



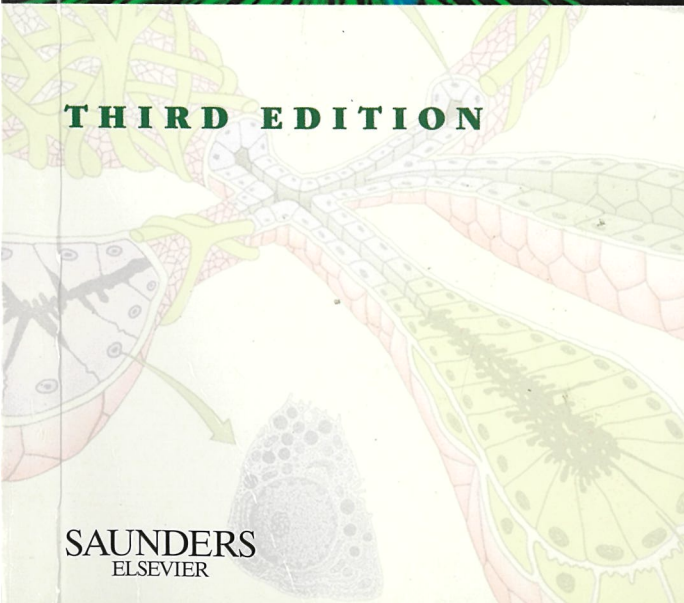
Leslie P. Gartner
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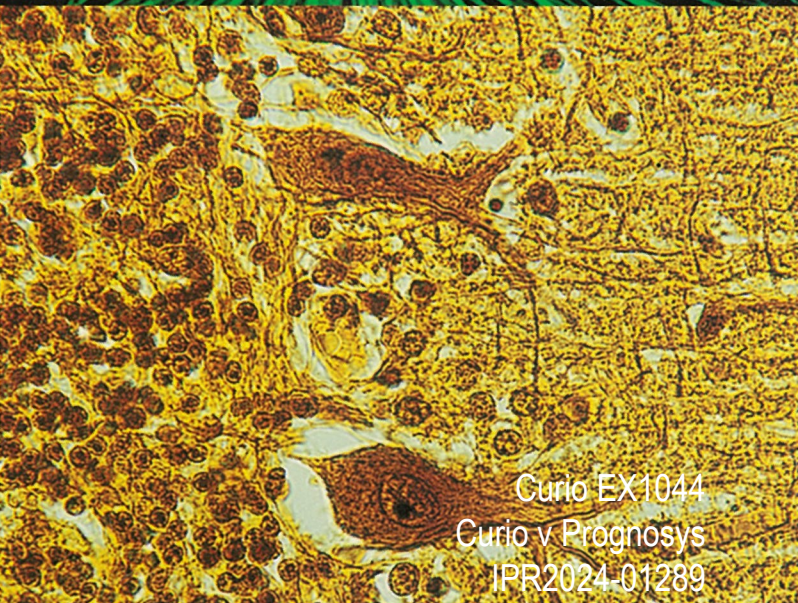
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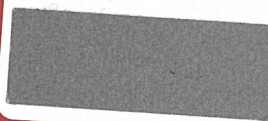
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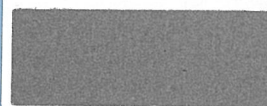
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Color Textbook of Histology

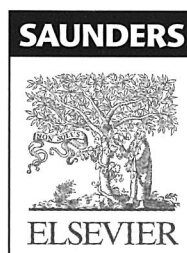
Third Edition

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*To my wife Roseann,
my daughter Jennifer,
and my mother Mary*

LPG

*To my grandchildren
Nathan David,
James Mallary,
Hanna Elisabeth,
Alexandra Renate,
Eric James,
and Elise Victoria*

JLH



Preface

Once again, we are gratified to release a new edition of a histology textbook that is well established not only in its original language but also in several other languages. The place of histology has changed as the biological sciences have progressed in the last half of the 20th century. It evolved from the purely descriptive science of microscopic anatomy to its current position as the linchpin between functional anatomy and molecular and cell biology.

