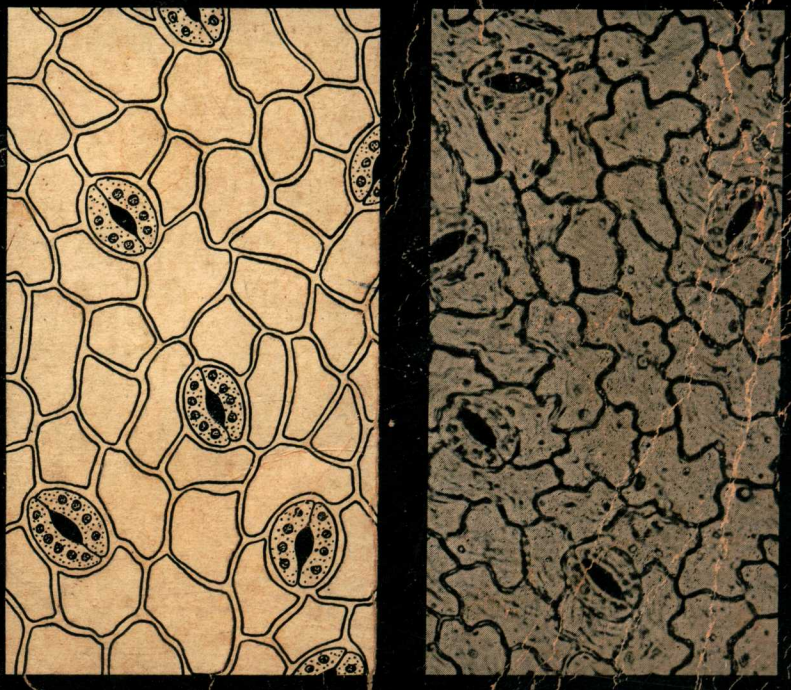
LABORATORY STUDIES *in* BIOLOGY

by ADDISON E. LEE

and OSMOND P. BRELAND



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PREFACE

The following laboratory studies have been designed to fit a weekly two-hour laboratory for a two-semester course in general biology. The coverage of subject matter is by no means comprehensive and many of the studies discussed in lectures have been omitted. The authors have tried to recognize the limitation on the amount of time which the student is able to spend in the laboratory and have carefully selected the examples for study on the basis of this factor as well as what these examples will teach and their practicability for elementary study by large groups. While it is desirable to have the lecture and laboratory studies as parallel as possible, the authors have found that in many instances this is either impracticable or impossible. The student will have to assume the initiative for contributing his share in coordinating the lecture and laboratory subject matter in these instances.

This manual has been written so as to require a minimum of lecturing to the class by the laboratory instructor, thus leaving a maximum of time for him to aid individual students. The organization of subject matter and the procedures suggested for the students have been developed after several years' use of the manual in the beginning biology class at the University of Texas. The authors are therefore greatly indebted to a number of their colleagues, laboratory instructors, and students who have contributed much in the development of this manual. We are particularly indebted to Dr. Charles Heimsch and Dr. John M. Cairns for many valuable suggestions.

ADDISON E. LEE
OSMOND P. BRELAND

December, 1953

TO THE STUDENT

In this laboratory manual the authors have attempted to give sufficient descriptions, explanations, and comments on each of the subjects included so that the instructor can devote a maximum of time to aiding you in your work rather than in lecturing to you. Important terms to be learned by you are in boldface. You are also expected to learn various concepts in the explanation of which many of these terms are used. *For this reason you are strongly advised to study each unit carefully before coming to the laboratory.* The laboratory should involve more than a quick look at a specimen and the labeling of a few drawings to be turned in to the instructor.

You will not be expected to make any detailed drawings, but you will be expected to provide label lines and labels for the diagrams. The labels to be included are listed at the end of a given study. *Make label lines end accurately on the structure being labeled.* Photographs are included in many instances to help bridge what you will see in the microscope on the one hand with the diagrammatic drawings on the other hand. No labeling is required on the photographs. In some instances you will be asked to prepare charts or graphs from various data which will be given. The following rules apply to all the work to be handed in to your instructor.

