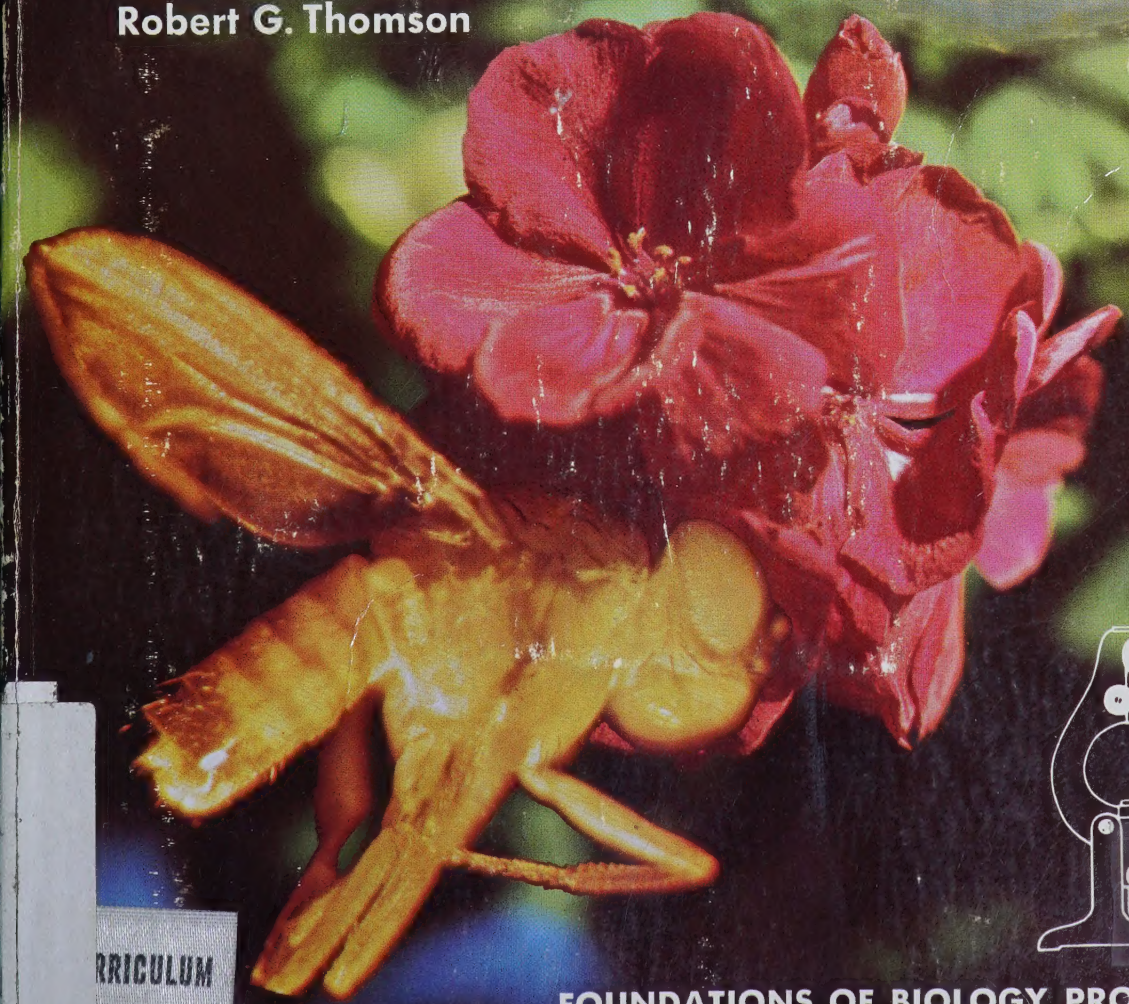
SPECIAL EDITION

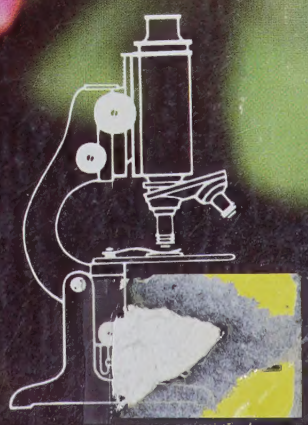
Investigations of Cells and Organisms

A LABORATORY STUDY IN BIOLOGY

Peter Abramoff

Robert G. Thomson





FOUNDATIONS OF BIOLOGY PROGRAM

Investigations of Cells and Organisms

A Laboratory Study in Biology

PRENTICE-HALL

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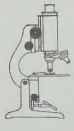


Investigations of Cells and Organisms
A Laboratory Study in Biology
Peter Abramoff and Robert Thomson

ABOUT THIS PROGRAM

A few years ago the series editors of this biology program were involved in the preparation of a paperback series entitled *Foundations of Modern Biology*. The success of that series led us to explore the possibilities of producing a similar series organized on the basis of a slightly different approach and organization. Extensive inquiry among biology teachers indicated that such a program would fill a real need. With that encouragement, we have planned the present FOUNDATIONS OF BIOLOGY PROGRAM.

Realizing that the subject matter and philosophy of biology are an extremely important part of the liberal education of every citizen, we felt that a biology program should be varied, yet pertinent. It should also do the following: 1. convey something of the meaning, scope, and excitement of biological science as a significant perspective from which to view the world; 2. provide an acquaintance with the world of living things, and of the relationships of one organism to others; 3. provide a knowledge of the structure and function of organisms and of populations; and 4. provide a knowledge of man: his history as an organism, his relation to other organisms, his rise to a position of dominance in the biological world, and the ways in which he functions as an animal and as a human being. In general, these are our goals in the four parts comprising this program.

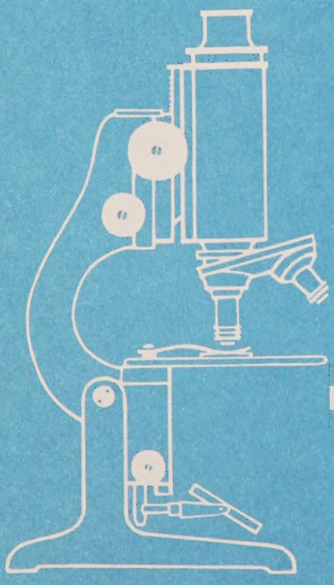
The FOUNDATIONS OF BIOLOGY PROGRAM also includes a separate volume, *Investigations of Cells and Organisms: A Laboratory Study in Biology* by Dr. Peter Abramoff and Dr. Robert Thomson.

All of us—authors and editors alike—are grateful for the excellent advice and constructive criticism so generously offered by the many teachers who helped in the preparation of this program. Their familiarity with the varied needs of students has been extremely valuable to us. Those who have been particularly helpful, and who deserve our particular thanks, are EDWIN M. FIELDS, CAROL L. CROW, VINCENT J. SILLUZIO, ELIZABETH A. SIMENDINGER, IRWIN SPEAR, and R. W. VAN NORMAN.

—The Editors

Investigations of

A Laboratory Study in Biology



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Cells and Organisms

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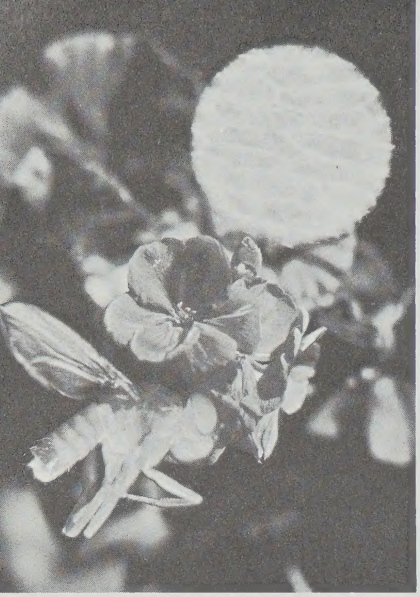

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ABOUT THE COVER

The theme of this laboratory manual is summarized photographically by the multiple exposure appearing on the cover. The study of plants is represented by the geranium in flower, genetics by the fruit fly, which is enlarged about 50X, and cell biology by the group of onion epidermal cells stained in a weak solution of iodine.

FOUNDATIONS OF BIOLOGY PROGRAM
William D. McElroy and Carl P. Swanson, Editors

INVESTIGATIONS OF CELLS AND ORGANISMS
A Laboratory Study in Biology

Peter Abramoff and Robert Thomson

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ABOUT THIS LABORATORY MANUAL

Laboratory work is an essential part of any biology program. The exercises in this book have been designed to give you both experience and understanding of the main principles and concepts of modern biology. Throughout, we have made special efforts to encourage cooperative activities of the imagination, accurate and logical reasoning, and careful observation.

We do not believe that routine memorization of unrelated facts is a sound approach to biology, or to any other science. You will not be asked to set such a task for yourself here. However, you will be asked to come to grips with detailed information when that information is required to document, illustrate, or dramatize a basic biological principle.

The experimental approach we have used in this manual has made it necessary to omit some of the traditional materials and experiments found in other manuals. We have deliberately excluded descriptive material, except when such material is needed to illustrate the principles in question. The routine study of prepared slides, for example, the preparation of highly detailed drawings, dissections, and study of life cycles have not been emphasized.

It is our hope that the approach we have taken in preparing this manual will enlarge your awareness of science as a method of investigating the world around you. It is also our hope that as your awareness grows you will also come to understand the role of science in society.

PETER ABRAMOFF
ROBERT G. THOMSON

Milwaukee, Wisconsin

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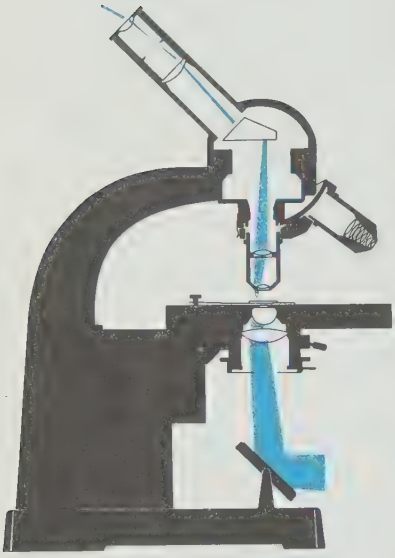
Investigations of Cells and Organisms

A Laboratory Study in Biology

1 THE MICROSCOPE



INTRODUCTION

The microscope is one of the principal tools of the biologist. As you learn to use this instrument you will understand its great importance. Without the microscope the cell theory would not have been developed, and we would lack most of our present knowledge of living things too small to be seen by the unaided eye.

The following exercises are designed to familiarize you with the use and care of the microscope, and to acquaint you with some of the great variety of plant and animal life that you will be able to see with the microscope. There are many types of microscopes in use today with a wide range of magnifications from as low as 2 or 3 to over 100,000 times. In your laboratory work you will be using mainly two kinds of microscopes: the student compound microscope with magnifications in the range of 40 to 450 times, depending on the particular make, and the stereoscopic dissecting microscope with magnifications in the range of 4 to 60 times.

One of the first men—perhaps the first—to study the miniature world of life in a drop of water was the Dutchman, Antony van Leeuwenhoek. Here is his description of what he saw when he first examined a drop of ". . . rain which had stood but a few days in a new tub."

> Of the first sort that I discovered in the said water, I saw, after divers observations, that the bodies consisted of five, six, seven, or eight very clear globules, but without being able to discern any membrane or skin that held these globules together, or in which they were inclosed. When these animalcules bestirred 'emselves, they sometimes stuck out two little horns, which were continually moved, after the fashion of a horse's ears. The part between these little horns was flat, their body else being roundish, save only that it ran somewhat to a point at the hind end; at which pointed end it had a tail, near four

times as long as the whole body, and looking as thick, when viewed through my microscope, as a spider's web. At the end of this tail there was a pellet, of the bigness of one of the globules of the body, and this tail I could not perceive to be used by them for their movements in very clear water.

These little animals were the most wretched creatures that I have ever seen; for when, with the pellet, they did but hit on any particles or little filaments (of which there are many in water, especially if it hath stood some days), they stuck entangled in them; and then pulled their body out into an oval, and did struggle by strongly stretching themselves, to get their tail loose; whereby their whole body then sprang back towards the pellet of the tail, and their tails then coiled up serpent-wise. This motion, of stretching out and pulling together the tail, continued; and I have seen several hundred animalcules, caught fast by one another in a few filaments, lying within the compass of a coarse grain of sand.

SELECTED READINGS

Anderson, M. D. *Through the Microscope.* New York: The Natural History Press. The American Museum of Natural History, 1965.

Benford, J. R. *The Theory of the Microscope.* New York: Bausch and Lomb, 1960.

Eddy, S., and A. C. Hodson. *Taxonomic Keys.* Minneapolis: Burgess Publishing Co., 1958.

Palmer, M. C. *Algae in Water Supplies.* Public Health Service, Publication No. 657, Washington, D.C., 1959.

EXERCISE 1

USE AND CARE OF THE COMPOUND MICROSCOPE

The microscope has undoubtedly been an important tool in a wide range of biological discoveries. It has, more than any other instrument, become a symbol of the activities of biologists.

For many of you, this exercise will be an introduction to a new dimension. Indeed, for some individuals it has stimulated a curiosity that has led to a lifetime of work in biology.

The observations that are possible with a microscope depend on proper use and care of this delicate instrument. The suggestions listed in this exercise should be learned thoroughly by those of you using a microscope for the first time.

PROCEDURE—The Parts of the Microscope

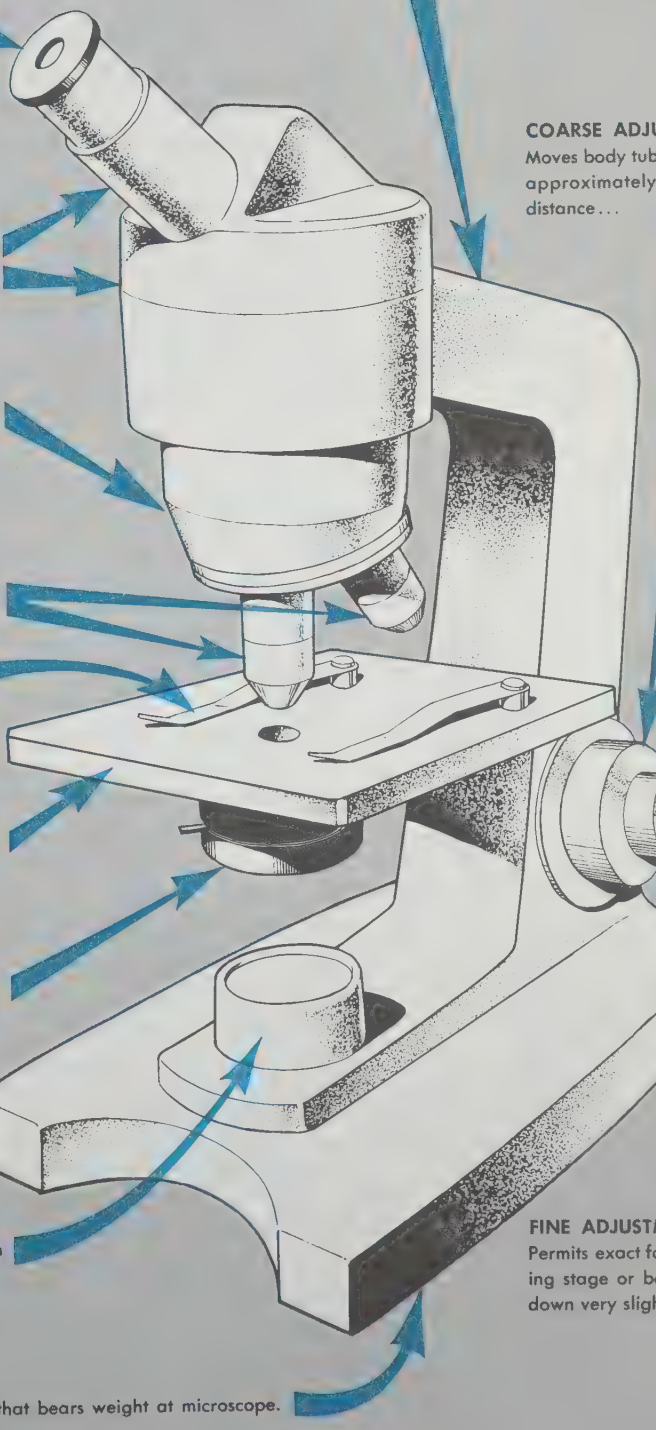
Using Fig. 1.1 as a guide, locate and learn the parts of your microscope. It will be to your advantage to know these parts since they will be referred to at various times during laboratory work. **1-A What is the magnification of: a) the ocular, b) the low power objective, and c) the high power objective? 1-B How do you determine the total magnifying power of a microscope? 1-C What is the total magnification of your microscope: a) when the low power objective is in position, and b) when the high power objective is in position?**

PROCEDURE—Use and Care of the Microscope

- Remove the microscope from its case or cabinet by grasping it firmly with one hand on the arm and the other hand under the base.
- Place the microscope on your table.
- Each time you use the microscope you should follow these preliminary steps:
  - Make sure that the low power objective is in position.
  - Open the diaphragm to its largest opening.
  - Look through the ocular, and by adjusting the angle of the mirror so that it reflects light through the opening in the stage, obtain an evenly and well illuminated field of view. If your microscope has a lamp in place of a mirror, no further adjustments are necessary. It is important to realize that objects are viewed with the compound microscope by transmitted light rather than by reflected light. Accordingly, it is necessary that light from the source travels upward through the specimen to your eye.
  - Develop the habit of cleaning the ocular and objective lenses each time you use the microscope.

CAUTION: Use only lens paper. Never use filter paper, paper toweling, or any other material that may scratch the lenses.

FIG. 1.1 THE PARTS OF A COMPOUND MICROSCOPE



OCULAR
Contains lenses to increase magnification. It may be replaced with another of lower or higher magnification.

ARM
Supports body tube and coarse adjustment.

COARSE ADJUSTMENT
Moves body tube up and down approximately to the correct distance...

BODY TUBE
Holds lenses of ocular and objectives at the proper working distance from each other.

NOSEPIECE
Permits interchange of low- and high-power objectives.

OBJECTIVES
Contain lenses of different magnifications; here the shorter, low-power objective and the longer high-power objective magnifiers.

STAGE CLIPS
Hold slide firmly in place.

STAGE
Supports slide over opening that admits light from mirror or lamp.

DIAPHRAGM
Regulates amount of light passing through the specimen.

LAMP
Directs light upward through diaphragm and hole in stage.

FINE ADJUSTMENT
Permits exact focusing by moving stage or body tube up or down very slightly.

BASE
Firm support that bears weight at microscope.

▶ Place a small section of newspaper containing the letter "h" or "g" on a clean microscope slide. The letter should be in a reading position in the center of the opening in the stage. Using the coarse adjustment turn the low power objective down as far as it will go.

▶ While looking into the ocular, slowly raise the objective by turning the coarse adjustment until the letter comes into focus. It may be necessary to move the slide slightly back or forth, or to the left or right, in order to locate the letter.

▶ It may then be brought into sharper focus by using the fine adjustment. **1-D Is the letter in the same upright position when you view it through the microscope as compared to when you view it with the naked eye? 1-E How does it appear to you when viewed through the microscope?**

▶ Move the slide a slight distance to the left. **1-F In what direction does the letter appear to move? 1-G In what direction does the letter appear to move when the slide is moved toward you?**

▶ Swing the high power objective into position. Do this slowly, making sure that as the objective moves into position it does not hit the stage or the slide. **1-H How does changing from low to high power affect the brightness of the field of view?**

▶ When you use the high power, check to make sure that the diaphragm is wide open. Open and close the diaphragm several times until you locate the position that provides the best light for viewing.

Many microscopes today are **parfocal,** that is, once the image is brought into sharp focus under low power, it will remain in focus when you change to high power. If the image is not in sharp focus when changing from low to high power it may be brought into focus by a slight turn (in either direction) of the fine adjustment. **1-I Why should you never use the coarse adjustment when the high power objective is in position? 1-J How did changing to high power affect the size of the field of view? 1-K Is it larger or smaller than the field of view seen with the low power objective in position? 1-L What is the basis for your answer?**

▶ When your observations are completed turn the low power objective into position and return the microscope.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Why is the image inverted when viewed through a microscope?
2 How could you increase the magnifying power of your microscope?
3 Why can't you examine opaque objects with a compound microscope?

EXERCISE 2

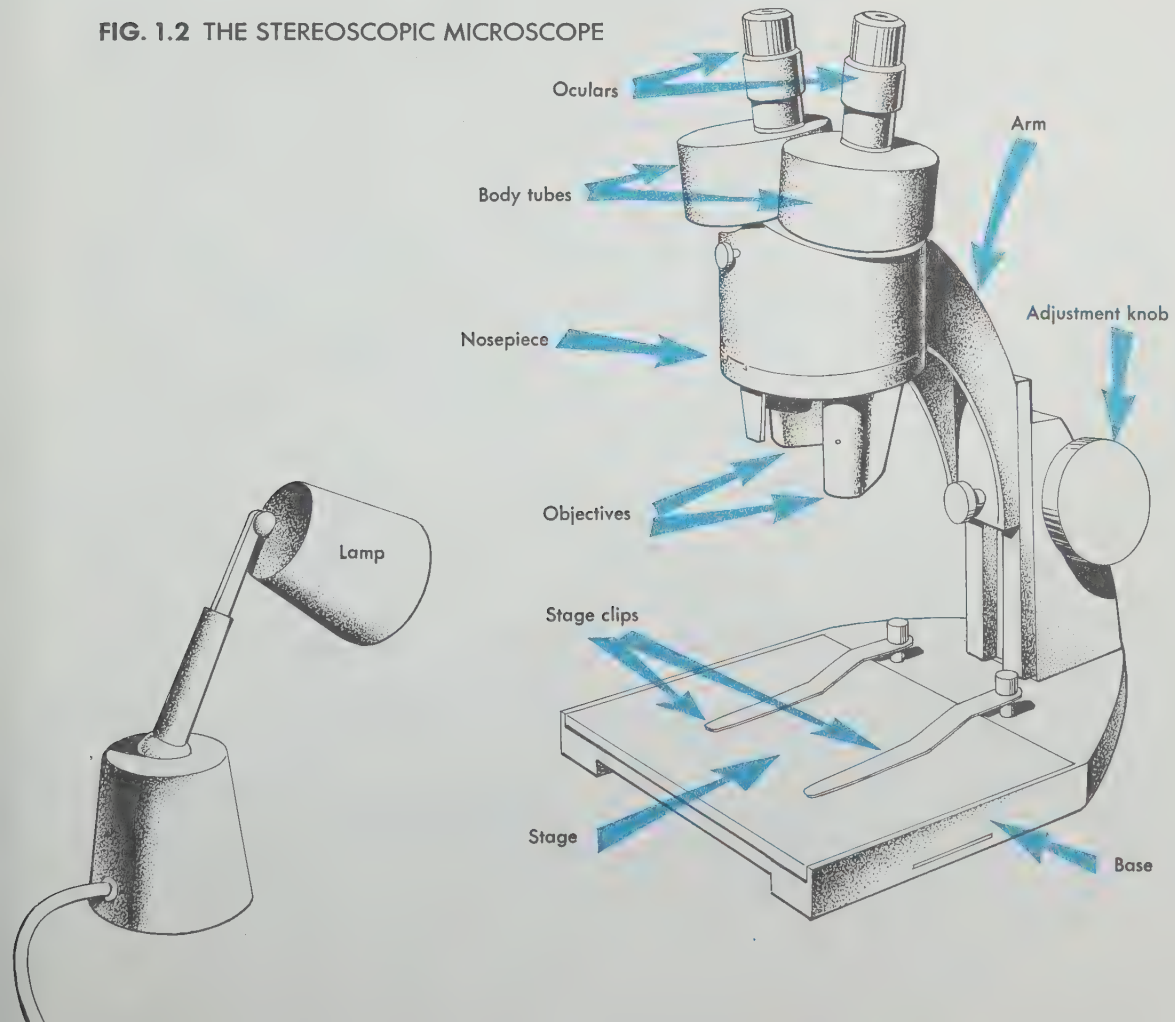
USE OF THE STEREOSCOPIC DISSECTING MICROSCOPE

The stereoscopic dissecting microscope (Fig. 1.2) has two distinct advantages over the compound microscope. 1. It will enable you to observe some objects that are too large or too thick to see with higher magnifications, but too small for the unaided eye, and 2. it will give you an opportunity to observe objects in three dimensions. Magnifications obtained with the stereoscopic microscope commonly range between 4 and 60 depending on the lens combinations used.

PROCEDURE

▶ Remove the microscope from its storage cabinet and place it on the desk. With the stereoscopic microscope you will usually observe the object by reflected light, although some models may have a mirror for observation by transmitted light.

FIG. 1.2 THE STEREOSCOPIC MICROSCOPE



▶ Illuminate the stage of the microscope with a gooseneck lamp or other similar light source.

▶ Start your observations with the lowest magnification of your microscope. **2-A What is the low power magnification of your microscope? The high power? 2-B If there are any intermediate magnifications obtainable, what are they?**

▶ This binocular type of microscope is essentially two microscopes in one; a monocular microscope for each eye. The distance between the two oculars can be adjusted by moving the ocular tubes towards or away from each other. Looking through the microscope, adjust the oculars to fit the distance between your eyes so that each eye views the same even circular field of light.

▶ Place a coin (or other object as directed by your instructor) on the center of the stage.

▶ Turn the objective downward as far as it will go. Looking through the oculars raise the objective until the object comes into focus. It may be necessary to focus separately for each eye. If your microscope has separate focusing devices for each ocular, focus first with one ocular, and then the other. However, some microscopes have one ocular fixed in position. With this type of microscope you will first need to look through the ocular that cannot be individually focused. Then focus for this eye by turning the adjusting knob until the object is sharply outlined. Adjust the ocular for the other eye until the object is in focus for both eyes.

▶ Move the object away from you. **2-C As you look through the microscope which way does the image move? 2-D When the object is moved to the side, which way does the image move? 2-E How does the direction of movement compare with that of the compound microscope?**

▶ Next observe the object at higher magnifications. **2-F As you increase the magnifications how does the area of the field of view change?**

▶ To get some practice with the stereoscopic microscope, observe various objects that are provided by your instructor. Try manipulating the object with dissecting needles while observing it.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 How does the stereoscopic microscope enable you to see in three dimensions?
2 When is it preferable to use a stereoscopic dissecting microscope instead of a compound microscope?
3 Suggest uses of the dissecting microscope in fields other than biology.

EXERCISE 3

HOW CAN MICROSCOPIC OBJECTS BE MEASURED?

It is often important for the professional biologist to know the dimensions of the object he is observing under the microscope. Described below are two methods you can use to obtain estimates of the size of microscopic objects. Method A is simple, but less accurate than Method B; nevertheless, it should be adequate for the beginning student.

Method A will enable you to obtain an approximate estimate of the size of an object by comparing it with the diameter of the field of view. To do this it will first be necessary to measure the size of the field. For this measurement, as well as others we will be making in subsequent laboratory work, the metric system will be used.

PROCEDURE

▶ Place a short, clear plastic rule on the center of the stage so that the scale is visible through the microscope (Fig. 1.3A).

▶ Line up one of the vertical lines so that it is just visible at the left side of the circular field of view (Fig. 1.3B).

▶ The distance from the center of one line to the center of the next line is 1 mm. Count the number of millimeters included from one side of the field to the opposite side. If the right side of the field does not coincide with one of the lines you will have to estimate the fraction of a millimeter involved. **3-A What is the diameter in (millimeters) of the low power field of view of your microscope?** For most microscopic measurements we need a much smaller unit than the millimeter. Scientists use the micron, which is one thousandth of a millimeter (0.001 mm). The symbol for the micron is the Greek letter mu (μ). **3-B What is the diameter of your low power field in microns?**

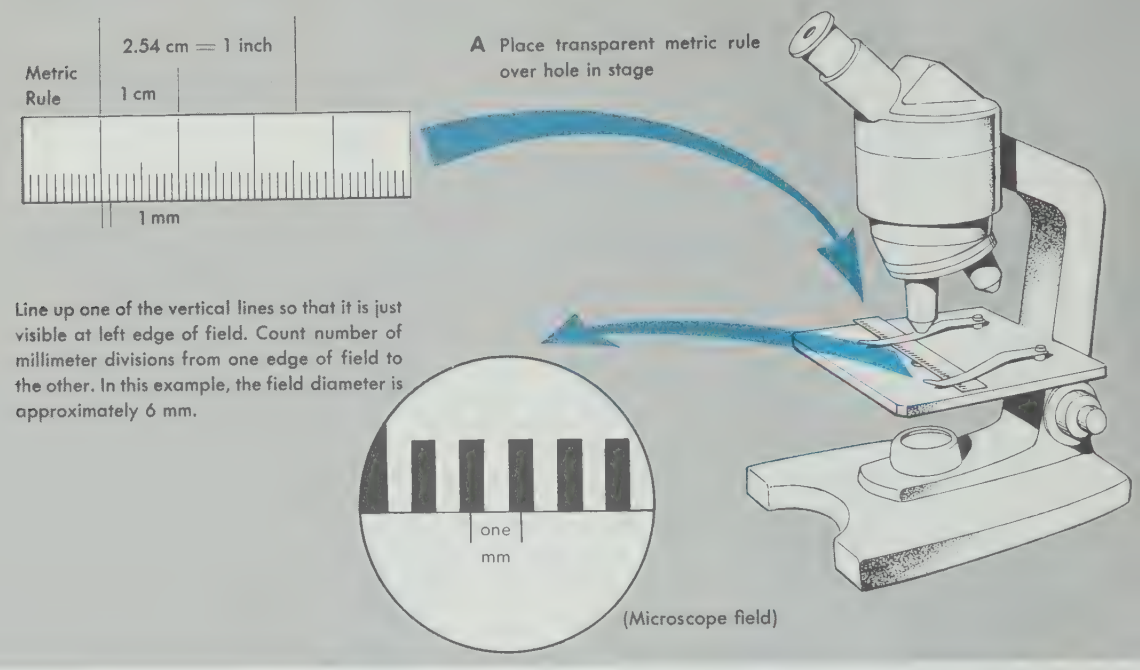
▶ Turn the high power objective into place. You will observe the field of view is less than 1 mm. Instead of measuring this field directly it will be more accurate to obtain the diameter by the following method: divide the magnifying power of the high power objective by the magnifying power of the low objective. Then divide this quotient into the diameter of the low power objective field previously obtained. **3-C What is the diameter of the high power field in millimeters? In microns?**

▶ Your instructor will supply you with various prepared slides of cells or other objects for you to measure. In each case find the length and width of the object in microns. For

The Metric System

The metric system of measurement is convenient because its different units are related on the basis of multiples of ten. The standard metric unit of length is the *meter* (m). The meter is divided into 100 *centimeters* (cm), and 1000 *millimeters* (mm). Fractions and multiples of the meter are designated by the prefaces *deci* (one-tenth), *centi* (one-hundredth), *milli* (one-thousandth), *deca* (ten) and *kilo* (one thousand). In order to relate these units to those of the English system with which you are more familiar, it will be helpful to remember that one inch is equal to 2.54 centimeters, and one meter is equal to 39.37 inches. In the metric system the standard unit of volume is the *liter,* which is the volume of a cube having edges 10 cm long. The standard unit of weight is the *gram* (454 grams are equal to one pound).

FIG. 1.3 METHOD "A" FOR DETERMINING THE SIZE OF A MICROSCOPE FIELD



example, if the object's diameter is about ⅓ that of the low power field, and you have calculated the diameter of the field as 1200μ, then the diameter of the object is approximately 400μ.

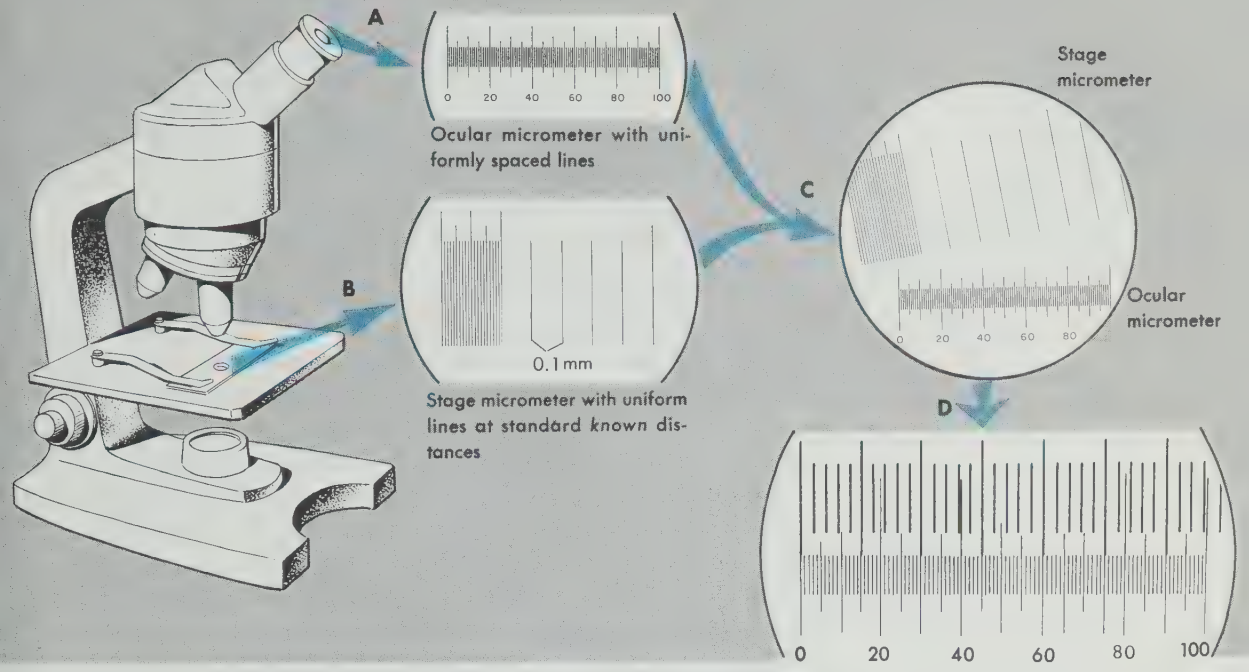
Method B: For more accurate measurements a microscope equipped with an ocular micrometer can be used. This is a small glass disc on which is etched uniformly spaced lines. It is inserted into the eyepiece of the microscope (Fig. 1.4A). When an object is observed with a microscope having an ocular micrometer, the micrometer acts as a rule which is superimposed on the object being measured. However, since microscopes vary in their actual magnification, it is first necessary to calibrate the micrometer for your specific microscope.

PROCEDURE

▶ Obtain a stage micrometer from your instructor; this is a special glass slide with uniform lines etched at standard known distances. When you observe the stage micrometer with your microscope without the ocular micrometer in place, it will appear as in Fig. 1.4B. If you observe the stage micrometer with the ocular micrometer in place it will appear as shown in Fig. 1.4C.

▶ Turn the ocular in the tube until the lines of the ocular micrometer are parallel with those of the stage micrometer. Match the left edge lines of the two micrometers by moving the stage micrometer (Fig. 1.4D).

FIG. 1.4 METHOD "B" FOR DETERMINING THE SIZE OF A MICROSCOPE FIELD



▶ Calculate the actual distance in microns between the lines of the ocular micrometer by observing how many spaces of the stage micrometer are included within a given number of spaces on the ocular rule. Use the following formula: 10 spaces on the ocular micrometer = (X) spaces on the stage micrometer. Since the smallest spaces on the stage micrometer are known to be 0.01 mm apart, then

$$1 \text{ ocular micrometer space} = \frac{(X) \text{ times } 0.01 \text{ mm}}{10}$$

$$= \_\_\_\_\_ \text{ mm or } \_\_\_\_\_ \text{ microns.}$$

▶ With this information you now can use this ocular micrometer in your microscope to measure various microscopic objects available in your laboratory.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 How many microns are there in a meter?
2 Knowing the diameter of the low power field of your microscope, calculate the area of the field. What is the advantage of this information to the biologist?
3 Given that the diameter of the average human red blood cell is 7.5μ, how many such cells can be lined up across the diameter of your microscope as seen under low power?

EXERCISE 4

WHAT FORMS OF LIFE CAN BE FOUND IN A DROP OF POND WATER?

In your laboratory work many of your observations with the microscope will be made on living organisms or on tissues or parts of organisms that you will want to keep alive. To allow them to dry out would greatly distort them, to say nothing of the effect death would have on a study of their movements. For observations of living material you will be making **wet mounts.**

A pond or roadside ditch is an ideal source of living organisms to use for your first observation. A pond is a vibrant, living community of plants and animals, ranging from microscopic forms invisible to the naked eye to the myriad forms of fish, turtles, crustaceans, water plants, and so on. In a community such as this the micro-organisms serve as the food source for the larger animals, which in turn are eaten by still larger forms, establishing what is called a "food web."

PROCEDURE

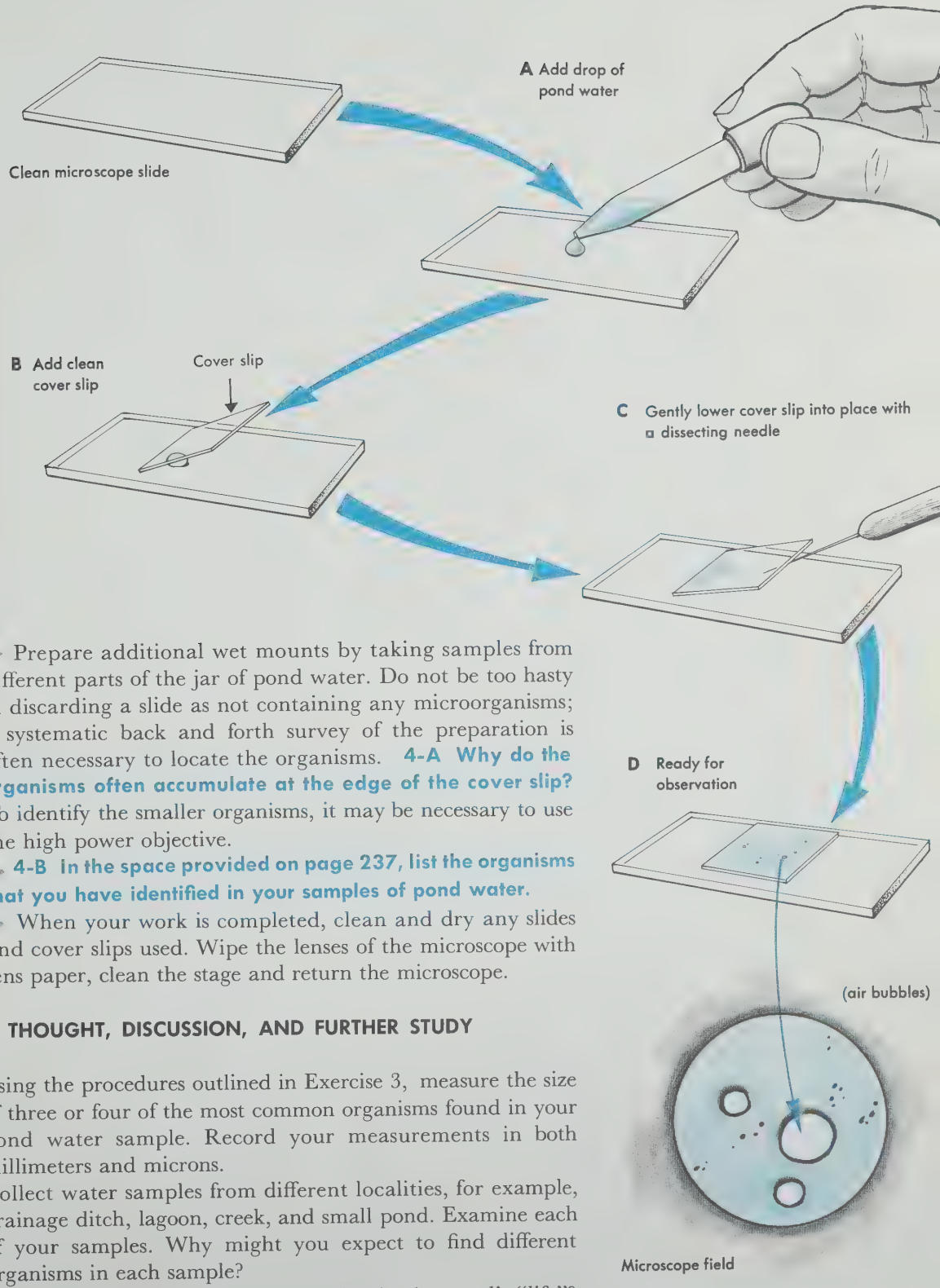
▶ To prepare a wet mount, first obtain a clean microscope slide and a cover slip (Fig. 1.5). The cover slip is very thin so that the objective lens of the microscope can be brought as close as possible to the specimens.

▶ Using an eyedropper, add a drop of the pond water to the center of the slide. The cover slip should be placed on the drop of water in the following way:

- Lower one end of the cover slip so that it touches one side of the drop of water at about a 45° angle.
- After the water has spread across the edge of the cover slip, carefully lower it by supporting the free end with a dissecting needle or the tip of your pencil. If this is done carefully it will prevent the accumulation of air bubbles under the cover slip. Air bubbles, which interfere with good observation, can be distinguished from other objects by their even, round shape and their heavy, dark outline.
- Excess water at the edges of the cover slip can be soaked up by carefully placing a piece of paper toweling to the edge of the cover slip. However, if your preparation begins to dry out while under observation, add one drop of water at the edge of the cover slip.

▶ Under *low power* and with reduced light, make a survey of the drop of pond water. Identify as many of the organisms as you can. Carefully study their differences in structure and their method of movement. Figures 1.6 and 1.7 should help you identify what you see.

FIG. 1.5 PREPARATION OF A WET MOUNT



▶ Prepare additional wet mounts by taking samples from different parts of the jar of pond water. Do not be too hasty in discarding a slide as not containing any microorganisms; a systematic back and forth survey of the preparation is often necessary to locate the organisms. **4-A Why do the organisms often accumulate at the edge of the cover slip?** To identify the smaller organisms, it may be necessary to use the high power objective.

▶ **4-B In the space provided on page 237, list the organisms that you have identified in your samples of pond water.**

▶ When your work is completed, clean and dry any slides and cover slips used. Wipe the lenses of the microscope with lens paper, clean the stage and return the microscope.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Using the procedures outlined in Exercise 3, measure the size of three or four of the most common organisms found in your pond water sample. Record your measurements in both millimeters and microns.

2 Collect water samples from different localities, for example, drainage ditch, lagoon, creek, and small pond. Examine each of your samples. Why might you expect to find different organisms in each sample?

3 What role might green organisms play in the pond's "life"?

FIG. 1.6 SOME REPRESENTATIVE POND WATER PLANTS

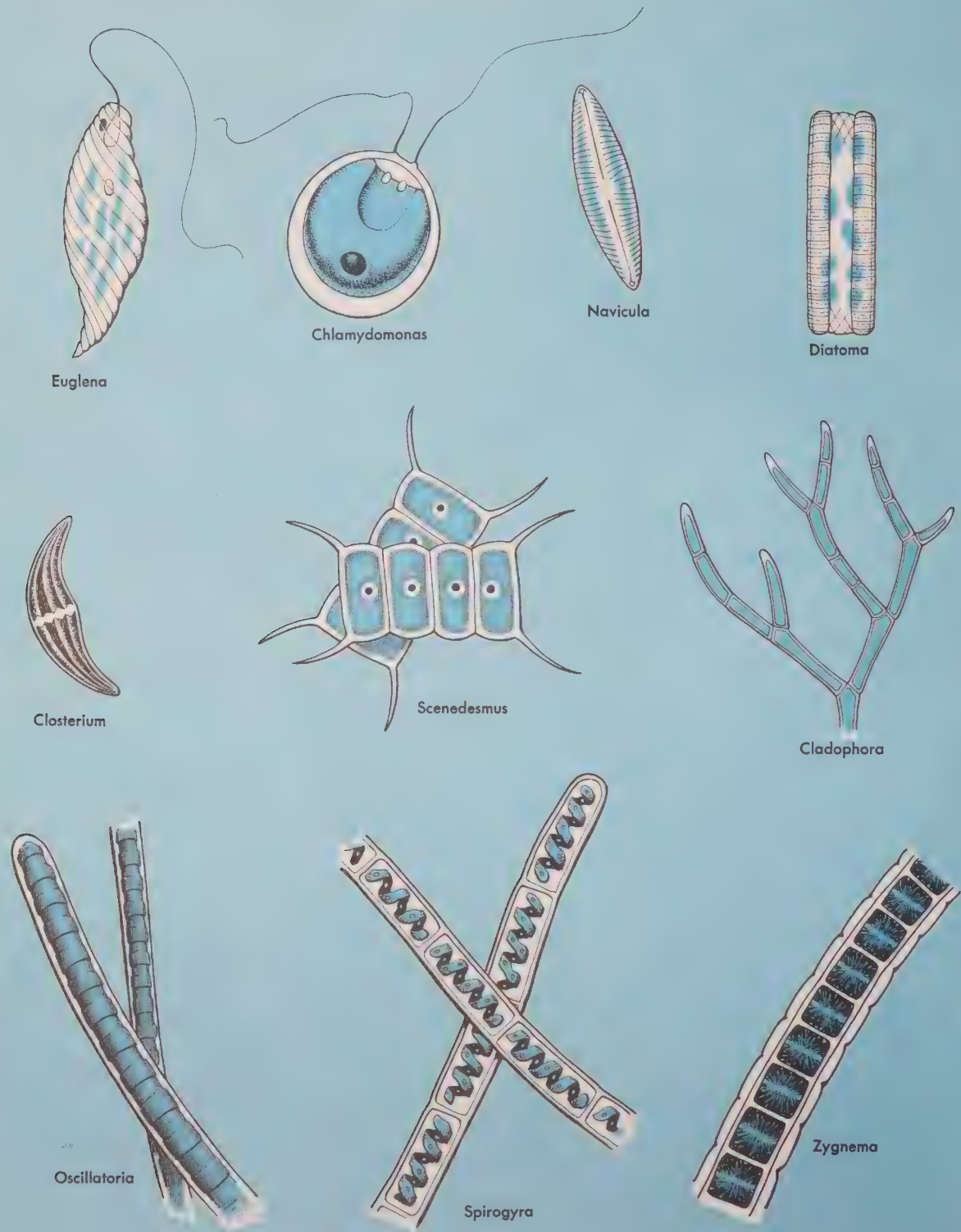
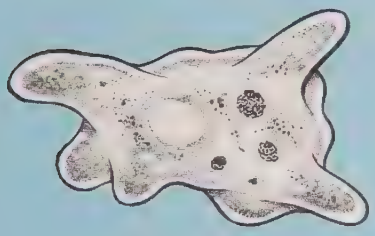
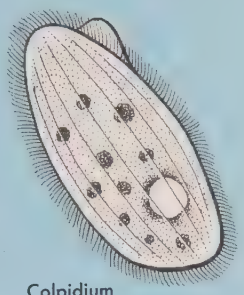


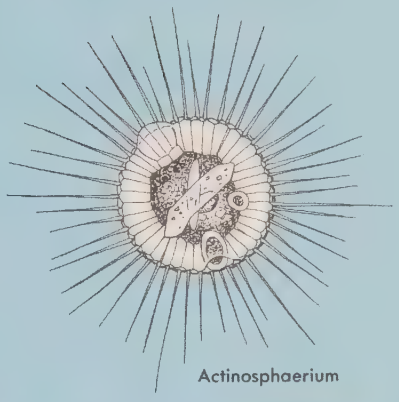
FIG. 1.7 SOME REPRESENTATIVE POND WATER ANIMALS



Ameba



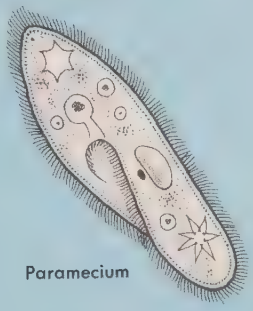
Colpidium



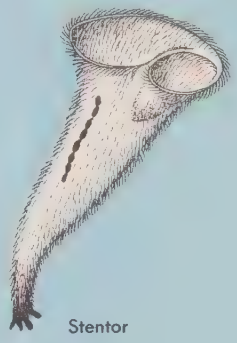
Actinosphaerium

Stylonychia

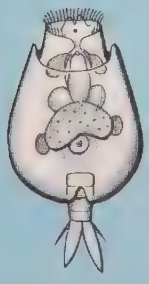
Vorticella



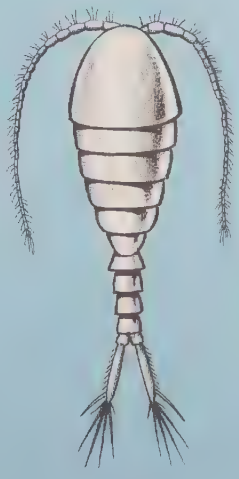
Paramecium



Stentor



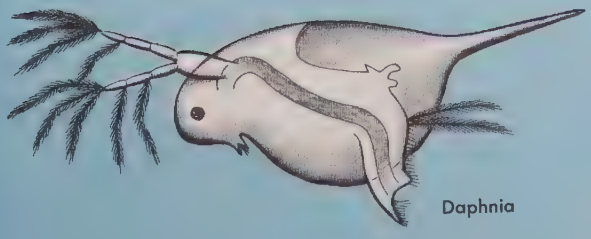
Rotifers



Cyclops

Diaptomus

Copepods



Daphnia

2 CELLS: THEIR STRUCTURE AND FUNCTION

INTRODUCTION

About 300 years ago, Robert Hooke of London, while examining slices of cork with his primitive microscope, saw structures that reminded him of a bee's honeycomb. He called the cavities of the honeycomb "cells." Here is how Hooke described what he saw:

> I took a good clear piece of Cork, and with a Pen-knife sharpened as keen as a Razor, I cut . . . an exceeding thin piece of it, and placing it on a black object Plate, because it was itself a white body, and casting the light on it with a deep plano-convex Glass, I could exceeding plainly perceive it to be all perforated and porous, much like a Honey-comb, but that the pores of it were not regular; yet it was not unlike a Honey-comb in these particulars. First, in that it had a very little solid substance, in comparison of the empty cavity that was contained between. Next, in that these pores, or cells, were not very deep, but consisted of a great many little Boxes, separated out of one continued long pore.

It was not until the early part of the 19th century, however, that it became commonly accepted that all animal and plants were composed of cells. This **cell theory,** first set forth by the German biologists Matthias Schleiden and Theodor Schwann, has served as a "base line" for all subsequent investigations into the nature of the cell.

Just as life is diverse in form, so are the forms and functions of the various cells that make up living organisms. A single cell can be a free-living organism capable of carrying on an independent existence. Such single-celled organisms as the ameba, *Euglena,* or paramecium are examples of this kind of cell. Some cells live as part of a loosely organized colony of cells that move from place to place (such as *Volvox*). Others are immovably fixed as part of the tissues of higher plants and animals and depend on the closely integrated activities of other cells for their exist-

ence. Cells vary greatly in size. A cell, such as the pleuropneumonia microbe, is 1/250,000ths of an inch. On the other hand, the yolk of an ostrich egg is the size of a small orange. Some cells, such as red blood cells transport oxygen and carbon dioxide. Other cells have different specialties. Whatever its form or function, the cell is recognized today as the basic unit of living matter and contains all those properties and processes that we collectively call **life.**

The following exercises will help you to better understand the nature of the cell, both as a free-living independent unit and as a part of an integrated organism.

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EXERCISE 5

CELL STRUCTURE—ONION CELLS

Although cells show great diversity in form and function, all cells are built to a fundamental design and share certain common features. A basic knowledge of *cell structure* is indispensible to the understanding of the cell as an independent unit and in its role in the life processes of higher plants and animals.

PROCEDURE

▶ Cut an onion bulb into quarters (Fig. 2.1). The fleshy "scale leaves" will readily separate from each other.

▶ Hold one of the leaves so that the concave side faces you and snap it backwards. The transparent, paper thin *epidermis* is usually seen as a ragged edge on the broken leaf.

▶ Using a forceps remove a small piece of the epidermis and place it in a drop of water on a slide. Add a cover slip.

▶ Examine this wet mount with the low power objective (10X). Adjust the diaphragm on your microscope to provide the best contrast. **5-A What is the shape of the cells as seen in surface view? 5-B Make a drawing showing one of the cells in three dimensions.** Next examine the cells under high power. The "lines" forming the network between the individual cells are nonliving cell walls composed chiefly of cellulose. The **cell wall** immediately surrounds the **cell membrane** (also called **plasma membrane**) which encloses the cytoplasm. The central part of many plant cells is taken up by a fluid-filled **vacuole** containing mostly water and salt.

Locate the **nucleus,** which appears as a rather dense body in the translucent cytoplasm. In some cells the nucleus seems to be lying in the central part of the cell and will look circular. In other cells, it appears compressed and pushed against the cell wall. **5-C. Explain this apparent discrepancy in the shape and position of the nucleus.** The cytoplasm is separated from the central vacuole, nucleus, and cell wall by membranes, but the membranes are difficult to observe in this preparation.

5-D Draw five or six epidermal cells, each about one inch long. In one of the cells draw and label only those structures that you were able to observe with your microscope.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

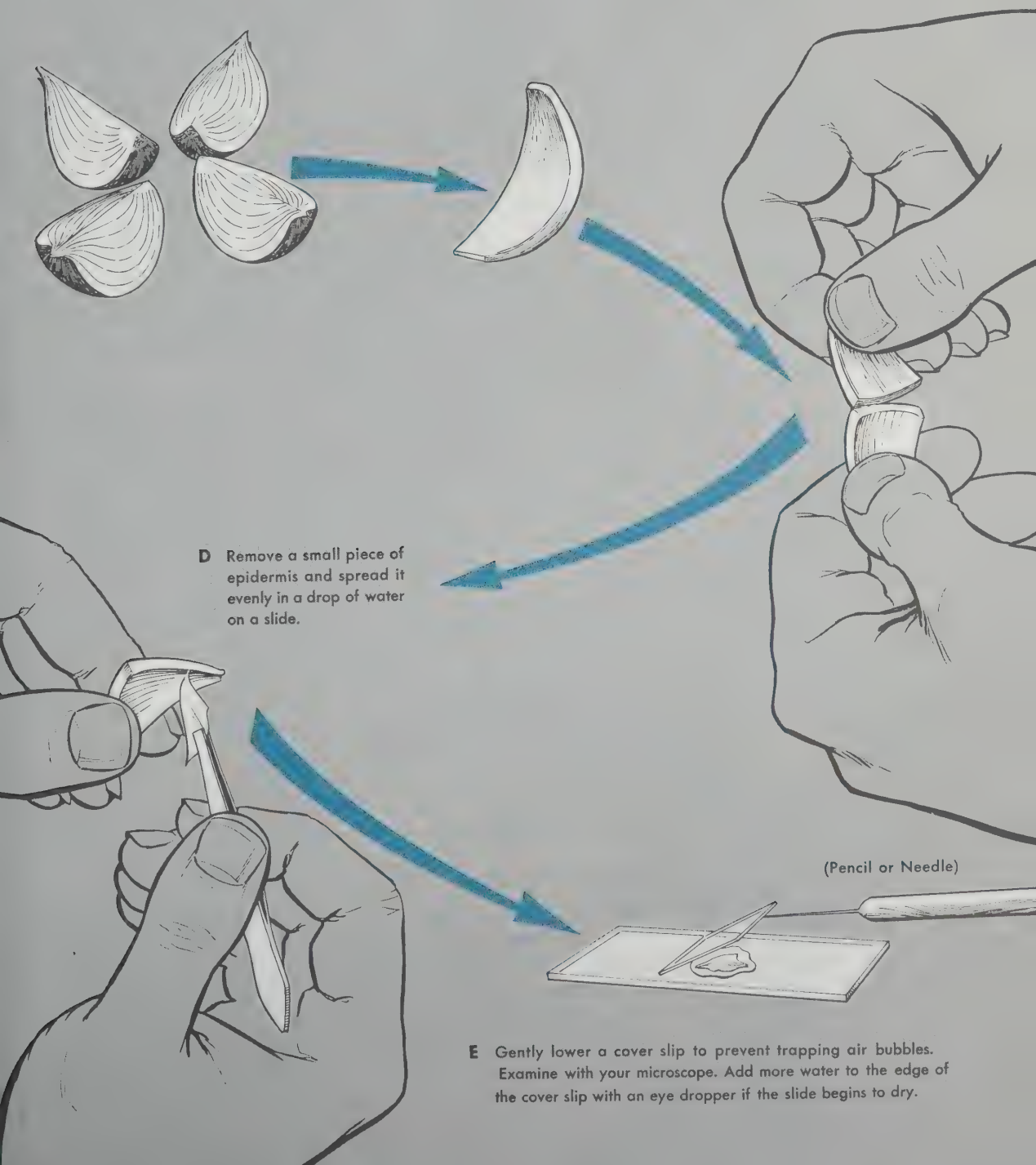
1 What important functions does the plant cell wall perform?
2 Suggest a function for the central vacuole.
3 Some algae and fungi consist of only one cell. Would you consider these to be an exception to the cell theory? Explain.

FIG. 2.1 OBTAINING LIVING PLANT CELLS FROM AN ONION BULB

A Cut an onion bulb into quarters.

B Remove one of the fleshy "scale" leaves.

C Snapping the "leaf" backwards usually provides a ragged piece of epidermis.



D Remove a small piece of epidermis and spread it evenly in a drop of water on a slide.

(Pencil or Needle)

E Gently lower a cover slip to prevent trapping air bubbles. Examine with your microscope. Add more water to the edge of the cover slip with an eye dropper if the slide begins to dry.

EXERCISE 6

CELL STRUCTURE—ELODEA CELLS

In this exercise you will examine cells from the leaf of a water plant commonly called Elodea. These cells, as you will see, are green because they contain a pigment called **chlorophyll.** By a process known as **photosynthesis,** this pigment is able to absorb light energy and convert it into chemical energy. (You will learn more about this important process in later exercises.) In this exercise you will determine where the chlorophyll is located in these cells. In addition, you will determine a relationship that exists between the cell membrane and the outer nonliving cell wall.

PROCEDURE

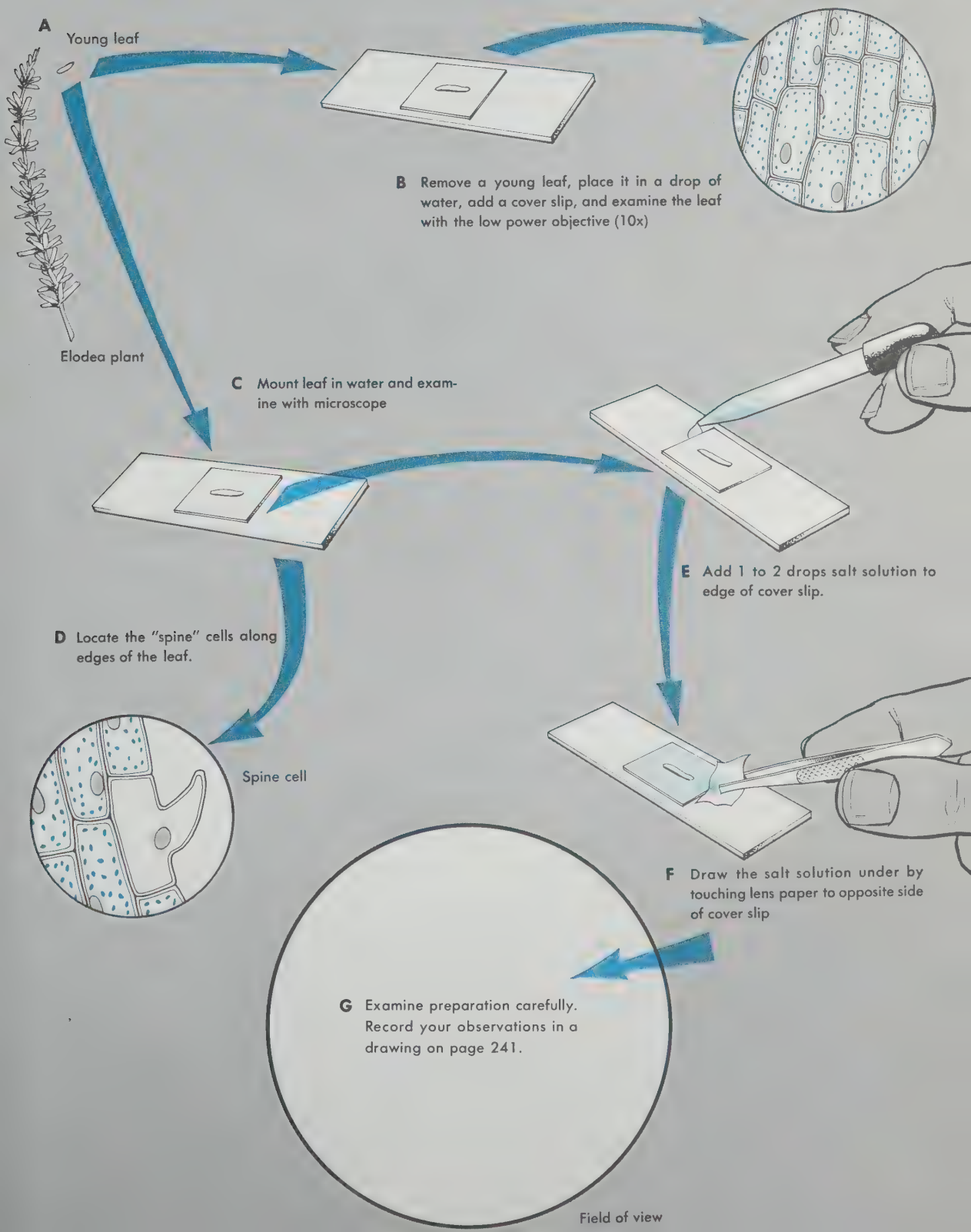
- Remove a young leaf from the tip of an Elodea plant (Fig. 2.2A). Place the entire leaf in a drop of water on a slide and add a cover slip.
- Examine the preparation with the low power objective (10X) in position. Locate the nucleus, cytoplasm, and cell wall. Label these structures in B of Fig. 2.2.
- Examine a group of cells near the center of the leaf. Carefully switch to high power. Note that the green pigment is located in small structures in the cytoplasm. These structures are called **chloroplasts.** As you examine the chloroplasts, you should see them moving in the cell. **6-A Since chloroplasts have no means of moving by themselves, how would you account for the movement observed?**

You are now familiar with the fact that the plant cell is enclosed by a nonliving cellulose cell wall. You also know that the cytoplasm is surrounded by a cell membrane that is difficult to observe because it is pushed very tightly against the inside of the cell wall. You can make this membrane easier to see, however, by placing the cell in a salt solution that is more concentrated than its cytoplasm. The salt solution causes water to move out of the cell. This causes the cell to shrink away from the wall, thus exposing the membrane.

PROCEDURE

- Select another young Elodea leaf, mount it in a drop of water and add a cover slip (Fig. 2.2C).
- Examine the preparation with the low power objective. Along the edges of the leaf locate "spine" cells (Fig. 2.2D). Switch to high power and study the cell. Note that the cell membrane cannot be distinguished from the cell wall.

FIG. 2.2 PREPARATION OF ELODEA CELLS FOR MICROSCOPIC EXAMINATION



▶ Add one or two drops of a concentrated salt solution to one edge of the cover slip. Then touch the liquid on the opposite side of the cover slip with a piece of lens paper (or paper toweling) so that the paper draws up the liquid. This will cause the drops of salt solution to be drawn under the cover glass and replace the liquid withdrawn by the paper (Figs. 2.2E,F).

▶ Repeat the step above two more times to be sure that the original water has been replaced by the salt solution.

▶ Examine the "spine" cell closely. Describe your observation in a drawing on page 241.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 When the cell membrane pulled away from the cell wall, what substance filled the space between the wall and the membrane?
2 The leaves of numerous submerged water plants are frequently two to three cell layers thick. Leaves that grow in air are usually many cell layers thick. Suggest an advantage that each of these leaves has in the environment in which it is found.
3 Lettuce wilts if left out of the refrigerator. It can be made crisp again by placing it in water for a short period of time. Suggest a reason for this.

EXERCISE 7

CELL STRUCTURE—HUMAN EPIDERMAL CELLS

You may have seen that the typical plant cell is characterized by a nonliving cell wall composed chiefly of cellulose, and may contain chloroplasts that function in photosynthesis. In this exercise you will examine some human epidermal cells and will find that although animal cells look very different from plant cells, they have many features in common.

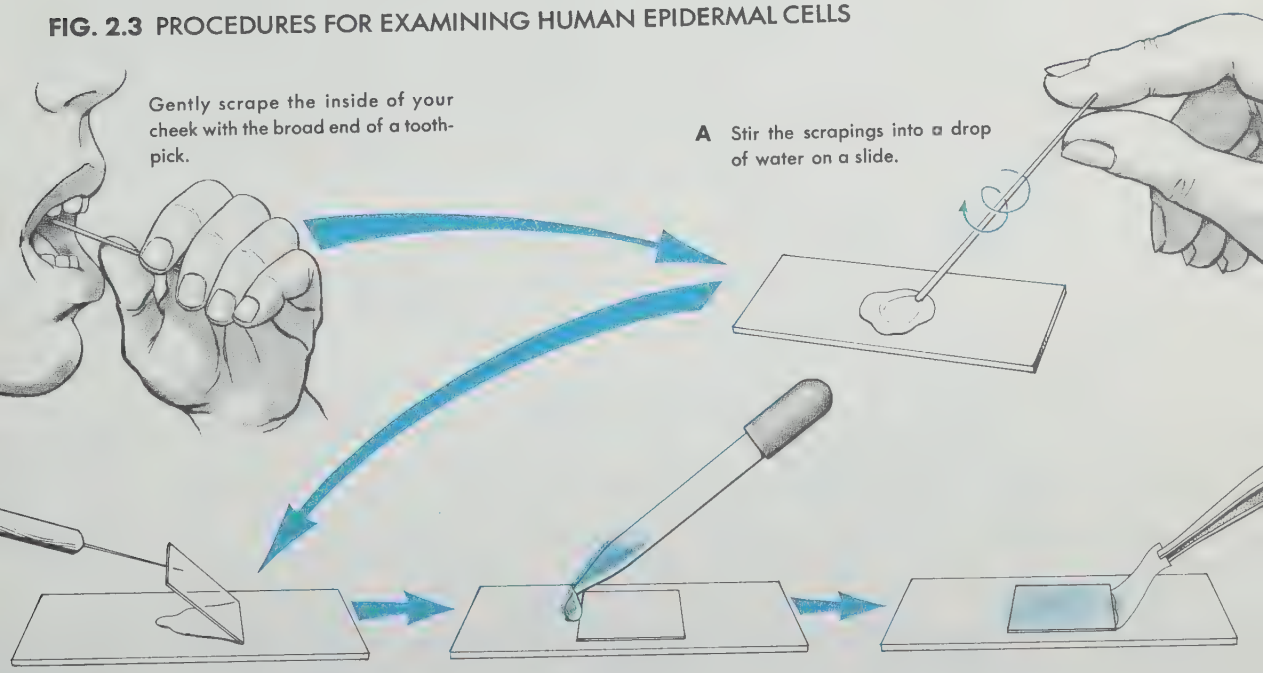
PROCEDURE

▶ Gently scrape the inside of your cheek with a broad end of a toothpick.

▶ Add a drop of water to a clean slide and then spread the scrapings on the toothpick in the water. Discard the toothpick. Add a cover slip and examine with the low power of your microscope (Figs. 2.3A,B). The cells will appear as small clumps of transparent, granular material.

NOTE: It will probably be necessary to close the diaphragm to cut down the light and provide some contrast.

FIG. 2.3 PROCEDURES FOR EXAMINING HUMAN EPIDERMAL CELLS



B Add a cover slip and examine with your microscope.

C To observe more cellular detail, add a drop of methylene blue or other stain to the edge of the cover slip.

D Draw the stain under the cover slip by touching a piece of lens paper to the opposite edge of the cover slip.

▶ Locate the cells under high power and examine them carefully. **7-A What do these epidermal cells have in common with the onion and Elodea cells? 7-B In what ways are they different?**

▶ Since it is difficult to observe the structure of living cells, they are frequently stained with dyes to bring out cellular detail. Add a drop of methylene blue to the edge of the cover slip and draw it under as shown in Fig. 2.3C,D. **7-C What cell structure has been stained by this dye?**

▶ If time permits, make new preparations of your cheek cells and treat them with other stains provided by your instructor.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Some of the epidermal cells may have had their edges folded over. What does this indicate about the thickness of the cells?
2 What is meant by "cell specialization?" Name some specialized cells in plants and animals.
3 What is a tissue? How are tissues related to organs?
4 What keeps plant and animal cells from separating from each other?
5 The mature human red blood cell does not have a nucleus. Is it then proper to call it a cell? Explain.

