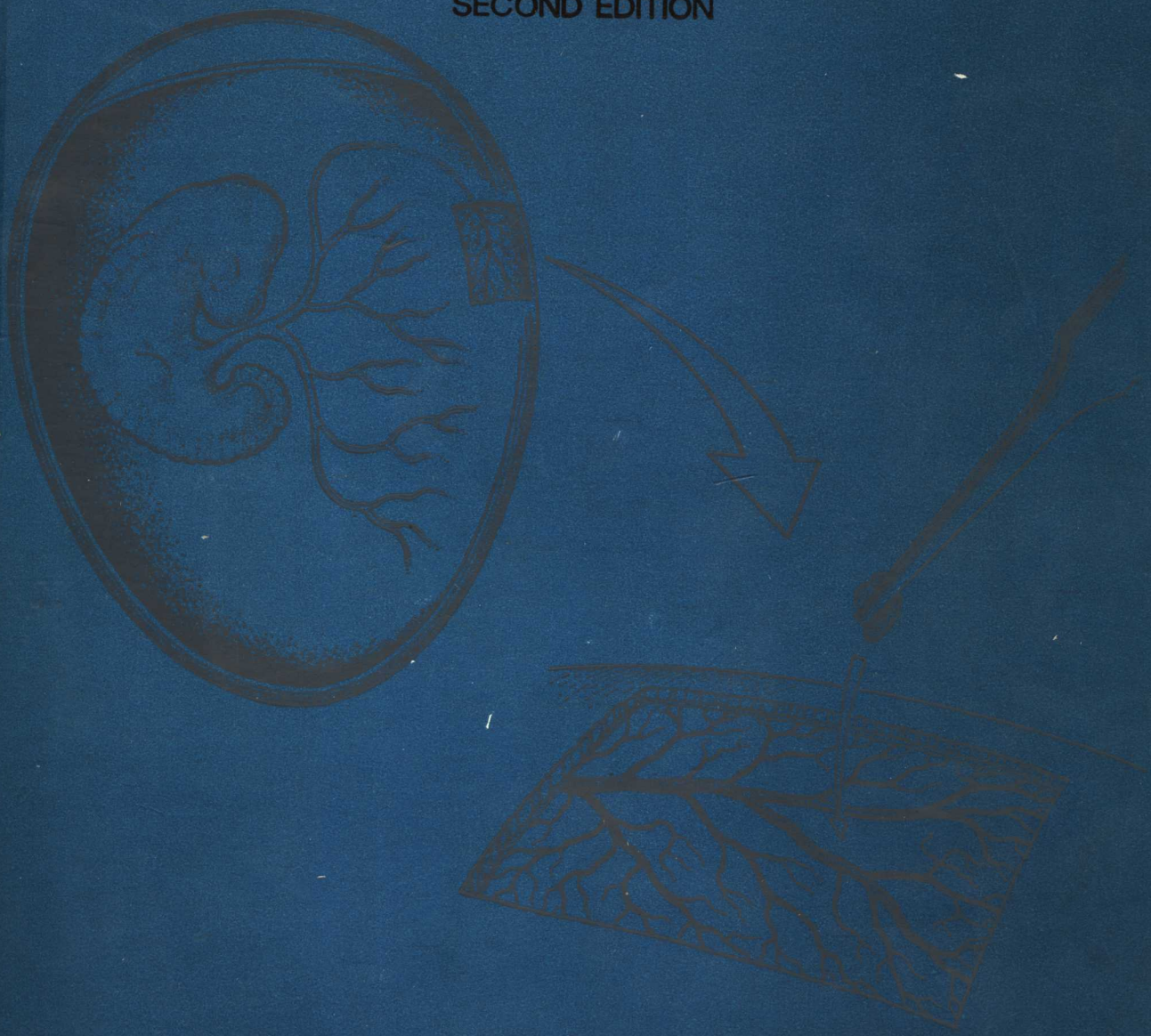
An Experimental Approach to

BIOLOGY

PETER ABRAMOFF/ROBERT G. THOMSON

SECOND EDITION



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PETER ABRAMOFF/ROBERT G. THOMSON
MARQUETTE UNIVERSITY

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Preface

The laboratory experience is an integral part of a biology program and should be complementary—not subservient—to the lectures. Furthermore, the opportunity for the student to practice scientific inquiry should be a significant part of any laboratory program. Therefore, it should be an objective of laboratory work to lead the student to a deep and intelligent understanding of the basic principles and concepts of modern biology through the interaction of imagination, accurate and logical reasoning, and precise direct observation. Thus these laboratory studies have been designed to acquaint the student with the fundamental principles and concepts of living systems through an experimental analysis of these systems. It is also their purpose to introduce the student to the more important techniques and procedures of modern biological research.

The second edition of *An Experimental Approach to Biology* is designed for those introductory biology laboratory courses that stress an investigative approach to biological principles. The exercises include very little descriptive material, concentrating instead on the molecular, cellular, and physiological aspects of biology. We assume that the student will have taken a course in chemistry, or will be taking one concurrently, and that the school will have available certain laboratory equipment, including colorimeters and centrifuges. Many of these exercises are well suited for undergraduate courses in cell biology, cell physiology, physiology, developmental biology, and molecular biology. All exercises are available as Laboratory Separates.