1. Each page turned in to the instructor should have the following data at the top of each right-hand page: *your name, section, date.*
2. Print all the labels. Use a 3H drawing pencil.
3. All labels should be parallel to the top of the page.
4. All labels on the same side of a drawing should be begun a uniform distance from the edge of the page.
5. Make label lines as nearly parallel to the top of the page as possible. See Fig. 2 as a sample.
6. Do not cross label lines.
7. Erase all sketch lines and unnecessary marks.

CONTENTS

UNIT 1

The Structural Unit of Organisms—Cells

THE MICROSCOPE

The microscope is one of the most expensive and indispensable tools used by biologists. There are several types of microscopes, each of which is designed for a particular purpose. The two types used most commonly by beginning students of biology are the **compound microscope** and the **dissecting microscope** (Figs. 1 and 2). The compound microscope will be used most frequently in this course and you should therefore become familiar with its parts and learn thoroughly the procedure required for its proper manipulation.

Parts of the Compound Microscope. Look at Fig. 2 and the microscope which has been issued to you and find the various parts mentioned in the following description.

A removable tube called the **ocular** or **eyepiece** is located at the top of the microscope. The tubular part of the microscope into which the ocular is inserted is called the **body tube.** A round disk-shaped turntable called the **revolving nosepiece** is located at the lower end of the body tube. Two or more smaller tubes called **objectives** are attached to the revolving nosepiece. The shorter one is the **low-power objective,** and the longer one is the **high-power objective.** Most microscopes have two pairs of adjustment knobs on either side just back of the body tube. The location of these adjustment knobs may be slightly different on different microscopes and some microscopes may have only one knob. When there are two knobs, the larger one is called the **coarse adjustment knob** and the smaller one the **fine adjustment knob.** The curved part of the microscope frame on which these knobs are located is called the **arm.** The platform **stage** of the microscope is attached to the arm and is under the objectives. When an objective is in its proper position under the body tube, it is directly over a hole in the stage in the microscope. A slide with an object to be studied should be placed on the stage so that it is directly over this hole. The slides may be held in place by **stage clips** which are on the top of the stage.

Microscopes differ as to the kind and number of parts there may be under the stage. A common type has a **condenser** below the stage. This is usually a round frame containing several lenses which concentrate light on the object being studied. Beneath the condenser and usually built into the same framework is an **iris diaphragm** that can be opened and closed by means of a small lever below the condenser lenses. In some microscopes the condenser and diaphragm can be adjusted up and down. Other microscopes may not have a condenser; and instead of the iris diaphragm a disk containing holes of different sizes may be attached under the stage. This disk can be turned to different positions. Either the iris diaphragm or the movable disk controls the amount of light that is transmitted through the microscope. The arm of the microscope is attached to the **pillar,** which is a projection of the **base** of the microscope. The **mirror** is under the diaphragm and is attached to the pillar. The mirror

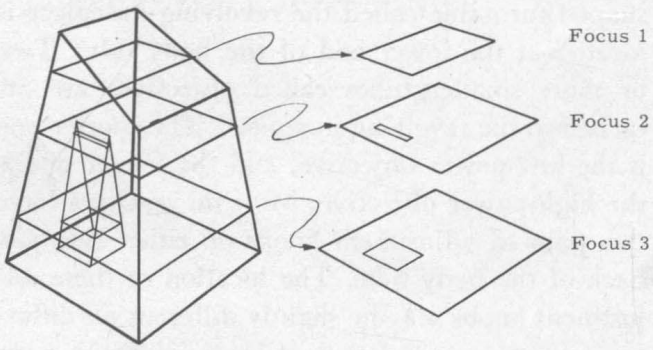
is movable and can be adjusted to reflect light up through the microscope. The concave side of the mirror should be used if the microscope has no condenser. If daylight of the proper quality is not available, artificial light may be used. Direct sunlight should not be used. In some microscopes a light bulb is built into the base, and no special adjustment is necessary to get the proper amount of light. Special lamps have been devised to fit under the stage of the microscope when the mirror has been removed. Artificial light from a desk lamp or ceiling lamp can be used, but in this case the mirror must be adjusted so as to reflect the light properly.