EXERCISE 8

THE CELL AS AN INDEPENDENT ORGANISM

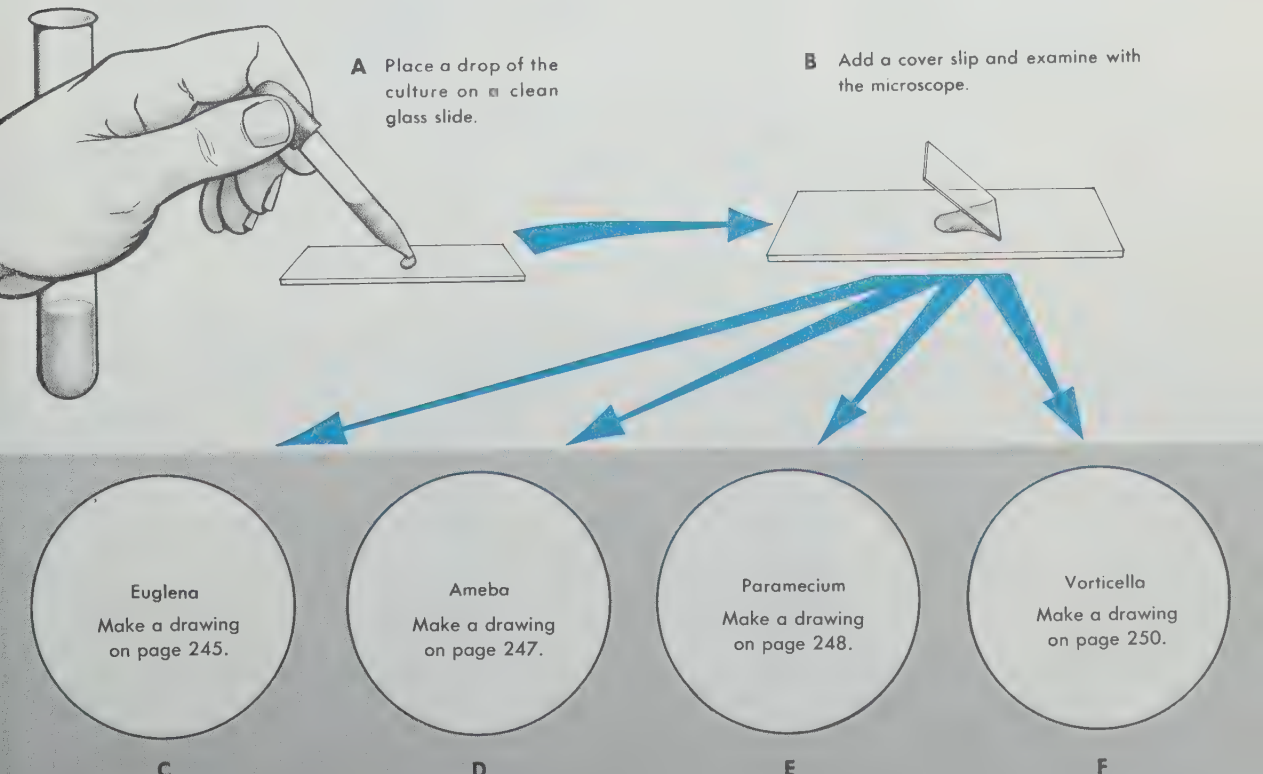
The cells of multicellular organisms are **differentiated,** that is, they are specialized to carry out certain specific activities. For example, red blood cells transport gases, kidney cells excrete waste products, and nerve cells conduct stimuli. These differentiated cells are organized into tissues and organs. The cells of multicellular organisms are integrated, interdependent parts of the organism.

This multicellular condition is, however, but one type of cellular organization. A second type is **unicellularity,** a condition in which the whole organism consists of a single cell. The cell in this condition is capable of living as an independent unit and displays all the activities of "life" found in multicellular organisms. The ameba is an example of such a single-celled organism.

PROCEDURE

Prepare wet mounts of the following free-living cells provided by your instructor (see Fig. 2.4). The names of these organisms are given for identification purposes only. It is not important that you make any effort to memorize them. As each organism is studied, sketch it in the workbook section.

FIG. 2.4 A STUDY OF SOME SINGLE CELLED ORGANISMS



▶ **Euglena.** **8-A What is the shape of this organism? 8-B How can you determine which is the anterior (front) end of *Euglena*? 8-C Describe any changes in shape which may occur as you observe this organism. 8-D How does *Euglena* obtain its food? Explain.** Although it is a relatively simple organism, *Euglena* can "see." Near the anterior end you should be able to observe a small reddish **eye spot** that is apparently sensitive to light. **8-E How would such an "eye" be of help to *Euglena*?**

▶ **The Ameba.** This one-celled organism may be difficult to find unless the diaphragm is adjusted to cut down on the amount of light entering the microscope. After you have located an ameba, watch it closely for a few minutes. **8-F Describe the shape of this cell.** Since an ameba does not contain chlorophyll, as *Euglena* does, it obtains food by another method. **8-G Based upon your observations, suggest a way in which this organism feeds.** In fresh water ameba, the cytoplasm contains a higher salt concentration than the fluid in which the ameba lives. Because of this difference, water tends to enter the cell. Unless water is removed from the cell periodically, the ameba will soon burst. A unique structure, the **contractile vacuole** performs the function of "pumping out" excess water. Observe the surface of the ameba closely and locate a contractile vacuole. **8-H How frequently does this organelle empty?**

NOTE: To observe movement in *Paramecium*, reduce the amount of light.

NOTE: To slow this movement of *Paramecium* add a drop of methyl cellulose before adding the cover slip.

▶ **Paramecium.** **8-I Describe the shape of this organism. 8-J What is the most obvious difference between *Paramecium* and *Euglena*? 8-K Suggest a way that this cell obtains its food. 8-L What is the mechanism for movement in this organism?**

▶ **Vorticella.** **8-M Describe the shape of this organism. 8-N In what ways is *Vorticella* different from the other organisms studied? 8-O Suggest how this organism obtains its food.** While observing this highly specialized cell through the microscope, gently tap the slide with the tip of your pencil. **8-P Describe the response of the organism.**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Considering the organisms you have observed as single cells, would you correctly refer to them as "simple?" Give reasons.
2 Design an experiment to demonstrate that the eye spot of *Euglena* is indeed light-sensitive.
3 Would you expect amebas that live in the ocean to have contractile vacuoles? Explain.
4 What environmental factors (for example, temperature) might affect the rate of contraction of the contractile vacuole? Design an experiment to measure the effects of one factor.

EXERCISE 9

THE INTER-RELATIONSHIP OF CELLS

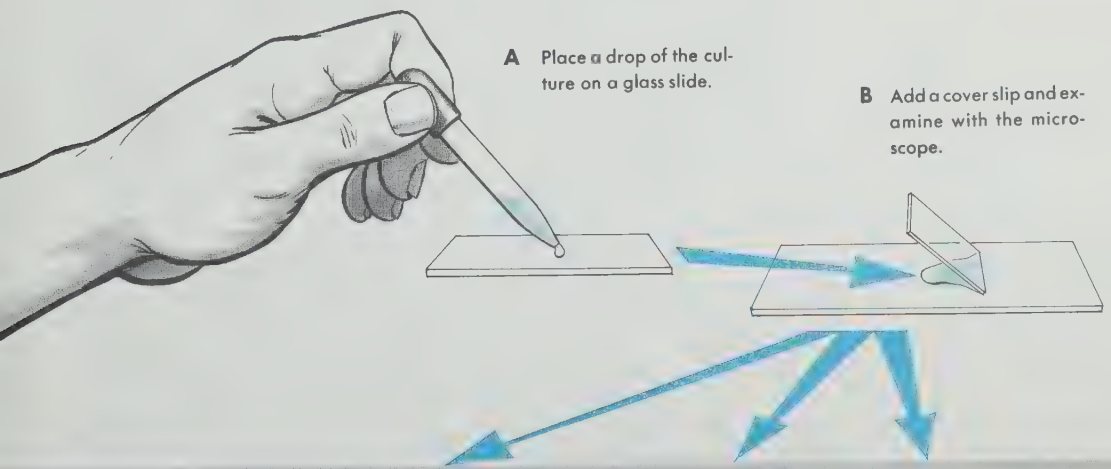
In the previous exercise you saw a few examples of cells that are self-sufficient organisms capable of carrying on an independent existence. A second path of development by cells has resulted in a loosely organized community of cells. In this exercise you will examine some "simple" multicellular organisms in which a moderate amount of cell specialization may be seen.

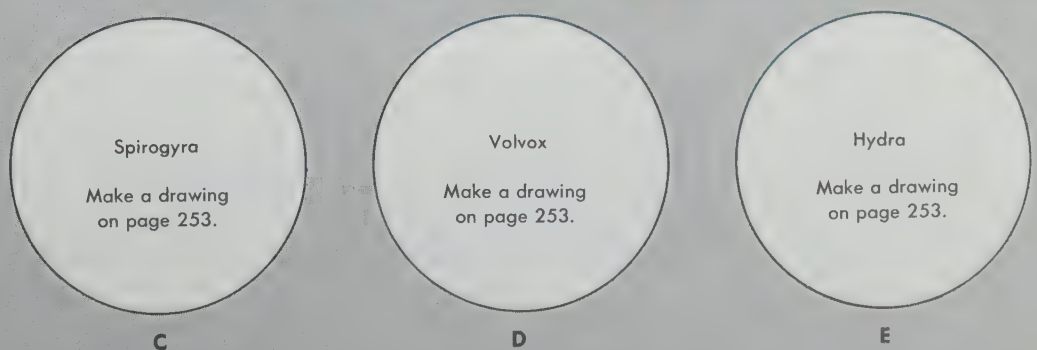
PROCEDURE

Prepare "wet mounts" of the following organisms (Fig. 2.5). **9-A After examining each specimen, sketch it in the space provided on page 253.**

▶ **Spirogyra.** One of the simpler associations of cells will be seen in this organism. **9-B In what way, if any, are individual cells of this colony different from each other? 9-C What is the shape of the chloroplast? 9-D Why do you think this organism was named *Spirogyra*?**

FIG. 2.5 A STUDY OF SOME MULTICELLULAR ORGANISMS





This organism stores part of its food in the form of starch which is associated with special structures in the cell. Add a drop of iodine to the edge of the cover slip. Wait a few minutes and then examine the cell again. In the presence of iodine, starch turns a deep blue black. **9-E Where in the cells of *Spirogyra* is starch stored?**

▶ **Volvox.** In *Volvox,* hundreds of individual cells are united within a single jelly envelope. Unlike *Spirogyra,* which is a very superficial union of cells that do not have any close relationship with each other, *Volvox* presents a case where a **division of labor** is becoming evident among cells. For example, certain cells at one end of the colony have larger eye spots than cells at the other end. These cells are responsible for "steering" the colony to brightly illuminated parts of the pond. **9-F Why is this desirable?** Certain other cells of the colony function as sex cells—gametes; some become eggs and others become sperm. Look at the colony closely. **9-G What indication is there that fertilization has occurred and resulted in the development of new colonies?**

NOTE: *Volvox* is best examined if you use low power and no cover slip.

▶ **Hydra.** From the modest beginnings observed in *Volvox,* a more extensive division of labor among cells will be seen in the hydra. Place a hydra in a watch glass (or Syracuse dish) containing pond water. Examine with a dissecting microscope. **9-H Why do you think the person who first discovered this organism named it *Hydra*? 9-I What response is seen when you touch this multicellular organism with a toothpick? 9-J What characteristic of "life" does *Hydra* thus exhibit?** Add several washed brine shrimp close to the hydra. Watch closely for several minutes. **9-K Describe what occurs when the shrimp comes close to a hydra.**

Next, examine *Hydra* with the low power (10X) objective of your compound microscope. Note the wart-like protrusions over the entire body. **9-L Where are they most numerous?** These are stinging cells that contain a long, very fine coiled thread. Adjust the diaphragm to reduce the light. While observing *Hydra*—and specifically the stinging cells—add a drop or two of iodine (or vinegar) near the organism. **9-M Describe what happens to the stinging cells. 9-N Based upon your observations of *Hydra,* how does this organism obtain its food?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Which of the organisms that you observed in this exercise would you call animals? What basis did you use for your decision?
2 Under what circumstances is a cell called an organism?

EXERCISE 10

HOW DO CELLS REPRODUCE?

In order for an organism to grow and survive, the cells of which it is composed must be able to reproduce. Cells reproduce by the process of **cell division.** In a large majority of organisms cell division involves two stages. In the first—called **mitosis,** or sometimes **karyokinesis**—the nucleus undergoes a series of changes with the end result that the nucleus divides into two. This is followed (but not always) by a division of the cytoplasm (**cytokinesis**) in which the cell divides so that each new **daughter cell** receives one nucleus. The entire process of cell division is one of constant, integrated change, with one stage leading into another. It is best studied in tissues that have been killed and stained so that cell structure may be studied.

PROCEDURE: Mitosis and Cell Division in Plant Cells

▶ Obtain a prepared slide of the onion root tip. Hold the slide slightly above a sheet of white paper. The series of dark streaks that can be seen are very thin sections cut lengthwise through a root tip (Fig. 2.6).

▶ Examine one of the root tips with the low power objective (10X) of your microscope. Locate the rounded end where most of the dividing cells will be found (Fig. 2.6A). For detailed examination, high power must be used.

▶ Many of the cells in your preparation will be in a so called "resting stage" between divisions. These cells will show the characteristic features of the typical plant cell you studied in the previous exercises. Locate these cells on your slide (Fig. 2.6B). **10-A Why is "resting stage" a poor term to describe these cells?**

NOTE: Since each section is very thin, not all of them will be equally good for studying cell division. Be prepared to examine other sections on the slide, or even change slides in order to locate and study the series of events described here.

▶ In the early stages of mitosis, the membrane surrounding the nucleus breaks down and the **chromosomes** (thread-like structures in the nucleus that carry hereditary information) become visible and randomly distributed throughout the cytoplasm (Fig. 2.6C). At first the chromosomes are elongated, but later become shortened. Although not readily seen by inexperienced eyes, each chromosome has duplicated itself. **10-B Why would you expect duplication of each chromosome to occur in mitosis?**

▶ The chromosomes soon lose their random orientation and arrange themselves near the center of the cell (Fig. 2.6D). Very thin strands (called **spindle fibers**) may be seen extending from the chromosomes to opposite ends of the cell.

▶ Shortly following the lining up of the chromosomes in the center of the cell, the previously duplicated chromosomes

FIG. 2.6 MITOSIS AND CELL DIVISION IN PLANT CELLS

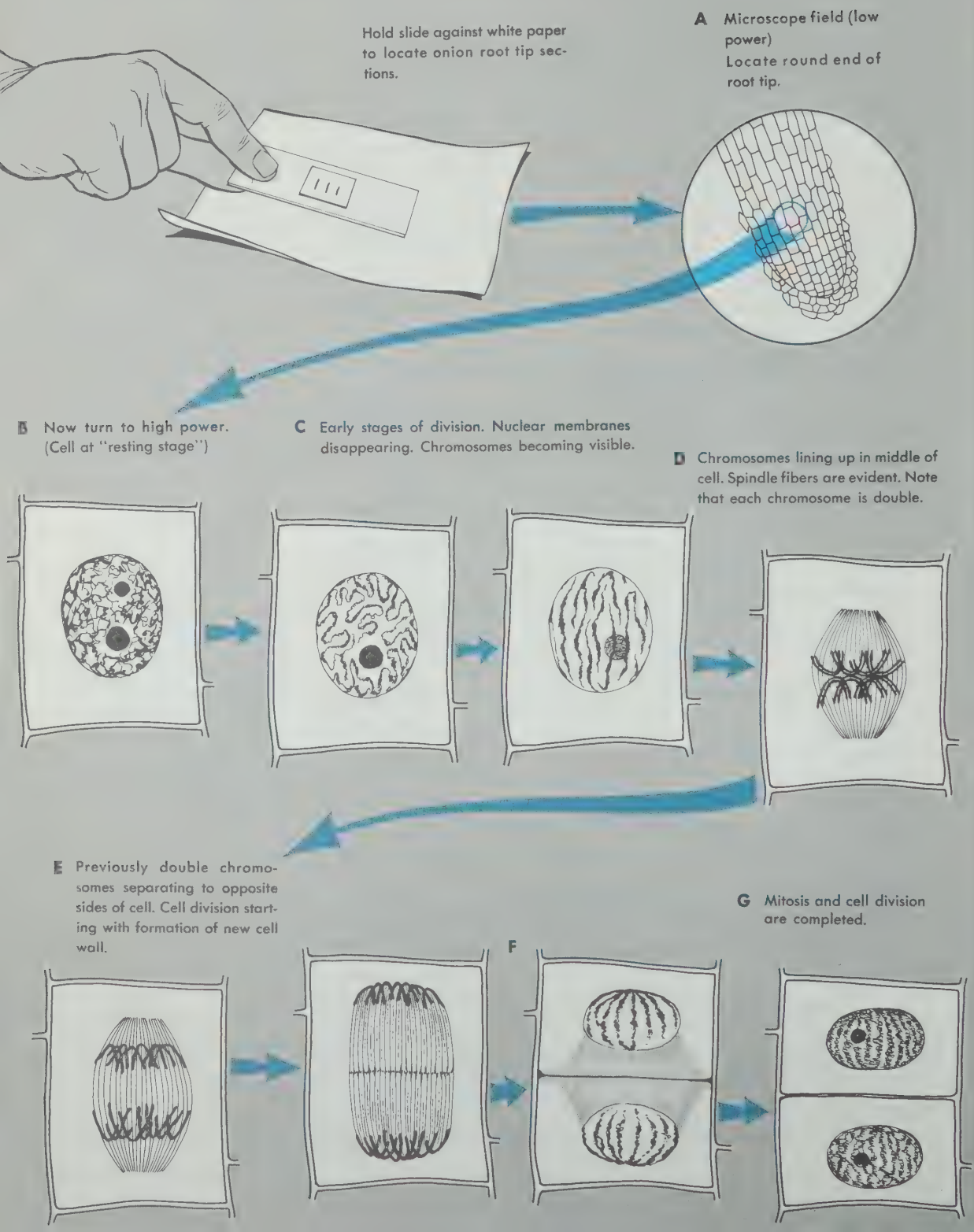
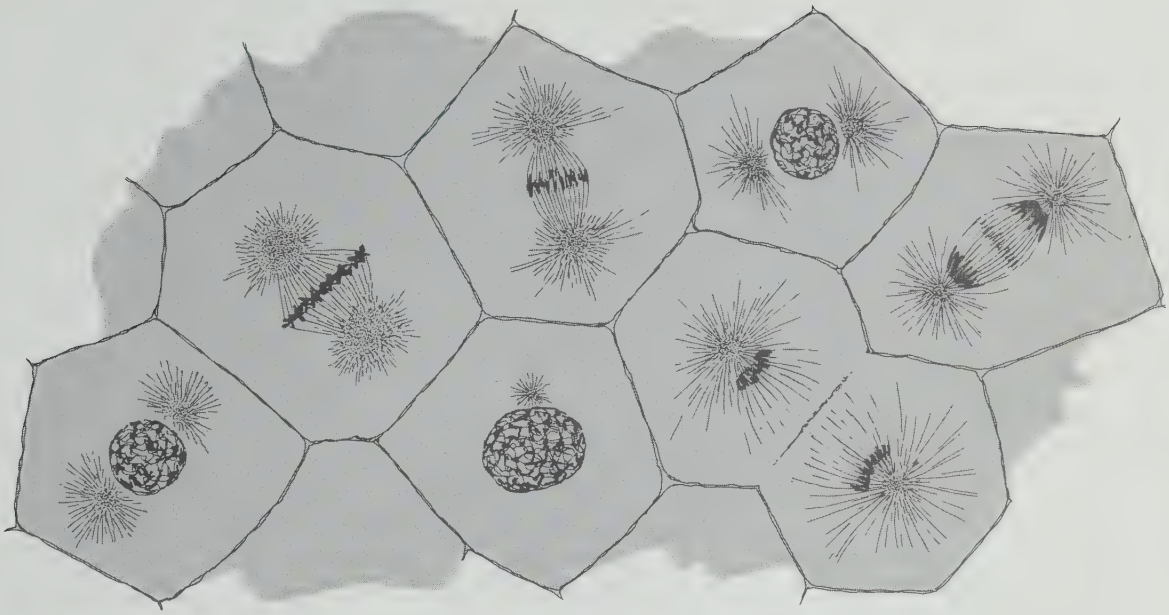


FIG. 2.7 CELL DIVISION IN ANIMAL TISSUE



separate from each other. One member of each pair goes to one side of the cell and the other toward the opposite side (Fig. 2.6E). After the chromosomes have migrated, a new nuclear membrane forms around each group. This completes nuclear division. At about the same time, a cell wall begins to form across the center of the parent cell (Figs. 2.6F,G). The formation of this wall completes cell division.

Mitosis and Cell Division in Animal Cells

Although plant and animal cells show structural differences, the process of cell division is essentially the same. One major difference, however, is seen in the way the cytoplasm is divided. In plant cells, a new cell wall is laid down after the chromosomes separate from each other. In animal cells, the division of the cytoplasm is brought about by a **cleavage furrow**—a pinching in of the cell membrane—that divides the cell.

Your instructor will provide you with slides of animal cells that were in division at the time they were prepared. Each slide will contain several small circles of tissue. With the aid of Fig. 2.7 locate the various stages of cell division in this tissue.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Where, in the human body, would you expect to find large numbers of dividing cells?
2 Chromosomes are generally not observed in nondividing cells. Does this mean that they are newly formed for each cell division? Explain.
3 If the parental cell has 12 chromosomes, how many chromosomes will each of the daughter cells have?
4 Suggest a function for the spindle fibers during mitosis.

EXERCISE 11

AN ARTIFICIAL CELL

Movement, irritability, and reproduction are three of several characteristics that can be used to define life. Once a definition of life has been made it is fairly simple to construct a model to satisfy the definition—yet the model certainly will not be alive. You can construct a model of a cell and use it profitably to show that some characteristics of living organisms can be mimicked by nonliving systems.

PROCEDURE

▶ Place a clean watch glass (or Syracuse dish) on a piece of white paper. Fill the dish with about ⅛ inch of dilute nitric acid (Fig. 2.8).

CAUTION: Do not spill any mercury. Small particles may be inhaled or absorbed by the body and cause serious illness.

▶ Add a drop of mercury, about ¼ inch in diameter, to the dish. To do this, place the tip of the medicine dropper containing the mercury under the surface of the acid.

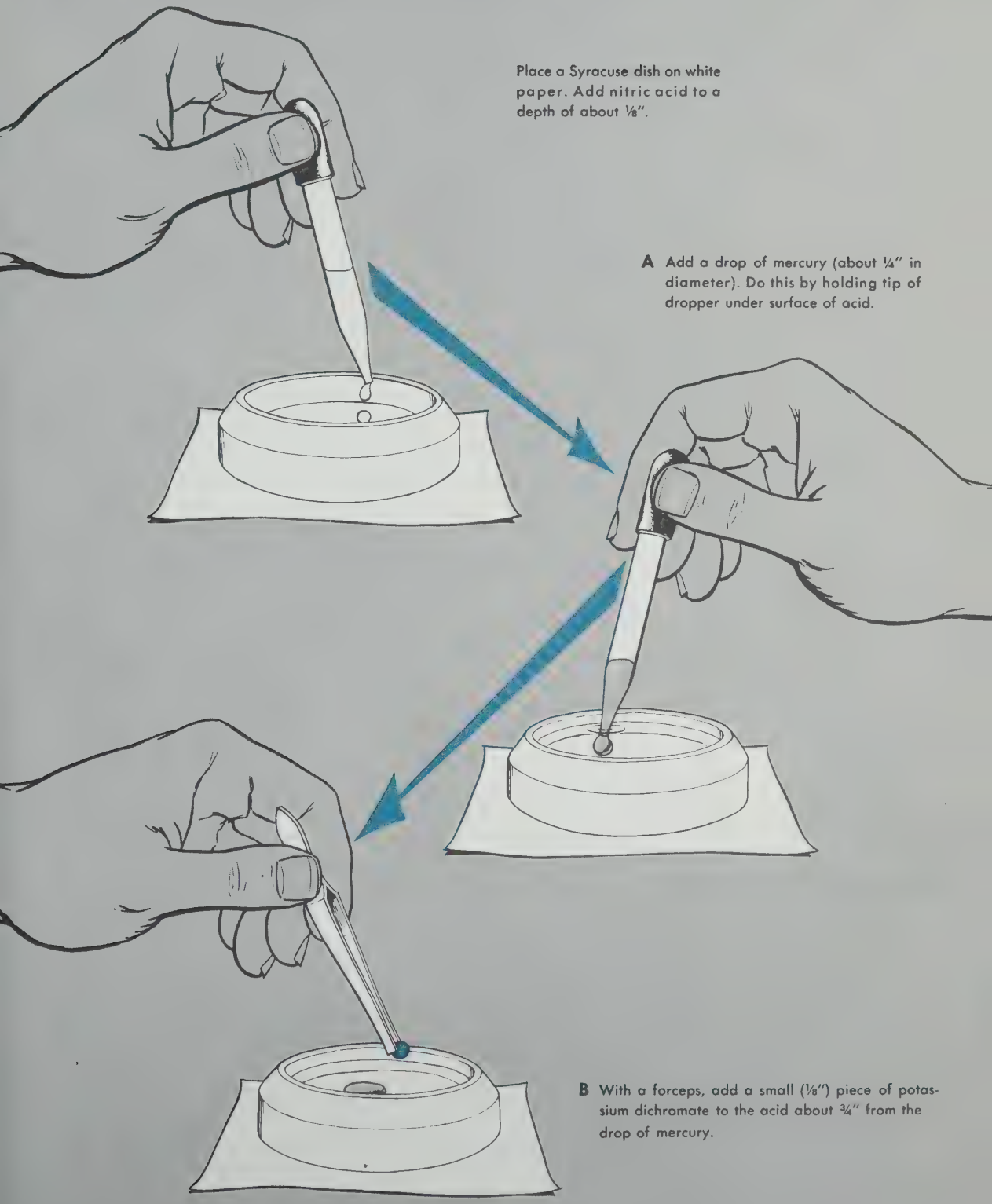
▶ With a forceps place a small piece of potassium dichromate (⅛ inch in diameter) into the acid so that it is about ¾ of an inch from the drop of mercury. You should see the potassium dichromate dissolving in the acid in all directions. **11-A Watch closely as the dichromate (yellow) reaches the drop of mercury and describe what happens.**

When you have completed this exercise, remove the mercury from the dish with an eye dropper and pour it into the container provided. *Do not drop any mercury on the floor.* If any is spilled by accident, it can be brushed onto a dust pan using a small wet paint brush.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 In addition to movement, irritability, and reproduction, what are some other characteristics that can be used to define "life?"
2 Based on your observations in this study, what characteristics of life were imitated by this artificial cell?

FIG. 2.8 METHOD FOR MAKING AN ARTIFICIAL CELL

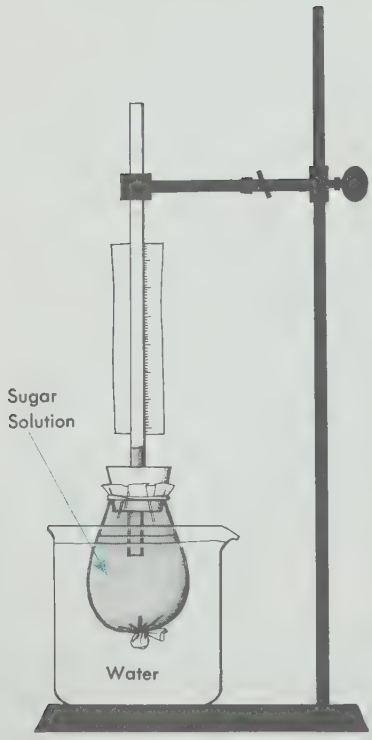


Place a Syracuse dish on white paper. Add nitric acid to a depth of about ⅛".

A Add a drop of mercury (about ¼" in diameter). Do this by holding tip of dropper under surface of acid.

B With a forceps, add a small (⅛") piece of potassium dichromate to the acid about ¾" from the drop of mercury.

3 MOVEMENT OF MATERIALS ACROSS CELL MEMBRANES



INTRODUCTION

All cells have cell membranes by which they are able to regulate the movement of materials in and out of their cytoplasms. A fundamental problem in biology is an understanding of the methods by which materials are able to move across such membranes.

Since not all dissolved substances can penetrate the cell membrane with equal ease, the membrane is said to be **selectively permeable.** This selectivity is vital in maintaining the life of a cell. Although the cell membrane is the major structure safeguarding the cell's internal environment, other parts of the cell, such as the nucleus and mitochondria, are also bounded by selective membranes that control their internal environments.

Whenever two different solutions are separated by a selectively permeable membrane, an **osmotic system** is established. Each cell, therefore, represents an osmotic unit. **Osmosis** may be defined as the movement of water molecules through a selectively permeable membrane. In cells, water is exchanged between the cytoplasm and the solution surrounding each cell. Take a simple osmotic system in which water is separated by a selectively permeable membrane from a solution of sugar. In this case, both the **solute** (sugar) and **solvent** (water) tend to diffuse from an area of higher concentration to an area of lower concentration. In a perfect system, where the membrane keeps the sugar isolated, only the water is able to penetrate. The two solutions can reach **equilibrium,** that is, a state where further change does not occur, only by the transfer into the sugar solution.

Since the water concentration on one side of the membrane is higher than on the other side, water molecules will pass into the sugar solution and increase the volume of water. Eventually,

the pressure of the water on the sugar solution side of the membrane will stop the entrance of more water. This pressure is called **osmotic pressure.**

However, the cell membrane is not a passive organ. While simple diffusion and osmosis account for much of the exchange of material between cells and surrounding fluids, the cell membrane also plays an active part in the exchange process. Many marine algae, for instance, accumulate iodine to a concentration more than a million times greater than that of the sea. Such situations cannot be accounted for by the simple laws of diffusion or osmosis alone. Cells, therefore, have a way of forcing molecules of a particular substance to move in a direction opposite to that dictated by the laws of diffusion. This is the process of **active transport.**

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EXERCISE 12

WHAT FACTORS AFFECT THE RATE OF DIFFUSION?

The constant motion (**kinetic energy**) of the molecules of gases and liquids results in their moving from regions of higher concentration to regions of lower concentration until they become uniformly distributed throughout the space available to them. For example, a gas set free in one corner of a room will, in time, become equally distributed throughout that room. Similarly, a crystal of salt dropped in a glass of water will dissolve and its molecules will gradually become uniformly distributed in the water. This movement of molecules from a region of high concentration to one of low concentration is called **diffusion.** The rate at which molecules diffuse depends on several factors, such as size and concentration of the molecules, and the physical nature of the substance in which the molecules are diffusing.

Brownian Movement

When viewed under the high power of a microscope, minute particles appear to move in a constant and erratic course. At first it was believed that this was due to some "living activity" of the particle or liquid until it was discovered that boiling failed to stop it. It is now apparent that such random motion is due to the continual bombardment of these particles by the fluid's molecules, which are in continuous motion. Thus, the energy that keeps a particle larger than a molecule in motion is the kinetic energy of molecules.

PROCEDURE

▶ Place a small drop of very dilute India ink or an aqueous suspension of carmine on a glass slide. Add a cover glass. Examine with the high power of your microscope. Observe and diagram the motion of one of the India ink particles.

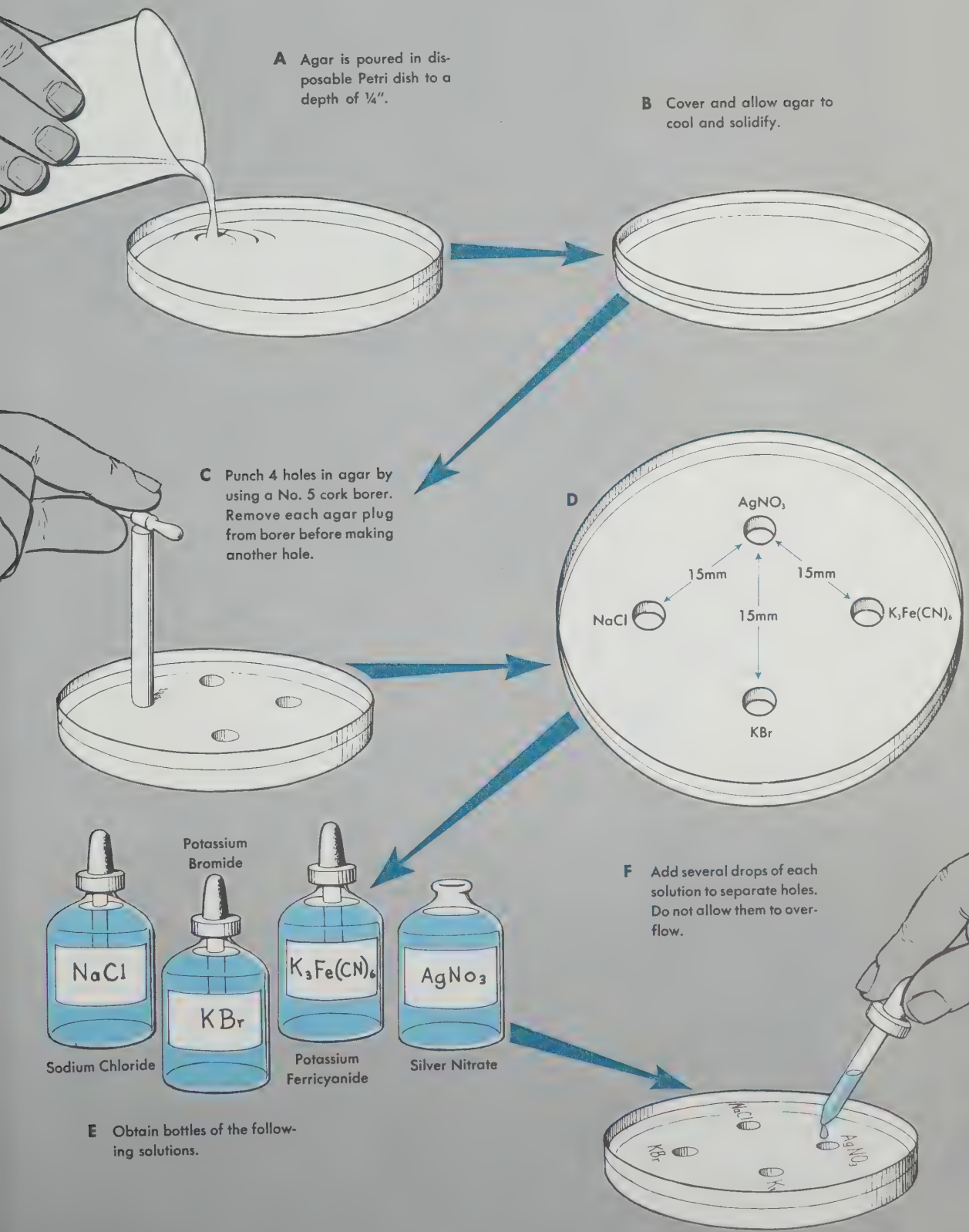
Diffusion

Diffusion can be defined as the net movement of the molecules of a substance, under their own kinetic energy, from a region of greater to a region of lesser molecular activity.

PROCEDURE

▶ Your instructor will give you a disposable petri dish containing a film of agar (Figs. 3.1A,B). Agar is a substance obtained from certain sea weeds.

FIG. 3.1 PROCEDURE FOR DETERMINING RATES OF DIFFUSION



▶ Using a No. 5 cork borer, punch four holes in the agar as shown in Figs. 3.1C,D.

▶ Fill each of the holes with a small amount of a 1 Normal solution of sodium chloride, NaCl; potassium bromide, KBr; potassium ferricyanide $K_3Fe(CN)_6$; and silver nitrate, $AgNO_3$.

▶ **12-A Periodically examine the petri dishes and record your final observations on page 261. 12-B From this study what can you conclude about the relationship of the rate of diffusion of a molecule to its molecular weight?** The approximate weights of each of the migrating groups that are formed when these substances are placed in solution are as follows: Chloride anion, Cl—35; Bromide anion, Br—80; Ferricyanide anion, $Fe(CN)_6$—212; Nitrate anion, NO_3—112.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Design experiments to measure the effect of concentration and temperature on the rates of diffusion.
2 In what way is diffusion important to animals? To plants?
3 Based upon what you know, or have read, about diffusion, suggest how an artificial kidney might work.
4 Give an example of a gas diffusing in a gas, a liquid diffusing in a solid, and a liquid diffusing in a liquid.
5 What role does Brownian movement play in diffusion (if any)?

EXERCISE 13

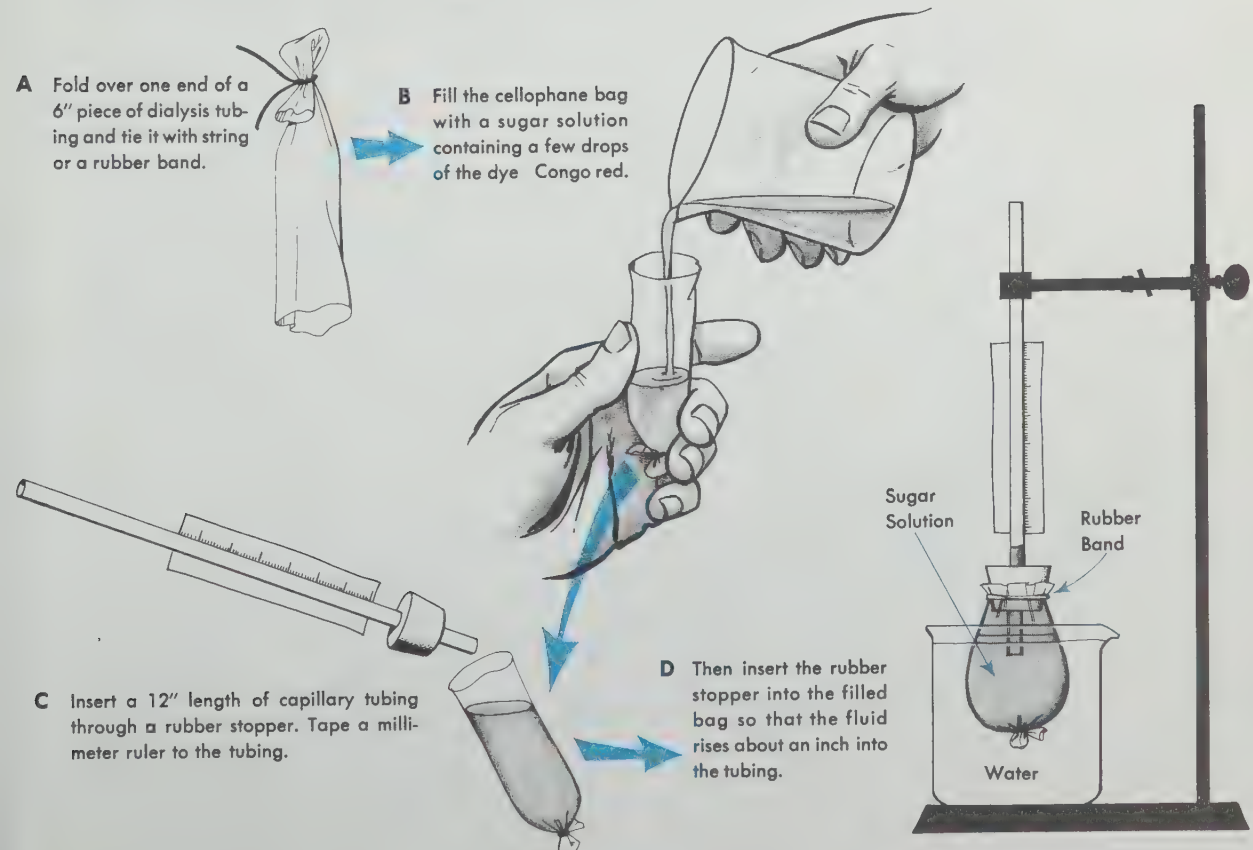
HOW CAN THE RATE OF OSMOSIS BE MEASURED?

Molecules move from regions of high concentration on one side of a membrane to regions of low concentration on the other side of the membrane, provided that the membrane is permeable to that molecule. In order to be permeable, the membrane must have submicroscopic holes through which the molecules can pass. In living cells, most membranes are **selectively permeable,** that is, they allow the passage of some molecules while preventing, or at least slowing down, the passage of other molecules. Dialysis tubing is such a selectively permeable membrane that can be used in the laboratory to study osmosis.

PROCEDURE

▶ Tightly seal one end of a six-inch piece of seamless dialysis tubing (previously soaked for several hours in distilled water), using string or a rubber band (Fig. 3.2A).
▶ Fill the bag with a sucrose solution containing a few drops of the dye Congo red (Fig. 3.2B). Other groups of students

FIG. 3.2 PROCEDURE FOR DETERMINING THE RATE OF OSMOSIS



should set up similar **osmometers** containing sodium chloride or egg albumin of the same concentration as the sucrose solution.

▶ Insert a 12-inch piece of capillary tubing to which is attached a millimeter ruler and rubber stopper into the open end of the tubing (Fig. 3.2C). Tightly seal the rubber stopper into place with a rubber band. The stopper should be sealed so that some of the fluid in the bag rises into the capillary tube.

▶ Attach the osmometer to a ring stand and suspend the dialysis bag into a beaker of water (Fig. 3.2D). If the Congo red dye is observed coming out of the osmometer a leak is indicated and the osmometer should be reassembled.

▶ Record the level of the fluid at the beginning of the experiment and then measure its level again at five-minute intervals thereafter. **13-A Record these data in the table on page 263. 13-B From your own data and those of other groups in your class, plot the results on the graph on page 264 for each of the solutions studied. 13-C From this study what can you conclude about the relative rates of osmosis of each of the molecules studied?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Define osmosis.
2 How does osmosis differ from diffusion?
3 In the winter people often throw salt on their sidewalks. In the spring, they may then find that the grass along the edges of the walk is dead. How do you account for this?
4 Red blood cells burst when placed in distilled water (water that has had its minerals removed). Explain why this occurs.
5 A differentially permeable membrane was made into a bag and filled with a 5 per cent sugar solution. The bag is impermeable to the sugar solution. The bag was then placed into a glass containing 10 per cent sugar solution. Will the bag burst? Explain.

EXERCISE 14

WHAT ARE SOME FACTORS THAT AFFECT THE MOVEMENT OF MATERIALS ACROSS CELL MEMBRANES?

Red blood cells can be conveniently used to illustrate the factors which affect the osmotic relations of the living cell. The cell membrane of red blood cells is freely permeable to water, but differentially permeable to most other substances. The red blood cell increases in volume as substances such as water and dissolved solutes enter it from the outside, but it does not increase indefinitely. Instead, it reaches a limit of size, and then the cell membrane ruptures and the hemoglobin diffuses out of the cell, leaving only the cell membrane (*ghost*) behind. This phenomenon is called **hemolysis.**

Dilute suspensions of red blood cells transmit very little light but when these cells are hemolyzed, the solution becomes transparent. The rate at which a dilute red blood cell suspension becomes transparent in solutions containing different kinds of molecules can be used as a measure of the rate at which such molecules enter the cell. In this exercise you will determine the effect of molecular weight and lipoid solubility on the permeability of cell membranes.

PROCEDURE—Effect of Molecular Weight

▶ Add two drops of a red blood cell suspension to each of four test tubes containing 2 ml of solutions of urea, ethylene glycol, glycerol, and glucose, respectively (Fig. 3.3A). The molecular weights of these compounds are shown in the table on page 265 (14-A).

▶ Mix each of the tubes by holding your finger over the tube and inverting it once or twice (Fig. 3.3B).

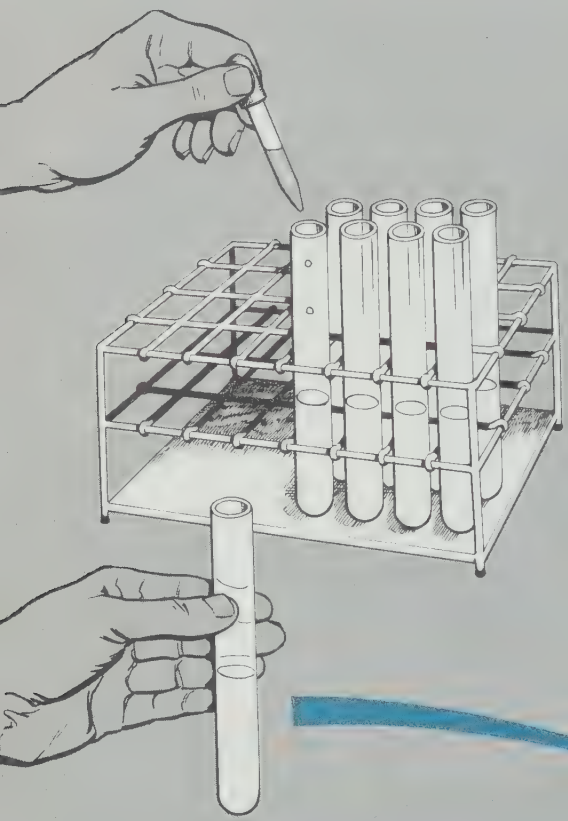
▶ Now hold the tubes flat against a sheet of printed paper (Fig. 3.3C). The time required for the print to become plainly readable through the dilute suspension of blood cells is taken as the time it takes for hemolysis to occur. A stopwatch or the second hand of your watch should be used to make these measurements.

▶ Determine the hemolysis time for each of these solutions and record your data in the table on page 265 (14-A).

NOTE: Test one solution at a time.

▶14-B Plot these data on page 265 (14-B). 14-C Which of the solutions appeared to enter the red blood cells the fastest and which ones entered the slowest? 14-D How do your results compare with those of the other students in your class? 14-E Why might you expect varia-

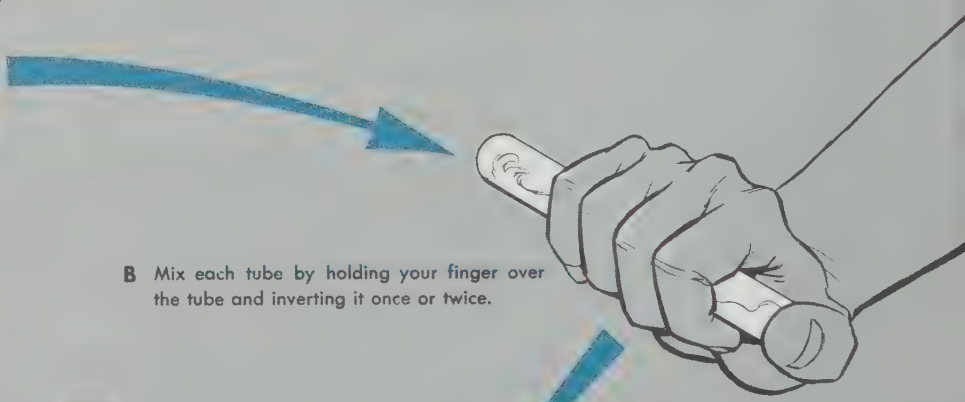
FIG. 3.3 PROCEDURE FOR DETERMINING HEMOLYSIS TIME



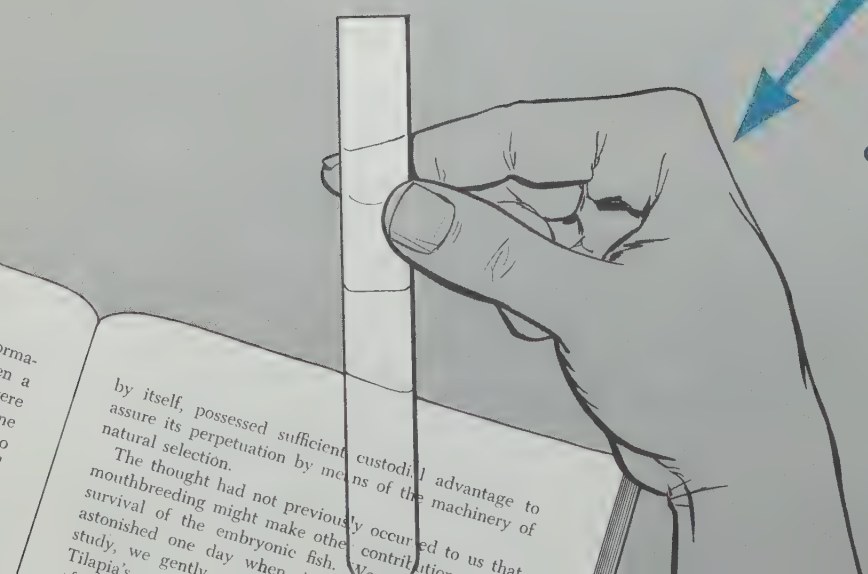
A Add 2 drops of a red blood cell suspension to separate test tubes containing 2 milliliters of:

EFFECT OF MOLECULAR WEIGHT
Tube 1—Urea
Tube 2—Ethylene glycol
Tube 3—Glycerol
Tube 4—Glucose

EFFECT OF LIPOID SOLUBILITY
Tube 1 — Methyl alcohol
Tube 2 — Ethyl alcohol
Tube 3 — Propyl alcohol



B Mix each tube by holding your finger over the tube and inverting it once or twice.



C Then hold each tube flat against a sheet of printed paper. The time it takes for the printing to become *plainly* readable is taken as the length of time it takes for hemolysis to occur.

tion in hemolysis time as determined from one student to the next?

14-F On the basis of your data what can you conclude about the effect of molecular weight on the permeability of cell membranes?

PROCEDURE—Effect of Lipoid Solubility

There are a number of compounds, such as the lipids (fats), that have a high solubility in fats or fat solvents such as alcohol and carbon tetrachloride, but a low solubility in water. In this experiment you will attempt to relate the ability of substances to enter cells to their solubility in fats or fat solvents (lipoid solubility).

NOTE: Test one solution at a time.

▶ Add two drops of a suspension of red blood cells to each of three test tubes, containing 2 ml of methyl alcohol, ethyl alcohol, and propyl alcohol, respectively (Fig. 3.3A).

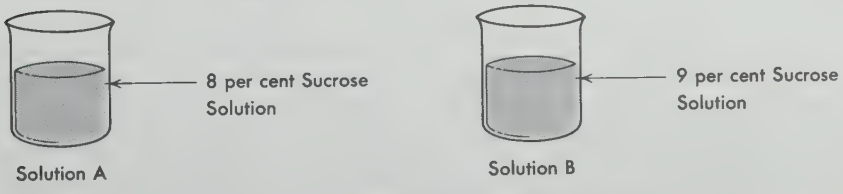
▶ Mix each of the tubes and then, as before, hold them flat against a sheet of printed paper and determine the hemolysis time for each of the solutions as described in Figs. 3.3B,C.

▶ **14-G Record your data in the table on page 267 (14-G). 14-H In which of the solutions did hemolysis occur the fastest and in which the slowest? 14-I In which of the alcohols measured do fats appear to dissolve the most rapidly? 14-J From these data what can you conclude about the chemical composition of the cell membrane?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is meant by the terms *Hypertonic? Hypotonic? Isotonic? Solute? Solvent?*

2 In the diagram, is Solution A hypotonic or hypertonic to Solution B? Explain.



3 In the lipoid solubility part of this exercise, the red blood cells eventually burst when placed in the different alcohol solutions. These alcohol solutions do not contain any solute particle. Why, then, do the blood cells burst?

4 Is the blood plasma hypotonic, isotonic or hypertonic to the red blood cells? Explain.

4 BIOLOGICALLY IMPORTANT MOLECULES

Richard F. Trump

INTRODUCTION

The bulk of the dry matter of cells consists of carbon, oxygen, and hydrogen variously organized into large molecules of four main types: proteins, carbohydrates, lipids (fats), and nucleic acids. These molecules, in turn, often combine and make even larger molecules of importance to the cell.

Carbohydrates, which are formed when a green plant captures light energy in the process of photosynthesis, play a key role in making this captured energy available to the cell. They also supply important carbon "skeletons"—carbon atoms linked together—for the manufacture of other biologically important molecules.

The basic units in carbohydrates are carbon, hydrogen, and oxygen. The term "carbohydrate" means *hydrate of carbon.* This name was used because it included many compounds that contain atoms of hydrogen and oxygen in the same proportion occurring in water—two hydrogens to one oxygen. So a carbohydrate can be described by the general formula $C(H_2O)$. Carbohydrates range from relatively simple molecules called sugars to the complex molecules of starch and cellulose.

Proteins determine, to a great extent, the structural and functional activities of cellular systems. These molecules make up a significant portion of the structure of cells and form a large part of most cellular membranes and organelles (for example, mitochondria, ribosomes, spindle fibers, chromosomes). Furthermore, proteins act as biological catalysts (enzymes), regulate cellular and tissue functions (hormones), and fight disease (antibodies).

Proteins are molecules of gigantic size, sometimes containing tens of thousands of atoms. They are tremendously complex and have no competitors in the diversity of roles they play. In addition to containing carbon, hydrogen, and oxygen, proteins

also contain nitrogen. They range in molecular weight from about 5,000 (insulin) to 40 million (tobacco mosaic virus protein).

The **lipids,** or fats, are extremely important because they serve as a concentrated storage source of energy and, indeed, supply more than twice as much energy to the cell as do proteins or carbohydrates. These molecules also form a large part of most cellular membranes, in which they control the movements of other lipids in and out of cells.

Lipids are classified into several subdivisions on the basis of their chemical and physical properties. All fats, such as butter, the fat on meat, and olive oil, are built up from carbon, hydrogen, and oxygen atoms, and consist of two major components—**fatty acids** and **glycerol.**

The **nucleic acids** are very large molecules that have the capacity to transmit information to other parts of the cell. The largest of these molecules, deoxyribonucleic acid (DNA) has the capacity to make copies of itself almost endlessly, and with remarkable exactness.

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EXERCISE 15

HOW CAN CARBOHYDRATES BE IDENTIFIED?

Sugar, starch, and cellulose are common examples of a group of molecules known as carbohydrates. The name carbohydrate means "hydrate of carbon," meaning that all of the molecules contain carbon, as well as hydrogen and oxygen in the ratio of two parts of hydrogen to one part of oxygen. Thus a carbohydrate can be described by the general formula $C(H_2O)$.

Most carbohydrates have a basic unit of six carbon atoms (sugars) which are linked together in various ways. On the basis of the number of these 6-carbon compounds that can be linked to make longer units, the carbohydrates are divided into three classes: the **monosaccharides,** which consist of a single 6-carbon molecule (for example, glucose); the **disaccharides,** consisting of two single sugar molecules linked together (cane sugar, or sucrose, is made up of glucose linked to fructose); and the **polysaccharides,** which consist of three or more sugar molecules linked together (starch and glycogen are long chains of glucose molecules). Starch and glycogen are storage forms of carbohydrates; starch is usually formed in plants, and glycogen in animals. They are insoluble in water and thus remain in cells until digested and used as sources of energy.

PROCEDURE—Test for Sugars

▶ Fill a test tube half full of Benedict's solution. Add about 10 drops of a dilute solution of glucose (a monosaccharide), as shown in Figs. 4.1A,B.

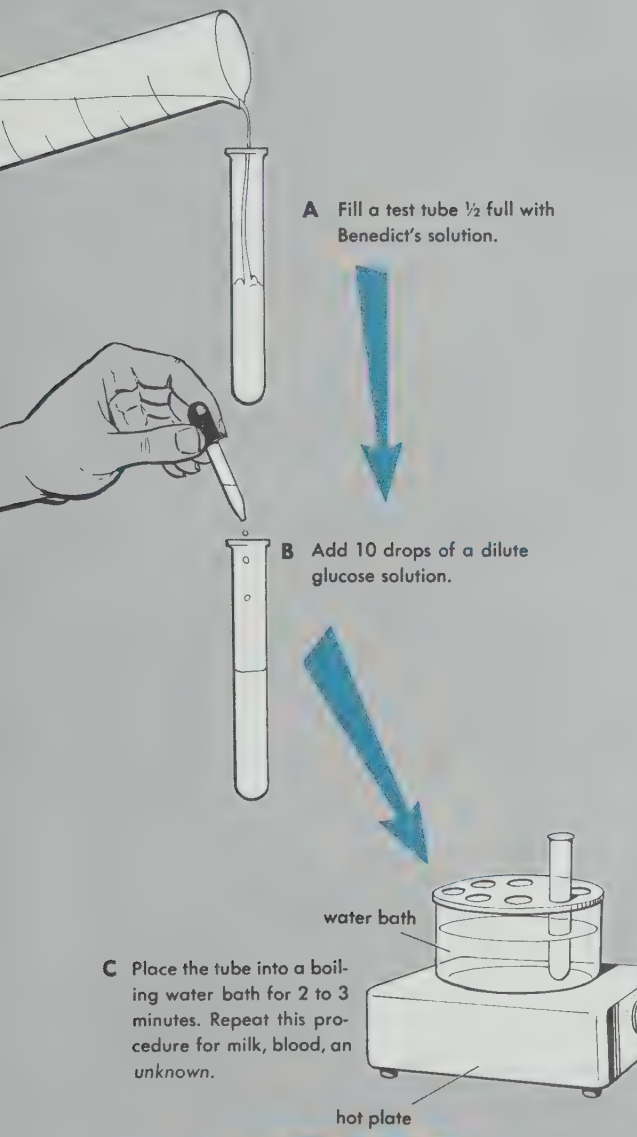
▶ Place this tube in a boiling water bath for two to three minutes and then allow it to cool. (Fig. 4.1C) **15-A What color change has occurred in the glucose solution? 15-B What is the appropriate control for this test?**

▶ Apply this test to samples of milk, blood serum, or unknown samples provided by your instructor. Tabulate your results by indicating the changes in color of each tube in Figs. 4.1E,F,G. **15-C Which of the substances tested showed a positive Benedict's test?**

PROCEDURE—Test for Starch

▶ Add several drops of a dilute starch solution to one of the depressions in a porcelain spot plate and several drops of water to another depression (Fig. 4.1H).

FIG. 4.1 PROCEDURE FOR TESTING FOR SUGAR AND STARCH



A Fill a test tube ½ full with Benedict's solution.

B Add 10 drops of a dilute glucose solution.

water bath

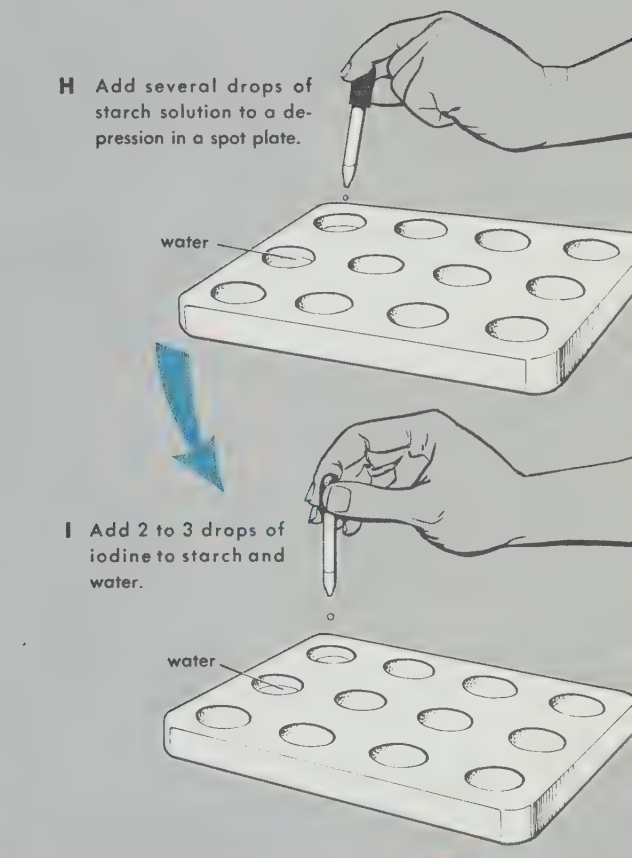
C Place the tube into a boiling water bath for 2 to 3 minutes. Repeat this procedure for milk, blood, an *unknown*.

hot plate

D **E** **F** **G**

Glucose Milk Blood *Unknown*

Record the color change that occurred in each tube.



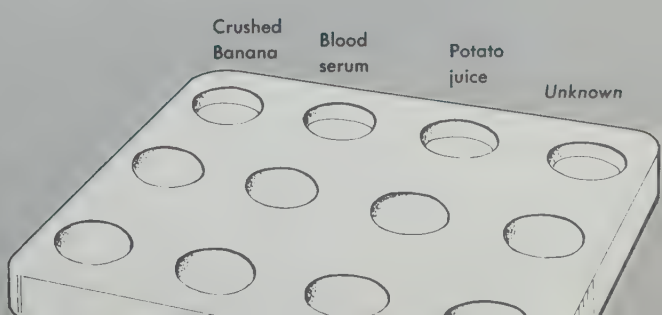
H Add several drops of starch solution to a depression in a spot plate.

water

I Add 2 to 3 drops of iodine to starch and water.

water

J Repeat this procedure on the following materials.



Crushed Banana

Blood serum

Potato juice

Unknown

▶ Add a few drops of iodine solution to each of these liquids (Fig. 4.1I). **15-D What color does the starch solution become? 15-E Is there any such color change in the water tested?**

▶ Apply this test to a sample of blood serum, potato juice, a small piece of crushed banana, or an unknown provided by your instructor. **15-F Record your results. 15-G Which of the substances showed a positive iodine test?**

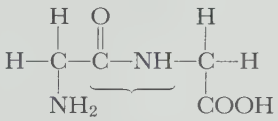
FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Name some foodstuffs high in carbohydrate content.
2 Although carbohydrates are a rich source of energy, can man survive on a diet consisting only of carbohydrates? Explain.
3 What biological process provides all of the carbohydrates consumed by man?
4 In what form is excess carbohydrate stored in an animal? In a plant?

EXERCISE 16

HOW CAN PROTEINS BE IDENTIFIED?

Proteins are molecules of gigantic size, sometimes containing tens of thousands of atoms. In addition to containing the chemicals carbon, hydrogen, and oxygen, proteins also contain nitrogen and sulfur. When proteins are broken down in the presence of water (hydrolyzed), they yield a mixture of simple molecules called **amino acids.** These amino acids, of which there are about 20 different kinds, all contain an amino group ($—NH_2$) and a carboxyl group (—COOH). In a protein molecule the amino acids are joined by a carbon-nitrogen bond (**peptide bond**) between the carboxyl group of one amino acid and the amino group of another.



Peptide Bond

Proteins give many striking color reactions with certain reagents. The compounds that give rise to these colors are formed not by the whole protein molecule but by certain of the amino acids present in the protein. In this exercise you will perform two specific tests for identifying the presence of a protein.

PROCEDURE—Millon Test

▶ Fill one test tube half full with egg albumin and a second tube half full with water. Add a few drops of Millon's reagent to each tube (Figs. 4.2A,B). **16-A What is the result?**

▶ Place the test tubes in a boiling water bath for two to three minutes, as in Fig. 4.2C. **16-B Record your observations. 16-C What color change occurs in the albumin solution?**

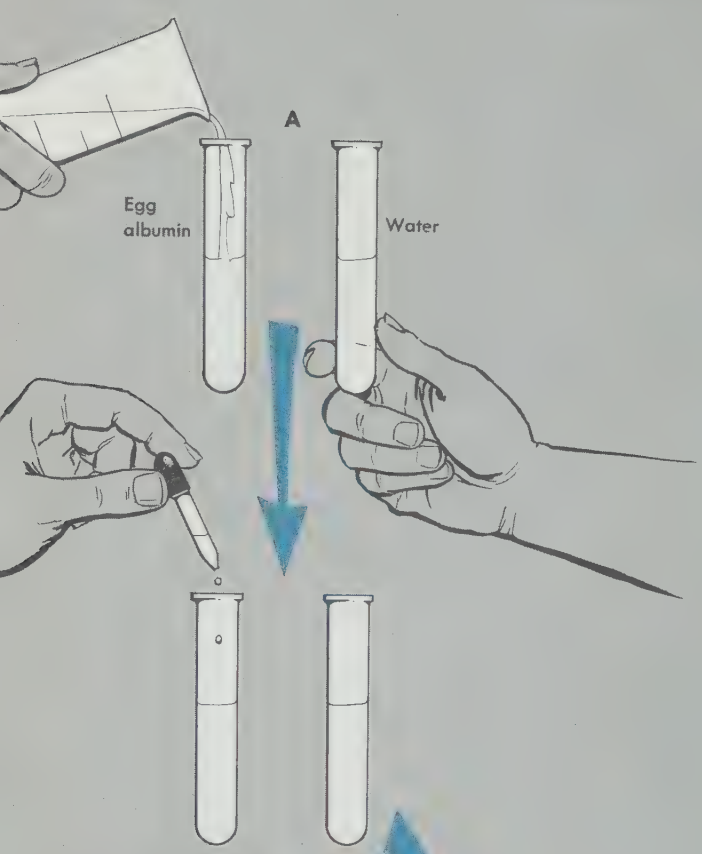
▶ Apply the Millon's test to small samples of milk, gelatin, blood serum or other unknown provided by your instructor. **16-D Which of the substances showed a positive Millon's test?**

PROCEDURE—Biuret Test

▶ Fill one test tube half full with water and a second tube half full with egg albumin (Fig. 4.2I).

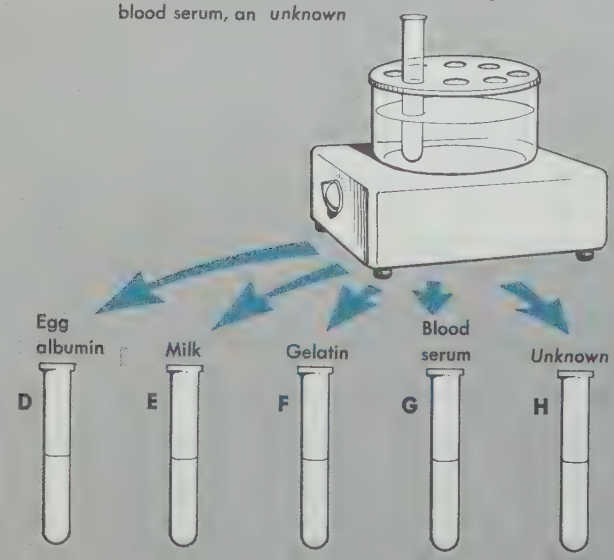
▶ To each tube add 10 drops of Biuret reagent (Fig. 4.2J). **16-E What color change, if any, occurs in each of the test tubes?**

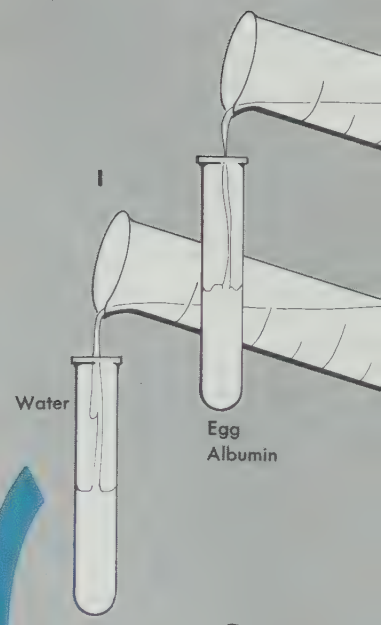
FIG. 4.2 PROCEDURE FOR TESTING FOR PRESENCE OF PROTEINS



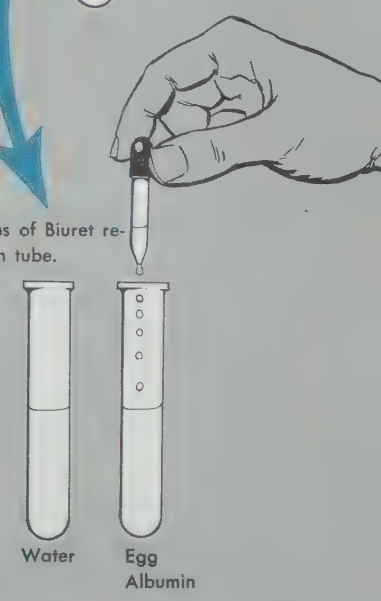
B Add 2 to 3 drops of Millon's reagent. What color change, if any, occurs?

C Place test tube in a boiling water bath for 2 to 3 minutes. Repeat this procedure for milk, gelatin, blood serum, an *unknown*

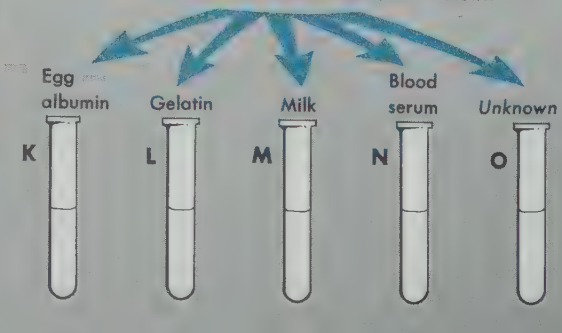




J Add 10 drops of Biuret reagent to each tube.



Repeat this test with gelatin, milk, blood serum, an *unknown*



▶ Apply the Biuret test to small samples of gelatin, milk, blood serum or other unknown provided by your instructor.

16-F Which of the substances tested showed a positive Biuret test?

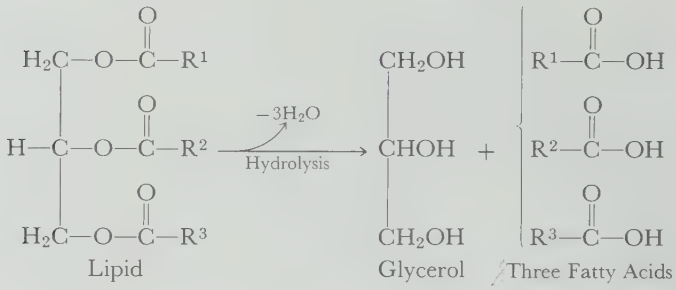
FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Are proteins as rich a source of energy for metabolism as carbohydrates or lipids? Explain.
2 What happens to proteins when they are exposed to very high temperatures?
3 What is meant by a "high protein" diet? Give an example of such a diet.
4 Why are athletes in training fed a "high protein" diet?
5 Contrast carbohydrates and proteins in relation to their chemical structure and their functions in the organism.
6 Why are such biologically important molecules as antibodies and enzymes composed of proteins and not carbohydrates or lipids?

EXERCISE 17

HOW CAN LIPIDS BE IDENTIFIED?

Lipids are a diverse group of substances that are classified together because they are all insoluble in water and are also soluble in the so-called fat solvents (for example, ether, acetone, carbon tetrachloride). The simplest lipids (butter fat, coconut oil, animal and plant fats) are composed of carbon, hydrogen, and oxygen atoms, and by hydrolysis yield glycerol and fatty acids.



PROCEDURE—Grease Spot Test

The simplest way to identify lipids is to make use of the fact that these compounds make translucent grease marks on paper.

▶ With a medicine dropper add a drop of salad oil to the corner of a sheet of brown wrapping paper, lunch bag, or any other unglazed paper (Fig. 4.3A). To the other corner add a drop of water.

