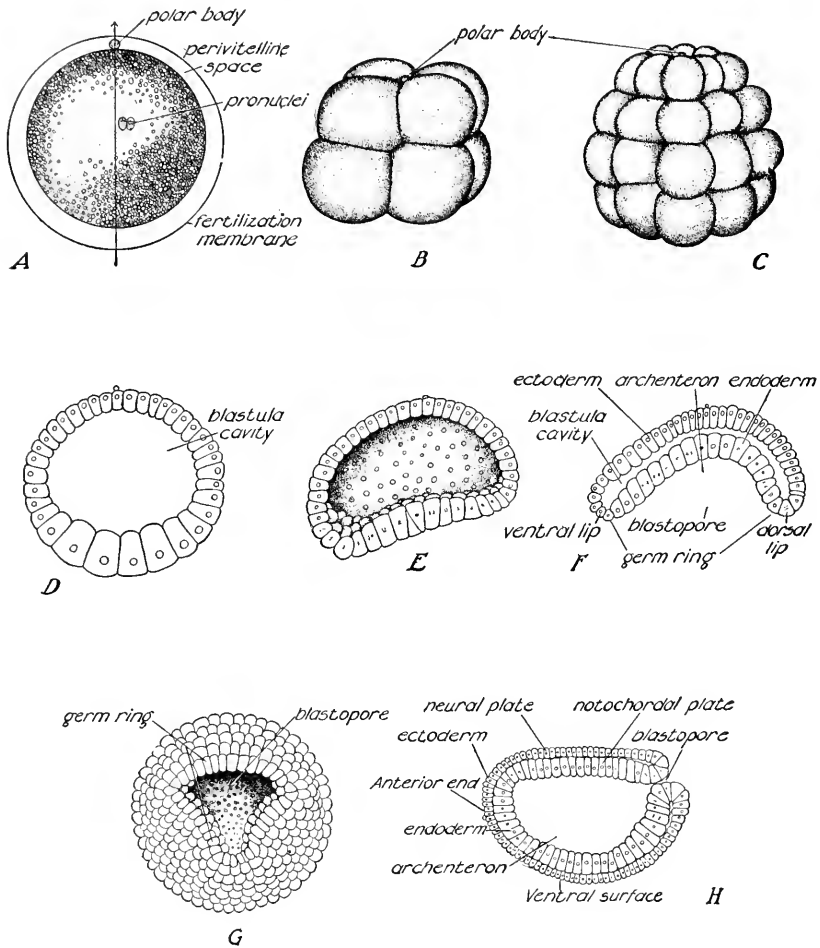


FIG. 31.—Early development in the amphioxus. *A*, one-cell stage, in section; the arrow indicates the egg-axis. *B*, eight-cell stage, from the surface. *C*, thirty-two-cell stage, from the surface. *D*, early blastula, in section. *E*, late blastula, as if cut in half. *F*, early gastrula, in section. *G*, gastrula, from the surface. *H*, late gastrula in section, oriented to show dorso-ventral and antero-posterior axes.

(*A*, *B*, and *G*, from E. G. Conklin, 1933, *Jour. Experimental Zoölogy*, vol. 64; *C-F* and *H* from E. G. Conklin, 1932, *Jour. Morphology*, vol. 54.)

frog's egg is total but unequal. Make two drawings of this stage, one from the side view and the other as seen from the animal pole, indicating the poles and furrows as before.

**Exercise 49.**—The Twelve-cell and Sixteen-cell Stages.

(h) When the eight cells divide it is obvious that the cytosomal constrictions in all of them cannot be in the same plane. Actually they appear in two vertical planes, so that we say there are two **fourth cleavage furrows**. The four upper cells, which contain less yolk than the lower cells, often complete their division before the furrows have appeared in the lower hemisphere. A twelve-cell stage results, with eight smaller cells toward the animal pole and four larger cells below. With the division of the four lower cells the sixteen-cell stage is produced. Examine eggs preserved in this stage and determine the exact outlines of the cells at each pole in several specimens. What forces determine the cleavage pattern at this stage? Draw a twelve-cell or a sixteen-cell stage from the animal pole, reproducing carefully the exact cell outlines of a typical specimen; number the furrows as accurately as possible.

**Exercise 50.**—The Thirty-two-cell Stage.

(i) The cytosomal constrictions appear in the division of the sixteen cells in such a way that there are two **fifth cleavage furrows** which are horizontal, one above and one below the third cleavage furrow. With the completion of these furrows, we have a **thirty-two-cell stage**. Why is it difficult, in studying any single preserved specimen of this stage, to assign numbers to the different furrows? A thirty-two-cell stage is a theoretical rather than an actual occurrence, because the cells about the animal pole now divide somewhat faster than the yolk-laden cells of the vegetal hemisphere. Why is there this difference in the rate of division? In the living egg the number of cells continues to increase by successive cell divisions, but from this time on it is impossible to recognize any uniformity in the pattern made by the furrows. Make a diagram of a thirty-two-cell stage from the side view and number the furrows. Draw a side view showing the exact appearance of an egg which is in about this stage of development.

**D. THE BLASTULA STAGE****Exercise 51.**—The Early Blastula.

(a) The thirty-two-cell stage is sometimes called the **early blastula**. More and more of the stored yolk is utilized in continued cell division, and the resulting **cleavage cavity**, or **blastula cavity**, becomes conspicuous internally. Take a preserved specimen in an early blastula stage and, after removing the jelly by rolling on damp filter paper, divide it into halves by a vertical sliding cut with a sharp scalpel or

a safety-razor blade. Several trials may be necessary but remarkably good preparations are often secured by this rough method. Study under water with the handlens and the low-power objective of the compound microscope with the mirror turned aside. What is the extent of the blastula cavity? Has it an external opening? What is the size of the cells? Have the cells begun to divide in planes parallel to the surface of the blastula? Draw the cut surface of the half blastula. Examine a demonstration section of this stage.

**Exercise 52.**—The Late Blastula.

(b) Examine older blastulae in which the surface cells have become much smaller. Is there any sign of differentiation anywhere on the spherical mass, other than in the distribution of pigment? Draw from a side view, showing the size of the cells by making a few cell outlines in each hemisphere.

**E. THE GASTRULA STAGE**

**Exercise 53.**—The Early Gastrula.

(a) Examine a stage several hours older than the last, using handlens and low-power objective. Can you recognize cell outlines at each pole? If a specimen is not found with the vegetal pole up, one can be secured as follows: cut a piece of filter paper a centimeter square and lay upon a slide; add enough water to saturate the paper and hold it in place but not enough to float it; using another piece of the paper, roll off the jelly from a single specimen and transfer to the above slide. The specimen can then be rolled into the desired position. If details are not clear under the low-power objective, add a cover glass, supported on one side by a pin, and run water under the cover glass with a pipette. Examine the vegetal hemisphere and the equatorial region. At this stage the pigmented cells of the animal pole begin to encroach upon the surface area of the lower, yolk-laden cells. This is the result of movement of cells following the onset of a more rapid rate of cell division in the cells of the equatorial region, which are now known as the **germ ring**. This movement of the cells of the germ ring is soon accompanied by an inturning of cells, the position of which can be seen on one side of the equatorial region as a crescent-shaped line separating the light and dark cells. The crescent-shaped line between the light and dark cells is really a narrow slit, the **blastopore**, which is the external opening of the **gastrula cavity**, or **archenteron**. The pigmented cells that border the blastopore constitute the **dorsal lip** of the blastopore; they are cells of the germ ring. Draw the early gastrula as seen from the vegetal hemisphere.

**Exercise 54.**—The Late Gastrula.

(b) Examine a late gastrula in which the cells of the germ ring have encroached still farther upon the exposed area of the yolk-laden cells. This gastrula is often called the **yolk-plug stage** because the yolk-laden cells appear as a small plug on the surface of an otherwise pigmented embryo. The crescent-shaped blastopore of the early gastrula has now become ring-shaped and has lateral and ventral lips. Cell movements continue until the yolk-laden cells are overgrown completely and the blastopore is a minute opening. Draw the late gastrula as seen from the vegetal hemisphere.

(c) Examine a demonstration stained section which shows the archenteron, or gastrula cavity, opening by way of the blastopore and the blastula cavity which is being obliterated. With your handlens try to determine the deepest region of the blastopore, that is, try to locate the dorsal lip of the blastopore on one of your specimens. Cut the gastrula into halves in a plane passing at right angles to the dorsal lip and examine the cut surfaces under water, using the low-power objective and reflected light. Good halves will show the cavities as clearly as in the demonstration specimen and, in addition, the third dimension can be seen. The cell movements of **gastrulation** which give rise to the gastrula cavity and tend to obliterate the blastula cavity bring about the first conspicuous localization of cells during development, namely, the formation of the **ectoderm**, an external layer of cells, and the **endoderm**, a layer of cells lining the archenteron. These layers are known as **germ layers** and are continuous with one another at the lips of the blastopore; they arise simultaneously during gastrulation. Since it is known from later development that the blastopore is at the posterior end of the embryo and the archenteron nearer the dorsal than the ventral surface, it is possible now to begin the use of the terms anterior and posterior, dorsal and ventral, as used for the adult frog. Draw the cut surface of a half gastrula, oriented in such a way as to place the future dorsal region toward the top of the page. The future anterior end may be to the right or to the left, but in subsequent figures the orientation here chosen must be continued.

**F. FORMATION OF THE ORGAN-SYSTEM PRIMORDIA****Exercise 55.**—The Early Neural-fold Stage.

(a) During the latter part of the localization of the ectoderm and endoderm certain cells pass into the region between these two layers. These cells make up the third germ layer, or **mesoderm**. At the same time, a rod of compact cells is separated from all three germ layers in



the mid-dorsal region between ectoderm and endoderm. This structure is the **notochord**, or primitive skeletal axis. What groups of animals possess notochords? Coincidentally with mesoderm and notochord formation the first localization of organ-system primordia begins in the ectoderm on the dorsal surface. After an increase in thickness by which the **neural plate** is distinguished from the remainder of the ectoderm, cell movements begin and produce a lifting of the edges of the neural plate to form the **neural folds**. Examine specimens in which these folds are forming. How are the right and left neural folds related to each other at each end of the neural plate? Find the blastopore, which now lies at the posterior end of the area enclosed by the neural folds. What is the position of the blastopore with reference to the neural folds? Notice that the neural folds are farther apart at the anterior end. Draw this stage from a dorsal view and make an outline of the jelly still surrounding the embryo. The jelly envelopes begin to disintegrate at about this time, and preserved embryos are likely to become separated from them before the time of hatching.

**Exercise 56.**—The Late Neural-fold Stage.

(b) Examine an older embryo. The neural folds have become higher and approached each other along the dorsal mid-line. What happens to the neural folds? What becomes of the blastopore? What general changes in the shape of the embryo are taking place? Understand what organ-system is differentiated from the cells of the neural plate which have now become localized. Draw from a side view, orienting the embryo as you did in Exercise 54 (c).

**Exercise 57.**—The Late Embryo.

(c) Conspicuous changes are beginning to appear in the shape of the embryo. Several specimens of slightly different ages are desirable, since the primordia are not easily recognized. Determine first the regions of the body in relation to the previous stage. The dorsal surface is less curved than the ventral. Where is the line of fusion of the neural folds? The head end is blunter than the tail end at this stage. On each side between the trunk and the head there is a slightly raised area, the **gill plate**. Anterior to this is the primordium of the **sucker**, recognizable as an unpaired U-shaped structure when seen from ventral view. Locate the **proctodeum**, or future anus, at the posterior end of the embryo. The specimens should be rolled over and studied from different angles. Draw one from a side view, oriented in the same way as the last figure.

(d) If living material is available the movement of the embryo

within its envelopes by means of the cilia which are now present on its ectoderm cells should be observed.

(e) The embryo of the frog is said to hatch when it frees itself from its jelly envelopes. Hatching time is not exactly correlated with the stage of development of the embryo, but varies with the condition of the surrounding envelopes. This is apparently determined by the quality of the water—pond, distilled, or deep well—in which the eggs have been developing. Consequently, the first tadpole stage is somewhat indefinite.

#### **Exercise 58.**—The First Tadpole or Hatching Stage.

(f) When the frog embryo has hatched it is known as a **tadpole**; it is a **larva**, that is, it is structurally different from the adult but self-sustaining. Examine specimens in this stage. The **head, trunk, and tail** are becoming conspicuous. The available specimens will probably be in slightly different stages and if a half-dozen are taken they can be oriented alike and arranged in order to show the developmental changes. Study such a series and follow the further differentiation of the parts shown in the last figure. The primordia of the **eyes** will now be seen. Draw a tadpole, which shows the head, trunk, and tail, the gill plates, the eyes, the sucker, and the proctodeum.

(g) Cut cross sections through the trunk region of an early tadpole, placing the specimen on wet filter paper and using a sharp scalpel or razor blade as with the blastulæ and gastrulæ. Examine under water, using the low-power objective and reflected light. Good sections will show the dorsal **neural tube**, the **notochord**, the **archenteron** lined with **endoderm**, the yolk-laden cells, the **dorsal mesoderm** on each side of the neural tube, the **intermediate** and **lateral mesoderm**, which will probably not be distinguished unless the **cœlom** is visible as the cavity of the lateral mesoderm, and the **superficial ectoderm**. Depending on the position of your cut, other structures, such as the **liver** and the **pronephroi**, or **head kidneys**, may be seen. Identify as many parts as possible and draw the cut surface. Examine a demonstration section of this stage.

#### **Exercise 59.**—The Second Tadpole Stage.

(h) Select a number of specimens in which the tail is beginning to differentiate into a **fin** and a **muscular axis**. Arrange a series as before. At one stage the tadpole will show metameric markings on each side in the dorsal region. These are the **mesodermal somites** which are formed from the dorsal mesoderm. Identify all the parts previously

studied and, in addition, the **external gills** which are appearing as a tuftlike growth on each gill plate. Draw, orienting as before.

(i) If living tadpoles are available observe their movement as they lie on their sides on the bottom of the dish. The ectodermal cilia are still present but will gradually disappear.

**Exercise 60.**—The Third Tadpole Stage.

(j) Up to this time the tadpole, although hatched, swims but little. Instead, it clings to the jelly and other objects by means of its sucker. Has the structure of the sucker changed? Now the activity becomes greater and in the living specimens swimming is much more in evidence. Study specimens with well-developed external gills, identifying all the parts as above and, in addition, the **stomodeum** which now appears as a median pit between the sucker and the eyes. It is seen clearly from the ventral view. To what does it give rise? Where is the proctodeum at this stage? The **brain vesicles** can be seen through the superficial ectoderm as swellings above the line between eye and gills. Look for mesodermal somites. What is the distribution of the pigment at this stage? Draw, orienting as before.

**Exercise 61.**—The Fourth Tadpole Stage.

(k) In older tadpoles look for the **operculum**, a membrane comparable to the lateral covering of the gills of a fish. It will be found overgrowing the external gills. The external gills are temporary respiratory organs and soon disappear with the growth of the internal gills which are in the region covered by the operculum and are comparable to the gills of a fish. Draw a tadpole, showing the external gills partially overgrown by the operculum.

### G. THE LATE LARVAL STAGES

**Exercise 62.**—External Features.

(a) Living specimens should be examined in an aquarium. Notice how they come up to breathe and how those in the more advanced stages are beginning to use their legs. For detailed study, preserved tadpoles of the leopard frog or of the bullfrog may be used. Select a specimen with hind legs just appearing. It should be placed under water in a dissecting pan. Identify the **nostrils**, the **eyes**, the **mouth** with its horny jaws which function as teeth, the **hind legs**, and, on the left side of the body, the **opercular opening** which leads into the gill chamber. Draw from a side view, orienting as in previous figures, and showing the opercular opening by a dotted outline if your drawing is from the right side.

**Exercise 63.**—The Gills and Viscera in Position.

(b) Fasten the specimen ventral surface up, by pinning through the tip of the head and tail. Remove the skin from the ventral half of the body wall, leaving the opercular opening intact. The coils of the **small intestine** may now be seen beneath the muscles, and just anterior to them the gill region. Are there any indications of metamerism? Carefully remove the thin layer covering the **gills**, and find the **fore legs** lying against the gills and within the **gill chamber**, or **opercular cavity**. Probe through the opercular opening and determine its relation to the gills. Find the **heart** between the right and left gill areas. Anterior to the gills are conspicuous bands of **muscle** connected with the jaws. Remove the muscles which cover the coils of the intestine. How are the opercular cavity and the **cœlom** separated?

(c) Pin out, beside the tadpole and in the same position, a small fish, preferably a catfish. Examine the gills and the operculum of each side, cutting anteriorly from the V where the two opercula meet. What is the relation of gills and **gill slits** to the mouth cavity and to the cavity beneath the operculum? Locate the heart by cutting along the mid-line in the angle of the V. Compare these structures in the tadpole and the fish. How many gills and how many gill slits in each? What is the relation of the gill slits to the mouth cavity in the tadpole? Using a tipped bristle, probe through the mouth cavity and out through the gill slits. Recall the earlier stages of the operculum as seen in Exercise 61. How does the operculum of the tadpole differ from that of the fish? Is the developing fore leg on the outside or inside of the body?

(d) Draw the entire tadpole ( $\times 2$ ) as thus dissected, showing the cut edge of the body wall, the partition between cœlom and opercular cavity, and the other structures observed. Show the relation between opercular cavity, gill slits, and mouth cavity by arrows. Spread the gills apart to show the gill slits.

**Exercise 64.**—The Cœlom and Its Contents.

(e) Lift up the mass of the intestine at its posterior margin and locate the **large intestine** against the dorsal wall on the left side of the body. Cut the large intestine, leaving a short stump. Lift the mass of the intestine and find the **esophagus** where it enters the anterior end of the cœlom. Follow the esophagus to the **stomach** and this to the **small intestine** where the latter enters the coil. Cut the intestine at this point and remove the coil after cutting its attachment to the dorsal mid-line. Look for the **spleen**, either on the dorsal surface of the coil as removed or still in the body. Uncoil the intestine and determine its

length. How does it compare with the length of the intestine in the adult? Is there anything in the feeding habits of frog and tadpole which is correlated with the different lengths of the intestine in the two? Examine the part of the digestive tract remaining within the coelom. Note, in addition to the parts above named, the **liver** with the **gall bladder** between its lobes, and the **pancreas** lying in the angle between the stomach and the intestine. Locate the **lungs**, **kidneys**, **fat-bodies**, and the rudiments of the **ovaries** or **testes**. Draw the above organs as they appear in position or slightly displaced to show as much as possible, making an outline of the tadpole and its coelomic cavity about the organs.

**Exercise 65.**—Transverse Sections.

(f) With a sharp scalpel, cut transversely through the body in the gill region and again at about the middle of the trunk. What is the condition of the skeleton and of the nervous system in comparison with those of the adult? If not clear, cut sections of a new specimen. Draw a favorable section or sections, as directed by your instructor.

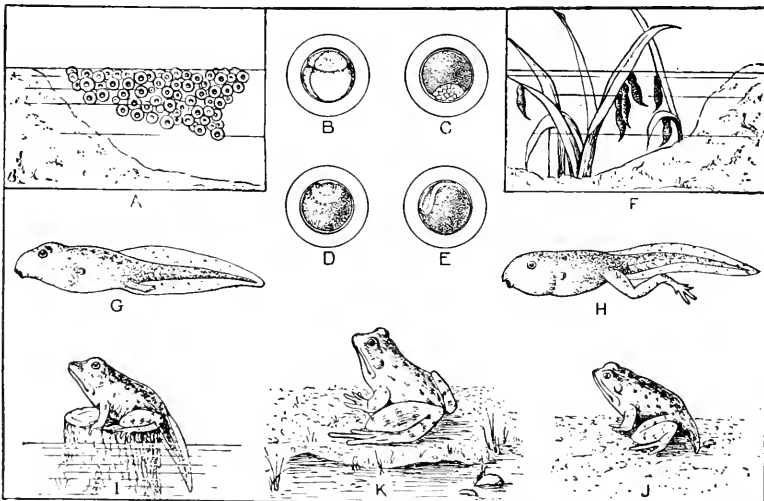


FIG. 32.—Development of the frog. *A*, eggs. *B*, *C*, *D*, and *E*, cleavage, gastrula, and neural-fold stages. *F*, newly hatched tadpoles. *G* and *H*, later tadpole stages. *I*, *J*, and *K*, stages in metamorphosis to juvenile frog.

**H. METAMORPHOSIS**

**Exercise 66.**—Late Tadpoles and Juvenile Frogs.

(a) Examine several preserved specimens showing stages in the **metamorphosis** or change from the larval to the adult condition (*cf.* Fig. 32). These transformations occur while the tadpole is active and

extend over a period which varies in length with different species. Note the changes in the shape of the body, in the mouth, the tail, the legs, and the coloration. What becomes of the operculum? Have the **tympanic membranes** appeared? Draw representative individuals.

#### I. DEVELOPMENT IN RELATED FORMS

##### **Exercise 67.**—The Tadpole of a Salamander.

(a) During the spring months the living tadpoles of the salamander *Amblystoma* may be obtained. Watch individuals as they swim in an aquarium and then examine them in a watch glass with a hand lens. Compare, part by part, with the tadpoles of the frog already studied. The suckers are long stalklike structures; otherwise the resemblance is obvious. Add a drop of ether or chloroform to the water, and when the larvæ become quiet examine with the low-power objective of the compound microscope. Note the pigmentation. Observe the circulation in the **capillaries** of the gills or tail. Can you recognize the blood cells? Is there a pulse?

(b) Older larvæ are often taken during the early summer in places where frog tadpoles are abundant. Examine preserved specimens, about 40 mm. in length, showing external gills attached to the operculum. Compare with demonstration specimens of the adult. Can you recognize mesodermal somites in larva and adult? What structures justify the statement that a salamander is a less specialized vertebrate than a frog? Draw the larva from a side view, orienting the same as the figures of the frog tadpoles.

##### **Exercise 68.**—The Unincubated Egg of the Hen.

(c) Take an unincubated hen's egg and, using scissors, cut open on one side a space about 25 mm. across, being careful that the points of the scissors do not injure the yolk. The opening may be further enlarged, if necessary, while the egg is resting upon cotton in a finger bowl. The familiar **yolk** and **white** of the egg will be observed. Find the **chalazæ**, or twisted cords of **albumen**, at each end. What relation have they to the yolk and to the shell? Find the two **membranes** that line the shell. These can always be seen at the larger end of the egg where there is an air space between them. At one place upon the surface of the yolk is a small whitish area, the **blastoderm**, the central part of which is known as the **area pellucida** and the peripheral part as the **area opaca**. Does this always appear at the top, however the egg is turned? Compare with the rotation of the frog's egg in its jelly envelopes. Compare such an egg with that of the frog. Under-

stand what stage of development has probably been reached. Draw ( $\times 1$ ) the egg as thus dissected.

**Exercise 69.**—The Twenty-four-hour Chick Embryo.

(d) Open an egg which has been incubated for twenty-four hours and, placing it on the cotton beside the one just drawn, compare the two. Record or make a simple sketch to show the changes which have taken place in the blastoderm during this first day of incubation. Before discarding this specimen, the existence of a delicate **egg-membrane** should be demonstrated by puncturing the yolk.

(e) Permanently mounted specimens of the unincubated blastoderm and the developing embryo may be used for the study of approximately the twenty-four-, thirty-six-, and forty-eight-hour stages, in their finer details. These should be handled with great care lest they be crushed by wiping and are to be studied with the handlens or the low-power objective of the microscope; do not use the high-power objective. Such slides are secured by removing the blastoderms, which are then killed, stained, and mounted in balsam. The first to be studied is the twenty-four-hour stage in which the following parts are to be made out: **neural folds**, **notochord**, **mesodermal somites**, **primitive streak**, **area pellucida**, and **vascular area**. Focus carefully to determine the vertical relationships of the parts and compare your results with what is shown by diagrams. Draw this stage as a full-page figure.

**Exercise 70.**—The Thirty-six-hour Chick Embryo.

(f) Open an egg which has been incubated for thirty-six hours and notice the changes which have occurred. With the aid of an instructor, inject some India ink into the cavity beneath the blastoderm and then harden the embryo by dropping strong alcohol upon the outside of the blastoderm. Compare, part by part, with a permanently mounted specimen of the same stage, placing the latter against a white background. Study with handlens and low-power objective the parts previously observed in the twenty-four-hour stage. Observe the mounted specimen further under the compound microscope and make out, also, the beginning of the **brain vesicles**, the **optic vesicles**, the **anterior amniotic fold**, the **omphalo-mesenteric veins**, the **heart**, and any changes in the size and proportions of parts. Here again, careful focusing and the comparison of what you see with models and diagrams are desirable for the proper understanding of the third dimension. Draw this stage in a figure similar to the last.

**Exercise 71.**—The Forty-eight-hour Chick Embryo.

(g) Examine a freshly opened embryo in the forty-five- to forty-eight-hour stage, comparing it with the last. Note the **blood vessels**, the pulsations of the heart, which should be counted per minute, and the extent to which the blastoderm has extended over the egg. Treat with India ink and alcohol as before. Study the specimen thus freshly prepared and a stained and mounted specimen of the same stage. Find all the structures previously observed in the thirty-six-hour stage, and, in addition, observe the **cranial flexure**, the **torsion** of the cephalic end of the embryo, the **lens** of the eye, the **auditory vesicles**, the tubular **heart** now bent into an S-shape, the **omphalo-mesenteric arteries** and the **sinus terminalis**, the **gill arches and slits**, and the extent to which the anterior and **posterior amniotic folds** have developed. Draw this stage, in a figure similar to the last.

(h) Understand from reading and from demonstrations of the later stages of the chick how the embryo is related in its several stages to the yolk mass and the origin and significance of the **yolk sac**, **amnion**, **chorion**, and **allantois**, which are called **embryonic membranes** (Fig. 33).

**Exercise 72.**—The 6-mm. Pig Embryo.

(i) Pig embryos about 6 mm. long from crown of head to rump and about eighteen days old correspond in stage of development to a chick embryo after four days of incubation. They are somewhat more advanced developmentally than a human embryo of comparable length and about five weeks old. Study pig embryos 6-7 mm. long which have been obtained from a slaughter house, preserved, and dissected from their embryonic membranes. Study in a watch glass containing dilute alcohol, using a handlens or the low-power objective with reflected light. Notice how the body is bent; the head appears triangular in shape because of the two flexures in that region. Identify the **eye**, **olfactory pit**, and rudiments of the **jaws** which form the anterior and posterior borders of the most anterior of four grooves along the side of the head. This anterior slit marks the position of the **mouth cavity**; the others are the grooves that would be perforated as gill slits in an aquatic vertebrate. The dorsal part of the groove just posterior to the mouth marks the position of the external ear of later development. Parts of the brain can be identified through the transparent covering of the head, especially the prominent **mesencephalon** where the most anterior flexure is noted and the thin-roofed **myelencephalon** which is dorsal to the grooves previously located. The head is folded against the **heart**, which is the most anterior of three swollen



regions in the trunk. Dorsally, the **auricle** can be distinguished from the ventral and thicker-walled **ventricle**. The position of the **liver** is indicated by the swelling between the heart and the **anterior limb bud**.

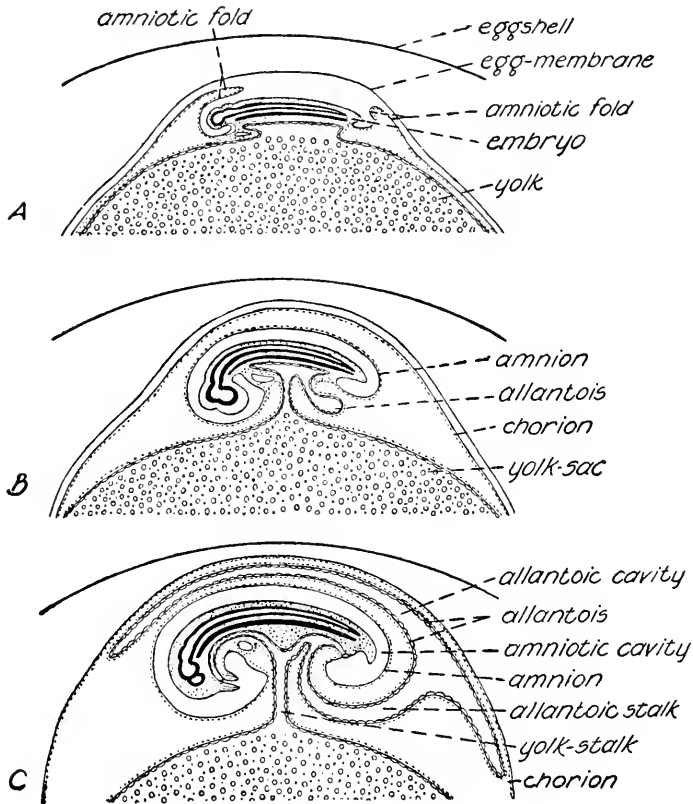


FIG. 33.—The embryonic membranes of the chick; diagrams of longitudinal sections of the embryo. *A*, by the end of the second day of incubation both the anterior and posterior amniotic folds, the fusion of which gives rise to the amnion and chorion, can be seen. *B*, by the end of the third day the amniotic folds have united and the allantois has arisen as an outpocketing from the gut and grown into the cavity between the amnion and chorion. Note that the yolk sac, or cellular membrane surrounding the yolk, is continuous with the lining of the gut. *C*, from the fifth day on, the allantois grows rapidly and fills all available space; its outer layer becomes closely applied to the chorion, while the inner layer lies next to the white of the egg.

(Redrawn with modifications from T. J. Parker and W. A. Haswell, "Textbook of Zoölogy," copyright, 1921, by Macmillan and Co., Ltd., printed by permission.)

Dorsal to the limb bud the **mesodermal somites** are conspicuous for the entire length of the trunk. Between the anterior and **posterior limb bud** the **mesonephros** forms a prominent mass. Posterior to the

hind limb bud the **tail** will be seen. Draw ( $\times 15$ ) an embryo as it lies on its side.

(j) The cut edges of the **amnion** and **yolk sac** will probably be found where they are attached to the embryo along the mid-ventral region of the trunk. The amnion, **chorion**, and **allantois** of the pig embryo are formed in the same way as in the chick but at a relatively earlier stage of development. The yolk sac is really formed at the gastrula stage of development but not distinctly separated from the gut until somewhat later. However, since the mammalian egg is not yolky the chief nutritive, as well as respiratory and excretory, functions are performed by the allantois in close association with the chorion. This **chorio-allantoic membrane** is closely applied to, but not fused with, the lining of the uterus. The association of embryonic membranes and the tissue of the mother constitutes a **placenta** by means of which exchange of products dissolved in the blood of either can occur by diffusion between mother and embryo.

#### **Exercise 73.**—Older Pig Embryos.

(k) A pig embryo of 9-12 mm. in length is about three weeks old and corresponds in stage of development to a human embryo of the same length which is about six weeks old. The features described for the 6-mm. embryo should be identified. The eye is now more easily seen and the beginning of the external ear can be observed. Note that the trunk of the embryo is somewhat straighter along the mid-dorsal line. This straightening is brought about by increased growth of the internal organs especially the heart, liver, and mesonephroi. The **belly stalk**, which corresponds to the umbilical cord of a human embryo, contains the stalks of the yolk sac and allantois which are connected to the gut of the embryo; part of the amnion forms the covering of the belly stalk. Draw ( $\times 10$ ) an embryo as it lies on its side.

(l) Pig embryos 15-20 mm. in length are 24-28 days old, that is, they have completed about one-fourth of their embryonic life. A human embryo of 17-18 mm. is about seven weeks old and is comparable to the pig in stage of development. In a pig embryo of this length note the **external naris**, the eye with its **lids**, and the **external ear** which is the only remaining trace of the grooves posterior to the mouth. It is still possible to identify the thin roof of the **myelencephalon** and a thicker ridge that is the roof of the **metencephalon** just anterior to it. Notice that, while the flexures in the head region persist, the trunk is much straighter dorsally than in the last stage. The very extensive growth of the liver, two lobes of which may be seen,

has been primarily responsible for this change in shape. Observe the primordia of five **digits** in the paddle-like limb buds. Recall that the pig at birth has only two fully differentiated digits and two that are rudimentary on each foot. How do you think this embryonic recapitulation of the typical vertebrate plan of appendage can be explained? Draw ( $\times 6$ ) an embryo as it lies on its side.

**Exercise 74.**—The Development of the Face.

(m) Since the ventral surface of the head is so closely pressed against the trunk, changes that occur can be seen only in heads removed from the trunk. Examine a demonstration of heads taken from embryos of approximately 6-, 10-, 15-, and 20-mm. crown-rump length. These heads should be observed from the ventral view. Notice the formation of the **lower** and **upper jaws**, the appearance of the **cerebral hemispheres** as seen through the head covering, the shifting contours of the region which change the position of the **external nares**, and the beginning of the elongation of the **snout**. The early molding of the facial region is brought about by proliferation of mesenchyme between the skin and the brain.

**Exercise 75.**—The Reproductive System of a Pregnant Sow.

(n) Examine a demonstration of the reproductive system which has been removed from a sow in early pregnancy. Notice the **ovaries** in which prominent **corpora lutea** and smaller **follicles** can be seen. The **oviducts** are not paired throughout their lengths as in the frog but are fused at their posterior ends to form the **cervix**, or neck, of the **uterus** which has two horns, each differentiated from one of the oviducts. Near the ovaries the oviducts are very small and are known as the **Fallopian tubes**, each of which has a funnel-shaped opening, or **ostium**, which closely surrounds the ovary. Eggs when shed from the ovaries pass directly into the Fallopian tubes.

(o) Depending on the stage of pregnancy the horns of the uterus may exhibit local enlargements. Note in one horn which has been opened the very elongated **chorionic vesicles**, each containing an embryo and its membranes; the covering of the chorionic vesicle is the outermost embryonic membrane, the **chorion**. The conspicuously vascularized **allantois** by its extensive growth is responsible for the dilation of the vesicle but does not extend to its ends.

(p) In an opened chorionic vesicle the **chorio-allantoic membrane** has been cut, and the delicate **amnion** surrounding the embryo can be seen. The **amniotic cavity** between the amnion and the embryo is filled with **amniotic fluid** in life. What is the function of this fluid? The **belly stalk** can perhaps be observed through the amnion.

**Exercise 76.—The Human Placenta and Associated Membranes.**

(q) Examine a demonstration of the discoidal human placenta and attached remnants of embryonic membranes. This is what is commonly known as the “after-birth.” The human **placenta** is formed by a fusion of a vascularized region of the **chorion** and the lining of the uterus; there is no continuity between embryonic and maternal blood channels. Remnants of the non-vascularized part of the chorion and of the thin **amnion**, which are closely applied to one another but not fused, will be found at the edges of the placenta. Note the **umbilical cord** which was severed at the time of birth. Embryonic blood vessels pass between the **fetus**, as the human embryo is called beginning with the third month of its development, and the chorionic capillaries in the placenta by way of this cord. Sometimes the vestige of the **yolk sac** can be found as a small bulb-like mass on a delicate cord near the junction of umbilical cord and placenta. The vestige of the **allantois** is within the umbilical cord. What are the functions of the placenta and how are they accomplished?

## HEREDITY AND VARIATION

### Exercise 1.—Study of External Features of *Drosophila*.

(a) The fruitfly *Drosophila* can be used to study the inheritance of obvious structural features. It is necessary to become familiar with the differences between males and females and to recognize the typical or wild-type qualities of the flies before beginning a breeding experiment. Obtain in your watch glass or on a piece of paper a wild-type male and female fly which have been etherized. Examine with the handlens and identify the head with the conspicuous red **eyes**, the **thorax** to which the single pair of **wings** is attached, and the **abdomen** which is banded with black. The wings extend beyond the tip of the abdomen when they are folded. In flies etherized too long the wings will be fixed at right angles to the body; the length can be checked on living flies in a culture bottle. The male fly is smaller than the female and has a rounded abdomen at the tip of which the black bands are wider than in the female and more conspicuous from the ventral surface. The tip of the abdomen in the female is somewhat pointed and a tuft of short bristles can usually be seen. More details of structure can be observed with a binocular microscope and, if available, demonstrations should be examined. Can you identify males and females as they move about in a culture bottle? Be careful not to release flies in the laboratory.

(b) As *Drosophila* has been bred under laboratory conditions numerous variations from the wild-type stock have arisen. Examine demonstrations of as many eye, wing, bristle, and body-color variations as may be available. Such characters reappear in the same form generation after generation so long as both parents exhibit the character in question.

### Exercise 2.—An Experiment in Monohybridization.

(c) In order to gain some information about the mechanism of heredity individuals that differ with respect to one character such as length of wing, shape of wing, color of eye, or color of body can be bred together. Obtain two culture bottles each of which contains a pad of sterile medium composed of cornmeal, syrup, agar-agar, and water; the piece of paper is provided for the fly's egg-laying. Carefully remove the cotton plug and, holding the plug between two fingers,

add 1-2 drops of a concentrated solution of compressed yeast to the surface of the food pad in each bottle; replace the plug. Now obtain pairs of flies that differ with respect to the character being studied in order to make the original or  $P_1$  cross. Both parent flies are from pure-breeding stocks. Half of the class should use wild-type males, and half, wild-type females. Understand that virgin females must be used and learn how they are obtained. Since the flies will move up and toward the light they can be transferred from the vials to the culture bottle. Remove the plug from the culture bottle and hold between two fingers or place on a clean sheet of paper. Unless you use care, mold spores will be introduced into your culture bottle from the plug and hinder or stop the production of flies. Hold the open end of the culture bottle away from the light and somewhat lower than the base. Tap the base of the vial containing the fly on the table to throw the fly away from the cork, quickly uncork the vial, and hold the open end of the vial within the open end of the culture bottle. The fly will crawl to the open end of the vial and can then be shaken off into the culture bottle in which the plug is immediately replaced. Repeat the process for the other fly; do not allow the first one to escape. Since virgin females are more trouble to obtain than males it is better to introduce the male into the culture bottle first. Because there are numerous reasons why cultures fail, each student is to start duplicate cultures. Make a label containing information about parents and date of beginning experiment and attach to each bottle.

(d) Keep the culture bottles at room temperature and examine each day to make sure that the parent flies are still alive and have not escaped. On the fourth day you should be able to see the small, cream-colored *larvæ* moving about in the food pad. When do you first find the pale **pupal cases** on the side of the jar or on the paper? Remove the parent flies on the seventh or eighth day. As soon as the pupal cases become dark—probably on the ninth day—carefully remove six to eight of them with a needle without puncturing and place in separate vials each of which contains a moistened piece of filter paper. When do you observe the first adults in the culture bottle? Eight or nine days after the first adults are seen transfer the flies from the culture bottle to an etherizing bottle. The mouth of this bottle should fit the mouth of the culture bottle, and the flies may be allowed to crawl up from one to the other or the transfer can be hastened by tapping on the culture bottle while it is held above, but in contact with, the etherizing bottle. Several drops of ether should be placed on the plug of the etherizing bottle. As soon as all the flies are in it

quickly separate the two bottles and stopper the etherizing bottle. When the flies have been quiet about a minute they can be emptied onto a piece of white paper. If the flies begin to move place them in the etherizing bottle again. Classify these flies of the **first filial** or **F<sub>1</sub> generation** with respect to the character with which you are dealing and as to sex. Make a record of the number in each class. If all the flies are alike with respect to the character being studied their appearance identifies the **dominant** expression of the character; the alternate state of the character, or the one which does not appear, is **recessive**.