Magnification of objects being studied under the microscope is accomplished by a system of lenses located in the ocular and objective. Note these lenses in Fig. 3. Most of the oculars will be marked "10x," which means that they have a magnifying power of ten times—usually spoken of as 10 diameters. The low-power objective may also be marked "10x," which means that it also magnifies 10 diameters. The high-power objective may be marked "43"—it will magnify 43 diameters. Low-power objectives and high-power objectives may also be marked "16 mm." and "4 mm." respectively. These designations refer to the equivalent focus of the lens system. The **equivalent focus** of a lens system is the same as the focal length of a comparable single lens. The **focal length** of a single lens is the distance from it at which parallel light rays passing through it are converged.

The size and position of the object under the compound microscope apparent to the observer constitute the **virtual image.** The object seen with the microscope will appear to be about ten inches away from the eye of the person viewing it. The magnification is the ratio of the virtual image to the actual size of the object. This is usually given in linear dimensions and is called the **linear magnification.** It is the product of the magnification of the objective and that of the ocular. For example, if the microscope you are using has a 10x ocular and a 10x or 16 mm. objective, the linear magnification of an object being viewed with this ocular and objective will be 100x. Compute the linear magnification for the microscope issued to you for both high- and low-power objectives. Follow the path of light rays through the various lenses and through the object being studied in Fig. 2. Note that the image is inverted as it reaches the eye. This is due to the way the lenses transmit the light rays through the microscope and to the eye. This is why a slide being pushed to the right appears to be moving to the left when it is viewed through a compound microscope.

The **depth of focus** is the thickness of the specimen that can be seen in focus at one time. If relatively thick objects are being studied, it is necessary to change the focus from time to time in order to study the entire thickness of the object. The depth of focus is much greater for low power than for high power; hence it is usually necessary to adjust the focus much more frequently for high power than for lower power.

In order to understand what you are studying under the microscope, it is necessary that you learn the parts of the microscope, their functions, and the working principle of the instrument. It is also necessary that you be able to build up in your mind the three-dimensional aspect of the object being studied. It is often the case that the complete object or structure can be reconstructed in the mind only after studying what is in focus at several depths. The accompanying diagram illustrates this problem. It shows an irregular-sided object within which another object is enclosed. The mechanics involved here are frequently found in biological material. If the overall dimensions of Object A were greater than you could see within the depth of focus at a given magnification, you could ob-



Object A

serve only a series of views including those shown as Focus 1, 2, and 3. From these you would have to build up the picture of this object in your mind. Cover Object A with your hand or a sheet of paper and see if you can visualize it from the series of views on the right. Of course, with the microscope you can get many more sections in focus than are shown here.

Drawing Instructions

Fig. 2, label: ocular, body tube, coarse adjustment knob, fine adjustment knob, pillar, arm, base, nosepiece, objectives, condenser, iris diaphragm, mirror.

Fig. 3, label: high-power objective, low-power objective.

The Dissecting Microscope. The dissecting microscope is used by beginning students to a lesser degree than the compound microscope. Fig. 1 illustrates a common type of dissecting microscope. It differs from the compound microscope in that its magnification is usually smaller than that of the compound microscope. Lower magnification results in a greater depth of focus and thus permits the study of larger and thicker objects and also increases the area that can be studied at one time. The compound microscope requires light which must be transmitted through the material being studied, and as a result its use is limited to the study of preparations that are thin enough to permit the light to pass through them. The dissecting microscope uses light directed to the object from the sides and above the object. This microscope also differs from the compound microscope in that the image is not inverted when seen through the instrument. All these features make the dissecting microscope a useful tool for the biologist because it allows him to make studies of entire small organisms or parts of larger organisms, to dissect such material, and to do other similar experimental work.

Operation of the Compound Microscope. In using the microscope there are certain steps that should always be followed rigidly in order to make a complete study and to prevent accidents to the lens system and working parts. It is important to carry out these steps in the order in which they are given below, even after you are experienced in using the instrument.