▶ Let the paper dry and then examine each spot by holding the paper up to the light. **17-A Which of the spots appears translucent?**

▶ Now apply this test to samples of milk, uncooked animal fat, blood serum, or any other substances you suspect might contain lipid material, (Figs. 4.3B,C,D). **17-B Which of the foodstuffs tested appear to contain lipid?**

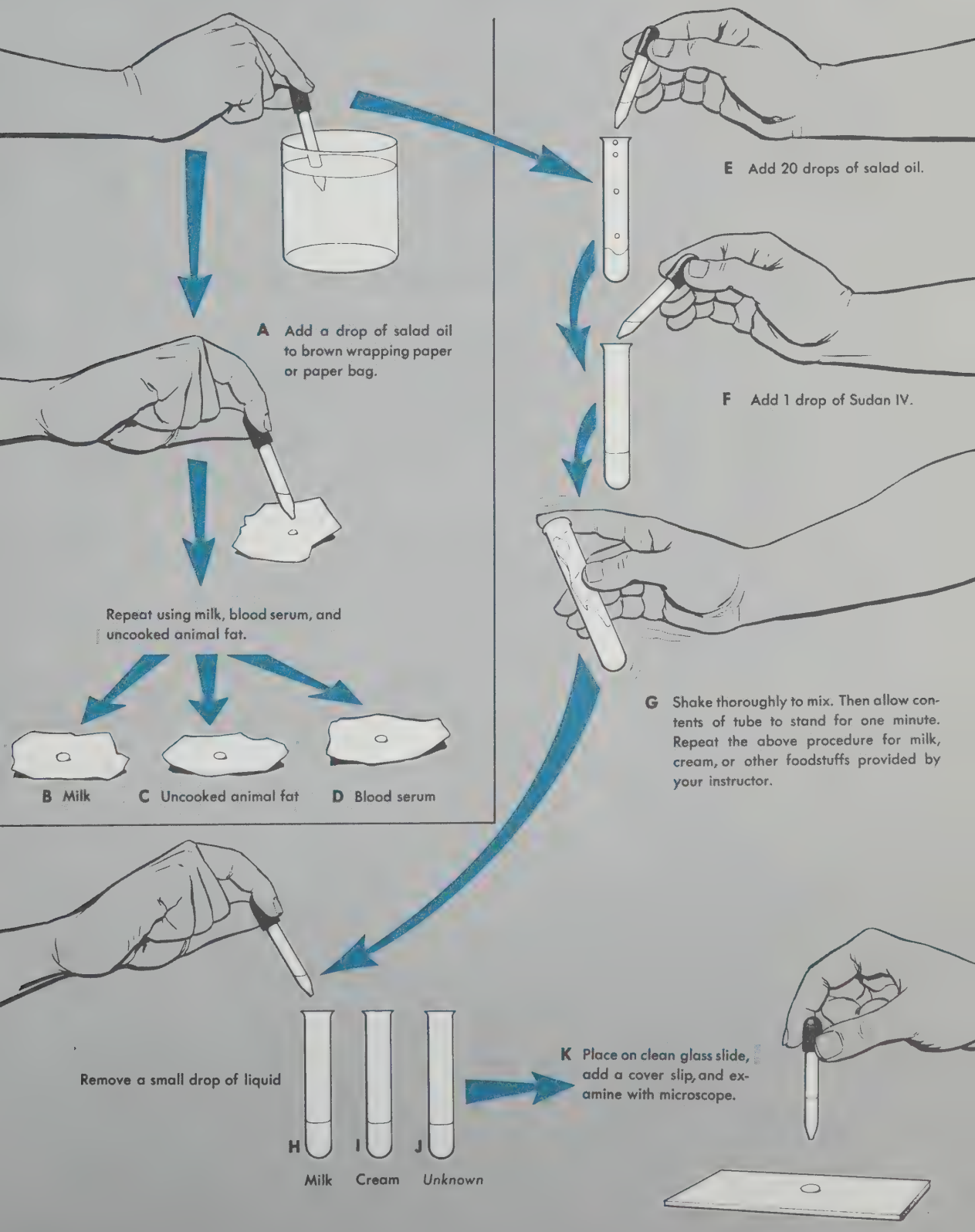
PROCEDURE—Dye Test

A more specific test for fats is their ability to take up the pigment of the dye Sudan IV.

▶ To 3 ml of water in a test tube, add 20 drops of salad oil and one drop of Sudan IV (Figs. 4.3E,F).

▶ Shake thoroughly. Allow to stand for one minute (Fig. 4.3G. **17-C What is the distribution of the dye with respect to the water and the salad oil?**

FIG. 4.3 PROCEDURE FOR TESTING FOR PRESENCE OF LIPIDS



▶ Now apply this test to several drops of milk, cream, or other foodstuffs provided by your instructor (Figs. 4.3H,I,J). After adding the Sudan IV, mix thoroughly by shaking, then remove a small amount of liquid from each tube, place it on a clean glass slide, and add a cover slip (Fig. 4.3K). Examine microscopically. In many liquids that contain lipids, the fat is contained within small globules. Examine the slides carefully. If lipid is present, the fat globules should take up the red dye. **17-D Which of the foodstuffs tested appear to contain a lipid?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is the difference between fats, oils, and waxes?
2 What are some important economic uses of fats, oils, and waxes?
3 What is the relationship between fats and soaps?
4 How does butter differ from oleomargarine in the kind of lipid that each contains?

EXERCISE 18

WHERE IS DNA LOCATED?

DNA (deoxyribonucleic acid) can be isolated from nearly every living organism—from viruses to man. No matter where it is found, DNA has much the same chemical and physical properties.

DNA is an extremely large molecule composed of thousands of repeating units called **nucleotides.** Each nucleotide consists of: **phosphoric acid,** a 5-carbon sugar called **deoxyribose,** and a nitrogen compound called a **base.** You will examine the detailed structure of DNA later in Exercise 60. In this exercise you will use a specific staining procedure to determine where DNA is located in cells.

PROCEDURE

Approximately one week ago your instructor placed several onions in water as shown in Fig. 4.4A. He then cut off 1 cm of the root tips that grew out from the onion and "fixed" them—a procedure that preserves the tissues in as nearly a natural state as possible (Fig. 4.4B).

▶ Place several of the "fixed" root tips in a vial of water. Wash them slowly in running tap water for five to 10 minutes (Fig. 4.4C). **18-A Why should the root tips be washed?**

▶ Carefully pour off the water in the vial (Fig. 4-4D) and cover the root tips with hydrochloric acid (HCl). Allow the root tips to remain in the HCl for five to eight minutes. **18-B What does this step accomplish?**

CAUTION: Do not get any HCl on your hands, clothes, or face!

▶ Wash the root tips in running tap water for five to 10 minutes to remove the acid (repeat steps C and D in Fig. 4.4).

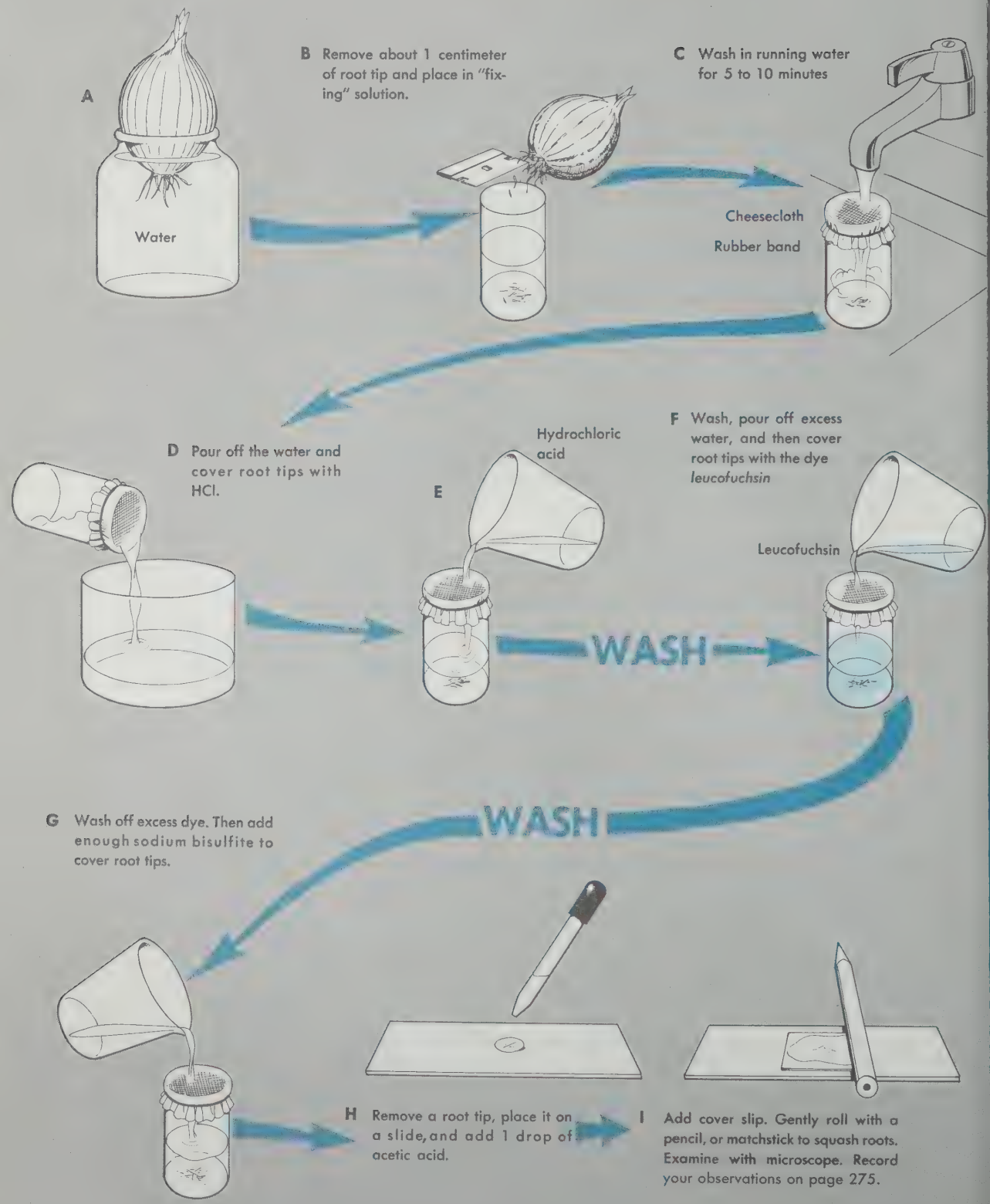
▶ Pour off the excess water and then add enough of the dye leucofuchsin (Schiff's reagent) to cover the root tips (Fig. 4.4F).

▶ Leave the root tips in the dye for 20 minutes. Then wash off all excess dye by washing the tips under tap water for three to five minutes (repeat as before).

▶ Now add enough sodium bisulfite to cover the root tips and leave them in this solution for one to two minutes (Fig. 4.4G). This step bleaches all parts of the cell which do not contain DNA.

▶ After bleaching, remove the root tips from the vial and place them on a microscope slide. Add a drop of acetic acid (Fig. 4.4H).

FIG. 4.4 PROCEDURE FOR IDENTIFYING DNA IN CELLS



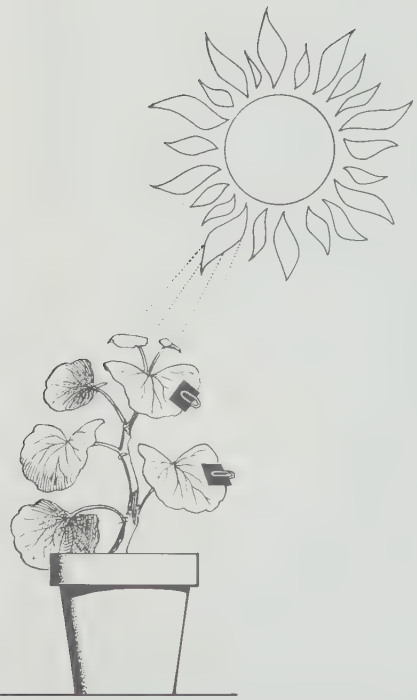
▶ Add a cover slip and with a wooden match, hardwood applicator, or your pencil, gently roll the preparation to squash the root tips and separate the cells from each other (Fig. 4.4I).

▶ Examine the slide with the microscope, first with low power and then high power. The dye used stains the DNA in the cell purple. **18-C Record your observations with a drawing. 18-D In what part of the cell is DNA located? 18-E What is the color of the cytoplasm? 18-F Can you identify individual chromosomes in any of the cells? 18-G Are there any cells in the process of dividing?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Why is DNA such a biologically important molecule?
2 Would you expect to find DNA in the cytoplasm of cells? Explain.
3 What happens to the concentration of DNA in cells undergoing mitosis? Explain.

5 PHOTOSYNTHESIS



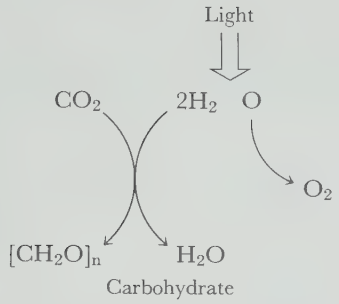
INTRODUCTION

The living world, with few exceptions, operates at the expense of the energy captured by the photosynthetic machinery of green plants. From the products of photosynthesis, and from a small number of inorganic compounds available in the environment, living organisms are able to build up the numerous, complex molecules that contribute to their cellular structure or, in other ways, are essential to their existence. Furthermore, the energy expended by living organisms has, as its ultimate source, the converted energy of sunlight that was trapped within the organic molecules that were synthesized during photosynthesis.

The term photosynthesis refers to the entire process whereby carbohydrates, usually glucose, are formed from CO_2 and H_2O by using solar energy. During this process the radiant energy of sunlight is converted into chemical energy that is captured in the bonds of the sugar molecule. Light, however, is required only in the first stages of the photosynthetic process. The reactions in which light energy is captured and transformed into chemical energy are called **light reactions.** The remaining reactions, in which the carbon skeleton of glucose is built up from CO_2 and H_2O, can proceed in the absence of light and are, therefore, called **dark reactions.**

The key component of green cells bringing about this energy conversion is the pigment **chlorophyll.** The net action of light is the splitting of H_2O, releasing oxygen and providing hydrogen which reduces the carbon dioxide and forms another molecule of H_2O. This is schematically shown on the next page.

In the exercises that follow you will examine the necessity of light, carbon dioxide, and chlorophyll in the photosynthetic process. Photosynthetic activity may be measured in terms of the amount of sugar or oxygen produced. In the introductory laboratory course there is no practical way of precisely deter-



mining the amount of sugar or oxygen produced in the cells or tissues as a result of photosynthesis. However, it has been shown experimentally that when a plant is actively undergoing photosynthesis much of the sugar produced in the leaves is converted into starch. Although starch is not a direct product of photosynthesis, we can use its presence in leaves as indirect evidence that photosynthesis has occurred.

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EXERCISE 19

IS LIGHT NECESSARY FOR PHOTOSYNTHESIS?

Light, as with other forms of radiant energy, varies in duration, intensity (brightness), and quality (wavelength). What techniques can we use to confirm the fact that light is necessary to photosynthesis?

PROCEDURE

Your instructor placed some geranium plants in the dark approximately 48 hours ago (Fig. 5.1A). Remove one of the plants from the dark and test one of the leaves for the presence of starch by using the following procedure:

CAUTION: Never heat alcohol directly over a flame or heat source. It readily catches fire. Use a water bath.

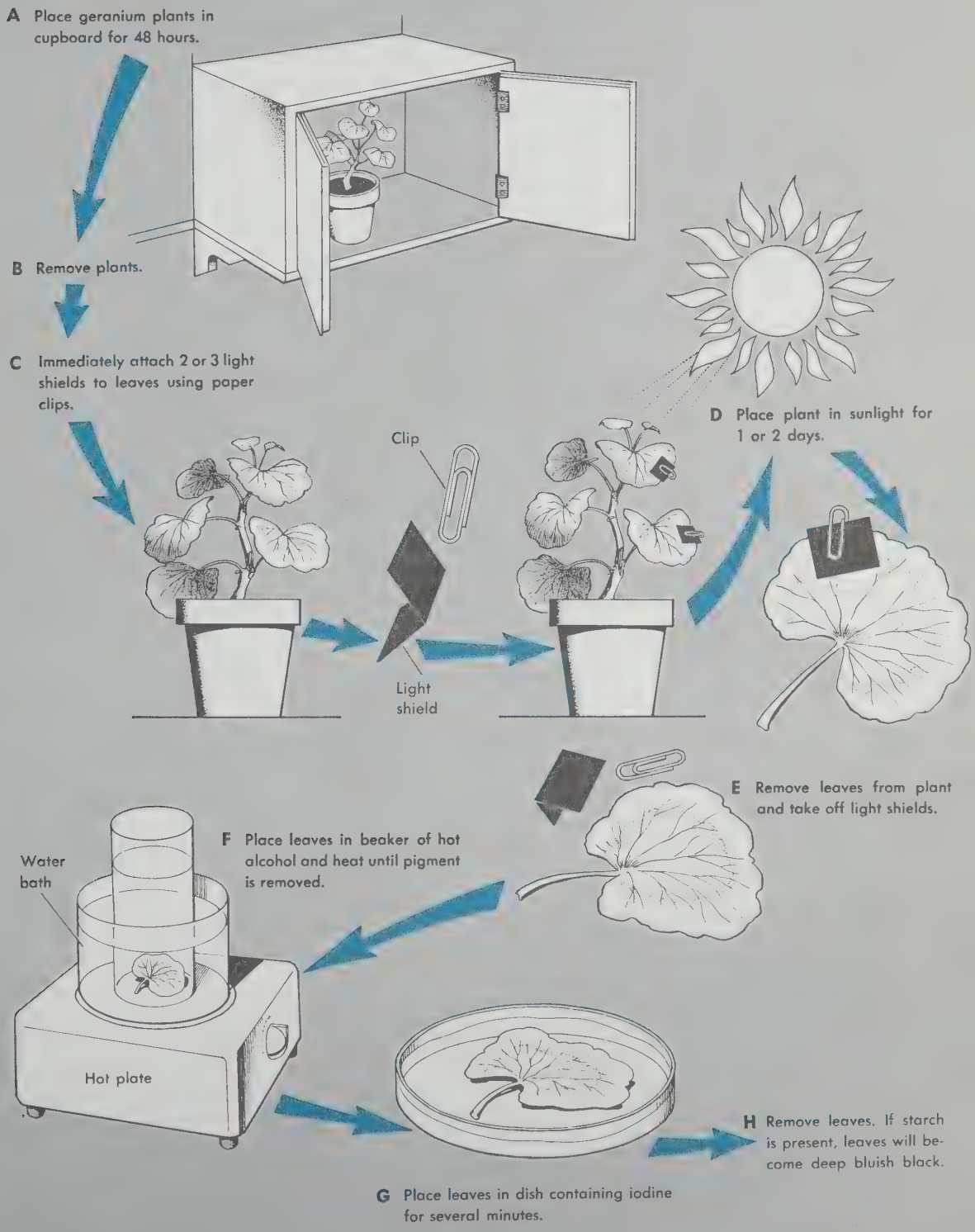
- Remove a leaf from the plant.
- Remove the pigment by placing the leaf in hot alcohol.
- Pour iodine over the leaf in a petri dish. If starch is present, the leaf will turn a deep bluish-black. **19-A Diagram the distribution of starch in this leaf.**
- Then place light shields provided by your instructor on two or three other leaves and place the plant under bright light (for example, on a window ledge) for two days (Figs. 5.1C,D). After 48 hours, test the leaves for photosynthetic activity by conducting a starch test on them (Figs. 5.1E,F,G,H).

19-B Diagram the distribution of starch in these leaves.
19-C Based on your observations, what conclusions can be drawn about the necessity of light for photosynthesis?

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 In the procedure for setting up this experiment, why were the plants placed in darkness for a period of time before attaching the light shields?
2 What is the reason for testing one of the leaves of these "dark" plants before putting on the light shields?
3 What would happen to animals if all plants were to die? Explain.
4 White light is composed of several wavelengths, each of which appears as a color when white light is passed through a prism (violet, blue, green, yellow, orange, red). Design an experiment to determine which of these colors (wavelengths) functions best in the photosynthetic process.

FIG. 5.1 PROCEDURE FOR DETERMINING IF LIGHT IS NECESSARY FOR PHOTOSYNTHESIS



EXERCISE 20

HOW DOES LIGHT INTENSITY AFFECT THE RATE OF PHOTOSYNTHESIS?

The intensity of sunlight striking the surface of the Earth varies from hour to hour as well as from one season to another. Since oxygen is a by-product of photosynthesis, oxygen production may be used in designing an experiment to measure the effect of variations in light intensity on photosynthesis. The production of oxygen may be demonstrated by placing a plant under water and then measuring the escape of oxygen bubbles. In this exercise, changes in the photosynthetic rate under different light intensities will be measured.

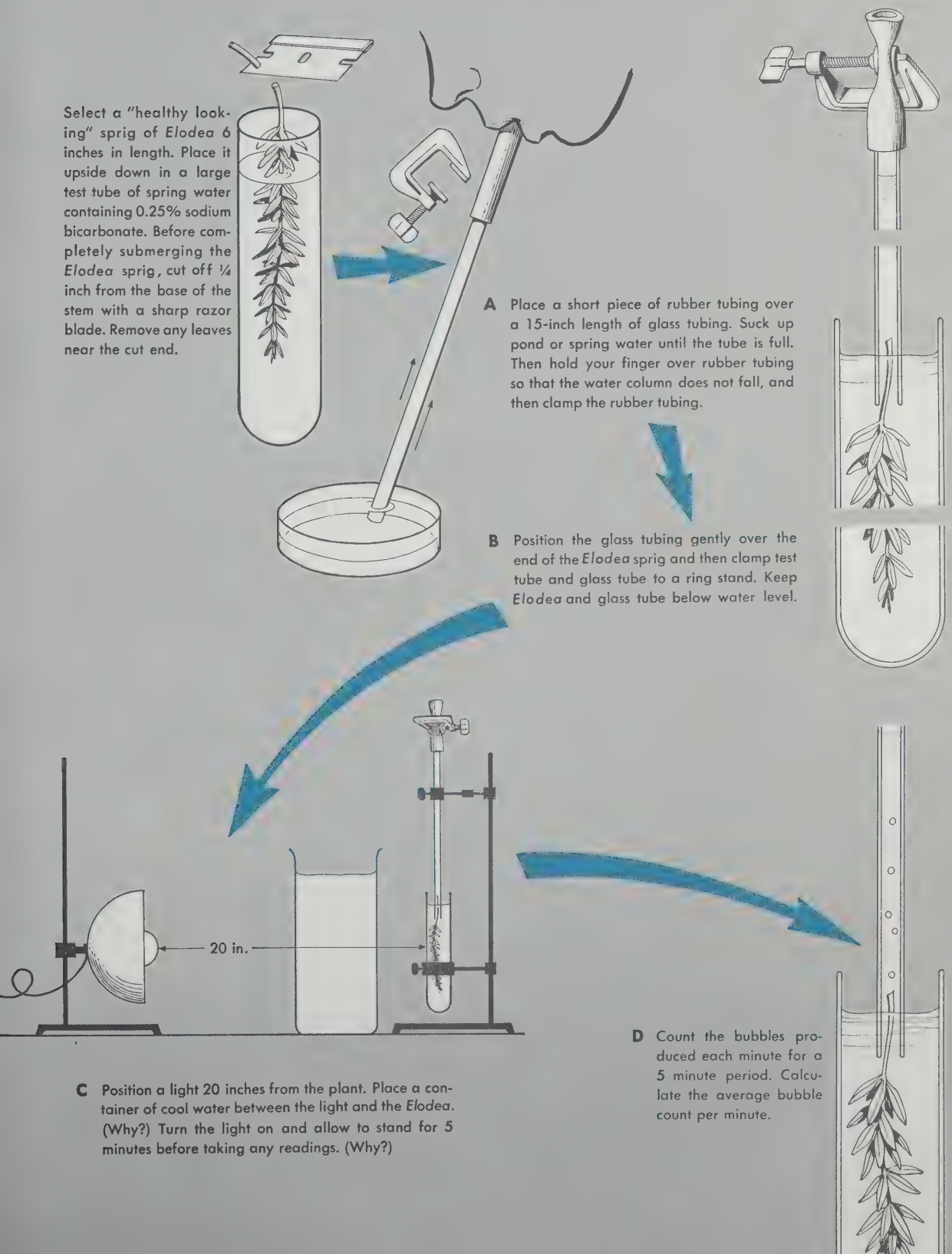
PROCEDURE

Following the method shown in Fig. 5.2, calculate the average number of bubbles produced per minute with the lamp 20 inches from the Elodea. **20-A Record your data in the table on page 279.** Move the lamp to a distance of 10 inches from the Elodea. Allow the set-up to stand for five minutes. **20-B Why? Determine the average bubble count at this distance (10 inches) and record your data. Repeat this experiment with the light source five inches from the Elodea and record your data in the table. 20-C Graph your results on page 279.**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 How can you prove that the bubbles given off during photosynthesis are composed of oxygen?
2 How has the intensity of the light been varied in the experiment conducted in this exercise?
3 What is the relationship between the amount of oxygen produced (as bubbles) and light intensity?
4 If you were able to increase the intensity of light indefinitely, would you expect the production of oxygen to continue to increase at the same rate? Explain.

FIG. 5.2 PROCEDURE FOR DETERMINING THE EFFECT OF LIGHT INTENSITY ON PHOTOSYNTHESIS



EXERCISE 21

HOW CAN YOU DETERMINE IF CARBON DIOXIDE IS NECESSARY FOR PHOTOSYNTHESIS?

The atmosphere is composed predominantly of nitrogen (approximately 78 per cent) and oxygen (approximately 21 per cent). In addition, it contains variable amounts of water vapor and small quantities of other gases. Carbon dioxide (CO_2) constitutes about 0.04 per cent by volume of the atmosphere.

PROCEDURE

Your instructor will provide you with several geranium plants that have been kept in the dark for 36 to 48 hours. Select a leaf from one of the plants and test it for the presence of starch (Figs. 5.3A,B). Return the plants to the dark during the time you are testing the leaves. **21-A Why?**

CAUTION: KOH or NaOH is extremely hazardous to use. Do not touch with your hands. Use tongs or a plastic spoon to transfer this chemical from its container.

If a strong, positive starch test occurs, select another plant and test the leaves until a negative or very weak starch test occurs. **21-B Why is this step necessary?**

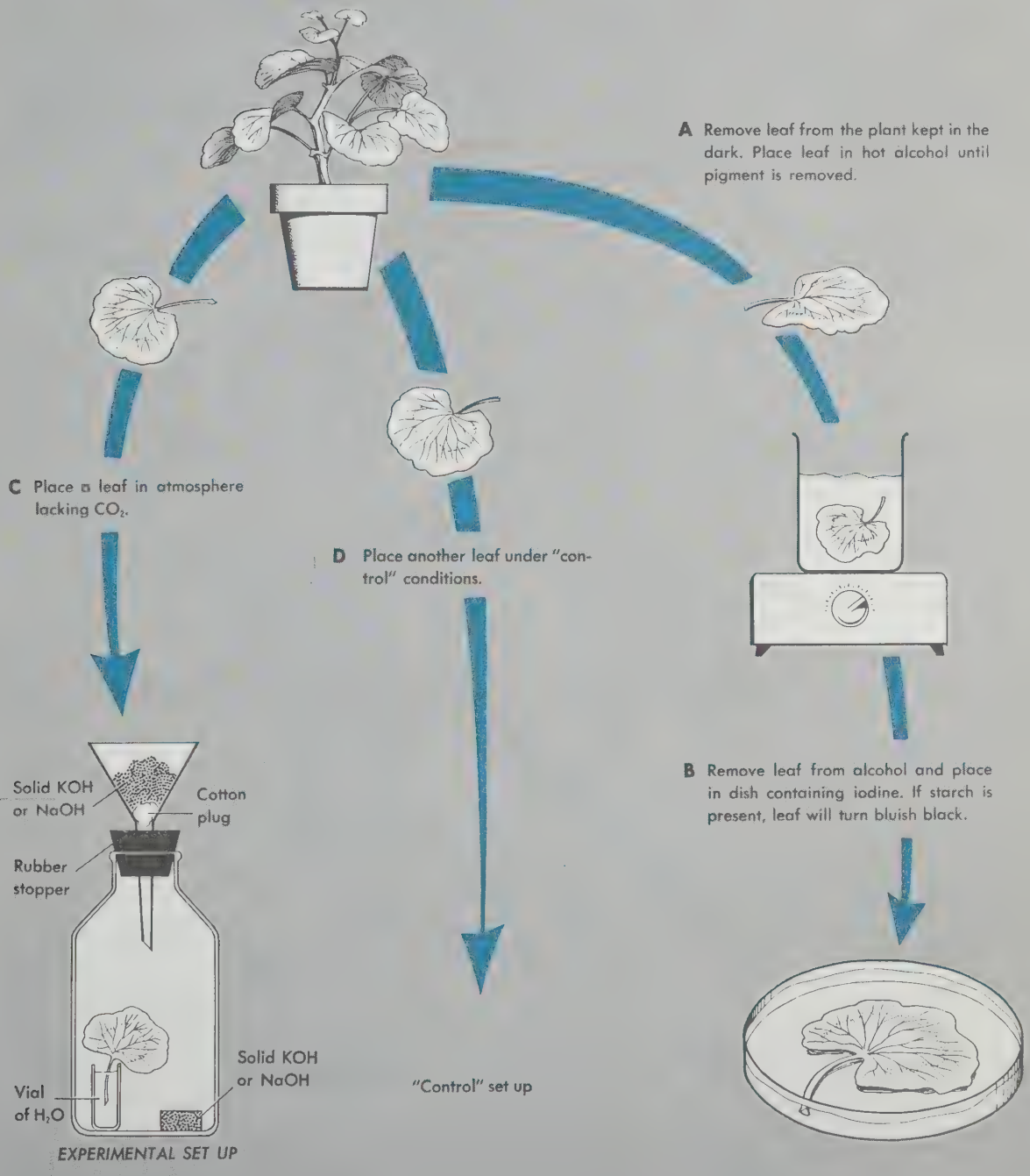
Set up the experiment as shown in Figs. 5.3C,D. This is accomplished by placing a geranium leaf in an atmosphere lacking CO_2. Potassium or sodium hydroxide (KOH or NaOH) effectively remove CO_2 from the air. **21-C What "control" should be set up so that meaningful conclusions can be made?**

Set up this "control" along with the experimental set-up and place the "control" under bright lights for 24 hours. Test for photosynthetic activity by testing the leaves for starch.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 In Fig. 5.3, why is potassium hydroxide (or sodium hydroxide) placed within the jar as well as in the funnel?
2 Based on the results of the experiment, what conclusions can be made about the necessity of carbon dioxide for photosynthesis?
3 Suppose you were to put a sprig of Elodea into a test tube completely filled with boiled (and cooled) water. You then seal the tube with a rubber stopper and place it under bright light. Would you expect photosynthesis to occur? Explain.
4 A solution of phenol red is orangish-red in the presence of carbon dioxide. The solution becomes yellowish in the absence of carbon dioxide. Devise an experiment to show that Elodea plants use CO_2 when photosynthesizing.

FIG. 5.3 PROCEDURE FOR DETERMINING IF CO_2 IS NECESSARY FOR PHOTOSYNTHESIS



Place "experimental" and "control" set ups under bright lights for 24 hours. Then test for starch as shown in steps A and B.

EXERCISE 22

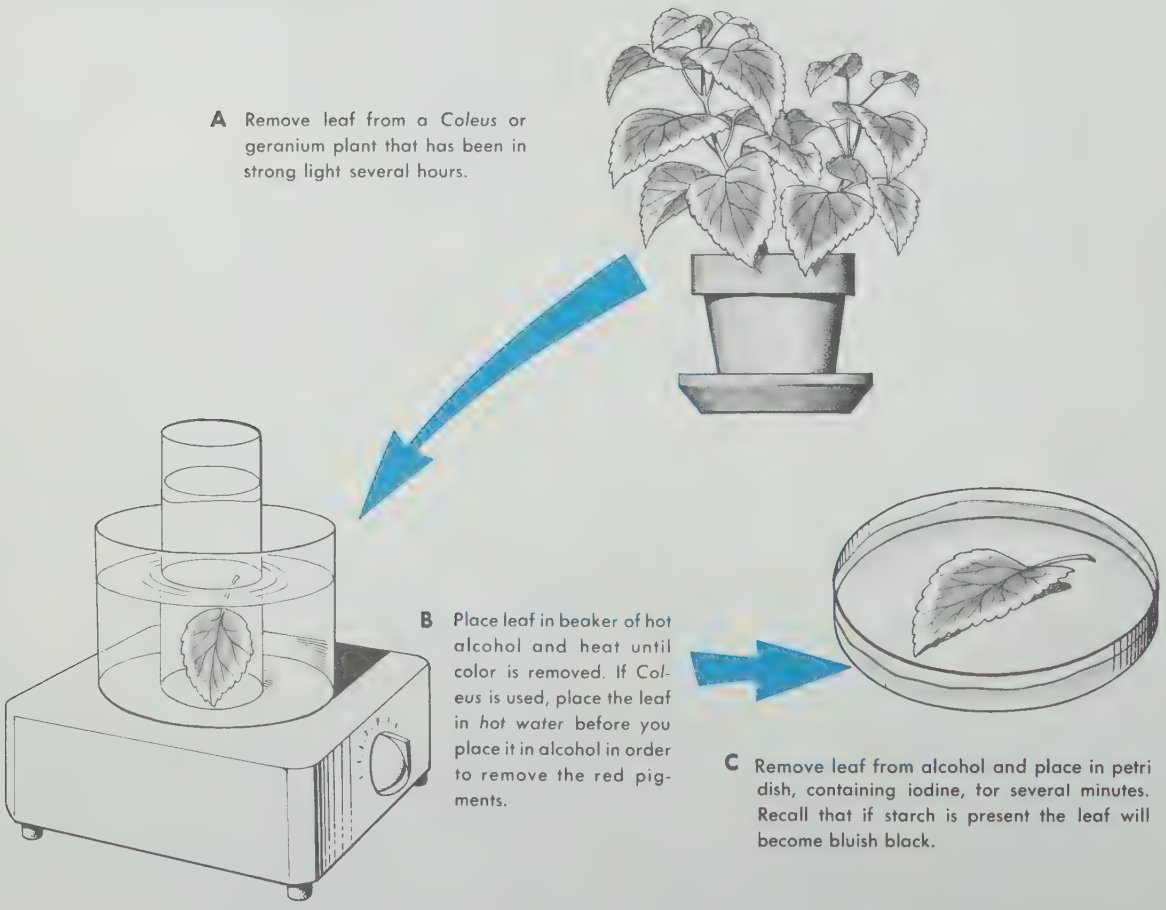
HOW CAN YOU DETERMINE IF CHLOROPHYLL IS NECESSARY FOR PHOTOSYNTHESIS?

The majority of common green plants with which you are familiar owe their color to a pigment—**chlorophyll**—that is contained in special cellular inclusions called **chloroplasts.** Although several different chlorophylls are known to occur in plants, chlorophylls *a* and *b* are more nearly universal in their distribution. In this exercise you will attempt to determine how it can be shown that chlorophyll is necessary for photosynthesis.

PROCEDURE

Obtain a leaf from a variegated (multicolored) *Coleus* or a silver-leaf geranium plant that has been in strong light for

FIG. 5.4 PROCEDURE FOR DETERMINING IF CHLOROPHYLL IS NECESSARY FOR PHOTOSYNTHESIS



several hours (Fig. 5.4A). **22-A Why not use a plant that has been in the dark?**

22-B Diagram the leaf and outline the distribution of chlorophyll (page 283). Next, extract the pigments and test for the presence of starch as shown in Figs. 5.4B,C. **22-C After testing the leaf for starch, diagram the leaf on page 283 and outline the distribution of starch. 22-D Is chlorophyll necessary for photosynthesis? Explain.**

NOTE: If you use a variegated *Coleus* leaf, place the leaf in boiling water for several minutes before placing it in hot alcohol. The water will remove the red anthocyanin pigments.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 There is a plant, called *Iresine,* that has blood-red leaves and stems. When the leaves of this plant are tested for the presence of starch, a positive test is obtained, suggesting that photosynthesis has occurred in the leaves. How is this possible?
2 Why do green leaves appear green to your eyes?
3 Some *red* algae are found hundreds of feet below the surface of the ocean. How are these plants able to obtain their food requirements?
4 What good use could be made of a chemical that would selectively destroy chlorophyll without harming other animal life?

EXERCISE 23

CAN CHLOROPLAST PIGMENTS BE SEPARATED?

Complex mixtures of chemical substances may readily be separated by a technique called **paper strip chromatography.** The separation of the mixture is based on differences in the solubilities of the constituents of the mixture in different solvents.

In this exercise you will attempt to determine if chlorophyll is one pigment or if it is composed of several different pigments.

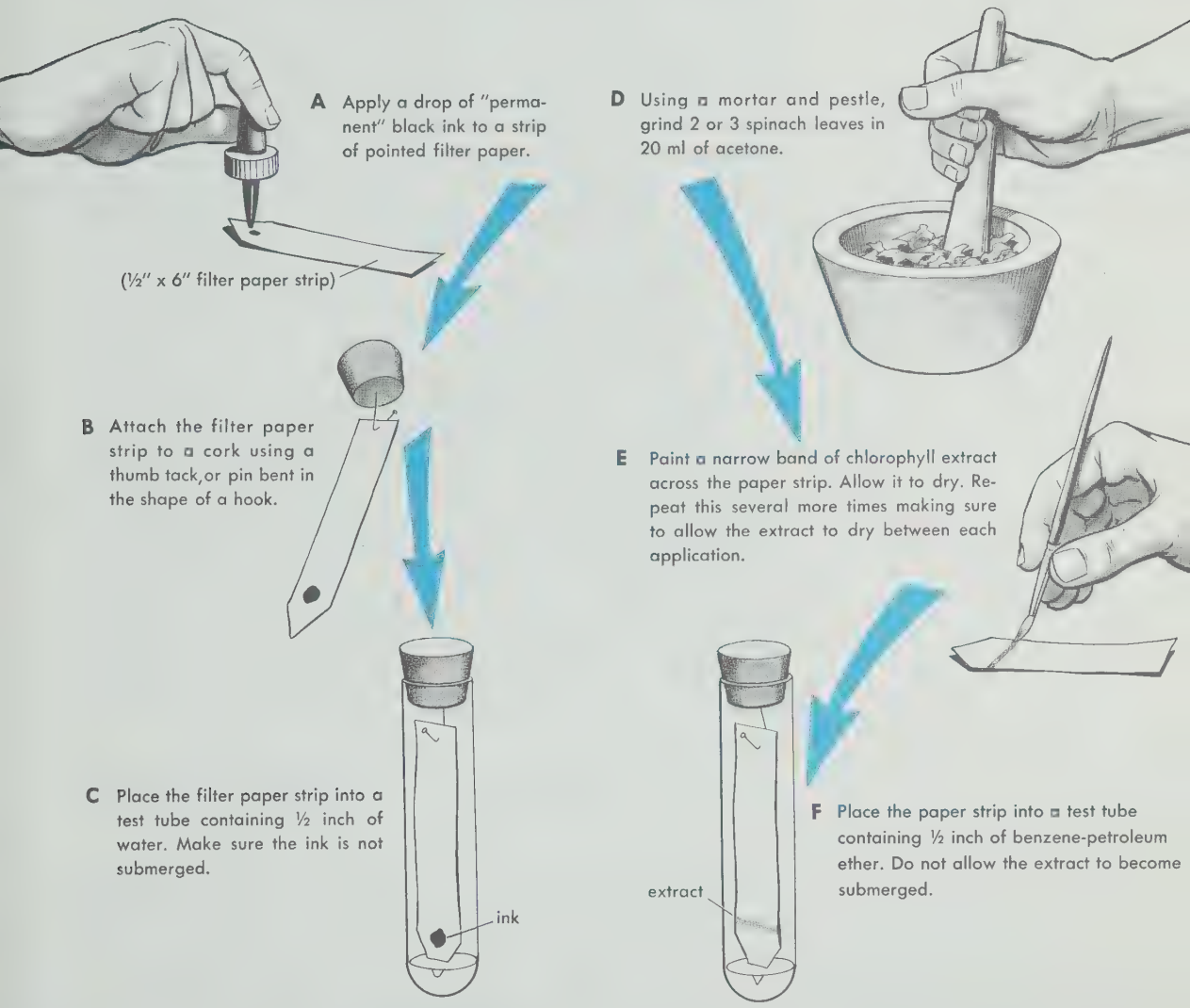
PROCEDURE

- Cut a strip of filter paper approximately ½ inch by 6 inches so that one end is pointed, then apply a drop of "permanent" black ink, as shown in Fig. 5.5A.
- Attach the paper strip to a cork or rubber stopper by using a thumb tack or pin bent in the shape of a hook (Fig. 5.5B).
- Place the strip in a test tube containing about ½ inch of water, making sure that the ink spot is not submerged in the water (Fig. 5.5C). Examine the filter strip after five minutes. **23-A Describe your observations.**
- If time permits, repeat this procedure, using your own fountain pen ink or ball-point ink. Allow the **chromatogram(s)** to dry and tape them into your laboratory book as a record of your results.
- Prepare a "chlorophyll" extract by grinding two or three spinach leaves in 20 ml of acetone (Fig. 5.5D). Adding a small quantity of quartz sand will make the grinding easier.
- Using a small paint brush, apply a narrow strip of chlorophyll extract to the filter paper (Fig. 5.5E). Dry thoroughly by blowing on the paper or waving it in the air. Apply the extract five or six more times. Make certain to let it dry thoroughly after each application.
- Place the strip in a test tube containing benzene-petroleum ether as shown in F of Fig. 5.5. Examine the chromatogram for the next several minutes. **23-B How many different bands of pigment can you see?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 If the "green color" of leaves results from more than one pigment, why aren't all of the pigments visible to the eye?

FIG. 5.5 PROCEDURE FOR SEPARATING PIGMENTS



2 Offer an explanation for the change in color of some leaves in the fall.

3 Why do you think you used water to separate the ink but not the chlorophyll?

4 Since there are several pigments in green leaves, how would you go about showing that chlorophyll is the pigment responsible for photosynthesis and not one of the other pigments that showed up on your chromatogram?

APPENDIX

PAPER STRIP CHROMATOGRAPHY

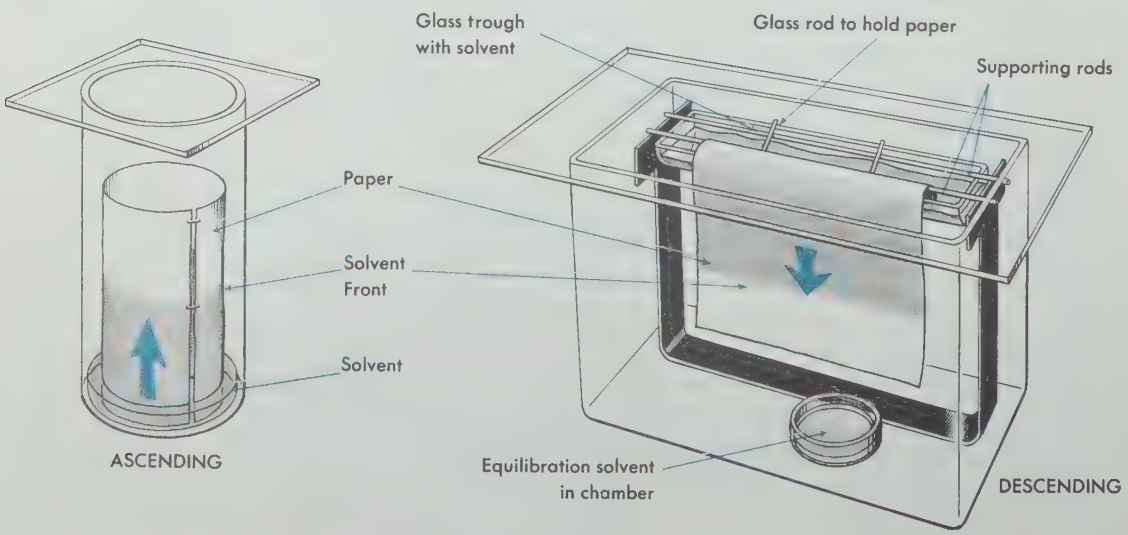
Along with the increasing complexity of chemistry has come the necessity for newer and more useful techniques of detecting and identifying small amounts of organic and inorganic substances. Among the most significant technical developments of recent years has been the development of paper strip chromatography. This technique permits the separation of mixtures on a very small scale. Something that no other simple method affords.

The fact that only microgram quantities of material are required makes paper strip chromatography an exceedingly useful tool for the study of many biological processes. For example, the metabolism of vitamins, hormones, and the action of antibiotics in test animals can be studied by analyzing blood and urinary extract of those animals after the agent in question has been fed or injected into the animal. The amino acid content of various proteins can be determined both qualitatively and quantitatively after hydrolysis. The complex mixtures of adrenal steroids can likewise be separated, and in fact have been separated by means of this technique.

The separation of mixtures of molecules by paper strip chromatography is accomplished by taking advantage of the differing abilities of the molecules in the mixture to be adsorbed on a given absorbent (in this case filter paper).

> The adhesion of a thin layer of molecules of a gas, or of a dissolved substance, or of a liquid or of particles to the surface of a solid substance

FIG. 5.6 APPARATUS FOR PAPER CHROMATOGRAPHY



is termed *adsorption.* An example is the familiar use of charcoal as an absorbent of gases and for the removal of undesirable colors from industrial solutions, such as sugar solutions.

The substance to be separated is placed as a small spot near one end of a length of filter paper. The position of the spot is called the *origin.* This end is then immersed in a solution (solvent) or mixture of solvents in which the molecules to be separated have different solubilities. The paper chromatogram can be set up in various ways. In *descending chromatography* the trough containing the solvent may occupy an elevated position within a large, covered rectangular jar (see Fig. 5.6), in a large battery jar, or under a bell jar. In this case, the solvent moves down the paper as a result of gravity as well as capillary action. In *ascending chromatograhpy* the solvent is placed at the bottom of the jar. In this case capillary action moves the solvent up the paper.

As the solvent travels the length of the paper, the molecules being separated are differentially absorbed onto the paper at different distances from the spot where they were applied to the paper (Fig. 5.7). After the chromatogram is dry the position of the materials that have been separated is identified by spraying with a reagent that combines with the substance to give a color that is characteristic of that particular substance. If the substances being separated absorb ultraviolet light, or if they fluoresce, the spots may be detected readily by examining the chromatogram under ultraviolet light.

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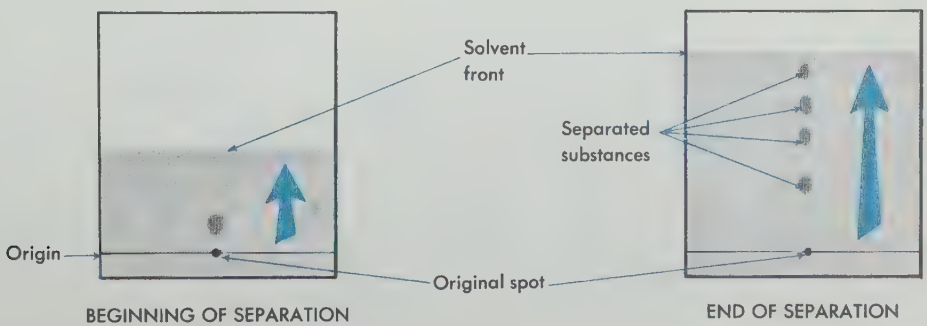
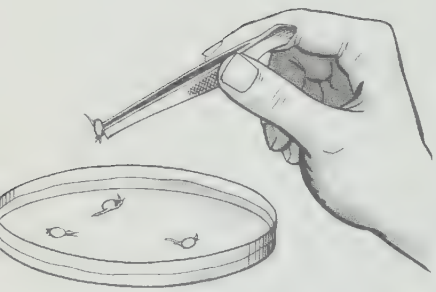
Stein, W. H., and S. Moore. "Chromatography." *Scientific American,* 1951.

FIG. 5.7 SEPARATION BY PAPER STRIP CHROMATOGRAPHY



6 CARBOHYDRATE METABOLISM



INTRODUCTION

In the broadest sense, metabolism includes all those events that occur in the cytoplasm of living cells. One of the more important results of these events is the formation of new cells. Along with new cell formation there is active synthesis and utilization of carbohydrates, proteins, lipids, and other complex organic materials. We will confine our studies to carbohydrate metabolism or, more specifically, to the digestion of starch.

Starch is a long-chain polysaccharide composed of glucose molecules, linked as shown here:

Starches are frequently stored by plants and animals for use as a potentially available energy source. The chemical conversion of starch into its component "sugar units" is called **digestion.** The important sugar unit we are concerned with is **glucose** because (1) it is a key compound in cellular metabolism (recall that it is synthesized from CO_2 and H_2O by photosynthesizing plants), and (2) it contains energy (found in the chemical bonds holding the molecule together) that can be released to do the work of the cell.

The digestion of starch is brought about through the activity of enzymes, called **amylases,** which cleave the starch molecule into smaller and smaller subunits until maltose, a reducing sugar, is obtained. Maltose is enzymatically converted to glucose, which may then be shunted into the glycolytic and Krebs cycles, where it is further broken down into carbon dioxide, water, and energy. This process, called **respiration,** will be considered in Unit 7.

Enzymes are proteins that change the rates of chemical reactions. Enzymes are, in effect, organic catalysts that are usually unchanged by the chemical reactions they catalyze. Most chemical reactions that occur in living cells require enzymes.

Living cells contain hundreds, perhaps thousands, of different enzymes. Each has a specificity for one, or a narrow class of, chemical compound(s). For example, specific enzymes are responsible for the formation of sugars from carbon dioxide and water. Other enzymes catalyze the formation of proteins and fats from other simpler molecules. One of the triumphs of modern biochemistry was the extraction of enzymes from living cells and having these enzymes perform, *in vitro* (in test tubes), the same reactions that they carry out in the living organism.

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McElroy, W. D., and C. P. Swanson. *Modern Cell Biology.* Englewood Cliffs, N.J.: Prentice-Hall, Inc., 1968.

EXERCISE 24

HOW CAN THE DIGESTION OF STARCH BE MEASURED?

Human saliva contains an enzyme called **salivary amylase,** which catalyzes the digestion of starch. The final product of the reaction is a sugar, **maltose,** that consists of two glucose units linked together. **Maltase,** which is secreted by the pancreas and the small intestine, digests the maltose.

The activity of salivary amylase can be followed by the iodine test. Iodine yields a deep, blue-black color with starch. As the starch is digested, repeated tests with iodine will give results that vary from the initial blue-black color of the undigested starch to a reddish hue for intermediate products in digestion. These intermediate products are called **dextrins.** When the digestion of starch by salivary amylase is complete, no change in the color of iodine will occur.

PROCEDURE

NOTE: The flow of saliva can be stimulated by chewing on a small piece of paraffin.

- Collect about a fourth of a test tube of saliva. Add an equal amount of tap water (Figs. 6.1A,B).
- Mix the saliva and water thoroughly by shaking the test tube. Then filter the mixture through a double layer of cheese cloth into a smaller beaker (Fig. 6.1C).
- Add 5 ml of starch solution to each of six test tubes numbered 1 to 6 (Fig. 6.1D).
- Follow the procedure for each tube as shown in the table on page 287 and in Figs. 6.1D–F. **24-A Write your observation of the reaction in each tube in the table on page 287.**

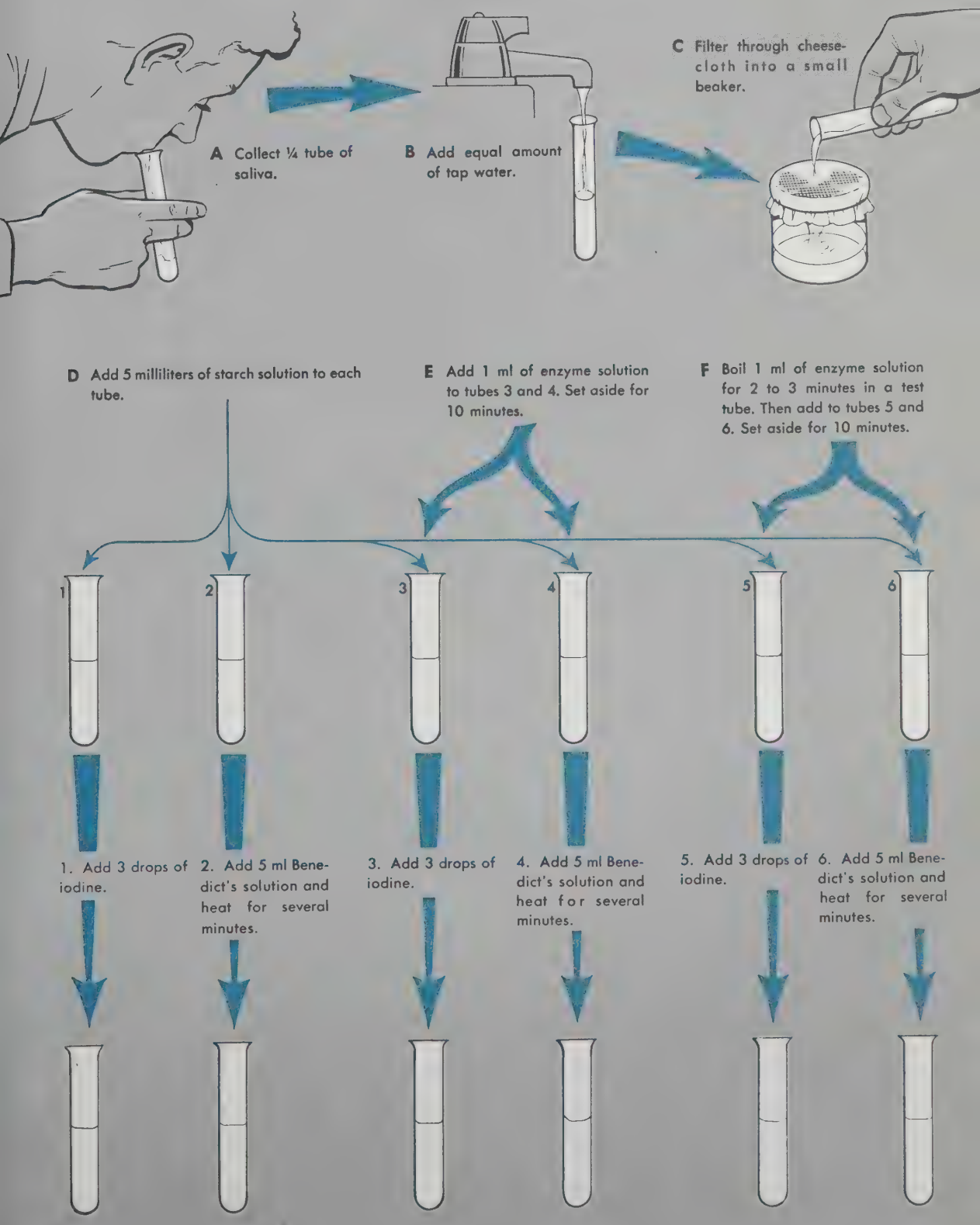
NOTE: If a Bunsen Burner is used to heat the contents of the tubes do not allow the solutions to boil. They will spurt out of the tube. As an added precaution, do not aim the open end of the tube toward anyone.

The presence of the sugar maltose can be shown by heating the sample solution for several minutes (in a boiling water bath) with an equal amount of Benedict's solution. If maltose is present the solution will become colored—green, red, orange, or brown—depending on the amount of sugar. Green denotes a small amount of sugar; brown, a large amount.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is the role of tubes numbered 1 and 2?
2 From the results of this experiment what can you conclude about the effect of temperature on enzyme activity?
3 Devise an experiment to determine the optimum temperature for salivary amylase activity.
4 Devise an experiment to adapt the iodine test to a quantitative measurement of starch digestion.

FIG. 6.1 PROCEDURE FOR STUDYING THE DIGESTION OF STARCHES



EXERCISE 25

WHAT IS THE EFFECT OF pH ON THE ACTIVITY OF SALIVARY AMYLASE?

In order to understand something about the chemical reactions that occur in cells and organisms, you must know something about acids, bases, and *p*H. A more detailed discussion can be found in the Appendix at the end of this exercise. Briefly, acids are substances that donate hydrogen ions (H^+). Bases are substances that combine with hydrogen ions. Finally, it is the concentration of hydrogen ions in an aqueous solution that determines the degree of acidity of the solution. One way of expressing the hydrogen ion concentration of a solution is in terms of the *p*H of that solution. On the *p*H scale, 7 is neutral —it is neither acidic nor basic. Anything less than seven is acid, anything greater is basic. The *p*H is important in cell metabolism and in regulating the activity of many large molecules.

PROCEDURE

▶ Collect about one-fourth of a test tube of saliva. Add an equal amount of tap water (Figs. 6.2A,B).

▶ Mix the saliva and water thoroughly by shaking the test tube. Filter the mixture through a double layer of cheese cloth into a small beaker (Fig. 6.2C). If more enzyme solution is needed, dilute whatever you collect with an equal volume of water.

▶ Fill four test tubes with 5 ml of solution having a *p*H of 3, 5, 7, and 9. Label each tube according to the *p*H of the solution.

▶ Now add an equal amount of starch solution to each test tube and mix the contents of each tube by shaking the tube.

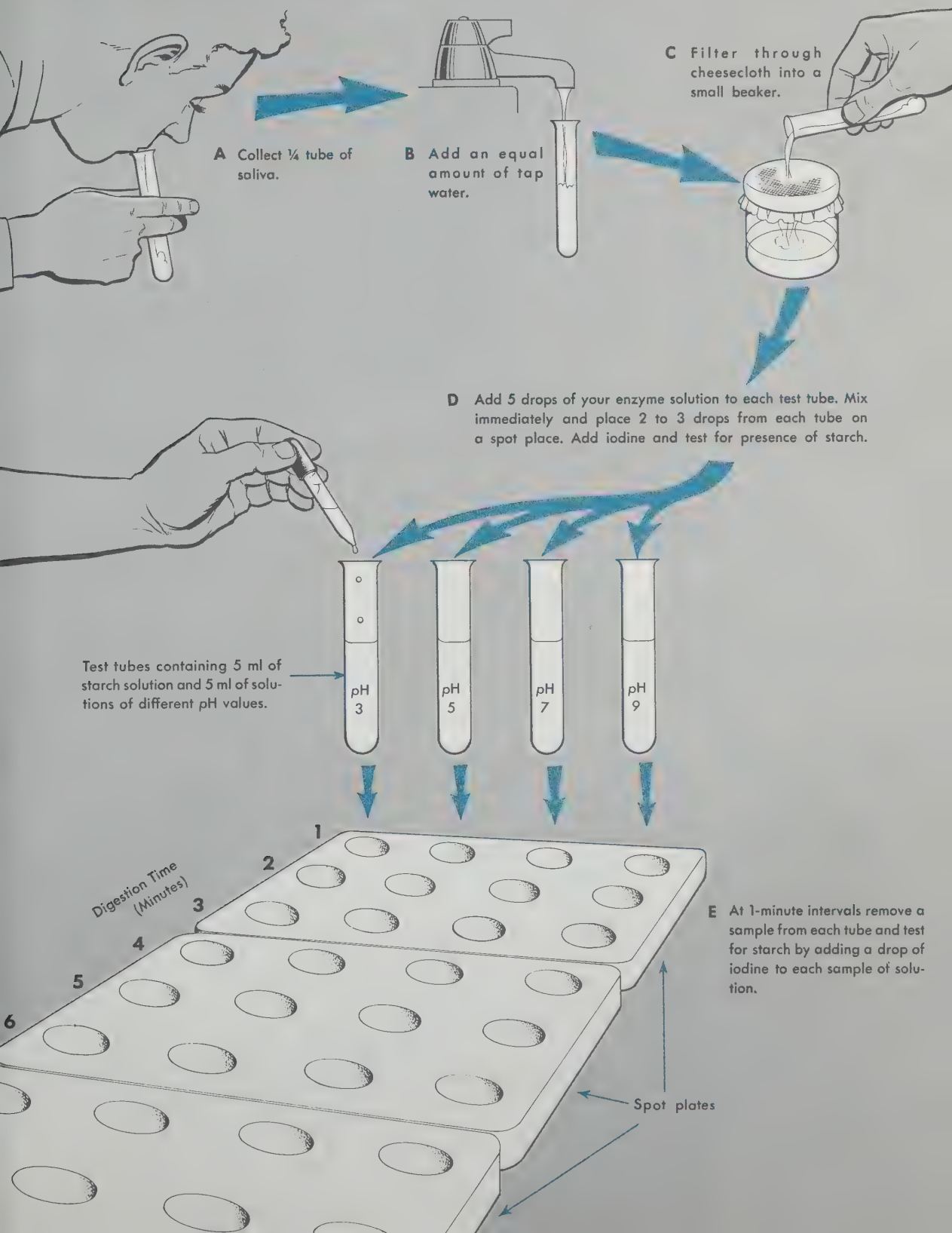
NOTE: Recall that starch plus iodine gives a deep blue-black color.

▶ Quickly add five *drops* of your enzyme solution to each tube (Fig. 6.2D). Shake each tube thoroughly. With an eye dropper, *immediately* remove a few drops of solution from *each* tube and test it for the presence of starch, using iodine.

▶ At one-minute intervals following your "0" reading, remove a sample from each tube and test for the presence of starch (Fig. 6.2E). In the first stages of digestion, starch is broken down to dextrins. These give a red color with iodine. When dextrin is digested to the sugar maltose, no color change will occur in the iodine test. The length of time it takes to reach this stage will be considered the length of time for complete digestion to occur.

25-A In the table on page 289 record a (+) sign if the starch test is positive, and a (−) sign if the test is negative.
25-B Graph your data (see page 289).

FIG. 6.2 PROCEDURE FOR DETERMINING OPTIMUM pH FOR SALIVARY AMYLASE ACTIVITY



FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Based upon your results in this study, what would you expect the *p*H of your saliva to be? How could you determine this?
2 Would you expect salivary amylase to be active in the stomach? Explain. Would you expect this enzyme to show activity in the small intestine? Explain.

APPENDIX

HYDROGEN ION CONCENTRATION (*p*H)

To understand something of the chemical reactions that take place in the cells of an organism, it is necessary to understand something about **acids** and **bases.** There are many kinds of acidic and basic substances in organisms.

Acids are substances that give up hydrogen ions (H^+). The concentration of the hydrogen ions determines the degree of acidity of a solution.

Bases are substances that can combine with hydrogen ions. For example, in an aqueous solution, hydrochloric acid (HCl) separates (dissociates) into H^+ and Cl^- ions.

$$HCl \rightleftharpoons H^+ + Cl^-$$

The chloride ion here acts as a base since it can combine with hydrogen ions.

Sodium hydroxide (NaOH) is a base and in an aqueous solution dissociates into sodium ions (Na^+) and hydroxyl ions (OH^-). The hydroxyl ion is basic and can unite with hydrogen ions to form water:

$$HCl + NaOH \longrightarrow \begin{matrix} H^+ + Cl^- + NA^+ + OH^- \\ H_2O + NaCl \end{matrix}$$

A basic substance, such as NaOH (or any substance that acts as a base) can remove hydrogen ions from solution. As the hydrogen ion concentration becomes smaller, the solution becomes less acid and more basic (greater *p*H than 7). The *p*H range is shown here:

← Acidic ——————— Neutral ——————— Basic →

0 1 2 3 4 5 6 7 8 9 10 11 12

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EXERCISE 26

DOES STARCH DIGESTION OCCUR IN GERMINATING SEEDS?

Seeds contain the embryo of a new plant. When seeds are placed in soil and given proper conditions of temperature and moisture, they germinate and give rise to seedlings having roots, stems, and leaves. As soon as the leaves are exposed to light the young plant can produce its own food by the process of photosynthesis. The organic compounds (usually sugars) that are formed as a result of photosynthesis can undergo numerous chemical changes in the cells of the plant. They may be converted into starch and stored or they may be broken down and provide energy. All of these reactions require the activity of enzymes. Therefore, we would expect to find enzymes in the developing plant. The embryo in the seed, however, has no way of producing its own food. It must rely on complex food molecules produced by the parent plant and stored in the seed. In this exercise we will attempt to find out how the embryo can make use of this stored food.

PROCEDURE

Before this laboratory meeting your instructor germinated corn seeds. Select two or three of the germinating seeds, each having a root about ¼ inch long.

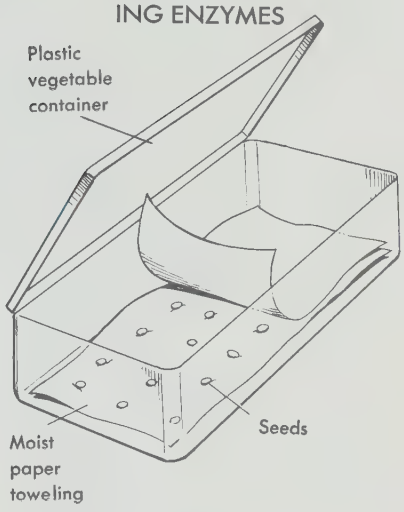
- Place the seeds on the surface of a starch-agar mixture in a petri dish so that the roots are pushed slightly into the surface (Fig. 6.3A).
- Set up the appropriate control for this experiment. Place the cover on the dish and wrap it in aluminum foil or set it in a dark place (Fig. 6.3B). After 24 to 36 hours, flood the surface of the agar with iodine solution (Fig. 6.3C). Let it stand for three to four minutes; then pour the iodine back into its container.

26-A Diagram your results on page 291. 26-B Do germinating seeds have starch digesting enzymes? 26-C What is the evidence for your answer? 26-D What evidence is there that partial digestion has occurred?

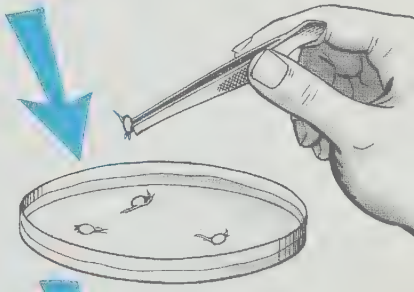
FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1. If amylase activity is present in the germinating corn seed, what role might it play in germination?
2. How could you use the procedure of this study to determine if the embryo in a seed is alive?

FIG. 6.3 PROCEDURE FOR DETERMINING IF GERMINATING SEEDS HAVE STARCH-DIGESTING ENZYMES

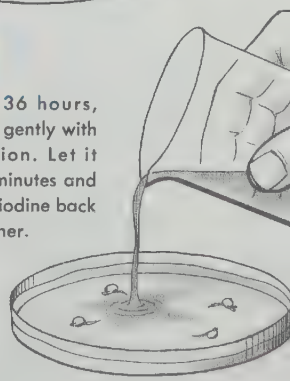


A Remove 2 or 3 seeds from germination tray and place them on the surface of the agar. Push the roots slightly into the agar.



B Cover the dish and place it in a dark place (or cover with aluminum foil) for 24 to 36 hours.

C After 24 to 36 hours, flood the dish gently with iodine solution. Let it stand 3 to 4 minutes and then pour the iodine back into its container.



EXERCISE 27

A MODEL OF LIFE: INGESTION, DIGESTION, AND EXCRETION IN AN ARTIFICIAL CELL

Cells, whether they exist as free-living, independent organisms, or as integrated parts of higher plants and animals, exhibit some degree of "choice" of materials they take in. For example, a protozoan such as an ameba, takes in (**ingests**) some particles of food but not others. Having taken in a potential food particle, the ameba **digests** it in part and expels (**excretes**) the indigestible remains.

Although the process of digestion is well understood, the mechanism by which cells take in some materials and discard others is only partly understood. The "model" you are about to examine can be visualized as a cell, a) taking in a particle which is partly food and partly indigestible material, and b) digesting the food and expelling the nonfood portion.

