FUNCTIONAL HISTOLOGY

THIRD EDITION

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Preface

This book was originally developed from a syllabus that has been distributed with favorable response to students of medicine and dentistry at Tufts University. Its value stems from a concise presentation of material that integrates histology with corresponding biologic functions. We have tried to establish a conceptual understanding of the histologic organization of cells, tissues, and organ systems through the use of representative diagrams. This book provides a manageable reading load in today's stacked curriculum, allowing the student to concentrate on lectures rather than on the production of copious notes that often must be interpreted later.

During the years following the first and second editions we received many comments, suggestions, and criticisms from both students and colleagues that have contributed significantly to the third edition. We believe that we have succeeded in producing a *practical* textbook, one that is both concise and comprehensive, functionally oriented, and well illustrated. The incorporation of new photomicrographs and additional diagrams is intended to complement the text and emphasize the link between structure and function. *Functional Histology* provides the student with a core of relevant information that can serve as a foundation for many other biomedical courses.

The reader who is familiar with past editions of *Functional Histology* will immediately recognize that, in addition to the revisions in the text, a question bank with annotated answers appears at the end of each chapter. The decision to include questions sparked a lively dialogue between the authors. The controversy centered around some students' tendency of using question banks to learn the subject rather than to assess whether the material has been learned. The student of histology is cautioned to use the self-examination questions for the latter purpose. In addition, it should be assumed that an unsuccessfully answered question is an indication that other related concepts may also be incompletely understood. Since the annotated answers may falsely reinforce the student's sense of security that learning from studying questions and answers is effective, the student is encouraged to return to the chapter and reread appropriate material to expand understanding in weak areas. To aid this process, occasional references are made to page numbers within the text for more information than can possibly be included in the annotations.

We are indebted to many people for their continued interest and dedication to the educational process. Bryan Toole, Ph.D., Chairperson of the Tufts Department of Anatomy and Cellular Biology, has been constantly supportive of our efforts and has done much to expedite the book's production through the use of departmental facilities. We gratefully acknowledge the original contributions of Joan Borysenko, Ph.D., and Alvar Gustafson, Ph.D., who were among the authors of the first edition but have now moved on to other worthwhile endeavors. Finally, we thank all those, too numerous to name, who have kindly provided us with diagrams and micrographs of their original work and who have given us help and encouragement in the evolution of this book.

M. B.
T. B.

Contents

1 A Brief Introduction to Histologic Organization and Techniques of Microscopy

Objectives

You will learn the following in this chapter:

General organization of biologic tissues

Preparation of biologic tissues for examination with the light microscope and electron microscope

Methods of tissue staining to enhance visibility of cellular and extracellular structures when viewed with the microscope

Principles of optics used in microscopy

Factors that affect microscopic resolution of cellular detail

OVERVIEW OF THE RELATIONSHIP AMONG CELLS, TISSUES, AND ORGANS

The Cell

Cytology is the study of the cell, which is the basic unit of living matter. Unicellular organisms like amebae or paramecia are capable of carrying out all metabolic processes and reproduction alone. Even in this simple context, however, cells begin to cooperate to enhance their chances of survival, as shown by the phenomenon of conjugation between paramecia. Simple cells may aggregate into colonies, such as volvox, to which the beginnings of multicellular organisms may be traced. As cells begin to cooperate and to share labor among themselves, different cells become suited to carrying out particular functions. This functional diversity is reflected by the diversity of cell structure (morphology). Even in a two-cell layered organism, such as a hydroid, some cells are differentiated into digestive cells, while others function as a combined muscle and covering cell. Certain of these two basic cell types differentiate further to become either reproductive cells or a special kind of defense cell. In this way, each cell type in the organism develops its own special functional priority. When structurally and functionally similar cells form groups within the organism, those groups of cells are known as distinct tissues. The different types of tissues become arranged into various organs. While these specialized cells have lost a multifunctional capacity in favor of fewer emphasized properties, their organization into tissues and organs allow the organism to perform biologic functions with greater economy.