This third edition, coming only a few short years after the second edition reached bookshelves, has been revised to reflect new information in cell and molecular biology that pertains to histology. While incorporating much new material we were mindful of the time constraints that students face due to an ever-expanding curriculum and an exponentially increasing information glut. We labored diligently to maintain readability and brevity. We revised many illustrations and added detail to figure legends.

The most visible, and we believe most valuable, addition for the student to this revision is the inclusion of a CD-ROM containing 21 brief PowerPoint presentations that give overviews of each chapter. They offer the student keys by which he or she can quickly form a basic understanding of the material.

The third edition of this textbook is bundled not only with the CD but also with access to Student Consult, a website developed by Elsevier publishing company that

permits the purchaser of this book to view the entire text plus all images online. The website also provides seamless integration to related content in other Elsevier books that the reader has purchased, if that book is a title that has been selected to be a Student Consult title.

As in the first two editions, we have conveyed information as efficiently as possible. Tables and figures summarize complex topics to promote acquisition of knowledge. The text is punctuated by bulleted sections that not only organize important aspects of functional histology but also alert the reader to their significance. Important terms appear in bold type to permit rapid review as the student prepares for examinations. Clinical Correlation boxes illustrate the relevance of histology to students of the health professions. We believe that these features emphasize an important tenet of modern day histology—that structure and function are intimately related.

Although we have made every effort to present a complete and accurate account of the subject matter, we realize that there are omissions and errors in any undertaking of this magnitude. Therefore, we continue to encourage and welcome suggestions, advice, and criticism that will facilitate the improvement of this text.

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We are truly grateful to Dr. Robert A. Bloodgood for providing us with an extensive list of suggestions for improvement. We also wish to thank Drs. Felipe A. Roberio and Joel Schechter for their helpful comments on topics related to their fields of expertise.

Histology is a visual subject; therefore, excellent graphic illustrations are imperative. For that we are indebted to Todd Smith for his careful attention to detail in revising and creating new illustrations. We also thank our many colleagues from around the world and their publishers who generously permitted us to borrow illustrative materials.

Finally, our thanks go to the project team at Elsevier for all their help, namely Inta Ozols, Jacqueline Mahon, and Joan Nikelsky.



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Introduction to Histology and Basic Histological Techniques

Histology is that branch of anatomy that studies tissues of animals and plants. This textbook, however, discusses only animal, and more specifically human, tissues. In its broader aspect, the word *histology* is used as if it were a synonym for microscopic anatomy, because its subject matter encompasses not only the microscopic structure of tissues but also that of the cell, organs, and organ systems.

The body is composed of cells, intercellular matrix, and a fluid substance, extracellular fluid (tissue fluid), which bathes these components. Extracellular fluid, which is derived from plasma of blood, carries nutrients, oxygen, and signaling molecules to cells of the body. Conversely, signaling molecules, waste products, and carbon dioxide released by cells of the body reach blood and lymph vessels by way of the extracellular fluid. Extracellular fluid and much of the intercellular matrix are not visible in routine histological preparations, yet their invisible presence must be appreciated by the student of histology.

The subject of histology no longer merely deals with the structure of the body; it also concerns itself with the body's function. In fact, histology has a direct relationship to other disciplines and is essential for their understanding. This textbook, therefore, intertwines the disciplines of cell biology, biochemistry, physiology, embryology, gross anatomy, and, as appropriate, pathology. Students will recognize the importance of this subject as they refer to the text later in their careers. An excellent example of this relationship will be evident when the reader learns about the histology of the kidney and realizes it is the intricate and almost sublime structure of that organ (down to the molecular level) that

is responsible for the kidney's ability to perform its function. Alterations of the kidney's structure are responsible for a great number of life-threatening conditions.

The remainder of this chapter discusses the methods used by histologists to study the microscopic anatomy of the body.