PROCEDURE

- Fill a test tube with about 3 inches of distilled water. Add seven or eight drops of chloroform (Figs. 6.4A,B).
- Shake the tube gently several times. Wait a few minutes for the drops of chloroform to fuse, forming a single drop about ⅛ inch across on the bottom of the test tube (Fig. 6.4C).
- From your instructor obtain a *clean* glass rod that has been drawn out to a fine tip. Try to put the tip of the rod into the chloroform drop (Fig. 6.4D). **27-A Describe what happens.**
- Thoroughly dry the glass rod and dip the tip (about 1/16 to ⅛ inch) into shellac. Blow on the shellac to dry it. Once again, try to insert the tip of the rod into the chloroform (Fig. 6.4E). Watch closely for several minutes and describe what happens. **27-B Does this "artificial cell" accept the "food particle" (glass rod)?**

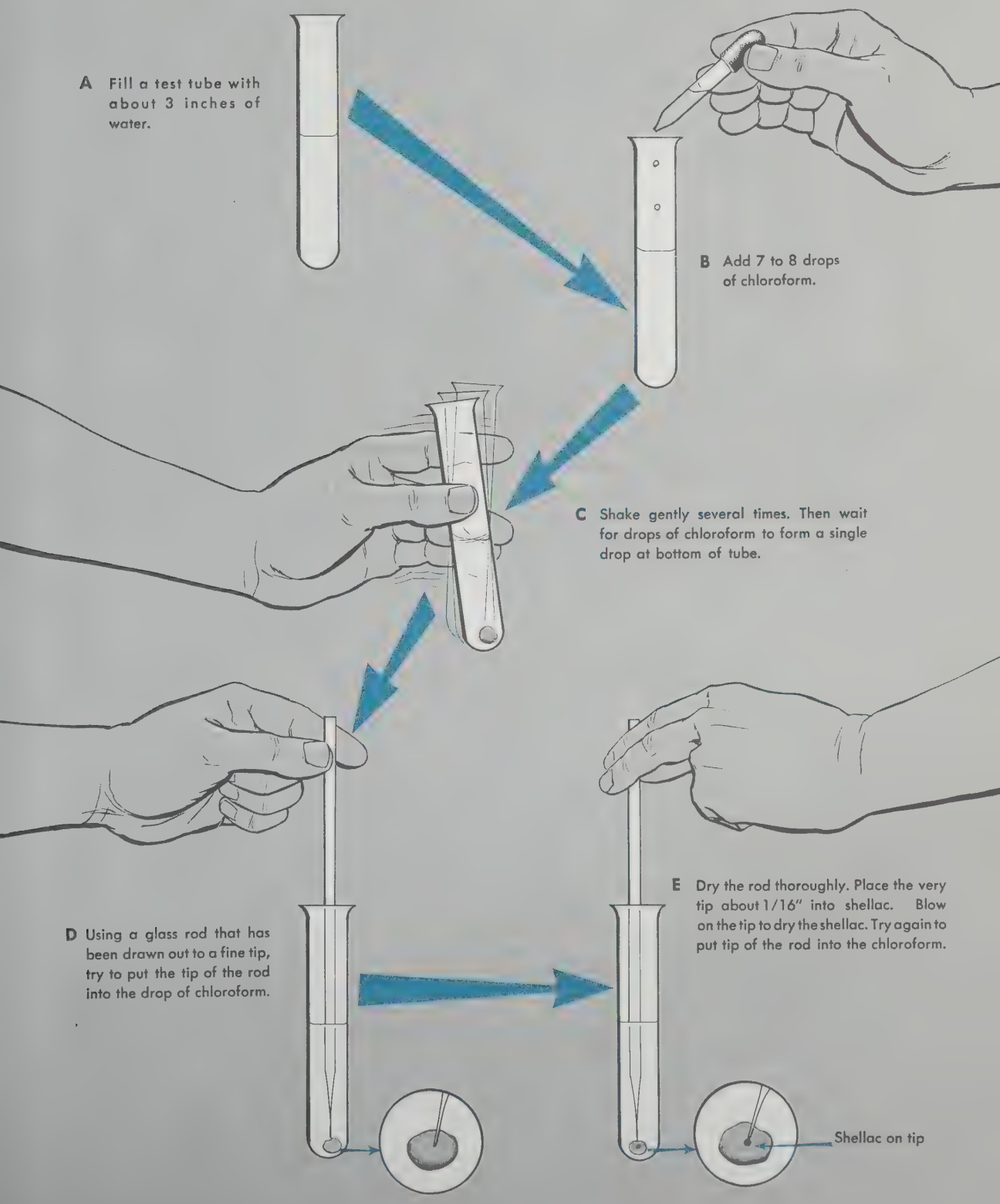
Your observations can be explained as follows: Chloroform, in water, tends to form a spherical shape because of surface tension.

Introduction of the glass rod, which is insoluble in chloroform, will increase the surface area of the drop and cause *a distortion of the spherical shape.* As a result, the rod tends to be repelled by the drop (or vice versa). When the glass rod is coated with shellac it is no longer repelled because it presents a substance (shellac) that is soluble in the chloroform. Eventually, however, the "cell" will expel the "food." **27-C Suggest a reason for this response.**

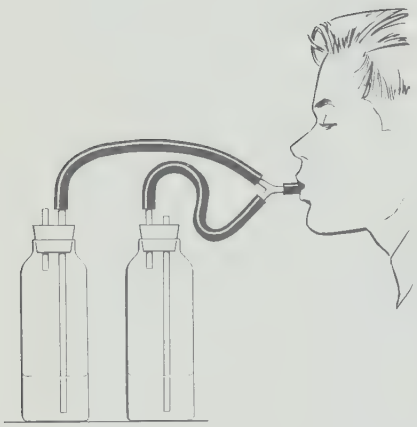
Surface Tension

Surface tension of fluids results when the molecules that make up the fluid attract each other more or less strongly, depending on the material of which the fluid is composed. At the surface the attraction is all directed to the inside, producing a tension that tends to contract the surface to a minimum. This is why fluids tend to assume a spherical shape and present the smallest surface for any given volume. Any distortion of the spherical form tends to be resisted by this surface tension.

FIG. 6.4 PROCEDURE FOR STUDYING SOME OF THE VITAL ACTIVITIES OF CELLS



7 RESPIRATION



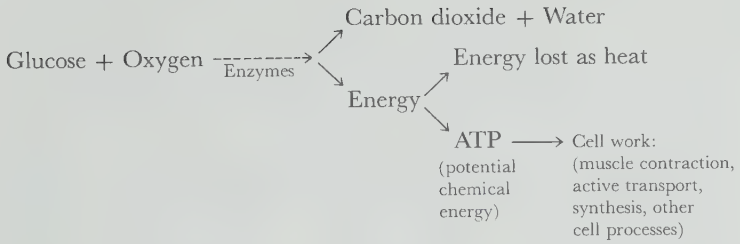
INTRODUCTION

All living things require energy to carry on their many activities. To obtain energy they need organic food substances that they can break down through the process of oxidation. This breakdown of food releases energy which is then made available for driving the other activities of the cells. In its broadest meaning, **respiration** includes all the processes involved in the release of this energy from food molecules.

It is necessary that the chemical energy in food be released in a slow, step-by-step process, not in one fast, wasteful surge as might happen if an organic molecule were burned in the laboratory. In this latter case most of the chemical energy would be transformed to heat which is quickly lost to the environment. In contrast, the energy released by respiration in the cell is converted step-by-step, releasing chemical energy which is available to the cell as needed.

The small packets of energy produced by the oxidation of glucose sugar are immediately used by the cell to change ADP (adenosine diphosphate) plus phosphate to ATP (adenosine triphosphate). These ATP molecules then become a sort of currency that can be cashed as needed. Their chemical energy can be transferred to other processes that require energy. Thus, these molecules serve as a storehouse of readily available energy.

Through respiration the cell is able to capture about 70 per cent of the chemical energy within the glucose molecule. In its energy conversion the cell shows a greater efficiency than your automobile motor which is able to convert only about 25 per cent of the energy in gasoline into energy useful in propelling the car. The use of glucose by the cell may be outlined as follows:



There are several ways in which we can demonstrate that respiration is occurring in an organism. One method measures the energy given off in the form of heat; another measures the amount of glucose used. Or we can experimentally determine that oxygen is being consumed, or that carbon dioxide is being given off as a waste product. In the following exercises we will study respiration by using several of these approaches.

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EXERCISE 28

HOW CAN OXYGEN CONSUMPTION IN RESPIRATION BE MEASURED?

When organisms are actively respiring, millions of molecules are involved. Sugar molecules are being oxidized, oxygen molecules are being consumed, and carbon dioxide molecules are being released. In this exercise you will measure the consumption of oxygen by living tissue by using a device called a **respirometer** (Fig. 7.1).

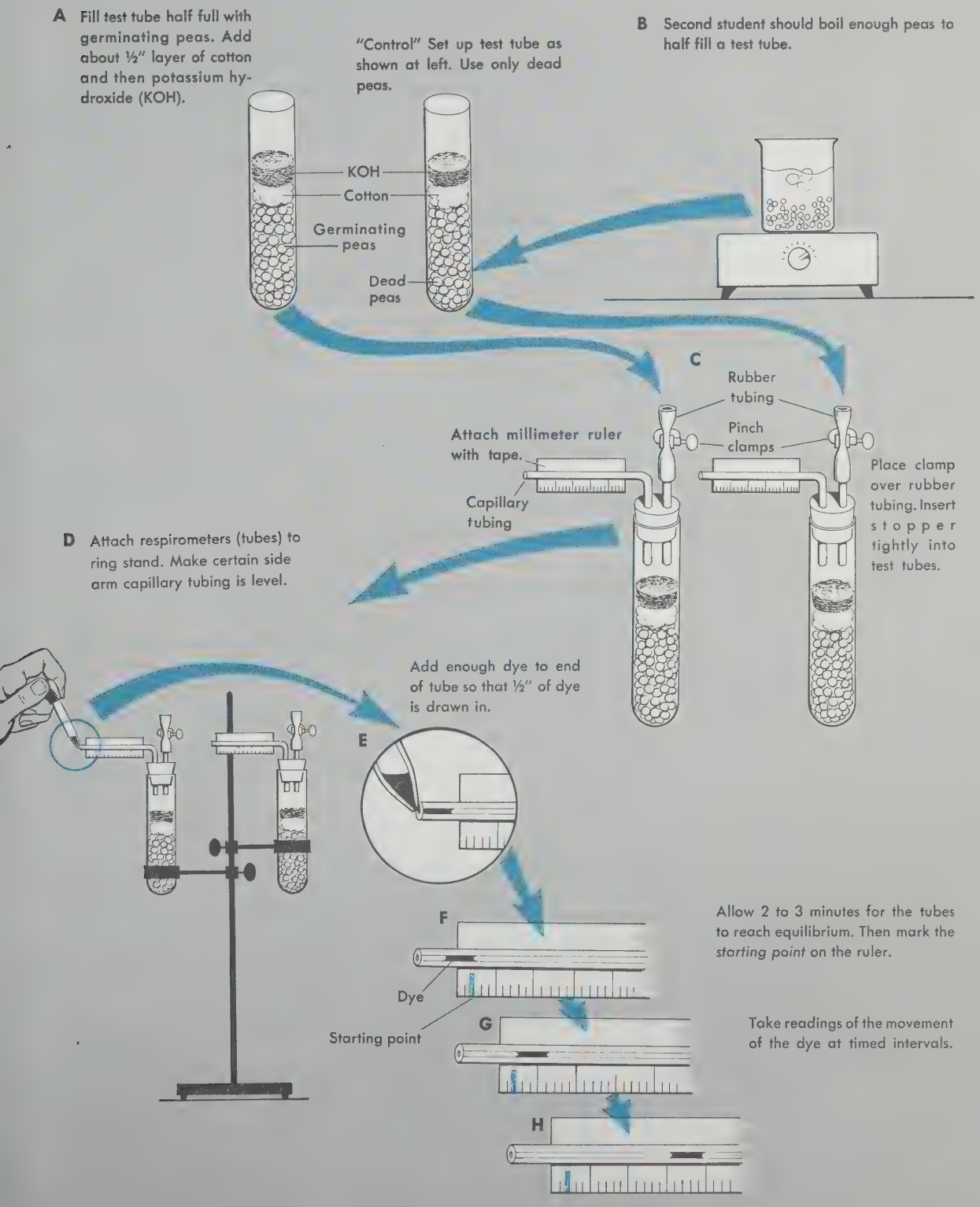
As the cells of the living tissue respire, pressure changes within the respirometer will be indicated by the movement of a dye or colored fluid in a capillary tube connected to the container. The fluid in the capillary tube will move toward or away from the respiration chamber in response to changes in the volume of the gases within the chamber.

PROCEDURE

- Half fill the test tube (respiration chamber) with germinating peas. While one student is assembling the respirometer, another student should boil an equivalent amount of germinating peas in water on a hot plate for five minutes (Figs. 7.1A,B). These peas will be used in the control experiment below.
- Place a loose wad of cotton over the peas in the respiration chamber.
- Add about a half inch of potassium hydroxide (KOH) pellets over the cotton (Figs. 7.1A,B). The cotton keeps the KOH from contacting the living seeds; it should not be packed tightly. KOH is a substance that will remove the CO_2 from the atmosphere of the tube as fast as it is given off by the respiring peas. **28-A Why is it necessary to remove the CO_2 from the tube?**
- Attach the pinch clamp to the rubber tubing to form a tight seal. Then insert the rubber stopper, with the attached tube, firmly into the test tube (Fig. 7.1C).
- As a control, assemble an identical respirometer, except that the peas have been boiled (killed). **28-B What is the purpose of using dead peas in the control?**
- Place the tube in a vertical position by clamping it to a ring stand (Fig. 7.1D).
- Using an eye dropper, add enough dye to the end of the capillary tube so that about a half inch of the dye will be drawn into the tube (Fig. 7.1E).

CAUTION: KOH is a strong alkali. Avoid direct contact with skin or clothing. Wash for several minutes with running water if you get KOH on skin or in the eyes.

FIG. 7.1 PROCEDURE FOR MEASURING OXYGEN CONSUMPTION IN GERMINATING PEAS



CAUTION: Since the respirometer is very sensitive to volume changes due to heat, it should be kept away from any heat sources (your hands, lamps, burners, etc.)

After allowing several minutes for the gas pressures to reach equilibrium, note where the outer end of the dye column reaches on the millimeter scale (Fig. 7.1F). **28-C Record this initial reading in the table on page 295.** For the next five minutes take readings of the location of the column at one-minute intervals (Figs. 7.1G,H). **28-D Why did the dye move toward the respiration chamber and not away from it? 28-E Under what circumstances might the dye move away from the chamber?** If the movement of the dye is fairly rapid, you must be prepared to take your readings at shorter intervals (20 or 30 seconds), or the column may reach the bent portion of the tube before you have enough readings to complete your data. The dye column can be returned to the outer end of the tube by opening the pinch clamp and tilting the capillary tube. **28-F Record the successive time and scale readings in the table (28-C). 28-G On page 296 graph the data you obtained for both the living and the dead peas.**

CAUTION: Dirt in the capillary tube may stop the movement of the dye.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Since there are several different gases in air, how could you prove that the gas being used in the tube was actually oxygen?
2 Why are the seeds soaked in water before the experiment is run?
3 What organic compound is being oxidized by the germinating peas?
4 Design an experiment that will measure (or at least indicate) oxygen consumption and carbon dioxide production at the same time.

EXERCISE 29

HOW CAN CARBON DIOXIDE PRODUCTION DURING RESPIRATION BE DETERMINED?

In single-celled organisms the gases involved in respiration (carbon dioxide and oxygen) are exchanged with the surrounding environment at the cell surface. In complex multicellular organisms (such as man) most cells are so far away from the external environment that they cannot rely on simple diffusion to provide this gas exchange. These organisms need a special system of organs, the respiratory system, to carry on this exchange with the external environment. In addition, they usually require a circulatory system to transport the gases between the respiratory organs and the cells.

In man, the main respiratory organs are the lungs where the exchange of gases between the organism and its external environment occurs. Because the tissues of the lungs constantly add to, or remove, certain gases from the air in the lungs, it is necessary that this air be periodically renewed. Breathing is the mechanical process by which the air in the lungs is periodically renewed. Thus, in man, respiration in the individual cell is dependent upon the breathing process.

PROCEDURE

▶ Add 100 ml of tap water to each of two gas-collecting bottles of 200 to 300 ml capacity (Fig. 7.2A).

▶ To each bottle add five drops of phenolphthalein (Fig. 7.2B). This chemical is colorless in a neutral or acid solution, but turns pink in a basic solution.

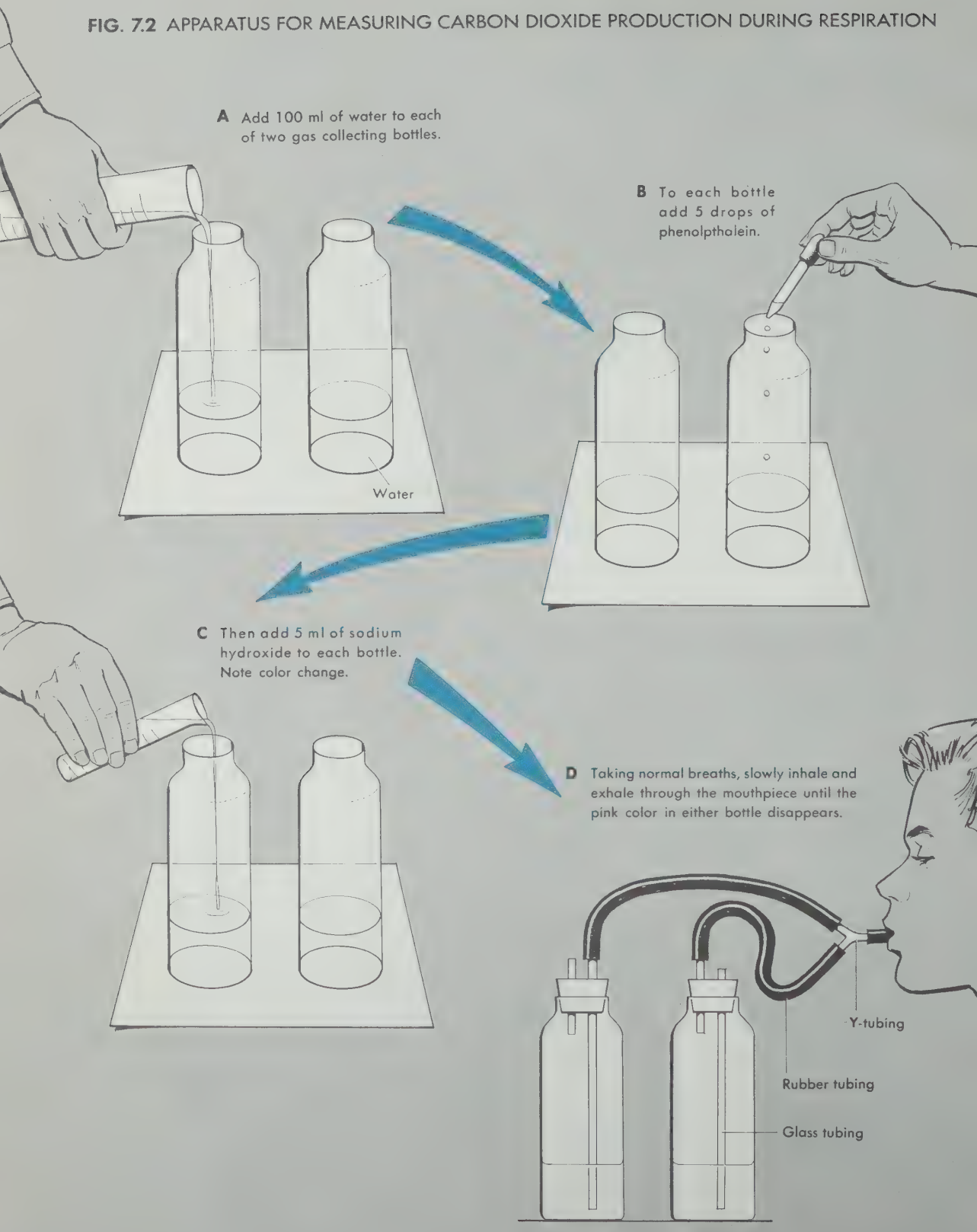
▶ To each bottle (Fig. 7.2C), add 5 ml of a 0.04 per cent solution of sodium hydroxide (NaOH). **29-A Note the color change. 29-B Is NaOH an acid or a base?**

▶ Insert two 5- to 6-inch pieces of glass tubing into a two-holed rubber stopper. Insert the stopper into the bottle so that one tube reaches well into the solution while the other tube extends only to the base of the stopper. Insert a similar stopper plus tubes into the other bottle (Fig. 7.2D).

▶ Connect three sections of rubber tubing about 6 inches long to a glass Y-tube. Then attach one of the rubber tubes from an arm of the Y to a glass tube that *extends into the solution* of one of the bottles. Connect the other arm of the Y to the glass tube that *does not extend* into the solution of the *other* bottle.

▶ Obtain a short piece of clean glass tubing and insert it into the free end of rubber tubing.

FIG. 7.2 APPARATUS FOR MEASURING CARBON DIOXIDE PRODUCTION DURING RESPIRATION



▶ Taking normal breaths of air, slowly inhale and exhale through the glass mouthpiece (Fig. 7.2D). Continue this procedure until you note the loss of pink color in either bottle. **29-C How many breaths of air are needed to cause the loss of color in this bottle? 29-D Has the solution that changed color become more acidic or basic?**

CAUTION: It is necessary that you slowly exhale and inhale. Hard breathing will force the solution out of the open tubes.

▶ Continue breathing through the apparatus for 20 or 30 more breaths. **29-E Does the pink color in the second bottle disappear? 29-F If you were to continue breathing through the apparatus would the color eventually change? Explain.**

▶ Remove the tube that served as a mouthpiece and place it in a soap solution as directed by your instructor. Remove the stoppers from the bottles and pour the solutions into the sink.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 The percentage of carbon dioxide in atmospheric air is 0.04 per cent and in exhaled air it is 4.5 per cent. Using these percentages determine the number of breaths needed to remove the color from the bottle receiving the inhaled air.
2 Why is the phenolptholein used in this experiment called an "indicator?"
3 How many breaths were needed by the members of your class to discolor the indicator? Discuss several reasons why individuals vary in their respiratory rate as indicated by the number of breaths needed to discolor the indicator.
4 What is the acid that is formed when CO_2 is blown through the water.
5 In order to use this experiment to compare the amount of carbon dioxide produced by different individuals, what variables would you need to control?
6 What is a basal metabolism test? What gases are measured in obtaining a person's basal metabolism rate?

EXERCISE 30

WHAT IS THE EFFECT OF TEMPERATURE ON RESPIRATION?

Many factors affect respiration. Among these are the general state of health and the degree of physical activity of the organism. The activity of certain hormones will also markedly affect respiration. In this exercise you will attempt to determine what effect temperature has on the respiratory rate of *poikilothermous* and *homeothermous* animals.

Poikilothermic animals are deficient in the necessary internal mechanisms for regulating body temperature. As a consequence, their respiratory rate tends to be directly related to the temperature of their environment. In contrast, homeothermic animals have the ability to maintain their internal temperatures and, therefore, are less affected by temperature changes in their environment.

In this exercise you will measure the volume of oxygen consumed during respiration by using a respirometer that consists in part of a **manometer** (Fig. 7.3). In its simplest form, a manometer is a U-shaped glass tube that is partly filled with water. Refer to the Appendix at the end of this exercise for a discussion of how the manometer is used to measure respiration.

A variety of animals are available in the laboratory. You are to measure their respiratory rates, and, using the data obtained, determine whether the animal you have tested is poikilothermic or homeothermic.

PROCEDURE

▶ Your instructor will determine the temperature you are to use in these experiments. Refer to Figs. 7.3A,B,C for the procedure to use to obtain the desired temperature.

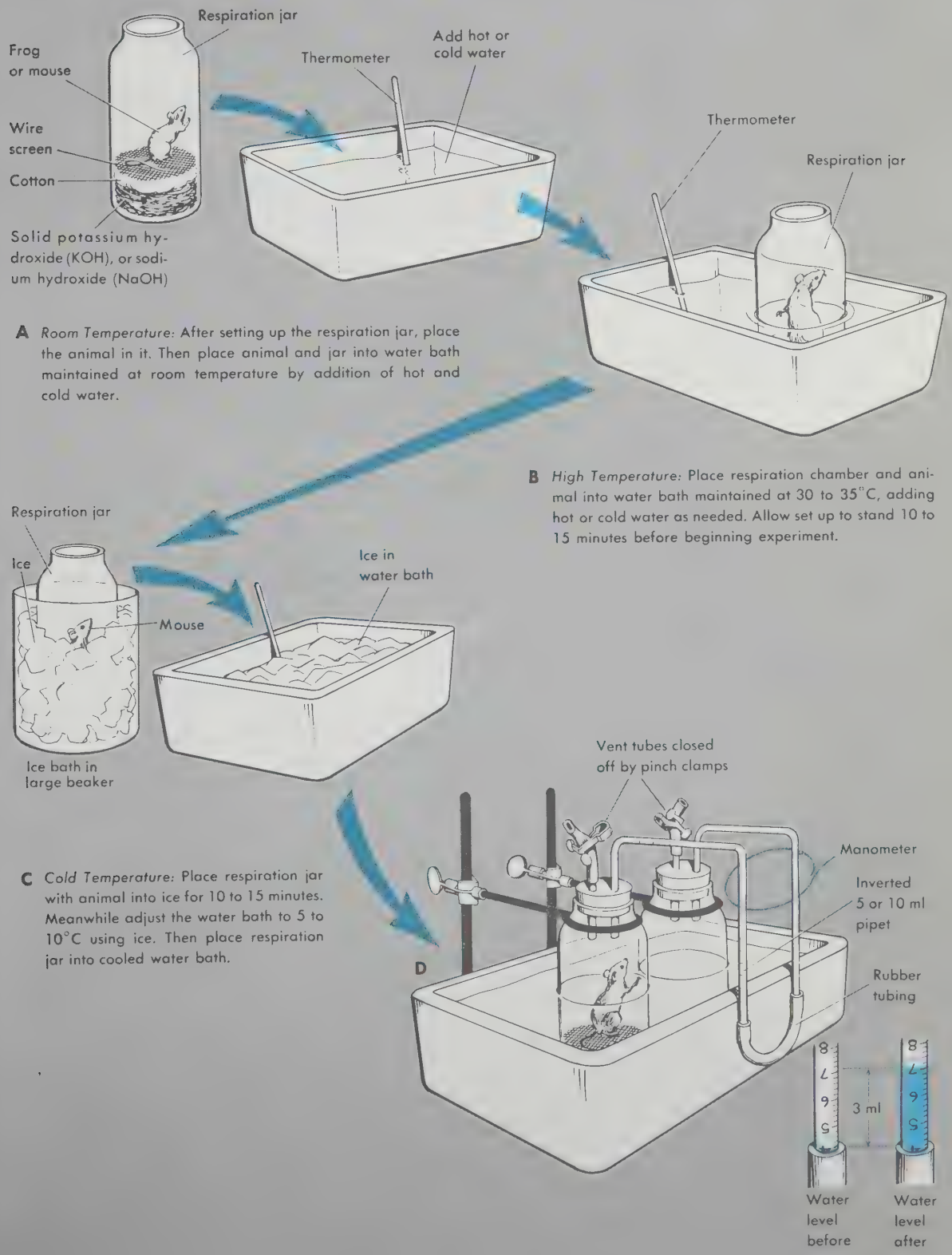
▶ Working in teams of two, weigh the animal to the nearest gram. **30-A Record the animal's weight in the table on page 299 in the temperature column you are going to work with. 30-B Since different animals and different temperatures are being used, the data collected should be exchanged with other teams and entered in the second table, (30-B).**

NOTE: If the balance you use is calibrated in pounds, convert the weight to grams.
1 pound = 454 grams
1 ounce = 28.5 grams

▶ After the animal and water bath are at the desired temperature, allow the set-up to stand for five to 10 minutes with the vent tube (Fig. 7.3D) open.

▶ Then attach a pinch clamp to each vent tube to seal them. Record the initial height of the fluid (in millimeters) in the pipet arm of the manometer (Fig. 7.3D). **Place this number in the table (30-A).**

FIG. 7.3 PROCEDURE FOR MEASURING RESPIRATION RATES OF ANIMALS



A *Room Temperature:* After setting up the respiration jar, place the animal in it. Then place animal and jar into water bath maintained at room temperature by addition of hot and cold water.

B *High Temperature:* Place respiration chamber and animal into water bath maintained at 30 to 35°C, adding hot or cold water as needed. Allow set up to stand 10 to 15 minutes before beginning experiment.

C *Cold Temperature:* Place respiration jar with animal into ice for 10 to 15 minutes. Meanwhile adjust the water bath to 5 to 10°C using ice. Then place respiration jar into cooled water bath.

D

NOTE: Choose a time interval in whole minutes to make succeeding calculations easier.

After five minutes, record the new position of the manometer fluid in the table (30-A). The time interval used will depend upon the respiratory rate of the different animals. Make it long enough so that the water column rises approximately one-third the height of the pipet.

After completing one such reading, remove the pinch clamps from the vent tubes. This will allow the fluid in the manometer to equalize.

Replace the pinch clamp and repeat the third, fourth, and fifth steps twice more. Calculate the average value and then determine how much oxygen was used per hour. Example:

$$\frac{12 \text{ milliliter } O_2}{\cancel{6}\ \text{minute}} \times \frac{\overset{10}{\cancel{60}}\ \text{min}}{1 \text{ hour}} = \frac{120 \text{ ml } O_2}{1 \text{ hour}}$$

$$= 120 \text{ ml } O_2 \text{ per hour.}$$

Then determine the respiratory rate of each animal in terms of the ml 0_2/kg/hour as follows:

$$\frac{\text{ml } O_2/\text{hour}}{\text{wt in gm}/1000} = \frac{120 \text{ ml } O_2/\text{hour}}{120 \text{ g}/1000} = \frac{120 \text{ ml } O_2/\text{hour}}{0.12 \text{ kg}}$$

$$= 1000 \text{ ml } O_2/\text{kg/hour}$$

Record your data in the table (30-A).

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Based upon your data, and the data from other students using the same type of animal, was the animal you used homeothermic or poikilothermic? Explain.
2 What is the difference between breathing and respiration?
3 Does respiration occur in plants? Explain your answer.
4 Why does the respiratory rate increase so dramatically during strenuous exercise?

APPENDIX

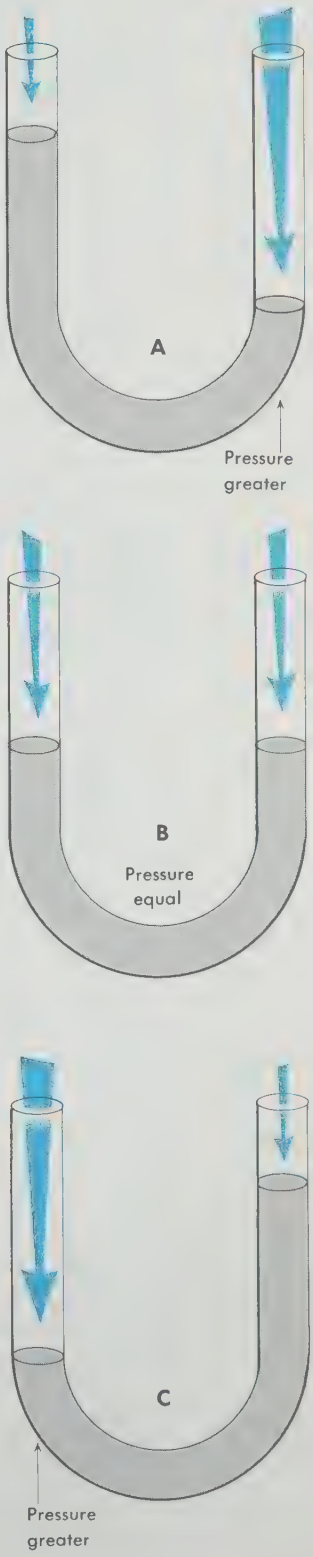
HOW A MANOMETER WORKS

In its simplest form, the manometer is a U-tube that is partially filled with water. The manometer measures the difference in the pressure between the two sides of the U-tube. For example, when the pressure on both sides of the tube is equal, the water level in both tubes are equal (Fig. B). However, when the pressure over one tube is greater than over the other tube, the water level will be lower in the tube having the greater pressure (Figs. A,C).

In the respirometer used in this exercise, the manometer is connected to two jars. These jars may be closed off from the pressures of the outside atmosphere with pinch clamps attached to the vent tubes (Fig. 7.3D). Any pressure changes that may develop in these jars will show up as a vertical movement of the fluid in the manometer tube. These changes may be measured if one arm of the manometer is composed of a measuring device called a pipet.

If a live animal is placed in one of the jars and the vent tubes are sealed, then the exchange of respiratory gases (O_2 and CO_2) will be restricted to the inside of the jar. The oxygen in the jar containing the animal will be used up and will be replaced by an equal amount of CO_2. Under these conditions, there will be little or no pressure change in the jar. If, however, some compound that absorbs CO_2 as it is exhaled is placed in the jar, then the CO_2 will be removed from the atmosphere in the jar. Consequently the total amount of gas in the jar containing the animal is lowered. This results in a decrease in the pressure in the jar containing the animal. **Why?** As a result, the fluid in the manometer tube moves. **In which direction?**

The empty jar attached to the other side of the manometer tube serves as a temperature-volume control. It provides an atmosphere that initially is identical with the jar containing the animal. Since no respiration is occurring in this empty jar, there will be no change in the gas pressure. When both jars are placed under varying temperature conditions, this arrangement insures that any changes in the height of the manometer fluid are due to pressure changes brought about by the respiratory activities of the animal in the respiration chambers.



8 BIOLOGICAL TRANSPORT

INTRODUCTION

The movement of water, metabolic wastes, food, hormones, minerals, gases, and many other materials within the organism is extremely important to higher plants and animals. Almost every metabolic activity of the organism depends ultimately upon the exchange of materials between cells, and between the organism and its environment.

Nutrient molecules must be absorbed, body wastes removed, and oxygen obtained to support aerobic respiration. A variety of substances including such diversified materials as chemical regulators (hormones) and defensive materials (antibodies) must be exchanged if the organism is to function coherently. Basically, exchange depends on physical processes such as diffusion, osmosis, pinocytosis (literally, "cell-drinking"), and biochemical processes such as active transport.

Since these phenomena can affect rapid movement over short distances, very small organisms (for example, protozoa) or organisms with most of their cells in contact with the environment (sponges) rely almost entirely on them to achieve exchange. Larger and more complex organisms require supplementary systems. With only a small fraction of their cellular mass in direct contact with the environment, exchanges based solely on diffusion would not occur rapidly enough to meet their needs.

In higher plants the vascular system, composed of **xylem** and **phloem** tissues, comprises a continuous network of conducting tissues. This network, aided by root pressure, transpiration, and gravity, moves water, minerals, and the products of photosynthesis to all parts of the plant body where they may be used or stored.

Circulatory systems in animals vary enormously in complexity. In the simplest cases movement of extracellular fluids is achieved

by the compressive action of body wall muscles or by ciliary activity. Usually, however, some type of pumplike heart is formed. The pump may consist simply of a group of pulsatile vessels, as is the case in the earthworm, or it may take the form of a simple membranous chamber, as in insects. In vertebrates and in many mollusca the heart is a complex, chambered structure. In any event, the evolution of a specialized heart is normally associated with the development of vessels that direct the flow of extracellular fluids to specific areas of the body. In insects and some other invertebrates such vessels form incomplete systems, since the fluids they carry are emptied into irregular cavities and return to the heart through the body spaces. Such circulatory systems are said to be "open" in type, and there is no distinction between the fluids within and outside the heart and vessels.

In vertebrates the circulatory system is completely enclosed in a continuous series of vessels. The blood carried in these vessels is distinctly different from the extracellular fluid in many respects. Blood flow takes place within this tubular system, and exchange between the blood and extracellular fluids occurs through the smallest vessels (capillaries) and irregular cavities (sinuses).

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EXERCISE 31

WHAT IS THE EFFECT OF VARIOUS ENVIRONMENTAL FACTORS ON TRANSPIRATION?

Most land plants obtain water from the soil. However, only a small amount of the water absorbed by the roots is used in growth and photosynthesis. The rest is lost through **transpiration,** a process in which water is lost (as water vapor) from the surface of leaves, or in some cases, from other aerial parts of plants.

In this exercise you will use an apparatus called a **potometer** to determine the effects of various environmental factors on the rate of transpiration.

PROCEDURE

▶ Completely cover the potometer flask (except for the openings) with aluminum foil.

▶ Using a 2-inch piece of rubber tubing, attach a 15-inch length of capillary tubing to the potometer flask. Support the capillary tubing in an *elevated position,* using a clamp and ring stand as shown in Fig. 8.1A. Attach a millimeter ruler to the back of the tubing with tape.

NOTE: The rubber tubing must fit tightly on the capillary tubing to prevent air leaks.

▶ Fill the flask to the brim with water provided by your instructor. Pour the water in slowly to avoid the formation of bubbles.

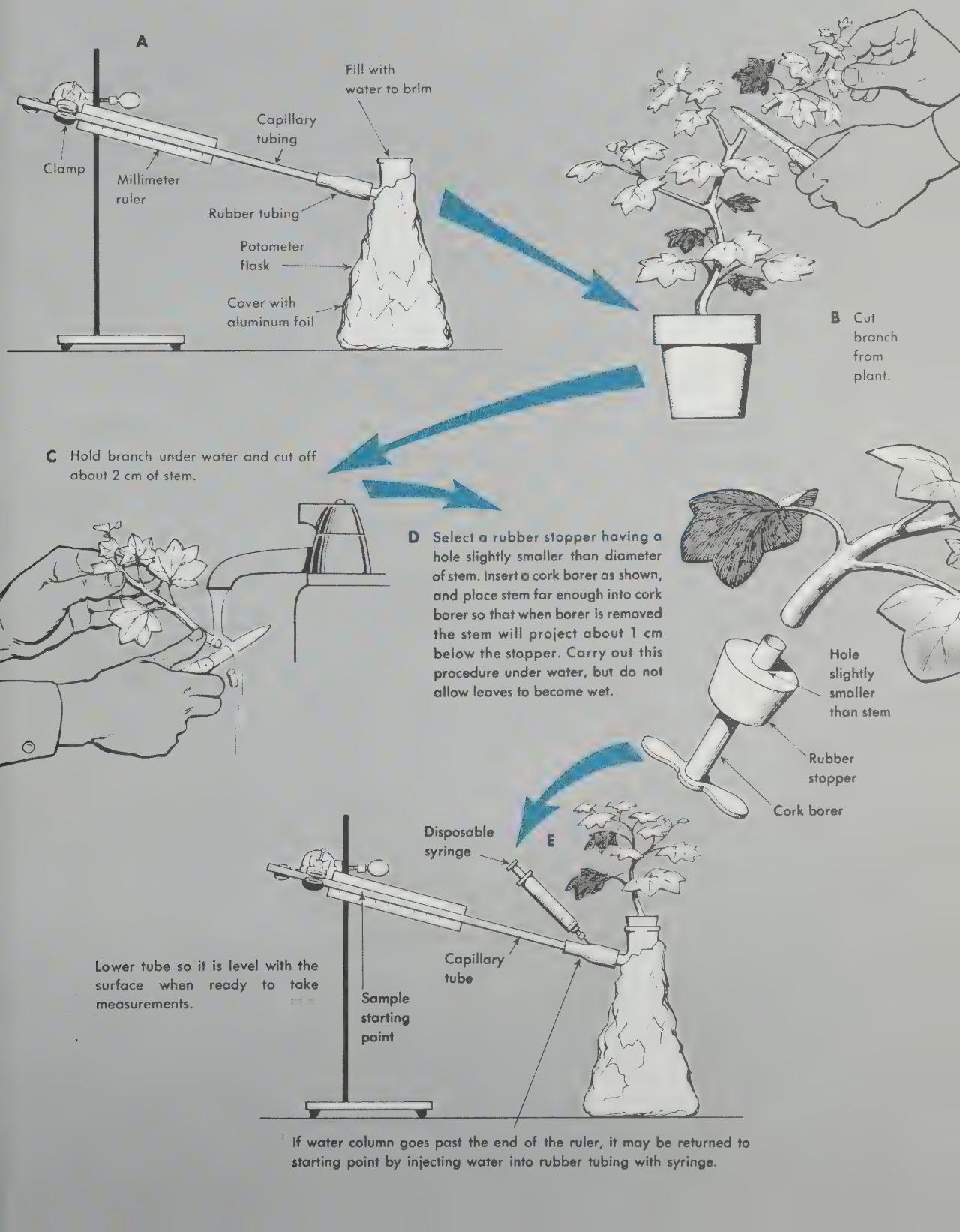
▶ Following the procedure shown in Figs. 8.1B,C,D, cut a branch from a geranium plant and insert it into a rubber stopper. *Keep the cut end moist, but avoid wetting the leaves.*

NOTE: After cutting the branch off, hold the cut end under a running faucet (avoid wetting the leaves!) and cut another inch off. The cut surfaces must be kept moist to prevent breaking the column of water in the vascular tissues of the branch.

▶ Slowly insert the rubber stopper and branch into the flask to avoid creating bubbles. (If this is done properly, water will be forced out of the end of the capillary tubing. When the pressure on the stopper is released, the fluid in the capillary tubing will tend to move back toward the flask. If this should occur, fill a syringe with water and insert the needle into the rubber tubing at the place where the capillary tubing and the flask join. *Slowly* inject water until it comes back out of the end of the capillary tubing.)

▶ Loosen the clamp on the ring stand and lower the capillary tubing so that it is level with the surface of your table or desk (Fig. 8.1E). If the apparatus has been properly set up, the water column in the tube will begin to recede toward the flask. **31-A What is responsible for this movement of water?** The rate at which the water moves is a measure of the rate of water uptake by the branch and may be used as a measure of the rate of transpiration.

FIG. 8.1 PROCEDURE FOR DETERMINING THE RATE OF TRANSPIRATION



NOTE: If the water column goes past the end of the ruler nearest the flask, it may be returned to your starting position by injecting water into the rubber tubing connecting the capillary tubing to the flask.

▶ Determine the transpiration rate by recording the distance the water column moves each minute for a period of 10 minutes (be prepared to change to shorter or longer intervals of time depending on the rate of water movement in the column). **31-B Record your results in the table on page 301. 31-C Graph your data on page 302.**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Under what conditions in nature would you expect a plant to have a high or low rate of transpiration?
2 Did you have a "control" for this experiment? If not, suggest one.
3 How do you think a scientist would proceed to measure the actual force of the transpirational pull in this experiment?
4 How is the movement of water and dissolved substances in a plant related to transpiration?
5 In this experiment, what parts of the apparatus represented the missing (cut off) parts of the whole plant?
6 In order for plants growing in a desert to survive, what are some of the adaptations of the leaves or other organs that you would expect to find?
7 If you used the procedure in this experiment, what would be the effect of the following on the rate of transpiration—light intensity, air movement, humidity, others? Enter your results in the table on page 301 and graph your data in Fig. 31-C.
8 Devise a method for estimating the volume of water lost in transpiration per unit area of leaf surface in a given time (using the apparatus of this experiment).
9 Of what value is this control of water loss to the plant?

EXERCISE 32

HOW CAN THE MOVEMENT OF MINERALS IN PLANTS BE MEASURED?

In recent years, the study of relationships between structure and function in plants and animals has been greatly aided by the use of radioactive substances called **radioisotopes** (see Appendix).

Radioisotopes can be used to trace the pathways of various substances during their movement throughout an organism. When used in this way they are called **tracers.** For example, research biologists using radioactive carbon dioxide ($C^{14}O_2$) have been able to identify the various intermediate compounds that the carbon becomes associated with during the various steps in the photosynthetic process.

In this exercise uranyl nitrate (a radioactive compound) will be used to determine if the minerals that are absorbed by roots are distributed throughout the plant.

PROCEDURE

- Carefully remove two young bean seedlings from their container (Fig. 8.2A). Try not to damage the roots.
- Wash the roots with tap water (Fig. 8.2B).
- Place one of the plants into a flask containing a solution of uranyl nitrate so that the roots are completely submerged (Fig. 8.2C). Label this flask "experimental."
- The second plant should be set up as your control (Fig. 8.2D). **32-A In what solution should the roots of the "control" plants be submerged?** Label this flask "control."
- Cover both flasks with aluminum foil and place them on a window ledge in the light for one to two hours.
- Next lift the plants out of the flasks just high enough so that the root can be cut off at the base of the stem. Allow the roots to fall back into the flasks (Fig. 8.2E). Return the containers to your instructor for disposal.
- Place each plant on a separate X-ray film pack. Spread the leaves so they do not overlap and tape the plants to the film packs (Fig. 8.2F). Mark each film pack with your name, the date, and indicate if "experimental" or "control."
- Now place each film pack between two pieces of blotting paper (or paper towels) and add a heavy weight (two or three heavy books) for 10 to 15 days.
- At the end of this time (they may be left for a longer period) develop each film according to the directions given by your instructor. Discard the plants and blotting paper in the container provided for this purpose.

NOTE: Use disposable plastic gloves when handling radioactive materials.

FIG. 8.2 PROCEDURE FOR PREPARING AN AUTORADIOGRAPH

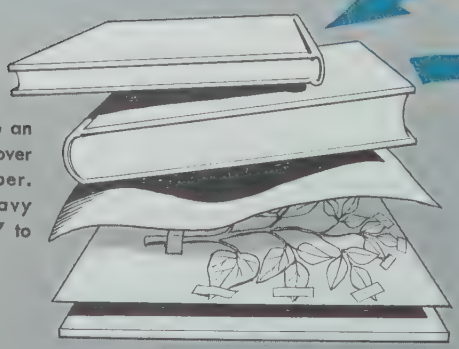
Remove 2 plants from the container and wash the roots under tap water to remove soil.

Cotton

C "Experimental" (Solution: Radioactive uranyl nitrate or P 32)

D "Control" (Solution: _____ _____)

E Cut the roots from the stem. Allow the roots to fall back into the flask. Give the flask to your instructor.



F Tape the plants to an X-ray film pack. Cover with blotting paper. Place 2 or 3 heavy books on top for 7 to 10 days.

G Develop film as shown by your instructor and record your results in a drawing on page 303.

The radioactive uranyl nitrate is emitting high energy particles. When these strike the emulsion of the film, silver ions in the emulsion are reduced to metallic silver, which produces a black spot on the film. In this way you will be able to show the distribution of the radioactive uranyl nitrate in the plant. This procedure is called **autoradiography.** The picture you obtain is an **autoradiograph.** **32-B Is the radioactivity uniformly distributed throughout the shoot (stem and leaves)? 32-C If not, in what regions of the shoot is it most highly concentrated?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is the source of the oxygen released through photosynthesis? Could you use radioisotopes to find out? Explain.
2 What is the pathway for the movement of minerals, water, and organic foods in plants? How could you demonstrate it?
3 Why is nitrogen important to plants?
4 What is **transpiration?** What role does transpiration play in the movement of water and minerals in plants?

APPENDIX

SAFETY PRECAUTIONS IN THE USE OF RADIOISOTOPES

Many radioactive compounds occur in a natural state in nature. Certain other basically stable elements can have their atomic structure rearranged to make them artificially radioactive. These are called **radioisotopes** and each has a distinctive radiation.

The high energy radiation emitted by radioactive substances are of three types: alpha and beta particles, and gamma rays. Gamma rays have the greatest power of penetration.

Because of the constant loss of particles, radioisotopes are said to "decay." The amount of radioactive material that remains will therefore be reduced with time. The rate of decay is known for many radioisotopes and is expressed as the **half-life** of the isotope. Half-life is the length of time it takes for a radioactive substance to lose half of its radioactivity. For example, the half-life of uranium-238 is 4,500,000,000 years. Phosphorus-32 is 8.0 days.

Because radiation is damaging to human tissues, any radioactive materials used in the laboratory must be handled with extreme caution. Radioisotopes should be handled with the same care given to harmful bacteria or strong acids. Remember, your "senses" cannot detect radiation. You *must,* therefore, use proper techniques and follow certain rules.

1 Radioactive materials should never be brought in contact with the skin, and certainly should not be taken into the mouth. Always use disposable plastic or rubber gloves when handling radioactive materials.
2 If available, a Geiger-Mueller counter should be used at the end of each work period to check your hands, clothes, work tables, equipment, and so on for contamination. Any radioactive material that is spilled should be washed up thoroughly in the presence of your instructor. Use aluminum foil to cover any work surfaces.
3 Contaminated materials and radioactive "wastes" should be placed only in those containers specifically provided for the materials. **If in doubt where to dispose of something, ask your instructor!**
4 All containers of radioactive material *must* be properly labeled (date and contents). If several bottles are to be transported, hold them at least 12 inches away from your body. Never carry a carton of radioisotopes in your lap. This could damage the reproductive organs.

EXERCISE 33

STRUCTURE AND FUNCTION OF THE HEART

The heart is an ideal organ for demonstrating a most important biological concept—the relationship of structure to function. Every part of the heart is marvelously constructed for the performance of some special function.

PROCEDURE

In the living organism the heart lies in a fluid-filled membranous sac called the **pericardium.** The pericardium has probably been removed from the specimen you are to work with.

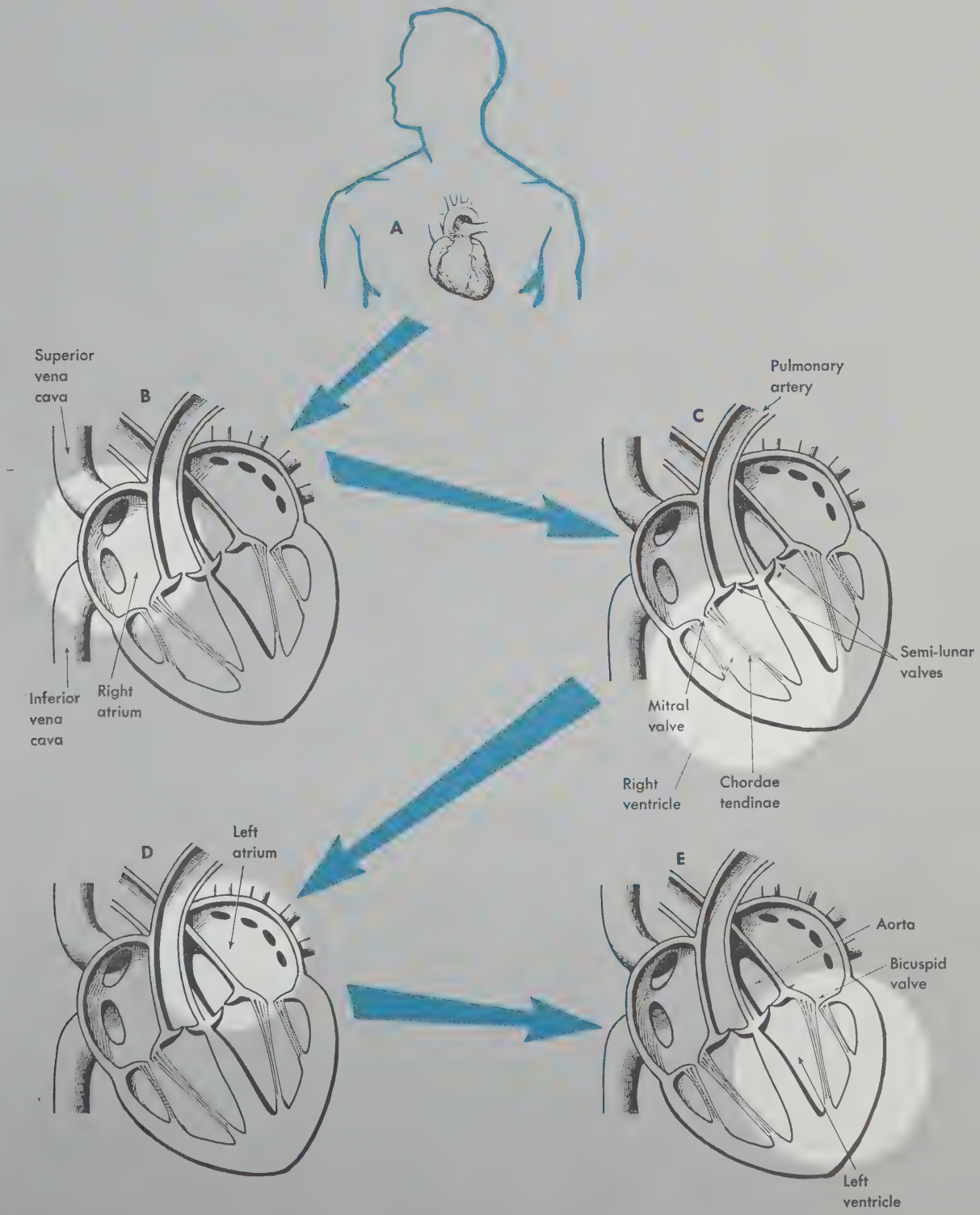
▶ Place the heart in a dissecting tray with the pointed end (apex) toward you. The sheep heart, like all mammalian hearts has four chambers—two **atria** (auricles) and two **ventricles.** The pointed apex consists entirely of the left ventricle. The walls of this chamber are considerably thicker than those of the right ventricle. Pick up the heart and feel the muscular walls of the ventricles. Locate the position of the left ventricle.

▶ Place the heart on the table in front of you so that the left ventricle is on your right. This is the position of the heart in the body (Fig. 8.3A). All of the steps that follow will be based upon this position. Have your instructor check the sheep heart to make sure you have oriented it correctly.

▶ Your instructor will demonstrate how he wants you to cut the heart. Follow his directions and dissect the heart. With the help of Fig. 8.3, locate the following:

- Right and left atria
- Right and left ventricles
- **Superior** and **inferior vena cavae** (Fig. 8.3B). These blood vessels enter the right atrium on the dorsal surface (the side facing the vertebral column when in the animals body). Push a glass rod, or wooden probe into each blood vessel and find their openings into the atrium. **33-A Are the vena cavae arteries or veins? Explain. 33-B Is the blood entering the right atrium high or low in carbon dioxide content? Explain.**
- Locate the **mitral** (or **tricuspid**) valve which consists of three membranous flaps projecting slightly into the right ventricle (Fig. 8.3C). Each "cusp" of this valve is attached to the walls of the right ventricle by muscular strands called **chordae tendinae.** **33-C What is the function of the mitral valve? 33-D What role do the chordae tendinae play in the functioning of this valve?**

FIG. 8.3 STRUCTURE OF THE MAMMALIAN HEART



- With your probe, locate the **pulmonary artery** in the upper corner of the right ventricle (Fig. 8.3C). **33-E To which part of the body does this vessel carry the blood?**
- The blood entering the pulmonary artery passes through **semilunar** (half-moon) valves. **33-F How do the semilunar valves prevent blood from flowing back into the right ventricle?** (You may have to cut into the artery to determine the answer.)
- Examine the inner wall of the left atrium. Using your probe, determine the number of blood vessels that lead into this chamber (Fig. 8.3D). **33-G What is the name of the blood vessels leading into the left atrium? 33-H Is the blood entering the left atrium high or low in oxygen content? Explain.**
- Locate the **bicuspid valve** between the left atrium and the left ventricle (Fig. 8.3E). **33-I What prevents blood from flowing back into the left atrium before the ventricle contracts?**
- Examine the walls of the left and right ventricle. **33-J Which ventricle has the thickest wall? 33-K How can you explain the difference in thickness?**
- Locate the **aorta,** the large blood vessel near the upper inner corner of the left ventricle (Fig. 8.3E). **33-L Describe the appearance of the valves associated with this blood vessel. 33-M What other blood vessel has similar valves?**

33-N Trace the direction of blood flow through the heart (see page 308). Indicate blood low in oxygen content with blue arrows and blood high in oxygen content with red arrows.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is the advantage of having the blood on the right side of the heart separated from that on the left side?
2 Defend or criticize this statement: "Arteries may be defined as blood vessels that carry oxygenated blood. Veins are blood vessels that carry blood low in oxygen concentration."
3 In the developing human fetus there is an opening in the wall separating the right and left atria. After birth, this opening is sealed over by tissue. What is the function of this opening in the fetus? Why is it closed following birth? What would happen if it did not close over?

EXERCISE 34

WHAT ARE THE PHYSICAL AND CHEMICAL FACTORS AFFECTING HEARTBEAT?

The rate at which blood flows through the blood vessels can be controlled in two ways: 1. the size of the vessels can be changed so as to allow more or less room for the blood to pass through; and 2. the rate of the heartbeat can be controlled so as to increase or decrease the amount of blood flowing through the vessels. In this exercise we will investigate the control of the heartbeat by various physical and chemical means. Before proceeding any further read the Appendix at the end of this exercise for instructions on how to use live animals in the laboratory.

PROCEDURE—Isolation of Living Frog Heart

▶ Prepare a spinal frog (that is, one whose entire brain has been destroyed) by the procedure of pithing described in the Appendix at the end of this exercise (see also Fig. 8.4A).

▶ Fasten the pithed frog to the pan by sticking pins through its arms and legs. With sharp scissors carefully cut the skin at the base of the abdomen marked "W" in Fig. 8.4B. Continue forward to the mouth ("X" in Fig. 8.4B). Then make horizontal cuts along the lines marked "Y" and "Z." The skin can now be pinned back toward each side.

▶ Cut through the sheet of muscle from the posterior to the anterior end; cut slightly to one side of the midline to avoid severing the large abdominal vein along the ventral side (Fig. 8.4C).

▶ Lift the sternum (breastbone) with your forceps and cut it free from the body by making a longitudinal cut through the bones at the base of each arm.

▶ Lift the sternum, cutting its attachment toward the throat end. The heart will now be exposed and can be seen beating within the pericardium (Fig. 8.4C).

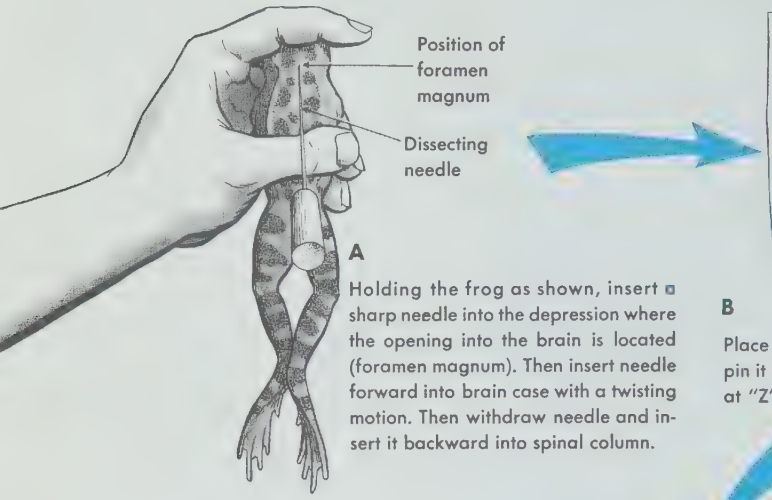
PROCEDURE—Preliminary Observations of the Heart

▶ **34-A Record the number of heart beats per minute in the table on page 309. 34-B Is the heart action under direct control of the central nervous system? Explain.**

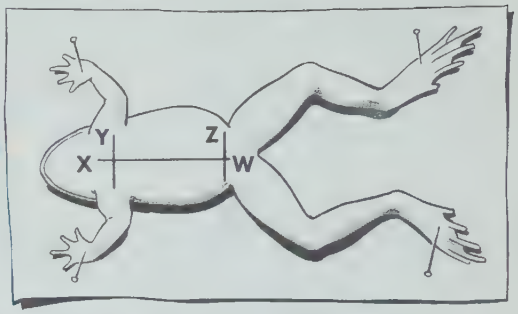
▶ Carefully slit open the pericardium. Note that the heart is composed of three chambers—two anterior thin-walled atria and one posterior muscular ventricle. **34-C Do the atria contract at the same time? 34-D Does the ventricle contract at the same time as the atria? 34-E If not, does it precede or follow the contraction of the atria?**

CAUTION: Keep the heart continuously moistened with Ringer's solution.

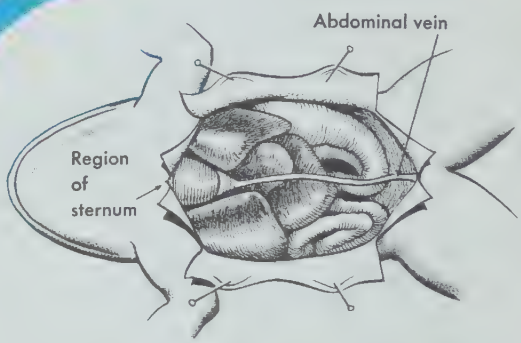
FIG. 8.4 PROCEDURE FOR DETERMINING PHYSICAL AND CHEMICAL FACTORS AFFECTING HEARTBEAT



A Holding the frog as shown, insert a sharp needle into the depression where the opening into the brain is located (foramen magnum). Then insert needle forward into brain case with a twisting motion. Then withdraw needle and insert it backward into spinal column.



B Place the frog belly-side-up in a dissecting pan and pin it down. Cut through the skin from "W" to "X" and at "Z" and "Y" (as shown).



C Cut through the sheet of muscle under the skin, using the same incisions as in "B." Then cut through and lift the sternum to expose the heart.

▶ On the underside of the heart, locate a large artery leaving the ventricle; it divides into two **aortic arches.** Major arteries carry blood from these arches to various parts of the body.

▶ With a glass rod lift the ventricle by its apex. Underneath the heart notice the thin-walled **sinus venosus** which carries blood from the body into the right atrium.

PROCEDURE—Effect of Temperature on Heartbeat

▶ With an eye dropper apply several drops of ice cold Ringer's solution on the heart. **In the table on page 309, record the number of heartbeats after such treatment.** After several minutes apply several drops of warm Ringer's solution (40°–50°C). **Record the number of heartbeats. 34-F From your data what can you conclude as to the effect of temperature on the heart? 34-G How might temperature bring about the effects observed?**

PROCEDURE—How do Certain Chemicals Affect the Heart?

▶ Chemicals having a physiological effect on organs or tissues are commonly referred to as drugs.

▶ Allow several minutes to elapse for the heart to return to its room temperature condition. Then apply a drop or two of 0.1 per cent adrenaline solution. **Record the number of heartbeats in the table on page 309.** Drugs that slow activity are known as **inhibitors** (or **depressants**). Those that speed up activity are known as **accelerators** (or **stimulants**). **34-H To which class of drugs does adrenaline belong?**

▶ Rinse the heart thoroughly with Ringer's solution and allow several minutes for it to return to normal.

▶ Then place a drop of acetycholine solution on the heart. **Record the number of heartbeats in the table.** **34-I Does acetylcholine appear to be an accelerator or an inhibitor?**

Your instructor may want you to make other observations on the frog, or preserve it for future work. If not, discard the frog as directed.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What causes the heart to beat so rapidly when one is frightened?
2 What is the "pacemaker" of the heart?
3 What is **adrenaline?** Why might adrenaline be administered to someone who suddenly experienced a drastic lowering of the blood pressure?
4 Where in the body is acetylcholine secreted? What is the function of this chemical?
5 What nerves regulate the heartbeat?

APPENDIX

THE USE OF LIVE ANIMALS IN THE LABORATORY

General Procedures. If this is the first time live vertebrate animals are being used in your class, it is important that you understand the purpose in bringing these animals into the laboratory. First of all, there must be a genuine reason for using live animals. Second, in the science laboratory we always treat live animals in a humane fashion, never causing them unnecessary

irritation or injury. Accordingly, if any organ or tissue damage may result from legitimate experimentation, the animal is first put under an anesthetic, or the nervous system is treated to make the organism insensible to pain, such as destruction of the brain by pithing. Try to avoid injuring the animal's tissues or making it bleed; such damage makes the animal less capable of "normal" reactions.

In handling exposed or removed organs or tissues, it is best to use a glass hook or a small camel's hair brush moistened with Ringer's saline solution. Never handle tissues with your fingers. When you need to lay down an excised organ or part, never place it on the table, or on dry paper; place it in a watch glass or other dish to keep it moistened. Apply Ringer's solution as often as necessary while working, to keep the tissue from drying out.

PROCEDURE—Pithing

NOTE: Always read the procedure carefully and have the apparatus for the experiment set up before beginning any work on the live animal.

- Wrap a frog in a moistened paper towel and hold its head upward in the left hand.
- Press the tip of the nose down with the left index finger (Fig. 8.4A). By holding the head at a right angle to your body, you will find it easier to insert a dissecting needle into an opening where the skull joins the vertebral column.
- With your fingernail locate the natural depression at this point. Then insert the needle into this depression, moving the point of the needle from side to side to cut across the posterior part of the brain.
- Next, push the tip of the needle forward into the brain case, and with a series of circular motions destroy the anterior part of the brain.
- Withdraw the needle and insert it through the same point, but backward into the vertebral column. Using the same circular motion, destroy the spinal cord.
- Lay the frog on its dorsal side on moistened paper toweling in a dissecting pan.

This method of destroying the central nervous system is called **pithing.** It should leave the frog without consciousness, and therefore incapable of feeling pain. If the frog makes any voluntary muscular effort, such as attempting to turn over, it has not been properly pithed. In a properly pithed frog many physiological activities are still being carried on and can be studied without interference from nerve control by the brain.

EXERCISE 35

HOW CAN THE HUMAN BLOOD CELL TYPES BE IDENTIFIED?

The blood of higher animals is a highly complex tissue. Using a centrifuge, however, blood can be fractionated into 1. a liquid, cell-free portion called **plasma,** and 2. a fraction consisting of various cells including the red blood cells (**erythrocytes**), white blood cells (**leucocytes**), and cells or parts of cells (**blood platelets**) that play a role in the clotting of blood. In this exercise you will examine some of the different cells that occur in blood.

PROCEDURE

▶ Sterilize the tip of the middle finger of your left hand (if you are right handed) with alcohol (Fig. 8.5A). If you are left handed, sterilize the tip of the middle finger of your right hand.

▶ With the sterile lancet provided by your instructor, puncture the finger lightly and squeeze out a drop of blood. Wipe off this first drop of blood with clean cotton.

CAUTION: Do not exchange lancets with other students.

▶ Squeeze out a second drop of blood and apply it to a *clean* glass slide about a half inch from an edge (Fig. 8.5B).

▶ Smear the blood on the slide as shown in Figs. 8.5C,D. Let the blood dry (Fig. 8.5E).

▶ Next cover the blood smear with 10 to 15 drops of Wright's blood stain (Fig. 8.5F). Leave the stain on the slide for three to five minutes.

▶ Next add distilled water, drop by drop, until a greenish metallic scum appears on the surface of the stain (Fig. 8.5G).

▶ Allow the staining to continue for another three to five minutes. Then wash the stain off the slide by holding it under a slow running stream of water for several seconds (Fig. 8.5H).

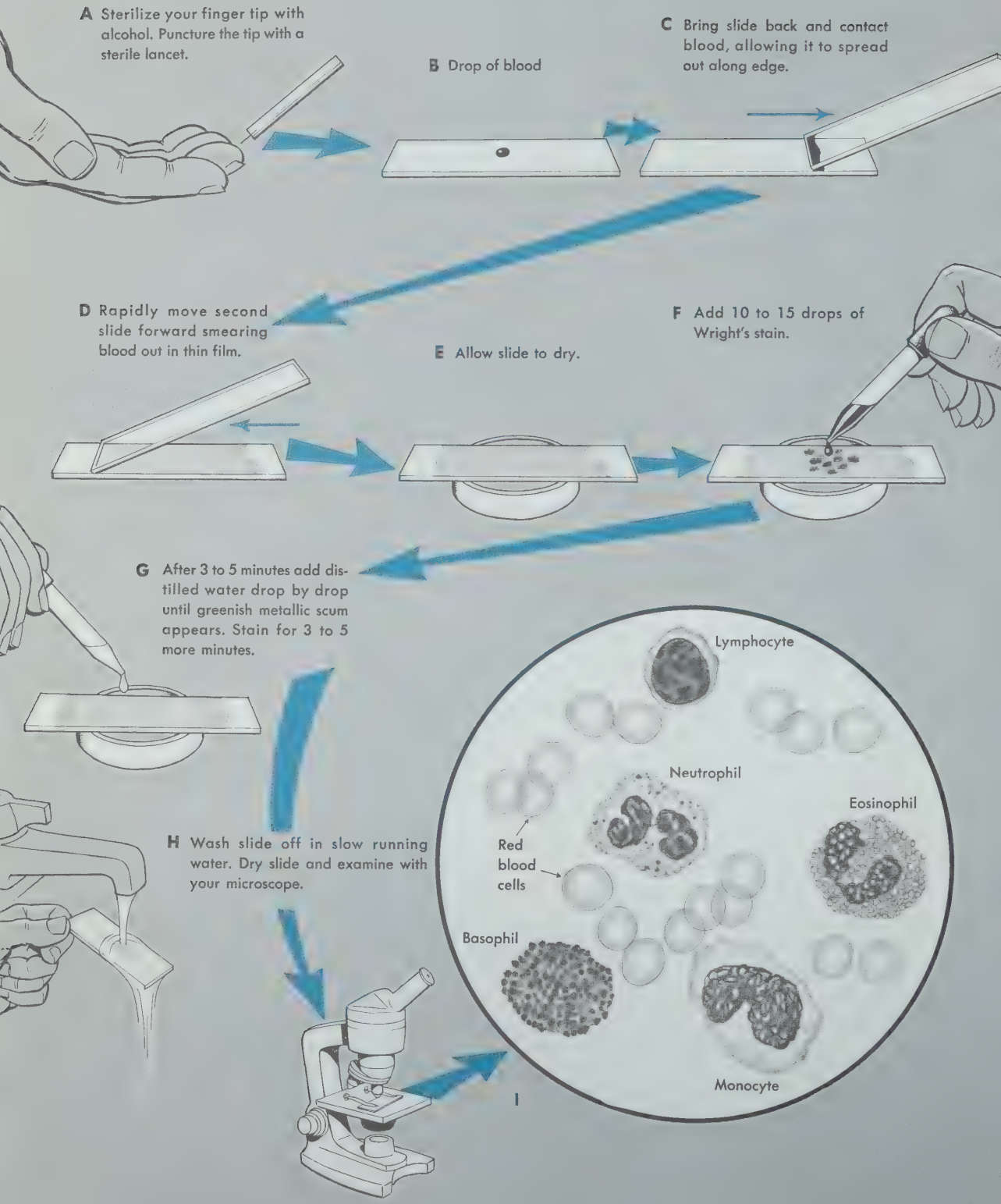
▶ Let the slide dry and then examine it with your microscope. If your blood has not stained well for some reason, obtain a blood slide that has been professionally stained. With the help of Fig. 8.5I, identify the various blood cell types listed below:

- **Red blood cells (erythrocytes)** are by far the most numerous type of blood cell. Their important function is to transport oxygen and carbon dioxide between the lungs and the cells throughout the body. They are flat, disk-shaped and thinner in the middle than at the edges.

35-A Do the mature red blood cells have a nucleus?

- **Neutrophils** are the most common type of white blood cell and have the function of getting rid of bacteria and other foreign cells that enter the body. Neutrophils do this

FIG. 8.5 PREPARATION OF A HUMAN BLOOD SMEAR



by engulfing the bacteria much in the manner that an ameba would. They are often called **phagocytes,** which means "eating cell." The neutrophil is larger than the red blood cell, and is readily recognized by its blue staining nucleus that consists of two or three lobes that are connected by fine strands (Fig. 8.5I).

- **Lymphocytes** are smaller than neutrophils and have a large blue-stained nucleus that almost fills the cell. These cells often migrate through the capillaries to sites of infection. The lymphocytes are largely concerned with the production of antibodies.
- A third type of white blood cell is called a **monocyte.** They resemble the lymphocyte except that the large nucleus is usually bean-shaped. This is another type of phagocytic cell.
- Several other types of white blood cells may be found occasionally in normal human blood. **Eosinophils** contain many bright red-staining granules in the cytoplasm. **Basophils** have blue-staining granules in the cytoplasm. The function of these rarer blood cells is unknown.

35-B How many blood cell types can you identify in your own blood smear? 35-C Which of the white blood cells appears to be the most common?