1. Before using the microscope, clean the ocular and the objective with lens paper. *Never use anything but lens paper.*
2. Turn the revolving nosepiece until the low-power objective is in place.
3. Adjust the mirror or lamp to get the maximum amount of light. The circular area you can see is called the **field**.
4. Open the iris diaphragm to capacity.
5. While looking *at* the microscope, *not through* it, turn the coarse adjustment until the low-power objective is about ⅛ inch from the slide.
6. Now while looking through the ocular move the body tube *upward* with the coarse adjustment until the image can be seen. Complete the focusing with the fine adjustment and readjust the iris diaphragm if necessary. It should be emphasized at this point that even though you can see the object, it is important to open and close the diaphragm while looking through the microscope to determine whether you can see the object better or more clearly with a different adjustment of the diaphragm.
7. If the high-power objective is needed, move the slide until the portion of the object to be studied is in the center of the low-power objective field. Have your eyes near the level of the stage so that you can watch the objective when the revolving nosepiece is turned. Then turn the nosepiece until the high-power objective is in place, being sure not to crush the slide. If the high-power objective even touches the slide, turn the coarse adjustment back until the objective clears the slide. Focus again with the fine adjustment and readjust the diaphragm. Do not use the coarse adjustment in the final focusing with the high-power objective.
8. After using the microscope, remove the slide from the stage, turn the low-power objective into position, clean the stage, and return the microscope to the case.

9. Be careful in handling the microscope. Never turn it upside down or swing it, because the ocular will fall out.

After you have learned the parts of the microscope and their functions, and the procedure to be followed in using the instrument, you should get some practice in operating it. Tear off a piece of lens paper about ¼ inch square and put it in a tiny drop of water on a clean slide. Place a cover glass (or cover slip) over the lens paper and do each of the steps listed above, one by one, until you have the lens paper in focus under the low-power objective. Move the diaphragm through its complete range of adjustments and note the difference in appearance at its various openings. Next, change to high-power magnification and repeat the diaphragm adjustments, again noting the changes in appearance.

Remove the slide and cover glass and lay another small piece of lens paper on the one already on the slide. Replace the cover glass and turn the revolving nosepiece so that the low-power objective is in place and repeat the procedure described above. This time, in addition to noting the differences caused by changes in the diaphragm opening, observe the depth of focus obtained with both the low-power and the high-power objective. Can you identify which layer of the lens paper you have in focus at a given time? Compute the linear magnification of the fibers which make up the lens paper under both high- and low-power magnification. While looking at these fibers try to imagine what their real diameter is. To help you understand the actual sizes involved, note that the diameter of the entire low-power field is about 1.5 mm. and that of the high-power field is about 0.35 mm.

When you have mastered the parts, the working principles, and the methods of using the microscope, you will be ready to use this instrument in your study of biological materials.

PLANT AND ANIMAL CELLS

All living things are made up of units called **cells.** The living substance of the cell is called **protoplasm.** The outer surface of the protoplasm is the **plasma membrane.** Outside the plasma membrane most plant cells have a **cell wall** composed chiefly of cellulose. The cell wall is a relatively rigid structure that is responsible for the more or less fixed shape of plants. Animal cells have a plasma or cell membrane but do not have definite cell walls. The protoplasm is made up of a **nucleus** and **cytoplasm.** The nucleus in living cells frequently has a similar refractive index to that of the cytoplasm and cannot be easily distinguished from it. It is easily identified, however, in stained preparations and is seen as a dense body surrounded by cytoplasm. The nucleus is surrounded by a **nuclear membrance.** In stained preparations the nucleus may contain one or more darker-stained bodies, each of which is called a **nucleolus.** The nucleus also has a number of bodies called **chromosomes** which control the inheritance pattern of the organism; the number is definite for each species. However, the chromosomes can be seen only after special preparation of cells that are in the process of division. You will not be able to see them in the cells selected for study in this unit, but you will be able to study them in a later unit. The cytoplasm varies in density but is generally more fluid than the nucleus. Certain materials and bodies (inclusions), both living and nonliving, may be present in the cytoplasm. Nonliving inclusions, such as fat droplets, are usually products of the physiological activity of the cell and may be localized rather definitely in **vacuoles.** A vacuole may be considered to be a fluid-filled sac within the cytoplasm; it is surrounded by a protoplasmic **vacuolar membrane.** Vacuoles vary greatly in number and size in different cells and usually appear as clear areas in the cytoplasm. As a rule, vacuoles are large and noticeable in plant cells but quite small or completely absent in animal cells. In green plant cells, green bodies of various shapes may be found within the cytoplasm of the cell. These bodies which are called **chloroplasts,** are of great importance in the food-making processes of the plant. The shape of the chloroplast is the same in a given species but may vary greatly from one species to another.