(e) The flies that emerge from the isolated pupal cases are to be mated in pairs in culture bottles as before in order to obtain a **second filial** or **F<sub>2</sub> generation**; make two cultures. Record observations on time of appearance of larvæ, pupæ, and adults. Again, remove the parent flies after seven or eight days. Eight or nine days after adults of the second generation first emerge, kill, classify for character and sex, and count the progeny as for the first filial generation.

(f) If time permits make two additional cultures, using for one parent a fly of the first filial generation and for the other a fly of the mutant or recessive stock which was used in the original cross. This is known as a **backcross** with the recessive type. Make observations and records as specified in (e).

(g) Tabulate the data gathered in this experiment in the form of a table as shown in Fig. 34.

The Inheritance of..... (Enter name of recessive character expression here.)

Crosses	Progeny						Grand total
	Enter dominant character expression here			Enter recessive character expression here			
	Males	Females	Total	Males	Females	Total	
Original or P <sub>1</sub> cross							
F <sub>1</sub> × F <sub>1</sub>							
F <sub>1</sub> × recessive							

FIG. 34.—Table for recording data collected in Exercise 2.

(h) Understand from reading and discussion how the concept of genes which can occur in alternate states and which exist in pairs in all zygotes but singly in mature germ cells can be derived from data of the kind you have collected. What is the **principle of segregation**? Why is it that your data do not conform precisely to the theoretical 3 : 1 and 1 : 1 ratios expected, respectively, in second filial and back-cross generations? Consider the effect that differences in viability would have on survival under natural conditions. Write an explanation of the course of inheritance of whatever recessive character expression you have studied.

**Exercise 3.**—Further Breeding Experiments.

(i) If time permits, flies which differ with respect to two characters conditioned by pairs of genes located on different autosomes can be crossed. When the  $F_2$  generation has been obtained the character expressions are found to be associated in all possible ways, that is, four classes of progeny occur. Understand how these results can be explained in terms of the **principle of independent assortment** and secondary hypotheses. Note again that perfect ratios are not obtained, and consider the various factors that contribute to the discrepancy.

(j) The progeny of the  $F_1$  and backcross generations of reciprocal crosses between individuals differing with respect to two characters conditioned by two pairs of genes carried by the X-chromosomes will demonstrate **sex-linkage**, **linkage**, and **crossing over**.



## THE AMOEBA

PHYLUM PROTOZOA

CLASS SARCODINA

### **Exercise 1.**—Occurrence and General Activities.

(a) Many species of the Genus *Amœba* and its close relatives occur in fresh water, others in moist soil, and some as parasites in the digestive tracts of larger animals. The fresh-water amœbæ are sometimes found abundantly in nature or in laboratory cultures, but such cultures are difficult to maintain, and for this reason the amœba is not so easily secured at specified times as are many other unicellular forms. Very old hay infusions, prepared originally for paramecia, may contain numerous amœbæ. Ooze from the bottom of a pond or stream, the floating scum, slime adhering to the stems and leaves of water plants, and similar material sometimes contain great numbers. In recent years special methods of laboratory culture have been developed. If you have opportunity, it is interesting to see the actual collection in the field of material likely to contain this protozoan and how the cultures are handled. In any case, examine the laboratory cultures that contain amœbæ, noting the general appearance, color, and odor.

(b) Secure a drop of material from a culture known to contain amœbæ; add small bits of a No. 1 cover glass so that the No. 2 cover glass placed on the drop will not crush the specimens. Before returning to your place in the laboratory, examine a demonstration having an amœba in focus in order to know what to look for with your own microscope. Use a small aperture in the iris diaphragm, and look with the low-power objective for small pale blue or gray translucent objects containing minute granules. When a specimen is located examine it with the high-power objective. Open and close the iris diaphragm and observe the result. Why is it necessary to use a small aperture in searching for amœbæ and similar organisms? Observe the granules closely. Are they in motion? Does the animal change shape? Is there any relation between the movement of the granules and the changes in shape? Does the animal accomplish definite locomotion? Make a series of four drawings showing the shapes assumed by a single individual at intervals of one minute during this **amœboid movement**. Omit all internal structures, show outlines only, and put

arrows on your figures to indicate the direction of flow within the organism.

**Exercise 2.**—General Structure.

(c) Begin a drawing at least 10 cm. in diameter, and add the various features as they are observed. The projections from the main mass of the cell are the **pseudopodia** or “false feet.” Observe their function in locomotion and if possible in feeding. The outer, clear region is the **ectoplasm**, and the inner granular portion of the animal is the **endoplasm**. Some of the larger masses in the endoplasm are within a clear drop of fluid. These spaces in the endoplasm, filled with fluid surrounding bodies of various sorts, are the **food vacuoles**, in which food is being digested. Can you identify within these vacuoles green plant cells or other organisms of the same kinds as found living in the cultures with the amœbæ? Such organisms can sometimes be observed within a vacuole and still moving. Examine the other granules of the endoplasm, determining their size and shape. Is

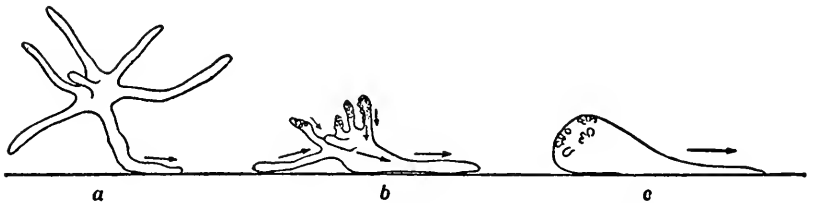


FIG. 35.—Reaction of an amoeba to contact with a surface. When the animal is allowed to sink slowly through the water pseudopodia are extended in all directions. If one of these comes in contact with the bottom or similar surface the cell responds in the manner shown. *a, b, c*, successive positions of same individual.

(From H. S. Jennings, 1904, Carnegie Institution *Pub.* 16.)

there a fixed boundary between ectoplasm and endoplasm? A single large, clear vacuole can be identified as the **contractile vacuole**, if it is seen to contract quickly and then to expand slowly. It is not easily recognized in the small specimens. This vacuole contains no granules, only liquid collected from the endoplasm. The liquid is discharged to the outside when the vacuole contracts. What is the function of the contractile vacuole? The **nucleus** is seen with difficulty in small amœbæ, but is recognizable in large ones as either oval or disk-like in contour, finely granular, and about the diameter of the expanded contractile vacuole. If the nucleus is not seen clearly in the living amœba, examine a demonstration of a stained and permanently mounted specimen.

(d) Examine amœbæ in a drop of water on a slide, without a cover glass and with the high-power ocular and the low-power objective. What can you make of the vertical dimension? Correct any errors in previous figures and make a clay model or a figure to show the superficial contours.

**Exercise 3.**—Special Activities.

(e) If individual amœbæ are carefully watched, it is sometimes possible to observe the manner in which food is ingested and fecal matter egested. Study also the contractile vacuole as it appears and disappears.

(f) If large specimens are abundant, study the currents within the endoplasm and the details of the process by which pseudopodia are formed and withdrawn. Do you find the ectoplasm to consist of an external membrane, the **plasmalemma**, enclosing a clear or **hyaline portion** of varying thickness in different parts of the cell; and the endoplasm to consist of an outer portion, the **plasmagel**, in which the granules are held as though embedded in a gelatinous material, and an innermost portion, the **plasmamol**, in which the granules move freely? Is this separation between hyaline ectoplasm, plasmagel, and plasmamol maintained at all times and in all parts of the amœba? Record your observations in a series of three drawings showing only the region of a pseudopodium.

## THE EUGLENA

PHYLUM PROTOZOA

CLASS MASTIGOPHORA

**Exercise 1.**—Occurrence and Activities.

(a) Species of the Genus *Euglena* are common in ponds and sluggish streams, being sometimes present in such numbers as to produce a reddish-green color in the ooze on the bottom or the scum on the surface. If you do not have opportunity to see the material collected, examine the cultures containing euglenæ, noting their general appearance and the parts of the vessels in which they are most abundant.

(b) Examine, on a slide, fresh material from a culture of euglenæ. Look with the low-power objective for elongated green bodies which may be at rest or moving about. Examine one under the high-power objective and observe the form and movements. Can you determine how locomotion is accomplished? Do the animals respond to light? Find individuals that are contracted and others that are expanded;

or better, observe the changes in shape of a single individual. These changes are termed **euglenoid movements**.

**Exercise 2.**—General Structure.

(c) Can you distinguish anterior and posterior ends? A slight notch in the profile of one end marks the opening of the **gullet** within which is the attached end of the **flagellum**, a long threadlike process difficult to see when in motion. Understand from textbook or lectures how this is used in locomotion. Make a clay model or a figure to show these external features.

(d) Begin a figure 10 cm. long, showing the outlines of an expanded euglena. Also make an outline of a contracted euglena on the

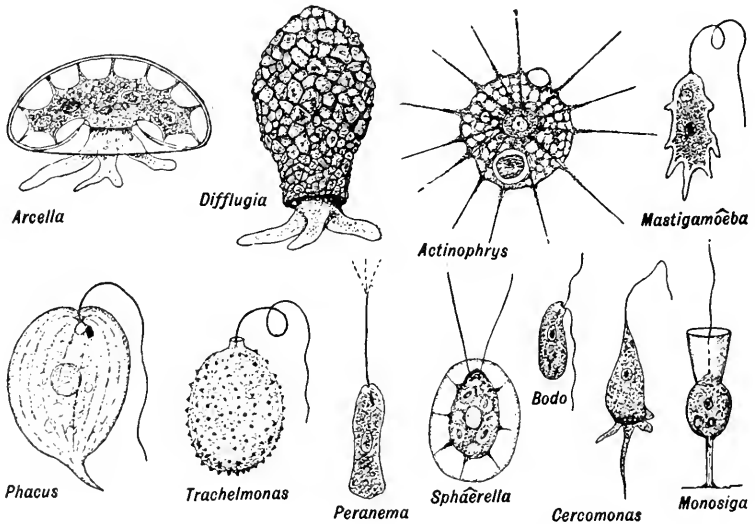


FIG. 36.—Representative Sarcodina (*upper row*) and Mastigophora (*lower row*). *Mastigamöeba*, which has a flagellum and pseudopodia, is classified as a mastigophoran.

(Drawn by C. E. Wilson.)

same scale. As you proceed, add details to the first drawing, labeling fully. The **chlorophyl**, or green coloring matter, is contained in bodies known as **chloroplasts**. Some of the larger species of euglena usually contain ringlike masses of **paramylum**, one of the starches. What can you infer, from the presence of chlorophyl, concerning the nutrition carried on by the euglena? Do you find a **nucleus**? What other structures can you find inside the cell or on its outer surface? There is a spot of red pigment, the so-called **eye-spot**, near the anterior end. The lighter spot near the anterior end of the cell is a group of **vacuoles**

which function as do the contractile vacuoles of other forms. Examine stained demonstration specimens showing the nucleus, and add it to your drawing.

(e) If the flagellum has not been observed, prepare another slide and stain with iodine or methyl violet. Find the flagellum attached to the anterior end of the cell and add this to the figure.

(f) Reproduction by longitudinal fission may be observed in the living material, or stained demonstrations may be studied. Draw dividing specimens if they are available.

### Exercise 3.—Encysted Phase.

(g) Encysted euglenæ are often found in cultures that have been standing for some days in the laboratory; or they may be collected in nature. Examine such material, and find the euglenæ surrounded by **cysts**. Note any structures identified in the active animal. Do you find individuals that have reproduced by cell division while encysted? Understand from lectures or textbook the relation between the active and encysted phases of the life-cycle. Draw several individuals in the encysted condition, including reproducing individuals if seen.

## THE PARAMECIUM

### PHYLUM PROTOZOA

### CLASS CILIATA

### Exercise 1.—Occurrence and General Activities.

(a) The paramecium is one of the unicellular forms most easily obtained. The species *Paramecium caudatum* is commonly studied. In nature, the animals are most abundant where the water is foul and ill-smelling, as in streams containing sewage and other decomposing organic material. Examine the laboratory cultures and note how they differ from cultures of amœbæ and euglenæ. Can the animals be seen without any magnification? Do they tend to collect at certain places in the culture jars?

(b) Fresh material will be distributed on slides. Do not add a cover glass at first; but examine with the low-power objective, noting the rapid movements and general behavior. Watch a single individual as it moves about. What determines the direction of its locomotion? Does the animal act as though it profited by experience? Determine its exact shape. How does the anterior end differ from the posterior end? Does the animal exhibit symmetry of any kind?

**Exercise 2.**—General Structure.

(c) Put a small number of absorbent cotton fibers, or of the fibers frayed from a piece of lens paper, on the drop containing the paramecia; or add a drop of thick tragacanth mixture to the drop of culture; then add a cover glass. The fibers will form pens enclosing the animals without crushing them or the tragacanth will prevent rapid motion, thus enabling you to observe the specimens under the high-power objective. Determine the shape and extent of the **buccal groove**, which extends backward from the anterior end on one side of the cell. How does it end posteriorly? The function of this blind pocket, the **gullet**, will be studied later. Observe the **cilia** on the surface of the cell. Are they the same length on all regions? Are their movements coördinated during locomotion? A clear vesicle near each end will be seen to enlarge slowly and suddenly disappear. These are the **contractile vacuoles**. Their activities will be observed more closely later. Numerous **food vacuoles** can be seen scattered throughout the cell; they contain various materials. Note that the cell consists of an outer layer of clear **ectoplasm** surrounded by a **cuticle** which appears as a firm line on animals that are compressed by the cover glass. The inner granular portion of the cell surrounding the food vacuoles is the **endoplasm**. Do you observe motion of the granules and food vacuoles?

(d) Draw off the water from the edge of the cover glass with filter paper until the animals are almost crushed. Look along the margin of the cell for rodlike bodies in the ectoplasm, the undischarged **trichocysts**. Draw off the water until the animal is crushed and the endoplasm flows out. Examine this carefully with the highest magnification to be obtained with your microscope. Examine the cuticle carefully. Can you see markings on its surface? Make a figure, 10 cm. long, with the buccal groove uppermost, and show the structural features observed so far.

(e) Make a fresh mount of the culture only and add a drop of methyl green. Determine the shape of the **macronucleus**. What is the difference in the reaction of the nuclear material and the cytoplasm to the stain? Does this indicate anything regarding their chemical or physical composition? Such crude staining methods do not ordinarily demonstrate the smaller **miconucleus** which lies in a depression in the macronucleus. Examine a demonstration showing the miconucleus and add these two structures to your previous drawing.

(f) Examine the margin of specimens stained with methyl green, iodine, or fountain-pen ink and look for heavy rodlike processes, much

longer than the cilia, extending from the surface. These are the trichocysts which are discharged as a result of contact of the animal with the stain. They are structures for defense. Show the discharged trichocysts on part of the margin of your figure.

**Exercise 3.**—Ingestion and Food Vacuoles.

(g) Take a very small drop of water, containing many paramecia, and add an equal amount of water containing India ink obtained by rubbing a bit of an ink stick against the bottom of a watch glass containing a little water; or finely powdered insoluble carmine may be used. Study the action of the cilia as they drive the particles about. Watch for a time and see how the particles get into the cell. Can you see the actual “gulping down” of the particles? Does feeding continue regardless of the amount of material ingested? Where are the particles found after ingestion? Trace the course of the food vacuoles as they are carried in the endoplasm. Where do they come to rest? Make a series of three outline drawings of a paramecium, showing details only in the region of the buccal groove and gullet, to illustrate this process, and write a brief explanation.

**Exercise 4.**—The Contractile Vacuoles.

(h) Mount some specimens in a very small drop of water, holding them in place, without crushing, by the weight of the cover glass. Study the formation and collapse of the **contractile vacuoles** and of the **canals** leading to each vacuole. Time the contractions. Compare with the observations of other students. Write an accurate description, accompanied by four drawings, explaining how the vacuoles and their canals function in discharging fluid from the cell.

**Exercise 5.**—Division and Conjugation.

(i) Individuals are often seen in the process of reproduction by cell division. This occurs by transverse **binary fission** or mitotic division of the cell into two equal parts. If a dividing specimen is found, it should be kept under observation continuously by moving the slide about. After some minutes it will be seen to separate into two independent cells, each of which then becomes a perfect individual. The nuclear changes during fission can be observed only in stained material. Examine and draw a series of demonstrations stained to show: (1) the beginning of constriction in the cytosome, elongation of the macronucleus, and the formation of a mitotic figure in the micronucleus; (2) further constriction of the cytosome and macronucleus with separation of the new micronuclei; and (3) a later stage in which division of the cytosome and macronucleus is almost complete.

(j) *Paramecia* may be found in the process of **conjugation**, but such pairs swimming about adhering together by their buccal grooves do not show the nuclear changes which are occurring. Examine and

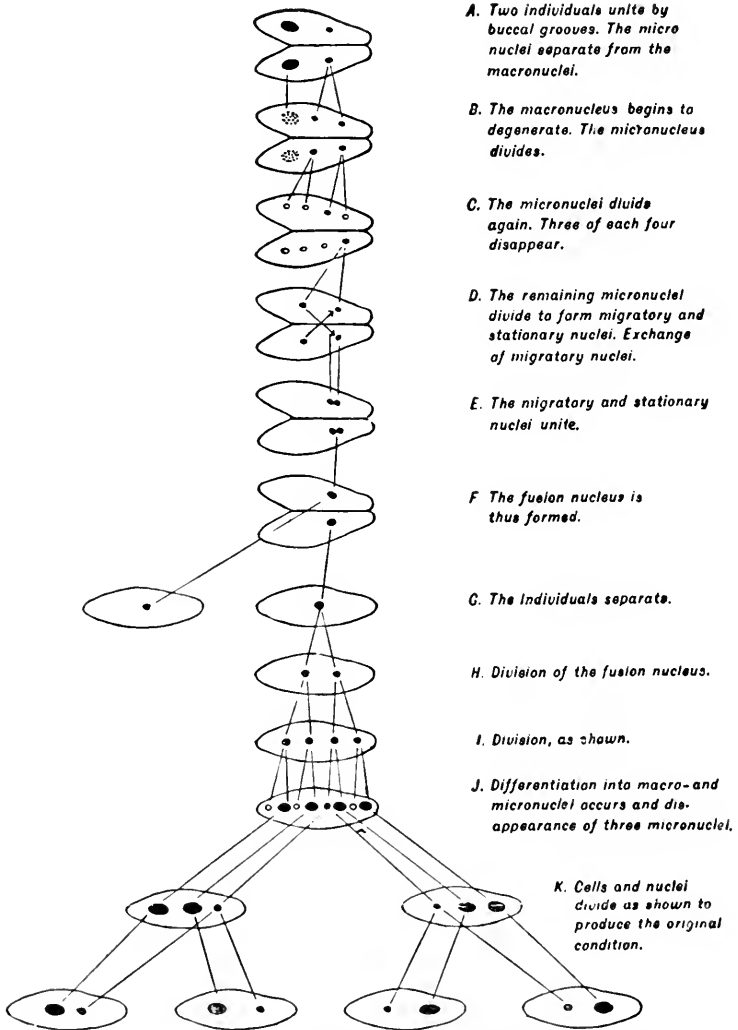


FIG. 37.—Conjugation in *Paramecium caudatum*; diagrammatic.

(Redrawn from H. S. Jennings, "Life and Death, Heredity and Evolution in Unicellular Organisms," copyright, 1920, Richard G. Badger, printed by permission.)

draw demonstrations showing: (1) A pair of conjugants, with a single micronucleus in each, in which the macronuclei are beginning to show some irregularity. (2) Conjugants in which the original micronuclei



have divided twice, followed by degeneration of three of the resulting micronuclei in each and the division of the remaining one to form a stationary and a migratory micronucleus in each conjugant; the macronuclei will be partly disintegrated. (3) An exconjugant in which the fusion nucleus has divided twice to form four nuclei; fragments of the old macronucleus may still be visible. Other intermediate stages may also be shown. Understand fully from textbooks all the changes that occur, and relate the process of conjugation in the paramecium to the syngamy of non-ciliated protozoans and to fertilization in the frog.

## OTHER NON-COLONIAL PROTOZOA

**Exercise 1.**—Free-living Species.

(a) Numerous species of Protozoa will have been observed in the cultures used for *Amœba*, *Euglena*, and *Paramecium*. The accom-

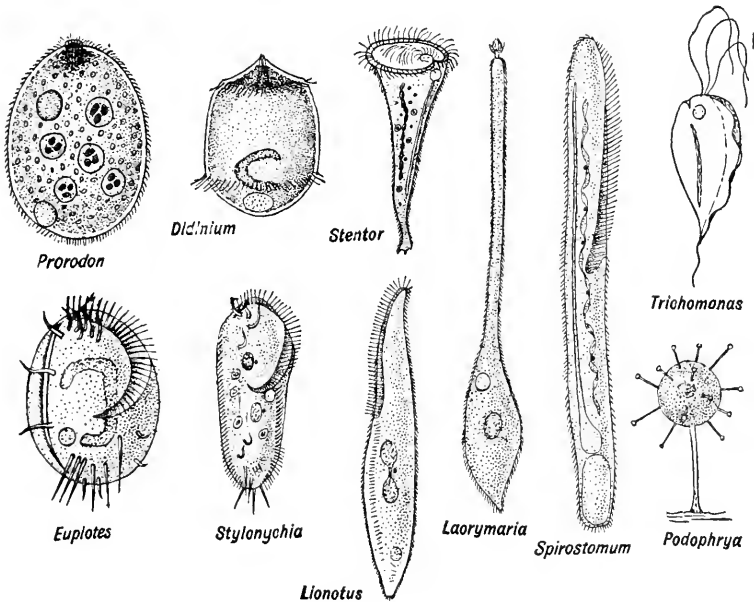


FIG. 38.—Common Infusoria and other Protozoa from fresh water.

(Drawn in part by Wiley Crawford.)

panying Figs. 36 and 38 show common and representative types seen in fresh water. It is instructive to examine a diversified culture and to list, with aid of your instructor, the different forms that are found. Especially favorable types may then be studied briefly for purposes

of comparison with the protozoans already studied in detail. Drawings should be made to illustrate the important structural features. Further examination of chart and textbook figures gives one a better idea of the number and variety of the types of these unicellular organisms.

**Exercise 2.**—Parasitic Species.

(b) Protozoa belonging to the Class *Sporozoa* live as parasites in the bodies of other animals. Notable among these is *Gregarina*, which occurs in the digestive tracts of arthropods. Material for this study may be obtained from the larvæ or adults of the meal beetle, *Tenebrio*. Take a living larva of the beetle on a glass slide and snip off, with scissors, the last segment of the body. With forceps, tear off the head, endeavoring to pull out the entire digestive tract attached to the head. Cut off the head and remove from the slide. The digestive tract can then be extended upon the slide, and the remnants of the cream-colored fat-body that may be adhering to the tract can be removed. Without adding a cover glass, examine the tract with the low-power objective. The gregarines, if present in numbers, will be seen within the tract as dark-colored bodies, two or three times longer than they are wide. Look for a digestive tract containing a considerable number of the gregarines. When found, such a tract may be cut into small pieces and these teased apart in a very small drop of 0.9% salt (sodium chloride) solution before the cover glass is added. Very little salt solution should be used, because the gregarines live longest when in the normal fluid of the gut cavity. A single well-infected tract may be used for the making of several preparations. As the infection with any parasite is largely a matter of chance, it may be necessary to examine a number of beetles or larvæ before satisfactory material is obtained.

(c) Study the preparation under the high-power objective. The gregarine has sharp outlines because of its firm cell membrane. The cell is divided into anterior and posterior parts. Where is the **nucleus**? Are there **ectoplasm** and **endoplasm**? Do the organisms move? How? Do you find more than one type of individual? Individuals are often found attached end to end. This is neither cell division, nor conjugation, but merely an association of the animals as they move through the thick fluid in which they live. Draw a figure or figures, 10 cm. long, to show the features observed. Compare the structure of *Gregarina* with that of *Paramecium*. How is the structure and activity of each related to its environment?

(d) Encysted stages of *Gregarina* are not readily obtained, but

cyts containing individuals of the Genus *Monocystis*, a sporozoan which lives in the seminal vesicles of the earthworm, can be studied (Fig. 39). Here the encysted stages are abundant in infected speci-

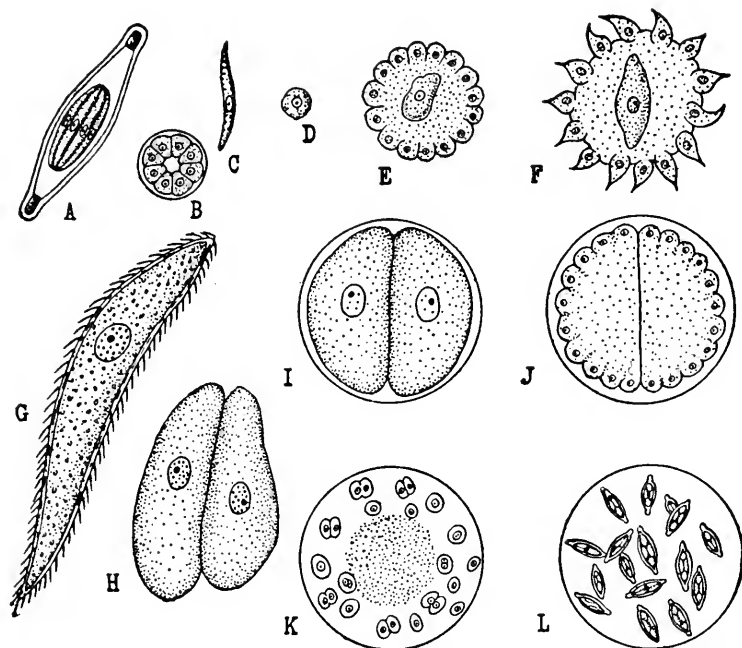


FIG. 39.—Life-cycle of the gregarine, *Monocystis*. *A*, spore consisting of a spore-case enclosing eight sporozoites. *B*, transverse section of same. *C* and *D*, liberated sporozoites. *E*, sporozoite after entering multicellular sperm-sphere of earthworm. *F*, growth in sperm-sphere to form a trophozoite which is later surrounded (*G*) by the remains of the sperm-sphere consisting of a thin envelope to which the tails of the degenerate spermatozoa adhere. *H*, two trophozoites that have become free of the degenerate sperm-spheres and united as gametocytes. *I*, encystment of gametocytes. *J*, reproduction by sporulation, or multiple division of the nucleus followed by cytosomal constrictions, to form isogametes. *K*, reproduction by union of the isogametes to form zygotes; the residual cytoplasm of the gametocytes is in the center of the cyst. *L*, cyst containing many sporozoites, formed by secretion of a spindle-shaped spore case around each zygote which undergoes sporulation to form eight sporozoites; these eventually become arranged as in *A* and *B*, in which state they are transferred to another earthworm.

(Drawn by Wiley Crawford.)

mens, while the active stages are less numerous. Take a bit of the seminal vesicle from a fresh earthworm, and tease it out in 0.9% salt (sodium chloride) solution on a slide. Add a cover glass and look for spherical bodies, containing two hemispherical cells or many smaller

spindle-shaped cells. These are the encysted stages of *Monocystis*. Look for active stages. The latter are large, spindle-shaped cells, either naked or surrounded by a layer of the earthworm's tissue from which protrude the flagella of degenerating spermatozoa. Is the cell divided as in *Gregarina*? Has it a nucleus? Are any structures visible which can be interpreted as related to feeding? By reference to Fig. 39, identify as many stages as your slide affords and draw the same arranged in order.

## COLONIAL PROTOZOA

### Exercise 1.—Types of Colonies.

(a) Certain kinds of Protozoa remain attached to one another after reproduction by cell division, thus producing colonies. In some colonies the cells are connected by cytoplasmic processes; in others the individuals are held together by a non-living matrix which they produce. Examine such colonial forms as may be available, preferably in the living state. As some species are seldom found in cultures, it may be necessary to study prepared slides. The following types of colonies occur (Fig. 40): **linear colonies**, such as *Ceratium*; **platelike colonies**, such as *Gonium*; **arboroid colonies**, such as *Carchesium*; **gregaloid colonies**, such as *Microgromia*; and **spheroid colonies**, such as *Pandorina*, *Eudorina*, *Volvox*, *Spondylomorom*, *Synura*, and *Uroglena*. Reference to charts or textbook figures will be helpful in supplementing this brief study.

### Exercise 2.—Spheroid Colonies.

(b) Study a series of spheroid colonies and compare them with one another and with non-colonial forms such as *Euglena*, *Chlamydomonas*, and *Chilomonas* which are independent and hence physiologically balanced cells. Particularly in the flagellates with plantlike characteristics, it is possible to arrange a series showing an increasing number of cells and a progressive specialization, and thus division of labor, among the cells of the colonies. For example, *Gonium sociale* is a platelike colony consisting of four cells arranged in one plane and held together by a gelatinous matrix; *Gonium pectorale* is a similar colony composed of sixteen cells. In these colonies each cell is physiologically balanced, although the cells of any colony are held together in the characteristic manner. Reproduction occurs by cell division and by the syngamy of isogametes. *Pandorina* consists of sixteen physiologically balanced cells forming a colony that is ovoid rather than spherical in shape. Reproduction occurs by cell division and by

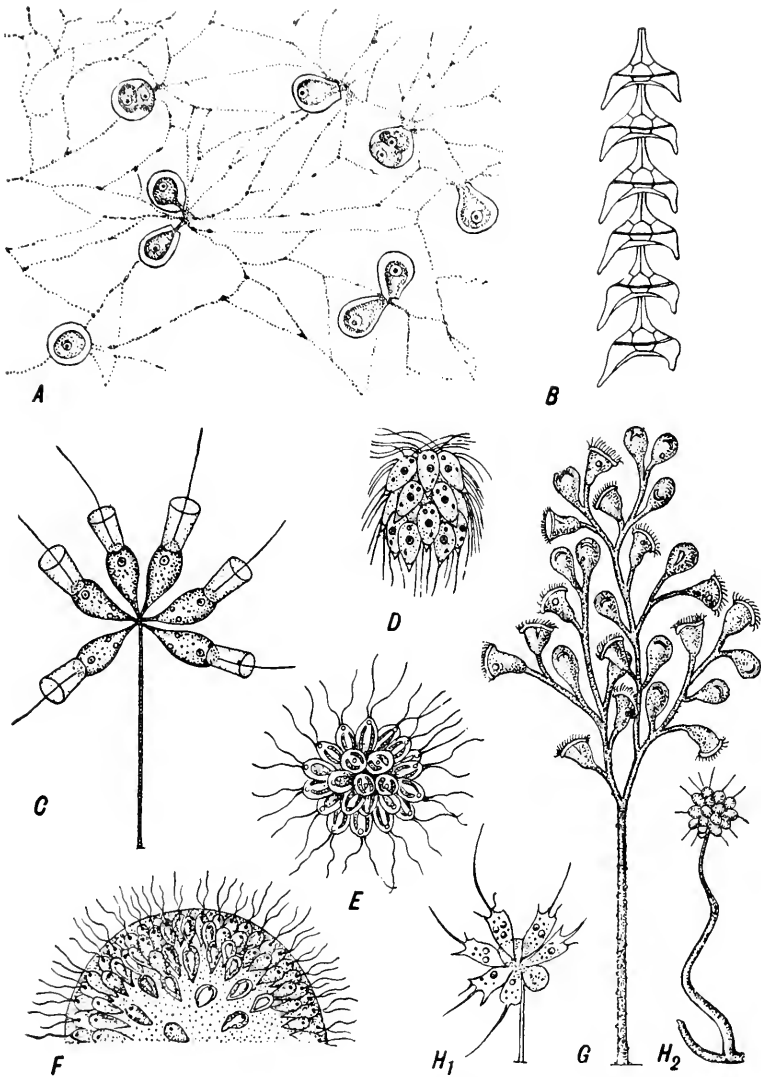


FIG. 40.—Types of colonial Protozoa. A, *Microgromia*, a gregaloid colony. B, *Ceratium*, a linear colony. C, *Codonosiga*, an arboroid colony. D, *Spondylomorom*, a spheroid colony. E, *Synura*. F, *Uroglena*. G, *Carchesium*. H<sub>1</sub> and H<sub>2</sub>, portions of *Anthophysa* colony.

(Figures redrawn as follows: A, from G. N. Calkins, "Protozoa," copyright, 1901, by The Macmillan Co., printed by permission; C, from E. A. Minchin, "Introduction to Study of Protozoa," copyright, 1912, by E. Arnold and Co., printed by permission; D, E, F, H<sub>1</sub>, and H<sub>2</sub> from B. Blochmann, 1895, "Die mikroskopische Thierwelt des Süsswassers"; G, from W. S. Kent, "A Manual of the Infusoria," vol. 3.)

gametes, but macrogametes and microgametes may occur instead of isogametes. *Eudorina* is a spherical colony, with more cells than in *Pandorina*; and there are two kinds of colonies, one producing microgametes and the other macrogametes. *Pleodorina* is a colony composed of thirty-two cells. Twenty-eight of these cells are physiologically balanced and larger than the other four; any one of these twenty-eight can reproduce by cell division to form a new colony. Reproduction by the syngamy of microgametes and macrogametes, formed by any of the twenty-eight cells, also occurs. The four small cells take no part in these reproductive processes and disintegrate when the reproduction, which occurs only among the twenty-eight cells, has been completed. It appears, therefore, that these four cells are not physiologically balanced, since they have lost the capacity of reproduction; hence they may be called **somatic cells**, or **body cells**, in contrast to the twenty-eight cells which may be called **germ cells**. In *Volvox* the number of somatic cells greatly outnumbers the germ cells which are the physiologically balanced cells, as shown by their capacity for reproduction by cell division to form new colonies or by the formation of microgametes and macrogametes which reproduce by syngamy. While differences occur in the several species of *Volvox*, the following characteristics are important for the present purpose. Three types of cells are found: numerous **somatic cells**; germ cells, called **parthenogonidia**, which divide repeatedly within the parent to form daughter colonies; and the **zygote-forming germ cells**, which differentiate into either **ova** or **spermatozoa**. When an ovum has been fertilized by a sperm, the resulting **zygote** secretes a cyst and after an inactive period emerges to divide and form a new colony. Drawings should be made as directed by the instructor. What eventually becomes of the cells in a colony of *Pandorina*? In *Pleodorina*? In *Volvox*? How does *Volvox* differ from a many-celled animal? How from a non-colonial protozoan? What is natural death? What is the individual in these colonial species?

## THE GRANTIA <sup>1</sup>

PHYLUM PORIFERA

CLASS CALCAREA

### Exercise 1.—Occurrence and External Features.

(a) *Scypha coronata* (*Grantia* sp.) is a sponge commonly found attached to wharf-piles or other objects just below the low-tide mark. If living specimens are available, add powdered carmine to the water containing them and determine the direction of water currents through the individual sponge.

(b) Examine in separate watch glasses a dried specimen of *Scypha* and one preserved in fluid. Observe the shape, region of attachment, and the large excurrent opening, the **osculum** (cf. Fig. 41). The many small openings on the surface are called **ostia**. Minute **spicules** project from the surface; note their arrangement about the osculum. Specimens with one or more **buds** should be examined as demonstrations, if not observed in your own or in a neighboring specimen. Draw one of your specimens ( $\times 4$ ) to show external features.

### Exercise 2.—General Internal Structure.

(c) With a sharp scalpel or a razor blade make longitudinal and cross sections of the dried specimen. The large central cavity from which the osculum opens is called the **cloaca**. The many small openings into the cloaca, called **apopyles**, lead from the **excurrent canals** which lie radially in the wall of the sponge. The ostia, previously observed upon the external surface, can be seen opening into the **incurrent canals** which also lie radially in the wall of the sponge. The minute openings which lead from the incurrent into the excurrent canals can be seen only in prepared sections. Draw ( $\times 10$ ) a portion of the wall to show the features observed. Indicate by arrows the course of water through the canal system.

<sup>1</sup> According to Libbie H. Hyman, *Science*, May 7, 1937, p. 454, the Woods Hole *Grantia* should be called *Scypha coronata* (Ellis and Solander) 1786, syn. *Spongia coronata* (Ellis and Solander). The word "grantia" has become so familiar to American zoölogists that its use, as the common name to designate this type of sponge, still seems justifiable.

**Exercise 3.—Microscopic Structure.**

(d) Stained sections of the grantia permit an examination of the cellular structure; in preparing such material the calcareous spicules are usually dissolved by acid to facilitate the sectioning. The cells of

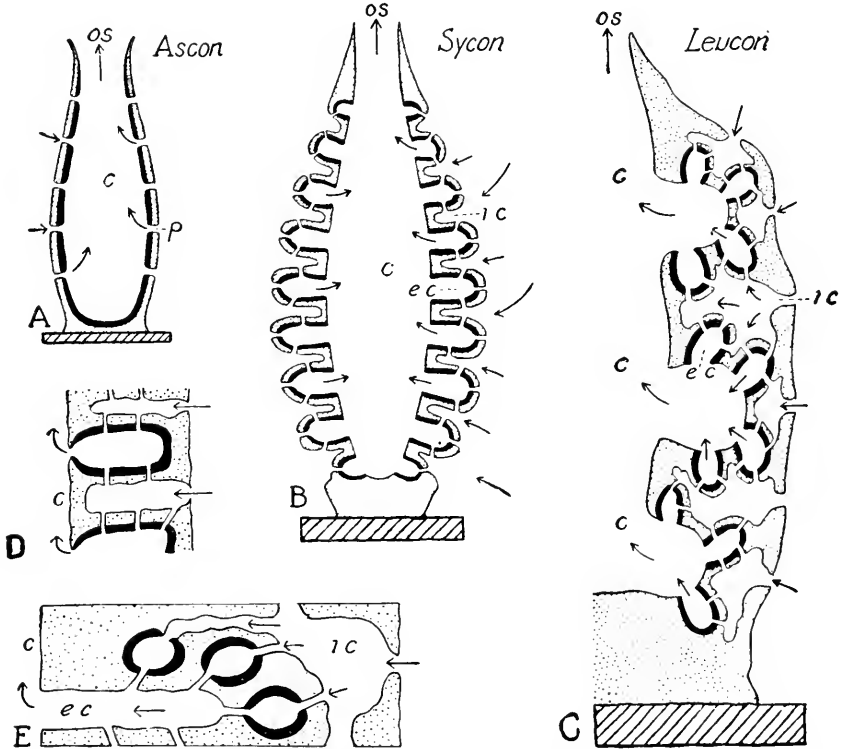


FIG. 41.—Canal systems of sponges. *A*, ascon type. *B*, sycon type. *C*, leucon type. *D*, a portion of the wall of a sponge showing a specialization of the sycon type. *E*, a portion of the wall of a sponge showing a specialization of the leucon type. In the sycon or leucon types, the openings into the incurrent canals (*i c*) are called ostia, the openings from the incurrent to the excurrent canals are called prosopyles and correspond to the pores (*p*) of the ascon type; arrows indicate the direction of the water currents. *c*, cloaca; *e c*, excurrent canal; *i c*, incurrent canal; *os*, osculum; *p*, pore.

(Redrawn from E. A. Minchin, in E. R. Lankester, "Treatise on Zoölogy," copyright, 1900, by A. and C. Black, printed by permission.)

sponges are so delicate that good sections are difficult to obtain. Covering the outer surface and lining the incurrent canals are the pavement-like **dermal cells**. Lining the excurrent canals are the **collar-cells**, or **choanocytes**; the collars and flagella of these may be retracted, as well as shrunken in the preservation, and hence not easily



recognized; refer to figures in textbook and Fig. 41. The beating of the flagella upon the choanocytes brings about the current of water through the sponge. Look for the **prosopyles** leading from incurrent to excurrent canals. In the gelatinous ground substance between the dermal and gastral layers are found several other cell types: large amoeboid cells, the **archæocytes**, which are capable of amoeboid movement; **scleroblasts**, which secrete the spicules; **collencytes**, or connective tissue cells; early **germ cells**, which resemble archæocytes; masses of **spermatozoa**; and fully developed **ova**. Sponges are commonly hermaphroditic, but the ova and spermatozoa may be produced at different times in the same individual. Fertilization occurs and the **zygotes** develop within the region between the two cell layers; **ciliated larvæ** are formed which leave the parent sponge by way of the excurrent canals, cloaca, and osculum. Draw ( $3-5 \times$  projected size), showing the cellular organization as observed in the preparation you have studied.