The Tissues

In more complex species, three cell (germ) layers develop in the embryo; these are endoderm (inner), mesoderm (middle), and ectoderm (outer) cell layers. These basic layers give rise to four functional groupings of similar cells, called **tissues.** Study of the four tissue types and their particular specializations is called **histology.** The tissue types and their basic germ layer derivations are as follows:

1. Epithelium: arises from all three primary cell layers
   - Endoderm: epithelium lining the digestive tract and its glands, epithelium of respiratory tract and its glands, epithelium of bladder and certain parts of the urinary and reproductive systems
   - Mesoderm: epithelium (endothelium) lining the blood vessels, mesothelium lining serous membranes (pleural cavity, pericardium, peritoneum), epithelium of a large portion of the urogenital system
   - Ectoderm: epithelium covering the body surface (skin), epithelium of the anus and oral cavity glands opening into the mouth, taste buds, enamel of teeth, and epithelium lining parts of the eye, ear, and nose
2. Connective tissue: primarily mesodermal, except some of the neuroglia
3. Muscle: mesodermal, except for the smooth muscle of sweat glands and pupillary muscles of the eye
4. Nerve: ectodermal

Each tissue can be further subdivided, resulting in variations on a basic theme. For instance, there are three types of muscle tissue that vary in the organization of the major contractile protein filaments, actin and myosin. The muscle proteins are best organized in the fast-contracting skeletal muscle fibers, whereas the slow, rhythmic contraction of smooth muscle is subserved by a more diffuse arrangement of component filaments. The intrinsic rhythmicity of cardiac muscle, however, relies both on the relationship between nerve and muscle cells, and also on the particular arrangement of protein filaments within the cells. In all cases, the important message to grasp is that **structure follows function.** Through the ages, organisms have evolved that are best able to adapt to the environment; this is also true on the cellular level. The various parts present within a cell reflect the function that these parts have evolved to perform. Therefore, simple examination of cell's component parts will reveal its particular function. When a functional approach is used in the study of histology, cell physiology will naturally unfold because the two together form a unified whole, and the need to memorize will be largely replaced by simple deductive logic.

The Organs

The four tissue types are further organized into **organs** and **organ systems.** For example, the digestive system is a series of hollow tubular organs with regional functional specializations of the component tissue types to form esophagus, stomach, and intestines. In addition, the large epithelially derived glands, liver and pancreas, as well as the gallbladder, contribute secretions to the digestive tract. The entire organ system functions in the intake, breakdown (digestion), and absorption of food. The human body is composed of several organ systems, which can be explored from the gross to the cellular level. Control and integration of the various systems are functions of both the nervous and endocrine systems. A basic knowledge of histology, therefore, provides both **morphologic** (structural) and **physiologic** (functional) understanding of the delicate homeostatic mechanisms involved in the interaction of an organism with the environment.

TECHNIQUES OF MICROSCOPY

The Light Microscope

The basic light microscope is constructed of three lenses arranged in sequence: the condenser lens, the objective lens, and the ocular