In order that you may observe these parts, study carefully the following types of cells:

Onion Cells. Strip off a piece of the epidermis or outer layer of tissue from the inner side of the fleshy leaf of an onion bulb. This is best done by taking a small section of the leaf, cutting it *to* the inner layer with a scalpel, and carefully peeling the epidermis from the rest of the leaf. Be sure not to include the underlying cells. Without allowing it to dry, spread this strip of epidermis on a glass slide in a drop of water and stain the cells by adding a drop of iodine solution. Carefully place a cover slip over the epidermis so that the tissue is held securely between the glass slide and the cover slip. Blot any excess fluid from the edge of the cover slip with lens paper. Place the slide on the stage of the microscope and focus first with the low-power, then with the high-power objective. Study the cells under high power. Using as a basis the diameter of the field under low power (1.5 mm.) and under high power (0.35 mm.), estimate the actual size of the cells.

Drawing Instructions

Fig. 4, label: cell wall, nucleus, nuclear membrane, cytoplasm, protoplasm, plasma membrane, nucleolus.

Living Green Plant Cells. The leaves of *Elodea,* a plant commonly found in small pools and ponds and frequently used to decorate fish bowls, are particularly suitable for the study of living plant cells. This is true because the leaves of this plant are made up of only two layers of cells and are therefore thin enough to transmit the light without any special preparation. Secure a young leaf from the tip of *Elodea,* mount it in a drop of water, and cover with a cover glass. Focus with low power, then with high power. Cut down the excess light with the iris diaphragm. Focus up and down with the fine adjustment and notice the two layers of cells arranged like blocks on top of each other.

The central portion of this plant cell is occupied by a large **vacuole**; the **cytoplasm** is restricted to a thin layer between the **cell wall** and the vacuole. It will be difficult to see a distinct boundary (**plasma membrane**) around the cytoplasm. If the **nucleus** is located on the side of the cell, the cytoplasm will appear to bulge into the vacuole at that point, but it is not likely that you will be able to see the nucleus in every cell. The small green bodies in the cytoplasm are **chloroplasts.** Determine the shape of the chloroplasts. Their color is due to a substance called **chlorophyll.** Move the slide around and find a cell in which the chloroplasts are moving. This movement is called **cyclosis,** or **protoplasmic streaming.**

The chloroplasts and cell wall in each of these plant cells are quite conspicuous; but in order to see the nucleus and to distinguish between the cytoplasm and the vacuole, you must follow carefully the instructions on using the iris diaphragm and keep in mind the depth of focus provided by the particular magnification you are using. After you have located the parts, estimate the actual size of the cells.

Drawing Instructions

Fig. 5, label: cell wall, chloroplasts, cytoplasm, nucleus, nuclear membrane, plasma membrane, vacuole.

Animal Epithelial Cells. Any cells that cover a surface, whether outer or inner, are called **epithelial cells.** To obtain these cells, wash your index finger and gently scrape the inner lining of your cheek with your fingernail. Spread the substance on a slide and set it aside for a few minutes to dry. Add a drop of methylene blue to stain the cells and allow it to set for one minute. Wash off any excess stain. Do not cover with a cover slip. Study under the high power and compare the shape and size of these cells with the plant cells you have already studied. Identify the parts of these cells. Note that they have no cell wall, chloroplasts, or large vacuoles. While it is important to remember that all cells are three-dimensional, note that these particular animal cells are much flatter or thinner than the plant cells studied above. However, the flatness or thinness of these cells is not characteristic of all animal cells. All cells, both plant and animal, exhibit a wide range in size and shape and all are three-dimensional.