(e) The spicules can be examined in thin sections of a dried grantia in temporary or permanent mounting. Observe their arrangement in the section and note the different kinds. Also examine the spicules as they appear in a drop of the residue obtained by boiling a *Scypha* in a solution of sodium hydroxide. Draw ( $3-5 \times$  projected size), showing arrangement of spicules to constitute the skeleton of the normal sponge.

## OTHER SPONGES

### Exercise 1.—Various Species.

(a) Stained sections of the sponge *Leucosolenia*, and pieces of the wall mounted whole, may be studied to illustrate the organization of a sponge having the **ascon type** of canal system (*cf.* Fig. 41).

(b) Examine: dried specimens of **glass sponges**, in which the skeleton is composed of siliceous spicules; and specimens of **horny sponges**, such as the bath sponge, *Euspongia*. The canal systems of the larger and more complex sponges may be compared with that of grantia as shown by Fig. 41.

(c) The fresh-water sponges, *Spongilla* and *Ephydatia*, are found in clear ponds and streams attached to stones or other submerged objects. They are easily collected but difficult to maintain in the laboratory. Actively growing specimens occur during the warmer months, but in winter only granular encrustations composed of **gemmules** which develop into new colonies the following spring.

## THE HYDRA

PHYLUM CŒLENTERATA

CLASS HYDROZOA

### Exercise 1.—Occurrence and Collection.

(a) Four species of *Hydra* are commonly found in the eastern and central United States:<sup>1</sup> *Pelmatohydra oligactis*, large, with a definite stalk in the body region, with tentacles much longer than the body, and brown in color; *Hydra carnea*, reddish brown or pinkish orange, but smaller than the first-named species and having tentacles shorter than the body, which is without a stalk; *Hydra americana*, no stalk, color white or tan, tentacles shorter than the body and held erect; and *Chlorohydra viridissima*, the small green hydra. The green ones are not so favorable for study. Hydrams live in clear ponds or slowly moving streams and are most easily collected from small objects such as plants and leaves. In the fall they are often found in great numbers attached to leaves that have settled to the bottom of a pond. They can be collected from these with a pipette, or the leaves can be brought to the laboratory and placed in aquaria where the hydrams can be seen attached to the vegetation or to the walls of the jars. Examine such cultures showing the animals under approximately natural conditions.

### Exercise 2.—General Structure.

(b) Study a hydra in a watch glass with sufficient water to allow the animal to expand fully. Use a handlens or the low-power objective of the compound microscope. The animal is attached by its **base** or **foot**. The free end terminates in the **hypostome**, at the end of which is the **mouth** which is closed except when the animal is feeding. **Tentacles** surround the hypostome. How many tentacles are there? Where do they arise? Note the knoblike clusters of **nematocysts**, or stinging capsules, on the tentacles. What is their function? Observe the movements and changes in shape of the animal; the handlens

<sup>1</sup> *Pelmatohydra oligactis*, *Hydra americana*, and *Hydra carnea* were formerly confused, and all were classified as *H. vulgaris*. The papers by Libbie H. Hyman in the *Transactions of the American Microscopical Society*, vols. 48, 49, and 50 (1929-1931), contain information concerning the identification of the hydrams of North America.

can be used more effectively now that you are familiar with the details as seen with the microscope. Make a drawing ( $\times 50$ ) of an expanded hydra to show the external features. Make also an outline drawing of a contracted hydra, looking down on the mouth and tentacles.

(c) Note that the body is hollow, containing a digestive cavity, the **enteron**, into which the mouth opens. Is there an anus? Does the enteron extend into the tentacles? The body wall consists of two layers of cells, the outer **ectoderm** which is thinner and more transparent, and the inner **endoderm**. In the green hydra the endoderm is

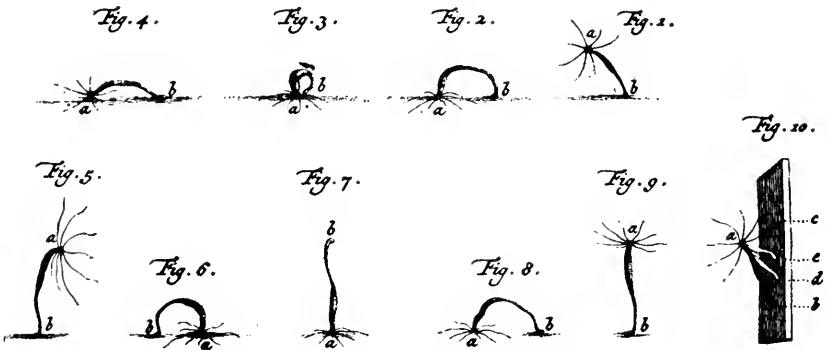


FIG. 42.—Locomotion in *Hydra*.

(Reproduced from the original by Abraham Trembley, "Polypes d'eau douce," Leyden, 1744.)

green because of the presence of symbiotic unicellular plants (*Zoöchlorællæ*) within the cells.

(d) Study prepared cross sections of hydra. Identify the ectoderm and endoderm and see that they are separated by the thin non-cellular **supporting lamella**. Make a drawing, about 3 inches in outside diameter, to show the layers but no cell details (state magnification). From your observations construct a diagram to show the structure as it would appear in a longitudinal section passing from end to end and including a tentacle.

**Exercise 3.—The Ingestion of Food.**

(c) Specimens of one of the larger species that have been made hungry by keeping them without food for at least twenty-four hours should be used. In nature, hydras often feed upon water-fleas; in the laboratory they will be more likely to take this natural prey if the fleas have been soaked in water in which several specimens of the annelid *Tubifex* have been crushed. Short pieces of *Tubifex*, bits of

flesh from a frog, crayfish, or water snail, raw beef, or liver may also be used for food. Place a very small piece of the food carefully against a tentacle with a clean needle. The hydra should be attached to the bottom of the watch glass. Observe the animal throughout the process, using handlens and lowest power of compound microscope.

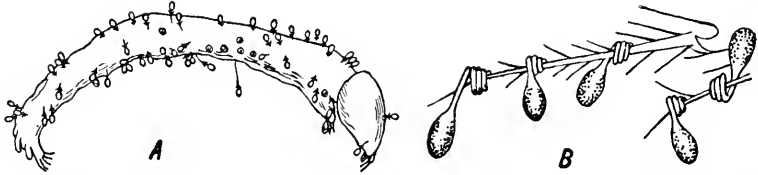


FIG. 43.—Nematocysts of *Hydra*. *A*, insect larva punctured by nematocysts of the barbed type. *B*, part of an appendage of a water-flea showing another type of nematocyst that seems to function like a grappling hook.

(*A*, redrawn from H. S. Jennings, "Behavior of the Lower Organisms," copyright, 1906, by the Columbia University Press, printed by permission; *B*, redrawn from O. Toppe, 1909, *Zoologischer Anzeiger*, vol. 33.)

Make a series of three drawings to show how ingestion is accomplished, and write a concise account of the process.

#### Exercise 4.—The Cellular Structure.

(f) Macerate a hydra with Bela Haller's fluid as follows: Place the animal on a slide with very little water, and add a drop of Bela Haller's fluid. After one-half minute, quickly remove the fluid with

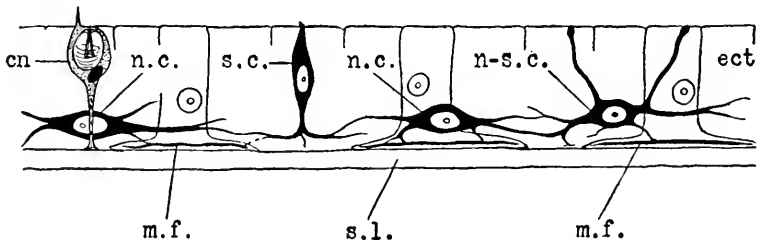


FIG. 44.—Diagram of the sensory-neuro-muscular mechanism of *Hydra*. One of the cnidoblasts (*cn*), which are independent effectors, is included. *ect*, ectoderm; *m. f.*, muscle fibril of large ectoderm cell; *n. c.*, nerve cell; *n-s. c.*, neuro-sensory cell; *s. c.*, sensory cell; *s. l.*, supporting lamella.

filter paper, and add at once a drop of methyl violet. After about two minutes, remove the stain with filter paper and add a drop of water. Put on a cover glass and tap on it gently. In this way the cells may be separated from one another while individual cells are left intact. It may be necessary to make more than one such preparation in order to find the maximum number of cell types; try adding the

cover glass without removing the stain. Before discarding any preparation make sure you have made the most of it.

(g) Separate your sheet of drawing paper into two areas by means of a light line. Mark one region for "Cells of the Ectoderm" and the other for "Cells of the Endoderm." As you find and identify the separated cells, draw them ( $5 \times$  measured size as projected to table level) in the correct region; indicate functions of cells in your labels. The following types of cells will be recognized in favorable preparations: (1) **Endoderm cells**, the largest ones present, usually elongated, with a conspicuous vacuole or vacuoles in the cytoplasm, and with **muscle processes** extending from the base. If green hydras are used, the endoderm cells will contain numerous green, unicellular plant cells, or *Zoöchlorellæ*. (2) **Gland cells**, elongated, slender, and frequently tapering toward one end, with deeply stained granular cytoplasm. (3) **Ectoderm cells**, similar in shape but smaller than the endoderm cells and bearing **muscle processes** which will usually be contracted. Why? (4) **Interstitial cells**, very small, rounded cells, with relatively large nuclei. (5) **Cnidoblasts**, or stinging cells, containing undischarged **nematocysts**. Recognize the nucleus, cytosome, and **cnidocil**. Find as many types as possible of discharged nematocysts. (6) **Nerve cells** from the nerve net are sometimes seen.

#### Exercise 5.—Regeneration and Reproduction.

(h) The phenomenon of regeneration is widespread but is most highly developed among those groups of animals which reproduce extensively by asexual processes such as budding and fission. Clean two watch glasses, filling one of them two-thirds full of water from a jar in which hydras have been living, and using the other as a cover. Take several hydras and cut each transversely into two or more pieces. Examine the pieces with the low-power objective of the compound microscope. Set aside and examine at subsequent laboratory periods until the regeneration is complete. Make sketches and briefly record the changes of the several pieces. Compare with the well-known powers of regeneration and vegetative reproduction in plants.

(i) Hydras reproduce by **budding** throughout the period when seasonal and food conditions are favorable. Budding is essentially reproduction by cell division, an asexual method. Observe and draw three progressive stages. Understand the relations between the layers of the parent and bud.

(j) Hydras reproduce by **syngamy** during certain periods. Gonads are formed in the ectoderm. The **ovary** contains a single **ovum**, which is exposed to the water when meiosis has occurred. The **testis**, of

which many may occur on one hydra, is a cone-shaped structure, containing numerous **spermatozoa** which are released by rupture of the testis. The sperm swim to the ova and the syngamy, or fertilization, occurs, after which cysts are secreted in which the **zygotes** develop. Draw hydras with gonads. Observe **hermaphroditic** specimens and sections of **differentiating gonads**.

## THE HYDROIDS AND HYDROMEDUSÆ

### Exercise 1.—Occurrence and General Structure of Colony.

(a) Hydroids are marine cœlenterates closely resembling the hydras in their general structure. They live attached to rocks, seaweed, and submerged woodwork. They differ from the hydras in that the individuals, instead of living singly, live together in **colonies** comparable to the colony that would be formed if a hydra budded many times and all the buds remained united to the parent body.

(b) For this study, preserved specimens of the hydroid *Obelia geniculata* will be used. Examine, with a handlens, a portion of a colony in a watch glass of water, and, also, museum specimens of entire colonies of this and other hydroids. In some of the colonies, the individuals are large enough to be recognized without a lens. Examine the obelia with the lowest power of the compound microscope. The **hydranths**, or feeding individuals, and the **blastostyles**, or reproductive individuals, are permanent members of the colony. In addition, the **medusæ**, or jellyfishes, appear on the blastostyles and constitute a third type of individual, which becomes detached. There is a division of labor among the individuals of the colony. The feeding individuals, which are the most numerous, are those with tentacles. Immature feeding individuals with rounded ends will be seen. The reproductive individuals are the blastostyles and medusæ. Notice how the upright stems are fastened at the base, and how rootlike, horizontal stems extend over the surface on which the colony is attached. Draw ( $\times 6$ ), showing these general features of a portion of a colony.

### Exercise 2.—Feeding Individuals, or Hydranths.

(c) Select a fully matured hydranth that is properly expanded, and identify the parts as found in the hydra: **body**, **hypostome**, **tentacles**, **ectoderm**, **endoderm**, and **enteron**. Notice that the body of the hydranth is continued downward as a slender stem, the **cœnosarc**, which is continuous with the common cœnosarc of the colony. The ectoderm, the endoderm, and the cavity of the hydranth are thus con-

tinuous throughout the branched stem and its rootlike extensions, the **hydrorhizæ**.

(d) The entire colony is protected and supported by a thin, transparent covering, the **perisarc**, which is not present in the hydras. The perisarc of the stem is continued upward to form a cup-shaped **hydrotheca** about each hydranth. Notice the shelf-like expansion of the hydrotheca on which the hydranth rests, the ringed form of the perisarc just below the hydrotheca, and certain places where the perisarc is greatly thickened. Do you find places where the ectoderm of the coenosarc is thickened? Explain. Draw a single hydranth, on a large scale, including its connection with the upright stem.

**Exercise 3.**—Reproductive Individuals, the Blastostyles, and their Medusæ.

(e) The blastostyles above noted are without mouths or tentacles. How are they nourished? The covering of a blastostyle is the **gonotheca**. How does it differ in shape from the hydrotheca? Attached to the blastostyle are numerous rounded bodies, the **medusa buds**. Each medusa bud becomes a **medusa**, or jellyfish, which is detached from the blastostyle and escapes into the water through an opening at the end of the gonotheca. The medusa may be considered a third type of individual of the colony, but unlike the other two types, hydranths and blastostyles, it is not a permanent member of the colony. What process in the hydra is comparable with the detachment of the medusa from its parent hydroid colony? Draw a single blastostyle on a large scale, showing its connection with the main stem and also the medusa buds in several stages of formation.

(f) Examine demonstrations of the medusæ of the obelia in the stages just after detachment, when they are swimming freely in the water. In the later stages of the medusæ, reproductive organs appear, as may be seen in a large hydroid medusa such as the species *Gonionemus murbachii*. Draw the medusa of *Obelia*.

**Exercise 4.**—The Life-cycle.

(g) The obelia colonies reproduce only by the asexual method of budding. They never have gonads as do hydras. The medusæ have gonads and reproduce by **syngamy**, or the union of **ova** and **spermatozoa** in fertilization. The offspring of the medusæ are not medusæ, like their parents, but are hydranths which, by extensive growth and budding, develop into a colony like the one just studied. The life-history of the obelia thus exhibits an **alternation of generations**, since the attached hydroid colony, reproducing by budding, alternates with the

free-swimming medusa, reproducing by syngamy. How could the life-cycle of an extensively budding hydra be modified to give stages

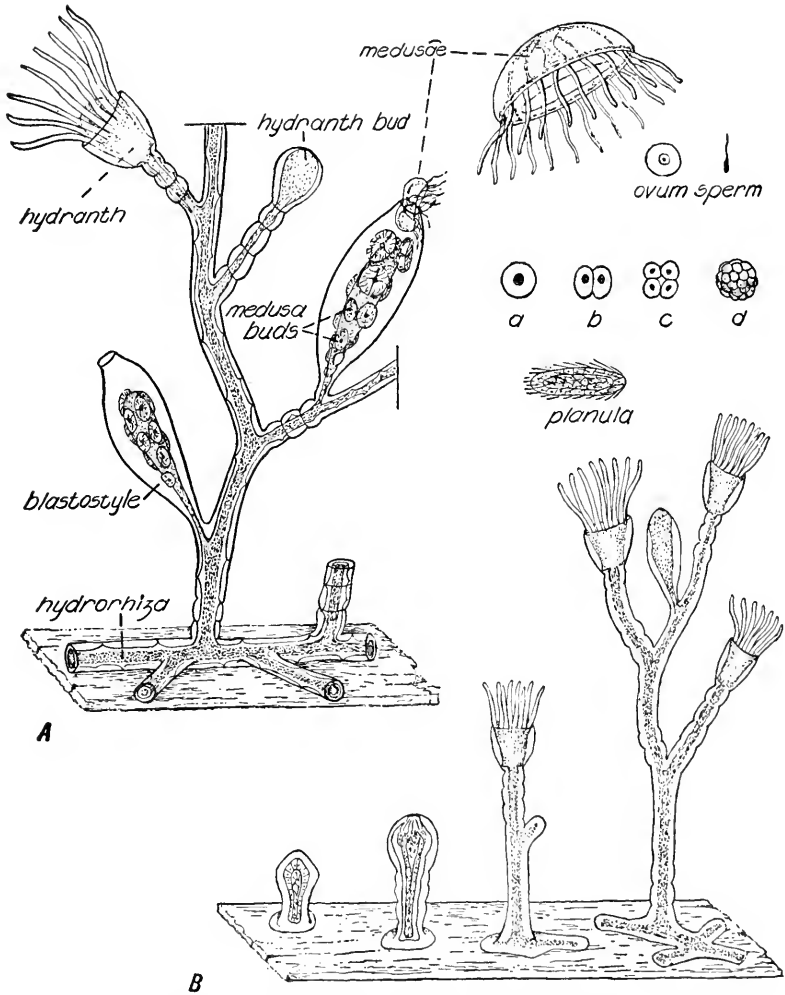


FIG. 45.—The life-cycle of *Obelia*, showing alternation of generations. *A*, portion of a colony of *Obelia*. *B*, development of a colony following attachment of the planula. *a*, zygote; *b*, two-cell stage; *c*, four-cell stage; *d*, blastula.

comparable with those in the life-cycle of the obelia? Construct a table or a full-page diagram comparing the life-cycles of the obelia and hydra.



**Exercise 5.**—Occurrence and Habits of a Large Hydromedusa.

(h) The medusæ of the obelia are very small and not easily studied. The larger hydromedusa, *Gonionemus murbachii*, which is almost identical in structure and essentially like the obelia in its life-history, is a more favorable specimen. The preserved medusa may be studied, under water in a watch glass, with handlens and lowest power of microscope. The appropriateness of the popular name jellyfish will now be appreciated. Handle the specimen carefully, and return it uninjured at the close of the period.

(i) This medusa lives in the shallow water of protected inlets. It originates by detachment from a simple hydroid colony which is attached on the bottom. Compare with the life-cycle of the obelia. In life the gonionemus is often seen "fishing," by swimming to the surface, turning mouth side uppermost, and slowly settling to the bottom with tentacles widely extended. If a small fish or similar animal comes in contact with the tentacles, it is quickly paralyzed by the nematocysts and drawn to the mouth. Individual medusæ sometimes show the enteron above the hypostome greatly distended with food. Compare with the hydra. What may be the advantage of such a free-living stage in the life-cycle of an attached animal?

**Exercise 6.**—Structure of a Large Hydromedusa.

(j) The medusa is umbrella-shaped, with the **hypostome** in the position of a short, thick handle. The margin bears many **tentacles** which are well supplied with stinging cells. The **velum** is a circular shelf projecting inward from the margin of the medusa, so that it partly closes the **subumbrellar cavity**. Notice the four much-convoluted gonads, **ovaries** or **testes** according to the sex. The hypostome is perforated by the **mouth** which communicates with a **stomach**, from which extend four **radial canals**, one above each gonad. The radial canals communicate with the **circumferential canal**, at the margin of the disk. At the base of each tentacle is a colored **eye-spot**. Between these organs, the **statocysts**, or organs of equilibrium, can be seen with the compound microscope as clear vesicles. The animal is covered on the outside with **ectoderm**, and the cavities entered through the mouth are lined with **endoderm** as in the hydra. Between the ectoderm and endoderm is a thick mass of gelatinous material, the **mesoglea**, which corresponds to a much-thickened supporting lamella such as is found in the hydra. Make a drawing ( $\times 4$ ) of the animal as seen from the oral or concave surface. Construct a diagrammatic vertical section ( $\times 4$ ) in the plane of two opposite radial canals.

## OTHER CŒLENERATES

**Exercise 1.**—Various Species.

(a) With the exception of the hydras, which are common forms, and a few other related species that have a very restricted distribution, the cœlenterates are exclusively marine animals. Familiar examples at the seashore are the jellyfishes and sea-anemones. The table of classification on p. 170 gives the several classes of the phylum. It will be interesting to examine such museum specimens as may be available, although even the best of preserved material gives a sorry picture of the beauties of form and color seen in many cœlenterates when alive. (1) Among the *Hydrozoa*, various hydroids and *Physalia*, the "Portuguese man-of-war," may be shown. (2) The *Scyphozoa* include the larger jellyfishes which are somewhat different in structure and life-cycle from the hydrozoan jellyfish *Gonionemus*. *Aurelia*, *Cyanea*, and *Dactylometra* are common genera along the North Atlantic Coast. (3) In the *Anthozoa*, various anemones, such as *Metridium*; sea-pens and sea-fans; expanded specimens of the coral *Astrangia* attached to its skeleton; and dry skeletons of various corals may be examined. Where skeletons alone are available, they should be compared with figures of the living animals. In any case, charts or the figures in a textbook should be consulted. (4) The *Ctenophora*, or sea-walnuts, are less abundant forms and so difficult to preserve that good figures are more likely to be intelligible.

## THE PLANARIAN

PHYLUM PLATYHELMINTHES

CLASS TURBELLARIA

### Exercise 1.—Occurrence and General Activities.

(a) Various kinds of planarians may be found on the under surfaces of stones, leaves, and other objects in ponds and streams. If pieces of liver are placed in the water, the worms will frequently collect upon them and may be washed off into a collecting jar; or the worms may be collected by gently detaching them from the stones on which they are found. *Euplanaria novangliæ* (*Planaria maculata*), *Euplanaria agilis* (*P. agilis*), and *Curtisia foremanii*<sup>1</sup> are suitable for the following study.

(b) Using a handlens and the low-power objective of your microscope examine a living planarian in a watch glass of water. How is locomotion accomplished? Turn the animal over with one point of your forceps and observe the movements in righting. Observe the responses to other types of stimuli. Can you discover any evidence of visual sense? Place small bits of crushed snails or other meat near the anterior end and observe what happens; or watch a number of worms in a larger dish after a strip of liver has been dropped upon the bottom. Does it appear that the animals possess a chemical sense? Record your observations, on the righting and feeding, by means of a series of drawings ( $\times 5$ ).

### Exercise 2.—Structure.

(c) The **anterior** and **posterior ends** and the **dorsal** and **ventral surfaces** are characteristic for the bilaterally symmetrical animal. Lobelike extensions of the sides of the head are the **auricles**. What seems to be their function as shown by the feeding experiment? What is the nature of the **eyes** as seen in the living animal? The tubelike structure that was protruded during feeding is the **pharynx**; when withdrawn it lies within the **pharynx sheath**. Where is the **mouth**? In sexually mature animals find the **genital aperture** posterior to the mouth. Living specimens will sometimes show the **digestive tract**. How does its shape justify the name **triclad** as applied to this order

<sup>1</sup>These names, with the older names in parentheses following them, are according to the classification of Libbie H. Hyman, *Transactions of the American Microscopical Society*, vol. 50, 1931.

of the *Turbellaria*? Put a small specimen on a slide under a cover glass and examine for **cilia**. Are they uniformly distributed? Can you detect flickering movements within the animal? These indicate the position of the **flame cells**, which are excretory in function. Do you find ducts leading from them to the outside? Draw ( $\times 10$ ), showing the features observed.

(d) Stained cross sections must be studied to make out the cellular structure. The outermost layer of cuboidal cells is the **epidermis**,

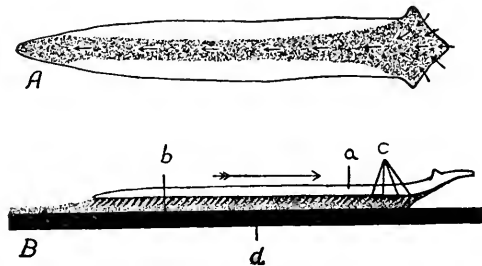


FIG. 46.—Locomotion in planarians. *A*, ventral surface of animal showing distribution of cilia; arrows indicate direction of ciliary currents; *B*, mechanism of gliding movement; *a*, planarian seen from side; *b*, layer of mucus (thickness exaggerated) secreted by animal; *c*, cilia; *d*, substrate; the arrow indicates the direction in which the organism is moving.

(From R. Pearl, 1903, *Quarterly Jour. Microscopical Science*, vol. 46.)

or **ectodermal epithelium**. To what layer of the hydra does it correspond? To what layer in the frog? The **gut wall** is composed of an **endodermal epithelium** made up of large columnar cells. Are they vacuolated? The region between the gut and epidermis is seen to be composed of a **syncytium** of stellate cells which contains spindle-shaped cells, the **formative cells**, and which is traversed by **muscle cells**. Small oval or rodlike structures, the **rhabdites**, may be seen in the peripheral regions of the syncytium and in the epidermis. The **nerve cords** will be seen on each side in the ventral region. Make a figure to show the regions as observed with the low-power objective. Draw under the high-power objective ( $2 \times$  projected size) to show the cellular structure of a narrow strip from the gut cavity to the outside. Label in such a way as to indicate the functions of all parts. What advance in cell specialization does the planarian show over the hydra?

### Exercise 3.—Regeneration, Reproduction, and Development.

(e) Using a sharp scalpel cut a specimen into two or three pieces. Keep the pieces in a wide-mouth bottle in some of the water in which

the animals have been living. Record by drawings the changes observed at daily intervals for one week. Understand the cellular changes that occur.

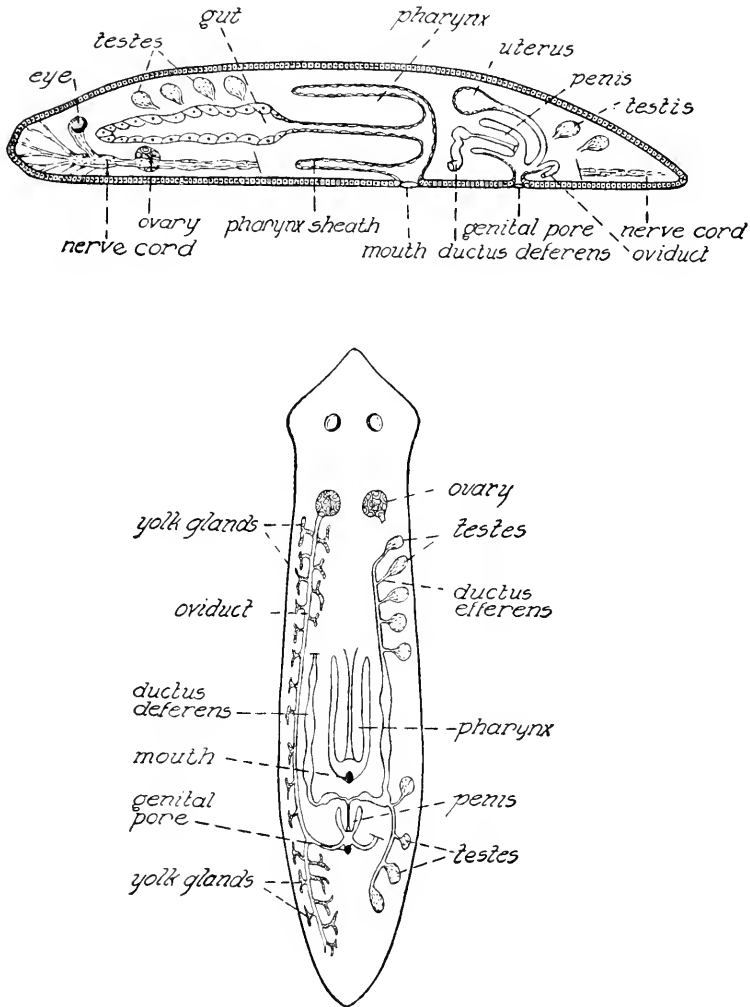


FIG. 47.—Structure of a planarian. Above, diagram of a planarian in lateral view. Below, diagram of the reproductive system of a planarian.

(f) The reproductive organs are seasonal in occurrence and cannot be studied in living planarians. If they are to be studied stained material will be furnished and further directions will be given. The animals are **hermaphroditic**. Fertilization is internal, following sexual

union; several **zygotes**, surrounded by thousands of **yolk cells** which serve as food, are enclosed in small **capsules** which are attached by stalks to the under surfaces of submerged objects. **Spherical embryos** are formed and these become transformed into the miniature worms,

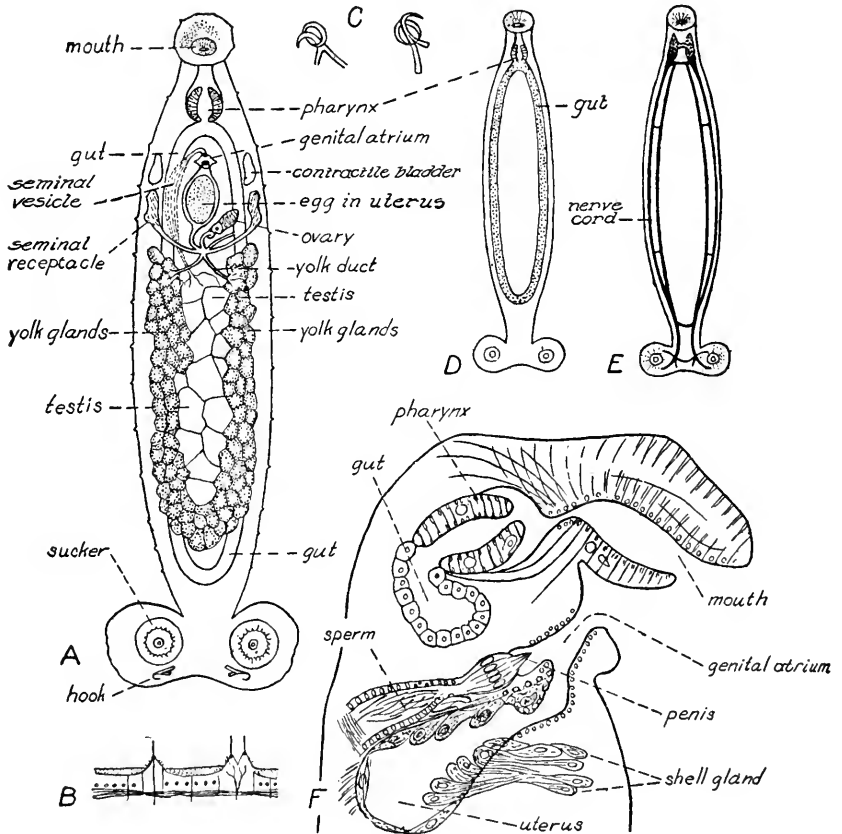


FIG. 48.—*Sphyranura osleri*, a monogenetic trematode parasitic on the amphibian *Necturus*. *A*, ventral view to show internal structure. *B*, section of surface membrane, with tactile structures. *C*, small hooks, which occur lateral to the suckers and are surrounded by chitinous rings at the point of projection from the surface membrane. *D*, digestive system. *E*, nervous system. *F*, median, longitudinal section of anterior end.

(Redrawn from R. R. Wright and A. B. Macallum, 1887, *Jour. of Morphology*, vol. 1.)

or **juveniles**, which hatch from the capsules. The capsules may be collected as laid by worms in laboratory cultures or in nature, and the later embryonic stages obtained by dissection from such capsules; the earlier stages can be effectively studied only in sections. Repro-

duction also occurs by **fission**. If dividing specimens are found in the laboratory, compare the regeneration following fission with the regeneration following artificial cutting.

## THE FLUKEWORM

### Exercise 1.—Structure of the Adult.

(a) The platyhelminths of the Class *Trematoda* are the external and internal parasites known as **flukes**, or **flukeworms**. For this study, specimens of the Genus *Pneumonæces*, from the lungs of the frog, or of the Genus *Clinostomum*, which is found in a somewhat immature condition encysted in the coelomic region of the frog, are excellent material.

(b) Examine living or preserved specimens and locate the **mouth** and **suckers**. How do the shape and behavior, if specimens are observed alive, compare with the same in the planarian? Locate the **digestive tract** and compare with that of the planarian. The **reproductive organs** are complex and of varied appearance in the different genera of flukes. If they are studied, special directions will be given by your instructor. The animals are **hermaphroditic**; **zygotes**, surrounded by **yolk cells** and an eggshell, accumulate in a terminal portion of the female organs, the **uterus**, and develop later when they are laid. The life-cycle is greatly complicated in correlation with the parasitic habits; understand from lectures or textbook. Make a figure ( $\times 10$ ), showing the features above noted.

## THE TAPEWORM

### Exercise 1.—External Features of the Adult.

(a) The platyhelminths of the Class *Cestoda* are known as **tape-worms**. They are parasitic forms, even more highly modified in relation to their parasitic habits than the flukes. The adults occur as parasites within the digestive tract of some vertebrate known as the **primary host**; the larval stages occur mostly within the tissues of a **secondary host**, upon which the primary host is likely to feed. Species of the Genus *Tænia* are found in many common mammals. *Tænia pisiformis*, which has the dog for its primary host and the rabbit for its secondary host, is excellent material for this study. Specimens may be examined alive in water, or after preservation in formalin.

(b) Examine the adult cestode in a pan of water. The smaller end has an enlargement, the **scolex**; the larger end has mature **proglottids**, as the segments composing most of the body are called. What

structures, adapted for holding fast to the host, are found upon the scolex? Count the proglottids, compare with numbers in neighboring specimens, and record. How and where do the proglottids seem to originate? If living worms are available, test the firmness of their attachment to the mucous membrane of the host. Why is it important that this attachment be maintained? Compare the external features of *Tania* with those of other tapeworms, such as *Moniezia expansa*, from the sheep, and *Crossobothrium laciniatum*, from the sand-shark, that may be available as demonstration specimens. Draw figures ( $\times 15-20$ ) of the scolex and representative stages in the development of proglottids.

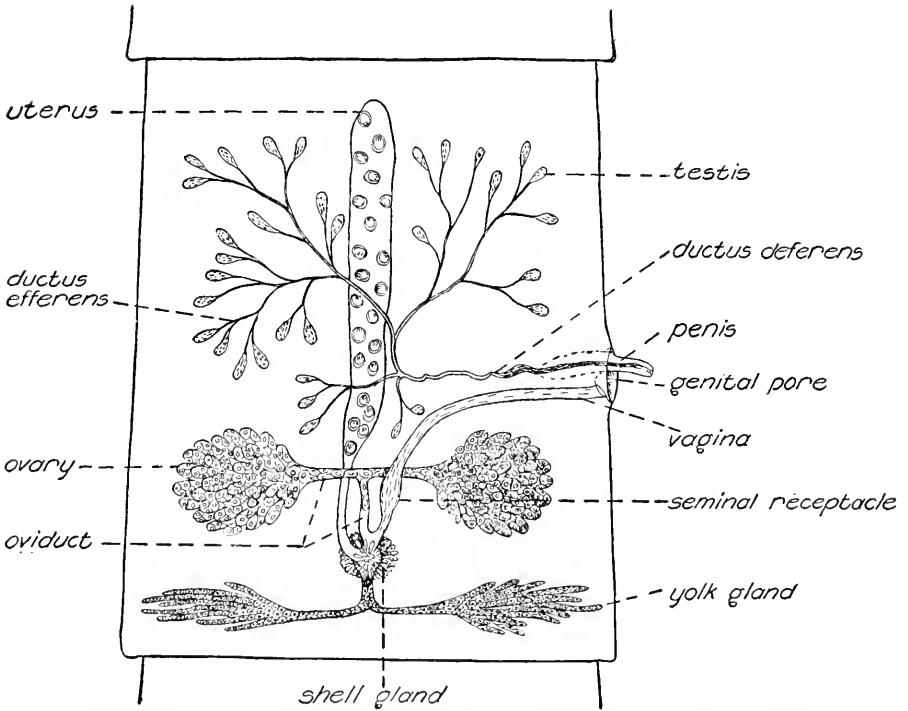


FIG. 49.—A proglottid of a cestode, showing the reproductive system; diagrammatic.

**Exercise 2.**—Internal Structure of a Proglottid.

(c) Mature proglottids of *Tania*, *Crossobothrium laciniatum*, or other favorable material stained to show the internal structure, will be provided and explained as necessary by the instructor. Recognize the **nerve cords**, **excretory ducts**, parts of the **reproductive system**,



granules of **calcium carbonate**, and the **cuticular membrane** covering the body. Where is the digestive tract? Which of these features are related to the parasitic habits of the animal?

(d) Tapeworms are **hermaphroditic**. Typically, each proglottid contains one complete set of male and female reproductive organs.

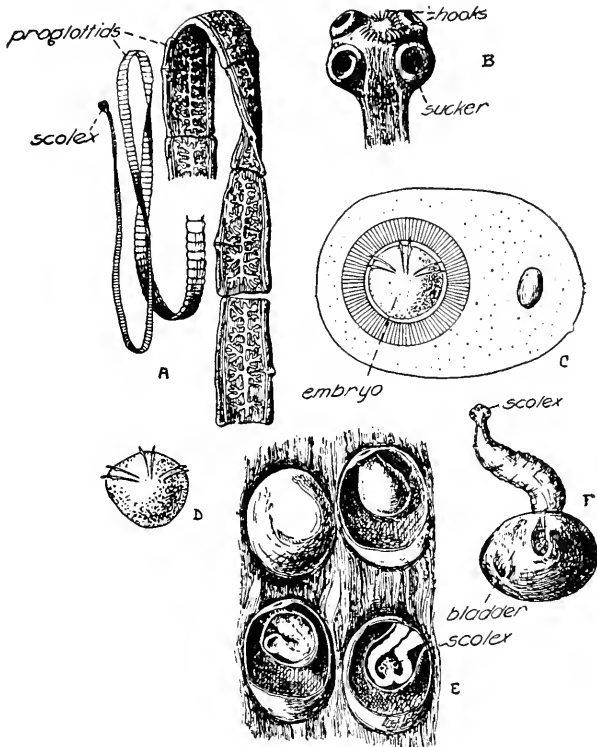


FIG. 50.—Life-cycle of *Tania solium*. A, parts of the worm, showing the youngest proglottids behind the scolex and the oldest (ripe) proglottids at the end of the worm. B, scolex of an adult tapeworm. C, six-hooked embryo inside its shell and the tough surrounding capsule. D, six-hooked embryo freed from its shell. E, portion of a muscle in which are embedded bladder-worms; successive layers are removed to show internal structures. F, juvenile tapeworm which arises from the bladder-worm by evagination of the scolex.

(From W. Stempel, "Zoölogie im Grundriss," 1926.)

The **male reproductive system** consists of numerous **testes** each of which discharges sperm through a **ductus efferens** (vas efferens). These ducts unite to form a single **ductus deferens** (vas deferens) which passes to the outside through the **penis**, located on one edge of the proglottid and protrusible from the **genital pore**. The **female reproductive system** consists of paired **ovaries** discharging ova through

**oviducts** which unite and open into a region surrounded by the **shell gland**. The ducts from the **yolk-glands**, and from the **uterus**, which is eventually distended with eggs and embryos, also open into this region, and from it the **vagina** leads to the outside by way of the genital pore. A **seminal receptacle** may be formed as a distended region of the vagina. Make a large drawing to show the features observed.