One-third of the exercises are new to this edition, and many of the others have been rewritten to reflect users' comments. Among the new material are experimental studies on chromosome morphology; sex chromatin; protein, nucleic acid, and enzyme extraction; bacterial and *Drosophila* genetics; bioluminescence; muscle physiology; sea urchin, frog, and chick development; plant-growth regulation; and bacterial and algal pollution of surface waters. The completely revised instructor's handbook includes detailed lists of the materials needed for performing the experiments, instructions

for preparing reagents and solutions, suggestions for handling live materials, and sources of helpful visual aids for the laboratory.

More exercises are included in this manual than are generally used in a one- or two-semester course in biology. Furthermore, individual exercises are not designed to meet the requirements of a specified number of laboratory hours. Most exercises are sufficiently comprehensive to allow subdivision and adaptation to any laboratory sequence, depending on the requirements of the course.

June 1975

Peter Abramoff
Robert G. Thomson

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EXERCISE 1

Cell Structure

Just as there is diversity of form in life, so there is in the form and function of the cells that make up living organisms. Single cells, such as *Amoeba, Euglena,* or *Paramecium,* can be free-living organisms capable of carrying on an independent existence. 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, depending on the closely integrated activities of other cells for their existence.

Cells vary in size. For example, the length of many bacteria is roughly 1 micrometer (μm), which is equal to 10^{-6} meter. On the other hand, the yolk of an ostrich egg, also a single cell, is the size of a small orange. Some cells, such as red blood cells, transport oxygen and carbon dioxide. Other cells have different specialities. Whatever its form or function, the cell is now recognized as the basic unit of living matter, containing all those properties and processes that are collectively called life.

A. MICROSCOPIC STRUCTURE OF PLANT CELLS

1. Onion Cells

Although cells have diverse forms and functions, all cells are constructed according to a fundamental design and share certain common features. A basic knowledge of cell structure is indispensable to understanding the cell as an independent unit and its role in the life processes of higher plants and animals.

Following the procedure outlined in Figure 1-1, prepare a wet mount of onion epidermal tissue. Examine this wet mount with the low-power objective (10×). (See Appendix A on the Use and Care of the Microscope.) 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