LIGHT MICROSCOPY

Tissue Preparation

Steps required in preparing tissues for light microscopy include (1) fixation, (2) dehydration and clearing, (3) embedding, (4) sectioning, and (5) mounting and staining the sections.

Various techniques have been developed to prepare tissues for study so that they closely resemble their natural, living state. The steps involved are **fixation**, **dehydration** and **clearing**, **embedding** in a suitable medium, **sectioning** into thin slices to permit viewing by transillumination, **mounting** sections onto a surface for ease of handling, and **staining** them so that the various tissue and cell components may be differentiated.

Fixation

Fixation refers to treatment of the tissue with chemical agents that not only retard the alterations of tissue subsequent to death (or after removal from the body) but also maintain its normal architecture. The most common fixative agents used in light microscopy are neutral buffered **formalin** and **Bouin's fluid**. Both of

these substances cross-link proteins, thus maintaining a lifelike image of the tissue.

Dehydration and Clearing

Because a large fraction of the tissue is composed of water, a graded series of alcohol baths, beginning with 50% alcohol and progressing in graded steps to 100% alcohol, are used to remove the water (*dehydration*). The tissue is then treated with xylene, a chemical that is miscible with melted paraffin. This process is known as *clearing*, because the tissue becomes transparent in xylene.

Embedding

In order to distinguish the overlapping cells in a tissue and the extracellular matrix from one another, the histologist must *embed* the tissues in a proper medium and then slice them into thin sections. For light microscopy, the usual embedding medium is paraffin. The tissue is placed in a suitable container of melted paraffin until it is completely **infiltrated**. Once the tissue is infiltrated with paraffin, it is placed into a small receptacle, covered with melted paraffin, and allowed to harden, forming a paraffin block containing the tissue.

Sectioning

After the blocks of tissue are trimmed of excess embedding material, they are mounted for sectioning. This task is performed using a microtome, a machine equipped with a blade and an arm that advances the tissue block in specific equal increments. For light microscopy, the thickness of each section is about 5 to 10 μm .

Sectioning also can be performed on specimens frozen either in liquid nitrogen or on the rapid-freeze bar of a cryostat. These sections are mounted by the use of a quick-freezing mounting medium and sectioned at subzero temperatures by means of a pre-cooled steel blade. The sections are placed on pre-cooled glass slides, permitted to come to room temperature, and stained with specific dyes (or treated for histochemical or immunocytochemical studies).

Mounting and Staining

Paraffin sections are mounted (placed) on glass slides and then stained by water-soluble stains that permit differentiation of the various cellular components.

The sections for conventional light microscopy, cut by stainless steel blades, are mounted on adhesive-coated glass slides. Because many tissue constituents have approximately the same optical densities, they must be stained for light microscopy, usually with water-soluble stains. Therefore, the paraffin must first be removed from the section, after which the tissue is

rehydrated and stained. After staining, the section is again dehydrated so that the coverslip may be permanently affixed by the use of a suitable mounting medium. The coverslip not only protects the tissue from damage but also is necessary for viewing the section with the microscope.

Various types of stains have been developed for visualization of the many components of cells and tissues; they may be grouped into three classes:

- Stains that differentiate between acidic and basic components of the cell
- Specialized stains that differentiate the fibrous components of the extracellular matrix
- Metallic salts that precipitate on tissues, forming metal deposits on them

The most commonly used stains in histology are **hematoxylin and eosin (H&E)**. Hematoxylin is a base that preferentially colors the acidic components of the cell a bluish tint. Because the most acidic components are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the nucleus and regions of the cytoplasm rich in ribosomes stain dark blue; these components are referred to as **basophilic**. Eosin is an acid that dyes the basic components of the cell a pinkish color. Because many cytoplasmic constituents have a basic pH, regions of the cytoplasm stain pink; these elements are said to be **acidophilic**. Many other stains are also used in preparation of specimens for histological study (Table 1-1).