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Would you expect to get a positive test for DNA in a mature human red blood cell? Explain.
2 Defend or criticize this statement: "Neutrophils, lymphocytes, and monocytes have the ability to move about; they are not carried passively in the blood stream. These cells then are single-celled organisms and therefore can be considered to be parasites of your body."
3 Would you consider the mature red blood cell to be a true cell? Explain.
4 What does it mean when a person is *anemic?*
5 What is *leukemia?*
6 Boils are frequently filled with a whitish fluid commonly called *pus.* What is pus composed of?
7 When you cut or scratch yourself the flow of blood usually stops after a few minutes? Why?

EXERCISE 36

WHAT IS THE FUNCTION OF HEMOGLOBIN?

The blood of higher animals contains a pigment called **hemoglobin** which is composed of a protein called **globin** and an iron-containing compound called **heme.**

In this exercise you will attempt to determine the function of hemoglobin and in what part of the blood it is found.

PROCEDURE—Function of Hemoglobin

▶ Obtain from your instructor two test tubes half filled with blood (Fig. 8.6A).

▶ Connect the rubber tubing from one tube of blood to a small hand pump and carefully *and slowly* pump air into the blood for five to six minutes (Fig. 8.6B).

▶ Into the second tube blow air from your lungs for one to two minutes. Do this *slowly* and by breathing at a normal rate (Fig. 8.6C).

▶ Compare the color of the blood in both tubes. **36-A Record your observations in the table on page 313.**

▶ Reverse the color of the blood in each tube. **36-B How would you do this? Record your results in the table. 36-C Suggest a reason for the color changes that occurred in the blood as a result of the different treatments.**

PROCEDURE—More About the Function of Hemoglobin

▶ Add about ½ teaspoon of baking soda to a test tube held in a clamp on a ring stand. Then *slowly* fill the test tube half full with dilute hydrochloric acid (HCl). Baking soda plus HCl will produce carbon dioxide (Fig. 8.6D).

▶ Quickly attach this "CO_2 generator" to the test tube of blood that had *air* from the hand pump bubbled into it. Allow the CO_2 to bubble through the blood for several minutes (Fig. 8.6D). In the meantime proceed with the next step.

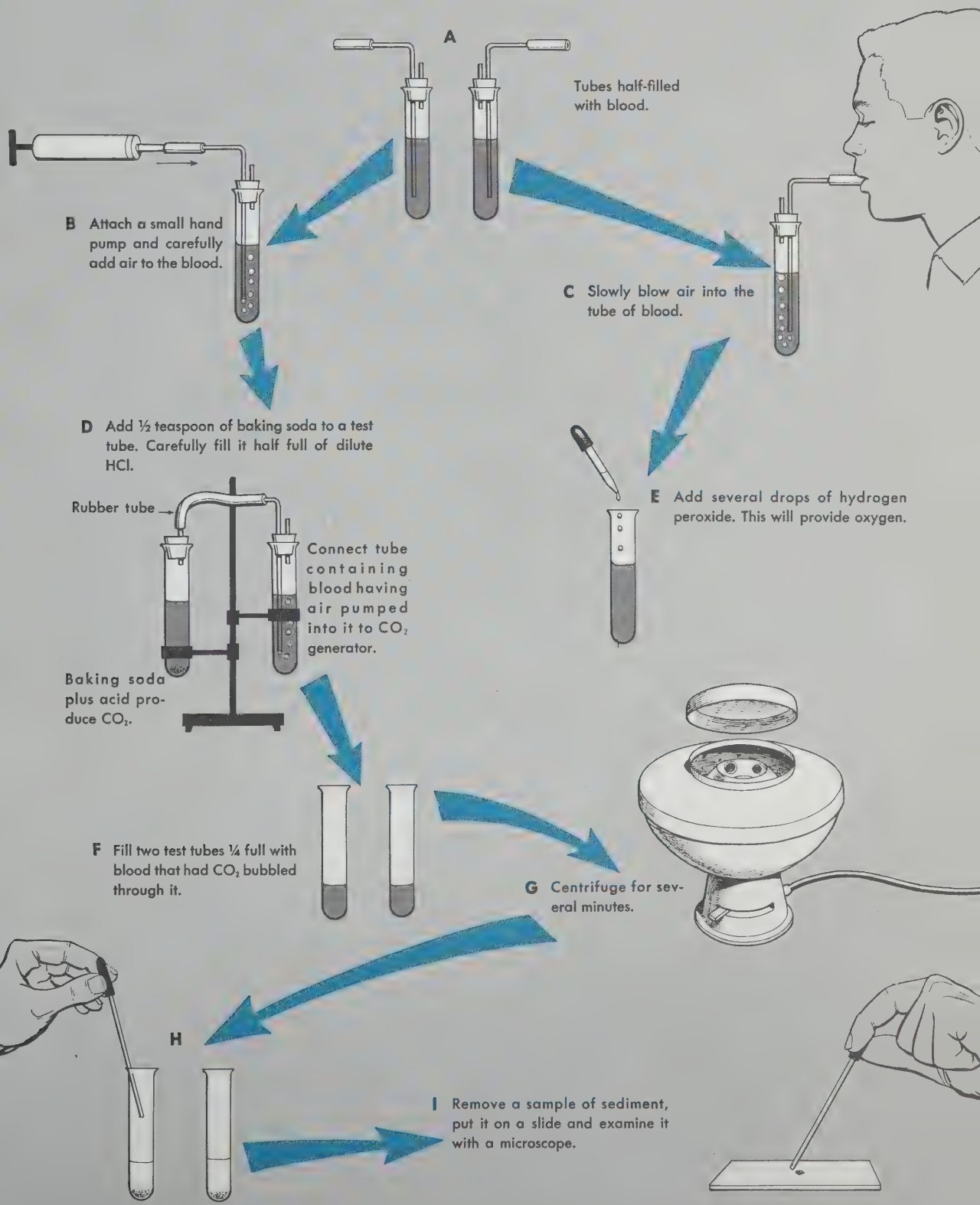
▶ Add several drops of a weak solution of hydrogen peroxide (H_2O_2) to the test tube containing blood that you bubbled your *breath* through (Fig. 8.6E). Hydrogen peroxide decomposes and gives off oxygen.

▶ Compare the color of the blood under each of the treatments above and record your observations in the table (36-A). **36-D Suggest a function for hemoglobin.**

PROCEDURE—Where is Hemoglobin Located in the Blood?

▶ Fill two test tubes about one-fourth full with blood that has had CO_2 bubbled through it (Fig. 8.6F).

FIG. 8.6 PROCEDURE FOR STUDYING THE PROPERTIES OF HEMOGLOBIN



CAUTION: The level in the tubes must be the same or they will be out of balance and may damage the centrifuge.

▶ Place the tubes opposite each other in the carrier of the centrifuge (Fig. 8.6G). Close the cover and adjust the speed to approximately 1000 rpm (revolutions per minute).

▶ After 10 minutes, turn the centrifuge off and allow the tube carriers to stop of their own accord. Then carefully remove the tubes (Fig. 8.6H). **36-E What physical changes have occurred as a result of centrifuging the blood? 36-F In what part of the tube is the hemoglobin located?**

▶ With a long-nose medicine dropper, remove a small amount of the sediment from the bottom of one of the tubes and place it in a drop of water on a slide. Add a cover slip and examine the sediment with the high power of your microscope. **36-G In what specific part of the blood do you think hemoglobin is located?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 We can breathe small quantities of carbon *di*oxide without any ill effects. Breathing the same amount of carbon *mon*oxide would result in death. Why is carbon monoxide so dangerous? List some conditions in which carbon monoxide is produced.
2 What pigment in plants is very similar to hemoglobin? What is a basic difference in their structure?
3 Do all animals that have circulatory systems also have hemoglobin? Explain.
4 Does hemoglobin have to be contained in cells to function? If not, give examples of animals that do not have hemoglobin in their blood cells.

EXERCISE 37

HOW DO THE CAPILLARIES FUNCTION?

In the early 1600's William Harvey, an English physician, discovered the pattern of circulation of blood. He subsequently concluded that the blood carries nutrients, oxygen, and various other materials to the body tissues, and carbon dioxide and other waste products away from the tissues. Harvey, however, never saw one of the more important parts of the circulatory system—the capillaries. These blood vessels are important because it is in the capillaries that the actual exchanges of various materials in the circulatory system and the cells take place. In this exercise you will examine the circulation of blood through the capillaries.

PROCEDURE

▶ Using a dip net, obtain a small (two-inch) goldfish from the aquarium in your room (Fig. 8.7A).

▶ Wrap the fish (except for the mouth and tail) in dripping wet cotton. Place it in the bottom half of a petri dish (Figs. 8.7B,C). (If the fish moves around too much, place it in a container of chloretone solution to anesthetize it. After a minute or two the fish will roll over on its side and will remain anesthetized for about one hour.)

NOTE: Do not allow the cotton around the fish to dry out.

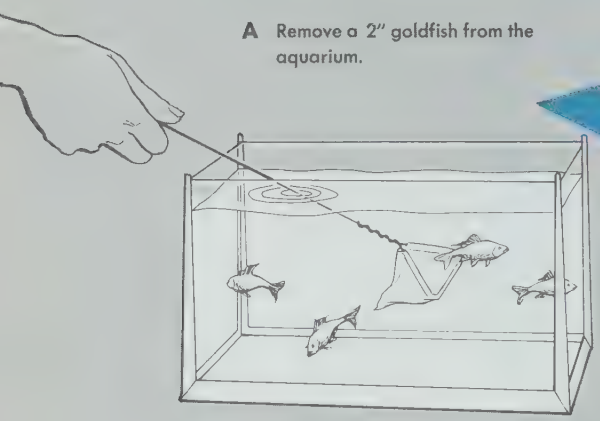
▶ Place a coverslip (or glass slide) over a thin region of the tail. Then position the petri dish on your microscope so that the fish's tail is over the hole in the stage (Fig. 8.7D).

▶ Focus on the tail with the low power objective of your microscope. Note the movement of blood through the blood vessels. The smallest vessels you see are the capillaries. They are just wide enough to permit the passage of a single file of blood cells. **37-A What is the advantage of having red blood cells pass through the capillaries in single file?** Change to high power to examine the capillaries and blood cells more closely.

▶ Blood enters the capillaries from small arteries called **arterioles.** Trace a capillary back to an arteriole. **37-B Does the blood flow more rapidly in the arterioles than in the capillaries?**

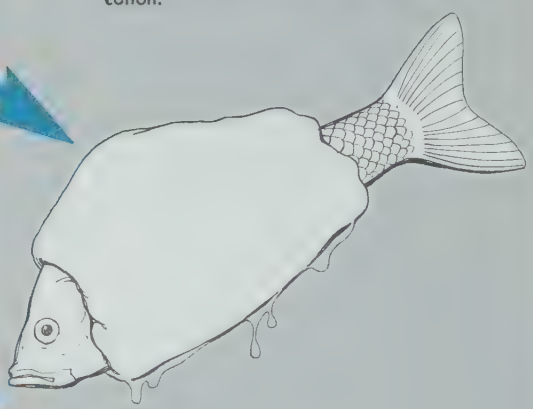
▶ Follow a capillary in the direction in which the blood is flowing. It will join a slightly larger blood vessel called a **venule.** **37-C Is the speed of the blood greater in the venules or in the capillaries? 37-D Record your observations of capillary circulation with a drawing on page 316.**

FIG. 8.7 PROCEDURE FOR EXAMINING CAPILLARY CIRCULATION IN GOLDFISH TAIL

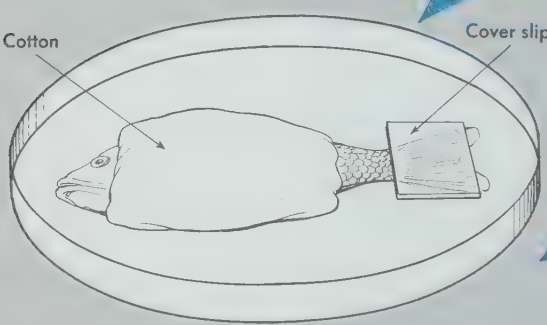


A Remove a 2" goldfish from the aquarium.

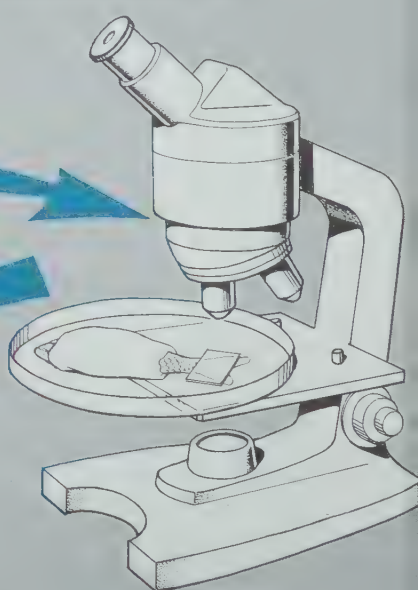
B Wrap the fish (except for head and tail) with dripping wet cotton.



C Place fish in bottom half of Petri dish. Place cover slip or glass slide over thin part of tail.



D Place dish on microscope so that fish's tail is over hole in stage.



Record your observations in a drawing on page 316. Label capillaries, arterioles, and venules.

E Examine with low and high power objectives of your microscope.

▶ When you have finished with your observations revive the fish by giving it "artificial respiration" in the special reviving tank. This is done by holding the fish between two of your fingers and pushing it rapidly through the water. Continue to do this until the fish swims out of your fingers. **37-E What is the purpose of this back and forth motion on the fish?** This is a modification of "shark walking" that is used by many large aquariums to acclimate the fish to its new surrounding.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Defend or criticize this statement. "The blood that leaves the capillaries and enters the venules has a high concentration of oxygen and a low concentration of carbon dioxide."
2 Capillary walls are very thin in comparison to the walls of arteries and veins. What is the advantage of this?
3 Would you expect muscle tissue to have an extensive capillary circulation? Explain.
4 Would you expect to find extensive networks of capillaries in the gills of a fish? Explain.

9 BIOLOGICAL COORDINATION

Wide World Photos

INTRODUCTION

As organisms become more complex and individual cells become more specialized, a need to coordinate the various activities within the organism develops. Furthermore, all organisms need effective ways in which to respond to physical and chemical changes in their environment. Thus, all organisms, whether plant or animal, must be adapted to receive various internal and external stimuli and to respond to these stimuli in a coordinated manner.

In higher animals coordination is maintained by information transferred from one part of the body to another by the secretion of specialized chemicals called hormones and by "messages" carried by nerves. In hormonal communication a specialized gland responds to certain internal or external stimuli by releasing hormones into the blood stream, which carries the hormone to all parts of the body. When a particular stimulus stimulates a hormone, the hormone affects only a specific organ or tissue even though the hormone is carried throughout the body.

Other stimuli are carried by nerves through the complex network of the nervous system. In some cases the messages carried by nerves go to the brain, which then sends specific instructions for action to various parts of the body by way of other nerves. In some cases the messages need to go to the spinal cord only.

Higher plants, however, do not have nervous systems, yet coordination of many activities is a necessity if the plant is to grow and develop. There are two types of mechanisms that bring about coordination in plants. One consists of a system of chemical messengers that directs the cells of the plant to carry out various functions. This group includes the various plant hormones and other growth regulators. A second coordinating

system involves various physical forces, for example gas-exchange gradients, electrical gradients, and metabolic or pressure differences throughout the plant. One end product of these controls is a highly complex plant body having localized areas of growth, special regions of photosynthetic activities, the ability to translocate and accumulate the necessary raw materials for growth and differentiation into reproduction, or other functions to assure the continuation of the species.

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EXERCISE 38

HOW DO PLANTS RESPOND TO ENVIRONMENTAL STIMULI? (1)

The ability to respond to a **stimulus** (a chemical or physical change in the environment) is a striking characteristic of living organisms. The response of the organism to any stimulus serves to coordinate the activities of the organism so that equilibrium with the environment is maintained. In this exercise you will be asked to determine the nature of the environmental stimulus that accounts for the results you see.

PROCEDURE

▶ Cut a small (three-by-five) white card to the size of a glass microscope slide (Fig. 9.1A).

▶ With a razor blade cut a narrow (1/16-inch) slit in the center of the card (Fig. 9.1B).

▶ Next prepare a wet mount of the green alga *Euglena*. Add a cover slip and examine the organism with the low power of your microscope (Figs. 9.1C,D,E). **38-A Diagram the random distribution of *Euglena* in the space on page 317.**

▶ Now slide the cardboard *under* the slide so that the slit is centered under the cover slip as you look down on the slide with your naked eye (Fig. 9.1F).

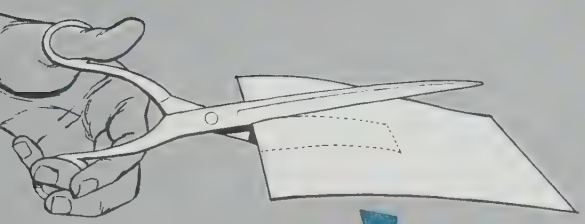
▶ With the low power objective of your microscope examine the organisms in the slit area (Fig. 9.1G). After five minutes, remove the cardboard from under the slide. Quickly re-examine the slide. **38-B Diagram the distribution of *Euglena*. 38-C Suggest what environmental factor is responsible for the response you observed. 38-D How is this environmental response of benefit to this organism?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

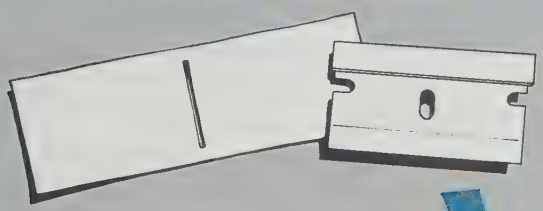
1 Define **tropism** and **taxis.**
2 Is the response of *Euglena* in this exercise a tropism or a taxis? Was the response positive or negative?
3 How does the response of *Euglena* in this study benefit the organism when it is growing in nature?
4 What are **turgor movements?** Cite two examples of this type of coordinated response.
5 Devise and conduct an experiment (adequately controlled) to determine the effect of the factor studied in this exercise on the growth of stems of a plant.

FIG. 9.1 EFFECT OF ENVIRONMENTAL STIMULI ON EUGLENA

A Cut a white card to the size of a glass microscope slide.

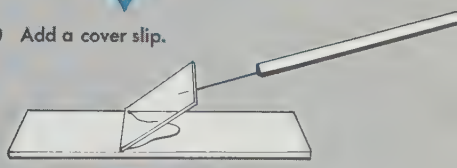


B With a razor blade cut a 1/16" slit in the center of the card.

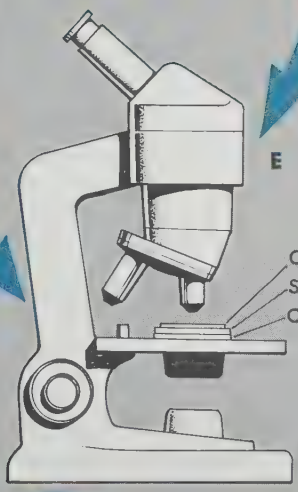


C Prepare a wet mount of *Euglena*.

D Add a cover slip.

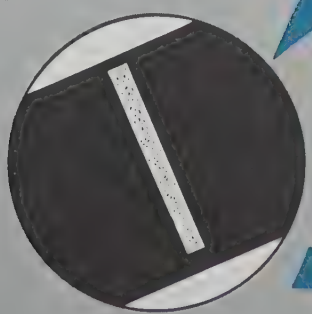


E Examine with the low power of your microscope.



F Slide the cardboard *under* the glass slide.

G Examine the organisms in the slit for several minutes.



Then remove the cardboard and examine the slide.

Diagram the random distribution of *Euglena* on page 317.

EXERCISE 39

HOW DO PLANTS RESPOND TO ENVIRONMENTAL STIMULI? (2)

Many animals, including man, react in spectacular ways to changes in environment. Plants, although generally anchored and not able to move about, also react to environmental stimuli, although in more subtle ways. In this exercise, you will try to determine what environmental factor provides the stimulus that results in the response you observe.

PROCEDURE

▶ Select four kernels and position them in the bottom of a petri dish with the embryo side down and the pointed ends toward the center (Figs. 9.2A,B).

NOTE: The region of the embryo shows up as a whitish area near the pointed end on one surface of the grain.

▶ Cut a piece of paper toweling to fit into the bottom of the petri dish (Fig. 9.2C). Place the paper over the corn. *Do not move the kernels out of position.* Thoroughly moisten the paper.

▶ Pack the rest of the petri dish with nonabsorbent cotton so that when the cover is put on, and the dish is turned on edge, the kernels will remain in position (Fig. 9.2E).

▶ Seal the cover in place with a strip of masking tape.

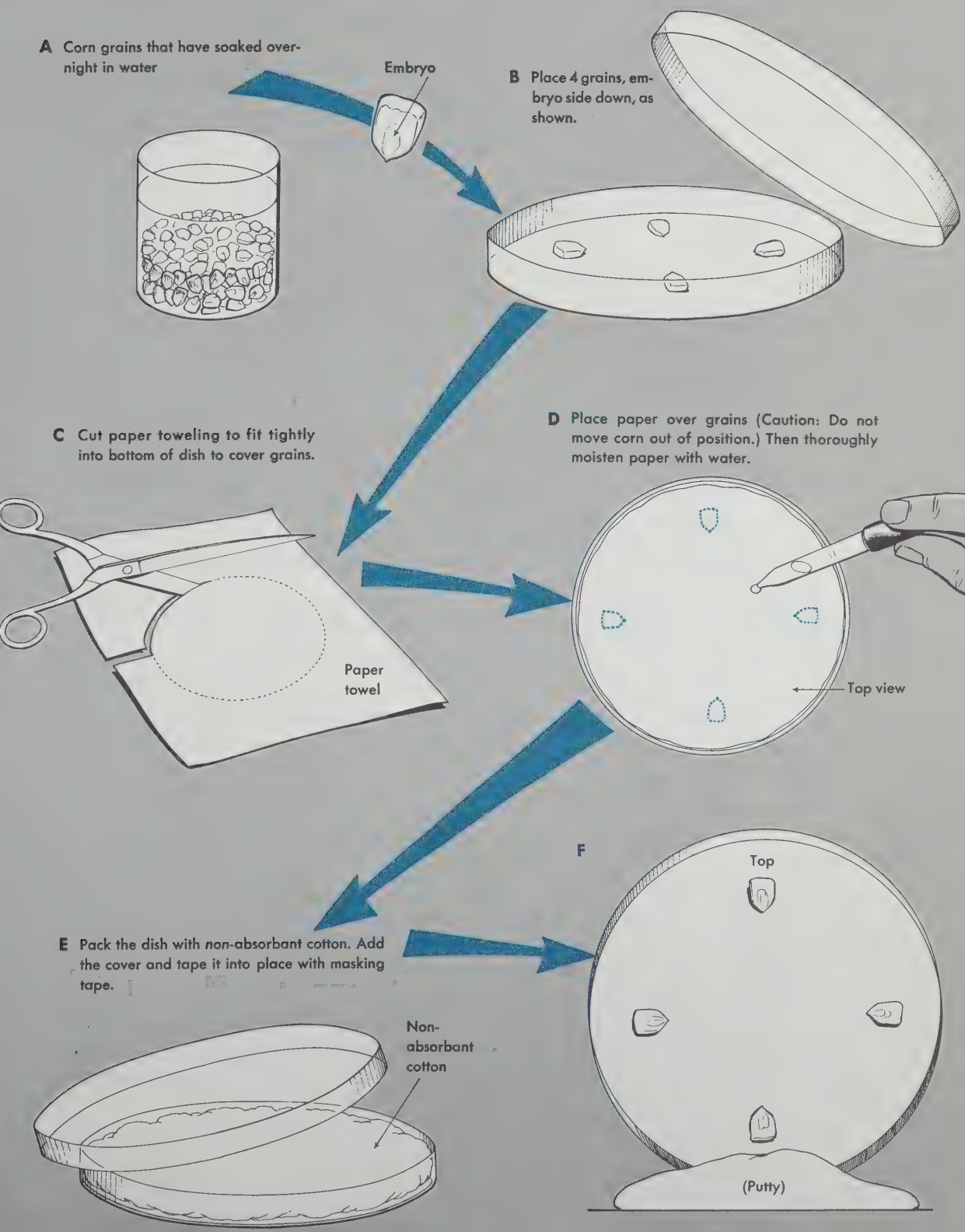
▶ Set the dish on edge in front of you so that one of the kernels is in the 12 O'Clock position. Mark the dish with the word *top*. Without changing the position of the petri dish, anchor it in place (Fig. 9.2F).

▶ Examine the kernels for the next two or three days, during which time they will germinate and send out a shoot and roots. **39-A At the end of the experiment (as determined by your instructor) diagram the positions of the roots and shoots for each kernel (see page 319). 39-B In what directions do the roots from each kernel grow? 39-C In what direction does the shoot of each kernel grow? 39-D To what environmental stimuli are the plants responding?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Devise and run an experiment (properly controlled) to determine the effect of the stimulus affecting root growth on the growth of stems.
2 Why do trees on mountains grow straight up (Diagram A) instead of straight out (Diagram B)?
3 Briefly discuss the suggested role of the plant hormone auxin in regulating the response observed in this exercise?
4 Would you consider the movements of the guard cells as a coordinated activity of the plant? Explain.

FIG. 9.2 EFFECT OF ENVIRONMENTAL STIMULI ON CORN KERNELS



EXERCISE 40

HOW DO MUSCLES RESPOND TO STIMULI?

It is a well known fact that muscles respond to external stimuli, such as heat and cold, by a characteristic reaction which draws the organism away from the stimulus. In this exercise you will measure the effect of several stimuli on the nerve impulse by using an isolated muscle-nerve preparation.

PROCEDURE—The Effect of Mechanical Stimulation

▶ Prepare a frog gastrocnemius muscle-nerve preparation (Fig. 9.3) according to the procedure outlined in the Appendix at the end of this exercise. Place the preparation on filter paper moistened with Ringer's solution.

▶ Using a glass rod, tap the sciatic nerve lightly near its outer end. **40-A What is the result? Repeat the procedure with a harder tap. 40-B Does the muscle respond differently from the way it responded the first time?**

▶ Pinch the nerve sharply with forceps about ½ cm from its cut end. **40-C How does this response compare to the tapping response?**

▶ Now stimulate the nerve 1 cm from the cut end. **40-D Explain the difference in the various responses measured in these experiments.**

PROCEDURE—The Effect of Thermal Stimulation

▶ *Very gently* touch the cut end of the nerve with the pointed end of a small glass rod so that there is no response. Now heat the end of the rod so that it is rather warm to the touch and again touch the nerve *as gently as before.* **40-E What is the response after heating the rod?**

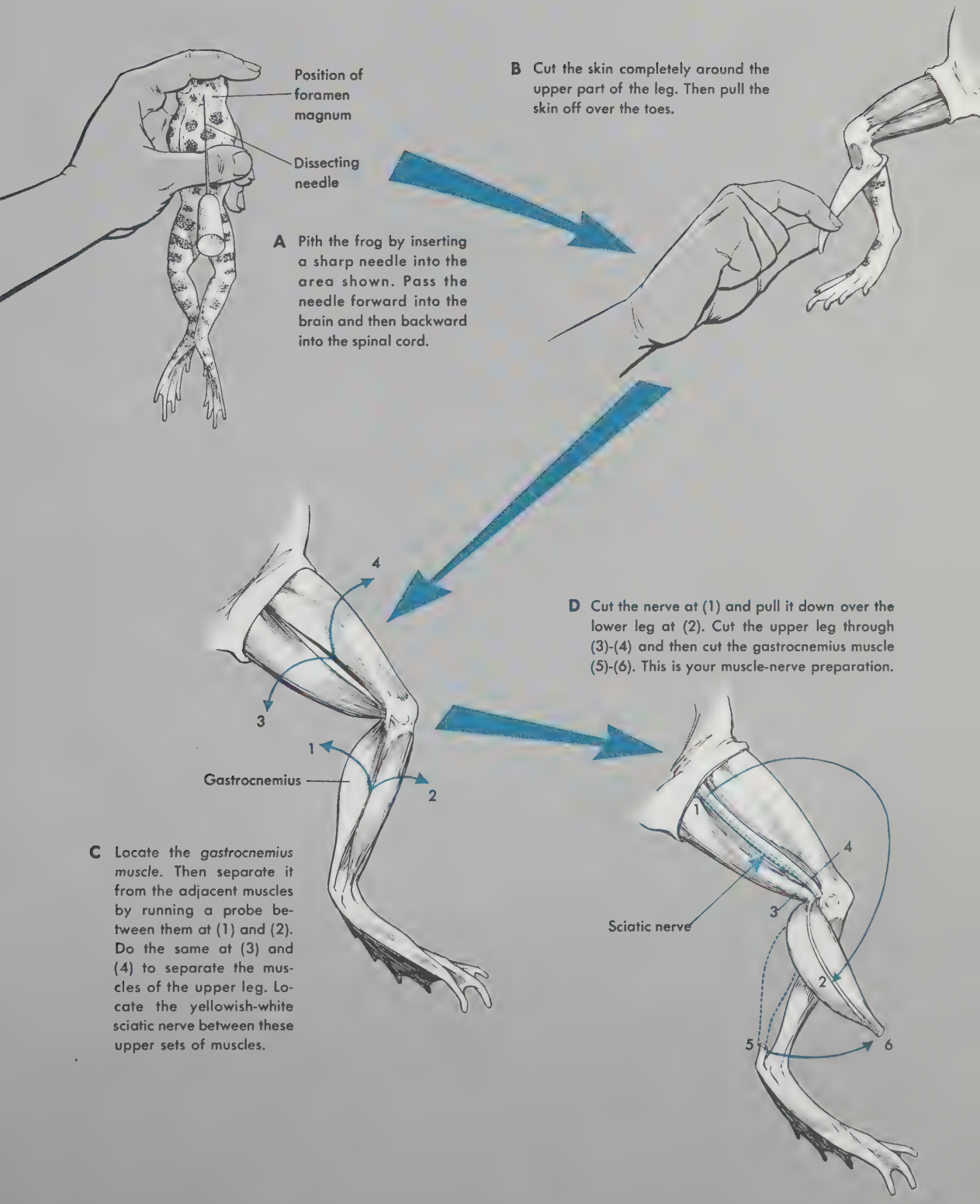
PROCEDURE—The Effect of Chemical Stimulation

▶ Cut off the part of the sciatic nerve that has already been stimulated. **40-F Why?**

▶ Now add a drop of HCl to the cut end of the nerve. **40-G What is the response?** Wash the acid from the nerve under tap water. Repeat all of the above experiments by stimulating the muscle directly.

40-H Do you get the same kinds of responses? Explain. 40-I. On the basis of these observations what can you conclude about the mechanism by which muscles respond to external stimuli?

FIG. 9.3 MUSCLE-NERVE PREPARATION



FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Would you consider that nerve tissue was a better conductor of stimuli than muscle tissue? Explain.
2 What chemicals are known to inhibit the stimulation of muscles by nerves?
3 What is the purpose of shivering when an individual responds to a cold stimulus?
4 What stimulus causes sweating? What is the purpose of this response?

APPENDIX

FROG MUSCLE-NERVE PREPARATION

▶ Destroy the brain and spinal cord of a frog (Fig. 9.3A) using the pithing procedure outlined in the Appendix of Exercise 34.

▶ Remove the skin by first cutting the skin completely around the upper part of one leg (Fig. 9.3B). The entire skin of the leg may then be pulled off in the same manner as you would remove a glove so that it turns inside out.

▶ Place the animal, back side up, on moist filter paper and keep it moist with Ringer's solution.

▶ Note the large **gastrocnemius muscle** of the lower leg. Run a probe between this muscle and the rest of the leg so as to expose the **sciatic nerve,** which is connected to the gastrocnemius muscle (Fig. 9.3C).

▶ Without pulling the nerve, remove or pull aside the muscles of the upper leg until the leg is exposed where it enters the hip (Fig. 9.3D1). Then with forceps, grasp the hip end of the nerve and cut it at this point.

▶ Carefully pull the nerve out from between the muscles of the upper leg and lay the free nerve down across the middle of the lower leg (Fig. 9.3D2).

▶ Now remove the lower leg by using coarse scissors, cutting it from the upper leg as shown on the dotted lines in Fig. 9.3D3,4.

▶ Cut the tendon that joins the gastrocnemius to the lower leg (Fig. 9.3D5,6). You have what is called a **muscle-nerve preparation.**

EXERCISE 41

SPINAL REFLEXES IN THE FROG

Behavior in vertebrates, including human behavior, is based to a great extent on **reflexes.** A nervous reflex may be defined as a coordinated response by **effectors** (cells specialized to transmit impulses from the brain and spinal cord to the muscles and glands) to an external stimulus received by **receptors** (cells specialized to receive external stimuli such as heat and cold). Thus, very simple reflex activity is brought about by two or three **neurons**—basic cellular units of the nervous system. These nerve cells are grouped in a **reflex arc** consisting of a **sensory neuron,** which receives and transmits the stimulus to an **effector neuron,** which responds in some characteristic fashion to the stimulus. The human knee-jerk reflex is an example of such a simple reflex arc.

PROCEDURE—Reflexes in the Living Frog

▶ Hold a live frog firmly in one hand by grasping the hind legs. Lightly touch one of its eyes with a blunt probe or a glass rod (Fig. 9.4A). **41-A How does the frog respond to this stimulus? 41-B Is the response more severe if you tap the eye with the probe? 41-C Does the strength of the stimulus affect the response? Explain.**

▶ Grasp the head of the frog and hold the forelegs firmly in one hand. Wash off any mucous that is present on the back of the frog. Now touch the skin on its back with a cotton-tipped toothpick or glass rod dipped in dilute hydrochloric acid (Fig. 9.4B). **41-D How does the frog respond to this stimulus? 41-E In what way is this response modified when very dilute acid is added to the frog's back? 41-F Do you get the same response if the dilute acid is placed on the left or right side of the back?** When you are through with this experiment wash off all the acid with water.

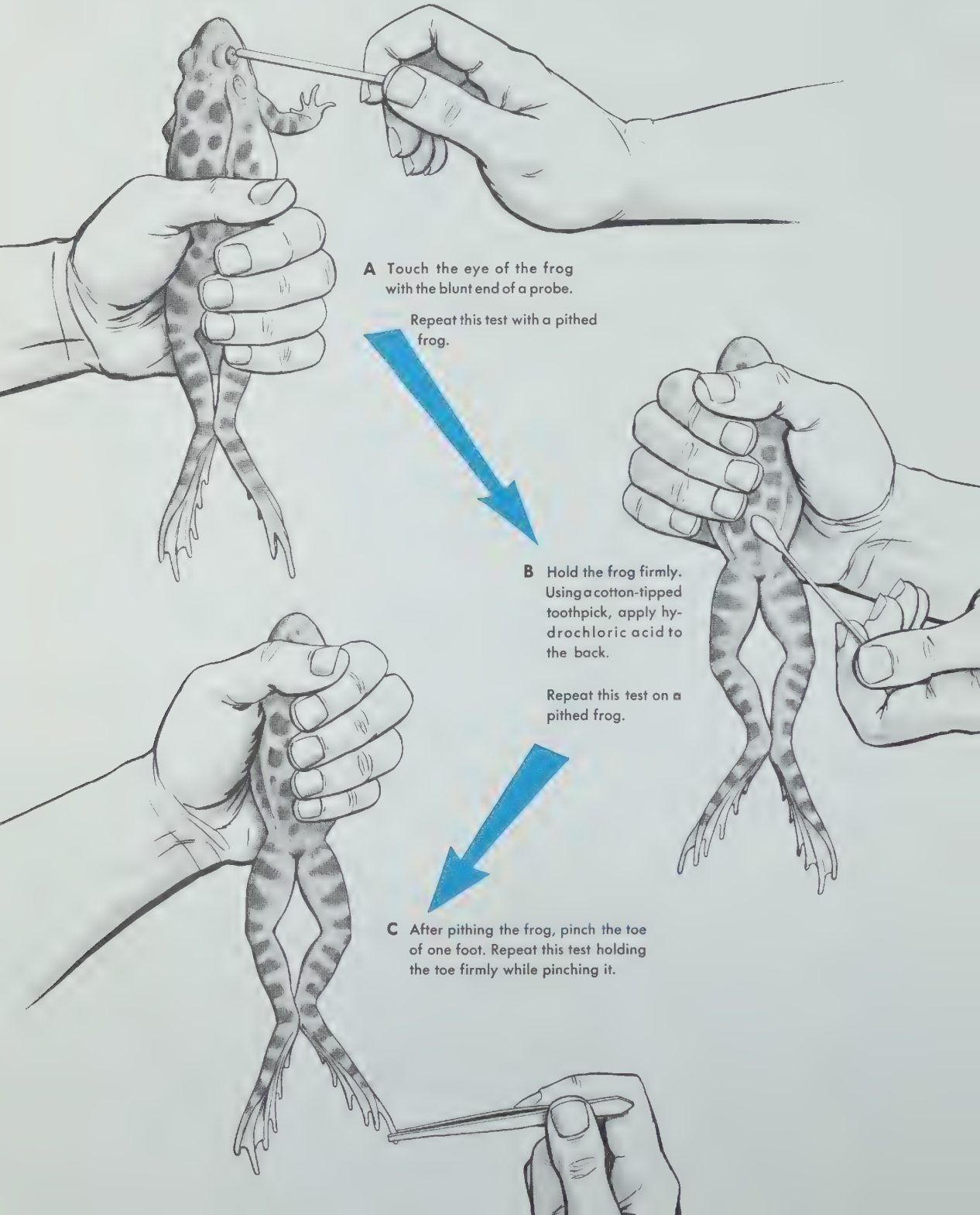
NOTE: Do not add so much acid that it drips.

PROCEDURE—Reflexes in the Pithed Frog

▶ Holding the frog firmly in the left hand, quickly sever the spinal cord using the pithing procedure described in the Appendix of Exercise 34. **41-G Is such a frog able to respond to conscious instructions from the brain? 41-H If not, where do the impulses directed to the effectors come from?**

▶ Test this frog for the eye and acid reflexes studied in the living frog (Figs. 9.4A,B). **41-I How does your pithed frog respond to these stimuli?**

FIG. 9.4 REFLEX ACTIVITIES IN LIVING AND PITHED FROGS



A Touch the eye of the frog with the blunt end of a probe.

Repeat this test with a pithed frog.

B Hold the frog firmly. Using a cotton-tipped toothpick, apply hydrochloric acid to the back.

Repeat this test on a pithed frog.

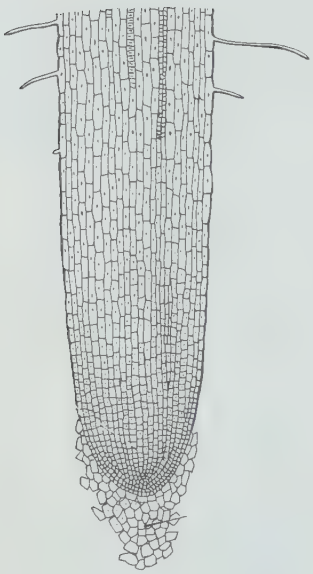
C After pithing the frog, pinch the toe of one foot. Repeat this test holding the toe firmly while pinching it.

41-J What happens when you pinch the toe of one foot of this frog? 41-K What happens when you pinch the toe of one foot while holding it tightly with forceps so that it cannot be withdrawn? 41-L How did the nerve impulse reach the unstimulated leg? 41-M Draw a diagram to show how the stimulus and impulse passed through the neurons involved in these reflex actions.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is a **conditioned reflex** activity? Give several examples.
2 How can some of the reflexes present at birth be modified in later life?
3 Are reflex actions completely independent of the brain? Explain.
4 To what types of external stimuli does man respond? What receptors (sensory organs) are most sensitive to each of these stimuli?
5 What sensory receptor in man is sensitive to gravity?

10 PLANT ANATOMY



INTRODUCTION

In order to comprehend the complex structure of higher plants and the inter-relationships between structure and function, it becomes necessary to study the anatomy of the organism involved.

Higher plants, like animals, are made up of groups of cells organized into tissues that become specialized for different tasks. However, the tissues of plants are different from those in animals in many respects. A striking difference is seen in the way they grow. Animals, in general, reach their full growth after a period of time and then stop growing. Plants, however, continue to produce new organs (for example, roots, leaves) throughout their growth period. Certain regions at the tips of the roots and stems remain embryonic and, through cell division, produce new cells that differentiate into the tissues of the stems, leaves, and roots. These regions of embryonic tissue are called **meristems** in contrast to the fully developed permanent tissues.

From a functional point of view, a plant consists of the following types of cells.

Meristematic cells have the ability to divide repeatedly and give rise to new cells of the plant. In general, meristematic cells are cube-like in shape, are small, thin-walled, and have many vacuoles. Their nucleus is quite large in comparison with the rest of the cell.

Parenchymatous cells are large, usually thin-walled, many-sided cells. They are relatively undifferentiated, and form the bulk of softer plant parts—most parts of the fruit, soft parts of the stem and leaves, and so on. They may contain chloroplasts, or may be adapted for the storage of water and reserve foods such as starch.

Supporting and conducting cells are all relatively long, but

otherwise are greatly different from one another. One group, whose walls have large quantities of lignin, are dead when they are mature. Another type of supporting cell, **collenchyma,** is characteristically thickened only at the corners of the cell.

Sieve tubes are the main pipeline cells of the phloem. They are strings of cells with perforated end plates. Such sieve cells do not have nuclei at maturity.

Protective cells have flattened surfaces with a waxy waterproof coating which prevents too much water loss from the plant's inner tissues. There are many different kinds of specialized cells that form the outer protective "skin" of a plant. Most important among these protective cells are those forming the **epidermis.**

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EXERCISE 42

ANATOMY OF THE ROOT

Characteristically, roots are described as those parts of the plant that grow beneath the surface of the soil. The main function of roots is the absorption of water and minerals and the movement of these substances to the above ground parts—the stem and leaves. Roots also anchor the plant in the soil and may store food, as in potatoes, carrots, and turnips.

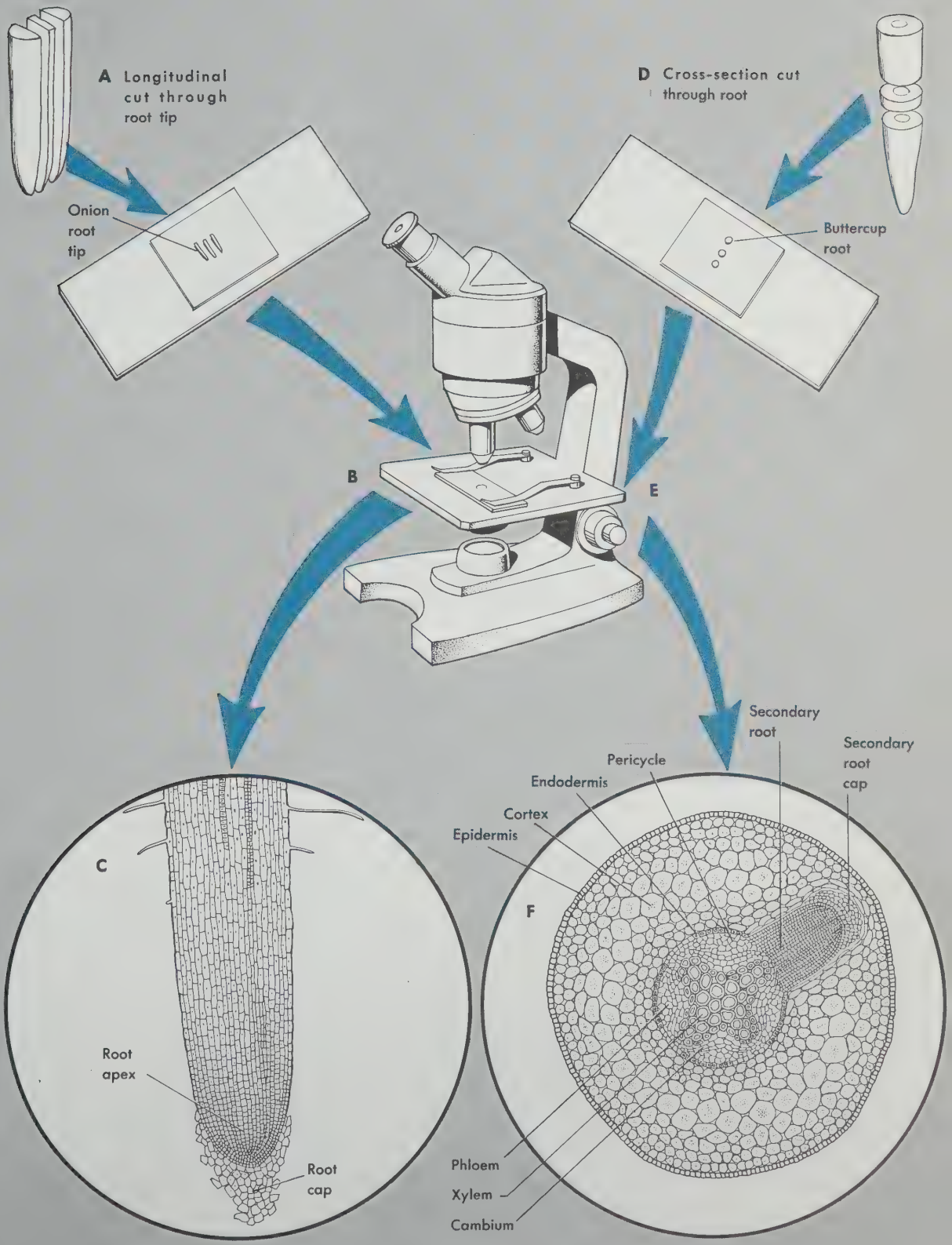
PROCEDURE

▶ Obtain a prepared slide of a longitudinal section through an onion root tip (Figs. 10.1A,B,C). With your microscope locate the **root cap;** a cone-shaped mass of cells covering the **root tip.** Examine the cells of the root apex area closely with low and high power. **42-A What do you see that would indicate that this area is involved in the growth of the root? 42-B Suggest a function for the root cap cells.**

▶ The root, as a specialized plant organ, is made up of various cells and tissues. Obtain a slide of a cross section through a mature region of a buttercup root. Using the low power of your microscope (and Figs. 10.1D,E,F), locate the following parts of the root.

- **Epidermis.** This is usually only a single cell layer thick. **42-C Suggest a function for this layer of tissue.**
- **Cortex.** In most roots this region consists largely of thin-walled cells and frequently functions as a storage tissue. Note the presence of purplish-stained starch grains in the cells of this region. Considered to be part of the cortex is a layer of cells called the **endodermis.** Its function is still not thoroughly understood. Note the thickened walls of these cells.
- The **vascular cylinder** consists of several different tissues occupying the central part of the root. The **pericycle** is a layer of cells next to the endodermis. The cells of this tissue have the ability to divide and are involved in the formation of lateral secondary roots.
- The **xylem,** which functions in the movement of water and dissolved minerals to the aerial parts of the plant, can be seen as three, four, or five starlike projections in the middle of the vascular cylinder. Alternating with the xylem arms, are groups of **phloem** cells. **42-D What is the function of phloem?**

FIG. 10.1 ANATOMY OF THE ROOT



• If the root is old enough, you may be able to see the **cambium,** a row of "brick-like" cells lying between the xylem and phloem. The division of these cells is responsible for the growth in diameter of many roots.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Describe how a root grows longer.
2 In diagram form, show how the cambium contributes to the growth in diameter of the root.
3 List plants that have economically important roots.
4 What are "root hairs?" In what way are root hairs important?
5 What are branch roots? Where do branch roots come from?

EXERCISE 43

ANATOMY OF THE STEM

The stem is that part of a plant which bears leaves and is commonly aerial and upright. Stems, like roots, have an epidermis, a cortex, and a vascular cylinder. Stems differ from roots in that they have lateral appendages called leaves.

Stems have several functions: 1. they provide the framework supporting leaves, flowers, and fruits; 2. they provide for the transport of water and dissolved minerals absorbed by the roots; and 3. they serve to transport the foods manufactured during photosynthesis to those parts of the plant where it is used or stored.

Some stems are green and carry out a limited amount of photosynthesis. Many stems function as food storage areas—sometimes to the extent of being economically useful.

PROCEDURE—The Shoot Apex

The stem, plus its attached leaves, is called a **shoot.** The tip of a shoot is known as the **shoot apex.** This region, like the root apex, is an area of active cell division.

▶ Examine a slide of the shoot apex of *Coleus,* a plant that is frequently used as an ornament around homes (Figs. 10.2A,B,C).

▶ Locate the shoot apex. The cells in this region look very much alike. In many of the cells, the nuclei may show different stages of mitosis.

▶ Projecting from the sides of the apex are **leaf primordia**—young leaves. The youngest leaves are found closest to the apex. These leaf primordia are attached to the stem at regions called **nodes.** The interval between two successive nodes on the stem is an **internode.**

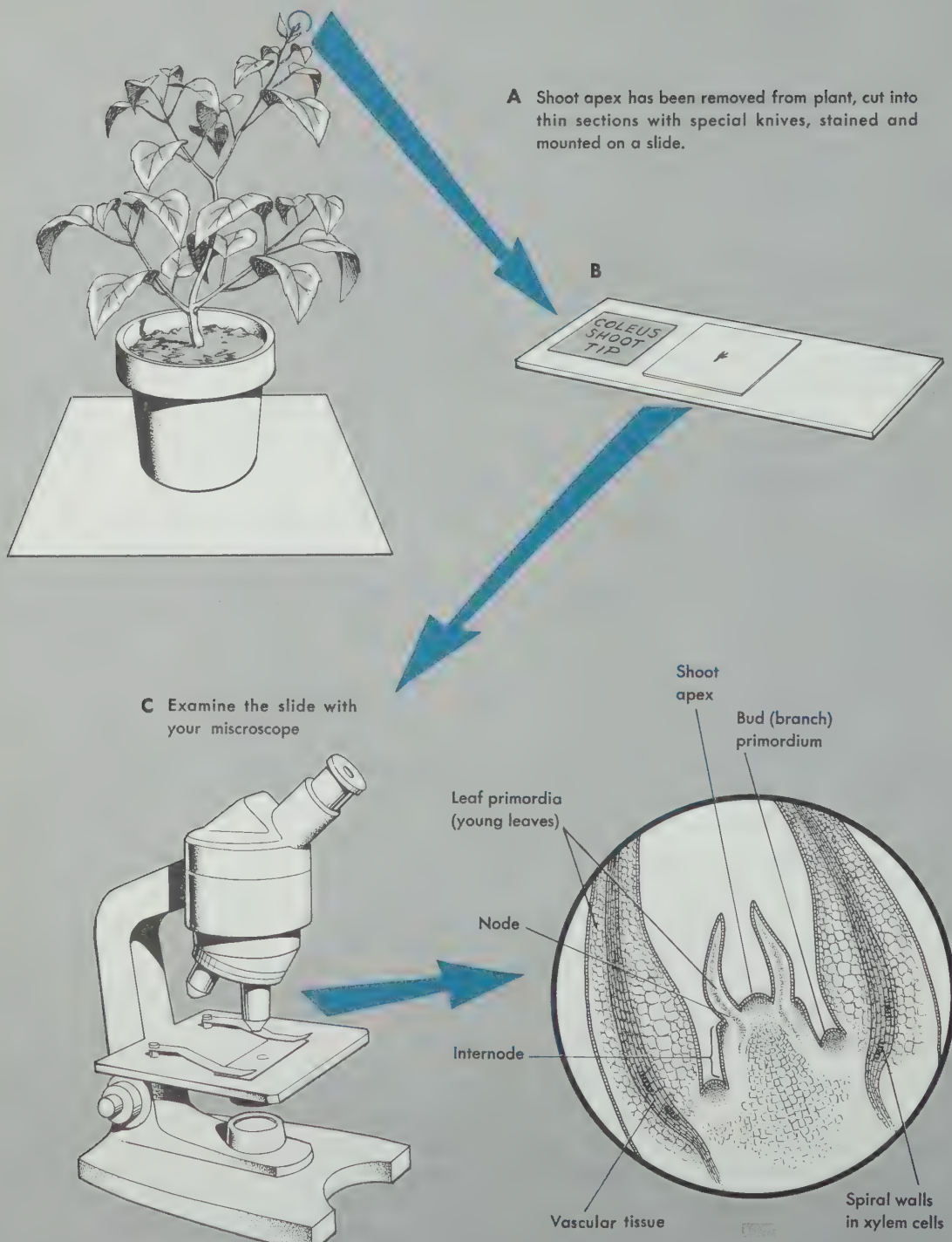
▶ Closely examine the larger leaf primordia. Locate reddish colored spiral shaped structures. These are thickenings in the cell walls of xylem tissue. **43-A What is the function of xylem?**

PROCEDURE—The Origin of New Branches

▶ In the angle that the leaf primordia make with the stem, you will find small rounded projections called **bud (branch) primordia.** These represent new shoot apices that will eventually produce new branches and leaves. Locate these structures on the slide of the *Coleus* stem tip.

▶ In numerous woody plants (the common trees and shrubs) the bud primordia enlarge and become enclosed in structures

FIG. 10.2 ANATOMY OF THE STEM



called **bud scales.** Obtain maple tree branches from your instructor. Locate a terminal bud at the tip of one of the branches. Using a dissecting microscope, and strong surface illumination, dissect and remove all the bud scales. **43-B What evidence is there that the bud of maple will develop into a new branch?**

PROCEDURE—Microscopic Examination of Stem Tissues

▶ Examine a prepared slide of a cross-section of an alfalfa stem (Figs. 10.3A,B,C). The outermost layer of cells makes up the **epidermis** (Fig. 10.3D). The outer walls of epidermal cells frequently become thickened and during their development secrete a waxy substance called **cutin** onto their surfaces. If you close the iris diaphragm you may be able to observe the layer of cutin. It will appear as a faint, pink layer covering the epidermal cells.

▶ The **cortex** lies beneath the epidermis and consists of two distinguishable tissues. One of these is a mechanical tissue that provides support for the plant. Locate this tissue in the rounded corners of the stem on your slide (Fig. 10.3D).

▶ Locate the vascular bundles encircling the stem (Fig. 10.3D). A mature vascular bundle consists of three main tissues—xylem, phloem, and cambium. The phloem is located toward the outside of the bundle and the xylem toward the center. Separating the xylem from the phloem is the cambium. Division of the cells of the cambium results in the formation of new tissues which increase the diameter of the stem.

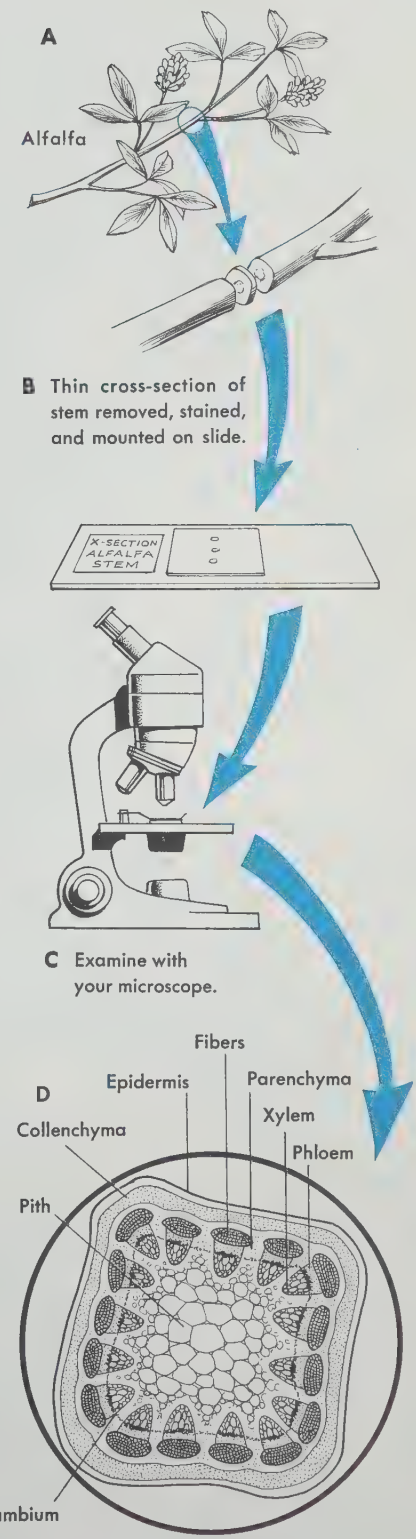
▶ On the outer surface of each vascular bundle is a group of thick-walled cells called phloem **fibers.** **43-C Suggest a function for these fibers.**

▶ The central part of the stem is made up of thin-walled cells. This region is called the *pith.*

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What are primary tissues? What are secondary tissues?
2 Bud scales are considered to be protective structures enclosing the bud. What do these scales protect the bud from?
3 Suggest a function for the layer of cutin that may be found on the surface of epidermal cells.
4 List some plants whose stems are commonly used for food by man.
5 Where does cork come from?
6 What is a "growth ring?"

FIG. 10.3
ANATOMY OF THE STEM



EXERCISE 44

ANATOMY OF THE LEAF

All forms of life, with the exception of some kinds of bacteria, depend directly or indirectly upon the process of photosynthesis. In the higher plants, most of this photosynthetic activity occurs in the leaves.

PROCEDURE—Gross Anatomy

▶ Examine the various plants on demonstration. Locate the following parts of a typical leaf:

- the expanded, flattened **blade.**
- a thin, stem-like **petiole** that attaches the blade to the stem. Some kinds of leaves do not have petioles. These are called **sessile** leaves. **44-A Which of the plants you are examining have sessile leaves?**
- small, paired, lobe-like structures found where the petiole is attached to the stem are the **stipules.** These are not present in all plants. **44-B Which of the plants have stipules?**

▶ Remove one of the leaves from the *Coleus* plant and look at the bottom surface. Note the numerous, somewhat raised strands. These are called **veins,** and are composed of vascular tissues. Leaves may be simply classified according to their type of **venation** (arrangement of veins).

- Leaves are **parallel-veined** when the larger vascular strands appear as lines running the length of the leaf. **44-C Which plants on demonstration have this type of arrangement?**
- Leaves are **net-veined** when the vascular strands form a network over the surface of the leaf. **44-D Which of the plants have the veins arranged in this fashion?**

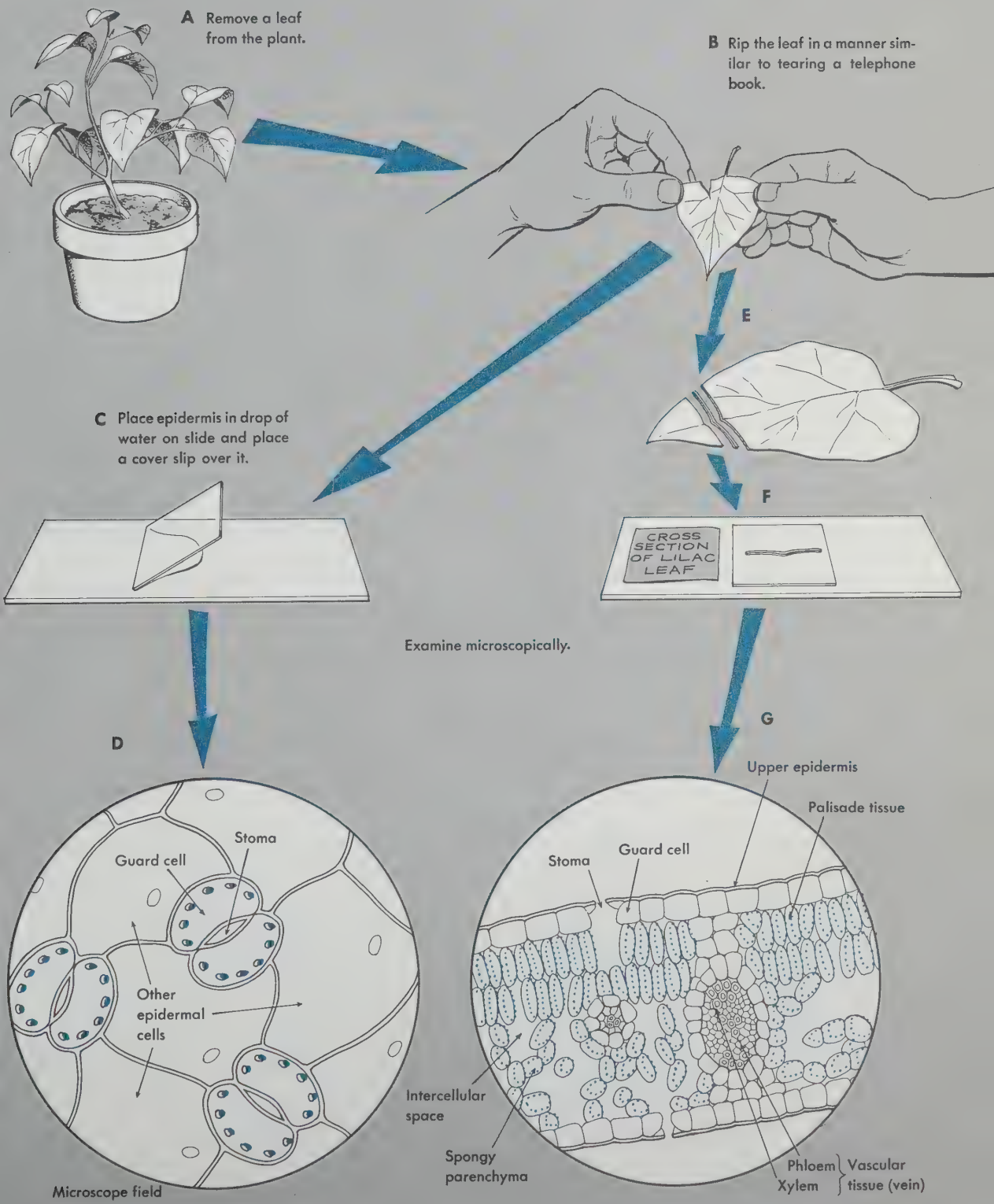
▶ Leaves may also be classified according to how they are attached to the stem.

- Leaves are **alternate** if only one leaf is attached to a node.
- Leaves are **opposite** if two leaves are attached to the node. **44-E Which of the plants has opposite leaves? 44-F Which of the plants has alternate leaves?**

PROCEDURE—Microscopic Anatomy

▶ Several tissues are present in the blade of a leaf. On the upper and lower surfaces are irregularly-shaped cells that make up the epidermis. Obtain a leaf from one of the plants provided by your instructor. Remove the lower epidermis by

FIG. 10.4 LEAF ANATOMY



ripping the leaf like you were tearing a telephone book (Figs. 10.4A,B). If this is done properly, a thin, transparent piece of tissue will be seen on the torn edge. Mount this tissue in a drop of water on a slide and examine with the low and high power of your microscope (Fig. 10.4C).

▶ Locate the numerous, kidney-shaped **guard cells** (Fig. 10.4D). The opening between the two guard cells is called a **stoma** (plural, **stomata**). It is through these stomata that the CO_2 in the air enters the leaf. The stomata are usually closed at night and open during the daytime. **44-G In what ways are these guard cells different from the other epidermal cells?**

▶ Obtain a prepared slide of a cross section through a lilac leaf. With the help of Figs. 10.4E,F,G, locate the following tissues of the leaf:

- The **palisade tissue,** located below the upper epidermis, consists of one or more layers of elongated cells. The palisade cells contain numerous chloroplasts. It is this layer in leaves in which most of the photosynthesis occurs. Locate the *stomata* and *guard cells* in the upper epidermis.
- **Spongy parenchyma** cells are located beneath the palisade cells. They are rounded and irregularly shaped. Note the presence of numerous spaces in this tissue. **44-H Suggest a role for these spaces in the functioning of the leaf.**
- **Vascular tissue (veins),** which appeared as raised strands on the bottom surface of the leaf, can be seen in the spongy parenchyma. The veins are continuous with the vascular tissue of the petiole and stem.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Describe the way in which the leaf is uniquely suited to carry out photosynthesis.
2 What color of light is least absorbed by green leaves?
3 How do the guard cells regulate the opening and closing of stomata?
4 What is the relationship between the stomata and transpiration?
5 List some plants whose leaves are commonly used for food by man.

EXERCISE 45

ANATOMY OF THE FLOWER

Thus far, the plant has been studied as an individual composed of roots, stems, and leaves. These structures develop and function in such a way to insure the successful existence of the plant. Ultimately, however, the plant dies. It is through a constant succession of new individuals that the species survives. This is accomplished by a process called **reproduction** in which the plant produces a group of offspring.

There are two main types of reproduction—**asexual** (or vegetative) and **sexual.** Flowering plants can reproduce asexually by numerous means. For example, the parent plants may split into two or more parts, each of which may then become independent of the others. In another case, the tip of a branch may arch down and come into contact with the soil, and develop leafy branches that root and form new plants. The most common method of reproduction, however, is by sexual means. To understand sexual reproduction in plants, it is necessary to study the anatomy of the flower.

PROCEDURE—Flower Anatomy

Your instructor will provide you with two or three different flowers that you may use for this exercise.

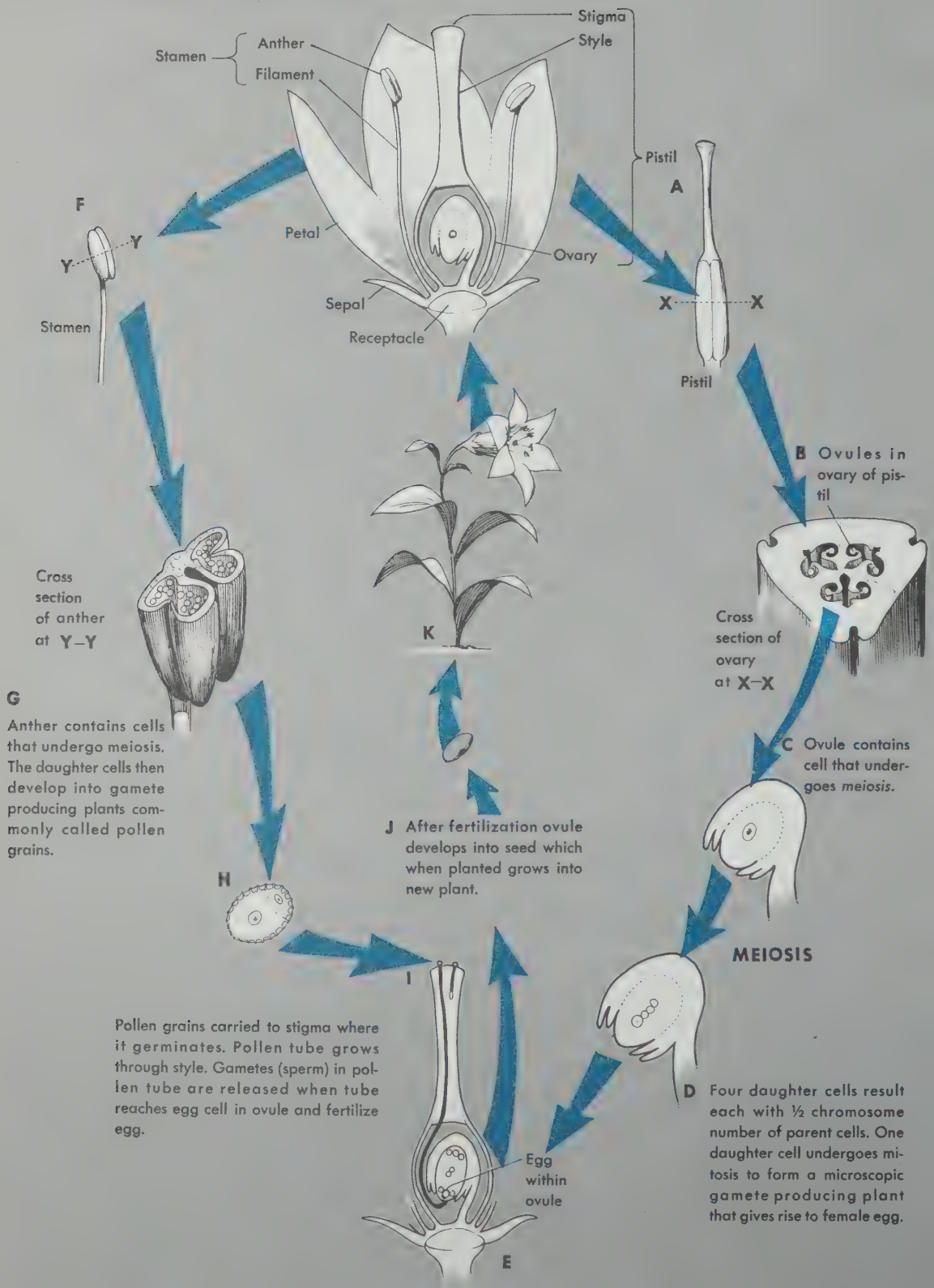
▶ With the help of Fig. 10.5 locate the following parts of these flowers:

- **Sepals** are modified leaves and are the outermost structures of the flower. They are typically green, although they may be colored. In some cases they may not be present.
- **Petals** lie to the inside of the sepals and are often brightly colored. Both the sepals and petals are attached to the enlarged end of the branch—the **receptacle.**

▶ Carefully remove the sepals and petals. In the center of the flower locate a stalk-like structure (Figs. 10.5A,B). This is the female part of the flower—the **pistil.** It is composed of a swollen base, the **ovary,** and an elongated **style** that is terminated by a **stigma.** There may be more than one pistil in a flower.