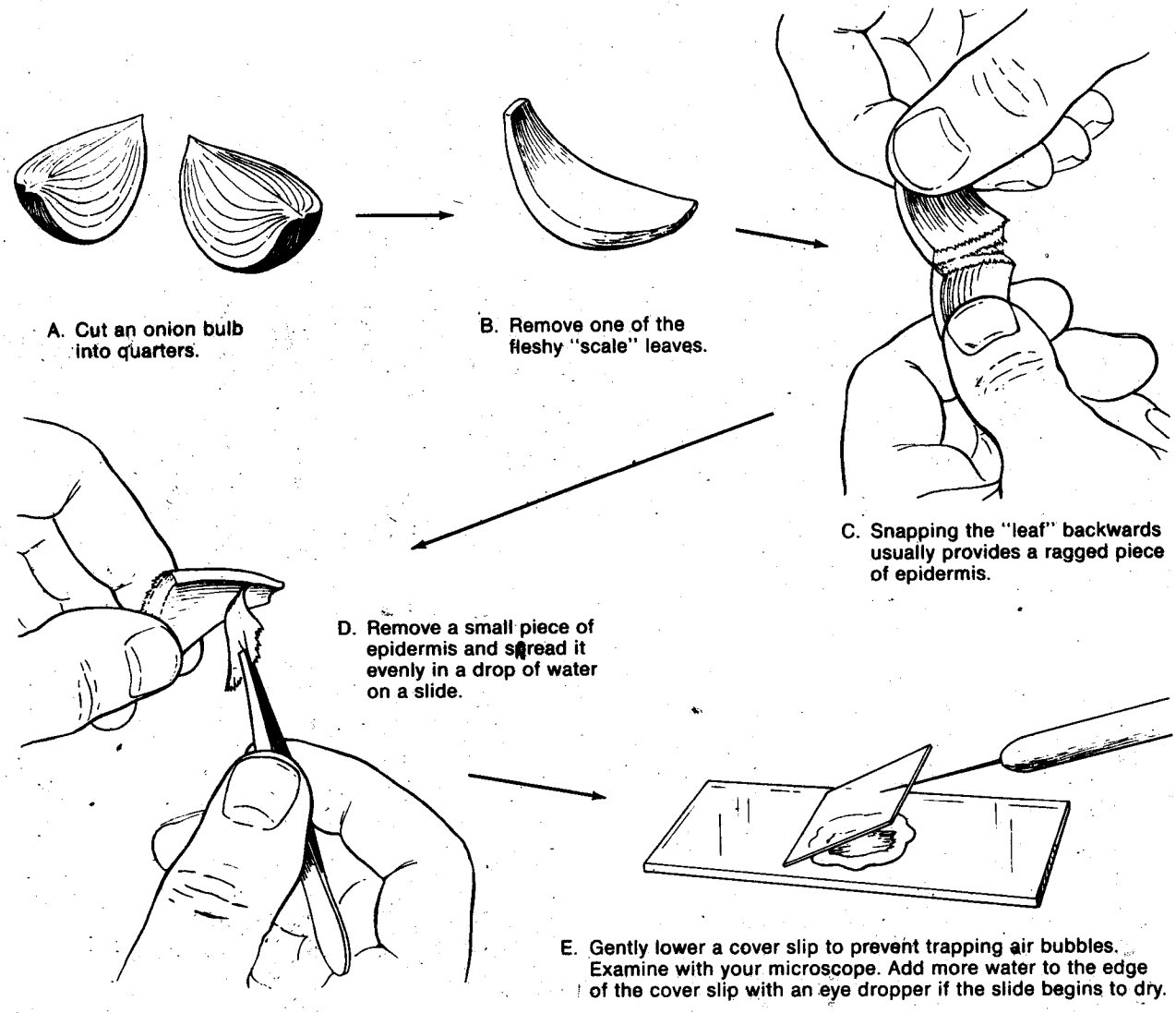

FIGURE 1-1
Procedure for studying living onion cells.

the **plasma membrane**), which encloses the **cytoplasm.** The central part of many plant cells (which is difficult to observe in living cells) is taken up by a fluid-filled **vacuole** containing mostly water and salts.

Locate the **nucleus,** which appears as a dense body in the translucent cytoplasm. In some cells the nucleus seems to be lying in the central part of the cell and looks circular. In other cells, it seems to be compressed and pushed against the cell wall. 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.

2. Elodea Cells

In this study you will examine cells from the leaf of a water plant commonly called Elodea. These cells are green because they contain a pigment called **chlorophyll.** In **photosynthesis** this pigment absorbs light energy and converts it into chemical energy.

Remove a young leaf from the tip of the plant (Fig. 1-2). Place the leaf in a drop of water on a

slide and add a cover slip. Examine the preparation with the low-power objective (10×) in position. Locate the nucleus, cytoplasm, and cell wall.

Examine a group of cells near the center of the leaf. Carefully switch to high power. By focusing up and down try to determine the number of cell layers.

_____

Are the cells in each layer the same size? _____ the same shape? _____ 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.

The plant cell is enclosed by a nonliving cell wall and a cell membrane that is difficult to observe because it is pushed tightly against the inside of the cell wall. You can make this membrane easier to see, however, by placing the cell in a saline (salt) solution that is more concentrated than the cytoplasm (hypertonic). The saline, being hypertonic to the cytoplasm, causes water to move out of the cell and results in the cell shrinking away from the wall, exposing the membrane.

Select another young Elodea leaf, mount it in a drop of water, and add a cover slip (Fig. 1-2C). Examine the preparation with the low-power objective. Along the edges of the leaf locate "spine" cells (Fig. 1-2D). Switch to high power and study the cell. Note that the cell membrane cannot be distinguished from the cell wall.

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 saline to be drawn under the cover glass, replacing the liquid withdrawn by the paper (Fig. 1-2E and F). Repeat the above step two more times to be sure that the original water has been replaced by the saline. Examine the "spine" cell closely. Describe your observations and account for the results you observed.

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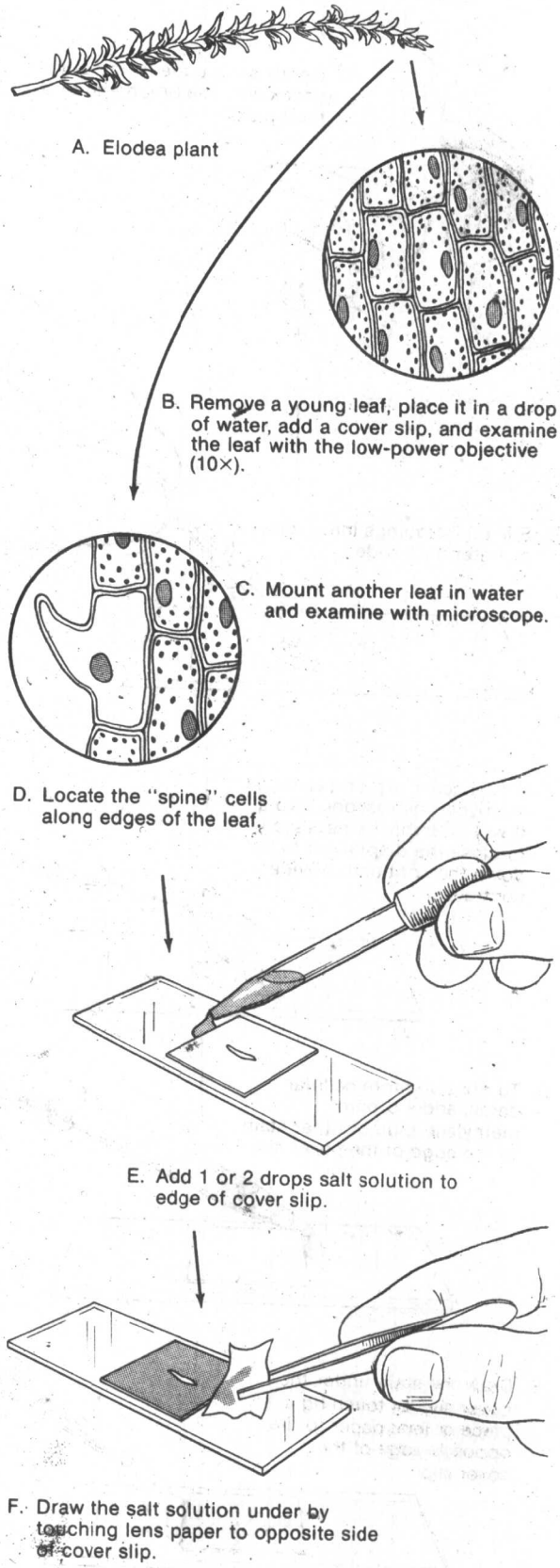