The principal differences between plant and

animal cells, as studied here, are as follows: (1) Plant cells have a cell wall, whereas animal cells do not. (2) Many green plant cells have chloroplasts which are not present in animal cells. (3) Mature plant cells have large vacuoles, whereas animal cells have very small vacuoles or none. There are other differences, but they are related to the detailed structure of certain inclusions in the respective cells and to their physiological activities.

Drawing Instructions

Fig. 6, label: plasma membrane, cytoplasm, nucleus, nuclear membrane, protoplasm.

TISSUES

As stated earlier, all living things are made up of cells. The preceding examples are but a very few of the many types of cells that make up the bodies of various organisms. The bodies of some organisms consist of only a single cell, whereas others contain many millions of cells. The most complex plants and animals (the so-called higher plants and animals) have cells that are highly specialized for the various functions they perform. Groups of cells which are specialized for a particular function make up what is called a **tissue.** Several tissues may work together in order to perform certain functions. Such a group of tissues is called an **organ.** In animals, which in general are more complex than plants, several organs may operate together in carrying out certain activities, thus forming an **organ system.** The coordinated activities of all the cells which make up the body of an organism, whether at the level of the single cell, tissue, organ, or organ system, constitute the life of the organism.

It will be the purpose of the next eleven units to study structures and functions in higher plants and animals. Plants will be considered from the standpoint of their principal organs, and organ systems will be the basis of study in animals.

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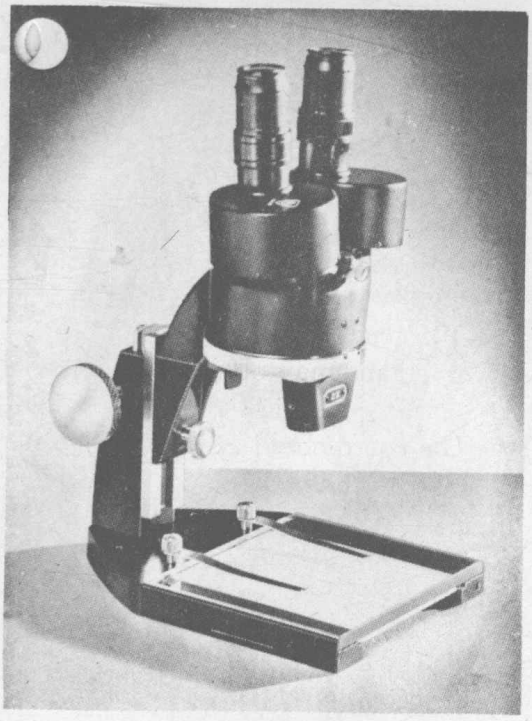
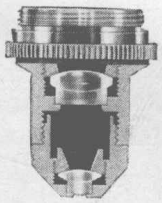


Fig. 1. Dissecting Microscope



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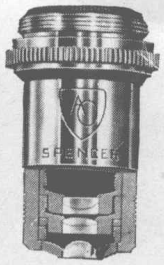

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Fig. 3.