**Exercise 3.**—The Six-hooked Embryo.

(e) In the mature proglottids the uterus is distended with eggs and embryos and only remnants of other parts are found. Each **zygote** is enclosed in an **eggshell**, together with **yolk cells** of which there are four in most cestodes. Within the uterus these zygotes develop into **six-hooked embryos**. Place in a watch glass containing a little water a mature proglottid, from a fresh specimen of the worm or from one preserved in formalin, and tease the proglottid into bits. Mount on a slide a little of the siltlike material thus liberated from the uterus and examine with the highest powers of your microscope. The embryos are now seen encased in their shells. What is the nature of these eggshells? Can you find the **six hooks**? Force embryos out of their shells by pressing gently on the cover glass with a needle. Can you make an estimate of the number of six-hooked embryos produced by a single proglottid; by the entire worm in the course of its lifetime? Draw ( $3\text{-}5 \times$  projected size), showing these features.

**Exercise 4.**—The Bladder-worm.

(f) The mature proglottids, with their six-hooked embryos, are detached and pass out with the feces of the host. The six-hooked embryos are then liberated by the rupture or disintegration of the proglottid and enter the secondary host with its food. After the shell disintegrates in the digestive tract of this new host, the six-hooked embryo bores through the mucous membrane into the submucosa and by entering blood vessels may be carried to tissues remote from the digestive tract. Within the tissue where it comes to rest the six-hooked embryo develops to a stage known as the **bladder-worm**. Examine living or preserved bladder-worms, and make out the **scolex** and **neck**, and their position with respect to the **bladder**. When the flesh of the secondary host, containing the bladder-worms, is eaten by the primary host, the scolex and neck are everted, the scolex becomes attached to the mucosa of the primary host, and the remnant of the bladder becomes digested. Soon the neck begins to grow and

produces proglottids so that a new adult is formed. Draw the bladder-worm ( $3-5 \times$  projected size).

**Exercise 5.**—Typical Life-cycles.

(g) Tapeworms, therefore, have two hosts. In the following cases of known life-cycles, which would be the primary and which the secondary host: man—pig; mouse—cat; housefly—hen; fish—bird; louse—dog; man—fish? Consider where and how the three stages above studied would occur in each case. Tabulate your conclusions.

## THE ASCARIS

PHYLUM NEMATODA

CLASS PHASMIDIA

### Exercise 1.—Occurrence and External Features.

(a) Various species of *Ascaris* and related genera are found parasitic in the intestines of pigs, horses, man, and other vertebrates. Living worms may be collected in packing houses or may be obtained in smaller numbers from a freshly etherized cat or dog. Specimens from the latter sources are usually small and not so desirable for study as the larger species from the horse or pig.

(b) Examine a preserved specimen of *Ascaris*, noting the general shape of the body. What kind of symmetry is exhibited? The dorsal and ventral surfaces are each marked by the narrow, white, **dorsal and ventral longitudinal lines**; broader **lateral longitudinal lines** are present along the sides. The **mouth** is at the anterior end with one **dorsal lip** and two **ventro-lateral lips**. The **anus** will be found ventrally near the posterior end. Bristle-like **penial setæ** project from the anus in the male. Mature males are smaller than the females and are further marked by a bend in the body near the posterior end. The **genital pore** in the female lies on the mid-ventral line about one-third of the worm's length from the anterior end. Make figures ( $\times 1$ ) to show the general shape of both the male and the female. Draw the anterior and posterior ends of each ( $\times 5$ ) to show the details of external features.

### Exercise 2.—General Internal Structure.

(c) Pin a specimen out, dorsal surface up, under water in a dissecting pan. Cut through the body wall along the dorsal mid-line, exposing the internal organs but being careful not to injure them. Can you make out the outermost layer, or non-cellular **cuticle**, of the body wall?

(d) The **digestive system** consists of a straight tube running throughout the length of the body cavity, or **pseudocœl**. The anterior end of the digestive tract is differentiated into a muscular **pharynx**. What is its function? The major portion of the tract is non-muscular and consists only of endoderm, bounded internally and externally by a thin, non-cellular **cuticle**. The posterior portion of the tract is

slightly muscular and is known as the **rectum**. Correlate the relatively undifferentiated digestive system with the food and habitat of the animal.

(e) What is called an **excretory system** consists of a longitudinal **excretory tube** along each side in the body wall. These tubes are

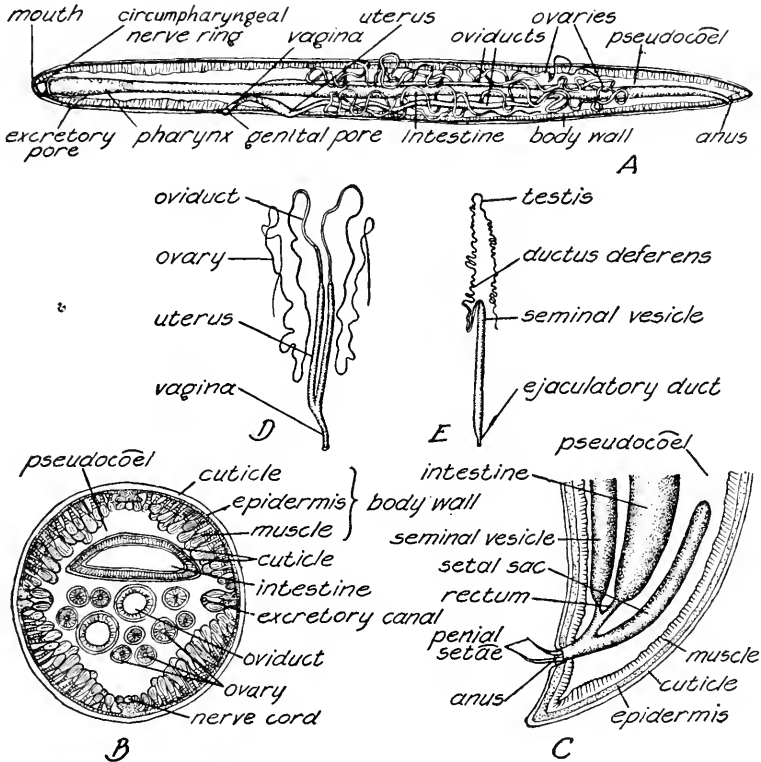


FIG. 51.—Internal structure of *Ascaris lumbricoides*. A, dissection of a female, from lateral view; diagrammatic. B, cross section of the body in the mid-region. C, longitudinal section of the posterior end of the male. D, the female reproductive system. E, the male reproductive system.

(Redrawn, with modifications, from R. Leuckart, "Die menschlichen Parasiten," 1876.)

closed at their posterior ends and open to the outside through a single ventrally located **excretory pore** near the anterior end.

(f) The **nervous system** consists of a circumpharyngeal **nerve ring** and dorsal and ventral longitudinal **nerve cords** with their smaller branches.

(g) The **reproductive system** of the male consists of a single coiled tube in which four regions are recognizable. The **testis** leads into

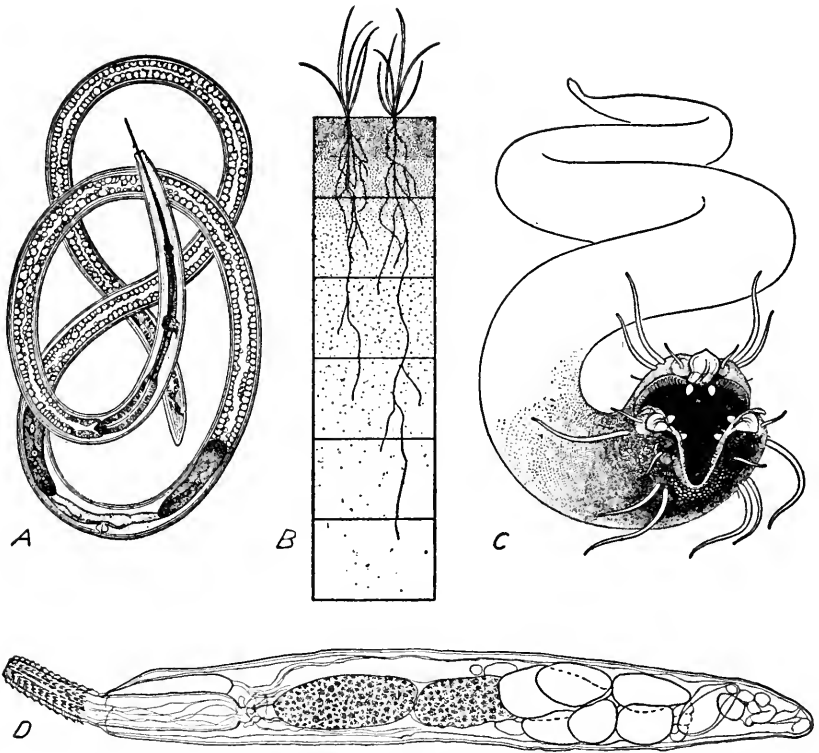


FIG. 52.—Representative roundworms. *A*, the dagger nematode, *Xiphinema*, an injurious form, which coils itself about the rootlets of plants in such a way that the spearlike structure at the anterior end can be thrust far into the tissue of the rootlet upon which the worm feeds. *B*, diagram showing by stippling the relative abundance of nematodes of all kinds in each successive two inches of a low-lying alluvial soil estimated to contain three billion nematodes per acre. Though shown distributed uniformly in each layer, the worms are really most numerous about the roots of plants. *C*, carnivorous nematode that feeds upon other nematodes. The three jawlike parts of the mouth are armed with teeth, and the head has tentaclelike projections which presumably are sensory. *D*, one of the Acanthocephala, the spiny-headed worm, *Echinorhynchus dirus*, parasitic in the intestine of certain fishes, showing the proboscis armed with hooks which serve for attachment to the host. The internal structures, which consist almost wholly of reproductive organs, are shown in optical section.

(*A*, *B*, and *C*, from N. A. Cobb, 1914, Yearbook U. S. Dept. Agriculture. *D*, from H. J. Van Cleave, 1931, *Transactions American Microscopical Society*, vol. 50. Drawn to different scales.)

a **ductus deferens** (vas deferens), a portion of which is expanded as a **seminal vesicle** serving for temporary storage of spermatozoa. This is followed by a short muscular region, the **ejaculatory duct**, which opens into the rectum. The **penial setæ** are also considered to be a part of the male reproductive system. The female reproductive system consists of paired **ovaries**, each of which is continuous with an **oviduct** which expands to form a **uterus**. The two uteri unite to form a single **vagina** which opens to the outside by way of the **genital pore** previously seen. Understand from textbook descriptions the reproduction and the life-cycle in *Ascaris*.

**Exercise 3.**—The Structure as Seen in Cross Sections.

(h) Study stained sections taken at various levels along the antero-posterior axis. Identify the structures previously observed, and compare layer for layer the structures seen with the layers of the body wall and gut wall of the frog. What kinds of cells do you find in each of the layers observed? Can you observe any advance in cell specialization over the platyhelminths? How is the body cavity different from that of the frog? What do your observations indicate regarding the kinds of movements possible by the animal? Compare with the planarian.

## OTHER NEMATODES

**Exercise 1.**—Other Parasitic Species.

(a) Observe demonstrations of **hookworms**, **trichina worms**, and such other parasitic nematodes as are available. From textbook figures and descriptions understand their life-cycles and the nature of their parasitic existence.

**Exercise 2.**—Free-living Nematodes.

(b) Study nematodes obtained from marine or fresh-water infusions or from moist soil, sandy beaches, and elsewhere. Note any structural modifications related to their free-living habits, and contrast their structure with that of parasitic species. Homemade vinegar often has numerous minute nematodes, the **vinegar eels**, *Anguillula aceti*, present in it. Such cultures may be maintained for several years by merely adding distilled water to maintain a constant volume of the fluid. *Rhabditis terricola*, which lives in the soil and is often abundant in decaying substances, is favorable for study. What structural features of nematodes resemble the structures with which you are familiar in other phyla? How do nematodes differ from these other forms in regard to cell specialization and division of labor among cells, tissues, and organs?

# THE EARTHWORM

PHYLUM ANNELIDA

CLASS OLIGOCHÆTA

## I. BEHAVIOR AND EXTERNAL FEATURES

### Exercise 1.—General Activities.

(a) The following directions apply to any of the species of *Lumbricus*. Place a vigorous, active worm upon wet filter paper in a dissecting pan and carefully observe the mode of locomotion. How does it elongate and contract? Is there a rhythm in these changes? Can you see the minute, stiff spines that project through the body wall? Can they be drawn in? Draw the worm through the fingers and feel the spines, or **setæ**. How many are there on each ring, or **somite**? Place the worm on its back. Does it right itself? Will it crawl backwards? Compare the **anterior** and **posterior ends**, the **dorsal** and **ventral surfaces**, the **right** and **left sides**. Which are alike? Touch various parts of the worm to find out which are the more sensitive. Note the movements of the soft lobe, the **prostomium**, above the **mouth**. On the mid-dorsal line look for the **dorsal blood vessel** which shows through the body wall. Does it pulsate? Which way does the blood move?

### Exercise 2.—External Features.

(b) For this study a preserved worm will be used. Is the number of **somites** the same in all specimens? On the ventral surface may be seen light-colored swollen areas, the **skin glands**. Notice the smooth swollen band, the **clitellum**, which passes around the animal. What somites are occupied by the clitellum? By means of the handlens, examine the setæ in different regions. Locate the **anus**. Look on the fourteenth somite, near the ventral setæ, for the minute **openings of the oviducts**, or ducts for the discharge of eggs. On the fifteenth somite, swellings mark out transverse slits on each side, the **openings of the ductus deferentes** (vasa deferentia), or ducts for the discharge of sperm. Make a figure ( $\times 2$ ), showing the anterior end as far back as a point just behind the clitellum, from ventral view. Number the somites and locate accurately all the structures observed. Make a similar figure of the posterior ten somites.



## II. INTERNAL STRUCTURE

**Exercise 3.**—The Digestive Tract and Cœlom.

(a) Fasten the specimen in a dissecting pan, dorsal surface up, by pinning through the first somite and again toward the posterior end. Make an incision through the body wall just back of the clitellum and on the mid-dorsal line. With fine scissors, cut toward the head end, using great care to *cut nothing except the body wall* and to *keep on the dorsal mid-line*. Spread out the edges of the cut body wall and pin them apart temporarily. Understand from Fig. 53 and others that

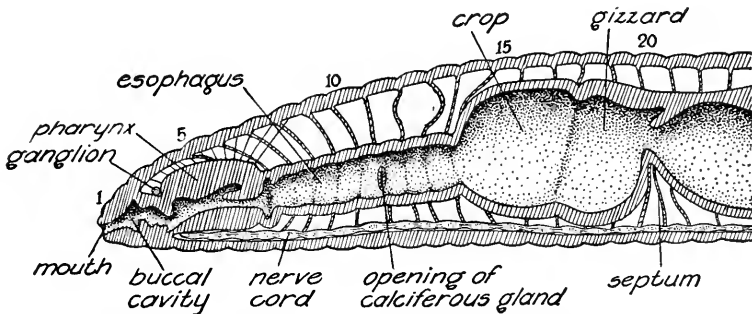


Fig. 53.—The anterior end of an earthworm, as if cut in the median, longitudinal plane.

(Redrawn with modifications from A. M. Marshall and C. H. Hurst, "Practical Zoology," copyright, 1895, by John Murray, printed by permission.)

may be available how the transverse partitions, or **septa**, which connect the inner surface of the body wall and the outer surface of the **digestive tract**, are related to the **cœlom**, the tract, and the body wall, and why they must be broken if the body wall is to be properly spread and the digestive tract exposed. Then break the septa from their attachment to the body wall and pin the wall flat in the dissecting pan; slant the pins outward to give room for fingers and instruments in working. Examine demonstration preparation or consult instructor if not sure your specimen is being properly dissected, since the proper execution of this initial step is very necessary for the success of the study that follows.

(b) You will now be able to see the brownish **stomach-intestine** and, on its upper surface, the large **dorsal blood vessel**. Toward the head, the gut becomes differentiated and is partly hidden by other organs which will be indicated presently. The cavity between the body wall and the digestive tract is the body cavity, or **cœlom**. With the handlens and a needle, observe and feel the edges of the septa which

divide the coelom into chambers one behind the other. What is the relative position of the septa and the external constrictions between the somites?

(e) Continue the cut forward as far as the second somite; carefully separate the edges of the body wall and pin the edges as before. How do the anterior septa differ from the others? Have they any different use? With the aid of Fig. 53 identify the following regions of the digestive tract, beginning anteriorly: **pharynx, esophagus, crop, gizzard,** and **stomach-intestine.** Check with instructor if you do not see each of these regions clearly. Other structures present in this region are parts of the **reproductive system.** The so-called **hearts,** or pairs of blood vessels which connect the dorsal vessel to the ventral blood vessel which lies beneath the digestive tract, are located in somites 7 to 11. Dorsal to the pharynx in somite 3 are two small white bodies, the **dorsal ganglia.** In all but a few of the anterior chambers of the body cavity, there are paired fluffy masses on each side. These are the

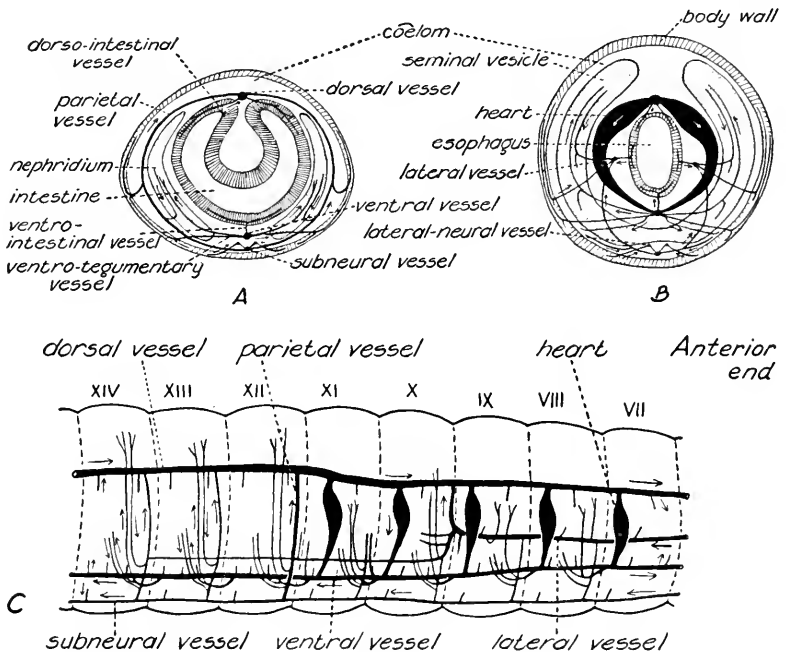


FIG. 54.—Circulatory system of *Lumbricus terrestris*; diagrams to illustrate the relations of the vessels and the course of the blood, as shown by arrows. A, cross section in the region of the stomach-intestine. B, cross section in the region of the hearts. C, lateral view of vessels in somites VII to XIV.

(Redrawn from J. B. Johnston and S. W. Johnson, 1902, *American Naturalist*, vol. 36.)

zard, and **stomach-intestine.** Check with instructor if you do not see each of these regions clearly. Other structures present in this region are parts of the **reproductive system.** The so-called **hearts,** or pairs of blood vessels which connect the dorsal vessel to the ventral blood vessel which lies beneath the digestive tract, are located in somites 7 to 11. Dorsal to the pharynx in somite 3 are two small white bodies, the **dorsal ganglia.** In all but a few of the anterior chambers of the body cavity, there are paired fluffy masses on each side. These are the

**nephridia**, or excretory organs. Look with a handlens for fine blood vessels on these organs. Turning a part of the stomach-intestine to one side, observe that these nephridial vessels are connected with the median ventral vessel. Beneath the ventral vessel is a conspicuous band, the **nerve cord**. Extend the cut through the body wall to the posterior end and carefully separate the various organs to see them more clearly. Which somites contain similar organs, and which ones are differentiated? Make a full-page drawing of the region from the prostomium to the beginning of the stomach-intestine to show all the organs thus far identified, and locate them in the proper somites.

(d) Lift up the esophagus with forceps, carefully cutting its attachments to the septa. Cut it across near the pharynx and pull it gently back, cutting the septa, but being careful not to remove any other structures from the worm. Continue this as far back as the beginning of the stomach-intestine, and lift up and remove the digestive tract in one piece. Examine this removed portion under water and correct any errors in your previous drawing. Find the **calciferous glands**, three pairs of lateral pouches on the esophagus.

#### **Exercise 4.—The Cœlomic Fluid.**

(e) Draw out a drop of the cœlomic fluid from a living worm by means of a capillary pipette. Place immediately upon a slide, adding 0.7% salt solution if necessary, and examine with the high-power objective. Are cells present? What are their characteristics? What organism do they resemble? Have they nuclei? Draw (3-5 × projected size) showing characteristic shapes.

#### **Exercise 5.—The Excretory System.**

(f) Using fine scissors, remove a part of a septum with a **nephridium** attached and examine with a microscope under both low- and high-power objectives. The nephridium is a convoluted tube, the walls of which contain many blood vessels. What is the function of these blood vessels? Look for the ciliated funnel, or **nephrostome**, and for the **bladderlike enlargement** at the outer end of the tubule. Understand the function and manner of action of the nephridium. If living worms are available, remove one of the nephridia, and look with the high-power objective for the flickering of the cilia within the tubule. Draw the nephridium as observed.

#### **Exercise 6.—The Reproductive System.**

(g) Wash out the anterior region of the worm by gentle currents from a pipette. Examine the posterior surface of the septum between somites 12 and 13 with a handlens; the two **ovaries** can be seen lying

one on each side and near the nerve cord. Immediately behind each ovary is an **oviduct**, seen as a whitish area on the anterior face of the septum between somites 13 and 14, and in somite 14 as a fine cord which is very short and passes diagonally to its place of exit through the ventral body wall. Locate these parts before pulling away the

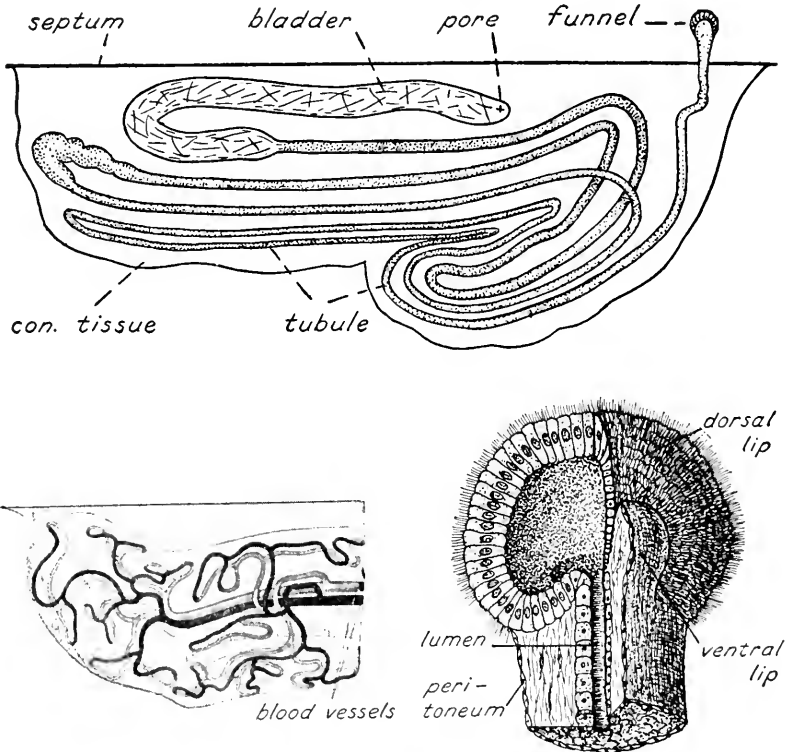


FIG. 55.—Nephridium of earthworm. *Above*, diagram of an entire nephridium, drawn with coils somewhat separated. *Below (left)*, portion of nephridium (*n*) showing blood supply; *(right)* the nephridial funnel, or nephrostome, by which the nephridium communicates with the coelom; the opening is between the dorsal and ventral lips.

(Redrawn with modifications from G. B. Howes, "Atlas of Zoötony," copyright, 1902, by Macmillan and Co., Ltd., printed by permission.)

remains of septa and nephridia, which may partially obscure them. Examine Fig. 56 and understand how the eggs pass from the ovary to the outside.

(h) In sexually mature specimens, the **seminal vesicles**, which should still be uninjured, consist of three lobes which extend dorsally on each side of the esophagus and which are united by a common median portion which lies against the ventral body wall. Careful

removal of the dorsal wall of this median region will disclose four large bodies, rather indistinct in outline, but different in texture from the vesicles and resembling crumpled bits of paper. These are ciliated funnels of the male ducts, or **ductus efferentes** (*vasa efferentia*). Look on the ventral body wall and find the ductus efferens which passes

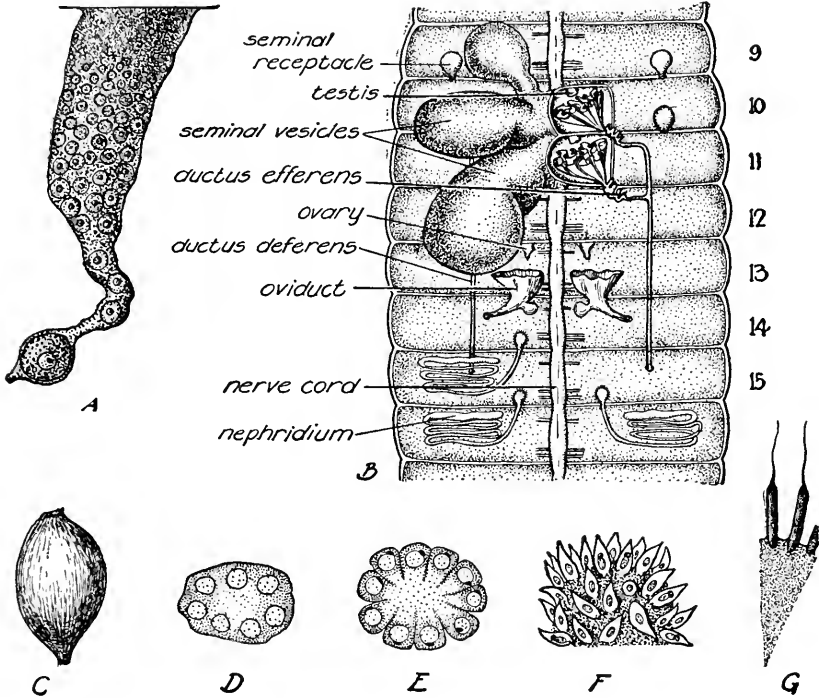


FIG. 56.—The reproductive system of the earthworm. *A*, an ovary with oocytes in various stages of growth, the terminal one about ready for detachment from the ovary. *B*, dorsal view of the reproductive system with the cavities of the seminal vesicles of the right side exposed. *C*, an egg-capsule. *D*, multinucleate cell which is detached from the testis and from which spermatozoa differentiate within the seminal vesicles. *E* and *F*, the spermatids on the surface of a central mass of cytoplasm from the original multinucleate cell (*D*). *G*, two spermatozoa as they appear attached to the surface of this spherical mass of cytoplasm.

laterally from the region of each funnel. The two ductus efferentes unite in somite 12 on each side to form the **ductus deferens** (*vas deferens*) which passes posteriorly to its opening on somite 15. The male germ cells begin their differentiation in the **testes**, bodies somewhat similar to the ovaries and in the same relative position in somites 10 and 11. Although the testes lie close to the funnels of the ductus

efferentes, the partially differentiated male germ cells upon leaving the testes do not at once enter the funnels, but pass into the lobes of the seminal vesicles. There they complete their differentiation, and the mature spermatozoa are then ready to enter the ductus efferentes and so pass to the outside through the ductus deferentes. The four testes and the four funnels have, therefore, a relation to the cœlom similar to the ovaries and their oviducts. The seminal vesicles, by enclosing both testes and funnels in a common cavity, prevent the spermatozoa from escaping into the cœlom and furnish a cavity in which spermatogenesis is completed. Compare the relationship to the cœlom of these male and female ducts with the relationship to the cœlom of the nephridia. In many annelids the nephridia function as the reproductive ducts.

(i) The **seminal receptacles**, which should not be confused with the seminal vesicles, are small whitish bodies attached to the ventral body wall on each side in the region of somites 9 and 10. They open to the outside only, and their function is to receive the spermatozoa obtained from another worm during sexual union.

(j) Consult a model, or a figure of the entire reproductive system, and then construct a large semidiagrammatic figure showing all these parts. Review their relationships by tracing the course of the ova and spermatozoa from gonads to the external openings of the oviducts and ductus deferentes.

(k) Cut out one of the ovaries and transfer to a slide. Add a drop of glycerin, put on a cover glass, and study under the low-power objective. The female germ cells will be seen in various stages of growth and differentiation. Where are they most advanced? The largest ones show a nucleus and nucleolus. Recall the stages of development of the ova in ovary of the frog. Make a drawing of the entire ovary, 6 cm. in length.

(l) Understand from lectures and textbook the functioning of the various parts in sexual union and egg-laying. Examine again the clitellum and the markings that extend forward from this glandular area to the openings of the ductus deferentes and the external openings of the oviducts.

#### **Exercise 7.—The Nervous System.**

(m) Lift the posterior end of the pharynx with forceps, and cut the muscles that connect it with the body wall. Trace the **nerve cord** anteriorly and find where it divides into right and left branches, the **circumpharyngeal connectives**, which encircle the pharynx and unite in the **dorsal ganglia**. Look for **nerves** from the brain and from the

collarlike circumpharyngeal connectives. Determine the number and place of junction of the nerves joining the nerve cord in the somites just back of the connectives and also in the somites toward the posterior end of the body. Cut across the nerve cord in the mid-body region and tear out a piece of it with a quick pull of the forceps. This will help you to determine the number of nerves in each somite. Make a drawing of the nervous system from dorsal view ( $\times 5$ ). Indicate the relation of the nerves and ganglia to the somites.

### III. STRUCTURE AS SHOWN BY SECTIONS

#### Exercise 8.—The Gross Structure.

(a) Take a piece of an undissected specimen about 3 cm. in length, and cut, with sharp scissors or a scalpel, transverse sections about one somite in thickness from the mid-body region. Sections previously cut and cleared in oil of wintergreen show structures better than freshly cut sections. It is important to have a specimen in which the stomach-intestine is not unduly distended. Study with the handlens, and identify the position of the stomach-intestine, coelom, nephridia, septa, nerve cord, and blood vessels. Can you distinguish the different layers in the body and gut walls? Notice the **typhlosole**, a fold of the dorsal wall of the stomach-intestine. How may it be of importance in digestion and absorption? Make a drawing about 8 cm. across, which will show the structures appearing in a region midway between septa.

#### Exercise 9.—The Cellular Structure.

(b) Study thin sections of the mid-body region as shown in prepared slides. Examine first with handlens and then with the low-power objective of the microscope and recognize all the parts. Examine carefully the cellular structure of each part with the high-power objective.

(c) The body wall is made up of four layers: (1) The outermost layer, or **epidermis**, is composed of columnar epithelial cells, some of which are gland cells in various stages of activity. Covering the outer surface of these cells is a thin non-cellular membrane, the **cuticle**, which is a secretion of the epidermis. Do you find perforations in it? (2) The **circular muscle layer** is composed of non-striated muscle cells which encircle the body and are, therefore, cut lengthwise in this section. Considerable fibrous connective tissue and numerous blood vessels can be seen. What is the function of these blood vessels? (3) The **longitudinal muscle layer** is the next and thickest layer of the body wall. It is made up of non-striated muscle cells running in a longitudinal direction and so arranged and held together by connective

tissue as to have a featherlike appearance in these transverse sections. What functions do these muscle layers perform? (4) The innermost layer of the body wall is the **peritoneum** which lines the body cavity; it is a single layer of squamous epithelium.

(d) The wall of the stomach-intestine is composed of three layers: (1) The outermost is the **chloragogue layer** which is composed of slender columnar epithelial cells containing numerous granules. This layer is a highly modified visceral peritoneum and is apparently concerned with the excretion of certain waste products. (2) Beneath the chloragogue layer is a very narrow layer, the **submucosa**. It contains some non-striated muscle cells, which are arranged so that some extend in a circular and some in a longitudinal direction. In addition, fibrous connective tissue surrounds numerous small blood vessels. What function do these vessels perform? (3) The innermost layer is the **mucosa**, a layer of ciliated columnar epithelium. Are gland cells present? How is the food mass moved along the length of the stomach-intestine?

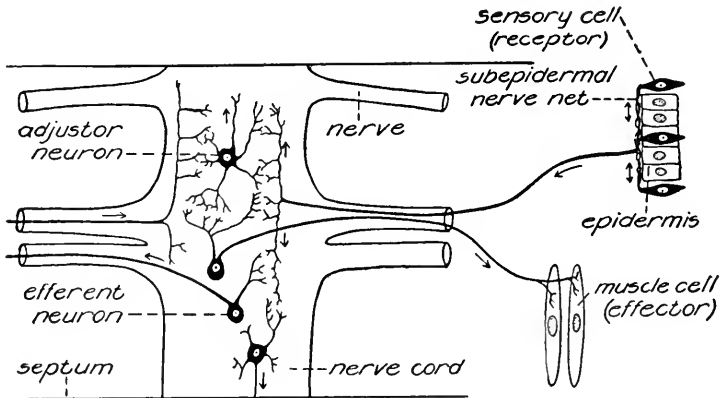


Fig. 57.—Sensory-neuro-muscular mechanism of the earthworm; arrows indicate the direction of transmission of nervous impulses.

(e) Examine the section as a whole again. What is the condition of the layers of the stomach-intestine in the region of the typhlosole? The contents of the coelom are likely to be confusing, because of the irregular manner in which the septa and nephridia are cut. Understand these parts and why they appear as they do in your section. Locate the **dorsal vessel**, **ventral vessel**, **subneural vessel**, and **lateral neural vessels**. Do you find branches of these blood vessels? Understand the direction of blood flow.

(f) Draw ( $\times 1$  projected size) a narrow rectangular strip from the gut cavity through to the outside of the body, showing accurately the



cellular structure of each of the layers. Label the layers, indicate what tissues are found, and the functions of all parts.

(g) Study the nerve cord in the cross section, and note the outer layer, containing **muscle cells** and **blood vessels**; the three large, clear areas, or **giant fibers**; the **nerve fibers** and the **nerve cell bodies**. Understand from lectures, textbook, and Fig. 57 the relationship between the central and peripheral portions of the nervous system and the cellular organization of the system as a whole.

**Exercise 10.**—Comparison of the Cell-layers and Tissues in the Earthworm, Hydra, and Frog.

(h) Compare the cellular structure, layer for layer, in corresponding parts of the earthworm, hydra, and frog. This may be done in tabular form, indicating the layers and tissues and their origin from the three germ layers in development. Write a short statement pointing out the similarities and differences in the body-plans and in the degree of somatic cell specialization found in these three animals.

#### IV. REGENERATION, REPRODUCTION, AND DEVELOPMENT

**Exercise 11.**—Regeneration.

(a) When earthworms are collected in nature individuals are often found with posterior ends showing a varying number of regenerating somites. Experimental study of this regeneration shows that many somites can be restored posteriorly and that in general the number thus regenerated is in proportion to the number removed. At the anterior end, however, the power of regeneration is restricted. Typically, only four somites can be regenerated and even these will not be normally restored if as many as fifteen somites have been removed. Some species of annelids have much greater powers of regeneration.

(b) The small fresh-water annelid, *Tubifex tubifex*, somewhat resembles the earthworm in its powers of regeneration. Examine, in water in a watch glass, recently collected *Tubifex* that show posterior regeneration; anterior regeneration is seen occasionally. If the regenerating individuals are abundant it should be possible to arrange a series that will illustrate stages of posterior regeneration. The stages of anterior regeneration cannot be easily observed in such living material. To study the posterior regeneration experimentally, remove the posterior halves of several individuals by a transverse cut made with a sharp scalpel; set aside the pieces, as directed by instructor, for subsequent examination. In the course of two weeks at room temperature the more important changes will occur. During such regeneration the cut end heals and forms a new anal opening within forty-eight

hours; cells called **neoblasts**, arise on the posterior faces of certain septa and migrate along the nerve cord to the posterior end where they may be seen differentiating into mesodermal tissues. Record in a table, or by means of drawings, such stages in this regeneration of *Tubifex* as may be observed.

**Exercise 12.**—Embryonic Stages.

(c) Sexual union in earthworms results in an exchange of **spermatozoa**, which are stored in the seminal receptacles. The **egg-capsules**, or "eggs," arise from the clitellum as a secretion which hardens and is passed along the body of the worm anteriorly; during this passage several **ova** and many spermatozoa are discharged into the space between the hardened secretion and the body. Thus the **zygotes** become enclosed in a capsule when the ends of the cylindrical secretion close as it slips off the anterior end of the animal. Worms kept in a tub of damp soil covered with a thin litter of leaves often lay egg-capsules; or capsules may be collected from the surface of ground, not covered by vegetation, where worms are abundant. Examine capsules and the **embryonic stages** that may be obtained by dissection from capsules. The worms hatch as **juveniles**, several individuals having developed within each capsule. Embryos and juveniles may be studied in water in a watch glass and internal features observed as in *Tubifex*. Examine whatever material is available and compare with textbook figures.

## OTHER ANNELIDS

**Exercise 1.**—Representative Examples.

(a) Examine any other annelids that may be available. There are many more species in the ocean than elsewhere, although there are many species of earthworms on land and many species of small annelids in fresh water. Some of the latter may have been pointed out in the study of Protozoa, as they are frequent inhabitants of such cultures. Such forms as *Tubifex*, *Dero*, and *Æolosoma* can be used in the living state without dissection to demonstrate the septa, nephridia, nephrostomes, functioning of the circulatory system, peristalsis, ciliary action in the intestine and the nephridia, coelomic fluid, blood cells, movements of the setæ, and other activities. These small annelids are available throughout the year in the mud at the margins or bottoms of ponds and streams; some species can be kept alive almost indefinitely in the laboratory.

(b) Among the marine types, the clamworm, *Nereis virens*, is a species that burrows in loose sand and swims freely in the water.

Notice the **eyes**, **tentacles**, and **jaws** on the head, and the pair of paddlelike appendages, the **parapodia**, on each somite. What differences in habits do these organs suggest, as compared with the earthworm? It is probable that the latter has descended from ancestors having greater external specialization and thus more like *Nereis*. What habits of earthworms may be related to such degeneration?

(c) There are also many marine forms that are specialized for living in tubes or burrows and feeding in various ways. *Serpula* (*Hydroides*), with its brilliantly colored tentacles, and *Chaetopterus*, with its parchmentlike tube, are examples. The **leeches** of fresh water and moist soil are representative of another group of annelids.

## THE FRESH-WATER MUSSEL

PHYLUM MOLLUSCA

CLASS PELECYPODA

### I. BEHAVIOR AND EXTERNAL FEATURES

#### Exercise 1.—General Activities.

(a) Many genera and hundreds of species of fresh-water mussels, or clams, are to be found in the streams, lakes, and ponds of the Mississippi Valley and elsewhere. These mussels live partially embedded in the sand or mud of the bottom and crawl about by means of a muscular **foot**, which can be protruded between the two **valves** of the **shell**. Living specimens and empty shells should be collected and brought to the laboratory for comparison with preserved material.