FIGURE 1-2
Preparation of Elodea cells for microscopic examination.

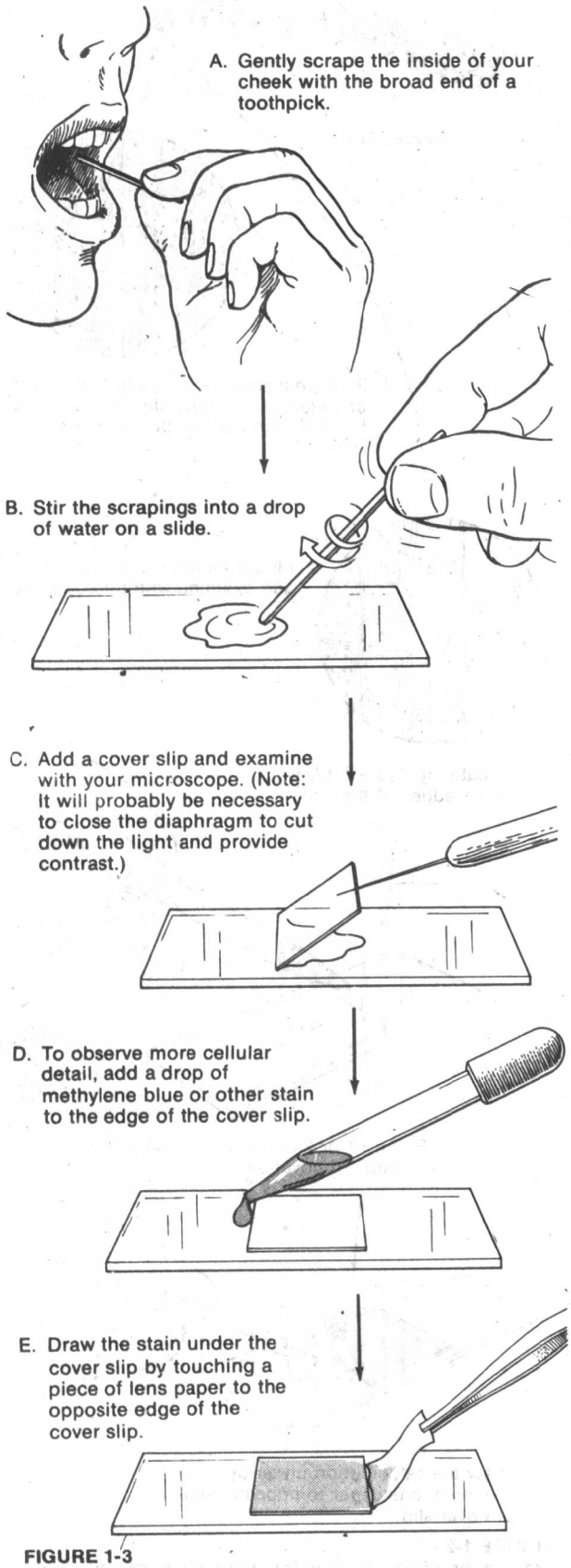

FIGURE 1-3
Procedure for studying human epidermal cells.

B. MICROSCOPIC STRUCTURE OF ANIMAL CELLS

The typical plant cell is characterized by having a nonliving cell wall and may contain chloroplasts. In this study you will examine human epidermal cells, which look different from plant cells, but the two kinds of cells have many features in common.

Following the directions outlined in Figure 1-3, examine cells obtained from the inner epidermal lining of your cheek. Locate the cells under high power and examine them carefully. What do these epidermal cells have in common with plant cells?

How are they different?

Some of the epidermal cells may have had their edges folded over. What does this indicate about the thickness of the cells?

Because it is difficult to observe structure in 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 Figure 1-3D and E. What structure in the cell has been stained by this dye?