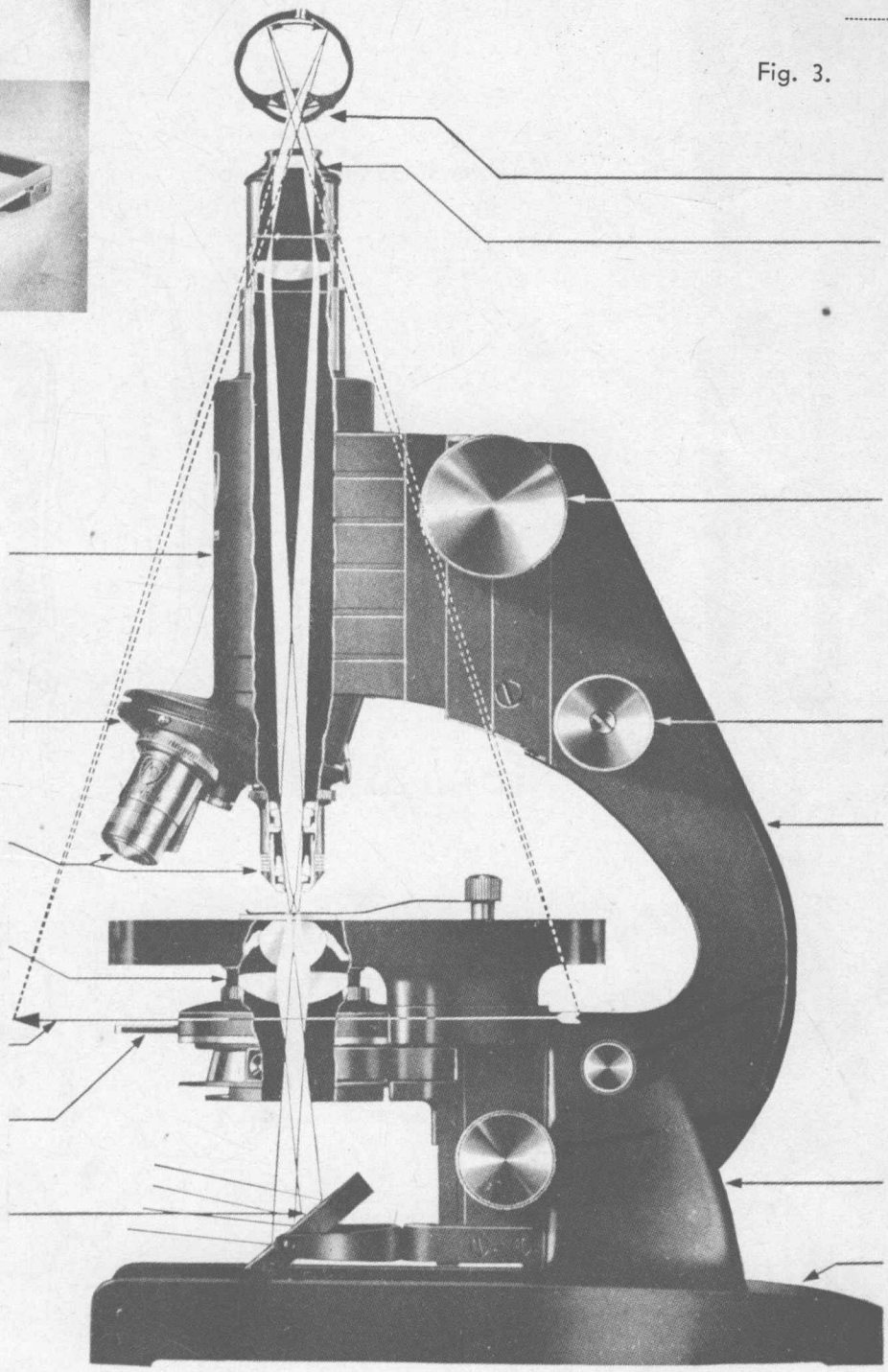
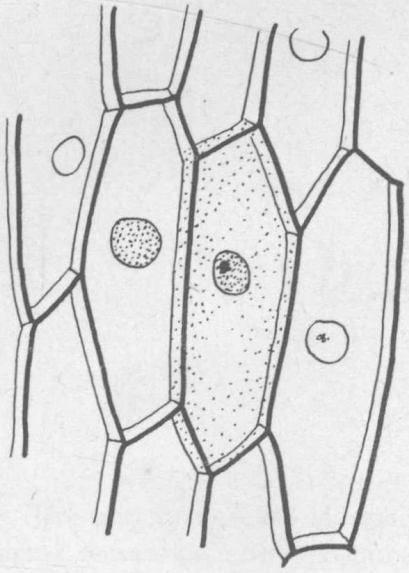


Fig. 2. Compound Microscope

Figs. 1, 2, and 3 are reproduced here by courtesy of the American Optical Company, Instrument Division, Buffalo, New York.

Name..... Instructor..... Date.....