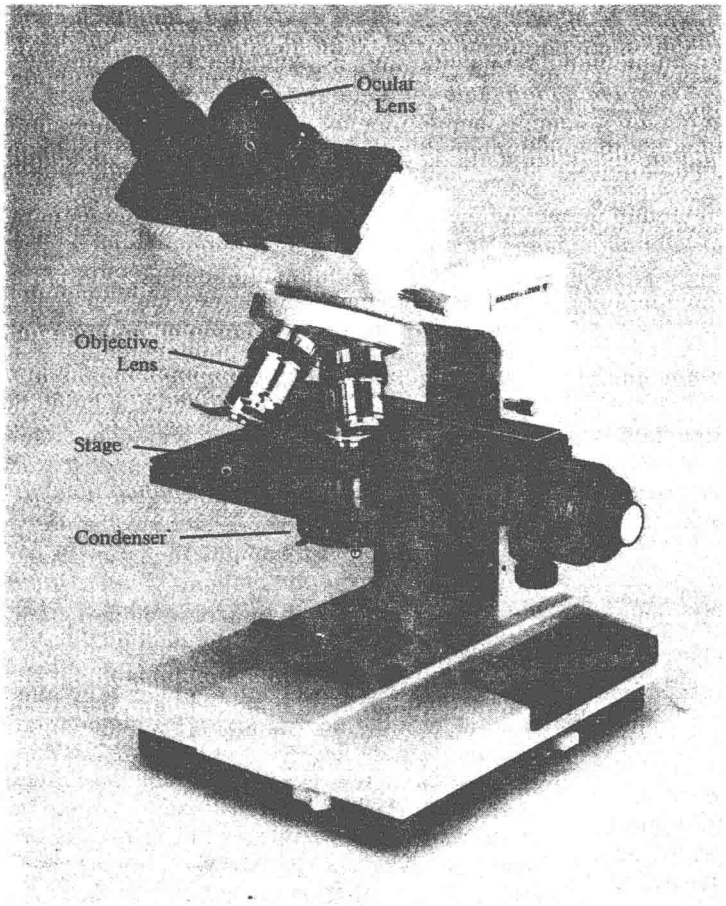

Fig. 1-1. The Galen III light microscope by Bausch & Lomb. The illuminating light source comes from the base of the microscope and rises in succession through the condenser, objective lens, and finally the optic lens. The microscope slide is mounted on the stage but not shown here. (Courtesy of Bausch & Lomb.)

lens. The condenser focuses the beam from a light source into a cone of light that shines upon the tissue section, which lays flat on a transparent glass slide. The correct positioning of the condenser imparts appropriate contrast to the visual image. The objective lens is deployed on the opposite side of the tissue section and collects light that has been transmitted through the section from the condenser lens. The objective lens magnifies the image of light and directs it to the ocular lens, which magnifies to a lesser extent and directs the image to the viewer's eye (in some cases, a photographic plate in a camera) (Fig. 1-1).

A variety of microscopes makes it possible to see not only cells but also their intracellular organelles that conduct various cellular activities. These different types of organelles often change their size in response to changing demands upon their functional roles in the cell at any given time. Consequently, references to their size are made frequently throughout this book according to a system of metric measurements listed in the scale below.

1 inch = 2.54 centimeters (cm)
1 cm = 10 millimeters (mm)
1 mm = 10^3 micrometers (μm; formerly micron, μ)
1 μm = 10^3 nanometers (nm; formerly millimicron, mμ)
1 nm = 10 angstroms (Å, no longer used in the international system of units)
1 Å = 10^{-7} mm

As a reference point appreciate that most human cells vary in size between 5 and 100 μm, although certain muscle and nerve cells have considerably larger dimensions. Furthermore, intracellular structures and organelles are even smaller. By comparison most bacteria have dimensions between 0.3 and 10 μm; and most viruses have dimensions between 1 and 50 nm. Since the smallest structure the unaided human eye can see is about 40 μm in diameter, the importance of magnification provided by the microscope is evident.

However, another characteristic of the microscope is more important than magnification, that is, **resolution.** Resolution is the smallest distance that can be seen between two visibly separate objects. For the light microscope the best effective resolution obtainable is about 0.25 μm. The equation defining resolution is:

$$R = \frac{0.61 \times \lambda}{NA}$$

The resolution (R) is directly proportional to the **wavelength of light** (λ) and indirectly proportional to the **numerical aperture** (NA). The most visible wavelength for white light is 0.55 μm, the yellow-green shade often used to make fire engines and ambulances more visible. The NA depends on the particular objective lens being used but it is defined as the sine of the angle of light entering between the middle and the edge of the objective lens. Each objective lens has its NA engraved on it, usually to the right of its indicated magnification. When these values for wavelength and NA are inserted into the equation for resolution, it becomes apparent that larger NAs produce better resolution. As the resolution improves, more details are visible in the image of the tissue specimen.

TISSUE PREPARATION

Biological tissues are prepared in a variety of ways on glass slides which pathologists and students study in schools of medicine and dentistry. They are prepared according to the following basic protocol.