C. CYTOPLASMIC STREAMING

In many cells the cytoplasm can be seen flowing throughout the cell. This phenomenon is called **cytoplasmic streaming** or **cyclosis.**

1. Streaming in Higher Plant Cells

Using a forceps, remove a stamen from the flower of *Tradescantia* (Fig. 1-4) and quickly place it into

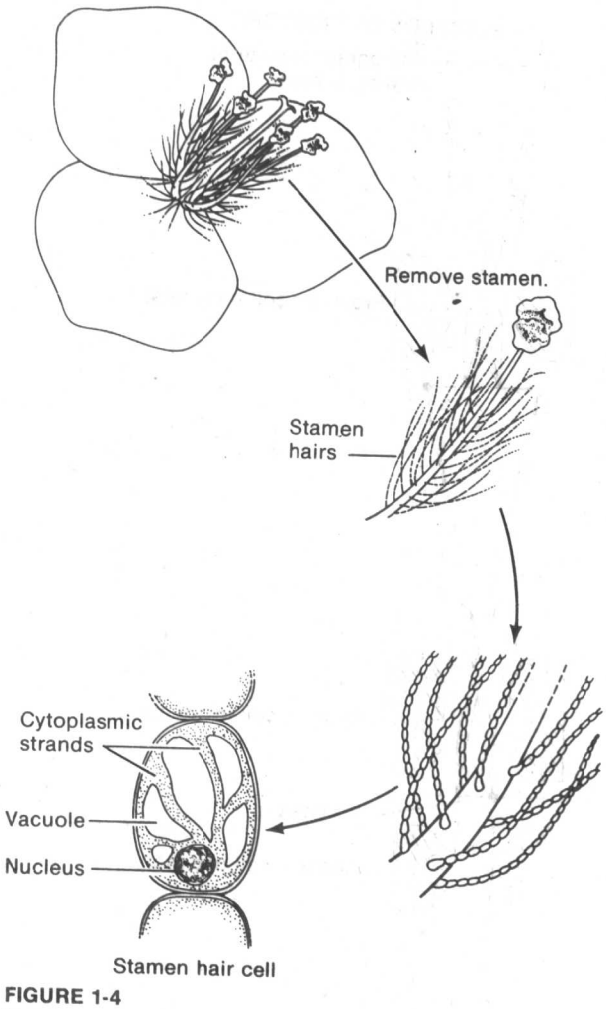

FIGURE 1-4
Procedure for studying cytoplasmic streaming in hair cells of *Tradescantia*.

a drop of water on a slide. Cut off the anther, add a cover slip, and examine under low power. Hairs are attached to the stamen and will appear as chains of "beads." Each "bead" is a single hair cell.

Select an undamaged cell and examine with high power. Oil droplets and other cellular inclusions make cytoplasmic streaming fairly easy to observe. Note the granularity of the cytoplasm and the prominent cytoplasmic strands. Try to distinguish between the vacuole, which contains a purple pigment called **anthocyanin,** and the cytoplasm. Unlike most plant pigments anthocyanins are water soluble and not localized in plastids. Where is the nucleus located in this cell?

_____

Where does cytoplasmic streaming occur in the stamen hair cell?

_____

Of what importance is cytoplasmic streaming to a cell?

_____

_____

_____

2. Streaming in a Slime Mold

The phenomenon of cyclosis is perhaps best observed in the plasmodium of the slime mold *Physarum polycephalum*. The plasmodium, or plant body, is a multinucleate mass of protoplasm that lacks a cell wall. This organism is easily propagated during its dormant stage when it becomes a hard, crusty structure, called a sclerotium. To do this, pieces of sclerotia are placed on a moist substrate (e.g., agar) containing nutrients. In a short time the organism begins to grow out over the surface. Channels of streaming cytoplasm become visible, usually after 72 hours of growth.

Examine a plasmodium of *Physarum* growing in a petri dish. Leaving the cover on, examine the organism carefully using a dissecting microscope. What appears to be unusual about cytoplasmic streaming in *Physarum?*

_____

_____

_____

_____

D. CELL ULTRASTRUCTURE

Progress in the life sciences has been dependent on man's ability to develop ever more refined tools and techniques. This has been especially true for cytology—the study of cells—in which adequate magnification has been a major problem to overcome. The problem of magnification is really one of increasing resolving power, which may be