(b) Observe living mussels in aquaria or shallow dishes containing water and enough sand to permit the animals to bury themselves. Note the normal positions assumed when at rest and when crawling. Does the animal bury itself completely? Note the openings, or **siphons**, which lie between the valves at the end that is exposed when the mussel is buried. With a pipette introduce powdered carmine or similar particles into the water near this end of the animal. The functions of the currents thus demonstrated will be understood after an examination of the internal structure. With a glass rod touch the margins of the two siphons and observe the result. Can you detect a difference in the sensitivity of these two openings? Are there **papillæ** on the siphonal margins? What is their function? Place a dish containing an active mussel in direct sunlight or under a strong light. When the animal has expanded pass a shadow over the siphons to test their sensitivity to light. Insert the end of the glass rod between the two valves. In what way is the response a protective reaction? The fleshy material, of which the siphons are composed and which lies between the free margins of the valves, consists of right and left parts adhering to the inner surfaces of the valves and is the edge of the so-called **mantle**.

#### Exercise 2.—The Shell.

(c) Examine the two **valves** of a shell. In life they are united by a **hinge**. Alternate periods of rapid and slow growth produce the

**lines of growth** seen on the outer surface. If these lines mark annual increments, how old was the animal when it died? What is the oldest part of the valve? This part is called the **umbo**. What region of the valve shows the most erosion? Why? Examine the broken edge of a valve and find the outer **periostracum**, the middle **prismatic layer**, and the inner **nacreous layer**, or mother-of-pearl layer. Do such sections show layers of growth? Sections ground thin for microscopic study will show further details of structure.

(d) The shell can be oriented according to the body axes of the animal that occupied it. The hinge is dorsal, the gape is ventral; the valves, therefore, are either right or left. A line through the umbo at right angles to the long axis of each valve divides it into a smaller anterior and a larger posterior portion. Orient the shell in the same position as your own body.

(e) On the inner surface of the shell near the hinge are the **teeth** which lock tightly when the valves are closed. What is their function? Roughened places or scars indicate the point of attachment of muscles which close the shell and move the foot. The largest scars are those of the **adductor muscles**. Which is the scar of the **anterior adductor muscle** and which of the **posterior adductor muscle**? Posterior to the scar of the anterior adductor muscle is the scar of the **anterior retractor muscle** of the foot. Below this is the scar of the **protractor muscle**. The scar of the **posterior retractor muscle** lies dorsal to the posterior adductor scar. How does the shell open, since no muscles for this purpose are present? Parallel to the margin of the shell and extending between the adductor scars is a line marking the line of attachment of the **retractor muscles of the mantle**. This is the **mantle line**, or "water line," often seen on pearl buttons. What is the relation at the edge of the shell of the three layers identified previously? Draw ( $\times 1$ ) the outer surface of the right valve and the inner surface of the left valve to show the features described above.

(f) Determine the effects of acids and of strong alkali on the shell. Of what is the shell composed? Why are mussels with thick shells found only in regions where limestone rock is abundant? What structure produces the shell? If an extensive collection of shells is available it may be used to illustrate the difference between a genus and a species, and between species of a genus. Examine the shells of a single genus, noting their peculiarities and features that all have in common. Examine other genera and compare with the first. The Genera *Quadrula*, *Lampsilis*, and *Anodonta* are valuable for this purpose.

**Exercise 3.**—The Mantle and Mantle Cavity.

(g) A specimen preserved in formalin or one just killed should be used. Remove the right valve by inserting the point of a scalpel between the valves and cutting the attachments of the muscles whose scars you have already identified. Being careful not to injure other structures, separate the valve from the mantle along the mantle line. Study the mussel from this right side as it lies in the other valve. This places the specimen in the same orientation as the drawings of the shell. Note the soft membrane, the **mantle**, which conforms to the

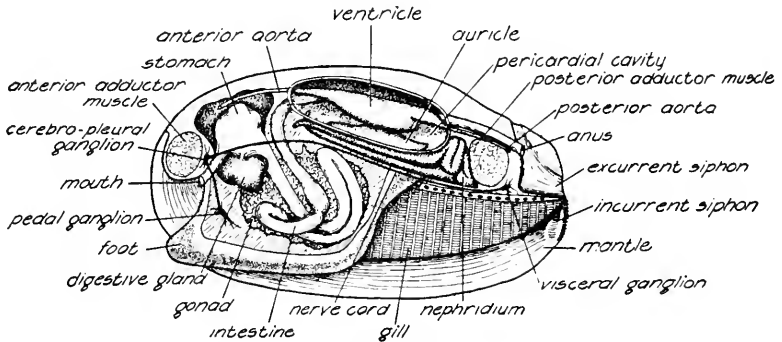


FIG. 58.—The internal structure of the mussel, *Anodonta*; semidiagrammatic.

inner surface of the shell. There will be no breaks in the mantle unless it has been mutilated. Find the ends of the following muscles, the scars of which have already been seen upon the shell: **anterior** and **posterior adductors**, **anterior** and **posterior retractors**, and the **protractor**. Find the line on the mantle which corresponds to the mantle line on the shell. The following internal organs can be more or less definitely recognized, according to the species or the method by which the specimen has been prepared: the **digestive gland**, a greenish-brown structure in the dorsal region near the anterior end, and the **pericardial cavity**, or **cœlom**, posterior to the digestive gland and containing the **heart**. The dark-colored **nephridium** may be visible through the pericardium. The **pericardial gland** (Keber's organ), of problematical function, is the darker region anterior and lateral to the pericardial cavity. Consult a chart, or Fig. 58, and understand the position of these structures even if you are unable to locate them at present.

(h) Determine the way in which the **incurrent siphon** communicates with the **mantle cavity**, which is the space between the right and left parts of the mantle. Lift the edge of the mantle on the right side

and remove part of the right half by cutting anteriorly from the middle of the incurrent siphon about 5 mm. below the attached dorsal edge. Taking care not to injure other structures, continue the cut neatly below the anterior adductor muscle. The **foot** and the four platelike **gills**, two on each side, are now exposed. Find the **palps**, a pair of leaflike organs on each side of the foot and attached to the mantle near the anterior adductor muscle. The **mouth** is a transverse slit between the foot and the anterior adductor; the **anus** is located at the termination of the intestine on the posterior face of the posterior adductor muscle.

(i) Using the dried right valve make an outline and draw the features observed thus far, limiting the drawing to what can be seen on the surface below the cut edge of the mantle.

#### **Exercise 4.**—The Gills.

(j) By looking into the uninjured excurrent siphon the posterior surface of the posterior adductor muscle will be seen. Below this on each side are two cavities extending anteriorly. Insert a tipped bristle into the outer one of these and, using the bristle as a guide, cut through the outer wall and expose the floor of the cavity. This is the **suprabranchial chamber** of the right outer gill. It opens posteriorly into the **excurrent chamber**, a region of the mantle cavity which is continuous with the excurrent siphon. Carefully cut away the entire outer wall of the excurrent chamber and of the suprabranchial chamber so that their extent can be seen. The floor of the suprabranchial chamber is marked by a series of transverse partitions, **interlamellar junctions**, which separate cavities, the **water tubes** of the gill. If the gill is not too shrunken, pass a bristle down any one of the water tubes to the ventral edge of the gill. How does the water tube end? Remove a piece of the outer gill, and cut sections, with sharp scissors or scalpel, at right angles to the water tubes. Examine under water with a handlens. The water tubes and interlamellar junctions will be seen cut transversely. It is in the water tubes that the zygotes of the mussel begin development, and you may find the gill distended with embryos. By looking forward from beneath the posterior adductor muscle and gently exploring with a bristle, it will be seen that the inner gill of this side and the two gills on the other side each have a suprabranchial chamber and water tubes, as in the gill just examined. Make a diagram ( $\times 2$ ), from the lateral view, of the suprabranchial chamber and excurrent chamber as thus exposed.

(k) You should now understand the course of the water current, from which the mussel obtains its food and oxygen and by which its

carbon dioxide and other soluble wastes are discharged. After entering by way of the incurrent siphon, the water bathes the organs of the mantle cavity. From here it passes through microscopic openings, the **ostia**, which lead from the mantle cavity through the inner and outer surfaces of all the gills into the water tubes. Passing upward in the water tubes, water enters the suprabranchial chambers and, flowing posteriorly in these, it reaches the outside through the excurrent chamber and excurrent siphon. The water that passes out has been strained of microorganisms and other bodies too large to pass through the ostia of the gills. Upon coming in contact with the surfaces of the gills, foot, or mantle, this food material is entangled in a mucous secretion and carried by cilia along definite lines which converge toward the palps. Passing between the inner and outer palp on each side, the food is at length delivered to the slitlike **mouth**, an opening which lies below the anterior adductor muscle and between the continuations of the inner and outer palps.

(1) Transverse sections, about 5 mm. thick, of whole specimens are valuable in helping you to understand the relationship of structures here and in your further dissection. If such sections are available, identify on them, as far as possible, the structures found in your dissection.

## II. THE INTERNAL STRUCTURE

### Exercise 5.—The Nervous System.

(a) Remove the right gills and palps by cutting near their attachments. Look on the ventral surface of the posterior adductor muscle for a pale yellowish body, the fused right and left **visceral ganglia**. Using your needles, carefully trace any nerves found extending from the ganglion on the right side. The paired **cerebral ganglia** will be found by carefully picking away the tissue below the base of the palps. From the cerebral ganglion trace a nerve, the **cerebro-pedal connective**, posteriorly and ventrally into the soft tissue in the upper part of the foot to find the **pedal ganglia**. The cerebral ganglia are also connected to the visceral ganglia by the **cerebro-visceral connectives**. These may be difficult to follow. Add these structures to your previous figure.

### Exercise 6.—The Circulatory System.

(b) Turn the specimen dorsal surface up and open the **pericardial cavity**, or **cœlom**, carefully by cutting with the scissors in front of the posterior adductor muscle. Remove the roof of the pericardial cavity and expose the **heart**, which consists of a median **ventricle**, wrapped



around the intestine, and thin-walled right and left **auricles** which lead from the sides of the pericardial cavity. Blood leaves the heart by

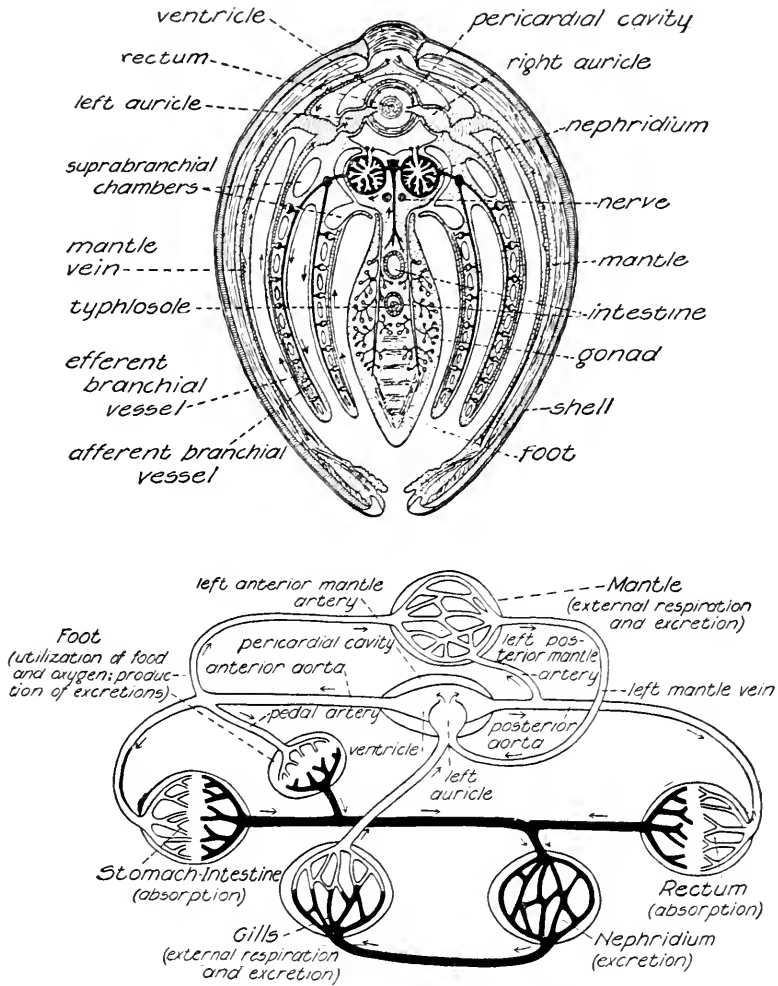


FIG. 59.—The circulatory system of *Anodonta*. Above, diagrammatic cross section of the mussel, showing the heart and principal blood vessels. Below, diagram showing the course of circulation; the arrows indicate the direction of blood flow.

(Upper figure from W. Stempel, "Zoölogie im Grundriss," 1926.)

way of the **anterior aorta** and the **posterior aorta**. The complete circulation should be understood from textbook figures and descriptions. Draw (× 3) the pericardial cavity and its contents.

**Exercise 7.**—The Excretory System.

(c) In addition to the gills, by means of which carbon dioxide is removed, a pair of special excretory organs, the **nephridia**, or **kidneys**, are present. Carefully open the suprabranchial chamber of the right inner gill from the side. The dark color of the nephridium can be seen through the medial wall of this chamber. Toward the anterior end of the chamber two small openings will be found; the dorsal one of these is the **excretory pore**, or external opening, of the right nephridium. Insert a tipped bristle into this opening and probe the cavity. Cut across the auricles, posterior aorta, and intestine, and turn these structures anteriorly, exposing the anterior wall of the pericardial cavity. Careful search along this wall with a tipped bristle should reveal two openings leading into the right and left nephridia respectively. Pass the bristle through the one leading into the right nephridium. Cut into this nephridium and observe that it is a U-shaped tube, of which the lower arm is spongy and glandular, and the upper arm is thin-walled. Note that fluid can pass from the pericardial cavity to the outside by way of the nephridia; compare with the relationship between cœlom and nephridia in the earth-worm. Make a diagram ( $\times 3$ ), from a lateral view, to show the structures disclosed by the foregoing dissection, and indicate the course of the fluid by arrows.

**Exercise 8.**—The Reproductive System.

(d) Identify the **genital pore** as the lower of the two openings seen previously on the median wall of the inner right suprabranchial chamber. This is the external opening of the **genital duct** which leads from the right **gonad**. The gonads, which are racemose glands located in the upper part of the foot among the coils of the intestine, will be observed when the digestive system is examined as directed in Exercise 9. The sexes are separate in most species of mussels.

**Exercise 9.**—The Digestive System.

(e) Look again into the excurrent siphon and recognize the **intestine**, lying on the posterior surface of the posterior adductor muscle and opening into the excurrent chamber by the **anus**. Follow the intestine along the muscle to the place where it was cut across in making the dissection of the nephridium. Using the handle of the scalpel, scrape the nephridia away from the top of the foot. Recognize and understand the function of the posterior retractor muscles of the foot which are now conspicuous and whose attachment scars have been noted on the shell. Leaving intact the parts of the intestine as cut,

free the foot and portions dorsal to it from the mantle of the left side. In this manner remove the foot and the **visceral mass**, as the region dorsal to the foot and containing the principal parts of the digestive system is termed. Remove the palps and any remains of the mantle and gills from each side of the visceral mass. Using a sharp scalpel, split the visceral mass and the foot as nearly into right and left halves as possible, leaving the free stump of the intestine attached to the left half. Examine the cut surface of this half pinned down under water in a dissecting pan. The visceral mass consists of a pasty substance, composed mainly of the gonads and digestive glands, in which parts of the digestive tract are embedded. Follow any of these parts that are cut by the section. From the **mouth** the flattened **esophagus** leads dorsally to an enlargement, the **stomach**, into which open the right and left **digestive glands**. The complete course of the tract should be noted as shown in Fig. 58. Make a diagram ( $\times 3$ ) of the digestive system to show the essential relations of its parts.

### III. REPRODUCTION AND DEVELOPMENT

#### **Exercise 10.**—The Glochidium.

(a) During the breeding season **spermatozoa** are discharged from the genital pores into the inner suprabranchial chambers and thence pass to the outer water through the excurrent siphon. They may then enter another mussel through its incurrent siphon. The **ova** are likewise discharged into the inner suprabranchial chambers where they are fertilized by sperm that have been derived from another mussel in the manner indicated. The **zygotes** then pass into the water tubes of the gills which thus function as brood-pouches. Development ensues to a stage, known as the **glochidium**, which is eventually extruded from the mussel through the excurrent siphon.

(b) Examine preserved glochidia or living specimens just removed from the brood-pouch. Note the **valves** of the **shell** and the single **adductor muscle**. Projecting from the tissue lining the valves are groups of **sensory hairs**. In one type of glochidium the valves are triangular and armed with so-called **hooks** (Fig. 61); another type is hookless (Fig. 60). With the exception of the shell, none of the adult parts is visible in the glochidium. Watch living glochidia for any movements. Are they capable of locomotion? When discharged from the parent the glochidium must become attached to a fish and live for some time as a parasite if it is to survive. Hooked glochidia normally become attached to the fish's fins; hookless glochidia become attached to the fish's gills (Fig. 60). The glochidium leaves the fish

as a juvenile mussel ready to begin life on the bottom (Figs. 60 and 61). Draw the glochidium on a large scale as it appears when gaping and from a lateral view when closed. Closure can be effected by

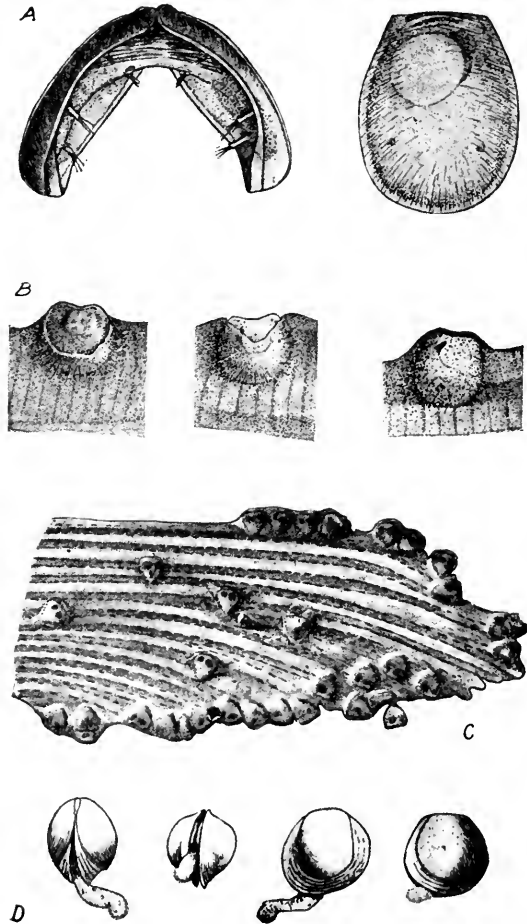


FIG. 60.—The development of fresh-water mussels. *A*, glochidium of *Lampsilis*; *left*, from posterior view, *right*, from lateral view. *B*, successive stages in the embedding of a glochidium of *Lampsilis* on a gill filament of a fish. *C*, fin of a fish with a number of embedded glochidia of *Symphynota*. *D*, juvenile *Lampsilis*, showing the persistent glochidial shell.

(From G. Lefevre and W. C. Curtis, 1910, *Bulletin U. S. Bureau Fisheries*, vol. 30.)

adding a drop of such a stain as methyl green to the water in a watch glass containing glochidia.

**Exercise 11.**—Infection of Fish with Glochidia.

(c) The glochidium of a particular species of mussel usually parasitizes only a few species of fish or even a single species. However, attachment often occurs on species of fish that will not carry the glochidia through their development. In such cases the glochidia are sloughed off within a few days, but a demonstration of the attachment to a host may be obtained from such material.

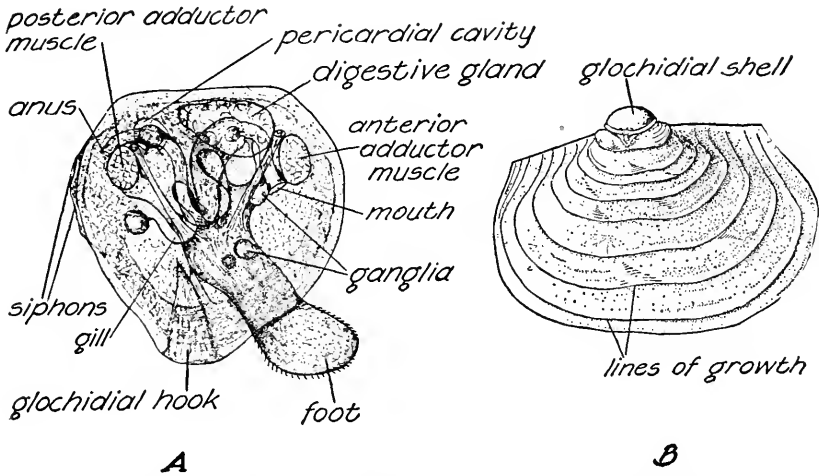


FIG. 61.—Juvenile stages of fresh-water mussels. *A*, *Symphynota*, shortly after leaving the fish. *B*, *Anodonta*, seven weeks after leaving fish.

(*A*, from G. Lefevre and W. C. Curtis, 1910, *Bulletin U. S. Bureau Fisheries*, vol. 30; *B*, from K. Herbers, 1913, *Zeitschrift für wissenschaftliche Zoologie*, vol. 8.)

(d) Take a considerable number of living glochidia in a finger bowl of clean water and put two small fish into it. Watch how and where the glochidia attach themselves. The water must be sufficiently agitated, either by the activities of the fish or by stirring gently, to prevent the glochidia from settling to the bottom. After five or ten minutes, take one of the fish and put it into an aquarium. Kill the other fish and remove its gills, fins, and tail without pressing on them. Examine them in a watch glass with a microscope. How and where are the glochidia attached? Draw one or more glochidia, attached to a gill or fin, on such a scale as to make the larvæ about 5 mm. in diameter. Make an estimate of the number of glochidia on this one fish; of the number produced by the single mussel.

(e) The fish that was placed in the aquarium after infection should be examined at succeeding laboratory periods, and the condition of the glochidia with reference to the tissue of the fish determined. Fish

infected twenty-four to forty-eight hours previously may be provided for examination on the same day as the foregoing. Record the condition of these glochidia and make figures. Glochidia of *Lampsilis subrostrata* remain on the sun-perch, *Lepomis pallidus*, for a period of ten to thirty days, according to the temperature, and then drop to the bottom as young mussels which crawl actively about (Fig. 60 D). They may then be seen with the unaided eye and collected without difficulty.

## OTHER MOLLUSKS

### Exercise 1.—The Pond Snail.

(a) For the following study the French snail, *Helix*, will be found very satisfactory, but the common pond snails, *Physa*, *Planorbis*, or *Lymnæa*, may be used to advantage. Observe the shell. Is there any division into valves? What is the general form of the shell? How many turns does it make? Each turn is known as a **whorl**. How do these whorls vary in size? Compare several specimens of the same species as well as several different species. If the coils turn to the right, the shell is **dextral**; if to the left, it is **sinistral**. Is the coiling loose or close, flat or conical? The **apex** of the shell is the oldest part. The wide opening of the shell is its **mouth**, or **peristome**. What is its shape? Is the margin smooth or toothed? Explain. One side of the peristome is drawn out into a spoutlike process in some species. What is its use? Do you find in any of the species an oval plate closing the opening? Such a structure is called the **operculum**. To what is it attached? Can you explain its use? The whorls make a line where they come in contact; this is the **suture**. Is the suture a smooth or an irregular line? Explain the lines that run parallel to the edge of the peristome as **lines of growth**. Is there any variation? The whorls coil around a central axis, the **columella**, as can be seen in a large shell that has been sawed lengthwise or from which the outer parts of the whorls have been broken.

(b) Fasten a specimen in a watch glass with wax so that the peristome of the shell is uppermost and barely covered with water. Study under the microscope. Or allow a small snail to become attached to a slide which may then be inverted, placed across the top of a watch glass, and examined under the microscope. How is the gliding movement effected? What is the shape of the **foot** and its relation to the rest of the body and to the shell? Can you observe **cilia**? Are there progressive waves of motion? The anterior region of the foot is the **propodium**; the posterior is the **metapodium**; and the mid-region is the **mesopodium**. Are these regions sharply marked off from one

another? To which part is the operculum attached when present? Anterior to the foot is the **mouth**. What is its shape? On each side are fleshy **tentacles**. Determine their size and shape? Touch them with a needle. What happens? Are there one or two pairs? Do you find small glistening spots, the **eyes**, on the tentacles? What is their position? Locate the **anal opening** on the right side anteriorly. In the air-breathing, or pulmonate, snails find the **respiratory opening** near the anal opening. Observe living snails in aquaria and notice their locomotion and feeding activities.

(c) If the internal structure is to be studied, large snails, such as *Busycon*, will be furnished, and additional directions will be given.

### Exercise 2.—Other Representative Mollusks.

(d) Refer to Fig. 74, p. 171, for classification within the Phylum Mollusca. Examine figures or demonstration specimens as follows: (1) *Amphineura*, such as *Chiton*, showing externally the segmented shell, foot, gills, mouth, and anus. (2) *Pelecypoda*, among which the small fresh-water *Sphaerium* may be examined alive in a watch glass and compared with the fresh-water mussel previously studied. It is viviparous, and young ones ready to escape may be seen through the shells of larger individuals. Marine **clams** and **oysters**, particularly their shells, may be studied. The shells of the clam, salt-water mussel, *Mytilus*, and oyster illustrate interesting types of modifications. In *Pecten*, the animal is adapted for swimming and has eyes and tentacles along the mantle margin. (3) *Gastropoda* have been studied alive as represented by the pond snail. The shells of some marine snails are modified in peculiar ways. If specimens are available, examine them for lines of growth and shape and try to determine how specialized types have been modified from the more usual forms. (4) *Cephalopoda* are exclusively marine. In contrast to the sluggish activities of most other mollusks, they are active free-living forms which capture fish and such animals for food. Examine a **squid**, *Loligo*. What structures are related to its active life? Compare the "head" with the corresponding region of a clam, a snail, and a chiton. The squid's tentacles are a modification of the foot. The shell is internal in the adult. The eye is almost as complex as that of a vertebrate, although it develops in a different manner. The *Octopus*, or **devil-fish**, is another cephalopod; also the *Sepia*, or **cuttle-fish**, the shells of which are sold as beak-sharpeners for canary birds. (5) The *Scaphopoda*, represented by *Dentalium*, show another type of specialization which may be visualized by thinking of a chiton as elongated dorso-ventrally and developing a conical foot.

## THE STARFISH

PHYLUM ECHINODERMATA      CLASS ASTEROIDEA

### I. BEHAVIOR AND EXTERNAL FEATURES

#### Exercise 1.—General Activities.

(a) The following directions may be used with *Asterias vulgaris* or *A. forbesi*; both species are found along the Atlantic Coast of North America. *Asterias vulgaris* is distinctly northern, ranging chiefly from Cape Cod to Labrador, although it may be found in colder or deeper waters as far south as Cape Hatteras. *A. forbesi* ranges from Maine to Florida. Both species vary greatly in color when alive and must be seen in the living condition if their beauty is to be appreciated. The most obvious difference between the two species is in the shape of the arms, which taper more rapidly and are more sharply pointed in *A. vulgaris* than in *A. forbesi*. Also, in *A. vulgaris* there is a median row of spines upon the aboral or upper surface of each arm which rarely occurs in *A. forbesi*. Living animals can be maintained inland in aquaria containing artificial sea water.

(b) If it is impossible to examine the starfish alive, some understanding of the animal's activities can be obtained from specimens preserved in various ways. Several dozen specimens, dried with no attempt to preserve them in the form desirable for dissection, will illustrate the diversity of shape that may be assumed in locomotion over irregular surfaces and in crevices among the rocks where the animals abound. Specimens preserved dry after having crawled into bottles, and masses of starfishes dried after they have "felt" together in a pail, may be examined. The consistency of the living animal is not unlike that of specimens preserved in formalin. The activities of various parts will be explained in the directions for study of their structure.

(c) If living animals are available, watch them as they crawl about in an aquarium. Are there anterior and posterior ends? Are the movements of the tube feet coördinated? Touch a glass rod to the ends of some of the tube feet. Pull the rod away. Do the tube feet exert suction? Frequently the tube feet become so firmly attached that they may be pulled off before releasing their hold. Can the ani-



mal crawl upon sand or upon a greased surface? The changes in shape of the arms are accomplished by muscle fibers in the body wall. Place an animal on its aboral surface and observe the righting movements.

(d) Place a mussel (*Mytilus*) or an oyster in the aquarium and see whether the starfish gives any evidence of a chemical sense. If feeding occurs, observe carefully and record what happens.

(e) Numerous minute structures, the pincerlike **pedicellariæ** and the fingerlike **papulæ**, occur upon the external surface (*cf.* Fig. 62). Hold a specimen just below the surface in a shallow pan of sea water and watch the pedicellariæ and papulæ with a handlens while you touch the surface gently with the point of a needle. Brush the surface gently with a camel's hair brush. Can you lift the starfish by means

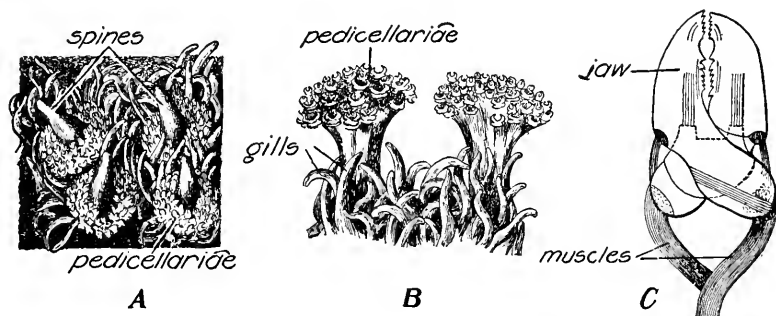


FIG. 62.—Papulæ and pedicellariæ of the starfish, *Asterias forrevi*. A, a portion of the external surface showing spines, the papulæ or gills, and retracted rosettes of pedicellariæ. B, portion of the surface of a starfish in which the rosettes of pedicellariæ are extended so that they cover the tips of the spines. C, the structure of a pedicellaria; diagrammatic.

(From H. S. Jennings, 1907, *University of California Publications in Zoology*, vol. 4.)

of the brush? Pull the brush away forcibly and examine the hairs with a handlens. What do these observations indicate concerning the functions of the pedicellariæ? Place a few small crustaceans, such as crabs, shrimps, or beach-hoppers, in a dish with a living starfish. How long are these small animals held fast if caught by the pedicellariæ? Some species of starfishes capture food in this manner and pass it along to the mouth by means of the pedicellariæ and tube feet. *Asterias forbesi* and *A. vulgaris* probably do not regularly employ this method of feeding. What is the distribution of the two kinds of pedicellariæ on the aboral surface? What is their distribution on the oral surface? Does this distribution suggest a protective function in any region? The presence of pedicellariæ apparently explains the clean external surface of the starfish and the absence of ectoparasites or

other attached forms which are so abundant upon the exposed surfaces of many marine animals.

(f) Inject a small amount of India ink or powdered insoluble carmine into the cœlom of a living starfish. Observe the surface carefully with a handlens or low-power of the microscope, or better with a dissecting binocular microscope. The injected particles can be seen, passing up one side of the cavity within a papula and down the other. The movement of the particles is caused by cilia. The action of cilia on the outer surfaces of the papulæ also may be demonstrated by India ink or carmine. These external cilia beat toward the tips of the papulæ. How may these internal and external cilia aid in respiration and excretion?

### Exercise 2.—External Features.

(g) Examine under water a specimen preserved in formalin. A dried specimen will be furnished for comparison as you proceed with the dissection of the formalin specimen. The main divisions of the body are the **disk** and the **arms**. On one surface of the disk between two of the arms is a conspicuous plate, the **madreporite**. Examine its surface with a handlens. The two arms nearest the madreporite are termed the **bivium**; the other three arms are the **trivium**. The surface on which the madreporite is found is the **aboral surface**. In the center of the aboral surface is the **anus**; it is doubtful whether the anus functions in egestion since it is an extremely small opening. Opposite the aboral surface is the **oral surface**. The **mouth** lies in the center of the disk on the oral surface surrounded by a membrane, the **peristome**. Specimens preserved in formalin may show a soft structure protruding from the mouth. This is part of the stomach, which is regularly everted in the process of feeding.

(h) Examine the **spines** on both surfaces of the animal. Are they actually on the surface or do they have a fleshy covering? What is their relation to the underlying skeleton? Note the **oral spines** around the mouth. What is their function? Examine this region in neighboring specimens. The grooves on the oral surfaces of the arms are the **ambulacral grooves** (*cf.* Fig. 63). Do they vary in width in different specimens? How is their width changed? Examine the cylindrical **tube feet** which protrude from the ambulacral grooves. These are locomotor organs by means of which the animals can crawl in any direction. How can the tube feet be protected from injury?

(i) Study the aboral surface, using handlens and keeping the specimen under water. Note the size and distribution of the spines and the soft fingerlike **papulæ** which have been previously examined

if you have studied the living starfish. Since their functions are respiratory and excretory, the papulae are sometimes called **dermal gills**. Among the papulae and surrounding the spines are minute white specks, some of which will be seen to be stalked; these are the **pedicellariae** as noted if you have studied the living animal. With a scalpel scrape off some of the pedicellariae, mount on a slide in glycerin, and examine under the microscope; certain structural features of the pedicellariae will be more clearly demonstrated if the tissue is macerated for a few

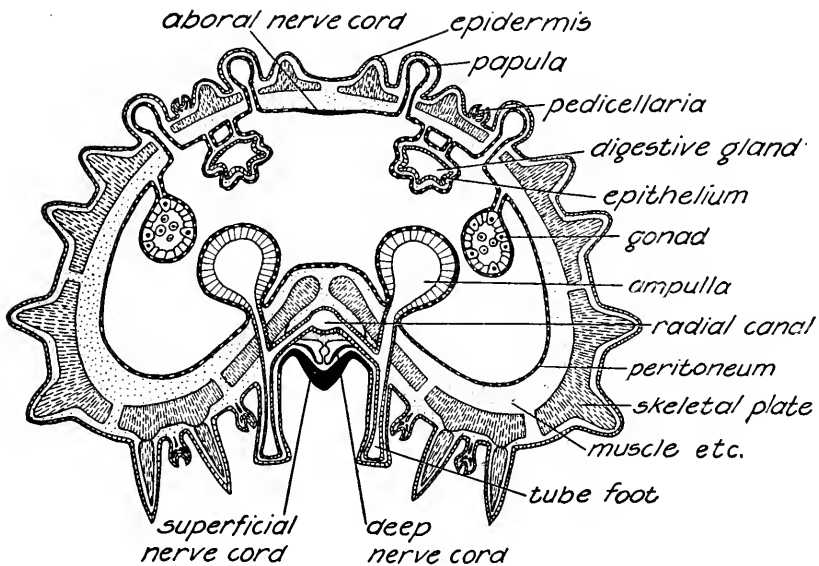


FIG. 63.—An arm of the starfish; diagrammatic cross section.

moments in caustic potash before the mounting in glycerin. Material from the dried specimen treated in this manner may also be used. How many kinds of pedicellariae do you find? How many parts has each? How do the jaws articulate? Where are the muscles attached? Gentle pressure on the cover glass will sometimes cause the jaws to open. Make a large outline of the starfish, to be completed as you proceed with your dissection. Use the distal half of one arm of the bivium for a drawing of the oral surface; and use the other arm of the bivium for a similar drawing of the aboral surface. Label carefully. Reserve the region of the disk and of the trivium for the drawing of internal parts.

## II. INTERNAL STRUCTURE

**Exercise 3.**—The Skeleton.

(a) Using the dried specimen, remove the aboral body wall from the disk without injuring the madreporite. Remove also the aboral body wall from one of the arms of the trivium. Pick away the dried remains of the internal organs and note the relation of the **skeleton** to the mouth. Examine the inner oral surface of the opened arm and find the openings through which the tube feet extend to the outside. Do they pass through or between the plates of the skeleton? Dried specimens macerated in caustic potash will demonstrate the nature of the skeleton. Compare the macerated material with the dried starfish and determine the body regions in which some of the types of isolated plates were located before maceration. Cut an arm of the dried specimen in transverse section and review the features already observed. Using a sharp scalpel make several sections of one arm of the formalin specimen. How thick is the body wall in the regions on the aboral surface where the skeleton is absent? Compare these sections with Fig. 63.

**Exercise 4.**—The Digestive System.

(b) Using the formalin specimen, remove the aboral portion of the body wall from the middle arm of the trivium, exposing the spacious coelom. First cut off the extreme tip of the arm and then with strong scissors cut toward the disk on each side, taking care not to injure any internal organ. The dissection should be made under water. As the cut proceeds, the aboral wall may be lifted, and the **mesenteries**, by means of which the greenish masses of the **pyloric caeca**, or **digestive glands**, are suspended from the aboral body wall, may be removed without injury to these organs. What is the nature of the mesenteries? Examine them in the transverse section of the arm previously made. Finally remove the aboral body wall of the arm by cutting across it near the disk. Cut around the margin of the disk above the arms until the cut is near the madreporite. Cut around the madreporite, being careful not to injure the internal structures, and continue the cut until the aboral surface of the disk is free from the remainder of the body wall. Carefully lift up the margin of this circular piece of the body wall so that you do not tear away its attachment to underlying structures. Determine the nature and extent of the pyloric caeca, and the way in which they are attached to the remainder of the digestive system. Observe that the saclike **stomach** is attached to the center of the disk by means of a short intestine from which the **anus**

opens aborally. The pair of lobed structures attached to the intestine are the **rectal cæca**. Remove the aboral wall of the disk except for a small piece around the anus. Leave all other structures intact.

(e) Now remove the aboral wall from the two remaining arms of the trivium, using the same precautions as before, but instead of cutting off the tips of the arms, cut outward from the disk on each side of the arms in such a way that the pyloric cæca will remain intact. Remove also the aboral wall from the proximal third of the two remaining arms without destroying the madreporite. To get a better view of the stomach, trim down the body wall in the angles between the arms but do not injure either the parts of the wall to which the reproductive organs are attached or the region beneath the madreporite.

(d) Cut across the bases of the pyloric cæca in the two arms of the bivium and, without destroying the ducts leading to the stomach, allow all five pairs to float out in the water. The stomach will now be seen to consist of two portions, an oral **cardiac region** and an aboral **pyloric region**. The cardiac portion is everted through the mouth in feeding. Where are the **retractor muscles** that withdraw this cardiac region? What muscles could produce the eversion? A diagrammatic figure of a vertical, longitudinal section through one arm and the disk should be consulted if available. Using the figure indicated at the close of **Exercise 2** (i), or making a new figure, show the cut wall as it actually appears over only a limited area along one side of an arm; for the remainder, a double line will suffice. Draw into this figure the digestive system, showing one pair of digestive glands in detail and the others cut off near the base. Cut off the digestive glands and free the cardiac region of the stomach by cutting all the retractor muscles. Float out this region and determine its oral attachment, after which it should be removed by cutting close to the peristome. Before discarding, reëxamine this central portion of the digestive system and check your drawing of the parts.

#### **Exercise 5.**—The Reproductive System.

(e) In specimens collected during the breeding season the reproductive organs are large and extend into the arms; at other times they are small. Five pairs of **gonads** will be found in the cœlom at the sides of the arms near the disk. How and where are these gonads attached? Each is a branched, lobed sac from which a duct opens to the outside through a perforated plate in the angle between neighboring arms. What is the exact location of this opening? Starfishes are either male or female, although the gonads have much the same

appearance in the two sexes. The ova and sperm are discharged into the water, where fertilization occurs. Show the reproductive organs in two of the arms in which the pyloric cæca are not shown on your previous drawing.