▶ Find the several **stamens** that surround the pistil (Fig. 10.5F). These are the male parts of the flower and consist of a terminal capsule—the **anther**—attached to a slender **filament.**

FIG. 10.5 ANATOMY OF THE FLOWER



PROCEDURE—Meiosis

The ovary contains one or more ovules. These may be seen if the ovary is cut lengthwise with a sharp razor, mounted in a drop of water on a slide and examined with the microscope. One of the cells in the ovule undergoes a special type of cell division called **meiosis** where the daughter cells that are formed have *one-half* the number of chromosomes that the parent cell had. One of the daughter cells develops into a microscopic plant that will produce the female **gamete,** the egg cell (Figs. 10.5C,D, E). **45-A Why is it necessary that meiosis occur during the formation of the gametes?**

The anthers contain cells that also undergo meiosis, producing cells that eventually develop into microscopic gamete producing plants commonly called **pollen grains** (Figs. 10.5G,H). Crush one of the anthers in a drop of water on a slide and add a cover slip. Examine it with your microscope and locate the pollen grains. During pollination, the pollen grains are transferred to the stigma—the sticky surface of the pistil. The pollen grain germinates and the **pollen tube** grows through the style to the ovary and enters the ovule (Fig. 10.5I). **45-B How does the pollen tube "know" that it should grow toward the ovary where the ovules are located?**

During the growth of the pollen tube, two male gametes (sperm) are produced. These are released when the tube enters the ovule. One sperm fertilizes the egg cell. The remaining sperm unites with the nucleus of another cell in the ovule. This second fertilization results in the formation of a special tissue—the **endosperm**—which you will learn more about in the next exercise.

Fertilization of the egg initiates extensive changes in the pistil. The ovary enlarges and develops into the fruit; in some cases, other floral parts also become part of the fruit. The ovules develop into seeds, which now contain the embryo of a new plant (Figs. 10.5J,K).

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 List some plants that can be propagated by asexual means.
2 Why might farmers want to use asexual propagation of plants instead of sexual propagation?
3 List several ways in which pollination is brought about.
4 In what way is flower color an aid to pollination?
5 In what way are seeds important to the plant? To animals? To man?

EXERCISE 46

ANATOMY OF SEEDS

Seeds are formed following the union of the sperm and egg in the ovary of the flower. Refresh your memory of the anatomy of flowers by referring to Fig. 10.6A. Since the process of development in higher plants begins with the seed, it would seem logical then to examine the structure and function of seeds.

PROCEDURE

Obtain previously soaked bean and corn seeds. Remove the seed coats from each and locate the following structures:

▶ **Cotyledons.** Scrape the inner surface of cotyledons of the bean. Add a drop of iodine and wait a few minutes. **46-A What appears to be the role of the cotyledons in the development of the bean seed?**

▶ The **epicotyl** gives rise to the shoot system of the mature plant. **46-B What evidence is there that this region will form stems and leaves?**

▶ The **hypocotyl.** Examine the bean seed closely (Fig. 10.6B). **46-C What part of the plant do you think will develop from the hypocotyl?**

▶ The **endosperm.**

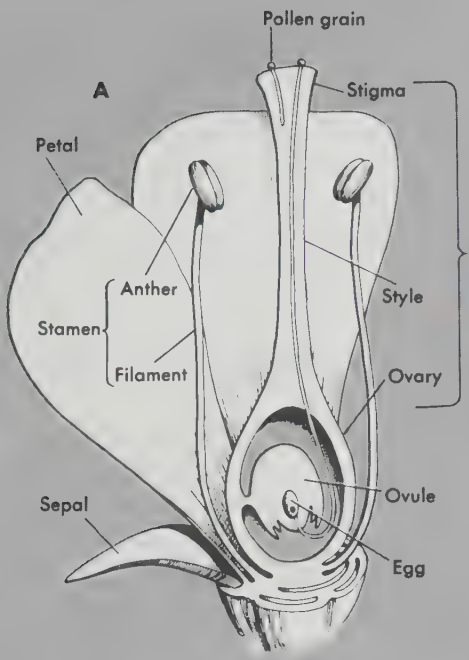
- Remove the endosperm from 10 corn seeds, and with a razor blade, cut them into small pieces (Figs. 10.6C,D).
- Divide the material into two equal piles. Add one pile to a test tube one-third filled with Benedict's solution. Heat the tube in a boiling water bath for several minutes (Fig. 10.6E). **46-D Describe what happens.**
- Add one or two drops of iodine to the remaining pieces of endosperm (Fig. 10.6F). **46-E What is the result?**
- **46-F On the basis of your results suggest a role for the endosperm in the development of seeds.**

▶ Several days ago your instructor planted some bean seeds. Remove one of the seedlings from the container it is growing in. Locate what was formerly the epicotyl and hypocotyl regions. **46-G What have these areas produced in the young plants?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

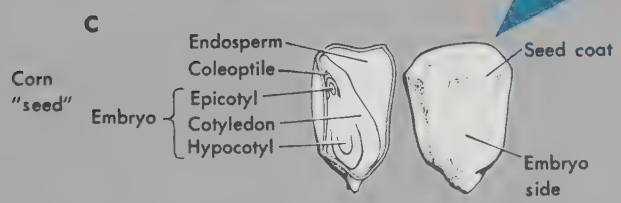
1 What is meant by **germination?**
2 List some factors that are sometimes necessary for seeds to germinate.
3 Describe an experiment you might carry out to determine the germination potential of seeds stored for 10 years.
4 How would you go about determining the optimum temperature for germinating bean seeds?

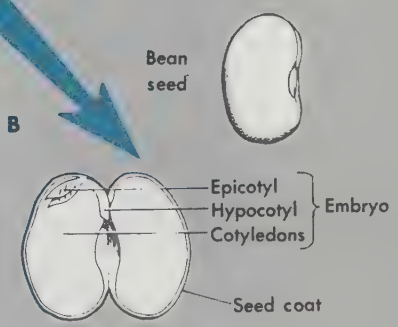
FIG. 10. 6 SEED ANATOMY



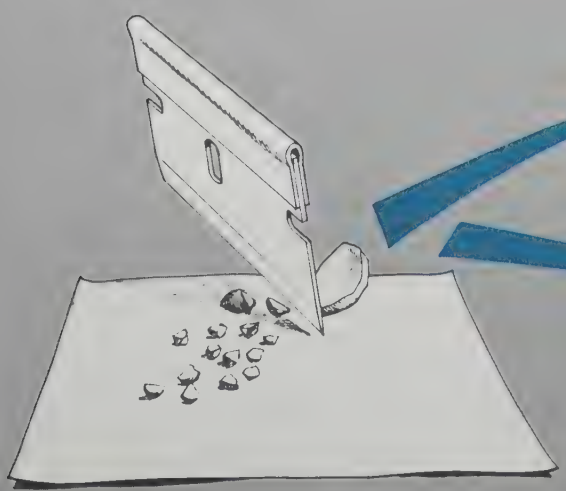
The stamens of a flower produce pollen in a terminal capsule called an anther. The ovary contains "seed-like" ovules which, when mature, contain the female gamete (egg). In the process of pollination, the pollen grains are transported to the stigma, where they may germinate and send a long tube down to the ovules. The pollen tube contains the male gametes (sperm).

When the pollen tube reaches the ovules it digests its way to the vicinity of the egg, then bursts and releases the sperm which fertilizes the egg. The ovule then undergoes a series of changes and develops into a seed.





D Remove the endosperm from 10 corn seeds, and cut them up into small pieces.

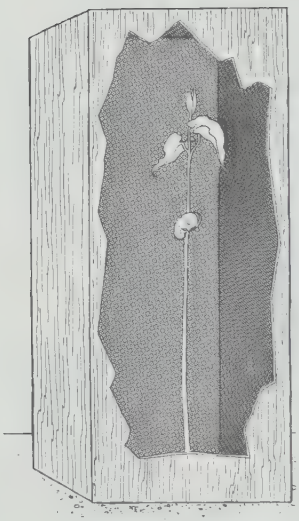


E

Add ½ of the cut-up endosperm to a tube ⅓ full of Benedict's solution. Heat in boiling water bath for several minutes.

F Add a drop of iodine to the remaining pieces of endosperm tissue.

11 PLANT DEVELOPMENT



INTRODUCTION

One of the more challenging problems facing biologists today is that of understanding the basic mechanisms that regulate the patterns of development in plants and animals. The systematic differentiation of millions of cells into various tissues and organs, and the development of specific form and shape, require precise coordination between the individual parts of the organism during each stage of growth.

The development of a seed into a mature plant is one aspect of this remarkable process. It involves growth by cell division and cell extension, differentiation of new organs such as roots, stems, leaves, and flowers, and a complex series of chemical changes. The final form of the plant is a blend of the plant's genetic "blueprint" and modifying effects of the environment. When the seed begins to germinate, or sprout, it absorbs large amounts of water, and the growing points begin cell division. For reasons we do not yet understand, the root almost always begins to develop before the shoot apex does. At both root and shoot ends of the seed new cells are formed by the meristematic (dividing) areas of the growing points, followed by elongation and differentiation of these cells.

Ultimately, of course, the control of growth and differentiation is in the DNA of the nucleus, which controls the production of hormones and other regulatory chemicals. We have made only a start in understanding just how the various patterns of growth and differentiation are regulated.

The role of hormones and other chemical regulators in controlling plant growth are now well established. Some of the growth regulating substances in plants are auxins, gibberellins, cytokinins, and various growth inhibitors.

Auxins are produced at the apices of roots and shoots and influence cell elongation, inhibit the growth of lateral buds,

promote the initiation of roots, and, in a few plants, regulate the differentiation of flower buds.

Gibberellins are highly complex substances that greatly affect the over-all growth and flowering responses of many plants.

Cytokinins are related to one of the chemical substances found in DNA, and promote cell division. **Inhibitors** of various kinds control such responses in plants as flowering, dormancy of buds and seeds, and rates of growth.

In the exercises that follow, the effects of certain environmental and chemical factors on the patterns of growth and differentiation will be studied.

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EXERCISE 47

IN WHAT PART OF THE PLANT DOES GROWTH OCCUR?

Growth may be defined simply as an irreversible increase in volume that is generally accompanied by an increase in weight. Merely measuring an increase in size or weight, however, does not tell us *where* growth occurs in the plant. In a root, does growth occur all along its length, or is it restricted to some specific region?

PROCEDURE

▶ Cover a glass plate (about three by four inches) with a moist paper towel. Then place it temporarily in a moist chamber made from a beaker lined with a wet paper towel. Place a large petri dish cover over the container (Fig. 11.1A).

▶ Select a pea that has a fairly straight root about 2½ centimeters long. Blot the root with a paper towel (Fig. 11.1B).

▶ Lay the root against a millimeter ruler. With a marking device provided by your instructor, carefully mark 10 lines, one millimeter apart, starting from the tip (Fig. 11.1C).

▶ Repeat this marking operation until five roots have been marked. As each root is marked, place the germinating seed between two pieces of moist towel (Fig. 11.1D).

▶ Remove the paper-covered glass plate from your moist chamber and lay the seedlings on it. Hold them in place with a rubber band so that the entire length of the roots is touching the moist paper towel (Fig. 11.1E).

▶ Place the plate and seedlings into your moist chamber, put the cover back on, and put the chamber in a dark place. After 48 hours measure the distances between the marks on each root. **47-A Record your data in the table on page 337.** Average the lengths for each interval. **47-B Graph your data on page 337. 47-C On the basis of your results in this study, where does most of the growth of the root occur? 47-D Providing that the marks you initially made were sharp and clear, how would you account for smudging of the first and possibly the second lines? 47-E If you were to cut a section lengthwise through the root and stain the cells, what would you expect to see that would account for the results you observed?**

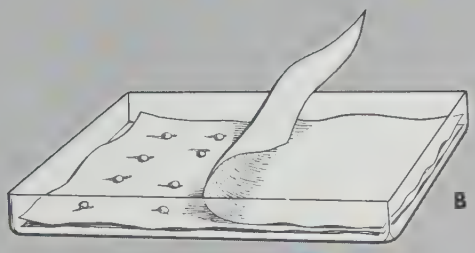
FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is a **meristem?** Where are meristems found in plants?
2 Briefly describe the internal structures of a typical root.
3 Contrast the terms **growth** and **differentiation.**

FIG. 11.1 PROCEDURE FOR DETERMINING WHERE GROWTH OCCURS IN ROOTS

A Prepare a moist chamber lined with wet paper towel.

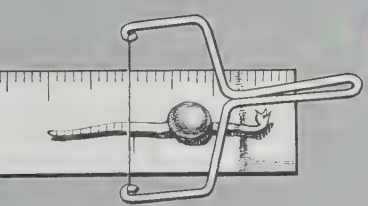
Petri dish cover



B Select a germinating seed with a fairly straight root 2 to 2½ cm long.

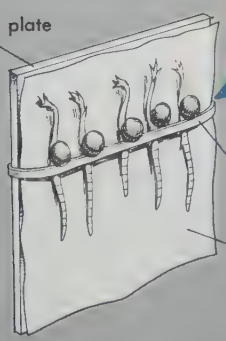
Blot the root with a paper towel to remove excess moisture.

C Lay the root against a millimeter ruler. Wipe excess ink from thread and carefully mark 10 lines, 1 mm apart, starting from the tip of the root.



D As each root is marked, place the seedling between moist towels to prevent drying.

Glass plate

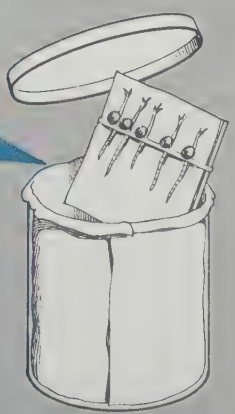


Rubber band.

Moist paper towel.

E Lay the seedlings on a glass plate and hold them in place with a rubber band.

F Place the plate into your moist chamber, cover, and set in a dark place for 48 hours.



EXERCISE 48

WHAT IS THE EFFECT OF LIGHT ON PLANT GROWTH?

As early as 1880, a plant biologist, using carbon arc lamps as a source of light, showed that artificially extended days promoted the growth of various plants. Following the invention of the electric light bulb, a large amount of data was accumulated on the effects of light on plant development. These ranged from studies on the effects of light on vascular tissue development to the recent establishment of the presence of a pigment called **phytochrome,** which apparently plays an important role in the plant's growth responses to light. In this exercise you will study the effect of light on plant development.

PROCEDURE—Plant Growth in the Absence of Light

▶ From your instructor, obtain two pots or trays that contain soil. Label one "dark" and the other "light." Include your name, the date, and section number on each label (Fig. 11.2A).

▶ Plant 10 bean seeds about ½ inch deep in each container. Water thoroughly and then place the pots where they will receive sunlight (Figs. 11.2B,C).

NOTE: Remember to water the trays each day.

▶ During the next three to four days, watch for the young seedlings to emerge from the soil. When the seedlings are about two inches tall place the "dark" tray in a closed cabinet or other dark place (Fig. 11.2). Leave the "light" tray where it is.

▶ After about 10 days examine the plants that have been growing in the dark. Compare them with the "light" grown plants. **48-A Record your observations in the table on page 339.**

▶ Then reverse the conditions under which the plants have been growing (that is, place the "dark" plants in the light and the "light" plants in the dark). Examine the plants after seven to 10 days. **48-B Describe your observations in the table on page 340.**

PROCEDURE—The Effect of Day Length on Flowering?

You are probably familiar with the fact that the length of the daily light period varies with the season. In the Northern Hemisphere the day length increases during the Spring and reaches a maximum on June 21. Thereafter, the day length decreases to a minimum on December 21st. The length of day is called the **photoperiod.** The responses of plants to photoperiods of varying lengths is called **photoperiodism.**

FIG. 11. 2 EFFECTS OF DARKNESS ON PLANT GROWTH

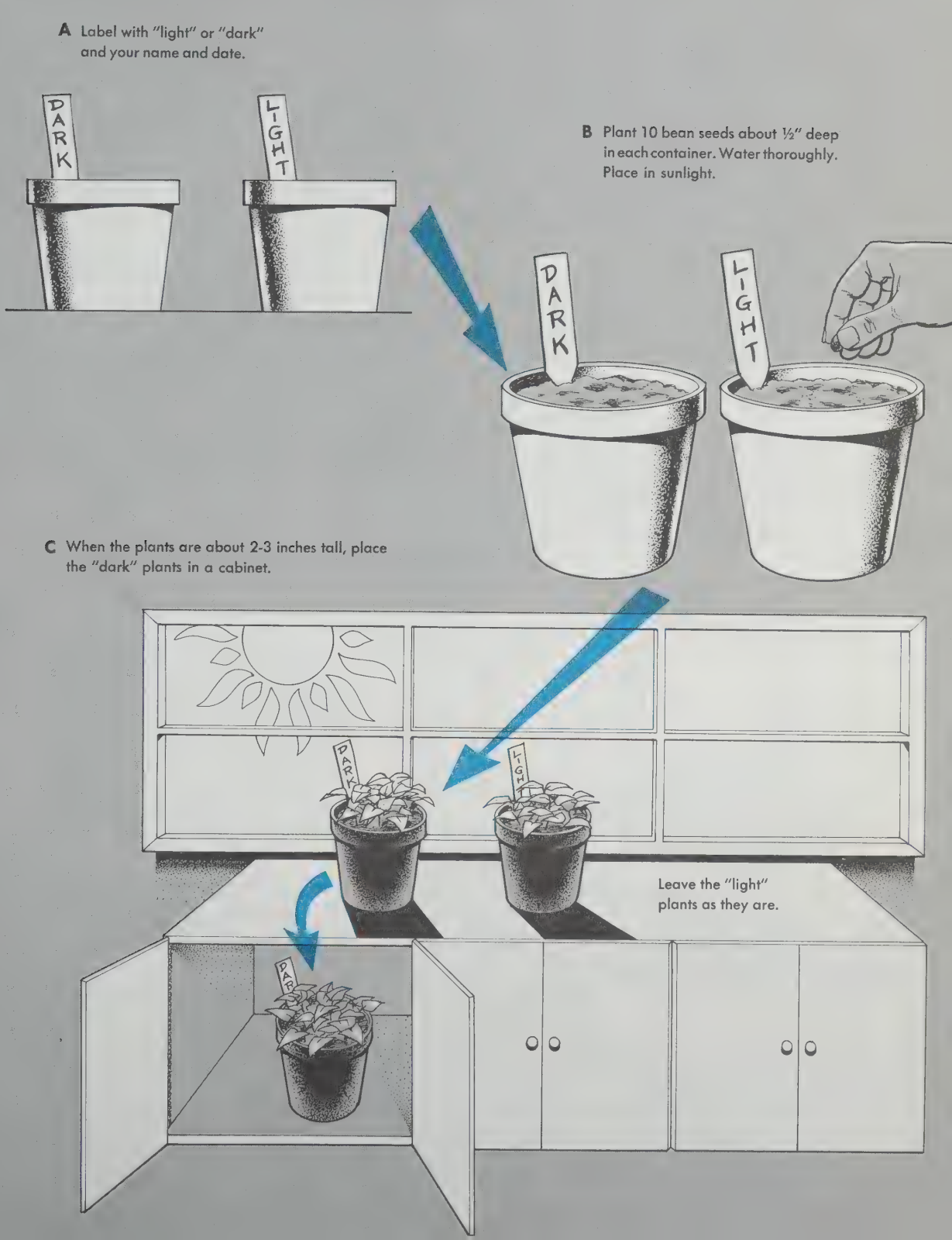
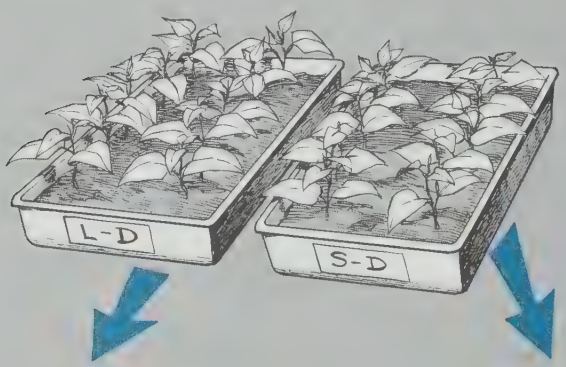


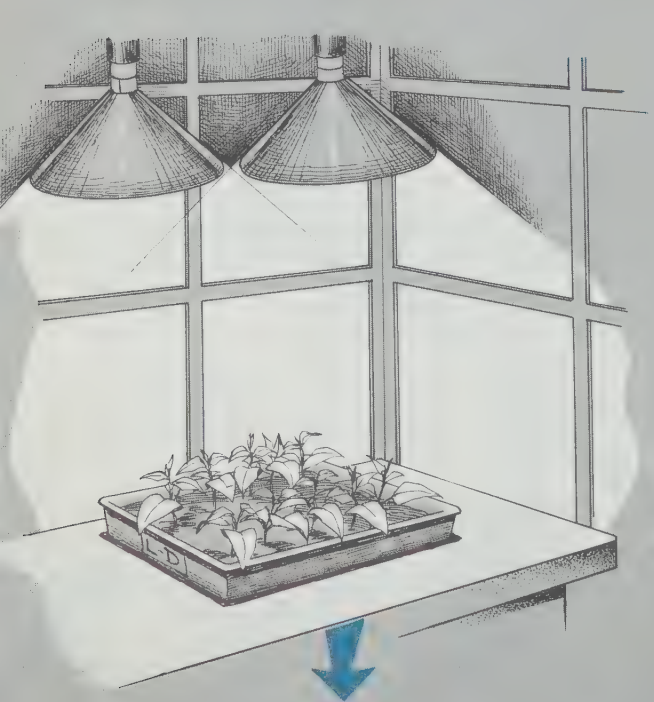
FIG. 11.3 EFFECT OF PHOTOPERIOD ON FLOWERING

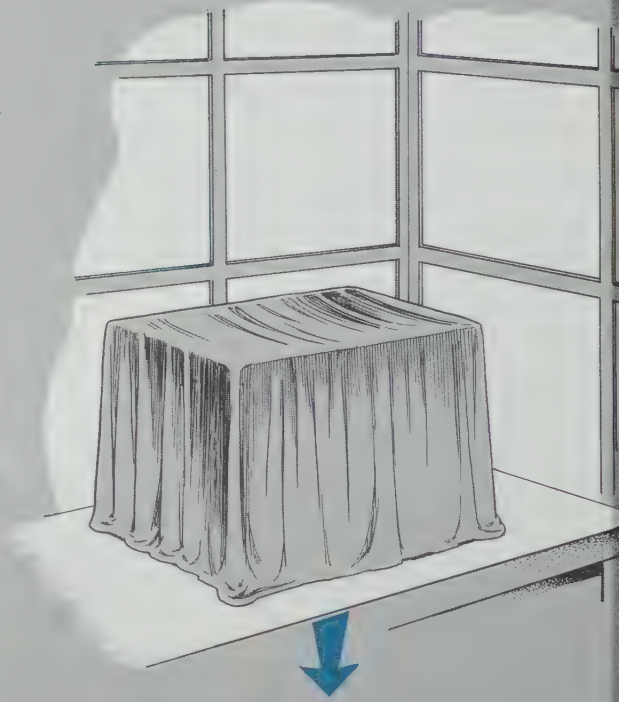
A Label with long day (L-D) or short day (S-D) along with your name, date, and section number.



B Place one group of plants under L-D conditions.

C Place the second group under S-D conditions.

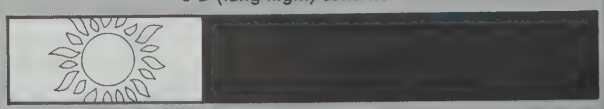




L-D (short night) conditions

8 hours of sunlight | 8 hours of artificial light | 8 hours of darkness

S-D (long night) conditions



8 hours of sunlight | 16 hours of darkness

D Examine the plants weekly for the appearance of flowers. After 3 to 4 weeks (if no flowers are visible) dissect the buds to determine if they have flowers or leaves in them.

There are basically two types of photoperiodic flowering responses. These are called **long-day** and **short-day** responses. Plants that exhibit these responses are called long-day (L-D) and short-day (S-D) plants. L-D plants flower when the photoperiod exceeds some critical value, which is generally in *excess* of 14 hours. At day lengths less than the critical value, the plant usually grows only vegetatively (that is, it produces leaves). An S-D plant (really a long-night plant) will flower when the photoperiod is *less* than some critical value, usually about 10 hours, and grows vegetatively when this critical day length is exceeded. Other plants, not responsive to day length, are called **day-neutral** plants. In this exercise you will examine the effects of the photoperiod on flowering in plants.

▶ Obtain 10 pots of the plant provided by your instructor. Label five of the pots "L-D" along with your name, section number, and date. Label the other five pots "S-D" (Fig. 11.3A).

NOTE: Two trays with 10 plants each are also acceptable.

▶ Place the plants under light conditions so that the L-D plants receive 16 hours of light and eight hours of dark (Fig. 11.3B). The S-D plants should receive eight hours of light and 16 hours of dark (Fig. 11.3C).

▶ Examine the plants weekly and look for the appearance of flower buds. Detecting flowers in their early stages of development is difficult so you will have to look closely. Avoid excessive handling of the plants.

▶ If at the end of three to four weeks flowers do not appear, remove the buds from each plant and place them in separate, labeled petri dishes that contain moistened paper.

▶ Using a dissecting microscope, carefully dissect the buds and determine if flowers or leaves are present. Your instructor will have "flower" and "leaf" buds on demonstration so that you may distinguish between the two. **48-C Are the plants you studied L-D or S-D plants? Explain.**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is meant by **etiolation?**
2 Assuming that in the second part of the exercise flowering is regulated by the photoperiod, how would you go about determining what part of the plant was perceiving the light stimulus?
3 What is **phytochrome?** What role does phytochrome play in the regulation of flowering?
4 What other aspects of plant growth are affected by light?

EXERCISE 49

HOW DO GIBBERELLINS AFFECT PLANT GROWTH?

Gibberellins are plant growth substances that were first isolated in Japan from a fungus that caused a disease called "foolish seedling disease." The Japanese scientists who studied this disease found that the fungus was producing chemical substances that were strongly affecting the normal growth and development of rice plants. Gibberellins are also produced by the higher plants, beans, for example.

In this exercise you will attempt to determine what aspect of plant growth is affected by this plant growth substance.

PROCEDURE

▶ Working in teams of three, obtain 40 bean seeds that have been soaking in water for several hours.

▶ Plant 20 seeds (about ½ inch deep) in moist vermiculite in a tray. Label the tray "Gibberellin treated" (Fig. 11.4B).

▶ Plant the remaining 20 seeds in a second tray labeled "Control" (Fig. 11.4B).

▶ Watch the trays for the next seven to 10 days. When the plants are several centimeters tall (about three inches), select 10 plants in each tray that are about the same size. Label each individual plant with a number (1, 2, 3, . . .) along with the date. Cut the remaining plants at the ground level and discard the parts you have cut off (Fig. 11.4C).

▶ Measure the height of each plant (in millimeters) from the soil to the tip of the shoot apex. **49-A Record the individual measurements in the table (page 341) under the column headed "Day 0."**

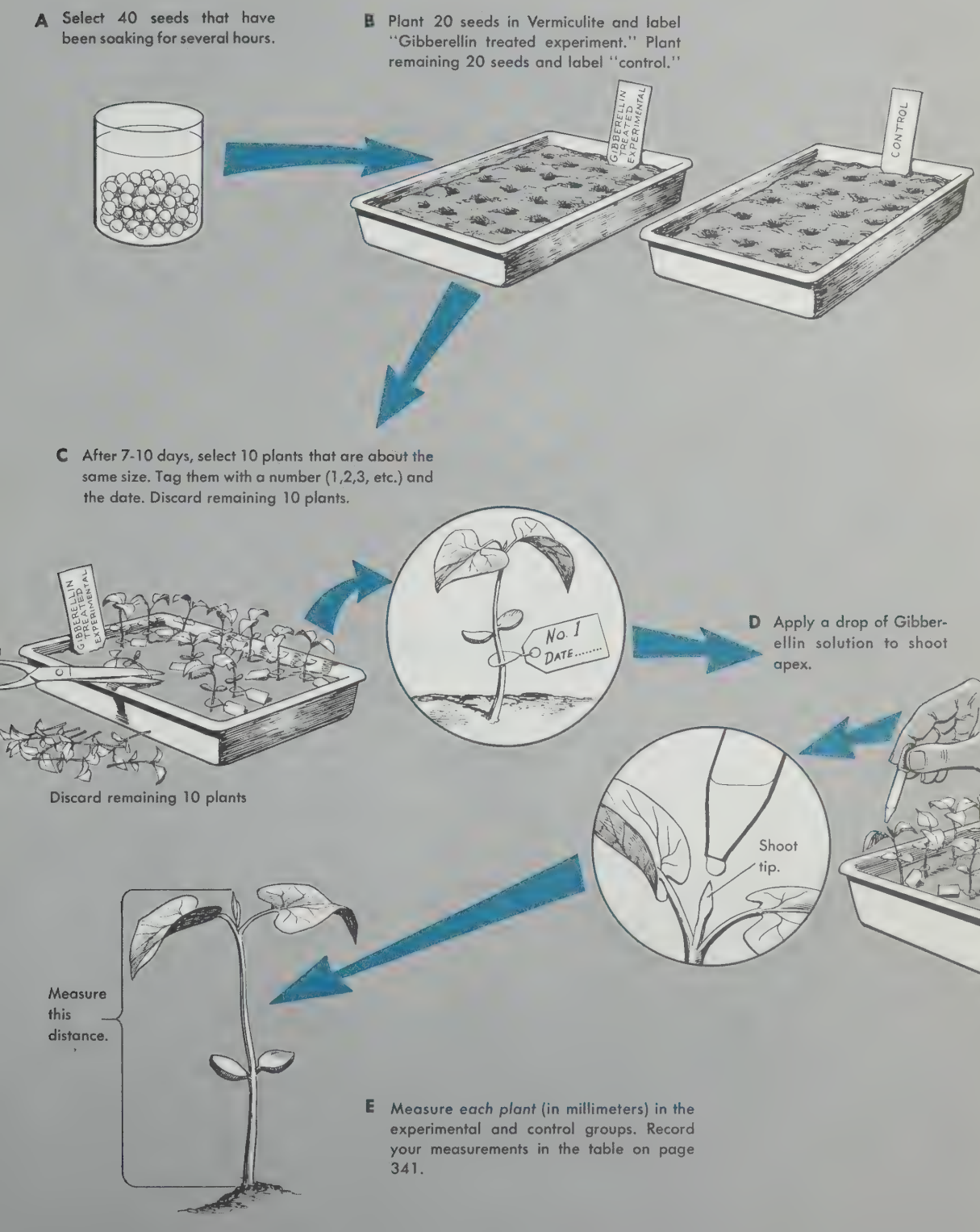
▶ Apply a drop of gibberellin to the shoot apex of each plant in the "G-A" tray (Fig. 11.4D). **49-B What will you apply to the "control" plants? (This procedure should be repeated in three to four days.)**

NOTE: This procedure may be modified if a weekend falls during the first 5 measurements.

▶ Measure the height of each plant in the "experimental" and "control" groups on each of five days following the initial measurement (Day 0) and on the eighth day (Fig. 11.4E). **Record the measurements in the table (49-A). 49-C Do the control plants respond to gibberellin in the same way as the experimental plants? If not, how do they differ?**

▶ Using the data in the table (49-A), calculate the *per cent* increase in length for each group on the first, second, third, fourth, fifth, and eighth day by using the following formula:

FIG. 11. 4 PROCEDURE FOR DETERMINING EFFECT OF GIBBERELLIN ON PLANT GROWTH



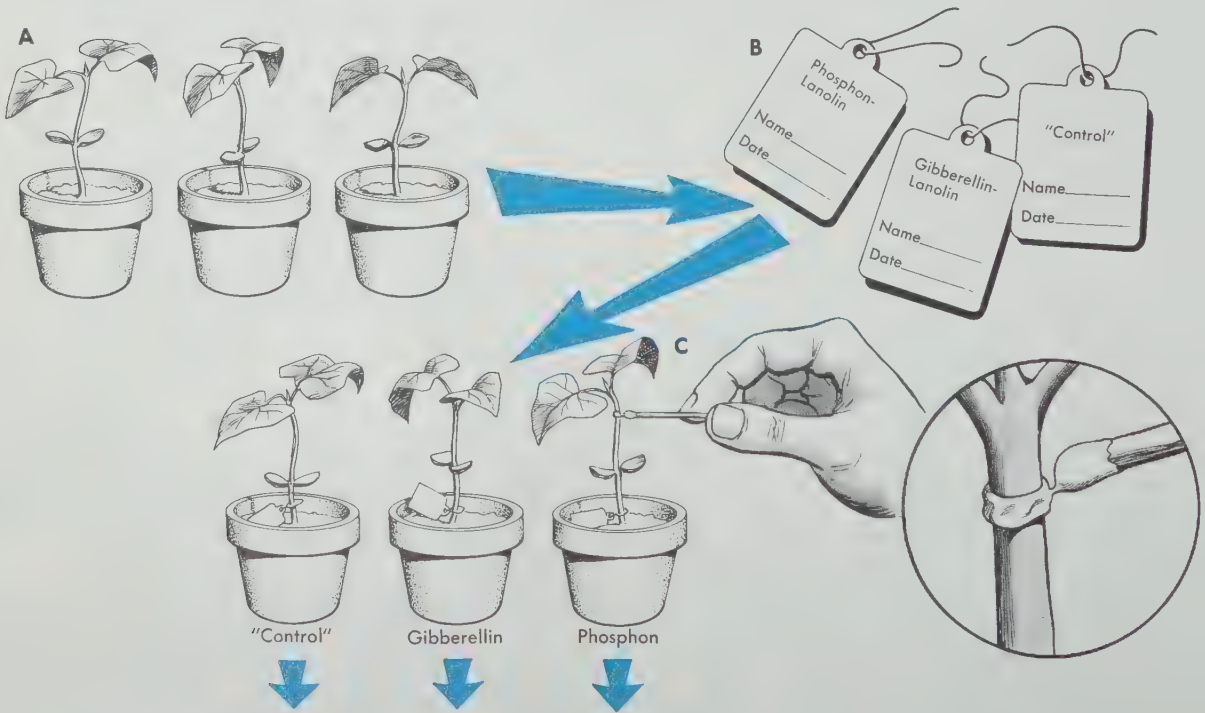
$$\frac{\text{Average length (day 1, 2, 3, etc.)} - \text{Average Initial Length}}{\text{Average Initial Length}} \times 100 = \% \text{ Increase in Length}$$

Plot these data in 49-D. Use a different colored pencil for the experimental and control group.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Based on the results of this experiment, what do you think the rice plants that have "foolish seedling disease" look like?
2 The peas used in this exercise are a dwarf variety whose dwarfness is controlled by a single gene. Suggest a possible way this gene might produce dwarf plants.
3 How would you go about determining where gibberellins are produced in the plant?

FIG. 11.5 EFFECT OF GROWTH INHIBITORS ON PLANT DEVELOPMENT



D Examine the plants every 2 to 3 days for the next 3 weeks. Record your observations in the table (on page 343) and by a drawing (on page 344).

EXERCISE 50

PLANT GROWTH INHIBITORS

The concept that inhibitors were important in the regulation of plant growth first gained importance in the late 1940's.

Experimenters found that dormant buds of ash trees contained large amounts of inhibitors and that when dormancy was broken the inhibitor concentration declined. More recently, British scientists have isolated a substance called **dormin** which they believe causes the plant to stop growing and enter into its dormant state. Surprisingly, dormin is identical with another substance that is responsible for causing leaves to fall off (absciss) plants late in the growing season. This substance is called **abscissin.** Thus a single substance isolated from different plants not only controls leaf fall but also regulates dormancy and, possibly, many other growth processes.

PROCEDURE

▶ Your instructor will provide you with a pot or tray containing several bean or sunflower seedlings. Select three plants that are in about the same stage of development (only the first two leaves should have expanded). Tag them (1) "phosfon," (2) "gibberellin," (3) "control," along with your name and date (Figs. 11.5A,B).

▶ Using a wooden match or similar applicator, apply a ring of phosfon-lanolin paste around the stem of the plant labeled "phosfon" about ½ inch below the first pair of leaves (Fig. 11.5C). Then place the plant in bright sunlight.

▶ Using a different applicator, apply gibberellin-lanolin paste to the pot labeled "gibberellin." **50-A If phosfon and gibberellin are prepared in a lanolin (wool-fat) paste, what should be applied to the "control" plant?**

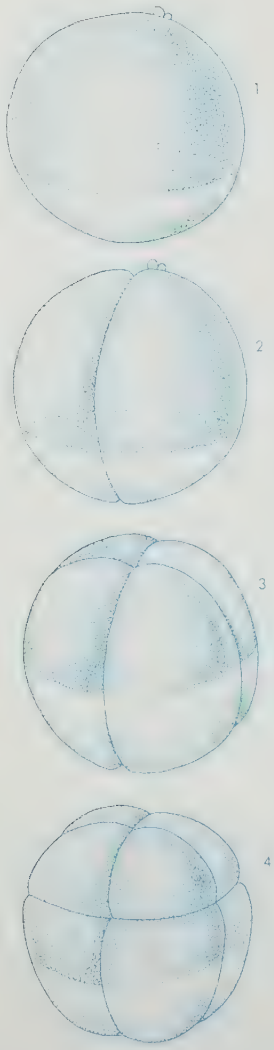
▶ Examine your plants and those of other students every two to three days for the next three weeks. **50-B Record your observations in the table on page 343. 50-C Which of the two chemicals tested is the plant growth inhibitor? 50-D At the end of the experiment draw the plants in the space provided on page 344.**

NOTE: Water the plants as necessary, but do not wet the leaves.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What is a **hormone?** Describe several plant hormones and the effects each has on regulating plant growth.
2 What uses could plant growth inhibitors have in agriculture?
3 What is **dormancy?** What advantages does the dormant condition provide?

12 ANIMAL DEVELOPMENT



INTRODUCTION

Development is most dramatically seen in the growth patterns of higher animals. A single egg becomes fertilized, undergoes a series of orderly changes, and becomes an embryo. The embryo develops further and changes into a young animal, which in turn matures into an adult. Thus, a single microscopic cell, by growth and replication, becomes an adult animal containing many millions of cells.

A significant feature of development is that every part of the organism increases in a carefully regulated fashion. For example, if a cell doubles in size various other parts of the cell (nucleus, cell wall, and so on) all increase in proportion to one another. Similarly, when a child becomes an adult, the organs of the body increase in harmony with each other.

Higher animals are composed of organs and tissues arranged in specific patterns and relationships to one another. The way in which these patterns are established, and the mechanism involved in the determination of form, is called **morphogenesis.** Development thus involves changes in the structure of an organism. A number of questions can be asked about this very basic phenomenon. How is growth initiated? Where does an organism obtain the raw materials and energy to carry out the necessary chemical reactions for the synthesis of new cytoplasm, nuclei, cell walls, and so on? Why does growth stop? What is the mechanism by which cell diversity is brought about?

The millions of cells that develop from a fertilized egg are not the same—some become muscle cells or nerve cells while others become skin cells. They are all different, each carrying out markedly different functions. This process of cell specialization is called **differentiation,** and presents yet another major problem to be solved by developmental biologists.

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EXERCISE 51

HOW CAN FROGS BE INDUCED TO OVULATE?

In most species of frogs, eggs are produced in the summer and are fully developed by late fall. However, they are usually kept in ovaries until the following spring when they are all released. The release of eggs is called **ovulation.** This usually results when the ovaries are stimulated by **gonadotrophic hormones** produced by the pituitary gland.

In this exercise you will inject a female frog with whole pituitary glands so that in two days you will have living eggs. You will also obtain sperm from male frogs and then use the sperm to fertilize the eggs collected from the female frog.

PROCEDURE—Removal of Frog Pituitaries

The number of glands that will be needed varies with the time of year. This is shown below:

Female Pituitaries Needed to Induce Ovulation

Sept.–Dec.	Jan.–Feb.	March–April
5–6	3–5	1–3

Female pituitaries are used because they are twice as potent as male pituitaries. Thus, if you used male frogs as a source of pituitaries you would need twice the number indicated above.

▶ From your instructor, obtain the number of female frogs necessary to provide the quantity of pituitaries shown above. Anesthetize a frog as described by your instructor.

▶ Remove the top of the head by cutting backward through the angle of the jaw to a point just past the tympanic membrane. Then make a transverse cut across the back of the head as shown by the dotted lines in Fig. 12.1A.

▶ Wash the top of the head clean with tap water and then place it, mouth side up, on your dissecting pan. With scissors remove the skin from the roof of the mouth to expose a cross-shaped bone under which the pituitary gland lies (Fig. 12.1B).

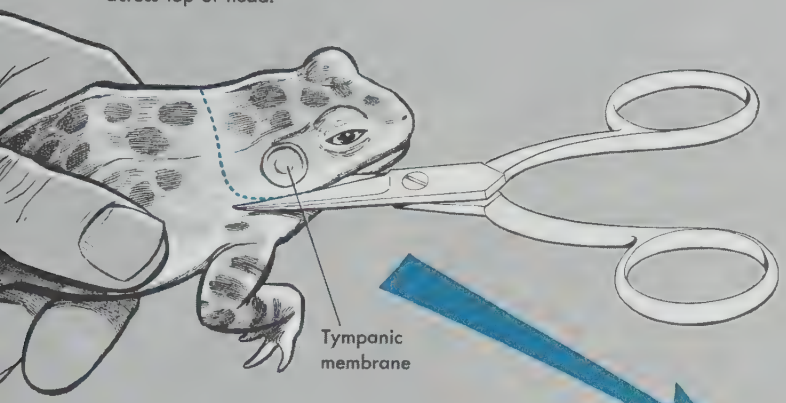
▶ Locate the **foramen magnum** in the back of the head—the opening leading into the brain cavity (Fig. 12.1C). Carefully insert *fine pointed* scissors into one side of the opening and cut through the roof of the mouth as shown by the dotted lines in Fig. 12.1C. Repeat on the other side.

NOTE: Occasionally the pituitary will adhere to the bony flap (Fig. 12.1D).

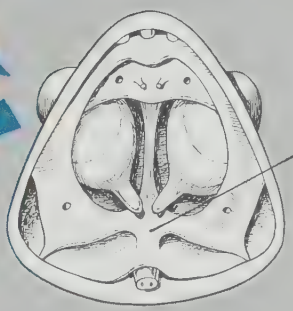
▶ Carefully lift the flap of bone with forceps. Locate the pituitary gland—a small pinkish body lying on the surface of the brain (Fig. 12.1D).

FIG. 12.1 PROCEDURE FOR REMOVING FEMALE PITUITARY GLANDS

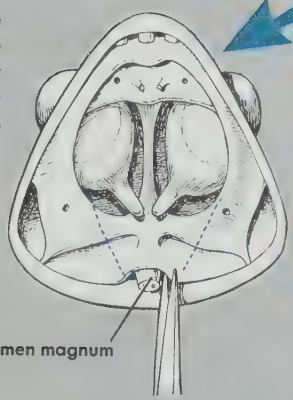
A Remove top of head by cutting through angle of jaw and across top of head.



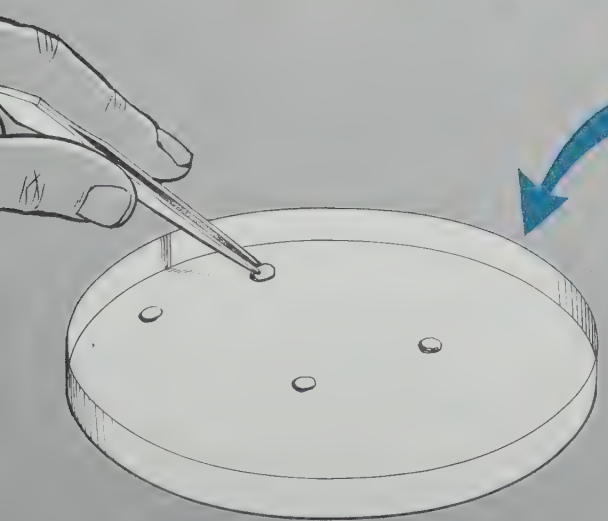
B Wash the heads clean and then, with scissors, remove the skin from the roof of the mouth to expose a cross-shaped bone.



C Carefully insert a fine pointed needle into the *foramen magnum* and cut through the roof of the mouth on an angle as shown by the dotted lines.

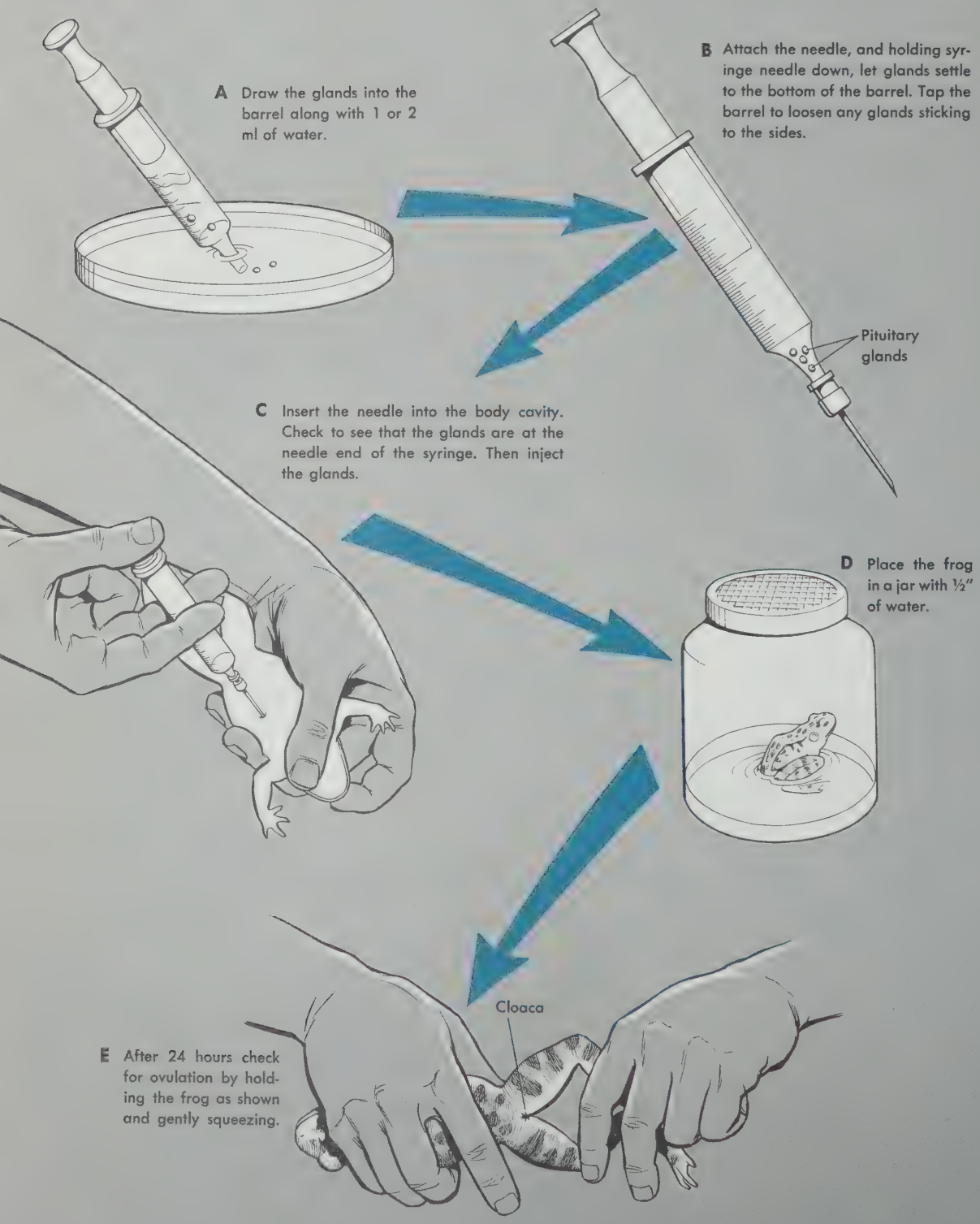


D Carefully lift the bony flap. Locate the *pituitary gland* in one or the other position shown.



E Place the glands in the solution provided by your instructor. Remove the whitish tissue associated with each gland.

FIG. 12.2 PROCEDURE FOR INJECTING PITUITARY GLANDS AND CHECKING OVULATION



▶ Using forceps, remove the pituitary by grasping the whitish tissue associated with it. *Do not touch the glands with your forceps. They are very fragile and break easily.* Place the glands in the solution provided by your instructor and carefully remove the whitish tissue adhering to the gland (Fig. 12.1E).

PROCEDURE—Induction of Ovulation

When you have the required number of pituitaries, inject a female frog. Use the following procedure.

▶ Remove the needle from a 2-ml syringe. Then draw the glands into the barrel of the syringe (Fig. 12.2A). Draw in enough fluid with the glands to obtain a volume of 1 ml.

▶ Attach the needle. Hold the syringe, needle down, so that the glands settle into the mouth of the needle (Fig. 12.2B). If any of the pituitaries stick to the sides of the barrel, gently tap the syringe until they all fall to the bottom.

▶ Holding the frog as shown, insert the needle into the body cavity just under the abdominal muscles. When the needle is deeply inserted, check to see if the glands are at the needle end of the syringe, and then inject the pituitaries. After a minute or so, slowly remove the needle and pinch the skin closed to prevent the loss of glands or fluid.

NOTE: Keep the needle parallel to the body wall to avoid injuring any internal organs.

▶ Then draw more solution into the syringe to see if any glands were stuck in the needle. If so, remove all but ½ ml of the solution and then inject the remaining pituitaries.

▶ Place the frog in a covered container (a mason jar) and keep the container at about 68°F (Fig. 12.2D).

▶ Check the frog after 24 hours to determine if ovulation has occurred, by gently squeezing the abdomen as demonstrated by your instructor (see also Fig. 12.2E). If ovulation has occurred place the frog in the refrigerator until needed. If ovulation has not occurred, check the frog again in 24 hours.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What internal and/or external mechanisms in nature determine when ovulation will normally occur in frogs?
2 Why does a frog release thousands of eggs when it ovulates, while the human female produces only one egg when she ovulates?
3 In the human, how does the quantity of sperm cells produced compare with the number of eggs produced? Why is there such a difference?

EXERCISE 52

FERTILIZATION OF FROG EGGS

In exercise 51 you injected female frogs with pituitary glands so that you could have living frog eggs available. In this exercise you will remove the male gonads (testes) to obtain sperm so that the frog eggs may be fertilized. In this way you will be able to examine the early division stages of the frog embryo and during the course of the next few weeks watch the changes that occur during the development of the frog.

PROCEDURE—Preparation of the Sperm Suspension

▶ Fill a petri dish with 20 ml of Holtfreter's solution (a mixture of chemicals which is very much like natural pond water).

▶ Pith a mature male frog as described in the Appendix of Exercise 34 (and in Figs. 12.3A,B,C,D).

▶ Then place the frog on its back in your dissecting tray. With sharp pointed scissors cut through the skin and belly muscles, remove the viscera, and then expose the testes as shown in Fig. 12.3E.

▶ Remove the paired testes, which are orangish-yellow bodies lying on top of the dark red kidneys (Fig. 12.3E).

▶ Clean away any blood and tissue adhering to the testes and place them in the petri dish of Holtfreter's solution.

▶ Using the blunt end of your probe, thoroughly crush the testes until a milky (sperm) suspension is obtained (Fig. 12.3F).

▶ Set this sperm suspension aside for 15 to 20 minutes to allow the sperm to become motile (show movement). As soon as you notice movement, put equal amounts of the sperm suspension into two or three petri dishes (Fig. 12.3G).

52-A How can you determine when movement is evident in the sperm suspension?

PROCEDURE—Fertilization of the Frog Eggs

NOTE: Your instructor will demonstrate how to strip the eggs from the injected frog.

▶ Holding the injected female frog as shown in Fig. 12.4A, squeeze the eggs directly into the sperm suspension in each dish. Line the egg masses in rows, or in a spiral in each dish so that all eggs are in contact with the sperm suspension. Gently swirl the dishes to ensure contact of sperm and eggs (Fig. 12.4B).

▶ Using forceps, remove several of the eggs. Place them in another petri dish and completely cover them with Holtfreter's solution. Examine these eggs with your dissecting

FIG. 12.3 PREPARATION OF SPERM SUSPENSION

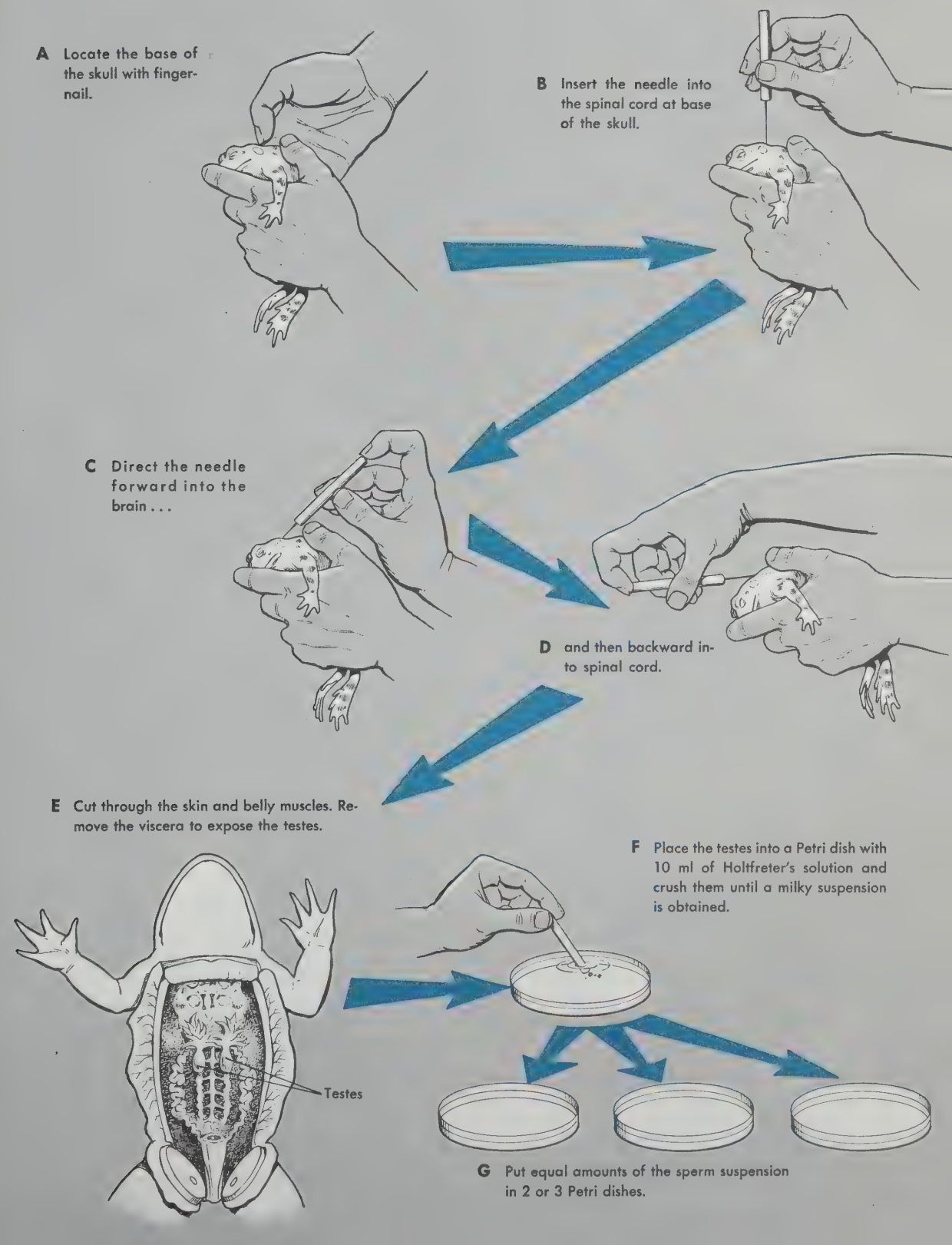
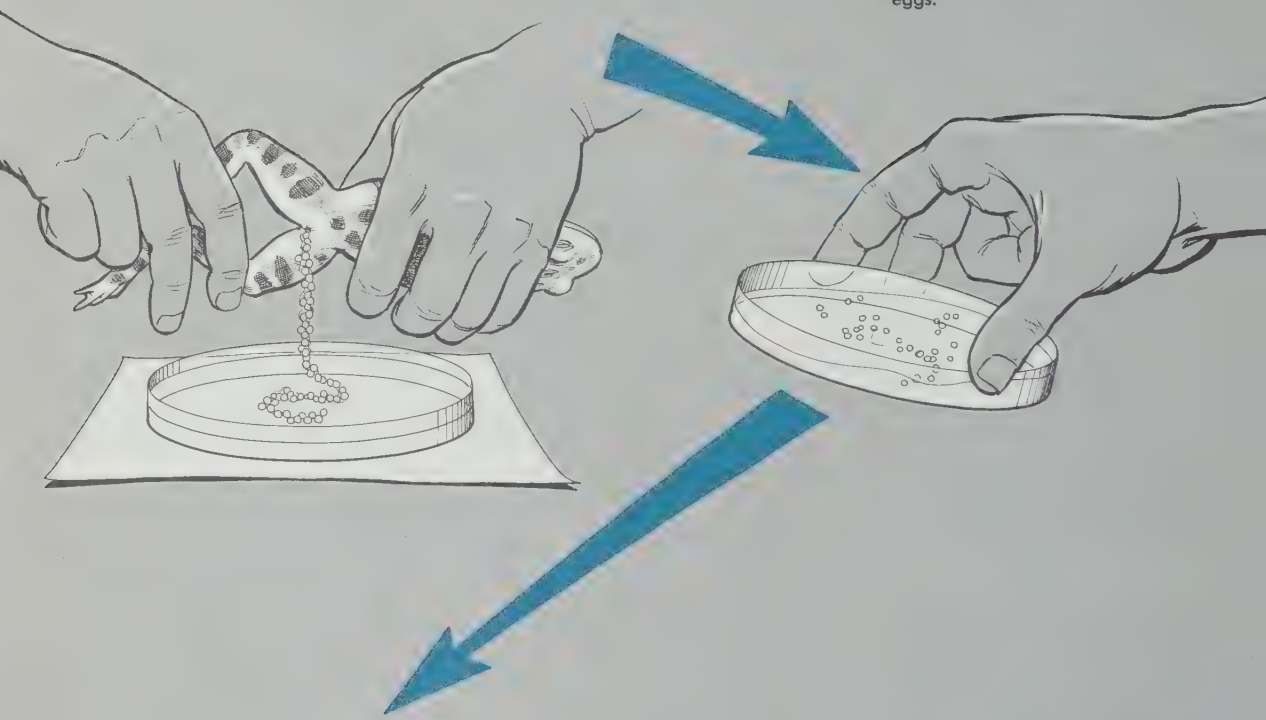


FIG. 12.4 FERTALIZATION OF FROG EGGS

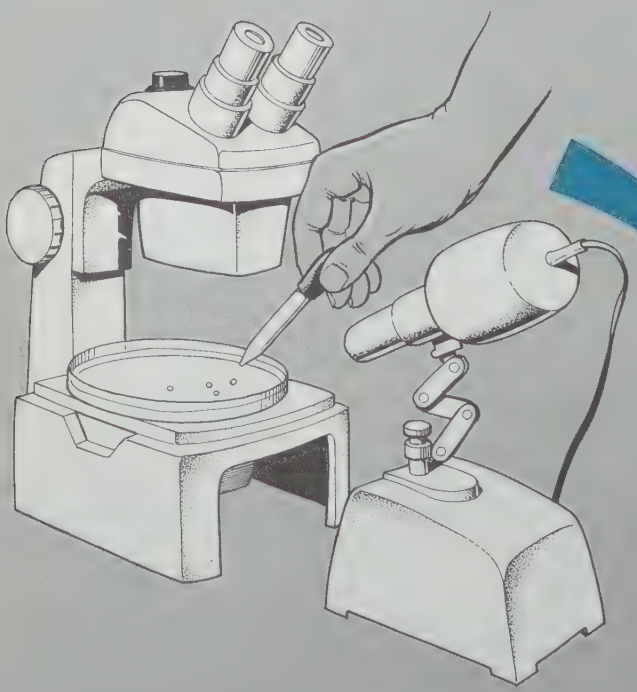
A Gently squeeze eggs from the frog into Holtfreter's solution in a Petri dish. Line the eggs in rows.

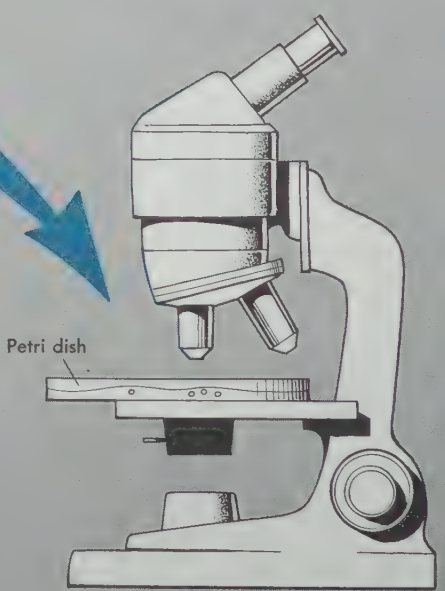
B Gently swirl the dishes to insure contact of sperms and eggs.



C Examine 3 or 4 eggs with a dissecting microscope.

D Then examine them with the low power of your compound microscope. How many jelly layers are present?





microscope. Use strong surface lighting. Note that the eggs are separated from each other by jelly layers. When first released from the frog they are clustered closely together. They separate from each other because the jelly surrounding each egg swells when the protein in the jelly absorbs water. **52-B How is this swelling important for the development of the eggs? Locate the pigmented animal pole and nonpigmented vegetal pole of the eggs.**

CAUTION: Do not place the eggs in a heaping pile in the dishes.

▶ Now examine these eggs with the low power of your microscope. **52-C How many jelly layers surround each egg?**

▶ After 15 minutes, carefully pour off the sperm suspension from the remaining "fertilized" eggs in the petri dish. Add enough fresh Holtfreter's solution to completely cover the eggs.

▶ Examine these fertilized eggs under your dissecting microscope. When an egg has been penetrated by a sperm, it secretes a proteinaceous substance. A membrane that was previously tightly bound to the surface of the egg is forced to expand when water is absorbed by the protein secreted by the egg. The vegetal pole, being heavier because it contains yolk, rotates downward and the animal pole becomes oriented upward. **52-D How many eggs in your dish have been fertilized? 52-E In nature, why is it an advantage to the developing egg to have the pigmented area uppermost?**

The first cleavage division of the egg will occur approximately two hours after fertilization. Fill several petri dishes with fresh Holtfreter's solution and transfer 10 to 12 of the fertilized eggs to each dish and cover the dish. Examine these eggs with your dissecting microscope (use strong surface lighting) as frequently as you can during the next day or two to observe the early cleavage stages. **52-F By means of a drawing on page 346, record your observations of the various divisions that occur (two cells, four cells, and so on) in the eggs.** Label each drawing with the amount of time elapsed after fertilization.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Differentiate between internal and external fertilization.
2 Where does fertilization normally occur in the frog in nature?
3 Compare the advantages and disadvantages of external and internal fertilization.
4 Where is the egg of a placental mammal fertilized?
5 Why is fertilization a necessary part of sexual reproduction?

6 What is the advantage of sexual reproduction of a species?
7 What is the **blastula** stage of development?
8 What is **gastrulation?**
9 Why is gastrulation an important stage in development?
10 What is meant by the term **induction?**
11 Give an example of induction during frog development.

EXERCISE 53

THE EARLY DEVELOPMENT OF THE CHICK

When a chicken egg is broken open you can see that it contains a yellow mass, the **yolk,** and a clear, sticky fluid called egg white, or **albumin.** Understanding how the remarkable transformation from fertilized egg to fully developed chicken occurs (in about 21 days) is one of the more exciting challenges in present day biology.

In this exercise, you will have the opportunity to examine some of the early stages in the development of a chick embryo.

PROCEDURE—The Unfertilized Chicken Egg

▶ Gently crack an egg into a bowl containing water (Figs. 12.5A,B,C).

▶ Identify the **yolk.** On the surface of this yellowish mass is a small white spot called the **blastodisc.** This region (which is the actual living material of the egg) plus the yolk make up the egg cell, or **ovum.** **53-A Suggest a function for the yolk.**

NOTE: If you cannot locate the blastodisc, gently turn the yolk over.

FIG. 12.5 STRUCTURE OF AN UNFERTILIZED CHICKEN EGG

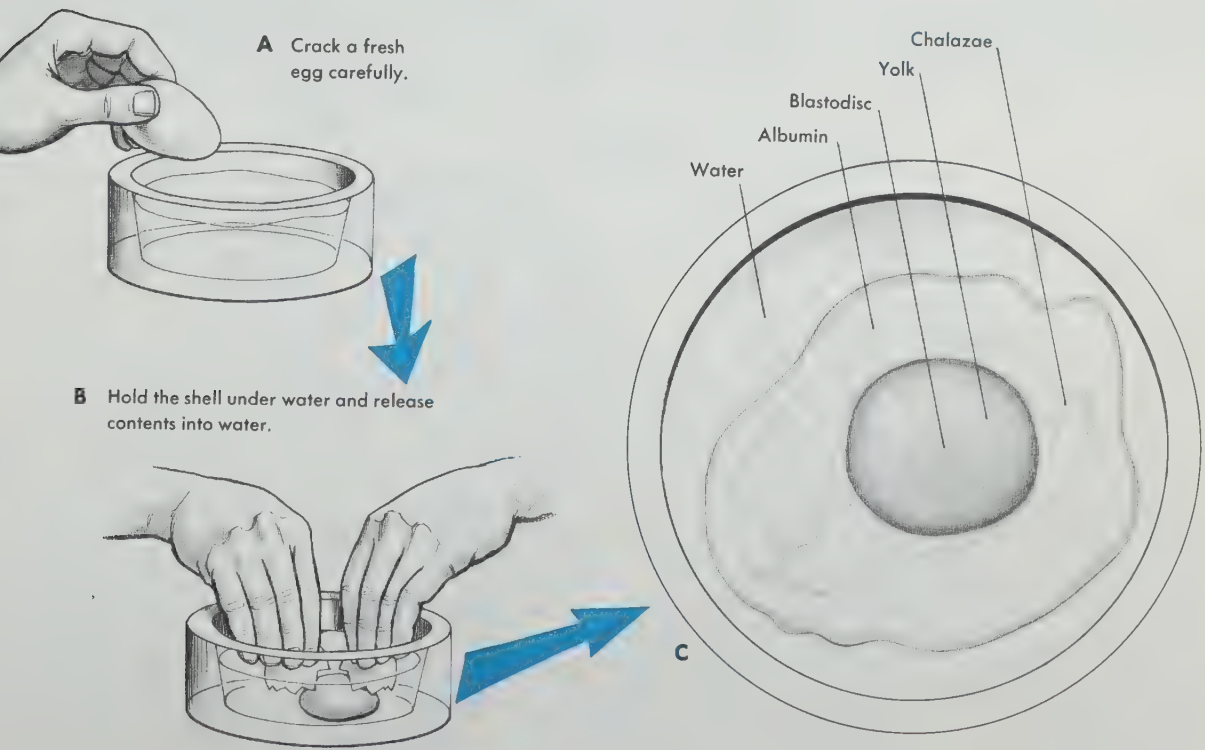
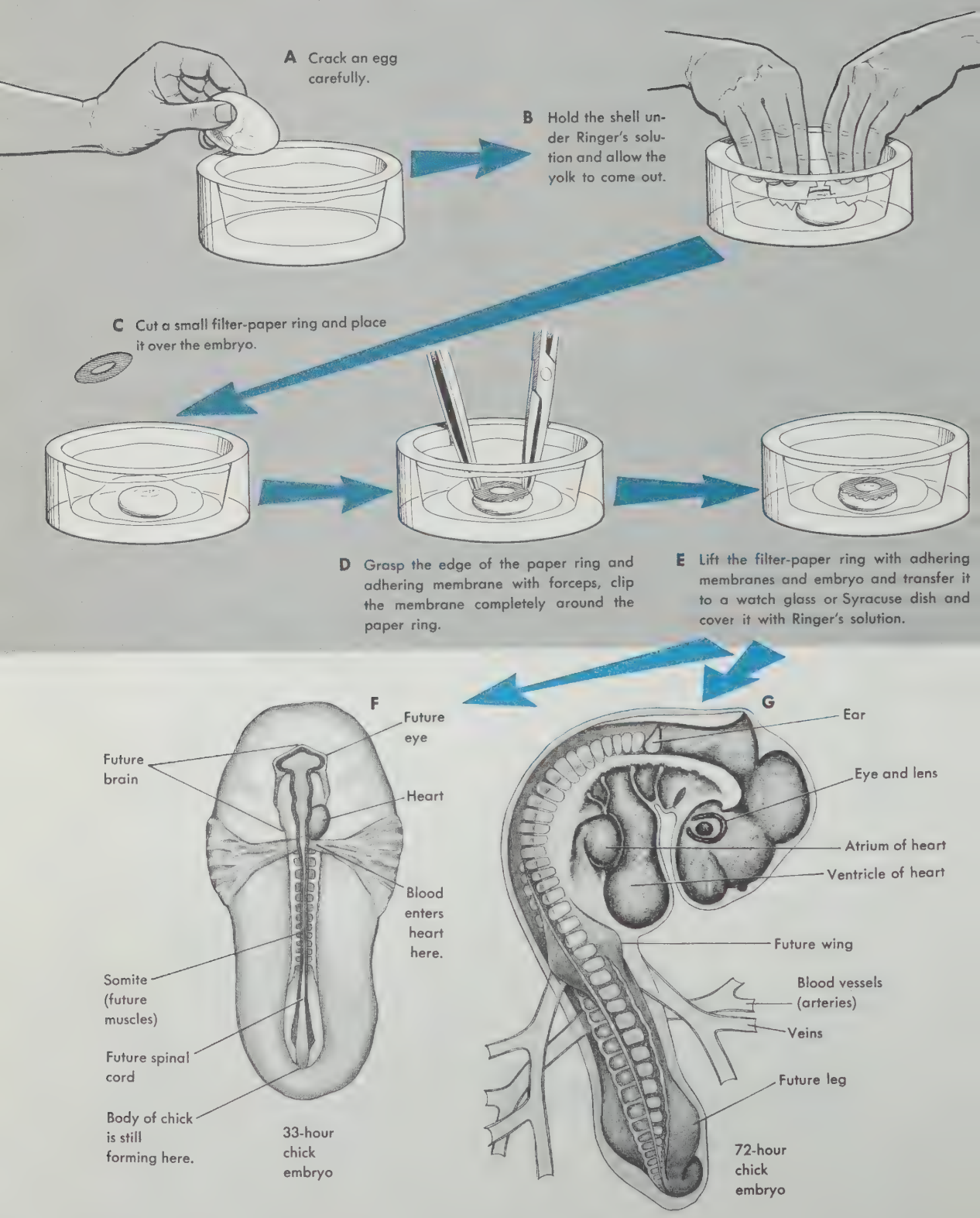


FIG. 12.6 33- AND 72-HOUR CHICK EMBRYOS



▶ Locate the whitish **albumin.** This proteinaceous substance is deposited around the ovum as it passes down the oviduct. **53-B Of what use might albumin be to a developing chick?**

▶ Identify the **chalazae.** Gently grasp one of these cords with forceps and pull. **53-C Suggest a function for the chalazae.**

PROCEDURE—Later Developmental Stages

About 33 hours before your laboratory period your instructor placed fertilized eggs into an incubator (or other high-humidity chamber) kept between 37° and 39°C.