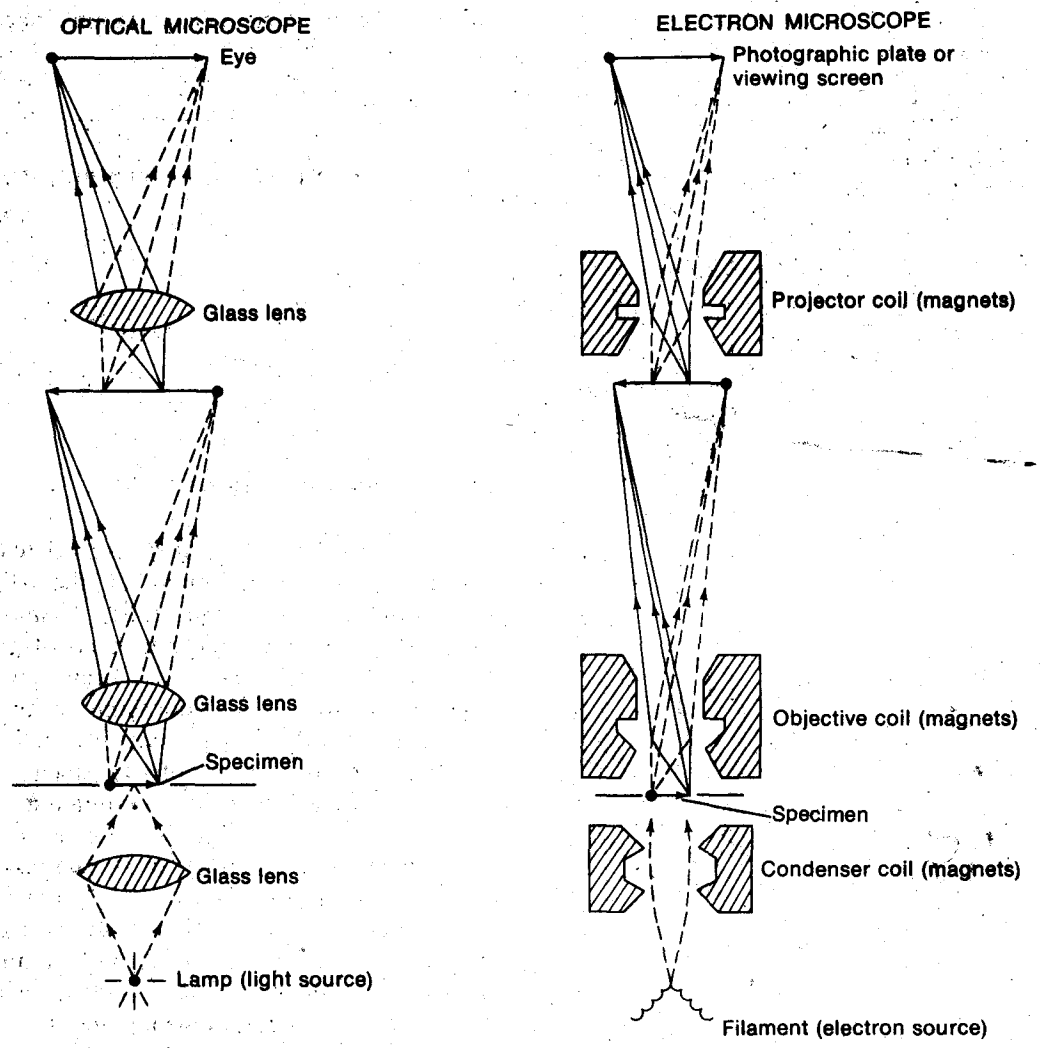

FIGURE 1-5
Comparison between optical (light) microscope and electron microscope.

defined as the ability of an optical system (the microscope in this case) to reveal details of structure.

The human eye has a resolving power of about 0.1 millimeter. Lines or objects that are closer together than this are seen as a single line. The resolving power of a microscope is limited by the kind of illumination used. Objects that are closer together than one-half of the wavelength of the light source will not be clearly distinguished in a light microscope. Therefore, if white light is used (average wavelength is 0.55 μm or 550 nanometers or 5500 angstroms), a microscope using 97× oil-immersion objective cannot resolve two discrete points less than 0.27 μm apart. Because many structures in the cell have dimensions smaller than this, their presence had gone undetected until a means was found for increasing the resolving power of microscopes.

The electron microscope provides increased resolving power by using a different type of illumination. High-speed electrons, the negatively charged particles of an atom, are used instead of light. As the electrons pass through the specimen being viewed, parts of the cell differentially absorb or scatter the electrons, thus forming, by means of lenses, an image of the specimen on an electron-sensitive fluorescent screen or photographic plate. The optical system is similar to that of a light microscope except that the "illumination" is focused by means of magnetic lenses instead of glass lenses (Fig. 1-5).

Under the high voltages used in an electron microscope (1,000,000 volts in the latest instruments), the electrons have a wavelength of about 0.05 Å. Thus an electron microscope could theoretically resolve objects of 0.025 Å. However, owing to the limitations imposed by the design of the instrument, the actual resolving power is closer to 8–10 Å. In approximate figures, then, the human eye can resolve down to 100 μm, the light microscope to 0.2 μm and the electron microscope to 0.0003 μm. The electron microscope, therefore, has enhanced our understanding of the structure and function of parts of the cell that were not previously visible to the cytologist. In the following study, you will examine electron micrographs of cells and become familiar with the structure and function of several structures that play a vital role in the life of the cell.

Figures 1-7 and 1-8 (on the next two pages) illustrate the basic ultrastructure of plant and animal cells, respectively. Carefully examine each photograph. What are some obvious similarities and differences between plant and animal cells?