**Exercise 6.**—The Ambulacral System.

(f) If the directions have been followed carefully, the specimen will now show the main parts of the ambulacral system, or water-vascular system with only a small amount of additional dissection. Specimens having the system injected with a colored mass are preferable, but almost all parts of the system can be recognized in an uninjected specimen. On the floor of the cœlom in each arm will be seen the **ampullæ** (*cf.* Fig. 63). Determine their arrangement in the specimen under observation and also by reference to the dry specimen. Cut the arm of the dry starfish transversely with a sharp scalpel and observe a conspicuous tube, the **radial canal** of the water-vascular system. Press one of the ampullæ and determine the effect on the tube foot with which it is connected. Examine several sections cut from the injected specimen and look for connections between the radial canal and the ampullæ and tube feet. After determining the anatomical relations of these parts, including the **valves** in the **lateral canals** by which the ampullæ and tube feet are connected with the radial canal, you should be able to explain the mechanism by which the tube feet are expanded and contracted. Traced toward the disk the radial canals will be seen attached to a **ring canal** which encircles the mouth and which is marked by nine protuberances, the **Tiedemann's vesicles**. Note their location with reference to the arms, and determine what region lacks a Tiedemann's vesicle. What relation to the ring canal has the **stone canal** which leads downward from the madreporite?

(g) Draw the ambulacral system ( $\times 1$ ) as seen from the aboral view. Outline the disk and one arm, showing the other arms cut off near their bases. Show only a few of the ampullæ and their connections with the tube feet and radial canal.

**Exercise 7.**—The Nervous System.

(h) It is difficult to observe all parts of the nervous system, but certain features may be observed that show its radial nature and relations to other structures. Examine the aboral surface of a dry specimen that has the ambulacral grooves well opened. Remove the spines guarding the groove and the peristome. In the median line of each groove is a dark line, the dried remains of a **radial nerve**. Traced toward the disk, these nerves are seen to arise from an **oral nerve ring**

around the mouth at the margin of the peristome. Distally the radial nerve ends in the **eye-spot** which can usually be seen by examining the outer end of the ambulacral groove with a handlens. These portions of the nervous system are in the epidermis. There are deeper portions of the system beneath the epidermis of the oral surface which are not visible in a gross dissection. These consist of paired radial nerve cords attached to a double oral nerve ring. In addition there is an aboral nerve cord located in the peritoneum in the mid-line of each arm and connected with an anal nerve ring. Draw ( $\times 1$ ) the parts of the nervous system that you can identify.

**Exercise 8.**—The Circulatory System and Cœlom.

(i) The extensive **cœlom** has been noted as a conspicuous feature of the internal organization. There is no well-developed circulatory system, but there is a system of cavities usually designated as the **blood system** or **hæmal system**. Against the outer surface of the stone canal is a fleshy body, the **axial sinus**. If these two structures are cut transversely in their middle region, and the cut surface examined under water with a handlens, the cavity of the axial sinus will be clearly seen. What is the appearance of the stone canal in this region? You may also be able to recognize the **axial organ** as a ridge on the inner surface of the axial sinus. The axial sinus joins a canal which encircles the mouth and from which vessels pass along the aboral surfaces of the arms between the epidermal radial nerves and the radial canal of the water-vascular system. Transverse sections of an arm will often show this radial vessel and may also show that it is divided by a vertical partition. Although the function of this canal system is not definitely known, it is generally considered comparable to that of a blood-vascular system.

(j) Reëxamine Fig. 63 in review of your study of the specimen. How does the starfish illustrate the triploblastic body-plan, when compared with the annelids and vertebrates? What are the essential similarities? In what ways does the structure of the starfish seem aberrant?

### III. REGENERATION, REPRODUCTION, AND DEVELOPMENT

**Exercise 9.**—Regeneration.

(a) Regenerating specimens may be studied alive, but where time is limited not much can be observed that is not as well shown by a properly selected series of dried specimens in various stages of regeneration. How does the process of restoring the normal proportions of the parts compare with that in a regenerating planarian? Examine

specimens collected in nature in which the number of arms is abnormal. How might such conditions arise?

**Exercise 10.**—Reproduction and Development.

(b) The **ova** and **spermatozoa** of the starfish and those of many other marine animals are discharged directly into the water where

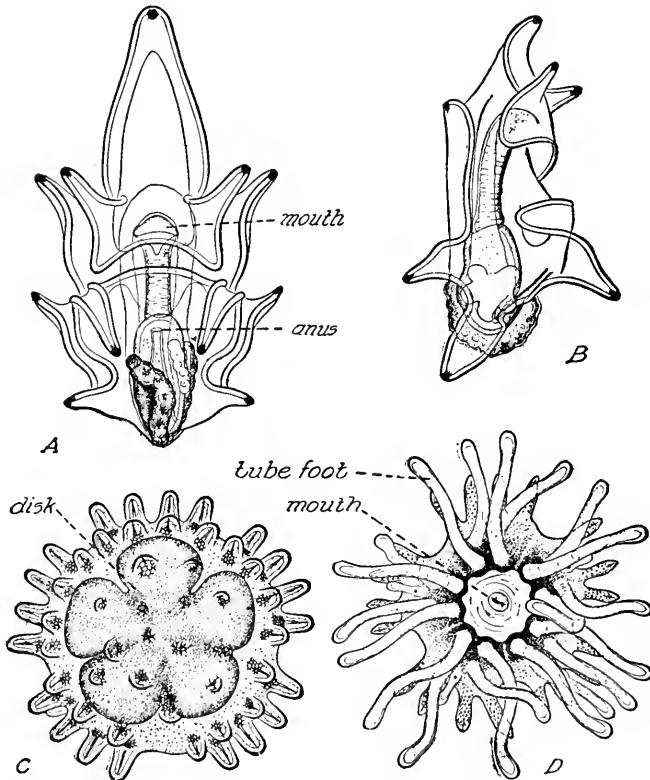


FIG. 64.—Development of the starfish. Bipinnaria from (A) ventral and (B) lateral view. Very young starfish from (C) aboral and (D) oral view.

(From W. K. Brooks, "Handbook of Invertebrate Zoölogy," copyright, 1882, by S. E. Cassino, reprinted by permission.)

fertilization occurs. Such ova are usually small and have but little yolk; they develop rapidly into **larvæ**, which swim and feed for a time and then take up the life of the parent upon the bottom. Since their normal environment of sea water is easily provided in the laboratory, these eggs are particularly favorable for study; they have become classic material for observations and experiments on fertilization, artificial parthenogenesis, and early development.



(c) The living eggs can be easily fertilized in a dish of sea water by addition of sperm and the development followed hour by hour. Instead of such living stages stained material may be used, either permanently mounted on slides or distributed in clearing fluid for temporary mounting as directed by the instructor. In the **one-cell stage**, or **zygote**, note the **fertilization membrane**, upon which the heads of many spermatozoa that failed to enter the egg may be visible. The **two-cell**, **four-cell**, **eight-cell**, later **cleavage stages**, and the **blastula stage**, will be recognized. Find late blastula stages, showing the origin of **mesenchyme cells** at one pole. Is the wall of the blastula of uniform thickness? Can you recognize, even in the blastula, the region that will invaginate during gastrulation to form the **archenteron of the gastrula**? The opening of the archenteron is the **blastopore**. After gastrulation the cells that have remained on the outside are known as **ectoderm**, and those that line the archenteron are known as **endoderm**. The mesenchyme cells and other cells which arise from the inner end of the archenteron constitute the **mesoderm**. In this manner the **germ layers** are formed. Make a series of outline figures illustrating these developmental stages; size, 5 to 10 × the measured size projected to table level.

(d) As development proceeds, the blastopore of the gastrula becomes the **anus** of the larva. The **mouth** of the larva is formed by an invagination, the **stomodeum**, which unites with the blind end of the archenteron. A larva which is strikingly bilateral and which is called a **bipinnaria** is formed within a day or two depending upon the temperature (Fig. 64). This larva becomes further specialized with respect to its free-swimming existence near the surface of the ocean. Other types of echinoderms have comparable bilateral larvæ. From these larvæ, by a curious **metamorphosis**, the radially symmetrical adult echinoderms are formed. The existence of such larvæ constitutes the main evidence for the belief that the present radially symmetrical echinoderms have descended from bilaterally symmetrical ancestors. Compare with the inferences drawn from the existence of fishlike stages in the frog and chick.

## OTHER ECHINODERMS

### Exercise 1.—Other Starfishes.

(a) Other species of starfishes should be examined with reference to modifications of the body outline, skeleton, and number of rays. This study should be supplemented by the examination of chart and textbook figures.

**Exercise 2.**—Other Classes.

(b) Examine specimens of brittle-stars, *Ophiuroidea*; of sea-urchins, *Echinoidea*; of sea-cucumbers, *Holothuroidea*; of sea-lilies, *Crinoidea*; and specimens of fossil crinoids. Compare them as to the five-parted, radial symmetry; position of the body with reference to environment; and other features. In no other phylum is there such diversity in the relationship of body axis to the environment. Compare diagrams of the internal structure of starfish, sea-urchin, and sea-cucumber.

# THE CRAYFISH

PHYLUM ARTHROPODA

CLASS CRUSTACEA

## I. BEHAVIOR AND EXTERNAL FEATURES

### Exercise 1.—General Activities.

(a) These directions apply equally well to any of the common species of crayfish of the Genus *Cambarus* and can be used with slight modifications for the lobster, *Homarus americanus*. Watch crayfishes in shallow pans of water, or in aquaria, and study their manner of walking and swimming. Are they sensitive to touch? How acute is their sense of sight? Do they respond to stimuli as well when removed from the water? Are they able to right themselves when placed dorsal surface down?

(b) Place some powdered carmine in the water on each side of an individual that is not moving about and determine the direction of any currents. Watch carefully and try to ascertain what produces these currents. Hold the crayfish out of water and note the bubbles produced at the anterior end of the animal. Selecting a small individual, or one having a very clean shell, look on the outside of the body, just above the base of the great claw and in line with the eye, and see if you can detect a flickering motion beneath the semi-transparent shell. You should be able to explain these observations when you have studied the gill chambers described on pages 144-146.

(c) Observe crayfishes in large aquaria containing stones and other objects. Note their habits of concealment, modes of swimming, reactions to other crayfishes and to small bits of meat dropped into the water. Do they show a preference for particular places in the aquarium?

### Exercise 2.—External Features.

(d) The body of the crayfish is made up of segments, or **somites**, each one of which bears a pair of jointed appendages. There are three general regions of the body: the **head**, consisting of five somites; the **thorax**, consisting of eight; and the **abdomen**, consisting of six. The **telson**, or median part of the tail-fin, is not considered to be a somite. In the abdomen the somites are distinct and movable; in the remainder

of the body they are covered dorsally and united by the **carapace** into a rigid portion, the **cephalothorax**. Note the pointed **rostrum** between the stalked **eyes**. Compare **anterior** and **posterior ends**, **dorsal** and **ventral surfaces**, and **right** and **left sides**. Is there any departure from strict bilateral symmetry? Examine the paired **appendages** from anterior to posterior end. Is the entire animal covered by the **exoskeleton**? What is the nature of the skeleton at the **joints** of the body and of the appendages? After looking carefully at the proportions of the abdomen, draw ( $\times 1$ ) an outline of an ideal cross section through this region to show the shape of the dorsal and ventral parts of the shell and the shape and attachment of the appendages; do not actually cut across the specimen.

(e) Find the **mouth** bounded by lateral toothlike jaws and the **anus** on the telson. Note the **excretory pores** on the bases of the longer **feelers**, or **antennæ**. Find on the dorsal surfaces of the smaller feelers, or **antennules**, the clear flat areas which mark the position of the **statocysts**, or **organs of equilibrium**. Examine the bases of the walking legs for the **genital pores**, or openings of the reproductive ducts; they are found on the last pair in the male, and on the second from the last pair in the female. In the male of *Cambarus* the two first pairs of abdominal appendages are modified to form, when pressed together, a **copulatory organ** along which the spermatozoa pass after leaving the genital pores. What is the structure of the corresponding appendages of the female? In the female there is a slitlike **seminal receptacle** on the ventral surface between the bases of the last pair of walking legs. Place a male and a female side by side and note the difference when they are viewed dorsally.

## II. RESPIRATORY ORGANS

### Exercise 3.—The Gills.

(a) Note the extension of the shell from the back down over the bases of the walking legs to form the carapace. Lift up the free ventral edge of the carapace and see the spongy mass of the **gills**, or **branchiæ**. Taking care not to injure the gills and using your scissors, remove the carapace from the left side, thus exposing the full extent of the gill cavity. Do not cut too far dorsally and injure other organs. Cut off the four walking legs, or **pereiopods**, and the large claw, or **cheliped**, of this side a short distance from their bases. Submerge the specimen in water in a dissecting pan and, by floating up and carefully parting the mass of the gills, determine what a single gill is like and where it is attached to the body. Move the stumps of

the legs and see how the outer gills are related to them. How many outer gills are there? To what appendages are they attached? What effect do you think the animal's walking has upon respiration? What structure do you find at the place where you saw the flickering movement under the shell of the living specimen? Back of this **bailer** is another, more delicate, blade which is the **epipodite** of the **first maxilliped**.

(b) From what you have observed about the gill cavity, its contents, and the water currents you have seen in the vicinity of a quiet animal, explain how the gills are bathed with a constantly changing supply of water.

(c) The outer gills are called **podobranchiæ**; note the significance of the name. Keeping the specimen entirely under water, and lifting

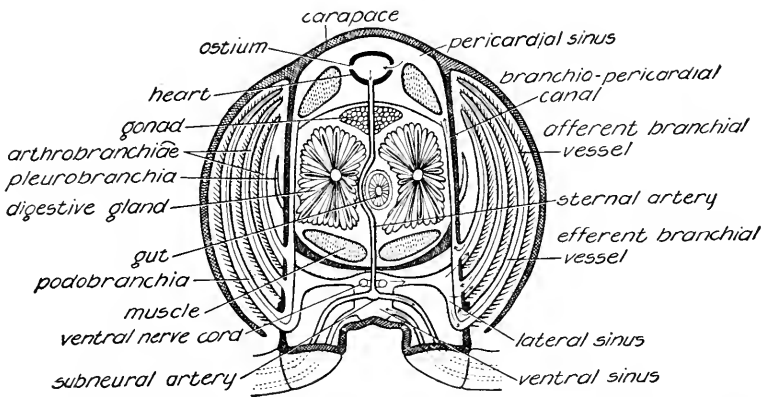


FIG. 65.—The thoracic region of the crayfish, in cross section; the arrows show the direction of blood flow.

the podobranchiæ one at a time to be sure you do not destroy any of the smaller gills which lie close beneath, remove all the podobranchiæ by cutting them off close to the appendages. Cut one across the middle with scissors and examine the section under water with a hand-lens. The **afferent** and **efferent branchial vessels** will be seen cut transversely.

(d) The inner layer of gills is now exposed. Are they attached to the legs? There are five pairs and a single one in front. Opposite which of the appendages are these gills located? They are called the **arthrobranchiæ**, or "joint gills." Note again the significance of the name.

(e) In the lobster and in some crayfishes there is another layer of gills lying beneath the arthrobranchiæ. Because they are attached

higher up and on the sides of the body, these last are termed the **pleurobranchiæ**, or "side gills." No pleurobranchiæ are present in the adult of *C. virilis*.

(f) Make an outline ( $\times 2$ ) of the cephalothorax from a side view. Show the stumps of the appendages and the places from which pleurobranchiæ have been removed. Put in all the arthrobranchiæ, and show also the bailer and the epipodite. Indicate the course of the water current by arrows.

(g) Examine specimens macerated in caustic potash and notice the delicate chitinous covering of the gills which has survived the maceration. Are the gills inside or outside the body? In answering this question, refer to Fig. 65.

### III. INTERNAL STRUCTURE

**Exercise 4.**—The Digestive, Circulatory, and Reproductive Systems.

(a) Using a freshly killed specimen or one that is preserved and injected, cut along the dorso-lateral surface on each side of the cephalothorax, taking care not to injure any of the organs lying immediately beneath the skeleton. Remove this dorsal part of the skeleton from the posterior margin of the thorax to a point just back of the eyes. Cover the specimen with water in a dissecting pan. The tops of the gills, which are exposed where you have cut into the gill cavity, are seen on each side. The **heart**, which may be still beating in a fresh specimen, lies on the mid-line in a cavity known as the **pericardial sinus**. Although the heart is soft and spongy, you should be able to distinguish the paired openings, or **ostia**, which are present in its walls. How many are there? The anterior part of the **gastric mill**, which is sometimes called the **stomach**, lies well to the front of the thorax. Note its thin and delicate walls and the two transverse bars of harder material, the **sclerites**, which are a part of the mechanism. When the specimen is intact, muscles pass from each of these bars to the inner surface of the dorsal skeleton. Find the remains of these muscles still attached to the shell which you removed, and also to the posterior sclerites. You should see the muscles arising from the anterior sclerite and attached to the inner surface of the shell just behind and between the eyes. These muscles form a part of the complex system by which the grinding action of the gastric mill is accomplished. Passing through the pericardial sinus are large muscles which diverge as they pass forward. If these pull on their forward ends as the fixed point, what movement will they bring about in the abdomen? You can

answer this if you understand how the somites of the abdomen are articulated to one another. The appearance of the region between the heart and gastric mill differs with the sex and sexual maturity of the specimen. In a female, if the **ovaries** are well developed, they will be seen as a paired mass in front of, and as a single median mass behind, the heart. In a male the **testes** are less conspicuous but have the same general Y-shape. In specimens that are immature or have recently shed their eggs or sperm, the organs are quite inconspicuous and need not be noted for the present. The paired **digestive glands**, which are a yellowish-green color in a freshly killed specimen, will be easily recognized, although in specimens with large ovaries they may be crowded out of sight and can be found only by pressing aside the latter organs.

(b) Cut off the tops of the gills, sever the **extensor muscles of the abdomen** at the level of the heart, cut back along each side of the abdomen as far as the terminal portion, or telson, and remove the dorsal skeleton of this region. The abdominal extensors will be found continued as two thin bands of muscle lying close under the skeleton. They may have been removed with the skeleton. The **intestine** lies in the abdominal region along the mid-line. Beneath and to the sides of the intestine are masses of muscle, which are the **flexor muscles of the abdomen**. Compare the bulk of these flexors with that of the extensors. Why should there be such a difference in the size and hence the power of these muscles? You will perhaps make out, lying on top of the intestine, a very small transparent thread that is colored in injected specimens, the unpaired **posterior aorta** (dorsal abdominal artery), which gives off branches to the muscles, intestine, and to all the abdominal appendages except the most anterior pair. At the anterior end of the abdomen the median portion of the reproductive organs may be found; or, if these are immature, the posterior ends of the digestive glands. Review all the foregoing items of internal structure, noting the appearance of the digestive and circulatory systems when viewed laterally (Fig. 66). Make an outline ( $\times 2$  or  $3$ ) of the cephalothorax and abdomen. Put into this the organs as they lie in place.

(c) The heart, posterior aorta, and branchial vessels have been identified. Other parts of the circulatory system (Fig. 66) which can be seen by pushing the organs aside without further dissection in injected specimens include: the **median anterior aorta** (ophthalmic artery) to the head; paired **lateral cephalic arteries** (antennary arteries) to the stomach, green glands, and anterior muscles; paired **lateral visceral arteries** (hepatic arteries) to the gonads, anterior part

of the intestine, and the digestive glands. Lift the heart carefully and observe the single **sternal artery** (descendant artery) passing ventrally. It can be seen to connect with the **subneural artery** which carries blood both anteriorly and posteriorly in the ventral region. Determine if possible exactly what structures are supplied by the branches of the subneural artery. From all these arteries blood escapes into intercellular spaces from which it flows back into an unpaired **ventral sinus** (sternal sinus). In the thoracic region this is joined to paired **lateral sinuses** by five pairs of small canals. From these lateral sinuses **afferent branchial vessels** carry blood to the gills; **efferent branchial vessels** return blood from the gills by way

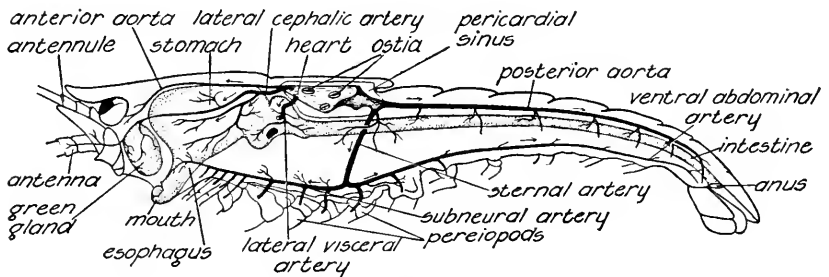


FIG. 66.—The digestive and arterial systems of the crayfish; arrows indicate the direction of blood flow (cf. Fig. 65).

(Redrawn with modifications from G. B. Howes, "Atlas of Zoöatomy," copyright, 1902, by Macmillan and Co., Ltd., printed by permission.)

of six pairs of **branchio-pericardial canals** to the pericardial sinus (cf. Fig. 65). It is impracticable to dissect out parts of the circulatory system other than the arterial portions. Add the observed portions of the circulatory system to your previous figure.

(d) Remove the heart and look for ostia on its ventral surface. Note the Y-shape of the reproductive organs, and find their ducts leading to the external openings previously noted. Remove the reproductive organs, being careful not to injure the digestive glands or the intestine. Trim off more of the gills and pull away the portions of the abdominal extensors that remain in the thorax. Make out the connection of the gastric mill with the intestine and the antero-posterior extent of the digestive glands. Cut in from one side and find the **esophagus**. It is very short and can be best located by noting again the position of the mouth. Trace the intestine to its posterior end, cut off close to the anus, and carefully free it up to its union with the stomach; also free the digestive glands. Cut across the esophagus, and remove the entire digestive tract and its appended glands in one



piece. Float out in water, and cut off the left digestive gland close to the tract. Note the region between the gastric mill and the intestine. Open the gastric mill along the ventral mid-line, find the **teeth**, work them together, and see how they grind.

(e) Draw a side view from the left ( $\times 2$  or  $3$ ), showing the digestive tract and the right digestive gland in position and the place where the left one opens into the tract.

**Exercise 5.**—The Nervous System and the Excretory Organs.

(f) Carefully remove all the museles and viscera from the abdomen. The **nerve cord** will be seen lying in the mid-ventral line. Notice the **ganglia**. How many do you count? In what somites do they lie? Notice the **lateral nerves**. How are these arranged with reference to the ganglia? In the cephalothorax, the nerve cord is concealed beneath transverse ridges of the ventral wall of the skeleton. Cut these with heavy seissors and expose the nerve cord, beginning at the posterior end of the cephalothorax and working forward. How many thoracic ganglia do you find? Just back of the esophagus is the large **subesophageal ganglion** from which two **circumesophageal connectives** pass to the **dorsal ganglion**, or **brain**, behind the eyes. Find the nerves passing from the brain to the eyes and to the antennæ and antennules. Draw a figure ( $\times 2$  or  $3$ ) of the nervous system thus exposed, showing accurately the ganglia, the somites in which they lie, and the lateral nerves. Compare the relation of ganglia to somites with that found in the earthworm.

(g) At the anterior end of the body, near the external openings already noted, find the excretory organs, or **green glands**. The thin bladder and underlying glandular portion of the organ can be readily distinguished. Refer to your textbook for further details.

#### IV. APPENDAGES

**Exercise 6.**—Serial Homology and Functional Modification.

(a) Examination of the appendages shows that they are obviously different in each region of the body. Anteriorly one finds that they have sensory functions. In the region of the mouth they are modified in relation to the seizing and mastication of food. In the middle region they serve for walking legs. In the abdomen the smaller appendages are modified with reference to respiration and reproduction, while the last pair and the telson constitute the powerful tail-fin. Comparison of their structure reveals a certain fundamental plan which will be appreciated as study progresses. Remove the appendages one at a time from the right side of the animal, placing each one

in a similar position for comparison or drawing in accordance with the instructions in the paragraphs that follow. Such orientation is important for correct understanding of the homologies between the various appendages. It is also important that the parts of each appendage drawn be completely labeled and that the smaller ones be drawn on a generous scale. If desired, the appendages may be properly oriented and glued to cardboard instead of being drawn.

(b) There are nineteen pairs of appendages. Beginning with the abdomen, count the number of pairs in this region and compare with the number of somites. The most posterior abdominal appendages are the **uropods**, or tail-feet; the others are the **swimmerets**. Note again the differences in the two anterior pairs of abdominal appendages in the sexes. Remove the right swimmeret of the fourth abdominal somite by cutting close to the body. A basal piece, the **protopodite**, bears two terminal pieces, an inner **endopodite** and an outer **exopodite**. No matter how much any of the other appendages may seem to differ from this plan of structure, all can be shown to be derived from such a fundamental type. The only exception is found in the case of the **antennules**, whose homologies are uncertain. Draw the above appendage with the end of attachment upward, the exopodite to the right, and the endopodite to the left. Use this same orientation in all your drawings of other appendages. Adjust scale of this drawing and of subsequent drawings of appendages to size of your specimen. It is desirable to have all these drawings on the same scale.

(c) Remove and draw the uropod of the right side. Orient and label as above.

(d) The thorax has eight pairs of appendages as follows: four pairs of walking legs, or **pereiopods**; the great claws, or **chelipeds**; and three other pairs farther forward which will be examined presently. Remove the right fourth pereiopod and the right cheliped, being sure to get all the parts of which each is composed. In the pereiopod the two proximal parts represent a divided protopodite, while the remaining five are divisions of the endopodite. In the embryo an exopodite is present (*cf.* Fig. 67). The great claws resemble the two anterior pairs of pereiopods save for the union of two of the divisions. Can you find where this has occurred? Note the modification of the distal end of the appendage. Draw this pereiopod in the same orientation as abdominal appendages, and show by a dotted outline the position the exopodite would have if present.

(e) Anterior to the great claws are three pairs of appendages, known as **maxillipeds**, or jaw-feet. The most posterior pair, the **third maxillipeds**, are large and easily recognizable. Before removal, the

right-hand member of this pair should be compared part by part with the walking leg just examined. It has the same parts, except that an exopodite is present. In one region two of the segments have fused to form a single one as in the cheliped. This third maxilliped is a very important appendage for purposes of comparison, because it still has the fundamental plan, and so can be compared with the simpler abdominal appendages; the structure of its endopodite shows how one may interpret the adult structure of the walking leg. Draw this appendage oriented as above.

(f) Examine, without removing, the **second maxillipeds** which lie in front of the third. They will be found to have parts similar to the

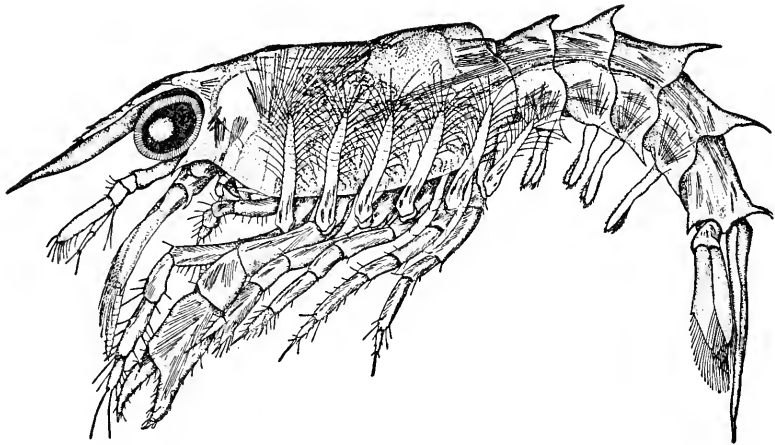


FIG. 67.—Young American lobster, *Homarus americanus*, at a stage when exopodites are present on the pereopods.

(From F. H. Herrick, 1895, *Bulletin U. S. Bureau Fisheries*.)

latter. Remove the right second maxilliped with care not to destroy the **first maxilliped** which lies close in front of it. Identify, without removing, the parts of the first maxilliped. There is a large **epipodite** which lies in the gill chamber just behind the bailer. Protruding toward the mid-line are two thin flaps which are outgrowths from the protopodite, and at about right angles to these are two other projections which are the exopodite and endopodite. Which is which? Remove the right first maxilliped and draw on a large scale, orienting as above.

(g) The remainder of the appendages occur on the head. Anterior to the first maxillipeds are two pairs of **maxillæ**, the parts of which should all be identified before removal is attempted. Each of the **second maxillæ** has a four-parted protopodite, a delicate endopodite,

and an exopodite which is fused with the epipodite so that it looks like a continuation of it. What is the function of the fused exopodite and epipodite? The **first maxilla**, the smallest of all the appendages, consists of three parts, the endopodite and a bilobed protopodite. Which is which? Remove the right second maxilla and draw, orienting as above.

(h) Remove and make a similar figure of the right first maxilla.

(i) The **mandibles** are now exposed. Against their posterior surfaces is a pair of lobes which are not true appendages. Each mandible consists of a heavy basal portion on the median surface of which is a cutting edge. This is shown by its development to be formed from the protopodite and to be comparable to the more delicate median outgrowths on the first and second maxillæ. The three-jointed **palp**, which protrudes from the heavy basal piece has its proximal joint formed from the protopodite and the other two from the endopodite. The exopodite is wanting in the adult. Where would it be if it were present? Remove and draw the mandible of the right side, orienting as above.

(j) The **antennæ** will show, when examined in place on the specimen, the typical exopodites, endopodites, and protopodites, and also the openings of the green glands. Remove the right one of this pair, orient, and draw.

(k) The **antennules** are the only appendages which do not show a true division into the three fundamental parts, although their two terminal portions suggest the endopodite and exopodite. Remove one and examine more carefully the region of the **statocyst**, or **organ of equilibrium**.

(l) Review the homologies indicated by the foregoing study. As a matter of definition how would you distinguish serial homology from the homology between the arms of a man and the wings of a bird.

## V. REGENERATION, REPRODUCTION, AND DEVELOPMENT

### Exercise 7.—Regeneration.

(a) Crayfish are often found in nature with one or more great claws or with walking legs missing or in process of regeneration. Examine such specimens if available. What is the relation of this regeneration to the process of molting? Sudden detachment, or **autotomy**, of the great claw is a common reaction when the claw is seized by an observer or by an enemy in nature. Try to demonstrate this by holding one or both the claws of a living crayfish. What is the nature of the so-called **breaking joint**?

**Exercise 8.**—Embryonic and Juvenile Stages.

(b) The **spermatozoa** are transferred from the male to the female during sexual union and retained in the seminal receptacle where they are available at the time of egg-laying. The **ova** are fertilized as laid,

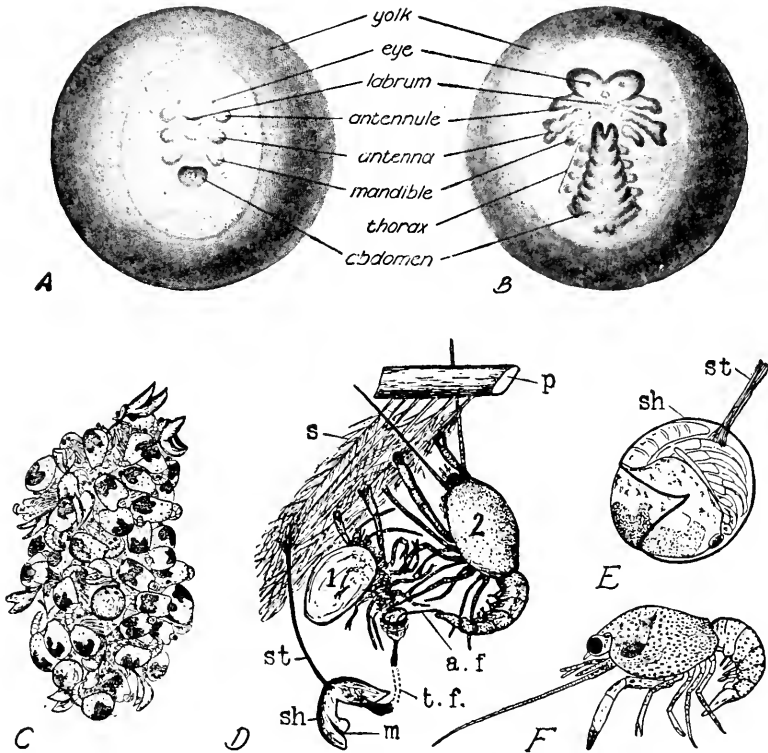


FIG. 68.—The development of the crayfish. *A* and *B*, ventral view of eggs, showing early stages in the development of the appendages and the principal divisions of the body. *C*, mass of young crayfish upon a swimmeret of the mother. *D*, second larval stage (2) attached by its chelipeds to hairs (*s*) on a swimmeret (*p*) of the mother. The molted shell of the first larval stage (1) is seen clinging by its chelipeds. The remains of the egg-membrane (*m*) and egg-shell (*sh*) are still attached to the hairs of the swimmeret by a stalk (*st*). When the first larva hatches it remains attached to the shell by a filament (*t. f.*) until its chelipeds can grasp a hair, and the second larva is similarly attached to the molted shell of the first by a filament (*a. f.*) By means of these filaments the young remain fastened to the mother during the periods of development when they might easily become detached. *E*, first larva hatching through a break in the eggshell (*sh*), which is attached to a swimmeret of the mother by a stalk (*st*). *F*, second larval stage.

(*A* and *B*, redrawn from G. B. Howes, "Atlas of Zoöatomy," copyright, 1902, by Macmillan and Co., Ltd., printed by permission; *C* to *F*, after E. A. Andrews, 1916, *Smithsonian Contributions*, vol. 35.)

and the **zygotes** become attached to the hairs of the abdominal appendages of the female. The pre-hatching, or **embryonic stages**, and also the post-embryonic, or **larval** and **juvenile stages**, can be observed if egg-bearing females are available. Examine in a watch glass of water the attached stages and juveniles about ready to leave the parent (*cf.* Fig. 68).

## OTHER CRUSTACEANS

### Exercise 1.—The Smaller Crustacea, or Entomostraca.

(a) The older division of the Class *Crustacea* into the subclasses *Entomostraca* and *Malacostraca* is still useful for the separation of certain smaller and simpler crustaceans from the larger and more familiar forms. **Water-fleas** and **copepods** are representative *Entomostraca*.

(b) The name water-flea was given by the early microscopists to the minute crustaceans of which the Genus *Daphnia* is an example. These are common in fresh water. They may be watched as they swim in an aquarium and studied in a watch glass with handlens and compound microscope. Addition of ether or chloretone produces a temporary anesthesia which facilitates the study.

(c) The **body**, consisting of **head**, **thorax**, and **abdomen**, and the **appendages** are covered laterally with a **carapace**, as if the carapace of the crayfish were extended ventrally and posteriorly so that the abdomen and appendages became enclosed. The organs of locomotion are enlarged **antennæ**. Do you find **antennules**? Look for the single **eye**, the **heart**, which should be still beating, the **digestive tract**, and any other features, recalling what has been seen in the crayfish. Under the dorsal portion of the carapace in the female is a **brood-pouch**, in which **eggs** and **embryos** may be found. The young are liberated as miniature adults. Make a drawing, 6-8 cm. in length, of the animal from a lateral view.

(d) The forms called copepods are another type of these small crustaceans, of which the Genus *Cyclops* is a fresh-water representative. Watch copepods swimming about in an aquarium; then study an anesthetized specimen on a slide and held lightly with a cover glass. The **thorax** with its **carapace** is shaped like a pear cut in half. The **abdomen** projects posteriorly and is tipped with hairlike processes. The single **eye** suggests the fabled "cyclops." As in *Daphnia*, the **antennæ** are the organs of locomotion. How are the remaining **appendages** distributed? Females may show an **egg-sac** projecting on each side of the abdomen. The young hatch as larvæ with three pairs

of appendages and are called **nauplii**. How do the males differ from the females aside from the egg-sacs? Internally the **digestive tract** will be seen. There is no heart. How may circulation of the body fluid be effected?

**Exercise 2.**—The Larger Crustacea or Malacostraca.

(e) Such crustaceans as the **amphipods** of fresh water, the **isopods** represented by the pill-bugs which are found under logs and stones on land, the edible **crabs**, **lobsters**, and **shrimps** are representative *Malacostraca*.

(f) Fresh-water amphipods may be examined alive in a small dish of water and their structure and activities noted. Terrestrial isopods may be similarly examined in a dish without water; do they “hide” under the litter when bits of damp paper are placed in the container? Do they “play possum”? A careful study would show that each of these types has the same fixed number of somites, and so appendages, as the crayfish.

(g) Examine a specimen of a crab and compare with the crayfish. The **abdomen** is greatly reduced and folded close against the ventral surface of the **thorax**. Understand the functions of the abdomen and its appendages in the two sexes. The **cephalothorax** with its **carapace** is extended laterally. **Pereiopods** and **chelipeds** will be recognized. There are the same appendages about the mouth as in the crayfish, but these are covered by the single large pair next anterior to the chelipeds. To which pair of appendages in the crayfish do these correspond? Note the characteristic positions of **eyes**, **antennules**, and **antennæ**. Dissected specimens may be demonstrated to show the **gills**, the **digestive tract**, and the **nervous system**, all of which are modified in conformity with the shortening of the body antero-posteriorly and its lateral extension. In life the crab scuttles about on the bottom or swims with paddle-like **fifth pereiopods**, going sidewise through the water. The **soft-shelled crabs** served as food are merely individuals that have recently molted. Larval crabs have a well-developed abdomen, but as development proceeds this becomes reduced and folded under the thorax. Examine any other representatives of the *Malacostraca* that may be available.

## THE LOCUST

PHYLUM ARTHROPODA

CLASS INSECTA

### I. BEHAVIOR AND EXTERNAL FEATURES

#### Exercise 1.—General Activities.

(a) Grasshoppers and locusts are the most common representatives of the Order *Orthoptera*. The large lubber grasshoppers are very favorable for study, but any good-sized local species can be used. Living individuals should be observed in glass jars containing grass and covered with a screen. How are the legs used in walking and jumping? Respiratory openings will be seen along the sides of the abdomen. Observe and time the intervals between the respiratory movements. Note the color and its distribution. Does this suggest an adaptation of the animal to its habitat? Offer bits of green vegetation to the animals and try to observe their mode of feeding. Touch the “feelers” of the head with a long piece of glass tubing having a plug of absorbent cotton in the end, and observe the sensitiveness of these organs as compared with other parts of the body. Moisten the absorbent cotton with some strong-smelling fluid, and bring it near a feeler without touching. Are these organs chemoreceptors? Can you determine whether other parts of the body have a similar function? Remove a specimen from the jar and examine the parts more closely. Note the “molasses” which is regurgitated from the mouth; this is a digestive fluid mingled with food. If a good-sized drop can be collected from one or more specimens and placed upon a slide, put a bit of fresh vegetation in this drop and note the result before the fluid evaporates. What may be the significance of this habit of regurgitating the contents of the digestive tract? If you have time, devise experiments to determine whether temperature or sensations comparable to fear in the higher animals influence the rate of respiratory movements.