Fixation

Initially the tissue must be fixed before or after removal from the body to arrest intracellular enzymatic degradation that would normally cause putrefaction of the tissue and destruction of cellular detail. Certain chemical fixatives, such as picric acid, preserve tissues but also denature proteins. These types of fixatives introduce alterations in intermolecular organization that can sometimes be visible with the light microscope but are tolerated for limited purposes of microscopy where resolution is not extremely important. One drawback to this type of fixation is that advanced histochemical procedures cannot be used to study cellular processes because denatured proteins lose their normal molecular functions.

More frequently these types of fixatives have been replaced by formalin, a concentrated formaldehyde solution, which causes less artifactual change in the tissue because it does not generally denature proteins or alter tissue and cellular structure as severely. This fixative is commonly used in hospitals for routine preserva-

tion of pathologic specimens. Researchers are more likely to use fixatives such as paraformaldehyde and glutaraldehyde. These fixatives cross-link proteins through their amine groups. They will also denature proteins but not to the same extent as formalin or picric acid. In many cases they retain the antigenicity of the tissue so that immunocytochemical studies can be conducted to localize some cellular protein that an antibody has been formed against. In order to preserve full enzymatic activity of a tissue it must be rapidly frozen in liquid nitrogen or liquid helium.

Dehydration, Clearing, Embedding, and Sectioning

Since tissue must be thin enough to transmit light to be examined with conventional light microscopes, it must be sectioned in thin slices from 1 to 20 μm in thickness, the exception being when a layer or two of cells grown on a coverslip in a culture dish is to be examined; the latter is already thin enough to transmit light. Sectioning requires that the tissue have support or it will be compressed during the cutting action of the knife. Therefore tissues are embedded in a support such as wax (paraffin) or epoxy. Epoxy is much harder and is used where sections of 1 μm are to be cut or where the much thinner sections required for electron microscopy are cut. First, however, the tissue must be dehydrated since water in the tissue would prevent the paraffin or epoxy from entering the tissue.

Dehydration is usually accomplished by immersing the tissue in a series of increasingly concentrated solvents such as ethyl alcohol. If the tissue is to be embedded in paraffin, the alcohol is followed by benzene (a clearing agent) since the benzene is miscible with paraffin whereas alcohol is not. If the tissue is to be embedded in epoxy the alcohol is followed by propylene oxide, which is miscible with epoxy. It should be recognized that these solvents extract most of the lipids from the tissue causing artifactually produced gaps where lipids had resided in the living state. This artifact is most prominent in fat cells, which lose their stored fat droplets during this dehydration step and will be seen in the microscope to have large, round vacant spaces where the lipid droplet was extracted. This is only one of many artifacts introduced into the tissue as a result of processing for microscopic viewing. Another artifact of dehydration is a certain amount of shrinkage, which is frequently observed in tissue sections as spaces where water has been removed and tissue components have pulled apart from each other. Once the tissue is embedded, it is sectioned with a microtome. The sections are then mounted on a glass slide. While on the glass slide the sections will be stained prior to viewing with the microscope.

Staining

Since the refractive index of different cell types and their intracellular organelles are very similar, they must be stained with dyes to help distinguish them and to make them more visible to the microscopist. Dyes absorb light in part of the visible spectrum and allow the complementary color to pass through the tissue. For instance, a blue dye absorbs yellow light and confers a blue color to any tissue element which the dye attaches to. Most dyes attach to cells by electrostatic attraction, which involves bonding between a dye and cell component of opposite charge. For this reason an acid dye and a basic dye of different colors are usually used in combination to color substances in the cell with different isoelectric points, which will have different affinities for the two dyes based upon charge and, consequently, different colors after staining. Recall that a protein that is found in a cell or tissue has a net neutral charge at its isoelectric point (pI). Above its pI a protein will be negatively charged; below its pI it will be positively charged. Consequently, a cationic dye (positive charge) will attach to negatively charged cellular substances; an anionic dye (negative charge) will attach to positively charged substances.