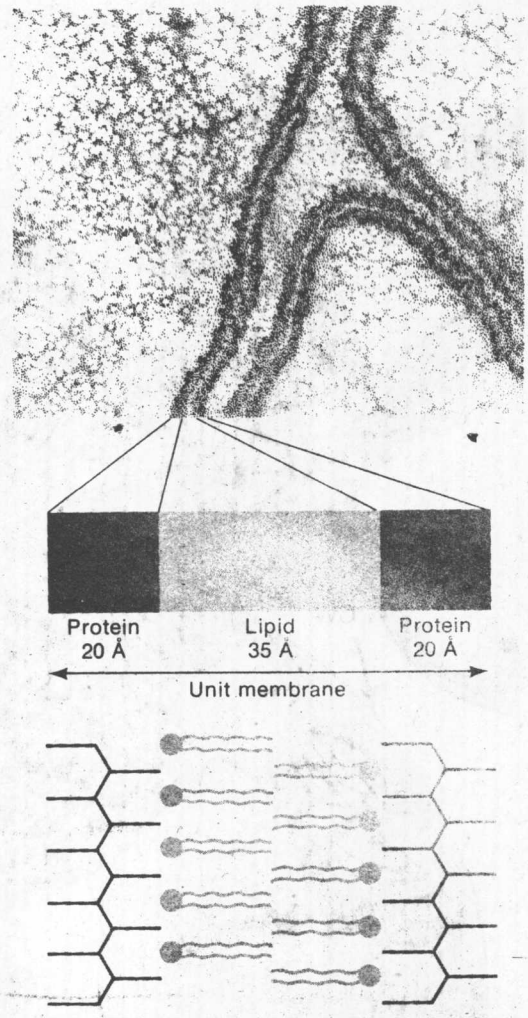

FIGURE 1-6
The structure of a single-unit membrane: each outer protein layer is 20 Å thick, and the inner lipid layer is about 35 Å thick.

1. Cell Membranes

Whereas the light microscope shows that the typical cell has only two or three membranes (the cell, nuclear, and vacuolar membranes in plants), the electron microscope has revealed that membranes are found throughout the cell and are organized into several structures.

When viewed under electron microscopic magnifications, the cell membrane, which appears as a fine line under the light microscope, is shown to consist of three distinct layers (Fig. 1-6). Sandwiched between the outer and inner dark layers is a light layer. The dark layers are composed of protein, whereas the middle region is composed of two lipid layers.

2. Endoplasmic Reticulum and Ribosomes

Internal to the cell membrane, the cell contains a complex, three-dimensional canalicular system that extends throughout most of the cytoplasm. This is the **endoplasmic reticulum,** or the ER as it is frequently called (Fig. 1-9).

The membranes of the ER have the same three-layered structure as the cell membrane and frequently are in continuity with the cell or nuclear membranes.

The membranes that make up the ER are of two types: rough and smooth. The rough ER is located in cells actively engaged in protein synthesis, which