#### Exercise 2.—External Features.

(b) The **body** has three main divisions: the **head**, **thorax**, and **abdomen**. Each of these divisions is made up of a number of more or less well-defined rings, called **somites**. The outer covering, or



**exoskeleton**, composed of a horny substance called chitin, protects the internal organs and serves as a place of attachment for the muscles. Bend the animal and observe that the exoskeleton is not absent but only thinner at the joints of both body and **appendages**, so that the entire outer surface is covered by a continuous armor. Recall the skeleton of the crayfish. Observe that on each abdominal somite the exoskeleton consists of a dorsal portion, the **tergum**, and a ventral portion, the **sternum**, joined by a thinner region below the line of the spiracles. Compare this sort of skeleton with that of the frog or man, which is an internal skeleton, or endoskeleton.

(c) The head is made up of several somites, as indicated by the fact that it bears several pairs of appendages, but these somites are so closely united that they cannot be distinguished. On the head are two large **compound eyes**. Examine the surface of one of them with a handlens and see that it is divided into a large number of small areas, each of which is the surface of one of the small independent visual units, or **ommatidia**, which make up the compound eye. There are also three **simple eyes**, or **ocelli**, which can be seen with the handlens. Two of these lie in front of and near the top of the two compound eyes; the third one lies in the median line somewhat ventral to the **antennæ**, or feelers. The skeleton of the top and front of the head is known as the **epicranium**. Below this is the **clypeus**, from which is suspended the **labrum**, or upper lip. The side of the head below the compound eye is the **gena**. The head bears four pairs of appendages, which will be identified and studied in greater detail later.

(d) The thorax consists of three somites: the **prothorax**, **mesothorax**, and **metathorax**, each of which bears a pair of **legs**. The dorsal surface of the prothorax has the form of a hood consisting of several fused plates and extending backward some distance over the mesothorax. Each leg of the grasshopper has five divisions: the **coxa**, a short segment by which the leg articulates with the body; the **trochanter**, also a short segment (not distinct in the large jumping leg); the **femur**, a long segment; the **tibia**, a long segment bearing **spines**; the **tarsus**, which is divided into several parts bearing **pads** below and ending in a pair of **hooks** and a little **pad**. The thorax bears two pairs of **wings**, a pair of tough, thick ones, and a pair of thin, membranous ones. To which somites do they belong?

(e) The abdomen includes ten somites and is without appendages. Somites 2-7 in the female, and 2-8 in the male, are complete rings and essentially alike. The first somite is interrupted at the sides by a backward extension of the metathorax, which bears the third pair of legs. The dorsal portion of the first somite bears the **tympanic mem-**

**branes**, sense organs whose function is supposedly auditory. The ventral part of the first somite is only indistinctly separated from the metathorax. The dorsal surfaces of the ninth and tenth somites are very narrow and only partially separated from each other. At the posterior end is the **supra-anal plate**, beneath which is the **anus**. At the sides of the supra-anal plate are the two triangular **podical plates**. At the base of each of these is a small projection, the **cercus**, which is sensory in function. In other respects, the terminal portion of the abdomen is different in the two sexes. In the female, the abdomen terminates in two pairs of stout, pointed structures which form the **ovipositor**, used for digging the holes in the ground in which the eggs are laid. In the male, the ventral surface of the ninth somite is prolonged backward and upward as the **genital plate**.

(f) On each of the first eight abdominal somites is a pair of **spiracles**, the openings of the **tracheæ**, or **respiratory tubes**. These are seen along the ridges on the sides of the abdomen. The spiracle on the first abdominal somite is just in front of the tympanic membrane. There are two pairs of spiracles on the thorax, located between the somites. These may be difficult to see but may be located by squeezing the specimen gently to press out the preserving fluid.

(g) Make a drawing of the insect as seen from the side ( $\times 4$ ). Spread out the wings above the back in such a position as to show their size and shape; arrange the legs in the position they assume when the insect is at rest; number the somites of the abdomen, and label all structures.

### Exercise 3.—The Mouthparts.

(h) Remove the **labrum** and clypeus and thus expose the **mandibles**, or **jaws**, of the insect. Each mandible is a heavy, strong structure with black toothed edges. The jaws of insects have been evolved from structures corresponding to the legs; in development they arise in the same manner as the legs and later assume their characteristic form. Is there any apparent advantage in having right and left instead of upper and lower jaws? Separate the two mandibles a little at the tips with a needle or point of a scalpel; then remove the right mandible by cutting with the point of a scalpel between the gena and the base of the mandible. Sketch the appendage as seen after removal ( $\times 10$ ), orienting it with the former attached end toward the top of the page (*cf.* Fig. 69).

(i) Posterior to the mandibles are the **maxillæ**, a pair of more delicate jaws, and the **labium**, which serves as the lower lip. Each maxilla has a basal portion which bears three other parts: (1) a short

segmented feeler, the **palp**, which is an organ of chemical sense; (2) the **galea**, a curved part with a rounded end; (3) the **lacinia**, a curved part which ends in sharp, black teeth. The labium has a basal portion, which bears right and left **palps**, which are sensory in function, and a flattened median portion which is partially divided into right and left halves. The labium is in reality composed of right and

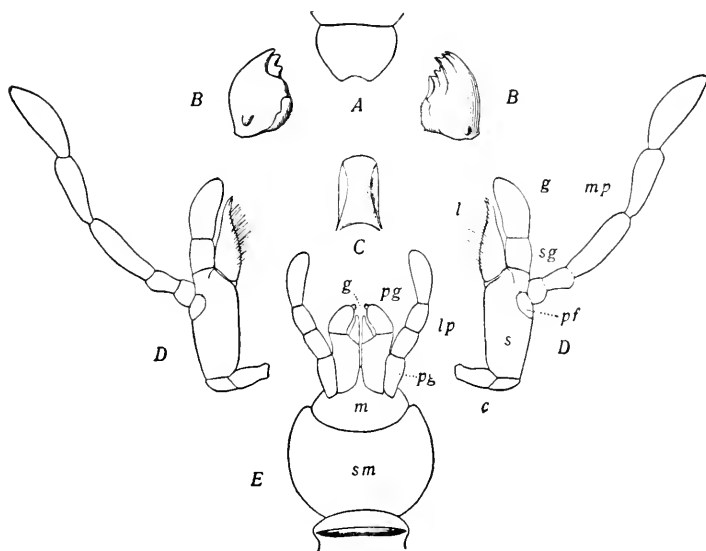


FIG. 69.—Mouthparts of a cockroach, *Ischnoptera pennsylvanica*. Mouthparts of insects show great diversity of structure in relation to feeding habits. All seem to have evolved from the simpler mandibulate types with jaws and other parts, such as are found in cockroaches and locusts. A, labrum. B, mandibles. C, hypopharynx. D, first maxillæ, each with basal portions (s and c), lacinia (l), galea (g and sg), and palp (mp and pf). E, labium, or fused second maxillæ, with basal portions (sm and m), glossa (g and pg) and palp (lp).

(From J. W. Folsom, "Entomology," copyright, 1906, by P. Blakiston's Son and Co., reprinted by permission.)

left appendages, which have become partly united in the median line. Remove the right maxilla by grasping it at the base with forceps and carefully pulling it away. Remove the entire labium in the same manner. Removal of these parts exposes the **hypopharynx**, or **tongue**, which is an important organ in the ingestion of food. The space surrounded by the mouthparts and from which food enters the esophagus is known as the **buccal cavity**. Draw these removed mouthparts ( $\times 10$ ), orienting with the former attached portions toward the top of the page.

## II. INTERNAL STRUCTURE

**Exercise 4.**—General Internal Structure.

(a) Cut off the legs. With scissors, cut through the exoskeleton along each side of the thorax and abdomen just above the spiracles, being careful not to cut deep enough to injure internal structures. Carefully remove the dorsal part of the exoskeleton, and before discarding it, look for the **heart**, which usually comes off attached along the mid-line. If the thin mass of muscles, which clings to the piece in the abdominal region, is carefully stripped away by grasping with forceps at the posterior end and pulling forward, the heart will perhaps be seen as a delicate tube lying upon the dorsal surface of the muscles. How far can you trace the heart anteriorly? Can you see the openings, or **ostia**, along its sides by using the handlens? Cut off the top of the head nearly as far down as the bases of the antennæ, being careful not to break the head from the body. Pin the animal down under water by two pins through the bases of the jumping legs and keep it under water for the following dissections of the internal organs.

(b) The **digestive tract** is now exposed in the thorax and anterior part of the abdomen. In the posterior part of the body, portions of the **reproductive organs** occupy a dorsal and lateral position and hide the digestive tract. These organs are somewhat obscured by the lace-like **fat-body**, which should be carefully picked away from the top of the digestive and reproductive organs.

**Exercise 5.**—The Reproductive System.

(c) If the specimen is a female, the **ovaries** will be seen as a large mass containing many good-sized, elongated eggs. If it is a male, the **testes** will form a compact mass in the posterior part of the abdomen. In both sexes, the **ducts** from right and left reproductive organs unite ventral to the digestive tract and discharge through an opening at the tip of the abdomen. These features are not easy to study, and the reproductive organs may now be removed to expose fully the digestive tract.

**Exercise 6.**—The Respiratory System.

(d) The respiratory organs of insects are air-tubes called **tracheæ**, opening to the outside by means of the **spiracles**, which have been previously observed on the outer surface of the body. The tracheæ may be seen as silvery-white tubes on the surface of the digestive tract, if they contain air, but if they contain fluid they will be hard to see.

Examine them carefully with a lens. Press the digestive tract to one side and find some of the larger tracheal tubes as they pass from the internal organs to the spiracles in the body wall. At places you may also see **air-sacs**, which are enlarged portions of the tracheal system. Take a bit of muscle from one of the legs of the insect, mount on a slide in water, tease apart, and study with the compound microscope. Notice the muscle fibers and the fine, branching tracheæ. Minute branches of the air-tubes extend to all parts of the body, running not only among the cells but actually passing into and through the cells, thus bringing the inspired air in intimate contact with the protoplasm. Compare the manner of distributing oxygen in the frog and locust. Make a drawing of part of such a preparation.

**Exercise 7.**—The Excretory System.

(e) Notice the numerous small, brown, crooked tubules on the surface of the digestive tract in the abdominal region; use the lens to distinguish them from the silvery-white tracheæ with which they are interlaced. These are the **Malpighian tubes**, which are the excretory organs of the insect. Where do they connect with the digestive tract?

**Exercise 8.**—The Digestive System.

(f) The **digestive tract** has the following parts: the **esophagus**, which lies in the head and cannot be seen fully until later; the **crop**, an enlarged portion in the thorax; the **gastric cæca**, several pairs of digestive glands which surround the tract and empty into it between the crop and the stomach; the **stomach**, which extends from the crop to the region where the Malpighian tubes are attached; the **intestine**, which extends to the anus. The intestine has a more slender part, called the **colon**, near its middle and terminates in a larger part, the **rectum**. Make a drawing of the digestive tract as seen from the side ( $\times 4$ ), surrounded by a simple outline of the entire animal.

**Exercise 9.**—The Nervous System.

(g) Remove the digestive tract by cutting it across at the esophagus and the rectum. The nervous system, which lies along the mid-ventral line, can now be seen in the abdominal region. It should be carefully exposed by picking away other tissues, and working forward until you have exposed the parts in the thoracic region and head. As in the crayfish, the nervous system consists of masses called **ganglia**, connected with each other by ventral, longitudinal nerve trunks. Each ganglion gives off **nerves** to surrounding parts of the body. The apparently single ganglionic masses are each composed of fused right and left

ganglia. Hence the nervous system consists of pairs of ganglia, united by transverse fusions, the **commissures**, between the members of a pair,

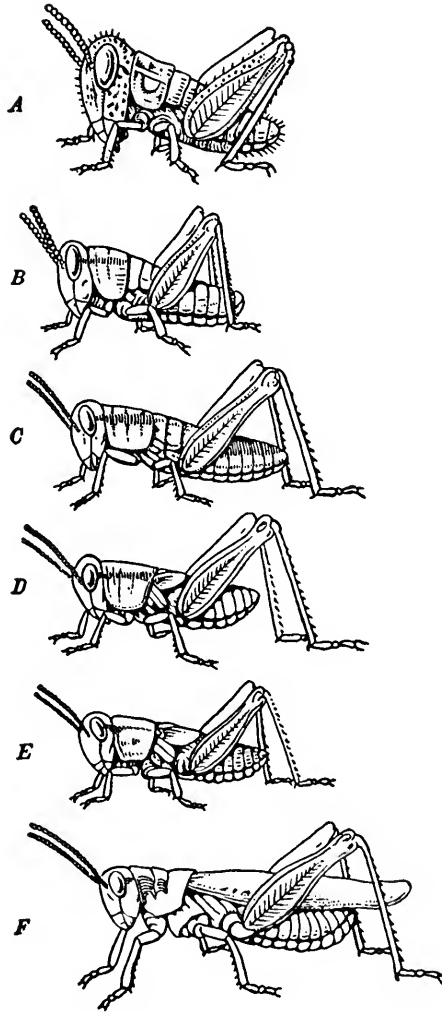


FIG. 70.—Growth and differentiation of the locust after hatching. A is recently hatched and more highly magnified than the other nymphs, which are shown after successive molts until the adult is seen at F. Note the appearance of wing-pads in D and E.

(After A. S. Packard, from H. R. Linville and H. A. Kelly, "General Zoölogy," copyright, 1906, by Ginn and Co., reprinted by permission.)

and by longitudinal **connectives**, between successive pairs. Nine of the ten ganglionic masses lie along the mid-ventral line close to the exo-

skeleton. The remaining mass, in which three pairs of ganglia are fused, constitutes the so-called **brain**, which lies dorsally in the front of the head close to the bases of the antennæ and sends large nerves to the compound eyes and smaller ones to ocelli and antennæ. A pair of **circumesophageal connectives** extends from the brain to the ventral nerve cord. Of the ventral ganglionic masses, five are in the abdomen, three in the thorax, and one in the head. The one in the head is termed the **subesophageal ganglion**. In the anterior part of the thorax the nerve cords pass under a part of the exoskeleton, the **tentorium**. Carefully insert the point of the scissors and cut the tentorium on each side so that it can be removed and thus expose the subesophageal ganglion. The connectives between it and the brain, and between it and the first thoracic ganglion, can now be found. Such a nervous system is characteristic of the entire Phylum *Arthropoda*, and also of the Phylum *Annelida*. Compare in position and structure with the nervous system in the frog and man. Draw the nervous system from a dorsal view ( $\times 4$ ), including a simple outline of the body and placing each ganglion in its proper somite. Note the position of the esophagus as it is now exposed and add to the drawing called for in paragraph (f).

### III. REPRODUCTION AND DEVELOPMENT

**Exercise 10.**—Eggs and Juvenile Stages.

(a) The **spermatozoa** are transferred from the male to the female during sexual union and retained in a seminal receptacle where they are available for fertilization of the **ova** before the egg-laying. Although the **zygotes** thus formed are laid in characteristic **egg-masses**, they are not easily collected; full-sized ova may have been seen in dissection of the reproductive organs. In spring and early summer **juvenile stages** of various locusts and grasshoppers may be collected by sweeping with an insect net the grass of fields where the adult insects abound (Fig. 70). Study as directed by instructor any of the foregoing stages that may be available.

### OTHER INSECTS

**Exercise 1.**—The Beetles.

(a) Any large beetle will do for this study, provided that it is not too highly specialized. Examining the animal from the ventral surface, locate the head, thorax, and abdomen, and note the number of somites visible in each. Look for antennæ, compound eyes, ocelli,

mandibles and other mouthparts, anus, and thoracic legs. Compare with what you have found in the grasshopper. Where are the wing-covers and the wings? When the latter are found, note how they fold up beneath their covers. Fasten down, dorsal surface up, by pinning through the prothorax, spread one wingcover out at right angles, and unfold the corresponding wing, which can be spread in the angle between the wingcover and abdomen. Raise the head, if it bends too far ventrally, and spread out the three legs on the side where wing

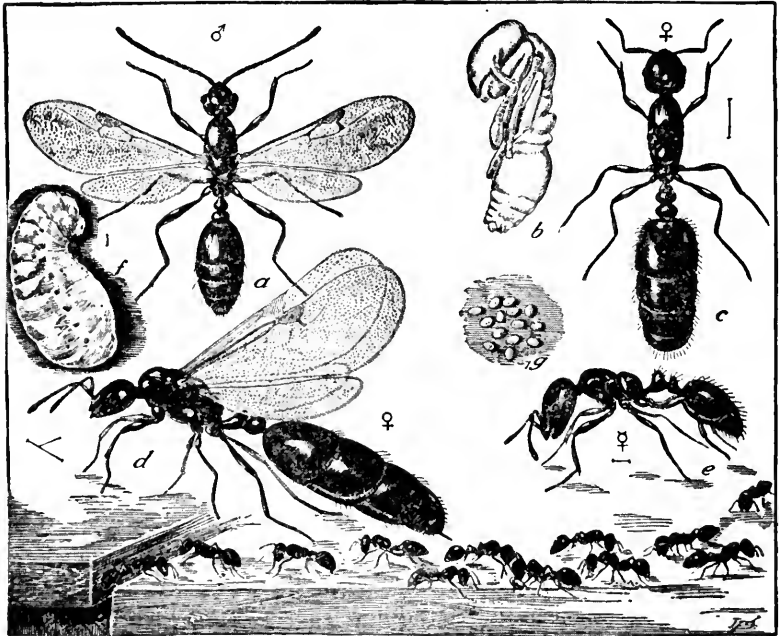


FIG. 71.—The little black ant, *Monomorium minimum*, one of the Order *Hymenoptera*. *a*, male; *b*, pupa; *c*, wingless female; *d*, winged female; *e*, worker, or sexually immature female; *f*, larva; *g*, eggs; group of workers in line of march below.

(From C. L. Marlatt, 1916, *Farmers' Bulletin*, No. 740, U. S. Dept. Agriculture.)

and wingcover are closed. Draw the specimen from this view and on such a scale as to make the figure about 10 cm. long. Show the plates of the skeleton with care, and number the somites of the thorax and abdomen.

(b) Examine, as directed by instructor, such additional preserved or living specimens of beetles and their larvæ as are available for individual study or demonstration (*cf.* Fig. 73 f). In the larva of a beetle, find the head, thorax, and abdomen, the mouth with its jaws,



and the anus. Count the number of somites, comparing with the adult of the same species. Draw such a larva ( $\times 3$  or  $4$ ) from a lateral view, showing these parts.

**Exercise 2.**—The Wasps and Ants.

(c) Wasps of the Genus *Polistes* are very common and are easily collected when they enter unscreened buildings with the approach of cooler weather in the fall. Head, thorax, and abdomen will again be recognized as in the other insects. How many somites in each? Look for antennæ, compound eyes, ocelli, mouthparts, and anus. At the posterior end of the female is the **sting**. The spiracles are a row of minute dots on each side of the abdomen. Compare the divisions of the thorax and of each of the legs with the corresponding parts of the grasshopper. To which somites are the wings attached? Draw a side view ( $\times 3$  or  $4$ ), with wings spread dorsally.

(d) Examine the “paper” **nests** of this wasp and others, if available. Observe artificial **ants’ nests** and the **eggs** and **larvae** recently taken from an **ant colony**. The most interesting facts regarding the ants, bees, and wasps are those connected with their social life in such colonies. It is possible to observe many of their activities in colonies kept in the laboratory in finely screened or glass-covered cages.

**Exercise 3.**—The Butterflies and Moths.

(e) Examine a good-sized butterfly, or moth, going over the features noted for other forms: the three main divisions of the body, eyes, antennæ, mouthparts, legs, and wings (*cf.* Figs. 72 and 73). Mount some of the “dust” from the wing surface and examine under a microscope. The **scales** observed are a modification of the hairlike processes seen on the bodies of many insects. Draw the entire animal from a dorsal view, with wings spread, making the figure about 10 cm. across; omit the color pattern.

(f) If available, the **eggs** of butterflies or moths will be shown as a demonstration. Understand to what species such eggs belong and where they are laid (*cf.* Fig. 73 c).

(g) Examine such **larvæ**, or **caterpillars**, as may be available. Where are the head, thorax, and abdomen? Do you find thoracic legs? There are paired structures on the abdomen which are not true appendages and which are known as **prolegs**. How many are there, and what is their structure as compared with the thoracic legs? Are there compound eyes, ocelli, antennæ, and mouthparts as in other forms? Do you find spiracles? Draw a side view on a large scale.

(h) During the proper season, living specimens of the larvæ of va-

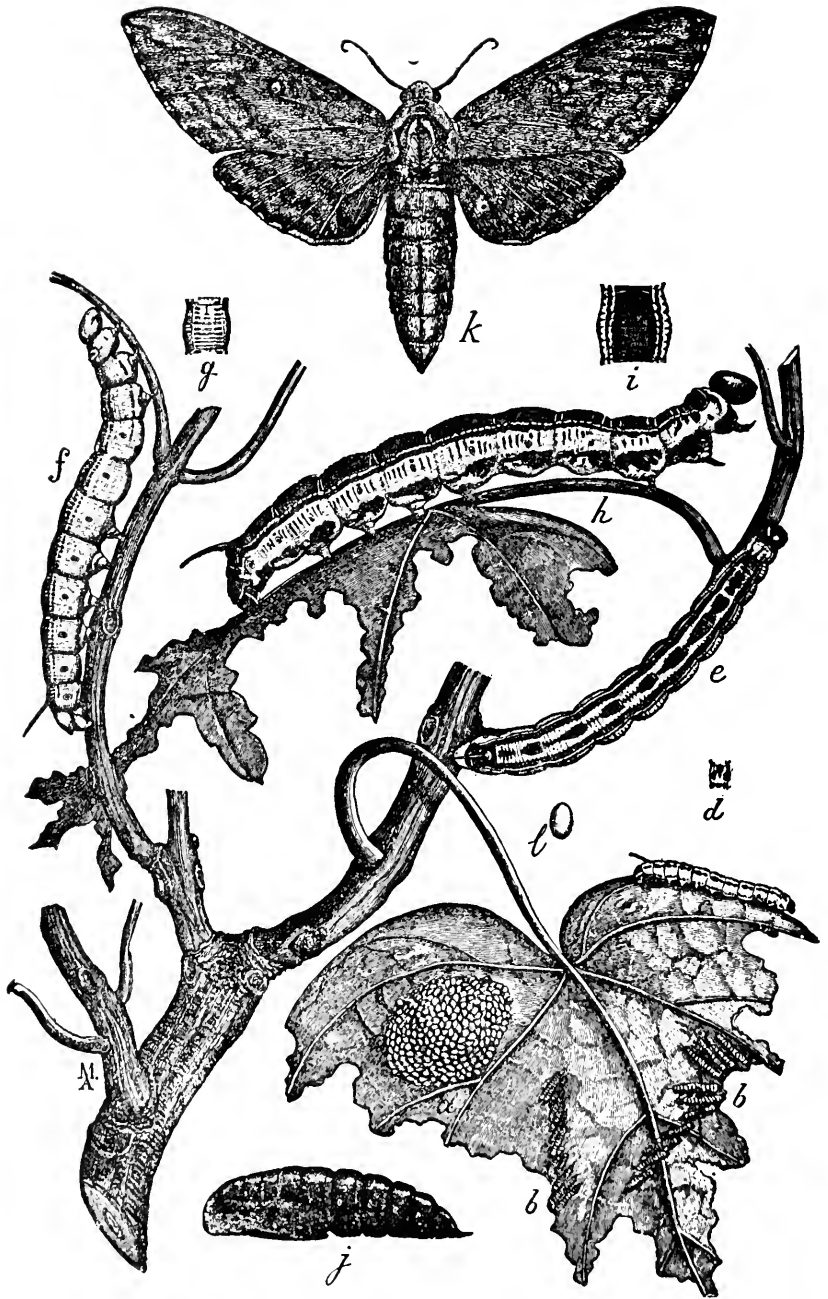


FIG. 72.—Life-cycle of the catalpa sphinx *Ceratomia catalpa*, one of the Order *Lepidoptera*. *a*, egg mass; *b*, *b*, newly hatched larvæ; *c*, larva one-third grown; *d*, dorsal view of a somite of *c*; *e*, *f*, two differently marked, nearly full-grown larvæ; *g*, dorsal view of a somite of *f*; *h*, full-grown dark larva; *i*, dorsal view of a somite of the same larva; *j*, pupa; *k*, moth; *l*, egg, enlarged. All natural size except *l*.

(From L. O. Howard and F. H. Chittenden, 1916, *Farmers' Bulletin*, No. 705, U. S. Dept. Agriculture.)

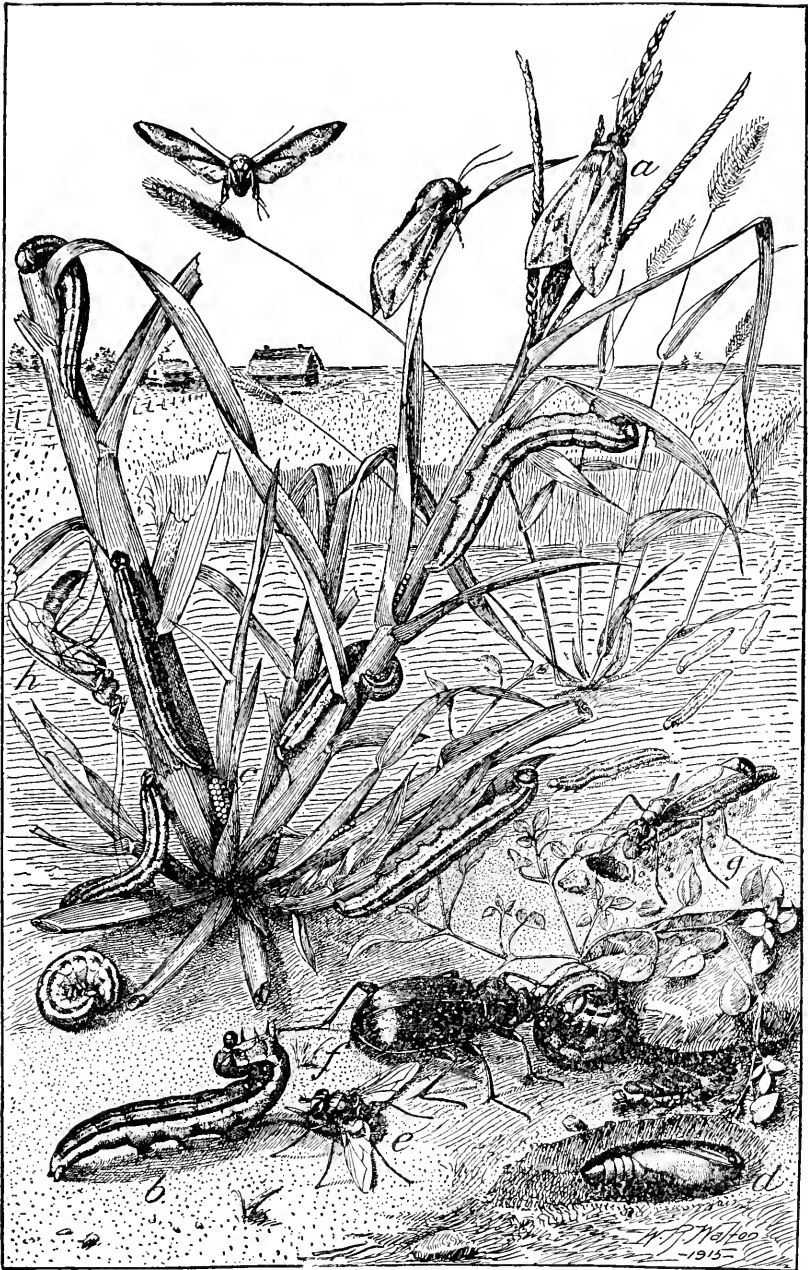


FIG. 73.—A study in ecology: the life-cycle and relationships to other species of the true army-worm, *Cirphis unipuncta*, one of the Order *Lepidoptera*. *a*, parent or adult moth; *b*, full-grown larva; *c*, eggs; *d*, pupa in soil; *e*, parasitic fly, *Winthemia quadripustulata*, laying its eggs on an army-worm; *f*, a ground beetle, *Calosoma calidum*, preying upon an army-worm, and, at right, *Calosoma* larva emerging from its burrow; *g*, a digger wasp, *Sphex* sp., carrying an army-worm to its burrow; *h*, *Enicospilus purgatus*, an ichneumon-fly parasitic on the larvæ of the army-worm. All about natural size.

(From W. R. Walton, 1916, *Farmers' Bulletin*, No. 731, U. S. Dept. Agriculture.)

rious forms can be brought to the laboratory for individual study or demonstration. Observe their way of moving and their voracious habits in feeding. How do the structure and use of the mouthparts differ in the larva and adult? The caterpillars of moths will often spin their **cocoons** in the cages where they are kept, or such cocoons may be collected and given out for study. Cut one open and find the **pupa**, or resting stage, within. Notice the silk of which the cocoon is composed. Such cocoons, or those collected during the late fall, if uninjured, may be kept in cages, and the emergence of the adult insect observed at some subsequent time. Cocoons are characteristic for the moths. The larvæ of butterflies typically have a naked pupa, called a **chrysalis** (Fig. 72 j), which is protected by a tough skeleton from which the adult insect emerges at the final molt. Understand the complete life-history in each of the orders studied and be able to explain the difference between the type of life-cycle in butterflies and moths and that found in insects such as the grasshopper. Examine carefully all the details of Figs. 72 and 73.

**Exercise 4.**—Other Orders.

(i) Of the remaining orders of the insects, three are more commonly known and recognized by popular names. These are the *Hemiptera*, or true bugs; the *Diptera*, or two-winged flies, of which the house-fly is our most common representative; and the *Odonata*, or dragon-flies. Observe as many representatives of the different orders as are available, noting structural features such as mouthparts, wings, and markings, which serve as taxonomic features.

## CLASSIFICATION OF ANIMALS

### Exercise 1.—Examination of Representative Forms.

(a) A sufficient number of representative animals has now been studied in detail to enable you to understand the typical modifications of structure as related to function in existing animals. Remember that although types of structure are varied, animals are essentially similar in their fundamental capacities. In your study of museum and other specimens as outlined below, you should keep in mind that structure is meaningless unless seen in its relation to function. Complexity of structure is correlated with increased number of cells, their relative location, and their specialization. The term **phylum** is applied to certain larger groups of animals, the members of which carry on their activities by means of similar structures.

(b) **Phylum Protozoa**.—The forms studied, *Amœba*, *Paramecium*, and *Euglena*, together with figures and demonstrations of other forms, sufficiently illustrate this phylum. The distinguishing characteristic is the unicellular state.

(c) **Phylum Porifera**.—Examine simple sponges such as *Scypha* (*Grantia*) and *Leucosolenia*. Note the **osculum**, or exhalant opening, and many small incurrent **pores** on the surface. The structure of larger sponges, such as the **bath sponge** and others, may be compared with that of the simpler types in terms of budding, folding, and vegetative growth (*cf.* Fig. 41, p. 84). Distinguishing characteristics of the phylum are: absence of a digestive cavity comparable to that of other Metazoa; attachment during the adult stage; absence of organs and true tissues, although there is a limited amount of cell specialization; a skeleton of fibers or spicules. The sponges are the simplest of the many-celled animals, with the exception of the small group known as *Mesozoa* which is sometimes called a phylum.

(d) **Phylum Cœlenterata**.—The examples studied are **hydras** and the **hydroïds** with their **medusæ** or **jellyfishes**. Examine other representatives, such as the larger **jellyfishes**, **sea-pens**, **sea-fans**, **sea-anemones**, **corals**, and **ctenophores**. Understand the relation between soft parts and skeletons. Distinguishing characteristics are: some degree of cell specialization and division of labor but no true tissues; radial symmetry; a single opening to the digestive cavity; two germ layers,

Phylum, <i>Protozoa</i> *	Phylum, <i>Rotatoria</i>
Subphylum, <i>Plasmodroma</i>	Class, <i>Seisonidea</i>
Class, <i>Sarcodina</i>	Class, <i>Belloidea</i>
Class, <i>Mastigophora</i>	Class, <i>Monogonata</i>
Class, <i>Sporozoa</i>	
Subphylum, <i>Ciliophora</i>	Phylum, <i>Nematoda</i>
Class, <i>Ciliata</i>	Class, <i>Phasmodia</i>
Class, <i>Suctorina</i>	Class, <i>Aphasmodia</i>
Phylum, <i>Porifera</i>	Phylum, <i>Nematomorpha</i>
Class, <i>Calcarea</i>	Class, <i>Gordidiidea</i>
Class, <i>Noncalcarea</i>	Class, <i>Nectonematoidea</i>
Phylum, <i>Cœlenterata</i>	Phylum, <i>Acanthocephala</i>
Subphylum, <i>Cnidaria</i>	
Class, <i>Hydrozoa</i>	Phylum, <i>Bryozoa</i>
Class, <i>Scyphozoa</i>	Class, <i>Entoprocta</i>
Class, <i>Anthozoa</i>	Class, <i>Ectoprocta</i>
Subphylum, <i>Acnidaria</i> <sup>1</sup>	
Class, <i>Tentaculata</i>	Phylum, <i>Brachiopoda</i>
Class, <i>Nuda</i>	
Phylum, <i>Platyhelminthes</i>	Phylum, <i>Annelida</i>
Class, <i>Turbellaria</i>	Class, <i>Archiannelida</i>
Class, <i>Trematoda</i>	Class, <i>Polychæta</i>
Class, <i>Cestoda</i>	Class, <i>Oligochæta</i>
Class, <i>Nemertea</i>	Class, <i>Hirudinia</i>
Phylum, <i>Gastrotricha</i>	Class, <i>Myzostoma</i>
Phylum, <i>Chaetognatha</i>	Class, <i>Echiurida</i>
	Class, <i>Gephyrea</i> <sup>2</sup>

<sup>1</sup> Often classified as a separate phylum, the *Ctenophora*.

<sup>2</sup> Sometimes classified as a separate phylum, the *Gephyrea* or *Sipunculoidea*.

FIG. 74.—Phyla of the Animal Kingdom and their principal subdivisions.  
representative zoologists

(A. S. Pearse, "Zoological Names," a list of Phyla, Classes and Orders

Phylum, *Echinodermata*Subphylum, *Pelmatozoa*Class, *Cystoidea*<sup>3</sup>Class, *Blastoidea*<sup>3</sup>Class, *Crinoidea*Subphylum, *Asterozoa*Class, *Asteroidea*Class, *Ophiuroidea*Subphylum, *Echinozoa*Class, *Echinoidea*Class, *Holothuroidea*Phylum, *Mollusca*Subphylum, *Isoplcura*Class, *Amphineura*Subphylum, *Prorhipidoglossomorpha*Class, *Gastropoda*Class, *Scaphopoda*Class, *Pelecypoda*Subphylum, *Siphonopoda*Class, *Cephalopoda*Phylum, *Arthropoda*Subphylum, *Branchiata*Class, *Crustacea*Subphylum, *Tracheata*Class, *Onychophora*Class, *Myriapoda*Class, *Insecta*Subphylum, *Arachnida*Class, *Arachnida*Phylum, *Chordata*Subphylum, *Hemichorda*Subphylum, *Urochorda*Subphylum, *Cephalochorda*Subphylum, *Vertebrata*Superclass, *Pisces*Class, *Agnatha*Class, *Chondrichthyes*Class, *Osteichthyes*Superclass, *Tetrapoda*Class, *Amphibia*Class, *Reptilia*Class, *Aves*Class, *Mammalia*Subclass, *Prototheria*Subclass, *Allotheria*<sup>3</sup>Subclass, *Theria*

Further subdivisions of the *Theria* are as follows:

Superorder, *Pantotheria*<sup>3</sup>Superorder, *Metatheria*Superorder, *Eutheria*Order, *Edentata*Order, *Cetacea*Order, *Carnivora*Order, *Ungulata*Order, *Chiroptera*Order, *Insectivora*Order, *Rodentia*Order, *Primates*<sup>3</sup> Extinct.

The names here adopted are mostly those preferred by a majority of the consulted in a recent survey.

prepared for Section F, Am. Assn. Adv. Science, Duke Univ. Press, 1936.)

or diploblastic structure; ectoderm and endoderm separated by a non-cellular, supporting lamella or by a thick gelatinous mass; tentacles about the mouth; stinging capsules, except in ctenophores; usually an attached stage in the life-cycle.

(e) **Phylum Platyhelminthes.**—Free-living representatives, the **planarians**, and the parasitic **flukeworms** and **tapeworms** may have been studied. Understand life-cycles, the degeneration of structures related to free life, and the specialization of structures and functions related to parasitism. Demonstration specimens of the **ribbon-worms**, or **nemerteans**, should be examined. Distinguishing characteristics of the phylum, as shown by its free-living members, are: greater cell specialization than in coelenterates; bilateral symmetry; a single opening to the digestive tract, except in the nemerteans; three germ layers, or triploblastic structure, but no coelom; excretory structures in the form of flame cells and ducts; mostly hermaphroditic.

(f) **Phylum Nematoda.**—**Vinegar eels** and other free-living roundworms of this type, as well as parasitic representatives, such as *Ascaris*, may have been studied. **Trichina worms**, **hookworms**, and **filarial worms** are other examples. Understand life-cycles of the forms examined. The almost universal distribution and the economic importance of the parasitic species are of interest. Free-living species abound in fertile soils (*cf.* Fig. 52, p. 106). Distinguishing characteristics of the phylum are: elongated cylindrical body; bilateral symmetry; digestive tract with mouth and anus well developed; three germ layers; body cavity; dorsal ganglion, nerve ring encircling the pharynx, and ventral nerve cord; sexes separate.

(g) **Phylum Nematomorpha.**—Demonstration specimens of the roundworm called the **horsehair snake**, *Gordius*, and its free-living larval stage should be examined. Understand the life-cycle. The distinguishing characteristics of the worms comprising this small group are so like those of nematodes that the Nematomorpha are often classified with the Nematoda in a single phylum.

(h) **Phylum Acanthocephala.**—Demonstrations of the roundworms called **spiny-headed worms** should be examined (*cf.* Fig. 52 D, p. 106). Understand the life-cycles of representative species. The distinguishing characteristics of the worms comprising this small group are so like those of nematodes that the Acanthocephala are often classified with the Nematoda in a single phylum.

(i) **Phylum Bryozoa.**—Demonstrations of the marine bryozoan, *Bugula*, and of fresh-water genera, such as *Plumatella* and *Pectinatella*, should be examined. Examine also the skeletons of encrusting marine types. These "moss animals" consist of **colonies** composed of



individuals called **zooids**. Some colonies have highly specialized protective members in the form of **avicularia** which are beaklike, or **vibracula** which are whiplike. The zooids feed by means of ciliated tentacles. The fresh-water representatives commonly reproduce by internal buds, called **statoblasts**. Distinguishing characteristics of the phylum are: mouth surrounded by tentacles, digestive tract U-shaped, and with anal opening; three germ layers; coelom within which germ cells are formed; a ganglion between mouth and anus, but no special nervous system or sense organs; skeletal secretion by ectoderm often conspicuous; mostly colonial.

(j) **Phylum Brachiopoda**.—Demonstrations of **brachiopods**, or **lamp-shells**, such as *Lingula* and *Terebratulina*, should be examined. You may be familiar with brachiopod shells as fossils, since these are abundant in many localities. The **shells** are paired dorso-ventrally, not right and left as in a clam, and the animal is attached by a **stalk**. Internally the most conspicuous feature is a feeding organ called the **lophophore** which occupies a large part of the space within the shells. Refer to figures and understand such details of the internal structure as time and material permit. Distinguishing features are: bilateral symmetry as shown by the characteristic dorso-ventral shells and stalk; short digestive tract with mouth and anus; three germ layers; a body cavity; a pair of nephridia; the lophophore.