NOTE: Perhaps you will want to do this part of the exercise by yourself.

▶ For observation of the living embryo, carefully crack an egg into a bowl containing Chick Ringer's solution (Figs. 12.6A,B). Remove the shell under water so that the unbroken yolk and the developing embryo float in the solution. **53-D What is so special about Chick Ringer's solution? Why not use plain water as before?**

▶ Remove the developing embryo from the yolk as shown in Figs. 12.6C,D,E. Then transfer the embryo to a watch glass or Syracuse dish and completely cover it with Ringer's solution (Fig. 12.6E).

▶ Examine this 33-hour-old embryo with your dissecting microscope or the low power of your compound microscope. Locate the parts of the embryo shown in Fig. 12.6F.

▶ Repeat the three steps above using eggs that have been incubated for 72 hours (Fig. 12.6G). **53-E What observable changes have occurred between the 33- and 72-hour stages of the chick embryos? 53-F Which organs appear to have developed more rapidly than others? 53-G Why should these organs develop more rapidly than others? 53-H What is the function of the large blood vessels leading from the yolk to the embryo?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What will the somites in the 72-hour embryo give rise to in the adult chicken?
2 How does the structure of the embryonic chicken heart at 72 hours of development differ from that found in the adult chicken?
3 What do you think would happen to a developing chicken inside an egg that was dipped in liquid wax? Explain.
4 How do you explain the fact that occasionally a single fertilized egg gives rise to two identical chickens?
5 What can cause abnormalities in limb development to occur?

EXERCISE 54

WHEN DOES THE ENZYME CYTOCHROME OXIDASE APPEAR IN THE CHICKEN EMBRYO?

There are several features that are characteristic of living things. Some of these are growth, reproduction, movement, response to stimuli, and an ability to adapt to changing environmental conditions. The collective activities of all of these comprise a "living" system. These life functions depend on enzymes, since enzymes catalyze all of the biochemical reactions that make these functions possible.

Until recently, studies on enzyme activity have depended on the use of bacteria or adult tissues. With present day techniques, we can now examine the biochemical changes that occur in a developing organism.

In this exercise you will examine chick embryos at early stages of development and try to determine how early the enzyme **cytochrome oxidase** makes its appearance. You will also determine if this enzyme is uniformly active throughout the embryo or if it appears to be active in localized tissues or organs. The presence of cytochrome oxidase may be detected by using NADI reagent. In the presence of oxygen, the colorless NADI reagent is oxidized to a blue color by the enzyme.

Cytochrome oxidase is an enzyme that specifically catalyzes the transfer of electrons and protons (H^+) to molecular oxygen and produces water. These electrons and protons are the end products of the metabolism of various substances taken in by the organism. Thus, the measurement of cytochrome oxidase activity is a measure of high metabolic activity.

PROCEDURE

▶ Remove the embryo from an egg that has been incubated for 48 hours, following the procedure as outlined.

▶ Place the 48-hour embryo into a Syracuse dish containing warm (about 37°C) Chick Ringer's solution (Fig. 12.7E). The solution will probably become cloudy from the yolk. Pipet off the cloudy solution and replace it with fresh warm Ringer's solution. Repeat until the solution is clear.

▶ Next, remove all of the Ringer's solution; note its approximate volume. Now add an equal amount of warm NADI reagent (Fig. 12.7F). **54-A Record the time when you add this reagent in the table on page 349.**

▶ While examining the embryo with a dissecting microscope, determine how long it takes for the blue color to appear. **54-B Record this data in the table (54-A).**

54-C Use a blue pencil to indicate the areas of the 48-hour chick embryo that show cytochrome oxidase activity in the drawing on page 350.

Continue your observations for the next 10 to 15 minutes and record any changes that occur.

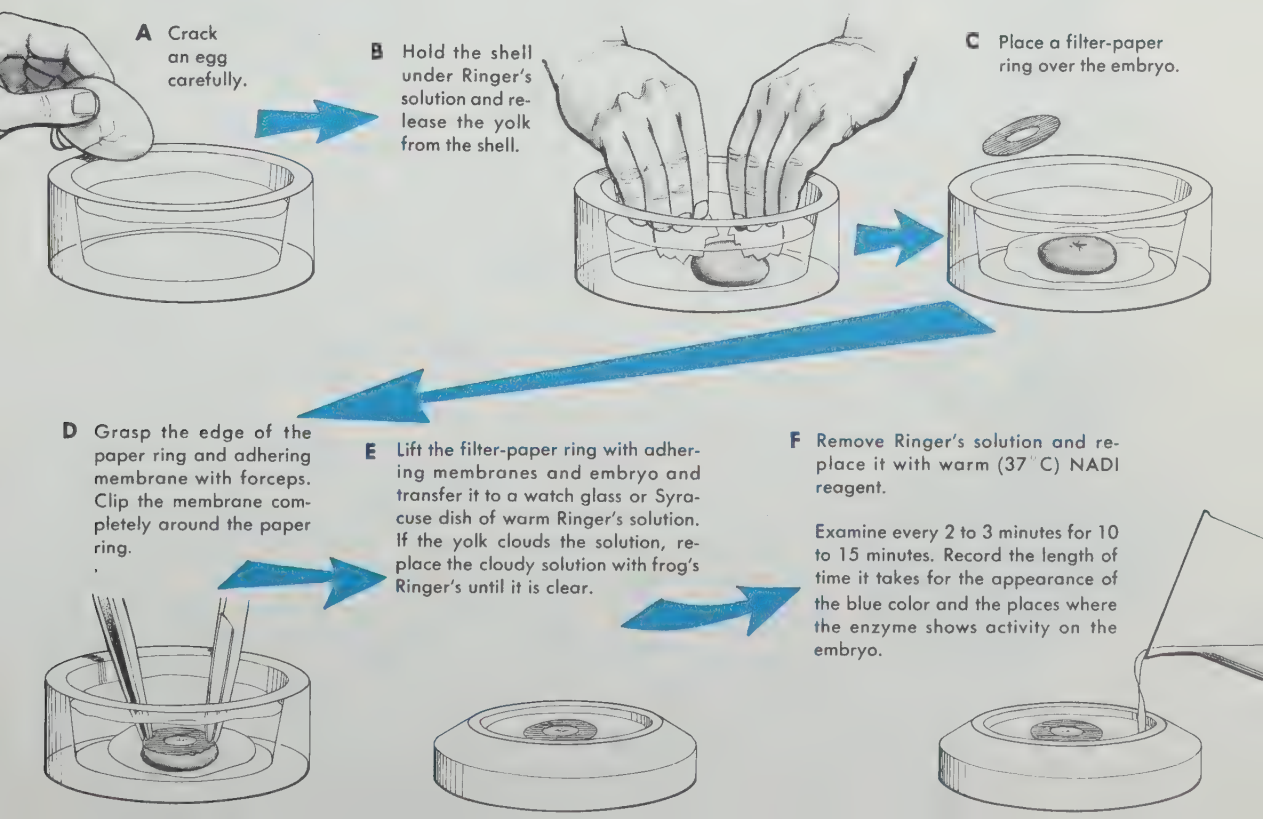
Repeat the first five steps with chick embryos approximately 33 and 72 hours of age. **54-D Show the areas of activity of this enzyme (if any) in the drawing on page 350. 54-E What areas of the embryo show the highest cytochrome oxidase activity? 54-F On the basis of what you know about this enzyme, why would you expect high cytochrome oxidase activity where you found it?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

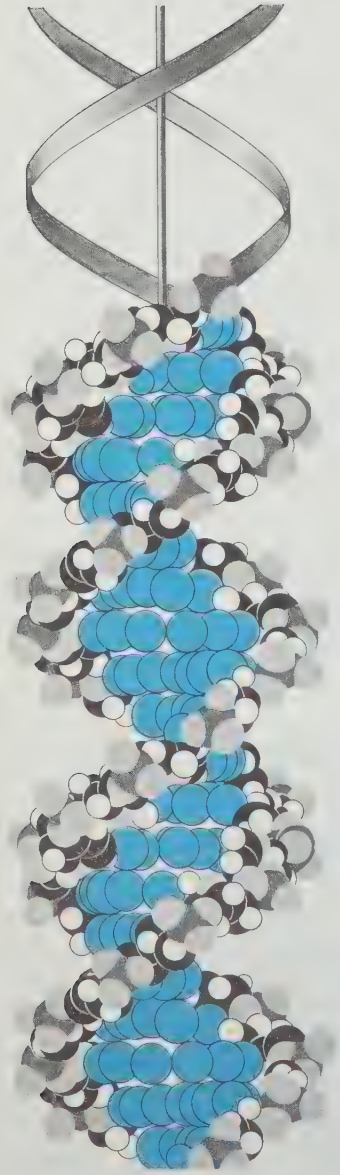
1 How could you determine that the blue color obtained was due to cytochrome oxidase and not to any one of the thousands of other enzymes known to occur in cells? (HINT: A chemical called sodium azide—NaN_3—inhibits the activity of cytochrome oxidase.)

2 Would you expect cytochrome oxidase activity to occur in anaerobic respiration (fermentation)? Explain.

FIG. 12.7 CYTOCHROME OXIDOSE ACTIVITY IN CHICK EMBRYOS



13 GENETICS



INTRODUCTION

The science of genetics has passed through several phases during the past sixty years. Each period was initiated by some important event.

The rediscovery of Mendel's papers at the beginning of the 20th Century began the first period of activity. The results of this period demonstrated the universal application of the laws of heredity. That is to say, the transmission of heritable characteristics, in both plants and animals, was brought about by the same mechanism regulating Mendelian segregation.

The second period, beginning around 1910, saw the introduction of a new "tool" to be used in genetic research—the fruit fly, *Drosophila melanogaster.* It was this period that produced the experimental evidence that genes are located in linear order on the chromosomes.

Further information on the organization of chromosomes and the discovery that radiation (such as X-rays) caused mutations (a chemical or physical change in the chromosomes) marked the introduction of the third period of activity. This information was developed further during the succeeding years.

The fourth period introduced micro-organisms in genetic studies and provided evidence that the genes regulated the activities of cells by controlling the production of enzymes.

The formulation of the Watson-Crick model of the molecular structure of deoxyribonucleic acid (DNA) has opened up the present exciting new era of molecular genetics. DNA is unique in three respects. First, it is a very large molecule, having a certain uniformity of size, rigidity, and shape. Despite this uniformity, however, it has infinite internal variety. Its varied nature gives it the complexity required for information-carrying purposes. The second characteristic of DNA is its capacity to

make copies of itself almost endlessly, and with remarkable exactness. The biologist or chemist would say that such a molecule can **replicate,** or make a carbon copy of itself, time and again with a very small margin of error. The third characteristic is its ability to transmit information to other parts of the cell. Depending upon the information transmitted, the behavior of the cell reflects this direction.

The past several years have shown that this genetic information (carried in the chemical structure of DNA) consists of a four-letter alphabet of which the "words" and "sentences" of the genetic message are written. The insights into the nature of genetic material and the role it plays in determining the structure of proteins will be regarded as one of the greatest discoveries of all time.

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EXERCISE 55

HOW ARE HUMAN BLOOD GROUPS INHERITED?

The presence of different blood groups in the population was reported for the first time in 1900 by Dr. Karl Landsteiner. His work led to the establishment of the presence of four basic blood types, A, B, AB, and O. These are determined by the presence or absence of antigens and antibodies in the blood. The factors that determine to which group a person's blood belongs are inherited. There are two different antigens called A and B that may or may not be present in the red blood cells of the individual, and their presence or absence determines your blood type. A person's blood cells may contain either the A or B antigens, or both A and B, or neither A nor B. The possibilities are outlined as follows:

HUMAN ABO BLOOD GROUPS

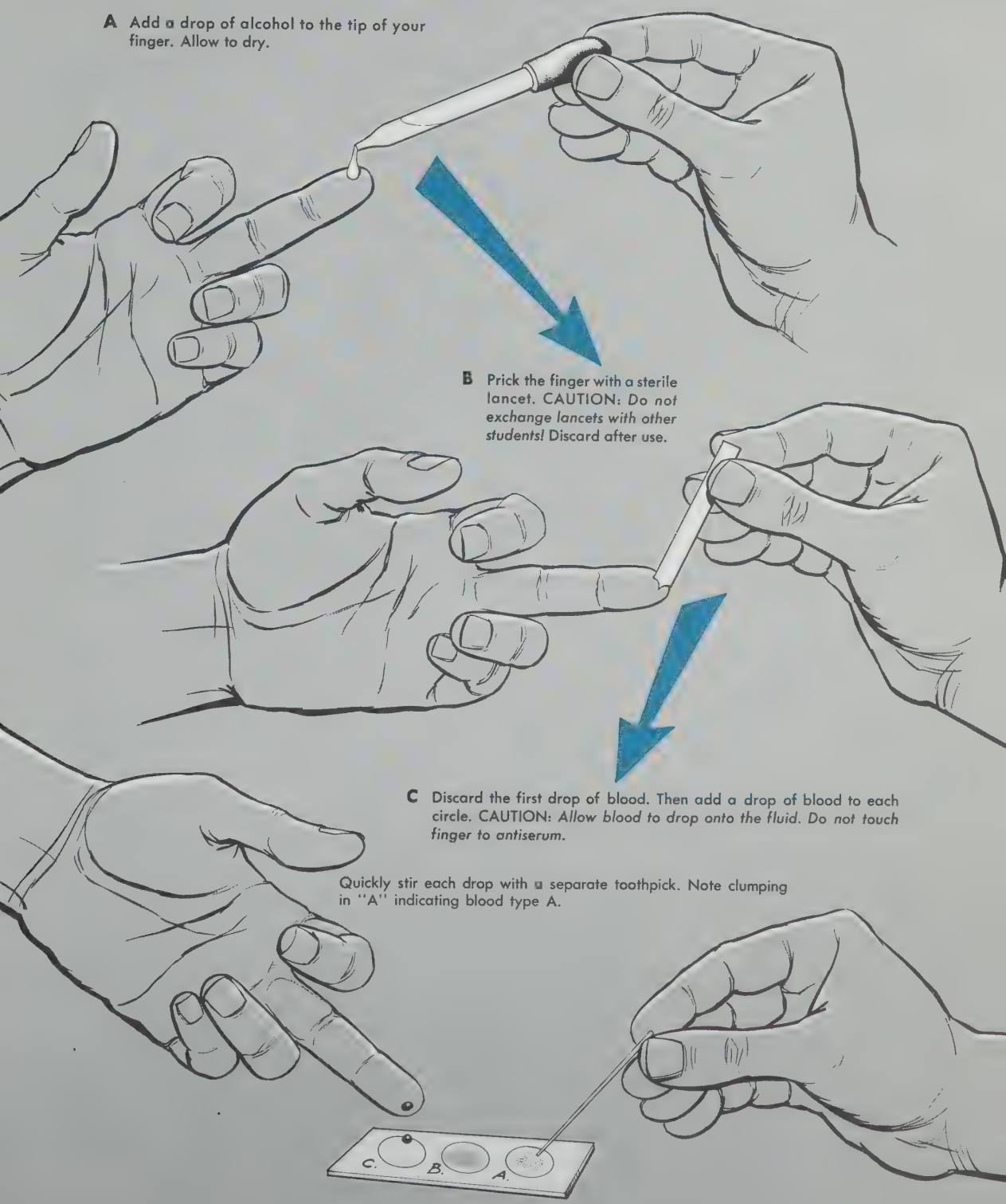
Blood Type	Antigen	Antibody
A	A	anti-B
B	B	anti-A
AB	A and B	none
O	none	anti-A and anti-B

Your blood may contain antibodies that will cause the clumping (**agglutination**) of red blood cells that are foreign to your blood. The distribution of these two antibodies (anti-A and anti-B) in the four blood types is also shown above. It should be noted that no individual's blood contains antibodies in the plasma that will agglutinate his own blood cells. Blood transfusions from one person to another can safely be made only when the blood groups are compatible. This means that a person cannot receive blood that contains antigens different from those of his own red blood cells. If new (foreign) antigens are introduced, then they will react with antibodies present in the recipient's blood. This reaction leads to agglutination of the introduced blood cells.

PROCEDURE—Blood Typing

To determine your own blood type it is necessary to have only two normal human sera, one containing anti-A antibody, and the other containing anti-B antibody. Each of the two antibodies should agglutinate the red blood cells containing the corresponding antigen. The table below shows the results that will be obtained if cells of the four different blood types are used. A plus (+) sign indicates agglutination of cells and a minus (−) sign indicates no agglutination.

FIG. 13.1 PROCEDURE FOR OBTAINING BLOOD SAMPLE



DETERMINATION OF HUMAN BLOOD GROUPS

Unknown red blood cells from individual	Serum used in reaction		Type Blood
	anti-A	anti-B	
1	+	−	A
2	+	+	AB
3	−	+	B
4	−	−	O

▶ Draw three circles with a wax pencil on a clean microscope slide. Label one circle "A" (for anti-A), one "B" (for anti-B) and the third "C."

▶ Add a drop of anti-A serum (colored blue for identification purposes) to circle "A," and the anti-B (yellow) to circle "B."

▶ Add a drop of 0.9% saline to circle "C" (Fig. 13.1). **55-A Why is this control needed?**

▶ Now place a large drop of blood in each circle (see next two steps). Quickly stir each drop with a separate toothpick to get a uniform mixture, and after a few minutes note if any reactions have occurred.

▶ The blood is to be obtained as shown in Fig. 13.1. Apply alcohol to the area of the finger to be pricked. Prick the tip of the finger with a sterile, disposable lancet provided by the instructor.

CAUTION: Discard the lancet after use; do not exchange lancets with fellow students.

▶ Squeeze the finger and wash off the first drop of blood. Continue to squeeze the finger and apply a large drop of blood to each circle. Quickly stir each drop with a separate toothpick to get a uniform mixture, and after a few minutes note if any reactions have occurred. After obtaining the blood, clean the finger with alcohol again.

CAUTION: Do *NOT* touch your finger to the antiserum. Allow blood to drip onto the fluid.

If agglutination has occurred only in "A," your blood type is A; if in "B," your blood is type B. If agglutination has occurred in both A and B, your blood type is AB; and if there is no agglutination in either "A" or "B," the blood type is O (see the Human Blood Groups table). **55-B Under normal circumstances would you expect agglutination to occur in circle "C?" Explain. 55-C What is your blood type? 55-D What antigen(s) does your blood contain? 55-E What antibody(ies)? 55-F Which blood type(s) can you receive in a blood transfusion? 55-G To individuals of which blood type(s) can you donate blood? 55-H Tabulate the blood types of your entire class in the table on page 354. 55-I What group has the largest percentage? 55-J What group has the smallest percentage? 55-K Compare the results obtained for your class with those shown in the table on p. 179.**

HUMAN BLOOD GROUPS IN THE UNITED STATES

	Incidence (%)			
Group	Caucasians	Negroes	Chinese	American Indians
O	45	48	36	23
A	41	27	28	76
B	10	21	23	0
AB	4	4	13	1

Inheritance of Blood Types

The antigens determining the four blood groups are the result of the expression of three allelic genes—O, A, and B, the latter two apparently being dominant to O. The genotype AA cannot be distinguished from AO, and genotype BB from BO, by chemical analysis and are classified as **phenotypes** A and B, respectively. Thus only four phenotypes (A, B, AB, O) can be recognized, although six genotypes occur (OO, AO, AA, BO, BB, AB).

55-L For the phenotypes of the parents listed in the Inheritance of Human Blood Types table on page 354 list all possible genotypes of the parents and of the children, and all possible phenotypes of these children.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What blood phenotypes are not possible from two AB or two O parents?
2 In a case of disputed paternity, the child was found to be group O, the mother group A, and the probable father, AB. What inference can be drawn from this knowledge? What if the probable father was blood group B?
3 On the basis of your study of the inheritance of human blood types, could it be proven that a child belongs to certain parents? Explain.
4 Can it be proven that a given child does not belong to certain parents? Explain.
5 It has happened occasionally that the identities of infants in a hospital nursery have become mixed. How could blood typing help to resolve this problem?

EXERCISE 56

HUMAN GENETICS

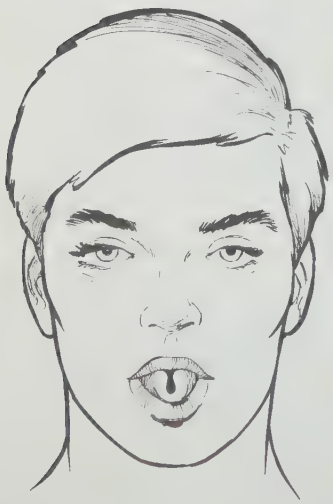
The purpose of this study is to examine certain, clear cut, genetically known human traits with a view to determining the extent of genetic variability in the human population of these traits.

Is the Ability to Roll the Tongue Inherited?

Most people can turn the sides of the tongue so that near the tip the sides nearly touch on top (Fig. 13.2). **Do you have this ability? 56-A When everyone in the class has tried, record the results in the table on page 355. Also record the data from other class sections.**

Percentages alone, however, will not tell you whether the ability to roll the tongue is inherited as a dominant or recessive gene. A study of the families of the other members of your class will help. To aid you in deciding on the method of inheritance it may be helpful to examine the inheritance of hair color in human beings as illustrated in Fig. 13.3. For example, it is possible for two parents that do not have red hair to have a child that does have red hair. However, it is not possible for two parents with red hair to have a child that does not have red hair.

FIG. 13.2 CAN YOU DO THIS WITH YOUR TONGUE? SOME PEOPLE CANNOT.



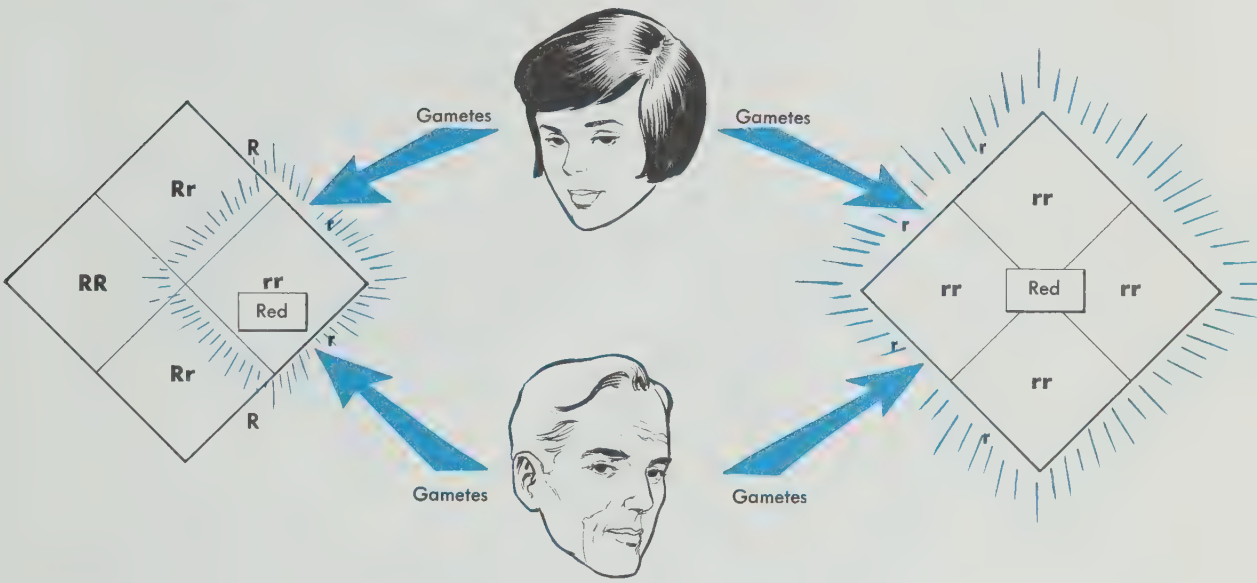
56-B To determine the nature of the inheritance of tongue rolling; fill in the chart (Inheritance of Tongue-Rolling) on page 356. Check the members of your family for this trait. Write the symbol (+) in the circle or square to indicate an individual who can roll his tongue. Use the symbol (−) if the person cannot "roll" his tongue. **56-C On the basis of the information you collect, is the ability to roll the tongue inherited as a dominant or recessive trait?**

Using "T" to represent the dominant character and "t" the recessive character, indicate the genotypes (that is TT, Tt, or tt) of each individual represented by the circles or squares in Chart 56-B.

Inheritance of a Physiological Character

Your instructor will give you a piece of paper which has been treated with phenylthiocarbamide (commonly called **P.T.C.**). On chewing this paper some people will detect a definite taste; others will taste nothing. **56-D Are you a "taster" or "nontaster?" 56-E When everyone in the class has done this, record the results in the table on page 357. Also record the data from other class sections in the same table.**

FIG. 13.3 INHERITANCE OF HUMAN HAIR COLOR



In order to determine the nature of inheritance of P.T.C. tasting, your instructor will give you several strips of P.T.C. paper which you are to take home. **56-F Gather as much information as you can in order to complete the taste inheritance table on page 358.** Use the symbol (+) to indicate a "taster" and (−) for a "nontaster." **56-G On the basis of the information you collected, is the ability to taste P.T.C. transmitted as a dominant or recessive gene?**

Using "T" to represent the dominant character and "t" the recessive character, indicate the genotypes (TT, Tt, or tt) of each individual tested in chart 56-F.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Could two parents who are "nontasters" have a child who is a taster? Explain.
2 Could two parents who do not have the ability to roll their tongues have children who could roll their tongue? Explain.
3 From a genetic point of view, what are the advantages and disadvantages of cousin marriages in the human population?

EXERCISE 57

HOW CAN THE SEGREGATION OF AN ALBINO MUTANT IN CORN BE DETERMINED?

Corn reproduces by means of seeds formed in large numbers on the ears of the mature plant. Each kernel or seed has been formed as a result of the fertilization of an egg cell by a sperm. Under proper conditions of light, temperature, and moisture each seed is capable of growing into a new plant.

Corn is known to have genes that regulate the production of chlorophyll. Therefore, if, during the development of the parent plant, a mutation occurs to the genes regulating chlorophyll synthesis, this mutation may be transmitted to the offspring and will result in plants that lack chlorophyll. Plants lacking this pigment are called **albinos.**

PROCEDURE

▶ Obtain from your instructor the special corn seeds needed for this experiment. Plant these as directed in flats containing sand or vermiculite (Fig. 13.4).

▶ Periodically water the flats so that they are kept moist but not saturated.

▶ Examine the flats in about a week. When the seedlings have emerged, count the number of green and albino shoots. **57-A Tabulate your results in the table on page 359.**

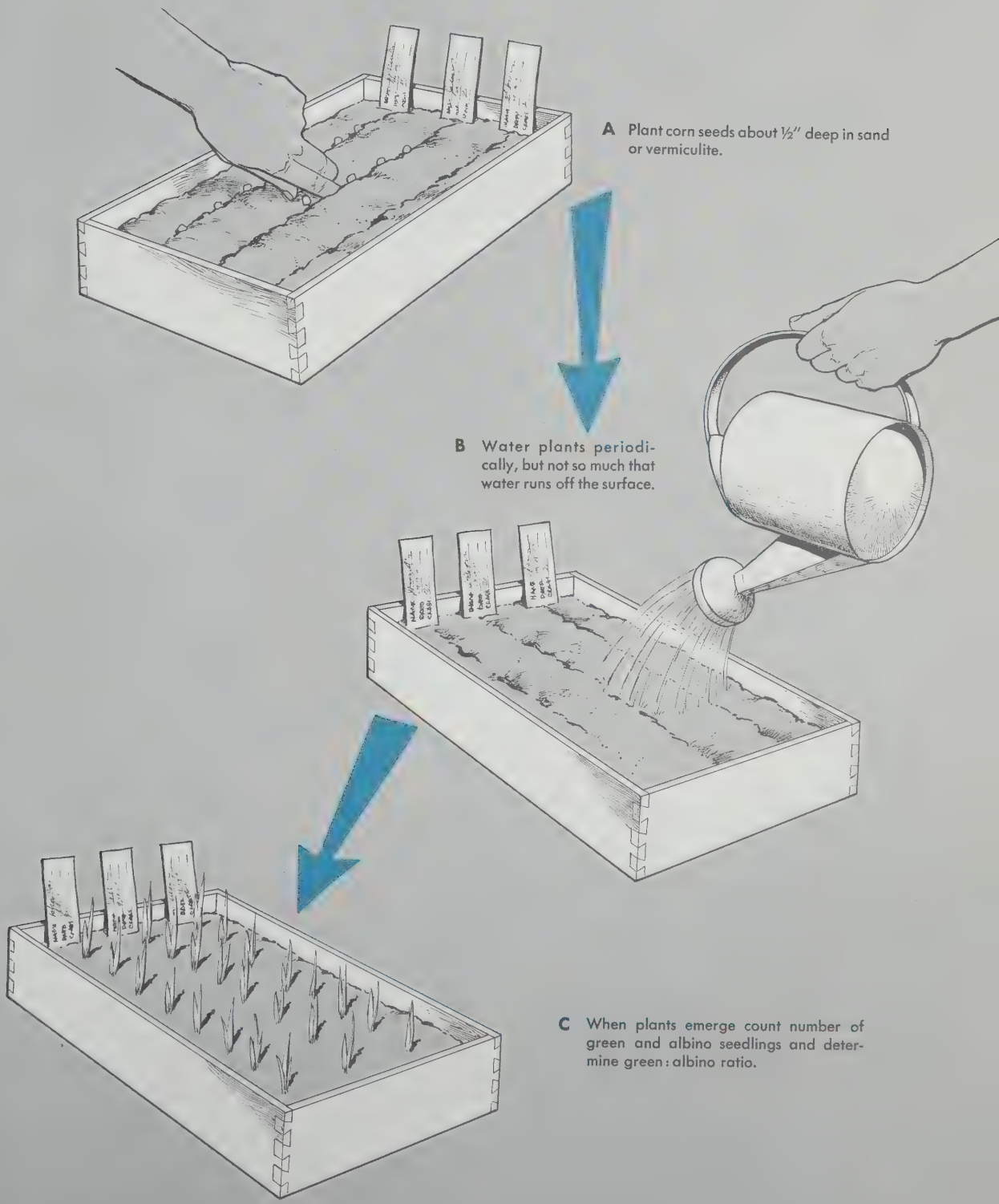
▶ In the last column determine the ratio of green to albino shoots. To obtain a larger sample, tabulate the results obtained by other biology sections. **57-B What is the green : albino ratio for your class section? 57-C How does the green : albino ratio for your section compare to the *total* green : albino ratio for all of the sections? Explain any differences.**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Given that the parents that produced the seed used in this exercise contained chlorophyll, outline the transmission of the albino character from the parental stock to the offspring. Use a capital "G" for the dominant gene, and a small "g" for the recessive gene. Label each individual according to its phenotype and genotype.

2 If it is true that the albino condition is lethal in nature, why is it that the potentiality for albinism persists in a population appearing generation after generation?

FIG. 13.4 PROCEDURE FOR STUDYING CHLOROPHYLL MUTANTS IN CORN



A Plant corn seeds about ½" deep in sand or vermiculite.

B Water plants periodically, but not so much that water runs off the surface.

C When plants emerge count number of green and albino seedlings and determine green : albino ratio.

EXERCISE 58

MUTATION IN DROSOPHILA

A **mutation** may be defined as a physical or chemical change in a gene, or chromosome, that is potentially transmissable to the offspring. Mutations very often cause such visible changes as loss of pigmentation, or they may result in very subtle changes, such as sensitivity to carbon dioxide concentration. In this exercise you will examine the "normal" fruit fly. You will also be given some "unknowns" and will be asked to identify the mutant traits they possess.

PROCEDURE

▶ Obtain a vial of normal, wild-type fruit flies.

▶ Tack a piece of cotton onto a cork that will fit the vial (Fig. 13.5A). Add two or three drops of ether to the cotton.

CAUTION: Ether is extremely flammable. Keep it away from open flame.

▶ Gently tap the vial to force the flies to the bottom.

▶ After tapping the flies to the bottom of the vial, replace the cotton plug with the cork and ether-soaked cotton.

▶ When all of the flies have stopped moving, dump them onto a white card and carefully examine them with a dissecting microscope or hand lens (Fig. 13.5C).

▶ The normal, wild-type fruit fly has dark red eyes, a tannish bristle-covered body, and long straight wings reaching just beyond the tip of the body (Fig. 13.5D). In mature male and female flies, there are also some obvious differences in body structure. **58-A Using Fig. 13.5 as a guide, examine your flies and fill in the table on page 361.**

▶ Now ask your instructor for the numbered vials containing a mixture of various mutant flies:

- **Vestigial.** A wing mutant characterized by highly reduced withered wings.
- **White.** A mutant whose eyes appear white.
- **Bar.** The chief effect shown in this eye mutant is the reduction in the number of facets of the eye, resulting in the appearance of a "bar" down the middle of the eye.
- **Black.** The body of this fly is black.

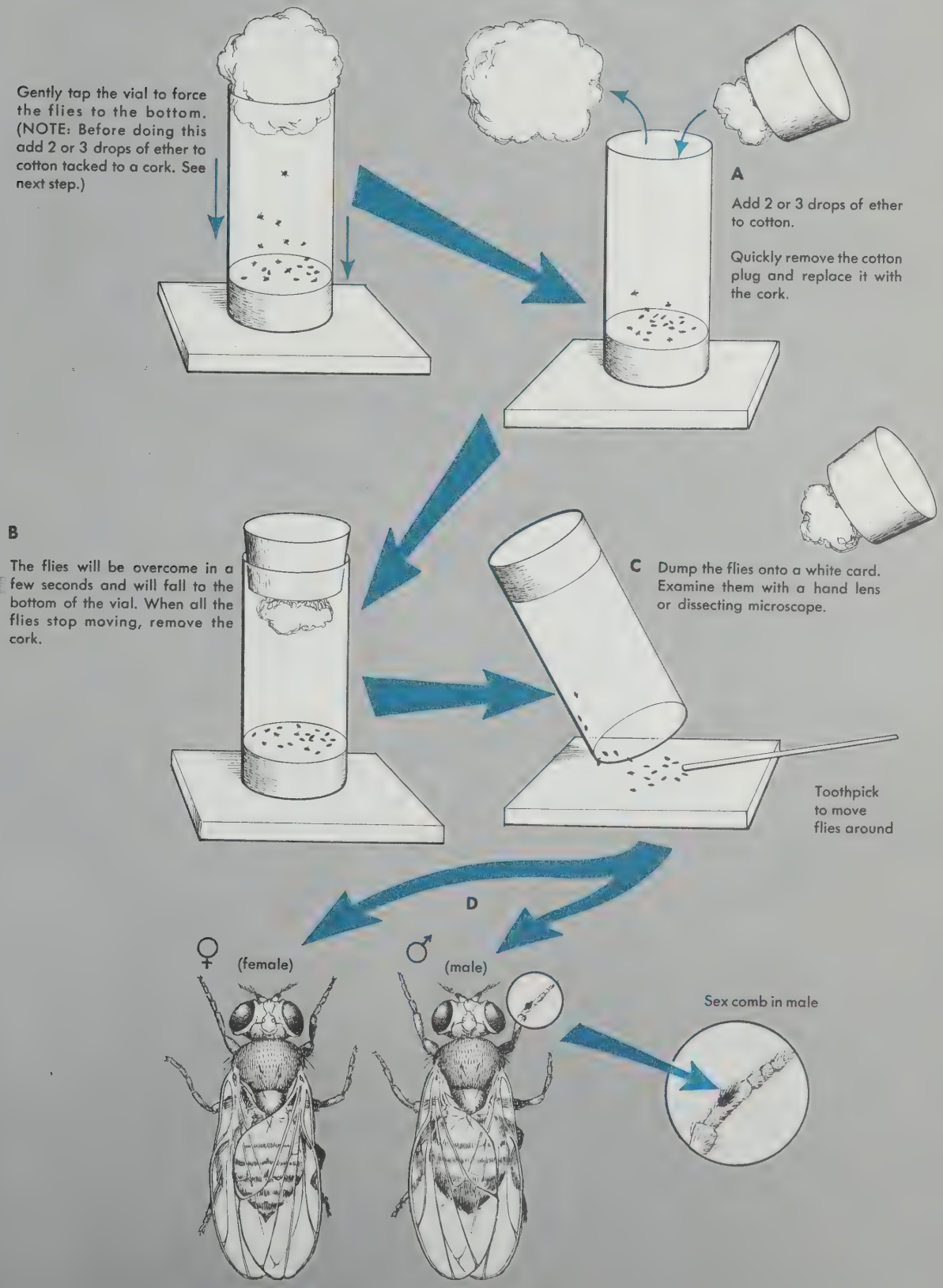
▶ Etherize the flies as shown in Fig. 13.5 and determine the nature of the mutant flies in your vial. **58-B Record the type of mutation and sex of the flies in the table on page 361.**

NOTE: Dead flies will have wings standing out at right angles to the body.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

How would you determine if different mutations are inherited as a dominant or a recessive trait?

FIG. 13.5 PROCEDURE FOR ETHERIZING FRUIT FLIES TO OBSERVE BODY FEATURES



EXERCISE 59

HOW CAN THE EYE COLOR MUTANTS IN DROSOPHILA BE DISTINGUISHED?

In your previous study of the fruit fly, *Drosophila melanogaster,* you examined the normal wild-type fly and several mutations involving the wings, body color, and eye pigments. It is known that the pigmentation of the eyes is genetically controlled. Some recent studies have shown that the "normal" eye color in this insect is due to the presence of a series of substances called **pteridines.** This term (from the Greek word *pteron* meaning wing) was chosen because these substances were first found in butterfly wings.

In this exercise you will attempt to detect the presence of these pteridines in the wild-type fly and in some eye color mutants to determine if the mutations, as expressed in eye color, are accompanied by changes in the kinds of pteridines that are present. You will do this using a research technique called paper chromatography (see Exercise 23).

PROCEDURE

▶ Get from your instructor a vial containing wild-type flies and several other vials, each of which contains a different eye mutant.

REMINDER: The wings of dead flies stand out at right angles to the body.

▶ Etherize the flies as shown in Fig. 13.6A. Allow the flies to remain in the vials until they are dead.

▶ Transfer the flies to a sheet of white paper. With the aid of a dissecting microscope or hand lens, select three male *or* female flies.

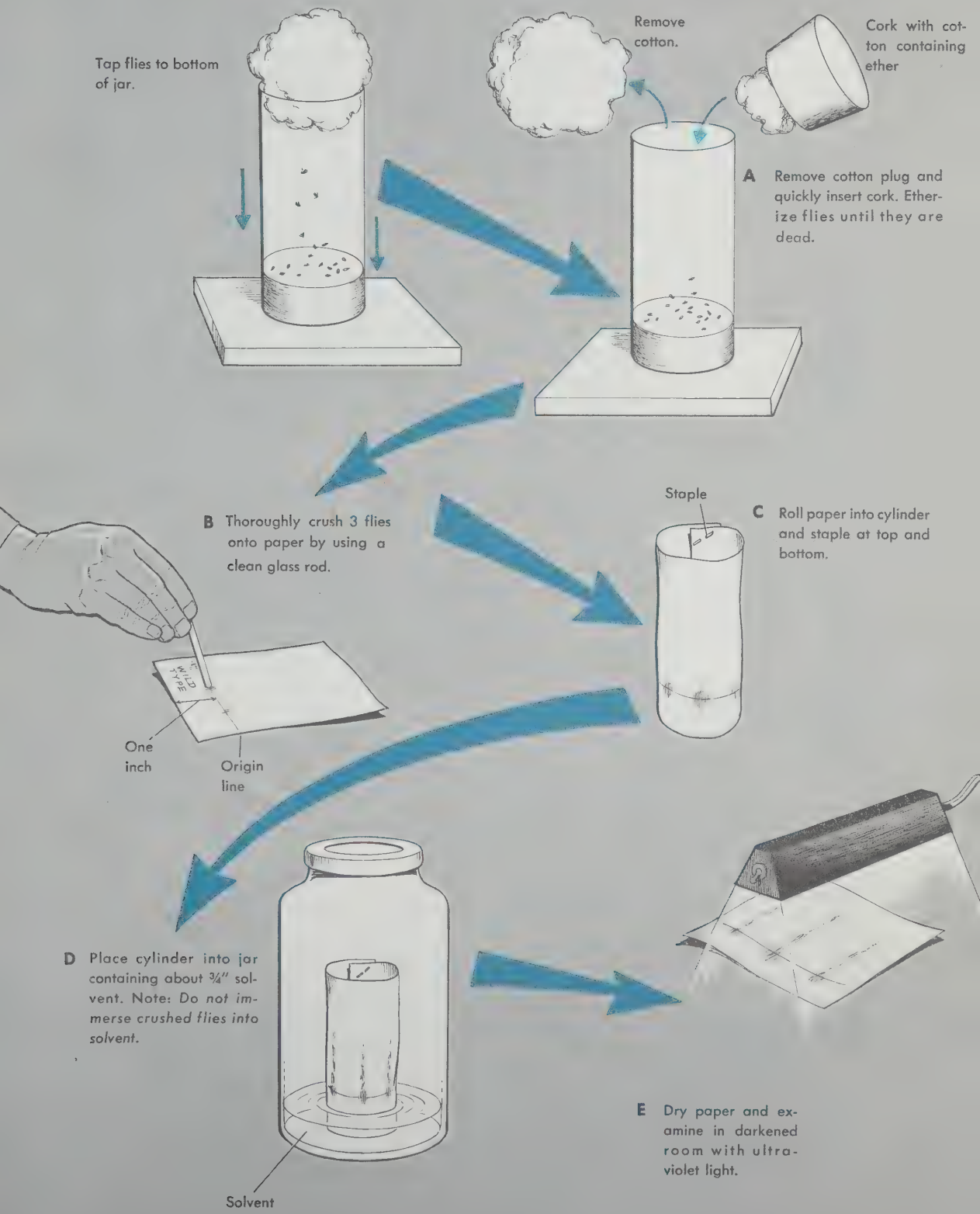
▶ Obtain a sheet of chromatography paper. Draw a light pencil line across the length of the paper, 1 inch from the edge. Lightly pencil in several evenly spaced dots along the line. The number of dots will depend on the number of different flies your instructor will provide.

▶ Place a wild-type fly on the first dot. Using a *clean* glass rod, crush the fly onto the paper. Place the second fly over the first and also crush it onto the paper. Repeat for the third fly. Label the paper below the crushed flies "wild-type" (Fig. 13.6B).

▶ Repeat each of the above steps for each mutant fly provided. Use a clean glass rod for each "mutant."

▶ After all the flies have been added to the paper, roll the paper into a cylinder and staple the top and bottom (Fig. 13.6C). Handle the paper by the edges as much as possible, since fingerprints on the paper may interfere with your results.

FIG. 13.6 PROCEDURE FOR SEPARATING PTERIDINES BY USING PAPER CHROMATOGRAPHY



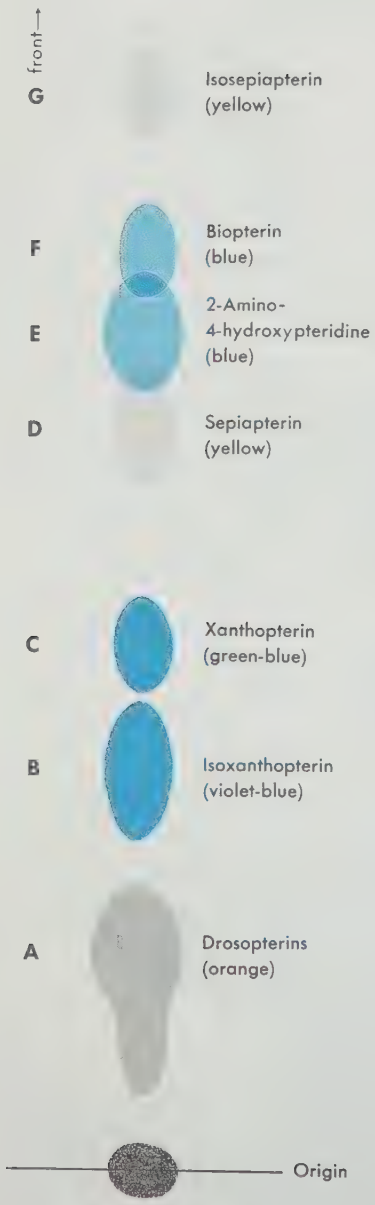


FIG. 13.7 PTERIDINES OF THE WILD-TYPE FRUIT FLY

▶ Place the paper cylinder into a chromatography jar containing about ¾ inch of a special solvent. **CAUTION: The "spots" must not be submerged in the solvent.** Place the set-up in a *dark place* for the period of time determined by your instructor. Then remove the cylinder and allow it to dry in a well ventilated place.

▶ When the cylinder is dry, remove the staples and place the flattened paper under an ultraviolet light (Fig. 13.6E). **CAUTION: Do not look directly into the UV lamp. It is very harmful to the eyes.** The pteridines will show up as different colored patches on the paper when exposed to ultraviolet light.

▶ Figure 13.7 shows the position and colors of several pteridines expected in the wild-type fly. **59-A Examine your chromatogram and in the table on page 363 list the pteridines that you find in your wild-type flies by placing a check (√) mark in the proper column. Also list the pteridines that are apparent in the mutant flies.**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 In this experiment, you were asked to use either all males *or* all females when testing for the presence of the pteridines. What do you think was the reason for not mixing the sexes?
2 Devise an experiment to determine when these pteridine pigments first show up in the developing fruit fly.
3 Are the mutations studied in this exercise (as expressed by pigment differences) lethal? Explain.

EXERCISE 60

WHAT IS THE MOLECULAR BASIS OF HEREDITY?

In the early 1940's, some ingenious experiments showed that, through the activity of certain chemical substances, the parental cell passes its own characteristics on to its daughter cells. That is, the daughter cells inherit the capacity to become muscle cells, or nerve cells from "information" provided by the parent cell. This "genetic information" was found to be contained within the structure of the chemical compound known as DNA (deoxyribonucleic acid).

The experiments that linked DNA and the "genetic material" used two different strains of bacteria—one having a smooth coat and one with a rough coat. The chromosomes contain a **nucleoprotein complex** consisting of DNA and several types of protein. By chemical means, it is possible to remove the nucleoproteins from bacterial cells and separate them into protein and DNA fractions. If these fractions are added to media in which other bacteria are growing, the "foreign" DNA and protein will be absorbed by the bacteria. Using this procedure, Avery, MacLeod, and McCarty (1944) set up the experiment outlined in Fig. 13.8. The results showed that the *smooth-coated*

FIG. 13.8 BACTERIAL TRANSFORMATION BY DNA

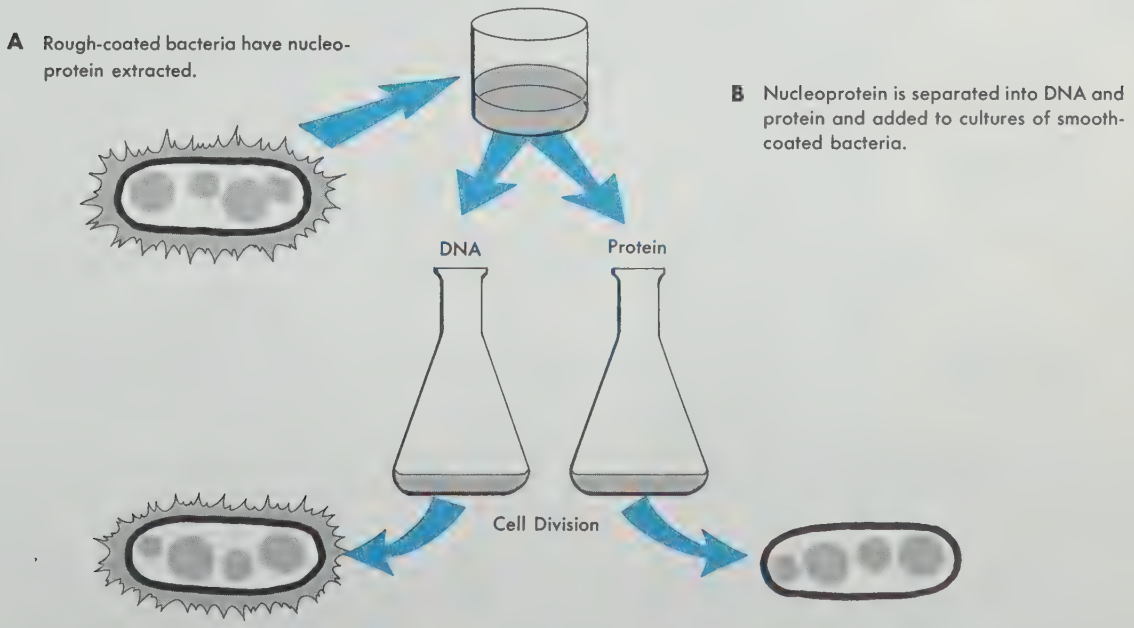


FIG. 13.9 MOLECULAR STRUCTURE OF DEOXYRIBONUCLEIC ACID (DNA)

bacteria, to which rough coated DNA had been added, acquired *rough-coated* characteristics. Moreover, the daughter cells of these bacteria gave rise to more rough-coated bacteria. This bacterial transformation was brought about specifically by DNA.

In the early 1950's, J. D. Watson and F. H. C. Crick formulated their classic model of the molecular structure of deoxyribonucleic acid. A three-dimensional model, based upon the Watson-Crick model is available to help you become acquainted with one of the most important molecules in living organisms.

PROCEDURE

▶ On the DNA model (and in Fig. 13.9A) note that the molecule is composed of nitrogen compounds, phosphates, and sugars joined in two coiling strands that form a double helix.

▶ Note that each strand has alternating sugar and phosphate molecules linked. Attached to each sugar, and directed to the inside, is a nitrogen base. The bases of the two strands are paired and are connected by hydrogen bonds. There are four different bases—adenine (A), thymine (T), cytosine (C), and guanine (G). Examine the model closely. **60-A What pattern of base pairing is evident?** The base-sugar-phosphate combination is called a **nucleotide** and is the structural unit of DNA (Fig. 13.9B).

▶ The distance between each of the nitrogen base pairs is 3.4 angstrom (Å) units. (One angstrom = 1/10,000 of a micron (μ) or 1 micron = 10,000 Å). **60-B How many base pairs are there in one complete turn of the helix? 60-C How many Å units would there be in one turn?** The model you are examining represents a very small part of the entire DNA molecule. Indeed, if the DNA from a single human cell was removed and stretched out as a single thread, it would be about three feet long! **60-D How many Å units would this be? (1 inch = 25 millimeters = 25,000 microns).**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 All multicellular plants and animals begin life as a single cell that undergoes mitosis and subsequently forms the adult organism. This suggests that the DNA molecule must also be able to duplicate itself. Suggest (by drawing) a method of replication.

2 Mutations may be simply described as physical or chemical changes in genes or chromosomes that are potentially transmissable to the offspring. In terms of your knowledge of the structure of the DNA molecule, suggest how mutation may occur at the molecular level.

14 BACTERIA

Courtesy The Nitrogin Company

INTRODUCTION

Despite their minute size and apparently simple structure, bacteria have become adapted to an extremely wide variety of environments. In fact, they are more widely distributed in nature than any other group of organisms.

Bacteria are especially abundant in the soil. Some species play an important role in the nitrogen cycle—where nitrogen gas in the atmosphere is converted into nitrogenous salts that are readily able to be used by plants for growth.

Bacteria are also responsible for the decay of organic matter, such as dead plants and animals. During the breakdown of this material carbon dioxide is released and may then be re-used in photosynthesis. While most bacteria are **heterotrophs,** some are **autotrophs** and can make their own sugars, fats, amino acids, and so forth. One group of bacteria, surprisingly, has the ability to obtain energy for their synthetic activities by converting light energy into chemical energy by the process of photosynthesis. They obtain their nitrogen from ammonia or nitrate, and their carbon from carbon dioxide in the air. Of all the biochemical processes in nature, photosynthesis is of paramount importance. The end product of photosynthesis is a reserve of chemical energy (carbohydrates) that serves as a sole source of energy for most living things.

A second group of autotrophic bacteria make their own organic cytoplasm from carbon dioxide, ammonia, or nitrate obtaining the energy for these syntheses from the oxidation of inorganic substances. For example, a bacterium present in the soil is capable of oxidizing ammonia to nitrate, thus generating useful energy. This organism is a typical chemosynthetic autotroph. Since its cells contain all the complicated carbohydrates, fats, proteins, nucleic acids, and vitamins, it represents a magnificent synthetic factory for making protoplasm.

Other bacteria are used widely in the commercial production of bakery goods, alcohol, vinegars, chemicals, enzymes, antibiotics, and many other products. These micro-organisms are also responsible for food spoilage, food poisoning, and for many plant and animal diseases including tuberculosis, scarlet fever, pneumonia, and diphtheria. Bacteria, therefore, directly or indirectly influence the survival of mankind.

The functional unit of these organisms is a single cell, the smallest of which is just visible with the light microscope. For many years bacteria were thought to reproduce **asexually** by a process called **fission,** during which the cell pinches in two. It is now known that bacterial cells also exchange genetic material through sexual reproduction. Although sexual mechanisms have been found in only a few species, future research in this area may reveal that sexual reproduction is actually a widespread occurrence among bacteria.

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EXERCISE 61

HOW CAN BACTERIA BE IDENTIFIED?

Bacterial cells generally assume three basic shapes: rods, (**bacilli**), minute spheres (**cocci**), and corkscrews (**spirilla**). In addition, the cells of many species tend to adhere to each other and form relatively simple colonies. However, each cell in the colony still functions as an independent unit and maintains the ability to carry out all of the processes necessary for survival.

In this exercise you will examine some bacteria and stain them to identify the three cell types listed above.

PROCEDURE

- Using the wide end of a clean toothpick that has been dipped in alcohol, scrape your teeth near the gums (Fig. 14.1A).
- Spread the scrapings in a thin film on a clean glass slide (Fig. 14.1B).
- Allow the smear to dry, then heat it gently by passing the slide back and forth several times over the flame of an alcohol lamp (Fig. 14.1C).
- Support the slide over a beaker or other small dish and apply several drops of crystal violet—a dye that stains bacteria (Fig. 14.1D).
- Leave the dye on the smear for one minute. Then wash off the dye under a *gentle* stream of tap water or water squirted from an eye dropper (Fig. 14.1E).
- Using paper towel, wipe off any dye that may be sticking to the *bottom* of the slide.
- Blot off the excess water from the upper surface, *avoiding only the smear* (Fig. 14.1F).
- Place a drop of water on the stained smear, add a cover slip and examine your stained slide with the high power of your microscope. **61-A Draw the various types of bacterial cells that you observe on your slide (see page 367). 61-B Which of the bacterial types is most common? 61-C Are motile (free-swimming) bacteria present? 61-D If these bacteria were living, how could you distinguish between true motility and movement due to Brownian motion?**

CAUTION: Do not overheat the slide. You should be able to place the slide on the back of your hand without flinching.

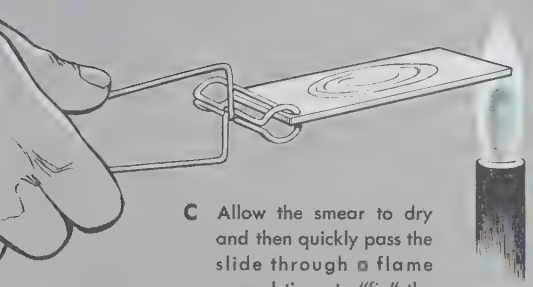
FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Name some specific examples of what could happen if the Earth would suddenly lose decay bacteria, nitrogen-fixing bacteria, and dentrifying bacteria.

FIG. 14.1 PROCEDURE FOR STAINING BACTERIA

A Using a clean toothpick, scrape the teeth near the gums.

B Spread the scrapings in a circular motion on a clean slide.

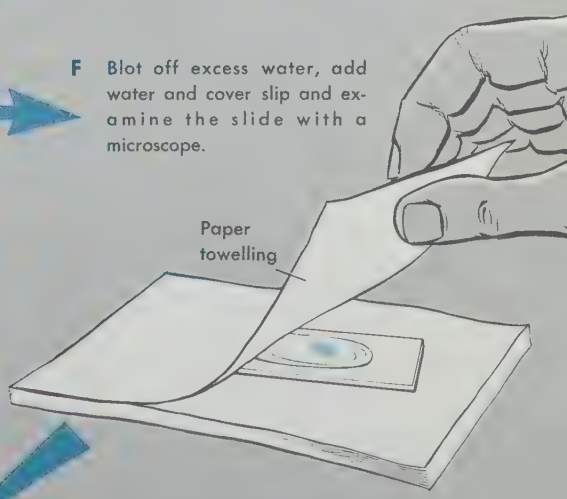


C Allow the smear to dry and then quickly pass the slide through a flame several times to "fix" the bacteria to the slide.

D Then supporting the slide over a beaker, cover the smear with several drops of crystal violet, a dye that stains bacteria.

E Leave the dye on the smear for 1 minute then rinse it off with water.

F Blot off excess water, add water and cover slip and examine the slide with a microscope.



G On page 367 draw the types of bacteria you observe.

2 What is meant by **heterotrophic nutrition, saprophyte, autotrophic, photoautotrophic, chemoautotrophic?**
3 With respect to their mode of nutrition, into which groups of Question 2 can bacteria be placed?
4 How has the electron microscope helped in providing a better understanding of the bacterial cells?

EXERCISE 62

HOW CAN BACTERIAL GROWTH BE CONTROLLED?

The control of bacterial infections has been, and still is, a major medical problem. The discovery and use of antibiotics, however, is one of the most spectacular developments in controlling infectious bacteria and other disease-causing organisms.

The story of antibiotics began in 1929 when the British biologist Alexander Fleming found that a petri dish containing a certain kind of bacteria had become contaminated by a mold called *Penicillium*. Looking closely, he noticed that the growth of the bacteria around the mold colony was inhibited. He concluded that the mold was producing a diffusable chemical agent capable of inhibiting bacterial growth. Fleming later isolated this antibacterial chemical and called it **penicillin.** Such chemicals, which are produced by living organisms and have the ability to retard the growth of some other organism, are called **antibiotics.** Among the wide variety of such antibiotics are streptomycin, chloromycetin, aureomycin, and terramycin. The use of such antibiotics has significantly lowered the occurrence of infections, which at one time in history would wipe out thousands of people in a single community.

In this exercise you will examine the effect of different antibiotics on the growth of one of the more common bacteria.

PROCEDURE

▶ Obtain a petri dish containing **nutrient agar**—a growth medium (a mixture of chemicals which is optimal for the growth of a given organism) for the bacteria you are going to study.

NOTE: You may wish to prepare the nutrient agar yourself. Your instructor will show you how.

▶ Divide each plate into four sections labeled 1, 2, 3, and "Control" by marking the bottom of the dish with black marking pencil (Fig. 14.2A).

▶ Lift the cover of the petri dish slightly and add 10 drops of the bacterial suspension (provided by your instructor) to the plate (Figs. 14.2B,C).

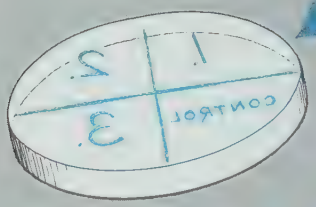
▶ Holding the plate at about eye level, tilt the plate, allowing the suspension to run to the far edge (Fig. 14.2D). Then, holding the plate level, jerk it toward you. Repeat this several more times, rotating the plate part way between each jerk (Fig. 14.2E). In this way the bacterial suspension will come to cover the entire surface of the agar.

CAUTION: Do not tilt the dish so far that the suspension runs over the edge of the plate.

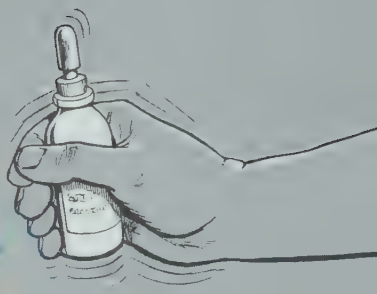
▶ Incubate the plate for 24 hours at 37°C before proceeding with the next step. **62-A Why might this incubation period be desirable?**

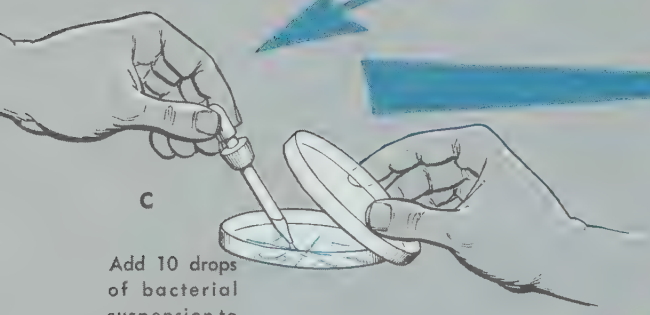
FIG. 14.2 EFFECT OF ANTIBIOTICS ON BACTERIAL GROWTH

A Mark the *bottom* of the dish with black marking pencil.



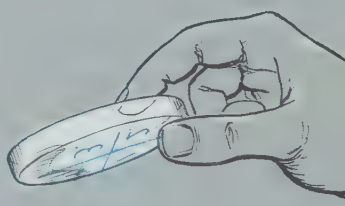
B





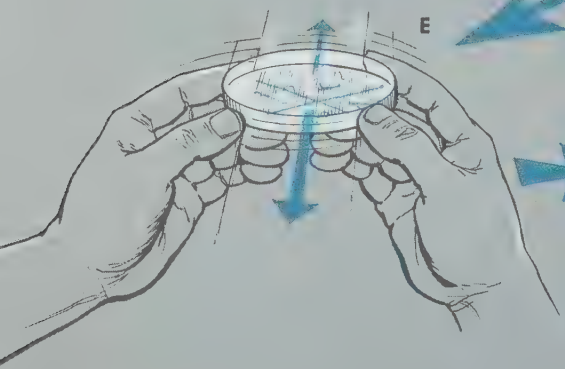
C

Add 10 drops of bacterial suspension to the surface of agar.



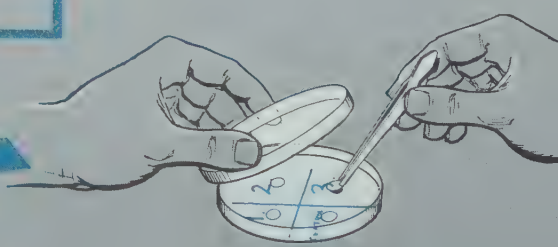
D Holding the plate at eye level, tilt the plate, allowing the suspension to run to the edge.

E



Incubate plate for 24 hours at 37°C or for 48 hours at room temperature.

F Add a different antibiotic disc to sections 1, 2, and 3. What will you place in the "control"?



G Incubate at 37°C or room temperature. Examine daily for the next 3 to 4 days. Record your observations with a drawing on page 369.

▶ After 24 hours, use forceps and, lifting the cover partially, place three antibiotic discs in the numbered sections of the plate (Fig. 14.2F). Use a different antibiotic in each section. **62-B What should be placed in the "Control" section of the plate?**

▶ Set the plate in the incubator. Examine it every day for the next three to four days. **62-C Record the effects of each of your antibiotics in a drawing (see page 369). 62-D Which of the antibiotics tested appeared to be most effective in controlling the growth of the bacteria tested?**

NOTE: In place of the antibiotics (or perhaps as a different study) you might want to use ½-inch discs of filter paper dampened with Lysol, mercurochrome, 1% iodine, or other antiseptics you may have at home.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 A species of bacteria that once was susceptible to the action of a particular antibiotic may suddenly cease to be affected by it. How can you explain this?
2 What is the mechanism by which the antibiotic penicillin controls the growth of bacteria?
3 Some bacteria have an enzyme called **penicillinase.** Would the growth of these bacteria be inhibited by penicillin? Explain.
4 What are some other antibacterial agents besides antibiotics?

EXERCISE 63

ARE THERE BACTERIA IN MILK?

Milk is the most nearly "perfect food" for man and it is also an excellent medium for the growth of bacteria. Therefore, great care must be used in its processing. If milk is cooled immediately after milking, the amount of bacterial contamination that occurs will be kept low. Any harmful bacteria that may be present will be killed by the process of **pasteurization.** In this exercise you will be asked to examine milk from different sources and determine the quality of the milk in terms of its bacterial population.

PROCEDURE

▶ Bring to class three to four samples of milk of various ages or from different sources (fresh, unopened milk, milk opened and in the refrigerator for one, two, three or more days, powdered milk, canned milk, and so on).

▶ Fill separate test tubes one-third full with each of the various samples (Fig. 14.3A). Label each tube for its contents. Then add 1 ml (20 drops) of methylene blue solution to each tube (Fig. 14.3B).

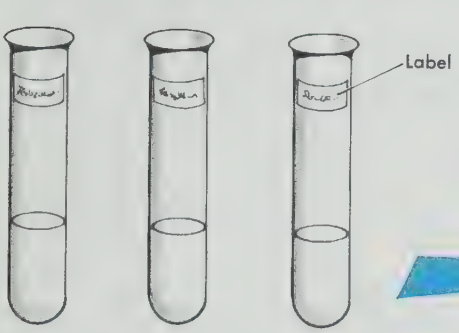
When bacteria are actively growing in milk, they consume oxygen. This reduction of oxygen can be detected by using methylene blue, which turns colorless in the absence of oxygen. If the number of bacteria present is high, the methylene blue solution will rapidly lose its color. If the bacterial population is low, a longer time will be required for decolorization.

▶ Plug each tube with sterile cotton and then place the tubes into a water bath maintained at 37°C (Fig. 14.3C).
63-A Record the time in the table on page 371.

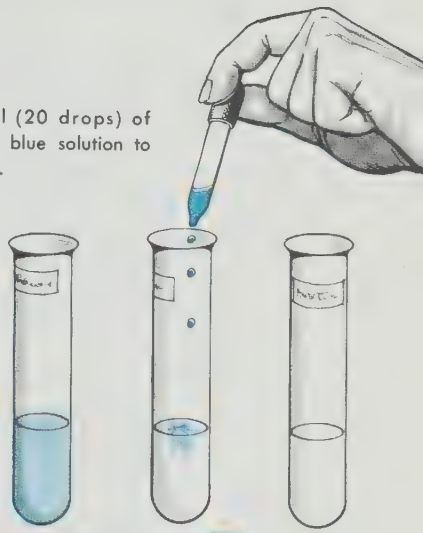
The quality of the milk, with respect to the number of bacteria present, can be rated by the time it takes to decolorize the methylene blue solution:

Time to decolorize methylene blue	Rating
Less than 20 minutes	Highly contaminated
20 minutes to 2 hours	Poor
2–5½ hours	Fair
5½–8 hours	Good
Greater than 8 hours	Excellent

FIG. 14.3 PROCEDURE FOR ESTIMATING BACTERIAL CONTAMINATION OF MILK

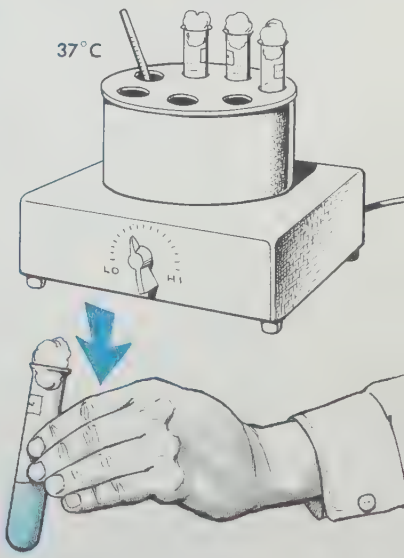


A Fill the test tubes 1/3 full with different samples of milk of various ages or different sources. Label each tube for its contents.



B Add 1 ml (20 drops) of methylene blue solution to each tube.

C Plug the tubes with cotton and place them in a hot water bath (37°C).



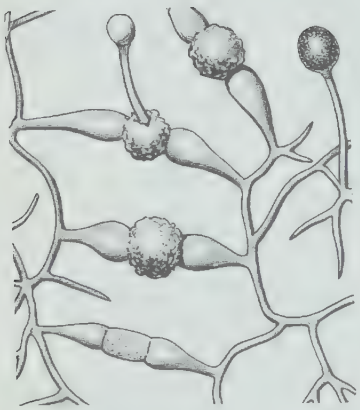
D Examine each tube periodically. Rate the quality of the milk according to its bacterial population as described in the procedure. Record your observations in the table on page 371.

▶ Periodically examine each of the milk samples. **63-B In the table on page 371 record the length of time it takes to decolorize each sample (that is, until the color of normal milk returns). Rate each of the samples according to the description given above. 63-C Which milk sample rated the highest in bacterial contamination? 63-D Which rated the lowest? 63-E From your results, what appears to lead to contamination of milk? 63-F What appears to reduce the contamination of milk?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Briefly describe how milk is pasteurized.
2 Why do you think that some cities require dairy companies to "date" milk and other dairy products sold in stores?
3 In a healthy cow the milk that is secreted into the milk ducts of the udder is sterile (bacteria free). List the possible sources of contamination from the time the milk leaves the cow until it is consumed.
4 What could be done to assure that bacterial contamination of milk would be minimized?
5 How could you account for a high bacterial population in supposedly pasteurized milk?
6 What is the importance of lactic acid bacteria?