The portion of a dye that absorbs light and im-

parts the color to a dye is the **chromophore.** The chromophore is part of a larger ionizable chemical structure which over a broad range of pH is either a basic dye (such as methylene blue) or an acid dye (such as eosin). A basic dye is composed of a cationic chromophore and a colorless acid radical; an acid dye is composed of an anionic chromophore and a colorless basic radical. Since methylene blue is blue and eosin is a reddish-orange, tissue components having different charges will stain different colors when these two dyes are used. In this case negatively charged tissue components staining blue would be referred to as **basophilic** because they stain with the basic dye methylene blue (a cationic dye); positively charged tissue components staining reddish-orange would be referred to as **acidophilic** because they stain with the acidic eosin (an anionic dye). Under conditions of pH where most staining is performed, examples of acidophilia are collagen, mitochondria, and the hemoglobin of erythrocytes. Examples of basophilia include nuclear chromatin and ribosomes. In the latter, binding occurs with the acidic phosphate groups of the DNA and RNA respectively. The extracellular matrix of cartilage also stains with basic dyes due to the high concentration of negatively charged sulfate groups present on sulfated proteoglycans.

Because hematoxylin is a commonly used dye but is somewhat different in character than the dyes described above, it will be described briefly. It must be oxidized to hematin before use and has no staining properties until it is complexed with a heavy metal such as iron. The iron is referred to as a **mordant.** The iron confers a positive charge to hematin which then allows it to act as a cationic dye. The iron chelates or binds to negatively charged tissue components. By convention, tissue components stained with iron-mordanted hematin are then referred to as basophilic. Hematin is a dark-bluish color and is usually used in conjunction with eosin as a counterstain.

Certain dyes such as toluidine blue are called **metachromatic** because they stain with a different color when they are grouped closely together from when they are dispersed. Toluidine blue is a basic dye which binds to negatively charged tissue components, staining them blue when these tissue components are dispersed. When bound to polyanions like heparin or nucleoprotein which have closely spaced negative charges, toluidine blue aggregates into a polymer which absorbs light at a lower wavelength emitting a reddish or purplish light instead of blue. Consequently the tissue containing closely spaced anionic charges will stain reddish instead of blue. This phenomenon is called **metachromasia.**

Other stains are described as they are encountered in applications throughout the text.

OTHER TYPES OF LIGHT MICROSCOPY

Fluorescence Microscopy

Fluorescence microscopy has become an extremely valuable research and clinical tool because it allows molecules that have been tagged with fluorescent markers to be located within the cell. One particularly useful application of this technique employs fluorescent-tagged antibodies which can be synthesized against any given protein that one wishes to study. Since the antibody will attach to the protein, the small fluorescent tag on the antibody allows a researcher to determine the exact location of that protein within the cell. This technique, when used in conjunction with a highly sensitive television camera fitted to the microscope, can effectively increase the resolution of the light microscope somewhat more.

Unlike conventional light microscopy, fluorescence microscopy uses ultraviolet (UV) light. An exciter filter or dichroic beam splitter is used to project a particular wavelength of UV light onto the tissue section. A particular exciter filter is selected to produce a wavelength of UV light which will excite a known fluorescent tag located somewhere in the tissue section. The fluorescent tag responds to this exciting wavelength by emitting a longer wavelength of visible light (fluorescence) which passes through the objective and

ocular lenses to be seen by the viewer. Since UV light damages the retina and can cause blindness, a barrier filter which eliminates any UV light but allows fluorescence light to pass through is placed in the final light path before reaching the ocular lens. Two excellent examples of fluorescence micrographs are found in Chapter 2 (see Figs. 2-27, 2-30).

Phase Contrast Microscopy

Although most conventional tissue sections are stained with different colored dyes to render cells and their organelles more visible and distinguishable from one another, a special pair of condenser and objective lens makes staining unnecessary. This type of optical system is referred to as phase contrast microscopy. It amplifies differences in refractive index between cellular organelles adding variable contrast to different organelles and increasing light intensity passing through areas with less refractive index. This technique is especially suitable for examining living cells in culture where staining may disturb cellular activity or even obscure it. An especially effective form of phase contrast microscopy is differential interference contrast (Nomarski optics). Examples of it are shown in Chapters 2 and 11 (see Figs. 2-38, 11-3).