(k) **Phylum Rotatoria**.—The **rotifers**, or **wheel animalcules**, are forms of microscopic size occurring principally in fresh water. They are often found in cultures and you may have seen many of them in studying Protozoa. Most genera are free-swimming; some are sessile; others live in tubes. Examine material from cultures rich in rotifers. Why were they called "wheel animalcules" by the early naturalists? Are the rotifers you have for study bilaterally symmetrical? Is the body obviously divisible into **head**, **trunk**, and **tail** regions? Can the animal become attached? How does the animal move and feed? A non-cellular **cuticle** is present in all rotifers, and in some genera this cuticle is heavy enough to be called a shell, or test. The **corona**, or ciliated region about the **mouth**, may be lobed or consist of a single circle of cilia. The **digestive tract** includes: a pharynx, containing a "milling" organ called the mastax; a stomach; and an intestine. The **anus** is non-functional in some species. Understand the foregoing parts and the **nervous**, **excretory**, and **reproductive systems**, as they may be identified in the species available. Note species with **degenerate males**; and the life-cycle with respect to the two kinds of **eggs**. Understand the possible significance of the rotifer type of structure in the evolution of invertebrates. Distinguishing

characteristics are: digestive tract with mouth and anus; three germ layers; body cavity ill-defined; excretory tubules with flame cells; no circulatory or respiratory systems; nervous system simple; the ciliated corona, in most species; the mastax; minute size; sexes separate; two kinds of eggs.

(l) **Phylum Annelida.**—The **earthworm** and fresh-water genera, such as *Tubifex*, are representative, although the more typical annelids are forms with lateral appendages on most of the somites and conspicuous sense organs in the head region. In these respects the **clamworm**, *Nereis*, is a better example. Examine charts or textbook figures of marine, tube-building annelids, such as *Serpula*. The **leeches** are greatly modified annelids; the **gephyrean** worms, such as *Phascolosoma*, are so different from other annelids that they are often classified as a small phylum. Examine specimens of leeches and gephyreans and understand their structure with aid of textbook accounts. Distinguishing characteristics of the phylum are: bilateral symmetry; obvious metamerism; three germ layers; an extensive coelom; paired nephridia in most of the somites; closed circulatory system; dorsal brain, circumpharyngeal ring, and ventral nerve cord; well-organized tissues and organ-systems, but very little head development or cephalization. The gephyreans present notable exceptions to these characteristics.

(m) **Phylum Mollusca.**—**Mussels, snails, limpets, chitons, squids, devil-fishes, and cuttle-fishes** may be examined. Understand from textbook accounts, or otherwise, the mode of life in each species thus studied; and note the modification of the molluscan type of structure that occurs in each class of the phylum. Distinguishing characteristics are: bilateral symmetry; no metamerism; three germ layers; a coelom and nephridia; open circulatory system, except in cephalopods; paired ganglia located in regions of greater bodily activity and joined by paired commissures and connectives; forms, such as the cephalopods, with a high degree of cephalization; a dorsal shell; a ventral foot; a mantle enclosing a cavity into which gills extend.

(n) **Phylum Echinodermata.**—Examine **starfishes** and **sea-urchins**, noting the radial symmetry. Compare these animals with a **sea-cucumber** and with figures of **crinoids**, noting the difference in relation of body axes to substratum. How would you homologize the outer surfaces of a sea-urchin, a sea-cucumber, and a starfish? The echinoderms are an aberrant group whose radial symmetry is considered to be superimposed upon a bilateral symmetry. Examine specimens or charts showing bilaterally symmetrical **larval stages**. Distinguishing characteristics of the phylum are: radial symmetry, mask-

ing a more fundamental bilateral symmetry; three germ layers; an extensive coelom which functions in circulation and respiration; a water-vascular system used for locomotion and peculiar to this phylum; an endoskeleton consisting of isolated plates embedded in connective tissue and frequently developed as spines; a relatively simple nervous system and few sense organs.

(o) **Phylum Arthropoda.**—Crayfishes, lobsters, crabs, insects, spiders, and scorpions, may be examined. Compare the principal regions of body, metamerism, appendages, eyes, antennæ, and other external features. In this phylum there is great diversity of structure but considerable advance in cell specialization and division of labor over the annulates. Cephalization is also more conspicuous. The existence of a coelom comparable with that of annelids is problematical; the ancestors of arthropods may have possessed a well-developed cavity of this nature. The circulation is called "open," since there is a heart with outgoing vessels from which the blood enters the open spaces of the tissues and so returns to the heart which it enters through ostia. Other distinguishing characteristics are: a continuous exoskeleton, thinner at the joints; bilateral symmetry; metamerism; typically, a pair of jointed appendages on each somite; compound eyes; three germ layers.

(p) **Phylum Chordata.**—The vertebrates, which are the conspicuous members of this phylum, are sufficiently familiar to be excluded from further description; representatives of the subdivisions of the Vertebrata should be reviewed at this point by means of textbook figures. Inconspicuous members of the phylum which should be examined are: the lancelets, *Branchiostoma* (*Amphioxus*); the acorn worms, *Dolichoglossus* (*Balanoglossus*); and the tunicates, or sea-squirts. Understand the mode of life in each species studied. The lancelet, or amphioxus, bears some external resemblance to simple vertebrates, such as the fishes, in its shape, metameric body, fins, mouth, anus, and other features. Gill slits, notochord, and a dorsal, tubular, central nervous system are present internally. Examine specimens or figures. The acorn worms and the tunicates suggest no such relationships in their adult state. *Dolichoglossus* is a wormlike, burrowing animal having a notochord present only in the proboscis. Examine specimens or figures. Most of the tunicates are adapted for a sessile life and possess inhalant and exhalant siphons that function in feeding and respiration like those of a clam; indeed the tunicates were classified as mollusks until the study of their development showed their larval stages to possess the chordate type of organization. Specimens and particularly figures of tunicates, showing the internal struc-

TABLE 6.—Larger subdivisions of the Animal Kingdom in relation to the phyla.

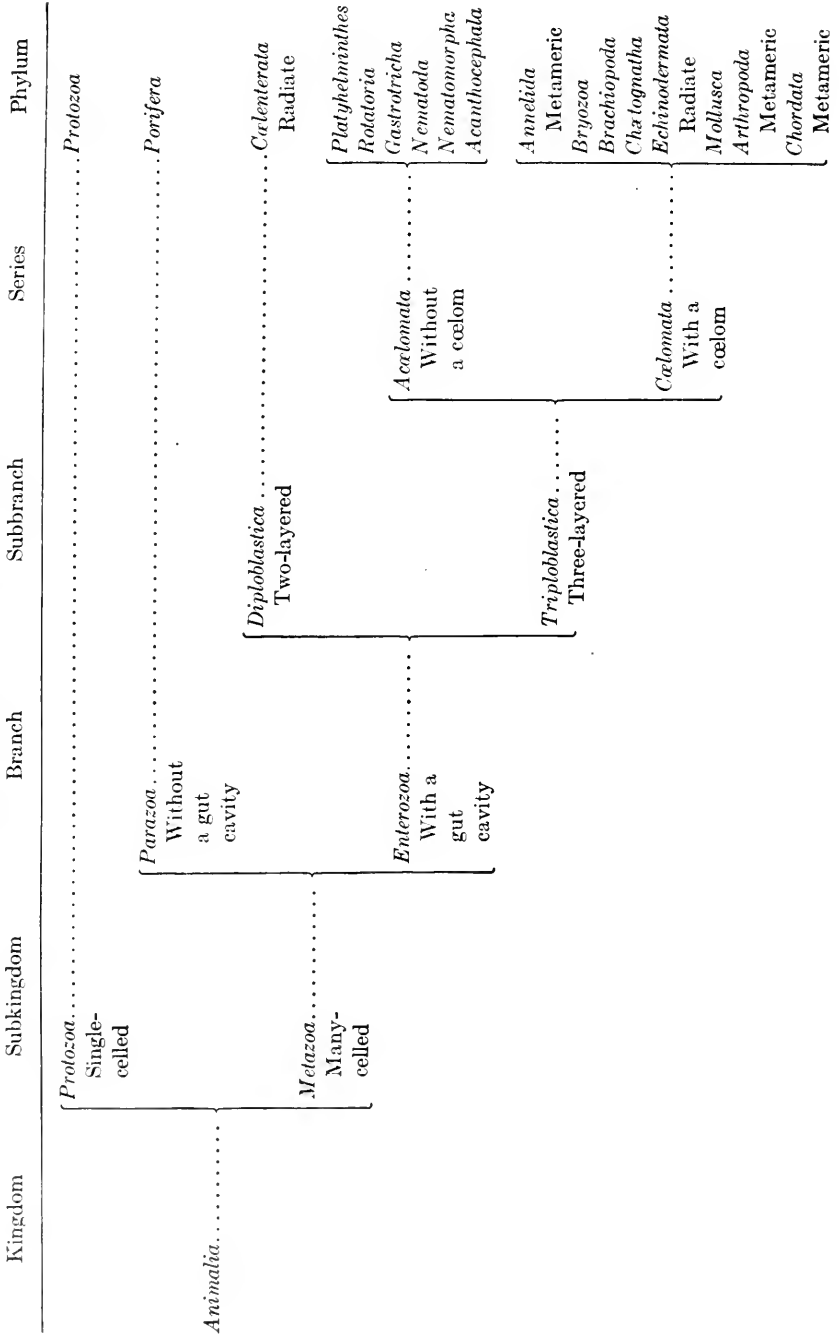


FIG. 75.—Larger subdivisions of the Animal Kingdom in relation to the phyla.

ture and stages in development, should be examined. Distinguishing features are: bilateral symmetry; metamerism; three germ layers; a coelom; a dorsal, tubular, central nervous system formed by infolding, as in the frog embryo; a notochord at some stage in the individual's existence, for example, in the immature stages only (frog) or throughout life (amphioxus); gill slits present in all embryos and in some adults.

(q) **Other Phyla.**—In addition to the types examined in paragraphs (a) to (p) several small groups of uncertain relationships are recognized and sometimes classed as phyla. If group names not included in the foregoing study are noted in Fig. 74, or otherwise, determine the organization of such forms and relate them to one or another of the principal phyla to which they might be appended instead of being classed as separate phyla.

**Exercise 2.**—Grouping of Phyla.

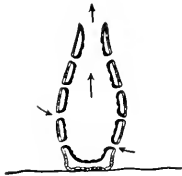
(r) With the foregoing review of representative types from the various phyla as a foundation, you can now examine the characteristics of the phyla with a view to arranging them into larger divisions on the basis of broad structural differences, such as the single-celled or many-celled condition; the presence or absence of a gut cavity; the diploblastic or triploblastic body-plan with its two or three germ layers; the presence or absence of a coelom; and a metameric or non-metameric organization. Examine carefully Figs. 75 and 76. Understand how such an arrangement as that shown in Fig. 75 suggests evolutionary relationships. What do you understand to be the meaning of natural classification? What is the significance of structural similarities among animals?

**Exercise 3.**—The Use of a Key.

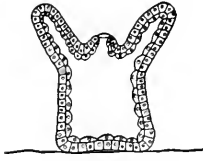
(s) Animals belonging to any phylum are grouped into smaller subdivisions on the basis of structural characteristics. The following key will enable you to understand the method by which the classification of any particular animal within the phylum can be determined. The material indicated is readily obtainable in most localities. For more extensive practice, use should be made of more detailed keys such as may be found in standard textbooks dealing chiefly with classification.

(t) Key to classes of Arthropoda.<sup>1</sup>

<sup>1</sup> From A. S. Pearse and F. G. Hall, "Laboratory Manual for General Zoölogy," University of Wisconsin, 1922.

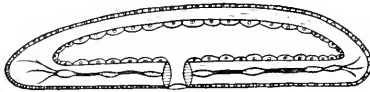


*Parazoa*

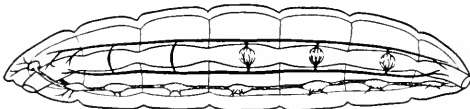


*Enterozoa*

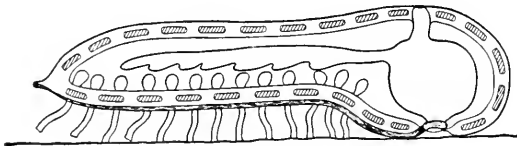
*Cœlenterata*  
*Diploblastic*  
*Radial*



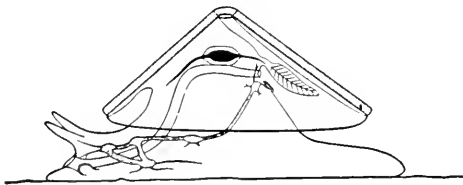
*Platyhelminthes*  
*Triploblastic*  
*Acœlomate*  
*Non-metameric*  
*Bilateral*



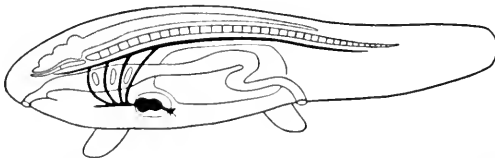
*Annelida*  
*Triploblastic*  
*Cœlomate*  
*Metameric*  
*Bilateral*



*Echinodermata*  
*Triploblastic*  
*Cœlomate*  
*Non-metameric*  
*Radial*



*Mollusca*  
*Triploblastic*  
*Cœlomate*  
*Non-metameric*  
*Bilateral*



*Chordata*  
*Triploblastic*  
*Cœlomate*  
*Metameric*  
*Bilateral*

FIG. 76.—Types of structure in the major phyla of the Animal Kingdom.

- 1 (2) With three pairs of legs, often with wings. . . . . *Insecta*
- 2 (1) Not with three pairs of legs, never with wings. . . . . 3
- 3 (4) With four pairs of legs, no distinct head. . . . . *Arachnida*
- 4 (3) Not with four pairs of legs. . . . . 5
- 5 (6) Legs similar throughout body. . . . . 7
- 6 (5) Legs usually not similar throughout body, usually aquatic. . . . . *Crustacea*
- 7 (8) A distinct head present. . . . . *Myriapoda*
- 8 (7) No head, tropical. . . . . *Onychophora*

(u) The following key is for the adults of the more important orders of aquatic insects. If you are trying to classify an immature insect, use another key designed for that purpose.

- 1 (2) Mouthparts are biting jaws which move sideways . . . . . 3
- 2 (1) Mouthparts form a sucking tube. . . . . 11
- 3 (4) Without wings, very minute. . . . . *Aptera*
- 4 (3) With wings . . . . . 5
- 5 (6) First (outer) pair of wings hard, meet in a straight line down the middle of the back; beetles . . . . . *Coleoptera*
- 6 (5) Wings membranous . . . . . 7
- 7 (8) Abdomen with long filaments protruding from posterior end; may-flies. . . . . *Ephemera*
- 8 (7) Abdomen without long filaments . . . . . 9
- 9 (10) Antennæ short; dragon-flies and damsel-flies. . . . . *Odonata*
- 10 (9) Antennæ very long; caddis-flies. . . . . *Trichoptera*
- 11 (12) Four wings; bugs. . . . . *Hemiptera*
- 12 (11) Two wings; flies, midges, etc. . . . . *Diptera*





## APPENDIX

### I. STAINS AND REAGENTS

#### A. STAINS.

##### 1. Iodine solution for staining flagella of euglenæ and spermatozoa.

Make a very strong solution of iodine crystals in 50% alcohol and test for each case, varying strength as necessary.

##### 2. Methyl green

Distilled water ..... 100 c.c.  
Methyl green ..... 1 gm. or less  
Glacial acetic acid, a few drops.

##### 3. Methyl violet

Distilled water ..... 100.00 c.c.  
Methyl violet ..... 0.05 gm.  
Glacial acetic acid ..... 0.20 c.c.

This dilute stain is better than a stronger one since it does not readily overstain. The acetic acid is essential, and if an old solution fails to stain the nuclei, the addition of a few drops of the acid will commonly restore its staining power.

To get the best results in staining blood, substitute for distilled water 0.7% sodium chloride solution for frog blood, and 0.9% for human blood. This prevents swelling and loss of hemoglobin from the red cells, which happens if more dilute solutions are used.

#### B. REAGENTS.

##### 4. Bela Haller's fluid

Distilled water ..... 50 c.c.  
Glacial acetic acid ..... 25 c.c.  
Glycerin ..... 25 c.c.

##### 5. Benedict's solution

Solution A

Sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ ) ..... 173 gm.  
Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) ..... 100 gm.

Dissolve with heat in 600 c.c. distilled water, filter, and make up to 850 c.c. with distilled water.

## Solution B

Copper sulphate ( $\text{CuSO}_4$ ) ..... 17.3 gm.

Distilled water ..... 100.0 c.c.

Dissolve and make up to 150 c.c. with distilled water.

Add B to A slowly with constant stirring. Keeps well. Gives a greenish-yellow precipitate (cuprous oxide) when heated with a solution of a reducing sugar.

## 6. Fehling's solution

## Solution A

Copper sulphate ( $\text{CuSO}_4$ ) ..... 35 gm.

Distilled water ..... 500 c.c.

## Solution B

Sodium hydroxide ( $\text{NaOH}$ ) ..... 160 gm.

Potassium sodium tartrate ( $\text{KNaC}_4\text{H}_4\text{O}_6$ ) ..... 173 gm.

Distilled water ..... 500 c.c.

Mix equal parts of A and B shortly before using. When heated with an equal amount of a reducing sugar, gives a brick-red precipitate (cuprous oxide).

## 7. Formalin solution (for preserving material)

Dilute stock formalin, which is a saturated solution (40%) of formaldehyde gas in water, to make 5 or 6 parts of this 40% solution in 100 parts of water. This is what is commonly meant by 5 or 6% formalin.

## 8. Iodine solution (for starch test)

Distilled water ..... 100.0 c.c.

Potassium iodide ..... 0.7 gm.

Iodine crystals ..... 1.0 gm.

## 9. Locke's solution (for mammalian tissues)

Distilled water ..... 1000.00 c.c.

Sodium chloride ( $\text{NaCl}$ ) ..... 9.00 gm.

Potassium chloride ( $\text{KCl}$ ) ..... 0.42 gm.

Calcium chloride ( $\text{CaCl}_2$ ) ..... 0.24 gm.

Sodium carbonate ( $\text{NaHCO}_3$ ) ..... 0.20 gm.

Glucose ..... 1.0-2.5 gm.

The glucose need not be added unless the tissue is to be kept alive for several hours.

10. **Ringer's solution** (for frog tissues)
- |  |         |      |
|--|---------|------|
| Distilled water                          | 1000.00 | c.c. |
| Sodium chloride (NaCl)                   | 6.50    | gm.  |
| Potassium chloride (KCl)                 | 0.14    | gm.  |
| Calcium chloride (CaCl <sub>2</sub> )    | 0.12    | gm.  |
| Sodium bicarbonate (NaHCO <sub>3</sub> ) | 0.20    | gm.  |
11. **Salt solution** (isotonic for frog tissues)
- |                        |        |      |
|------------------------|--------|------|
| Distilled water        | 1000.0 | c.c. |
| Sodium chloride (NaCl) | 7.0    | gm.  |
12. **Salt solution** (isotonic for mammalian tissues)
- |                        |        |      |
|------------------------|--------|------|
| Distilled water        | 1000.0 | c.c. |
| Sodium chloride (NaCl) | 9.0    | gm.  |
13. **Starch paste** (Exercise 9, Frog)
- Mix 10 c.c. of corn starch with 50 c.c. cold water.  
Pour into 750 c.c. boiling water and boil two minutes.

## II. SUPPLEMENTARY DIRECTIONS FOR PHYSIOLOGICAL EXPERIMENTS

### A. Gastric Digestion (Exercise 8, Frog)

#### *Equipment for students:*

- 4 test-tubes for each pair of students.
- 5 gummed labels for each pair of students.
- 1 glass jar with cotton for each pair of students.

#### *Equipment for general use:*

- Several pipettes for transferring solutions.
- Test-tube brushes.
- Wash-bottles containing distilled water.
- Fibrin: soak in water and then cut into very small pieces.
- Hydrochloric acid (concentrated).
- Pepsin solution: A very concentrated solution in tap water.  
This solution will not keep.

#### *Directions for students:*

- Students may work in pairs, but each must make the observations independently and write up the work.
- Clean the test-tubes with distilled water.
- Label each tube, telling what it is to contain, as directed in the manual.

Students get materials from center table to fill test-tubes. Be sure that everything is clearly labeled. Insist that students use care and get the right materials.

*Amounts of materials for students to take:*

Distilled water,  $\frac{1}{2}$  test-tube full, or less.

Fibrin, one small piece.

Pepsin,  $\frac{1}{2}$  pipette full (explain how pepsin is prepared).

Hydrochloric acid, 2 drops.

Have students wash test-tubes at end of experiment.

## B. Salivary digestion (Exercise 9, Frog)

*Equipment for students:* (students work singly).

3 test-tubes for each student.

3 gummed labels for each student.

*General equipment:*

Gas burners.

Test-tube racks.

Test-tube brushes.

Matches.

Pipettes.

Starch paste (formula on page 183).

Iodine (page 182).

Benedict's or Fehling's solution (pages 181-182).

*Directions for students:*

To test for starch: Put a drop of the solution to be tested on a clean glass slide and add a drop of iodine. A dark blue color means starch is present.

To test for sugar: Heat 10 c.c. of the solution to be tested in a test-tube with half as much of Benedict's (or Fehling's) solution. A greenish-yellow precipitate (or brick-red) means a reducing sugar is present.

Rinse test-tubes with distilled water.

Label each tube, telling what it is to receive.

Amounts of materials:

Starch solution, 10 c.c., or to a depth of about one inch in bottom of tube.

Saliva, 5 c.c., or to a depth of about half an inch in bottom of tube.

Test each tube first for *starch* and second for *sugar*.

Keep all tests till the experiment is finished.

Have students wash tubes at close of experiment.

### C. Absorption of food (Exercise 10, Frog)

(A demonstration to be made by the teacher.)

The apparatus should be set up and the materials put in about two days before the final demonstration to the students is to be given (*cf.* Fig. 77).

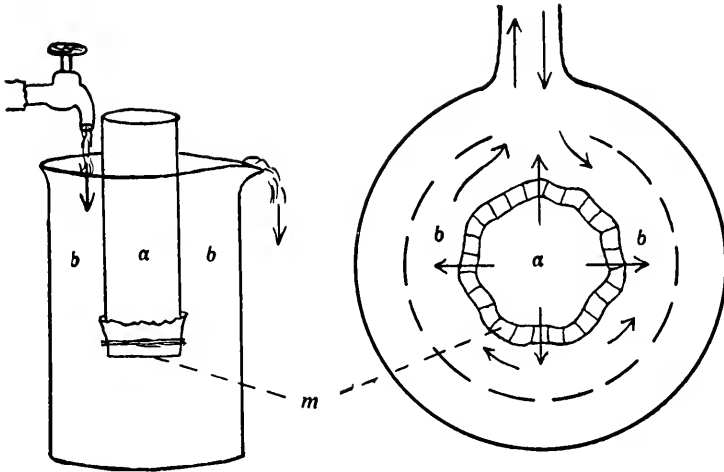


FIG. 77.—Diagram for comparison of the tubes separated by parchment and a cross section of the digestive tract.

#### *Equipment for setting up apparatus:*

- 2 tall glass cylinders (tube B).
- 2 smaller glass tubes (tube A).
- Parchment paper and thread.
- Starch paste.
- Sugar: Dextrose (grape sugar, glucose) should be used.
- Distilled water.

#### *Equipment for demonstrating to students:*

- Apparatus as set up.
- Several test-tubes.
- Test-tube holder.
- Test-tube rack.
- Gas burner and matches.
- Pipettes with long tubes to take material from jars.
- Benedict's or Fehling's solution.
- Starch solution.

#### *Tests:*

- Starch, blue color with iodine.
- Sugar, Benedict's or Fehling's test.

**D. Demonstration of Capillaries** (Exercises 15 and 34, Frog)

The animal may be prepared for demonstration without anæsthesia but such specimens are likely to be troublesome. If anæsthetized use ether, not chloroform, or preferably chloretone. For the latter make a solution of 4 parts Ringer's solution to 1 part 0.5% chloretone in water. Immerse the frog in this solution about 30 minutes or until quiet; do not leave longer than necessary. Then wrap the animal in wet cheese cloth, with care not to bind too closely, leaving one hind foot exposed. Fasten animal to a frog board and spread web of foot. Soft twine can be tied to the ends of toes and fastened to pins to avoid pinning through any part of the foot. Place the web so that observation can be made with the low-power objective. Keep the surface of the foot moist throughout the demonstration; a cover glass with a generous drop of water beneath it can be used.

**E. Functions of the Sensory-neuro-muscular System** (Exercise 21, Frog)*Equipment:*

Frogs, A, B, C, and D. See directions for operation.

The same frog may be used for B, C, and D.

Jar of water for frog to swim in.

Bell-jar to place over frog A.

Bunsen burner.

Ring stand.

String and bent pin or fish-hook.

Scalpel, scissors, forceps, dissecting needles.

Pithing needle.

Dissecting pan.

Watch glass.

Strong acetic acid.

Filter paper.

0.7% salt (NaCl) solution, or Ringer's solution.

*Directions for operation* (see Fig. 78):

Frog A. Normal. No operation.

Frog B. Cerebral hemispheres and diencephalon removed.

Cut through the roof of the skull at *a*, sever the brain, and pith forward. Or for a more precise operation, etherize frog, lay back skin, remove roof of skull, and carefully dissect out

the desired parts. After either operation, give the frog several hours to recover. About three frogs should be operated upon, and one chosen that best illustrates the points involved. The important point is to have the medulla and cerebellum left.

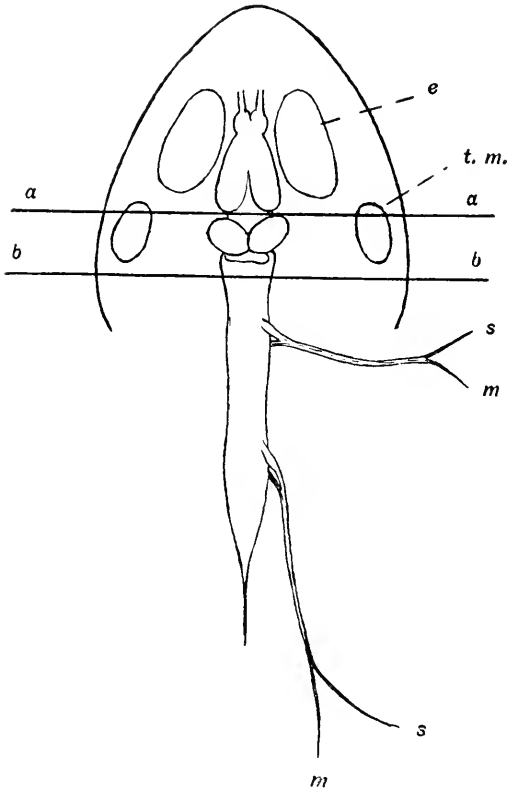


FIG. 78.—Diagram of the central nervous system of the frog, showing levels at which cuts are made in the experiments on the functions of the sensory-neuro-muscular system.

Frog C. Entire brain removed. Frog B may be used. Cut medulla at *b*, just back of the skull, pith forward and about 5 mm. backward.

Frog D. Brain and cord removed. Cut at *b* and destroy cord by pithing.

*Procedure for demonstration:*

The following are points that may be noted about the different frogs. Others might be added, but it seems better to call

attention only to those more striking points which are very definitely demonstrable.

*Frog A. Normal:*

Attitude, breathing, reactions in water (swims and stops), righting when laid on back, vision, reaction to tactile stimuli (response varies with intensity of stimulus), and croaking (particularly when touched behind fore legs).

*Frog B. Hemispheres and diencephalon removed:*

Attitude, breathing, reaction in water (swims until exhausted), righting when laid on back, vision, reaction to tactile stimuli (response varies with intensity of stimulus), and croaking (particularly when touched behind fore legs).

*Frog C. Entire brain removed:*

Attitude, breathing, reactions in water, righting, reaction to tactile stimuli, and croaking. Reflex acts: Hang up by tip of jaw. Touch at various places; pinch toe, first gently, then harder; acetic acid applied with bit of filter paper to ventral surface, side, and back; hold the foot used in attempted removal of acid; note purposive nature of these reflex actions and crossing over to other side of body under certain conditions. Call attention to diagram of cross section of cord and nerves (Fig. 24 A, p. 26).

*Frog D. Entire brain and cord destroyed:*

Make tests as for frog C.

To show that muscle and nerve have not lost power to function, cut across middle of body, remove viscera, and hang up hind legs. Apply chemical, thermal, mechanical, or electrical stimuli to the sciatic nerve. A muscle-nerve preparation can be used here. Refer to cross section of spinal cord and emphasize the central nervous system as a connecting link between the afferent and efferent nerves of the peripheral system.

The heart may be removed from the frog and allowed to beat in warm 0.7% salt (NaCl) solution, or Ringer's solution, as further material from which to discuss the meaning of life and death.

*Record of observations:*

The student should record his observations in the form of a table filling an entire sheet of drawing paper and hand it in as one of the regular laboratory exercises. The statements concerning localization of function, as specified in the exercise, should be written on a separate sheet.



### III. MISCELLANEOUS SUPPLEMENTARY DIRECTIONS

#### A. Maceration of Columnar Epithelium (Exercise 29, Frog)

Cut the anterior two-thirds of the small intestine of a frog recently killed by pithing into pieces 2-3 mm. long. Place in 30% alcohol for 8-12 hours before the meeting of the class. When the student obtains the material he should take a drop of the alcohol.

Another method which can be used to obtain a permanent stock of columnar epithelium is to cut the small intestine of *Necturus* lengthwise, wash it quickly if necessary, and cut into pieces about 1 cm. long. Place in 30% alcohol for 8-12 hours. Check to see if maceration is satisfactory and transfer to 80% alcohol. This should be changed, by decanting, after about 24 hours. The columnar cells can be shaken off and given out for study in drops of the fluid. Students need to be warned about the rapid evaporation.

#### B. The Effect of Ciliary Action (Exercise 30, Frog)

To demonstrate ciliary currents in the roof of the frog's mouth kill the animal by pithing and remove the lower jaw and floor of the mouth. Open the coelom and remove the viscera, leaving as much of the esophagus as possible. With the specimen pinned out in a dissecting pan, sprinkle fine particles of cork or lead filings on the anterior end of the ciliated roof of the mouth. They will be carried posteriorly to the esophagus and emerge through its cut end in the coelom. The epithelium must be kept moist with salt solution.

#### C. Maceration of Non-striated Muscle (Exercise 35, Frog)

Pieces of intestine can be cut about 1 cm. in length and placed in 35% potassium hydroxide (KOH). The length of time must be determined for the thickness of the wall; the best time for the rat is 15 minutes. Pour off the potassium hydroxide and cover the pieces with glacial acetic acid for an hour or more. Wash thoroughly in water and replace with 5% formalin. Shake at once to separate muscle cells, which can then be kept and given out for study by the drop. It is possible to remove most of the mucosa by scraping an opened intestine before the maceration.

**D. Artificial Ovulation (Exercise 45, Frog)**

Ovulation in the frog can be artificially induced late in the autumn and with increasing ease as the winter advances. Better results are obtained if large, mature females are selected for stimulation. Hypophyses should be obtained from large female donors beheaded after pithing. Insert the point of scissors into the foramen magnum, cut forward, and remove the floor of the brain case. The hypophysis will be seen lying against the brain somewhat posterior to the optic chiasma or will be found adhering to the removed bone. Take two glands in about 1 c.c. of water and draw into a hypodermic syringe. Attach a short needle with a bore adequate for passage of the glands and inject them into the posterior coelom of the host female; the viscera are less likely to be damaged if the needle is inserted obliquely. If only a small bore needle is available the glands can be mashed with the end of the syringe to form a coarse suspension which can be injected. Repeat the dose after twenty-four hours. Ovulation will occur on the third day. The eggs can be obtained by "stripping," or gentle pressure with thumb and forefinger downward from the region of the forelegs as the female is held vertically.

When eggs are known to be available a sperm suspension is prepared by teasing two testes from a mature, recently killed male in about 10 c.c. of pond water or an amount which will just cover a single layer of eggs in the dish which is to be used. Water from a pond where frog eggs are known to be laid and normally fertilized is the best medium for the sperm suspension and as a medium for early development. However, other water may by test be found to be entirely satisfactory. The sperm suspension is allowed to stand for about thirty minutes in order that the sperm may become fully active. Then the eggs are stripped into the dish until a single layer covers the bottom. When the eggs have rotated until the pigmented hemispheres are uppermost, which is about thirty minutes after insemination, the dish is filled one-half to two-thirds full of water. At normal room temperature cleavage should occur in two to three hours.

### E. Methods of Culture for *Drosophila* (Exercise 2, Heredity and Variation)

Flies can be reared in half-pint milk bottles plugged with cotton wads wrapped in cheese cloth. Ordinary milk-bottle caps which contain fine perforations can be used instead of cotton plugs but beginners may have more trouble with them. The culture medium is prepared by dissolving 15 gm. of agar-agar in 750 c.c. of hot water. When this is completely dissolved slowly sprinkle in 100 gm. of cornmeal, stirring continually in order to prevent lumping. Allow this mixture to come to a boil and then add 135 gm. of corn syrup (Karo red label is satisfactory). Boil slowly for five to ten minutes. Pour the medium into the milk bottles to a depth of one-half to three-fourths of an inch and immediately drop one end of a strip of paper toweling about an inch and a half wide and three inches long into the food pad; as the mixture solidifies the paper will be held in place and provide a suitable surface for egg-laying. Plug the bottles and sterilize for twenty minutes at fifteen pounds pressure. About twenty-five to thirty culture bottles can be prepared with the amounts stated. So long as the plugs are not removed and the food pad remains moist the bottles can be used successfully.

Selection of mutant stocks for crossing with wild-type flies should be done with the student's experience and equipment in mind. Character differences recognizable with a hand lens and not too much conditioned by the age of the fly are not easy to find. Vestigial wing is one of the easiest to identify; ebony body color is another. Both of these morphological effects are associated with somewhat lowered viability. Sepia and cinnabar eye-color are both readily distinguished from wild type if the flies are not freshly emerged, and viability is relatively good. Among sex-linked characters, which are probably rather confusing to use for a beginning experiment, white eye-color and miniature wing are very easy to classify.

Because most mutant stocks are less viable than wild-type flies perfect ratios are not to be expected. Better results will be obtained if culture conditions are good. Do not use crowded cultures or too scant or dry food; take every precaution against contamination by molds. The times stated

for student guidance are for development at 25° C. At lower temperatures development will be slower; temperatures above 28° C. are very unsatisfactory.

More experience in handling the flies will be gained by the student if flies are drawn off and counted more frequently than specified in the directions. However, unless all flies are removed every twenty-four hours counting should not be continued for longer than nine days because of possible contamination with flies of the next generation.

Virgin females can be obtained by isolating very dark, that is, old pupæ in vials containing a small piece of moist paper. Be sure that no drop of water is in the vial to trap the newly emerged adult. If it is desirable to keep flies obtained from isolations longer than twenty-four hours they can be fed drops of a solution of compressed yeast on the paper in the vial. To obtain virgin females from stock cultures remove all adults. Females that have emerged within ten hours after clearing the stock bottle will be virgin. Breeding stock obtained from culture bottles should be very lightly etherized and full recovery checked before it is issued to students.

Students should be told that the abdominal pigmentation is not strong on freshly emerged adults. This makes identification of sexes difficult. They should also be warned about over-etherization, especially if a wing-length character is being studied.

For etherizing bottles use ordinary wide-mouthed bottles selected to coincide with the mouth size of the culture bottles. Wrap a small amount of cotton in a piece of cheese cloth and fasten it, with a nail or wire, to the inner end of a cork which fits the etherizing bottle. Bottles should be used clean and dry; the ether is applied to the cotton.

One-fourth of a fresh cake of compressed yeast dissolved in about 100 c.c. of water is concentrated enough for the inoculation of culture bottles. Use two drops of this solution for seeding bottles just before introducing the breeding stock.

Six to eight pair matings can be made in running stock cultures. Care must be exercised to keep stocks uncontaminated.

## F. Methods of Quieting Ciliated Protozoa

*Formalin method.*—Add one drop of full-strength formalin to 50 c.c. of water filtered from the culture containing the paramecia. Place in a watch glass a measured amount of water taken from the culture and containing many paramecia. Pour into this an equal amount of the water to which the drop of formalin was added, making a solution of one drop to 100 c.c. The movements of the paramecia will gradually become slower until the animals die without distortion in about ten minutes. One thus sees the shape and movements very easily. The danger with a large class is that the student will continue the study of a single preparation too long, not realizing that the specimens are assuming an abnormal appearance. The method is excellent with a small group under close supervision by the instructor. Hypotrichs may be quieted in a similar manner. Some protozoans, notably *Didinium nasutum*, remain with cilia and general contour intact for many minutes after death. Trial of various dilutions of the formalin mixture should be made, as the optimum seems to differ slightly for different species and in different cultures.

*Gum tragacanth method.*—Solutions of various gelatinous substances may be used for quieting ciliates, the method being to place the animals in a fluid that impedes progress without at first causing distortion. Gelatin, gum arabic, and the jelly obtained by soaking quince seeds in water have been used by investigators. The authors have found none of these to be satisfactory for use with students. Recently, we have used tragacanth as follows: Flake tragacanth, obtainable at drug stores, is ground in a mortar and then placed in cold water to make a thick jelly. Dilute the stock thus obtained and add to water containing paramecia in a watch glass or on a slide. A few trials will show the viscosity most advantageous for impeding movement without immediate injury.

*Vaseline-sealed mounts.*—Spread a line of vaseline around an area corresponding to that of the cover glass that is being used and place a drop of the culture within the pen thus formed. Add cover glass so that a sealed chamber will enclose the water containing the paramecia or other protozoa. Practice is necessary to make such preparations

neatly and without undue smearing of the vaseline. Set the slides aside until the protozoa are anaesthetized by lack of oxygen. The time will vary with size of drop and particular cultures. When finished clean all such slides and covers with a strong soap or other solution.

#### G. To Discharge Trichocysts of *Paramecium*

Add a little fountain pen ink by touching point of the pen to edge of water containing *Paramecium* on a slide with or without cover glass. Various inks have been found effective, particularly Shaeffer's "Permanent Royal Blue" and Parker's "Quink."

#### H. Preservation of Tapeworms

Dogs living on the outskirts of towns or in the country where rabbits abound are frequently found to be heavily infected with *Tænia pisiformis*. Specimens of this parasite and similar tapeworms can be well preserved for class work in the following manner. Remove the worms from the host's intestine and wash quickly in two or three changes of tap-water until thoroughly clean. A large crystallization dish used against a black background is a convenient receptacle. Leave the worms in the clean tapwater for the few moments necessary to kill them but no longer; then lift them one by one and place in another dish containing 5% formalin or somewhat stronger. The worms will be well extended and will not contract to any great extent if left long enough in the tapwater; leaving them too long may result in maceration. By not crowding the specimens and by seeing that they are well laid out the worms can be preserved in an excellent state of extension. After several days when thoroughly hardened they should be transferred to fresh 5% formalin and stored without crowding in receptacles such as glass fruit jars. Worms so preserved can be examined by students in the water of a dissecting pan and used many times if students are cautioned against mutilation. The parts of the scolex show well with a lens or under the low power of a microscope; masses indicating stages in development of the reproductive organs as well as the outlines of the mature uterus can be recognized since formalin specimens, in contrast to those preserved in alcohol, are translucent. Mature proglottids can be cut into pieces and teased, as

directed on p. 102, for study of the six-hooked embryos which will be well preserved. In common with other material preserved in formalin the worms do not stain well if used for permanent mounts or for sections. For this purpose the specimens can be killed with the tapwater as directed and then transferred without delay to a good fixative such as Bouin's solution.