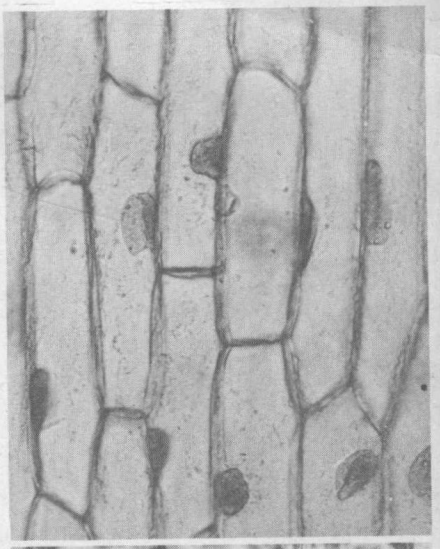
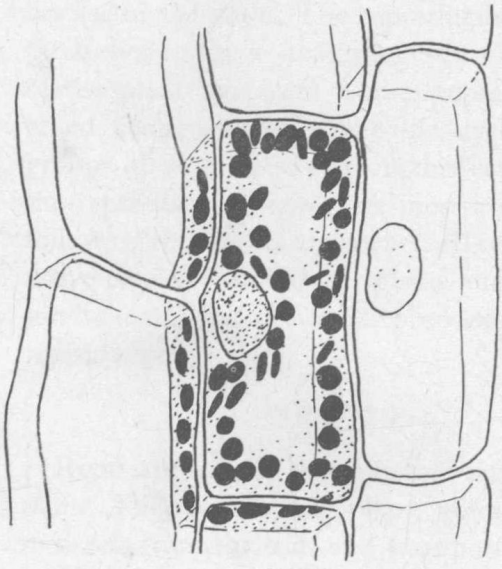


Fig. 4. Onion, Epidermis Cells



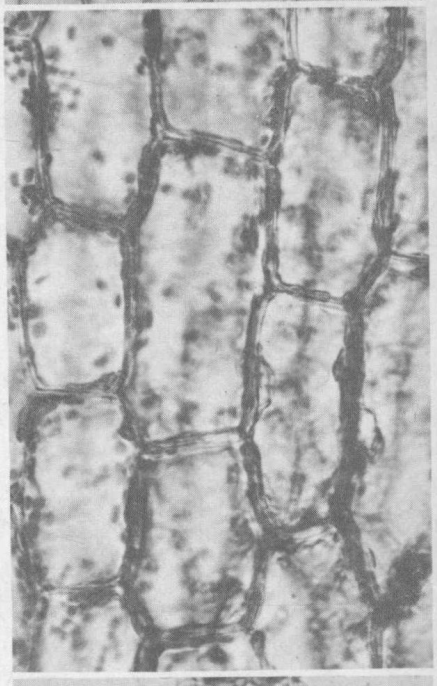
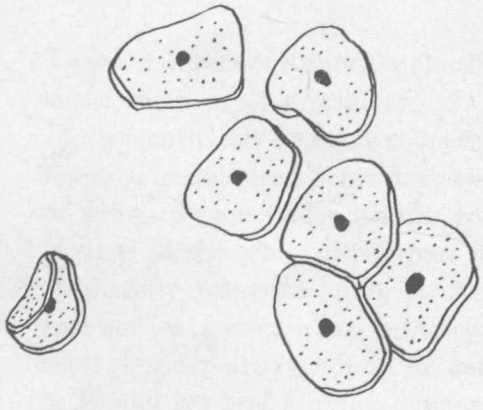


Fig. 5. Elodea, Leaf Cells



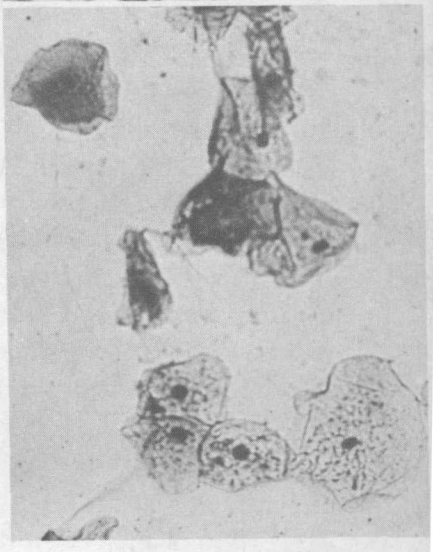


Fig. 6. Animal, Epithelial Cells