Electron Microscopy

TRANSMISSION ELECTRON MICROSCOPY

Unlike light microscopy, electron microscopy employs an electron beam to "illuminate" the tissue. There is a substantial improvement of resolution since the wavelength of an electron beam accelerated by 100 kV is about 0.04 Å. When working with biologic tissue the attainable resolution is about 1 nm although theoretically it is even smaller. Instead of using glass lenses as in the light microscope, the electron microscope shapes the electron beam with electromagnets to provide magnification (Fig. 1-2). Staining is accomplished by using heavy metals such as uranyl and osmium ions which obstruct the passage of electrons through the tissue section. Deflected electrons are eliminated by a metal aperture. Areas not specifically stained with the heavy metal allow the electron beam to pass through the section. Since the various parts of the cell in the tissue section have different affinities for the metal ions, cellular components exhibit different contrasts which generate a two-dimensional image of varying electron density. Since an electron beam is invisible to the eye, the beam, after passing through the tissue section, impacts on a fluorescent screen which is excited by the electrons. In this way a fluorescent image is generated to make the tissue image visible to the microscope operator. A permanent record of the image is made by exposing special photographic plates to the electron beam image to produce a negative from which black and white prints can be made.

Since the electron beam on conventional electron microscopes does not have high penetrating capabilities, tissue sections must be much thinner than for light microscopy. Consequently sections are cut as thin as 60 to 100 nm on a special ultramicrotome. The thinness of the section generates nearly a two-dimensional image. Although this characteristic has its limitations, the resolution of fine details is valuable. For examples of electron micrographs, see Figures 2-14 and 2-15 in Chapter 2. In high-voltage electron microscopy the electrons are accelerated at 1000 kV. This not only improves resolution, since the wavelength of the accelerated electron beam is smaller, but also improves penetrating power so that thicker sections comparable to those used with the light microscope can be viewed. The result is a more extensive morphologic picture providing more three-dimensional information than can be acquired from thinner sections.

SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy differs from transmission electron microscopy in that only the surface architecture of cells is visualized. A gold rep-

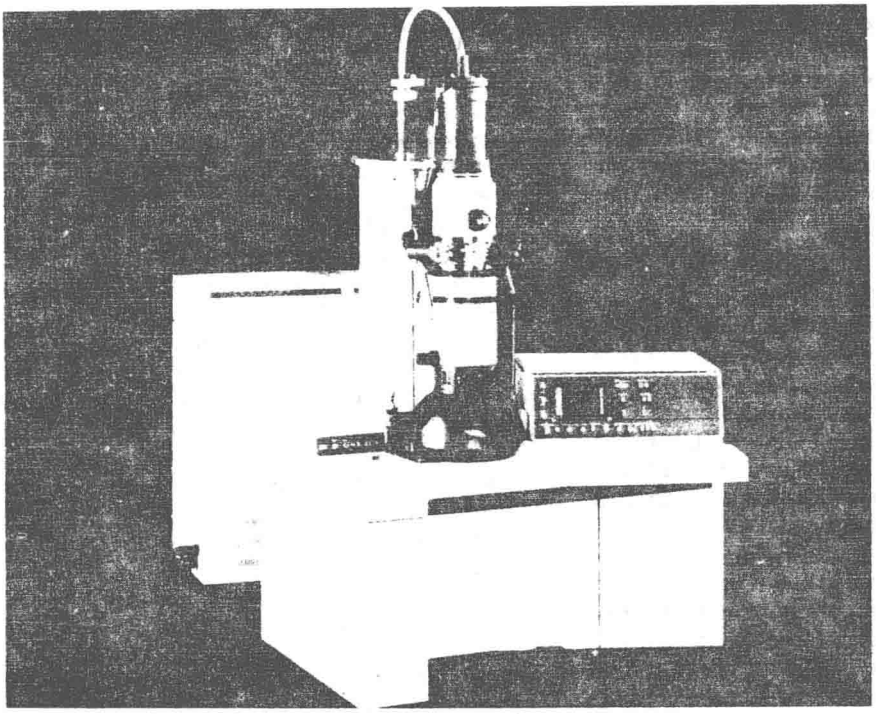

Fig. 1-2. The Philips CM10 transmission electron microscope. The specimen is mounted in the column. The electron beam is accelerated down the column from above. Magnification of the image is accomplished by electromagnetic lenses which shape the electron beam. (Courtesy of Philips Electronic Instruments, Inc.)