Molecules of some stains, such as **toluidine blue**, polymerize with each other when exposed to high concentrations of polyanions in tissue. These aggregates differ in color from their individual molecules. For example, toluidine blue stains tissues blue except for those that are rich in polyanions (e.g., cartilage matrix and granules of mast cells), which stain purple. A tissue or cell component that stains purple with this stain is said to be **metachromatic**, and toluidine blue is said to exhibit **metachromasia**.

Light Microscopes

Compound microscopes are composed of a specific arrangement of lenses that permit a high magnification and good resolution of the tissues being viewed.

The present-day light microscope uses a specific arrangement of groups of lenses to magnify an image (Fig. 1-1). Because this instrument uses more than just a single lens, it is known as a **compound microscope**. The light source is an electric bulb with a tungsten filament whose light is gathered into a focused beam by the **condenser lens**.

The light beam is located below and is focused on the specimen. Light passing through the specimen

Table 1–1 Common Histological Stains and Reactions

Reagent	Result
Hematoxylin	<i>Blue</i> : nucleus; acidic regions of the cytoplasm; cartilage matrix
Eosin	<i>Pink</i> : basic regions of the cytoplasm; collagen fibers
Masson's trichrome	<i>Dark blue</i> : nuclei <i>Red</i> : muscle, keratin, cytoplasm <i>Light blue</i> : mucinogen, collagen
Orcein's elastic stain	<i>Brown</i> : elastic fibers
Weigert's elastic stain	<i>Blue</i> : elastic fibers
Silver stain	<i>Black</i> : reticular fibers
Iron hematoxylin	<i>Black</i> : striations of muscle, nuclei, erythrocytes
Periodic acid–Schiff	<i>Magenta</i> : glycogen and carbohydrate-rich molecules
Wright's and Giemsa stains (used for differential staining of blood cells)	<i>Pink</i> : erythrocytes, eosinophil granules <i>Blue</i> : cytoplasm of monocytes and lymphocytes

enters one of the objective lenses; these lenses sit on a movable turret located just above the specimen. Usually four objective lenses are available on a single turret, providing low, medium, high, and oil magnifications. Generally, in most microscopes the first three lenses magnify 4, 10, and 40 times, respectively, and are used without oil; the oil lens magnifies the image 100 times.

The image from the objective lens is gathered and further magnified by the ocular lens of the eyepiece. This lens usually magnifies the image by a factor of 10—for total magnifications of 40, 100, 400, and 1000—and focuses the resulting image on the retina of the eye.

Focusing of the image is performed by the use of knurled knobs that move the objective lenses up or down above the specimen. The coarse-focus knob moves it in larger increments than the fine-focus knob does. It is interesting that the image projected on the retina is reversed from right to left and is upside down.

The quality of an image depends not only on the capability of a lens to magnify but also on its **resolution**—the ability of the lens to show that two distinct

objects are separated by a distance. The quality of a lens depends on how close its resolution approaches the theoretical limit of 0.25 μm , a restriction that is determined by the wavelength of visible light.

There are several types of light microscopes, distinguished by the type of light used as a light source and the manner in which they use the light source. However, most students of histology are required to recognize only images obtained from compound light microscopy, transmission electron microscopy, and scanning electron microscopy; therefore, the other types of light microscopes are not discussed.

Digital Imaging Techniques

Digital imaging techniques employ computer technology to capture and manipulate histologic images.

The advent of computer technology has provided a means of capturing images digitally, without the use of film. Although this method of image capturing cannot yet compete with film technology, it has several advantages that make it a valuable tool:

- Immediate visualization of the acquired image
- Digital modification of the image
- Capability of enhancing the image by the use of commercially available software

In addition, because these images are stored in a digital format, hundreds of them may be archived on a single CD-ROM disk and their retrieval is almost instantaneous. Finally, their digital format permits the electronic transmission of these images by e-mail or distribution via the Internet.