15 THE ECONOMY OF LIFE



INTRODUCTION

All living organisms require energy to maintain the complex organization and many chemical reactions that distinguish the *living* from the *nonliving*. Although the need for energy is a basic requirement of all living things, this does not mean that all organisms obtain their energy requirements by the same means.

Sunlight is the primary energy source for all organisms—including man. However, the energy in sunlight cannot be used directly. It must first be converted to chemical energy in the form of sugar or other organic compounds. This is accomplished by chlorophyll-containing organisms using the process of photosynthesis. These photosynthetic organisms, living both on land and in the water, are known as **autotrophs.** Autotrophic organisms are able to grow and multiply in a purely inorganic medium. In other words, they do not depend on an outside source for vitamins, amino acids, or other complex organic molecules. From a nutritional viewpoint, autotrophs are the least exacting group of organisms. They are the *producers* in the earth's aquatic and terrestrial environments.

The other major category of organisms are the **heterotrophs.** These consist of organisms that get their energy mainly from organic sources, such as carbohydrates. These organisms are the *consumers*.

Man is a very complex heterotroph. He must eat other organisms in order to obtain the growth factors he himself cannot make. He requires not only certain kinds of amino acids in his diet, but other required growth factors including a number of vitamins and certain fatty acids. Compared with many other organisms, the synthetic machinery of man is less versatile, hence less complex. The photosynthetic green plants, on the other hand, are remarkable in their ability to use the energy of

sunlight to make essentially all the known vitamins and amino acids. It is for this reason that many higher vertebrates, such as man, depend on plants as their primary supply of food—if not directly, then indirectly by eating other animals that eat the plants. Thus, the economy of life may be thought of as a "web" woven by the activities of producers and consumers.

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EXERCISE 64

PRODUCERS

Plants that contain chlorophyll are generally considered to be the primary producers of the world's food supply. The largest amount of organic materials produced is by the photosynthetic activity of microscopic aquatic organisms. To adequately carry out photosynthesis, these organisms have come to possess *morphological* or *physiological* adaptations which will assure them of maximum exposure to sunlight.

PROCEDURE

▶ Prepare a wet mount of an alga called *Euglena* and examine it with your microscope (Figs. 15.1A–D). **64-A What is the color of *Euglena*? 64-B What is the shape of this organism? 64-C Describe any movement observed.**

▶ Remove some of the water from under the cover slip by touching a piece of paper towel to one edge of the cover glass. **64-D Does any change occur in the way *Euglena* moves? Describe your observations.**

▶ Add a drop of iodine to the edge of the cover glass and wait a few minutes for it to diffuse under the cover slip (Fig. 15.1E). Again examine the slide with your microscope. **64-E Does the iodine cause a change of color in any part of the organism? 64-F If there is a color change, what does this tell you about the kind of "food" produced by *Euglena*?**

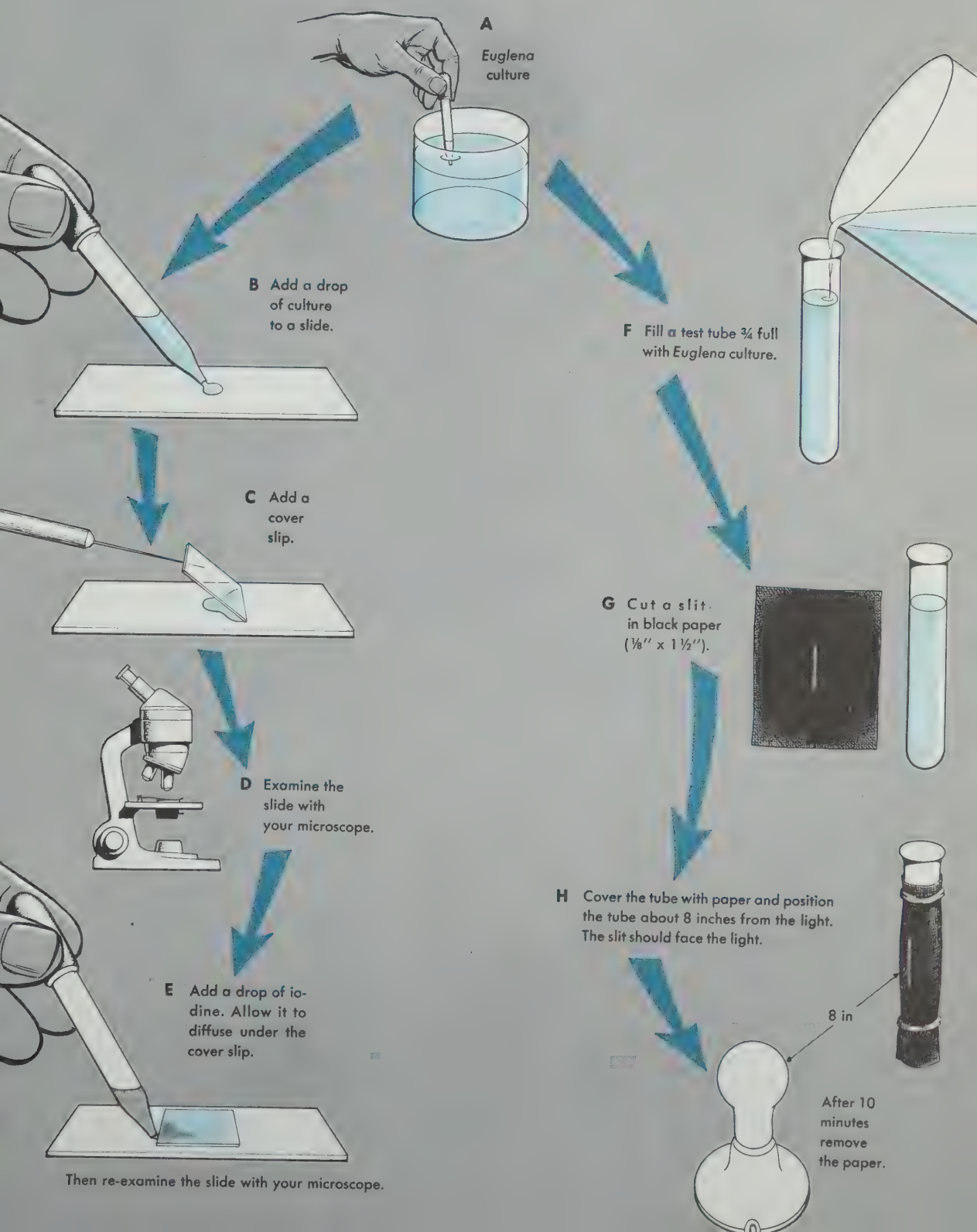
▶ The iodine will kill these organisms, but it will also help you to determine what causes the movement in *Euglena*. Examine *Euglena* closely. In order to do this reduce the illumination by closing the diaphragm. **64-G What structure do you see that gives motility to *Euglena*?**

▶ Fill a test tube half full with a sample of *Euglena* (Fig. 15.1F). Then cover the tube with a tightly-fitting cylinder of black paper which has a narrow 1- to 1½-inch long slit in it (Figs. 15.1G,H).

▶ Place the covered tube about eight inches from a light source (microscope illuminator or window ledge) so that the slit is facing the light (Fig. 15.1H).

▶ Allow the light to shine on the set-up for about 10 minutes, then quickly remove the paper cover. **64-H How are the *Euglena* arranged in the test tube? 64-I How is the response you observed advantageous to the *Euglena*? 64-J What happens to the arrangement of these organisms during the several minutes following the removal of the paper cover? 64-K How do you explain the observed response?**

FIG. 15.1 STUDY OF THE PRODUCER, EUGLENA



FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 Although green plants are primarily food producers, can they ever be considered as consumers? Explain.
2 What is the Venus fly trap? Would you consider this organism a *producer* or *consumer?* Explain.
3 What is the ultimate source of energy required by *producer* plants?
4 How is this energy "captured" by the producers?
5 What would eventually happen to the human population if the producer plants were to die?

EXERCISE 65

THE BREAD MOLD: A COMMON CONSUMER

The organisms commonly recognized as "consumers" generally do not have chlorophyll and thus are not able to synthesize the organic molecules necessary for metabolism. The consumers, therefore, depend on other organisms for the synthesis of organic compounds. In this exercise you will examine a type of fungus that obtains its organic nutrition from bread.

PROCEDURE

▶ Obtain a petri dish containing black bread mold. The mold is not growing on bread here, but on agar containing the organic compounds it needs for growth. If you wish, bring some bakery bread from home (not store bread because it contains mold inhibitors) and place a piece into a petri dish. Moisten it with several drops of water and then place a small piece of the mold from the agar onto the bread. Cover the dish and set it aside for one to two days.

FIG. 15.2 PROCEDURE FOR EXAMINING THE STRUCTURE OF THE BLACK BREAD MOLD

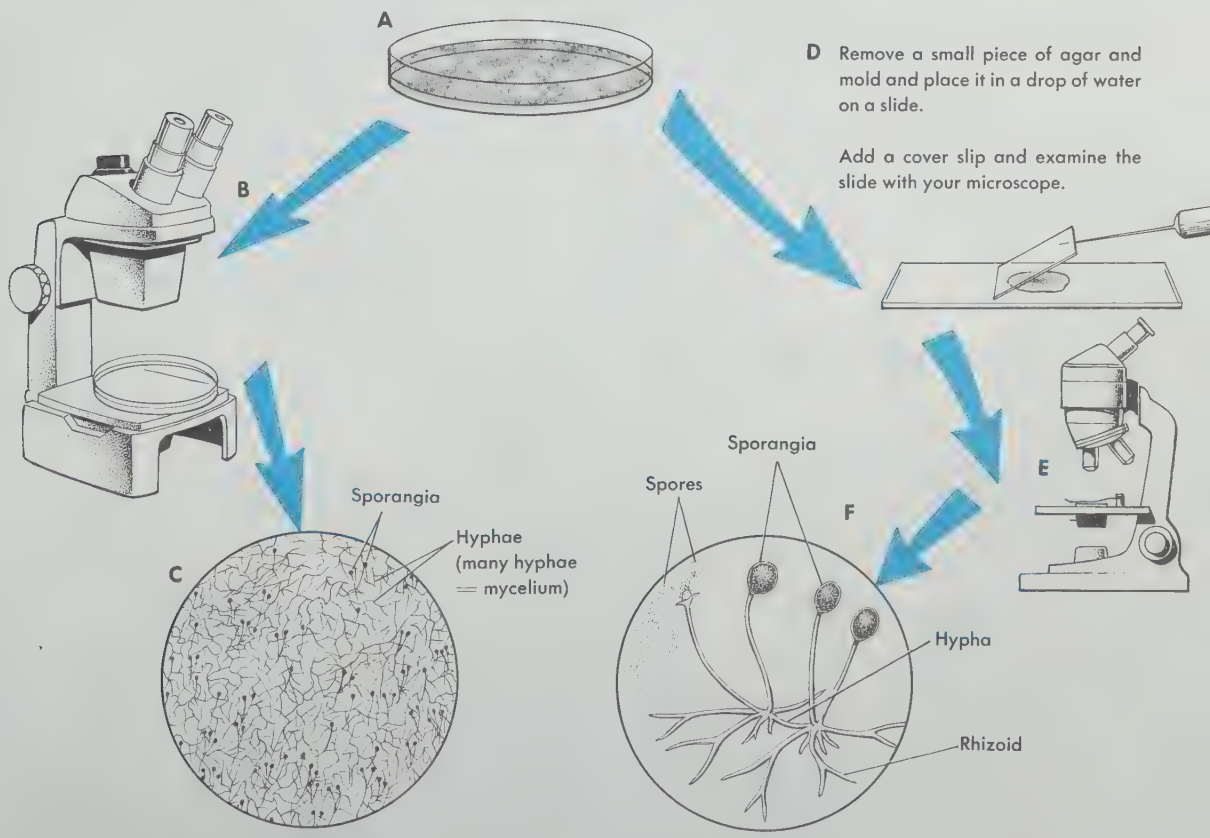
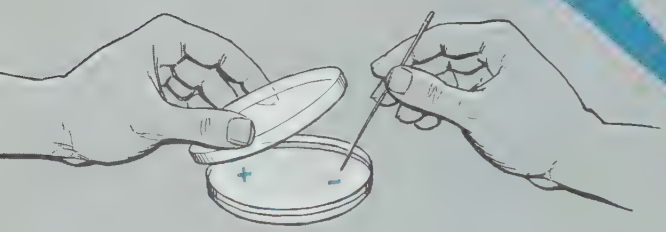
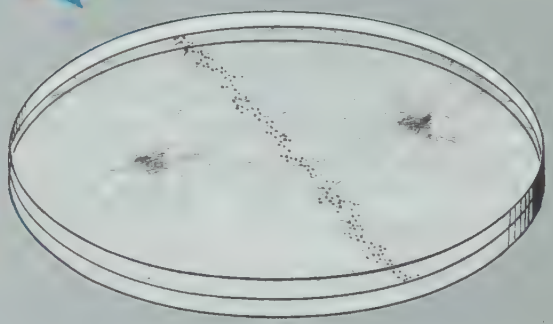


FIG. 15.3 SEXUAL REPRODUCTION IN THE BLACK BREAD MOLD

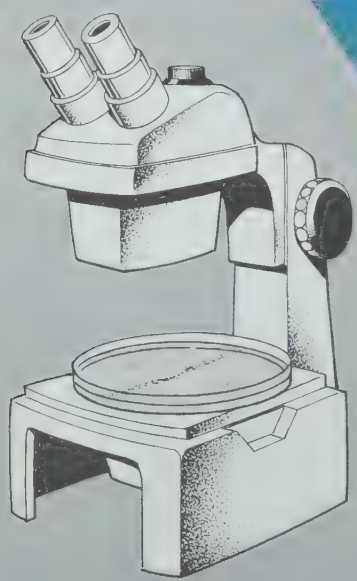
A Your instructor has earlier innoculated (+) and (—) strains of bread mold onto the agar of a Petri dish.



B When the two strains meet, gametes form and fuse, producing a line of dark zygospores.



C Examine the unexposed dish with your dissecting microscope and locate the zygospores.



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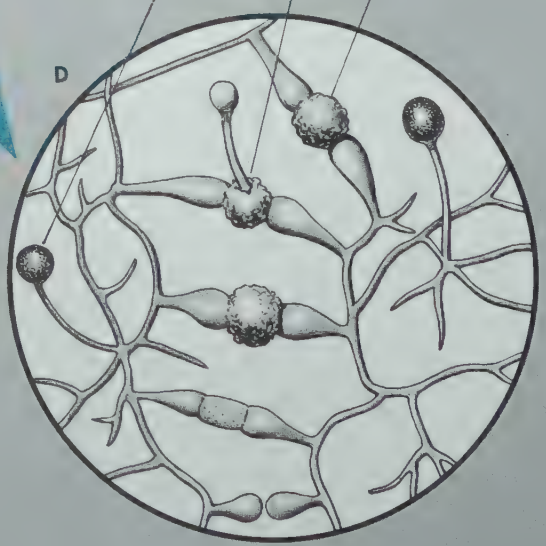


▶ Examine the mold with your dissecting microscope (Fig. 15.2B). Do not remove the cover! Note the whitish mass of thread-like filaments growing over the surface of the agar. Each separate filament is called a **hypha** (plural **hyphae**) (Fig. 15.2C). The total mass of hyphae is a **mycelium.**

▶ Some hyphae grow upward and form small black, globe-like structures called **sporangia** (Figs. 15.2C,F). Inside the sporangia are cells called **spores.** They are released when the sporangia open. **65-A What is the function of the spores?**

▶ Some of the hyphae penetrate the agar. Turn the dish over and focus downward through the agar until you locate small root-like hyphae called **rhizoids** growing into the agar. (Fig. 15.2F). **65-B What is the function of the rhizoids?**

▶ Remove the cover from the petri dish. With a razor blade, or scalpel, remove a small piece of the agar and mold. Place it in a drop of water on a slide (Fig. 15.2D). Add a cover slip and examine the slide with your microscope. Locate the sporangia, spores, hyphae, and rhizoids.

Black bread mold requires two different strains before sexual reproduction can occur. Your instructor has inoculated an agar plate with a (+) strain and a (−) strain (Fig. 15.3A). The growth of each strain has brought them into contact. At the points of contact, gametes are formed. Fusion of the gametes (**fertilization**) has resulted in the formation of black, thick-walled **zygospores.**

▶ Examine the "sexual" culture provided by your instructor. If the culture is old enough, you should see a black line of zygospores running across the culture (Fig. 15.3B).

▶ Keeping the dish closed, locate the zygospores with your dissecting microscope (Figs. 15.3C,D). After a period of time, the zygospore will break open and a hyphae bearing a sporangia will emerge (Fig. 15.3D). **65-C In what ways, if any, do the plus and minus strains *look* different? 65-D Would you consider each strain to have a different sex?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 What are some of the kinds of organic materials that "producers" supply to "consumers"?
2 What is a **food web?**
3 Describe a food web, beginning with the microscopic producers of the ocean and ending with man.
4 Would you consider elephants and men to be final consumers of a food web? Explain.

EXERCISE 66

THE SLIME MOLD: A UNIQUE KIND OF CONSUMER

The slime molds are a group of consumer organisms that exhibit the characteristics of both plants and animals. In one stage of their life cycle, the slime mold exists as a **plasmodium,** a multinucleated mass of protoplasm organized as a single cell. In this form, which may occupy several square feet in area, the slime mold moves about and ingests bacteria and other food much like an ameba. After a period of time, however, the plasmodium stops moving and forms fruiting bodies in which spores are formed. The spores act as gametes and fuse, giving rise to a cell that develops into a new plasmodium.

PROCEDURE

▶ Your instructor will provide (for each two students) a petri dish containing agar (Fig. 15.4A). He will also provide you with a small piece of filter paper containing some slime mold.

▶ Place the paper with the slime mold onto the agar near the center of the dish and sprinkle some crushed oatmeal flakes next to it (Figs. 15.4B,C). Add two or three drops of water to the oatmeal and slime mold (Fig. 15.4D).

▶ Replace the cover of the petri dish. Seal the dish with masking tape and then set it in a cool, darkened place.

▶ Examine the plates each day. **66-A Make sketches of the changes you observe in the slime mold.**

▶ After three or four days examine the culture with the low power of your compound microscope. **66-B What living activity do you observe?**

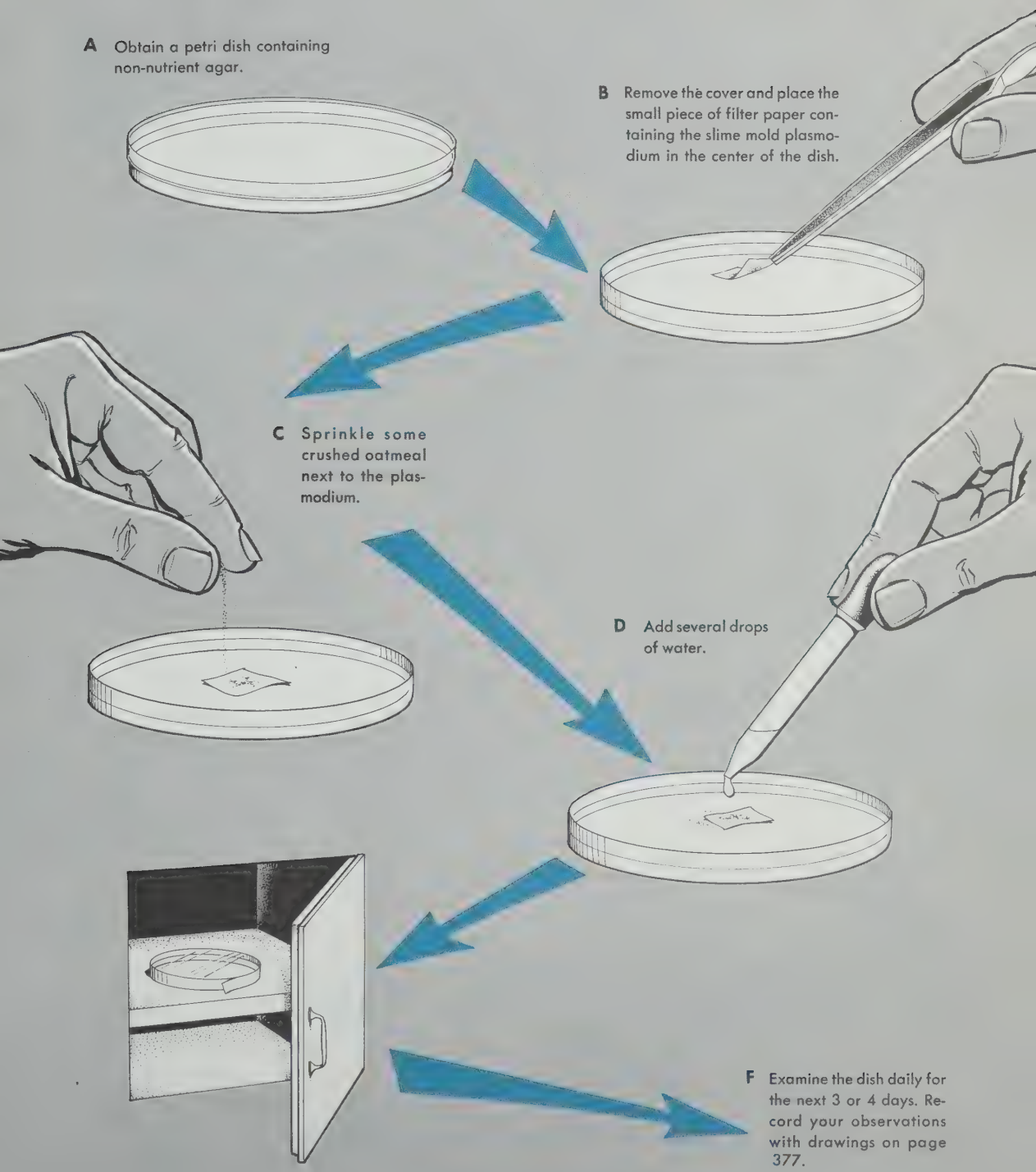
▶ Watch this "living" activity for several minutes. **66-C Describe what happens.**

▶ With a needle, puncture a branch of the slime mold and watch it carefully for several minutes. **66-D Is the hole that you made repaired?**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 How do slime molds differ from other molds, such as bread molds or blue-green molds?
2 Would you consider a slime mold a useful consumer? Explain.
3 Make a list of other consumer organisms. Divide your list into two parts: those that obtain their organic materials from living organisms; and those that obtain organic materials from nonliving organisms or products of organisms.

FIG. 15.4 PROCEDURE FOR GROWING A SLIME MOLD



EXERCISE 67

COOPERATIVES

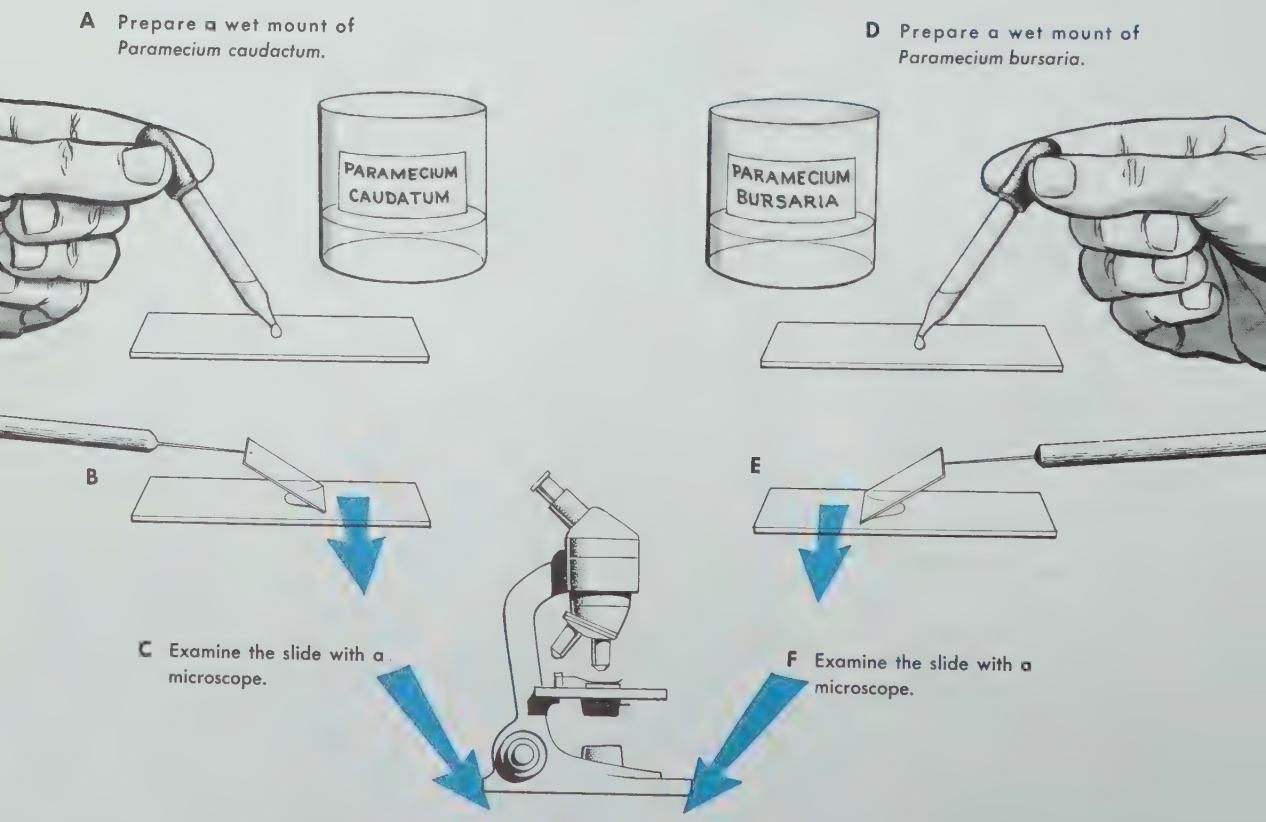
Relationships between different organisms are frequently unfriendly. Organisms struggle for a place to live; some eat others or live on them as parasites. Occasionally, however, there are intimate associations of different species that provide advantages to both parties. This relationship is called **symbiosis.**

PROCEDURE—Symbiosis With the "Consumer" an Animal

▶ Prepare a wet mount of the protozoan *Paramecium caudatum* (Figs. 15.5A–C). **67-A Describe the movement of this one-celled animal. 67-B What color is this organism?**

▶ Prepare a second slide of *Paramecium bursaria* (Figs. 15.5D–F). **67-C How does this animal differ from *Paramecium caudatum*? 67-D What are the "green bodies" in this single-celled organism? 67-E How does the "consumer" in this symbiotic relationship benefit? 67-F How does the "producer" benefit?**

FIG. 15.5 SYMBIOSIS IN PARAMECIUM



PROCEDURE—Symbiosis With the "Consumer" a Plant

A **lichen** is an organism consisting of two different kinds of plants—fungi and algae—living together in a symbiotic relationship. This is a unique union because the two plants living together form a structure that looks nothing like the individual alga or fungus.

▶ Examine the various living lichens provided by your instructor (Fig. 15.6A). **67-G Do they look anything like the fungi observed in exercises 65 or 66?**

▶ With a sharp razor blade, cut a *thin* section from the lichen and prepare a wet mount (Figs. 15.6B,C,D). Examine the section closely with your microscope. **67-H What do you see that indicates that the lichen contains a fungus? 67-I What tells you that an alga makes up part of the body of the lichen? 67-J In what ways do the alga and fungus benefit from this association?**

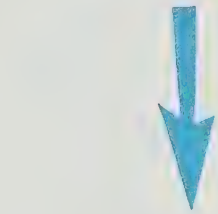
Recent experimental studies have shown that the fungus and the algal components of lichens can be grown separately from each other. Under these conditions, the forms of each are quite different than the lichen from which they came. When the alga and fungus are placed close together, another lichen is slowly formed. This association truly represents a new pattern of growth and organization.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

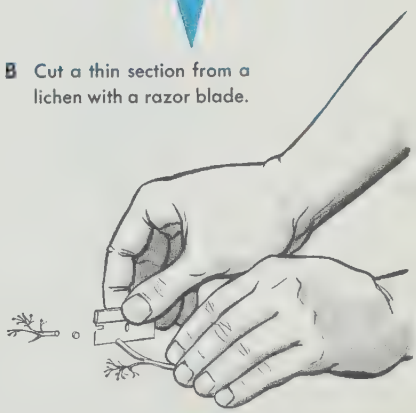
1 Give examples of some other symbiotic relationships and explain how each "partner" benefits from the association.
2 What is the difference between **mutualism, symbiosis,** and **parasitism?**
3 What is a **mycorhiza?**
4 Give an example of a symbiotic relationship between bacteria and higher plants (peas, for instance).

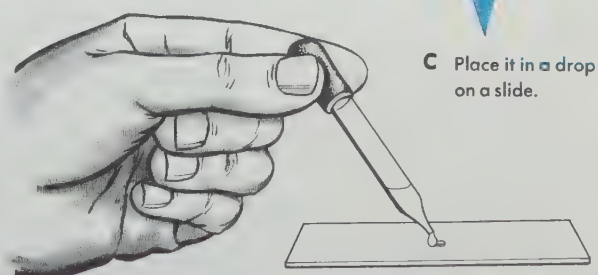
FIG. 15.6 PRODUCER-CONSUMER RELATIONSHIPS IN A LICHEN

A Examine various lichens on demonstration.

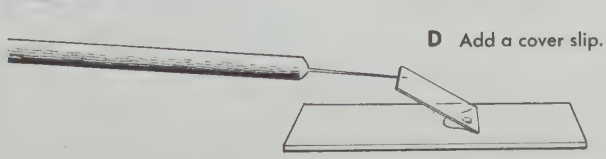


B Cut a thin section from a lichen with a razor blade.

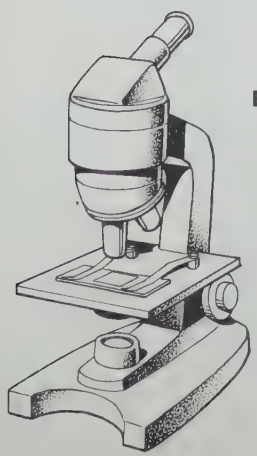




C Place it in a drop of water on a slide.



D Add a cover slip.



E Examine the slide with your microscope.

16 PATTERNS OF REPRODUCTION IN PLANTS

INTRODUCTION

Sexual reproduction in plants, as in animals, involves the fusion of two gametes, or at least the transfer of genetic material from one cell to another. Even the bacteria, which, for many years, were thought to reproduce only by asexual means, are now known to reproduce by a process very similar to the sexual process of higher organisms.

Why is sexual reproduction of such universal occurrence in living organisms? Undoubtedly, the reason lies in the advantage it provides in the organism's struggle for existence. This advantage lies in the **genetic variability** obtained from the new gene combinations that occur when the gametes unite at fertilization. When there is a wide variability of gene combinations present in a population, then the population is more likely to survive severe environmental changes. These organisms have a higher probability of having the genes that will enable them to grow and reproduce in the new environment.

In all organisms that reproduce by sexual means, the chromosome number is doubled when the nuclei of the gametes fuse. Consequently, somewhere in the life cycle, there must be a compensatory mechanism in which the chromosome number is halved again. This "halving" is achieved by the process of **meiosis.** Beginning students frequently confuse meiosis with mitosis. Mitosis is the division of the nucleus, usually followed by cell division, which results in new cells having the *same kinds and numbers of chromosomes* as found in the parent cell. Meiosis, on the other hand, results in daughter cells containing different numbers and kinds of chromosomes. Furthermore, meiosis is restricted to a particular part of the life cycle and to particular regions of the organism.

In animals, meiosis occurs during the formation of the gametes, which are the only **haploid** cells in the body. Most other

body cells are **diploid.** In plants, however, meiosis does not always lead to the formation of gametes. Frequently, the haploid cell that is produced from meiosis develops into a haploid plant that later produces the gametes. The union of gametes then results in a cell that develops into a diploid plant which at some point will again produce haploid cells by meiosis. This **alternation of generations** is common to all higher plants and to several algae. The haploid phase in the life cycle is called a **gametophyte.** The "alternate" diploid phase is the **sporophyte.** In the exercises that follow, the reproductive cycles of algae, mosses, and ferns will be examined. Although sexual reproduction is a common occurrence among the major groups of plants, the mechanisms and structures involved in producing gametes and getting them together varies considerably.

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EXERCISE 68

REPRODUCTION IN THE ALGAE

Algae are a diverse group of mostly aquatic organisms that range in size from microscopic one-celled individuals to giant marine forms that may grow to 100 feet in length. Algae have the green pigment chlorophyll although it may be "masked" by other pigments that give the various algae their characteristic color. As a result algae have been classified as blue-green, red, or brown, depending on the kind of accessory pigments present in the cells.

In the algae, new individuals are produced by sexual or asexual means. In this exercise you will study reproduction in the green algae in order to add to your understanding of the algae themselves and to gain an understanding of certain of the basic principles that will apply to the reproductive patterns that will be seen in other plants.

PROCEDURE—Reproduction in a Unicellular Alga

▶ Prepare a wet mount of *Chlamydomonas* and examine it with your microscope (Figs. 16.1A,B,C). **68-A Would you consider this organism to be a "producer" or "consumer"? Explain.**

▶ During **asexual** reproduction, the nucleus of this single-celled alga undergoes mitosis. A new cell wall is then formed around each nucleus. The daughter cells formed within the old parent cell become motile spores called **zoospores** and develop into new *Chlamydomonas* plants (Figs. 16.1D,E). **68-B The zoospore contains the haploid number of chromosomes. Is the original parent cell haploid or diploid? Explain how you determined this.**

Sexual reproduction in this alga is somewhat different from that of higher plants and animals. In these groups the gametes are specialized sex cells. In *Chlamydomonas,* the entire plant (which itself is a single cell) functions as a gamete. Further, since the individual cells are the same size, the gametes are the same size. However, in more advanced organisms the female gamete (egg) is considerably larger than the male gamete (sperm). Although the gametes of *Chlamydomonas* "appear" to be similar, they can, as a result of their behavior, be assigned to different mating groups that have been designated as "plus" and "minus."

▶ Your instructor will place a drop of the (+) and a drop of the (−) strain close together on your slide. *Do not mix the drops yet* (Fig. 16.1F).

FIG. 16.1 REPRODUCTION IN CHLAMYDOMONAS

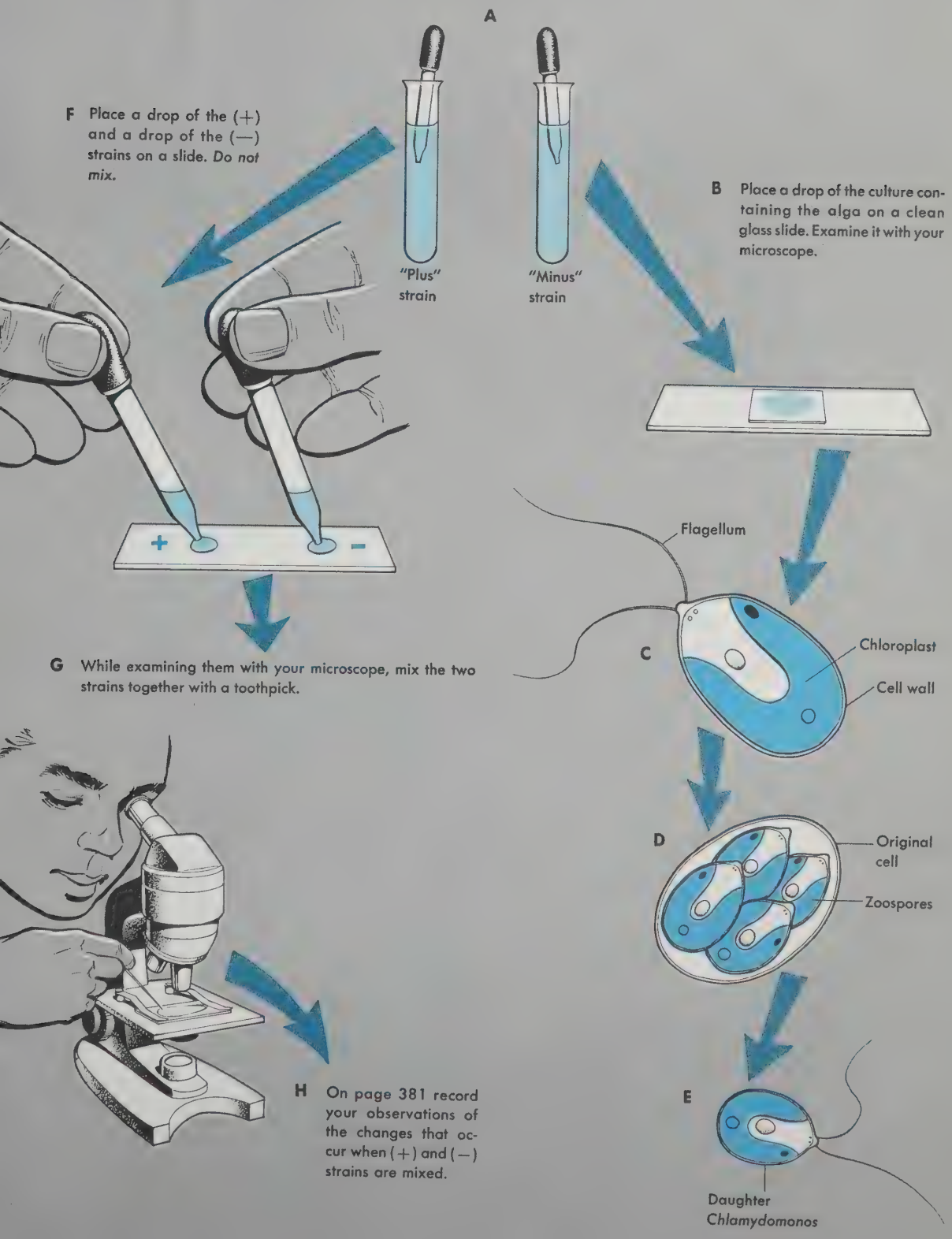
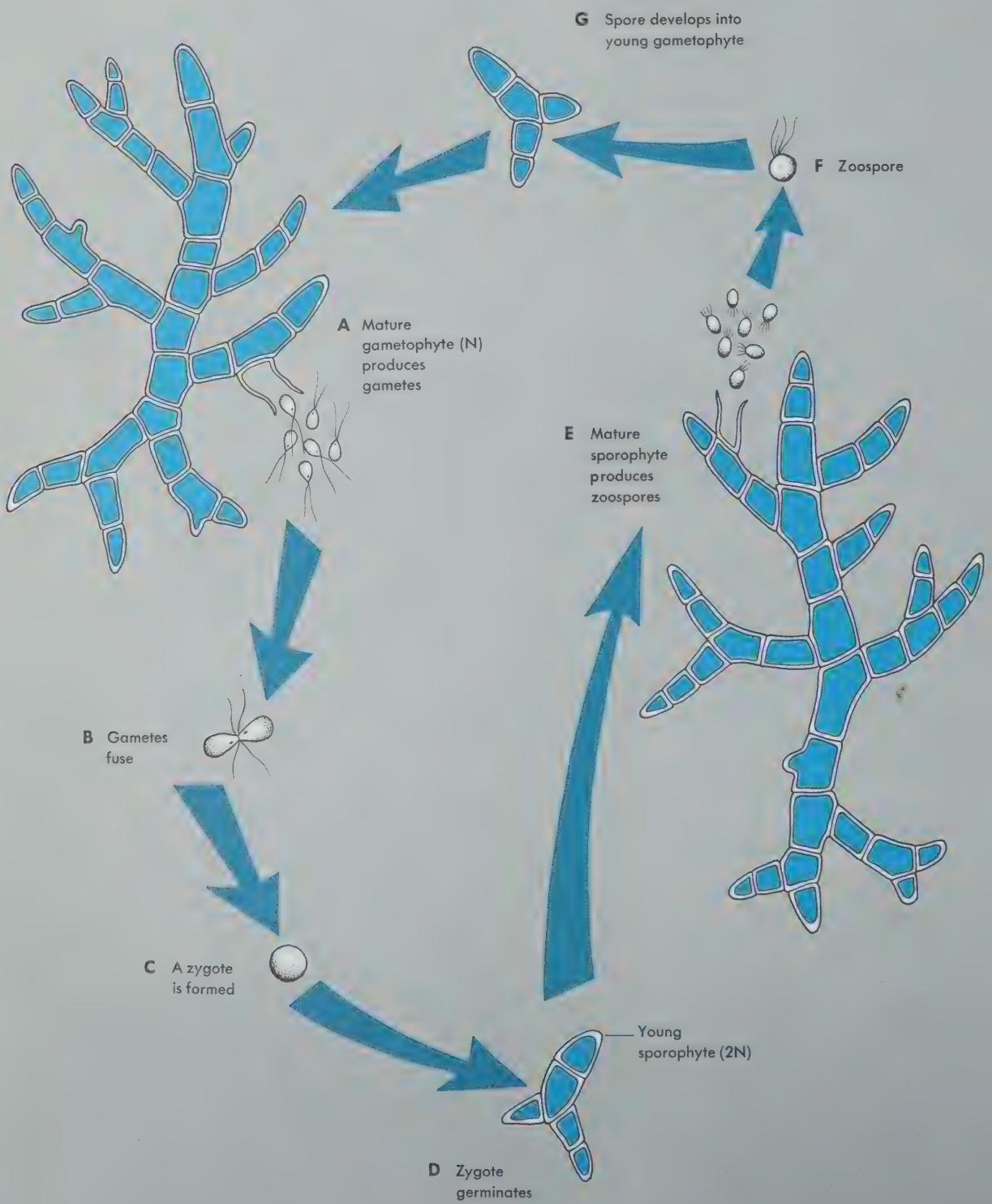


FIG. 16.2 ALTERNATION OF GENERATIONS IN THE GREEN ALGA CLADOPHORA



▶ While examining one of the drops with the low power of your microscope (a cover slip is not necessary), use a toothpick to mix the two drops together (Fig. 16.1G). Note the even distribution of the cells in the field of the microscope.
▶ Watch the cells *closely* for the next several minutes. **68-C What indication is there that the alga is undergoing sexual reproduction? 68-D Record your observations of the changes that occur by making a drawing on page 381. 68-E What is the name of the cell formed following the union of the gametes? 68-F Is this cell diploid or haploid? Explain. 68-G Does meiosis occur at any stage in the reproductive cycle of *Chlamydomonas*? Explain your answer.**

PROCEDURE—Alternation of Generations

▶ Prepare a wet mount of *Cladophora,* a green alga frequently found growing in abundance on rocks or piers along lake shores (Fig. 16.2).
▶ Examine *Cladophora* with your microscope. **68-H How does this alga differ from *Chlamydomonas*? 68-I How is it similar?**
▶ This organism exhibits a phenomenon in which one plant produces zoospores and a different plant produces gametes. The spore-producing plant is called a **sporophyte** and is diploid (Fig. 16.2E). The gamete-producing plant is called the **gametophyte** and is haploid (Fig. 16.2A). This phenomenon, called **alternation of generations,** occurs in all higher plants. Its presence in the green algae (along with the fact that the green algae have similar food reserves and pigments as the higher plants) suggests that this algal group represents the type of organisms that were the ancestors to the green land plants.

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 How do **isogametes** differ from **heterogametes** (**anisogametes**)?
2 Suggest a mechanism by which a (+) gamete "recognizes" a (−) gamete.
3 Why do you think that unicellular algae are more widely used for research on photosynthesis than are the higher green plants, such as ferns or pines?
4 What is **agar-agar? Algin? Carrageenin? Kombu?**
5 What is the **Sargasso Sea?**
6 What are **kelps?**
7 What is the role of algae in food chains?
8 How could algae be used in flights to outer space?

EXERCISE 69

REPRODUCTION IN MOSSES: PRIMITIVE LAND PLANTS

Although sexual reproduction probably occurs in every major group of plants, this does not necessarily mean that the process involves similar mechanisms and structures in the various groups. For example, widely different adaptations have been evolved by various plants to insure the union of the gametes during fertilization. Other adaptations provide for the nourishment and protection of the developing embryo. In this exercise you will study the reproductive cycle of a primitive land plant, the moss.

PROCEDURE

▶ Examine the mosses on demonstration. The "leafy" plants are the gametophytes and produce sex organs at the top of their branches (Fig. 16.3G).

▶ From your instructor, obtain "male" and "female" gametophyte plants. Remove as many of the "leaves" as possible from the top of the female gametophyte. Then crush the top into a drop of water on a slide. Add a cover slip and examine the slide with your microscope. Locate the female sex organs (Fig. 16.3G).

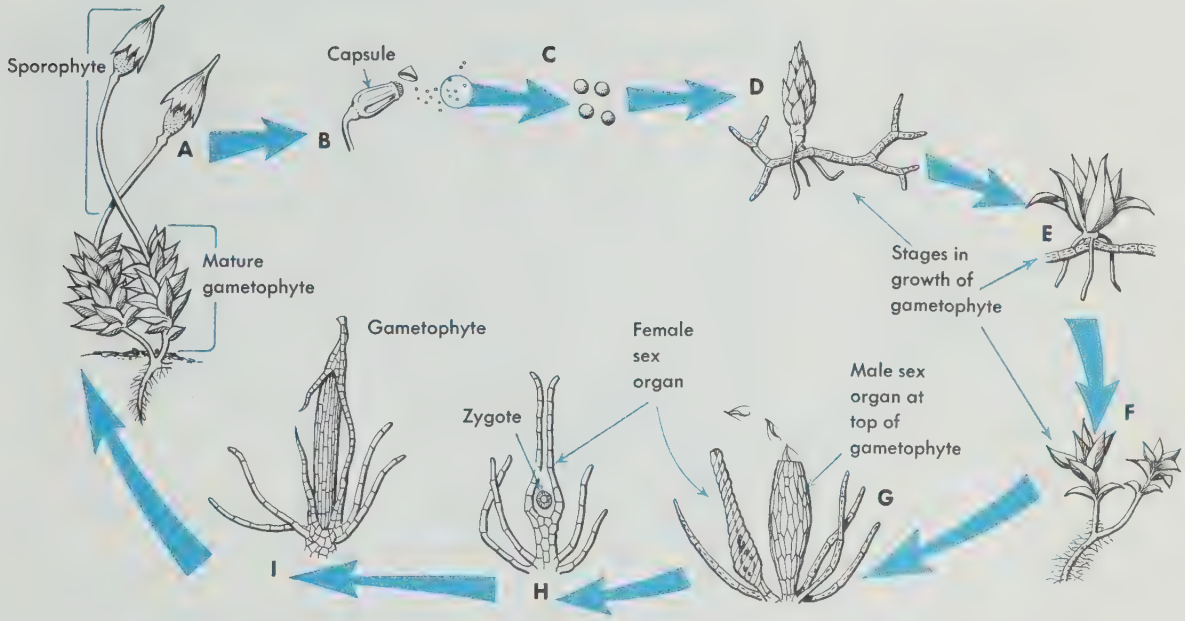
▶ Repeat the step above for the male gametophyte. The egg, which remains enclosed within the parental tissue, is fertilized by a motile male gamete. **69-A What environmental factor must be present to insure fertilization?** The **zygote** that is formed as a result of the fertilization process then divides and produces a multicellular embryo that becomes the sporophyte (Fig. 16.3I). The base of the moss sporophyte remains attached to the top of the gametophyte.

▶ Examine the mosses again and locate the sporophyte plants. **69-B Describe the sporophytes. 69-C Would you consider the young developing sporophytes to be parasitic plants? Explain.**

▶ Crush the small capsule at the top of the sporophyte into a drop of water and examine microscopically. **69-D What does the capsule contain? 69-E What will the "structures" found in the capsule develop into? 69-F Are these structures haploid or diploid? Explain.**

▶ Add the following additional labels to Fig. 16.3: "stage at which meiosis occurs;" "spores;" "young developing sporophyte;" "egg;" "male gamete;" "stage at which fertilization occurs."

FIG. 16.3 LIFE CYCLE OF A MOSS



FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 In the mosses, the male gametes must swim to the female sex organs, and swim down the passage to the egg before fertilization can take place. Suggest a mechanism that might be involved in attracting the male gamete to the egg.
2 What is a **protonema?** How does the structure of the protonema suggest that the mosses may have evolved from the algae?
3 Define **antheridium; archegonium; monoecious; dioecious.**
4 Is the moss plant diagrammed in Fig. 16.3G **monoecious** or **dioecious?** Explain.
5 In what way is **Sphagnum** (peat moss) economically important?
6 In the Northern Hemisphere would you expect to find mosses growing and reproducing on the south side of trees? Explain.

EXERCISE 70

REPRODUCTION IN FERNS: PRIMITIVE VASCULAR PLANTS

The ferns represent a large step upward in the plant kingdom. In these plants, we meet for the first time a system of specialized tissues that carry water, dissolved minerals, and other organic products throughout the plant. They are called **vascular tissues** and the plants that have them (ferns, pines, oaks) are called **vascular plants,** or **tracheophytes.** These plants are able to transport large quantities of water and minerals over long distances in relatively short periods of time. As a result, vascular plants have reached much larger sizes than the nonvascular mosses, algae, and fungi. In this exercise you will become familiar with the structure and reproductive patterns of the fern, a primitive vascular plant.

PROCEDURE

▶ Examine the fern plants on demonstration. One of them has been removed from the soil.

▶ Locate the *stem.* Unlike most plants, it is not vertical, but lies horizontally on or beneath the surface of the ground (Fig. 16.4A).

▶ Locate the young leaves (Fig. 16.4A). As they develop, they unroll in a curious fashion. **70-A Why are the young leaves called "fiddleheads?"**

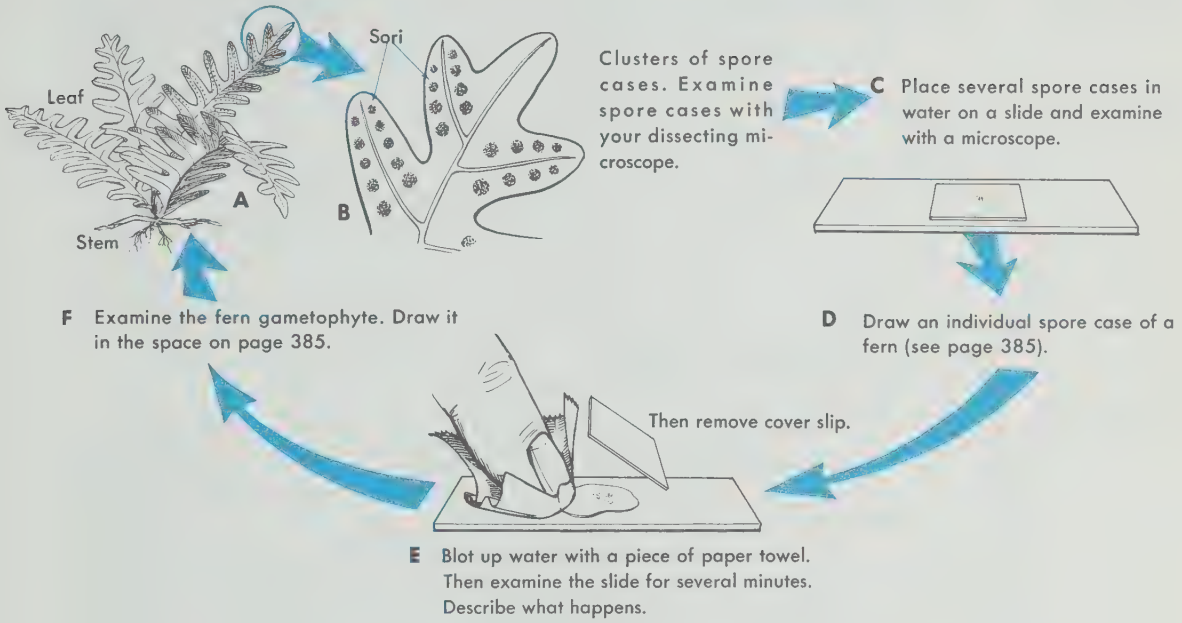
▶ Examine both surfaces of the older, expanded leaves. Notice that some leaves have small, yellowish-brown structures (**sori**) on them (Figs. 16.4A,B). Examine these structures with your dissecting microscope. They are made up of many spore cases.

▶ Scrape a few of these spore cases into a *small* drop of water on a slide and examine them with your compound microscope (Figs. 16.4C,D). **70-B Draw one of the spore cases.**

▶ Remove the cover slip from the slide and carefully blot up only the water with a paper towel. Now carefully re-examine the spore cases with the low power for several minutes (Fig. 16.4E). **70-C Describe what occurs. 70-D What are the small structures in the spore cases? 70-E What do these structures become? 70-F Based on what you have seen so far, describe the sporophyte of the fern.**

▶ Obtain mature fern gametophytes from your instructor. Examine them with your dissecting microscope (Fig. 16.4F). **70-G Describe the appearance of the gametophyte. 70-H Where does the gametophyte have its origin?**

FIG. 16.4 REPRODUCTIVE CYCLE OF A FERN PLANT



▶ Turn the gametophyte over. With your dissecting microscope examine the entire lower surface carefully. Locate small rounded structures protruding from the lower surface of the gametophyte. **70-I What do you think these structures are? 70-J What would you expect these structures to contain? 70-K Where does the fern sporophyte have its origin? 70-L Draw the fern gametophyte. Label it as directed by your instructor.**

FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

1 How does the fern sporophyte differ from the moss sporophyte in complexity, position, and nutrition?
2 Why are ferns normally not found growing in dry places, even though their sporophytes have vascular tissue?
3 What generation (in the alternation of generations) is initiated by the fertilized egg?
4 What is a **sporophyll?**
5 What is a **sporangium?**
6 In what ways does sexual reproduction in ferns differ from sexual reproduction in the algae? In what ways are they similar?

17 REGENERATION

INTRODUCTION

The individual organism is an elaborate and complex system, the structure and activities of which are highly organized. The organism is able to maintain its organization and activities for varying periods of time by the appropriate use of energy captured or otherwise obtained from its environment. Within limits, the organism can adapt its structure and behavior in such a manner as to remain adapted to changing conditions in its environment. Again, within certain limits, all organisms have the remarkable ability to replace destroyed, damaged, or defective tissue with new growth. This ability, called **regeneration,** shows considerable variability from one species to the next and, in man, and other complex animals, is highly limited.

In human beings regeneration is generally restricted to the replacement of tissue in broken bones, to the production of new blood cells, and to the healing of wounds. In sharp contrast, many of the lower animals, such as crabs, lobsters, and salamanders, can easily regenerate entire limbs. Some worms can grow entire new bodies from small fragments.

In those animals capable of regeneration, new tissue growth apparently originates from unspecialized cells. Like the cells of embryos, these can develop into any number of other kinds of cells. Just what regulates regeneration is not fully understood. Recent experimental work suggests that nerves and hormones play an important role in the process.

In the exercises that follow, you will examine the striking phenomenon of regeneration—the ability of the organism to repair or replace parts lost by disease or accident.

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Barth, L. J. *Development: Selected Topics.* Reading, Mass.: Addison-Wesley Publishing Co., 1964.

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Hamburger, V. *A Manual on Experimental Embryology.* Chicago: Univ. of Chicago Press, 1947.

Nicholas, J. S. "Regeneration, Vertebrates," in Willier, Weiss, and Hamburger (eds.), *Analysis of Development.* Philadelphia: W. B. Saunders Co., 1955.

Waddington, C. H. *Principles of Development and Differentiation:* New York: The Macmillan Co., 1966.

EXERCISE 71

HOW CAN REGENERATION BE INDUCED?

Numerous plants and animals have the ability to replace parts of their bodies that have been damaged or lost. This process, called **regeneration,** has long been of interest to biologists because regeneration essentially represents a return to the embryonic condition. A basic problem is to determine what causes apparently mature cells to become embryonic and initiate the production of new tissues and organs. In this exercise you will attempt to induce regeneration in two of the "simpler" forms of animals: planarians and hydras.

PROCEDURE—Regeneration in Planarians

▶ Transfer three planarians from the stock dish to a petri dish containing about ⅛ inch of pond water. Use a small brush or a tooth pick to make the transfer (Fig. 17.1A).

▶ Examine the animals closely with a dissecting microscope (Fig. 17.1B). **71-A Is the planarian motile? If so, suggest a mechanism for its movement. 71-B Do you think the organism can "see"? Explain. 71-C From what you see does the planarian have a mouth? If not, how do you think this animal obtains its food?** (You may have to examine several planarians before you will be able to answer this question).

NOTE: Wash the razor blade *thoroughly* and dry with a paper towel before using.

▶ Transfer one of the planarians to a clean glass slide (Fig. 17.1C). Wait until the planarian extends itself and then, with a *clean* razor blade, cut off the "head" as shown in Fig. 17.1D. Place the "head" in one of the petri dishes and label it "head piece" along with your name and date.

▶ Cut the remainder of the body into a "middle" and "end" piece and place them into the other dishes (Fig. 17.1E). Label each according to its contents.

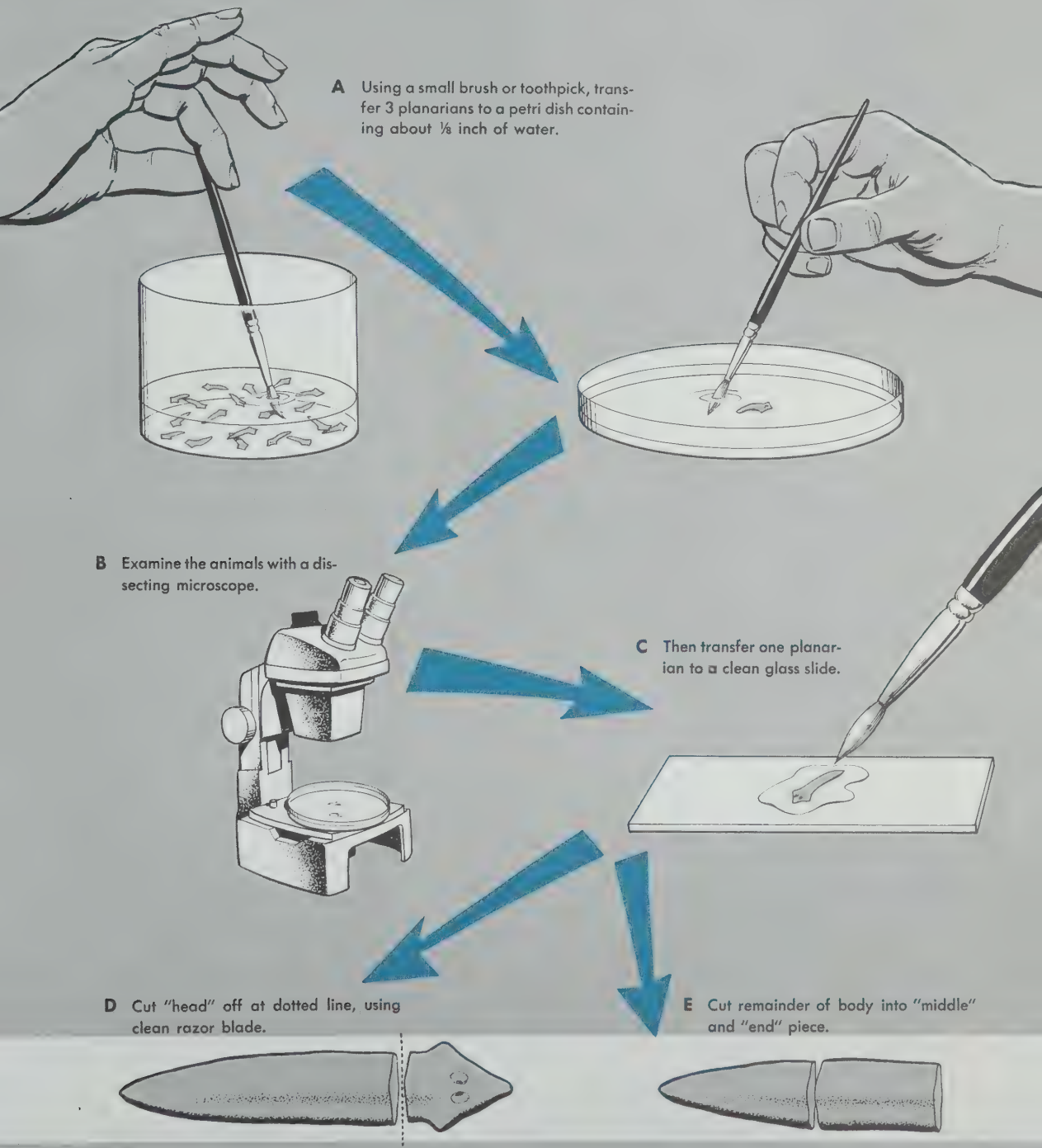
▶ Cut the remaining two planarians in the same way and place the "head," "middle," and "end" pieces into the appropriate dishes. **71-D Why do you think more than one planarian should be cut?** Next, cover the dishes and place them in a drawer, cabinet or other dark place.

▶ Examine the planarians every three to four days for the next three weeks. **71-E At the end of the experiment, make a drawing (on page 389) of what changes have occurred in each of the three cut pieces.**

PROCEDURE—Regeneration in Hydra

▶ Half fill three petri dishes with the special culturing solution provided by your instructor. With a marking pen label

FIG. 17.1 REGENERATION IN PLANARIANS



Place pieces in previously labeled dishes set aside in cool, dark place. Examine every 3 to 4 days for 2 weeks. Then on page 389 draw your results.

one dish "tentacles" and the second dish "body." Add your name and date (Fig. 17.2A).

▶ With an eye dropper, transfer five hydra from the stock container to the third unlabelled petri dish (Figs. 17.2B,C) and examine the hydras with your dissecting microscope.

71-F Why was this organism called "hydra" by the person who discovered it? 71-G Based on your reading, what is the function of the small knob-like projections on the tentacles?

NOTE: The cutting will be easier if a dissecting microscope is used.

▶ Transfer one of the hydras to a *small* drop of water on a clean slide (Fig. 17.2E). When the organism extends itself, cut it in two with a razor, as shown in Fig. 17.2F. Place the "tentacle" piece and the "body" piece into the dishes prepared in the first step (Fig. 17.2G).

▶ Now do the same to the remaining four hydras; cover the dishes, and set them in the place designated by your instructor. Examine the cut hydras daily for the next two weeks.

71-H At the end of this time, make a drawing of the changes that have occurred (page 390).

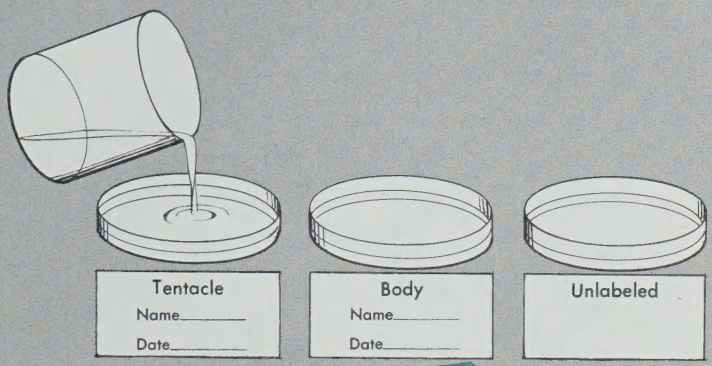
FOR THOUGHT, DISCUSSION, AND FURTHER STUDY

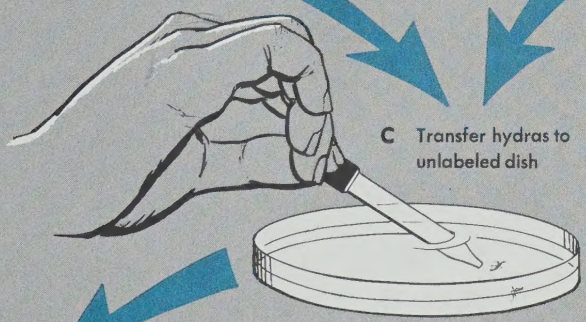
1 Design and carry out an operation that will result in a planarian having a head at both the front end and the rear end.
2 Design and carry out an operation that will produce a planarian having two heads at the anterior end.
3 Design and carry out an operation that will produce two planarians from one.
4 Carry out an operation to determine if one tentacle can give rise to an entire hydra.
5 Design and carry out an operation to determine how *small* a piece of hydra will regenerate an entire hydra.
6 It has been known for many years that if a sponge is cut up into small pieces and then forced through a sieve, the disaggregated sponge tissues will come together and reform the sponge. This phenomenon, called **reconstitution** has also been shown to occur with kidney tissues of chickens. Devise and perform an experiment to see if a disaggregated hydra will be able to reconstitute itself.
7 Obtain a *Coleus* plant from your instructor. Remove the terminal bud. Examine the plant every two to three days for the next two weeks. Does *Coleus* have any powers of regeneration? Explain.
8 List several examples of regeneration exhibited by plants.

FIG. 17. 2 REGENERATION IN HYDRAS

A Half-fill 3 petri dishes with special culture solution. Label as shown.

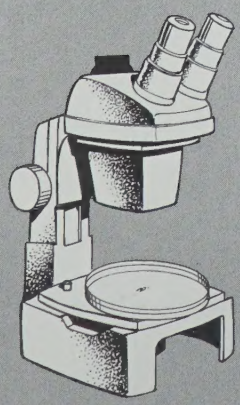
B With an eye dropper remove 5 hydras (one at a time) from stock dish.



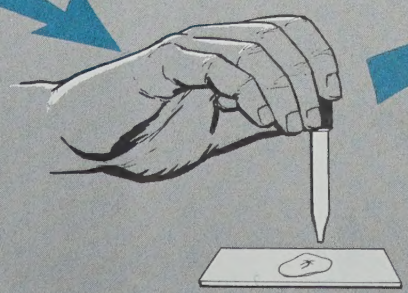


C Transfer hydras to unlabeled dish

D Examine hydras with dissecting microscope.

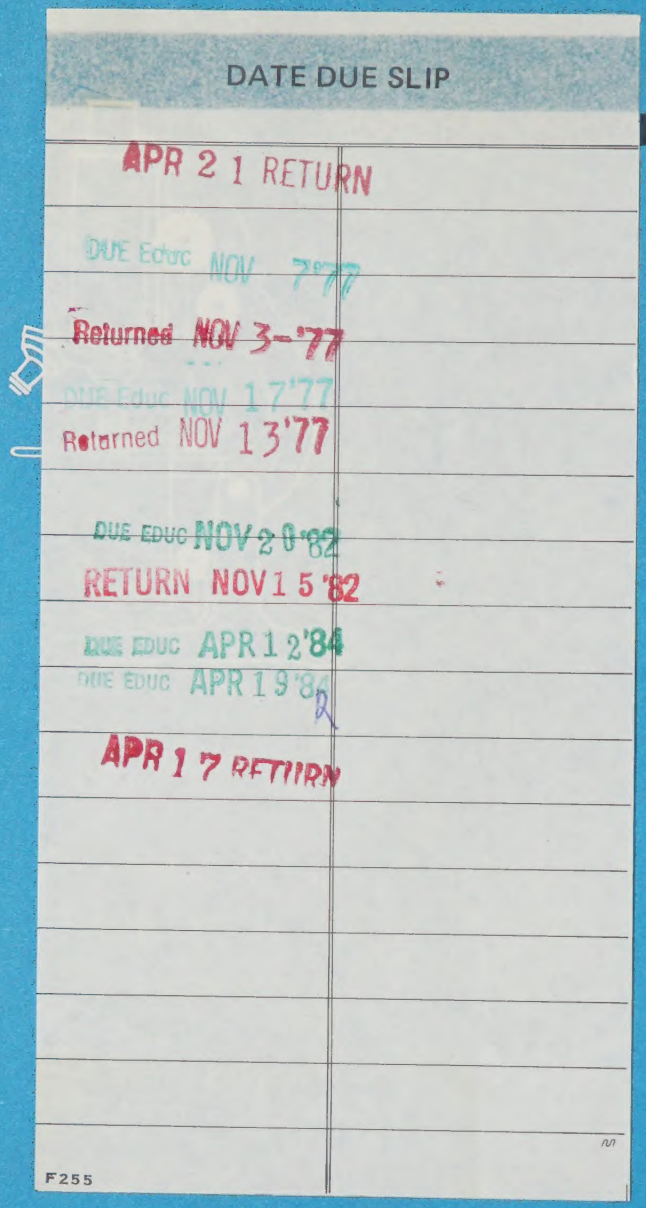


E Place a hydra into small drop of water on slide.



F Cut hydra in "tentacle" and "body" pieces. Repeat this procedure for remaining 4 hydras.

G Place pieces in appropriate culture dishes (Step A) and set aside. Examine daily for next 2 weeks.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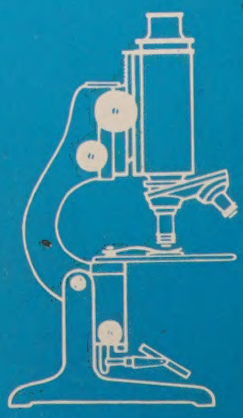
"Think you that a drop of water, which to the vulgar eye is but a drop of water, loses anything in the eye of the physicist who knows that its elements are held together by a force which, if suddenly liberated, would produce a flash of lightning? . . . Think you that the rounded rock marked with parallel scratches calls up as much poetry in an ignorant mind as in the mind of a geologist, who knows that over this rock a glacier slid a million years ago? The truth is, that those who have never entered upon scientific pursuits know not a tithe of the poetry by which they are surrounded."

—HERBERT SPENCER

"From my early youth I have had the strongest desire to understand or explain whatever I observed—that is, to group all facts under some general laws. These causes combined have given me the patience to reflect or ponder for any number of years over any unexplained problem. As far as I can judge, I am not apt to follow blindly the lead of other men. I have steadily endeavoured to keep my mind free so as to give up any hypothesis, however much beloved (and I cannot resist forming one on every subject), as soon as facts are shown to be opposed to it."

—CHARLES DARWIN



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