lica is made of the cellular surface by evaporating gold in a vacuum on the tissue. When the replica is viewed in the scanning electron microscope the electron beam provokes secondary emission of electrons which are detected by a cathode ray tube producing a three-dimensional–appearing image of the cellular surface. The resolution of the scanning electron microscope is about 5 nm, somewhat inferior to the transmission electron microscope but advantageous for generating a surface landscape of the cell. For a comparison of scanning and transmission electronmicrographs, see Figure 5-11 in Chapter 5.

FREEZE-FRACTURE TECHNIQUE

In recent years the freeze-fracture (freeze-cleave) technique has come into wide use. A tissue specimen is frozen in liquid nitrogen–cooled isopentane and then fractured with a blade; the resulting fracture face is replicated under a high vacuum with platinum (at a 45-degree angle) and carbon. The platinum shadowing creates an effect similar to a snowfall propelled by a stiff wind. The platinum, like snow, accumulates on the near side of elevations and is absent on the far side. Conversely, the near side of a depression would be empty, while platinum would pile up on

the far side. After the tissue has been dissolved away, the platinum replica can be viewed in the transmission electron microscope. When viewed in the transmission electron microscope, areas where platinum has accumulated will impede the flow of electrons and appear dark; areas devoid of platinum appear as white shadows. The carbon does not yield further electron density, but acts to stabilize the platinum-shadowed replica of the fractured surface after the tissue has been dissolved away. The advantage of this technique is that fracture occurs along the plane of least resistance, and in cells, one such area is between the hydrophobic acyl tails of membrane phospholipids. Thus the cell membrane is split down its middle exposing large macromolecular structures within the membrane (see Chap. 2, Figs. 2-2, 2-3).

NATIONAL BOARD TYPE QUESTIONS

Select the single best response for each of the following.

1. A cationic dye
   A. has a net negative charge.
   B. has an anionic chromophore.
   C. binds to positively charged tissue components.
   D. binds to negatively charged tissue components.
2. The resolution obtained with the electron microscope is superior to that obtained with the light microscope primarily because
   A. sections for electron microscopy are generally thinner.
   B. superior dyes are used in electron microscopy.
   C. the wavelength of the electron beam is shorter than the wavelength of visible light.
   D. the wavelength of the electron beam is longer than the wavelength of visible light.
3. Fixatives such as glutaraldehyde and paraformaldehyde
   A. act by cross-linking proteins through their amino groups.
   B. severely denature all cell proteins.
   C. completely fail to prevent intracellular enzymatic degradation.
   D. also impart a stain to the tissue specimen.
4. Occasionally, closely associated tissue components are stained a different color than the color of the dye attached to them. This phenomenon is
   A. mordanting.
   B. metachromasia.
   C. acidophilia.
   D. staining with acid dyes.
5. Which of the following is used to generate contrast in sections viewed with the electron microscope?
   A. Hematoxylin
   B. Eosin
   C. Methylene blue
   D. Uranyl and osmium ions

ANNOTATED ANSWERS

1. D. At the pH prevailing during staining a positively charged dye will have an electrostatic attraction to negatively charged tissue components.
2. C. Resolution is improved by shorter wavelengths. Read the discussion of resolution on page 4.
3. A. Covalent bonds formed, especially with intracellular enzymes, prevent enzymatic degradation of the cell after fixation.
4. B. Metachromatic dyes when closely packed absorb light at lower wavelengths than in the dispersed state.
5. D. Uranyl and osmium ions are large enough that their positively charged nuclei deflect electrons in the tissue where uranyl and osmium are deposited.

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I Cells and Tissues