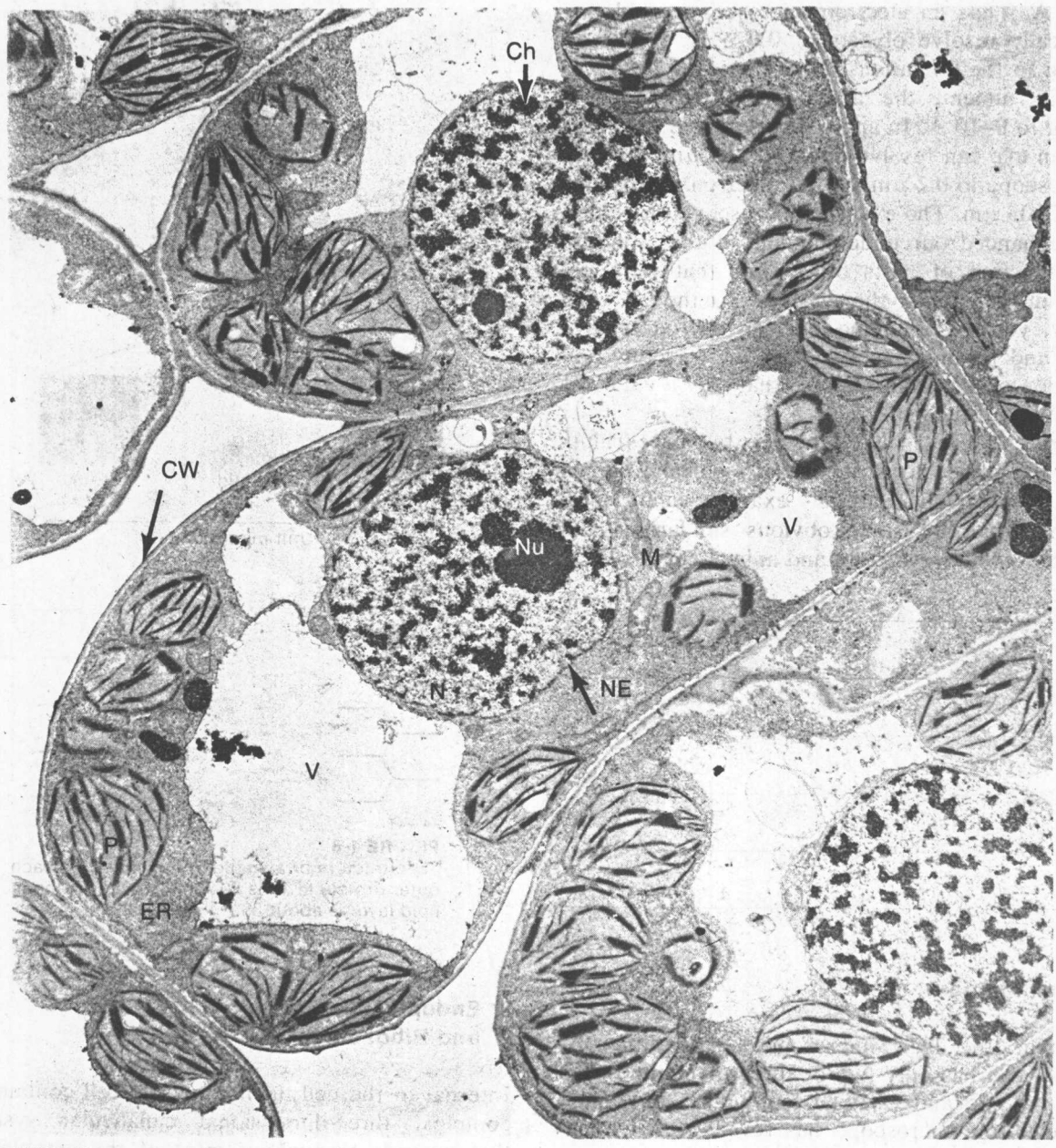

FIGURE 1-7
Structure of a higher plant cell: N, nucleus; Nu, nucleolus; NE, nuclear envelope; Ch, chromatin; CW, cell wall; V, vacuole; P, plastids; M, mitochondria; ER, endoplasmic reticulum. (Electron micrograph courtesy of Dr. Eugene L. Vigil, Marquette University.)

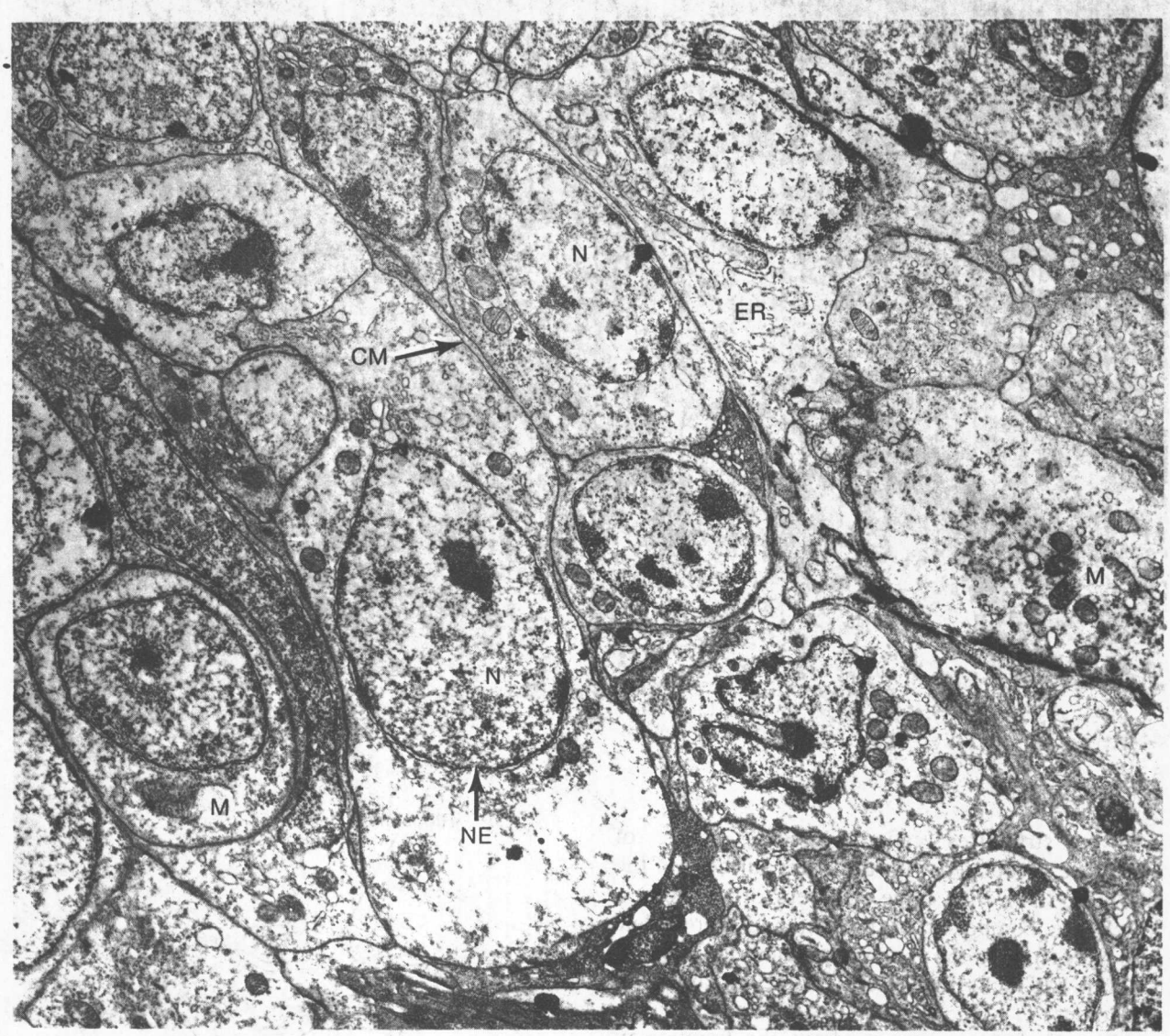

FIGURE 1-8
Fine structure of a typical animal cell: N, nucleus; NE, nuclear envelope; CM, cell membrane; M, mitochondrion; ER, endoplasmic reticulum.