Interpretation of Microscopic Sections

One of the most difficult, frustrating, and time-consuming skills needed in histology is interpreting what a two-dimensional section looks like in three dimensions. If you imagine a coiled garden hose and then take thin sections from that hose, you will see that the three-dimensional object is not necessarily discerned from any *one* of the two-dimensional sections (Fig. 1-2). However, by viewing all of the sections drawn from the coiled tube, you can mentally reconstruct the correct three-dimensional image.

Advanced Visualization Procedures

Histochemistry

Histochemistry is a method of staining tissue that provides information about the presence and location of intracellular and extracellular macromolecules.

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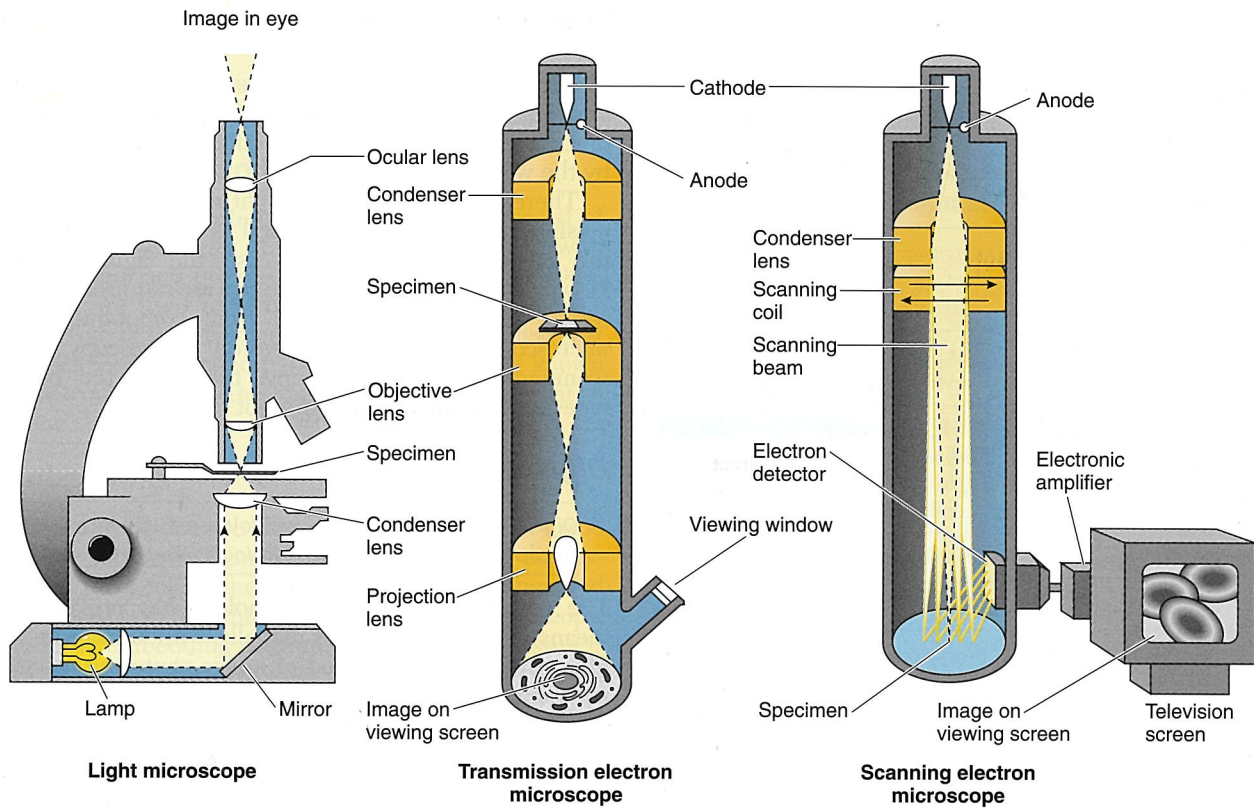


Figure 1-1 Comparison of light, transmission electron, and scanning electron microscopes.

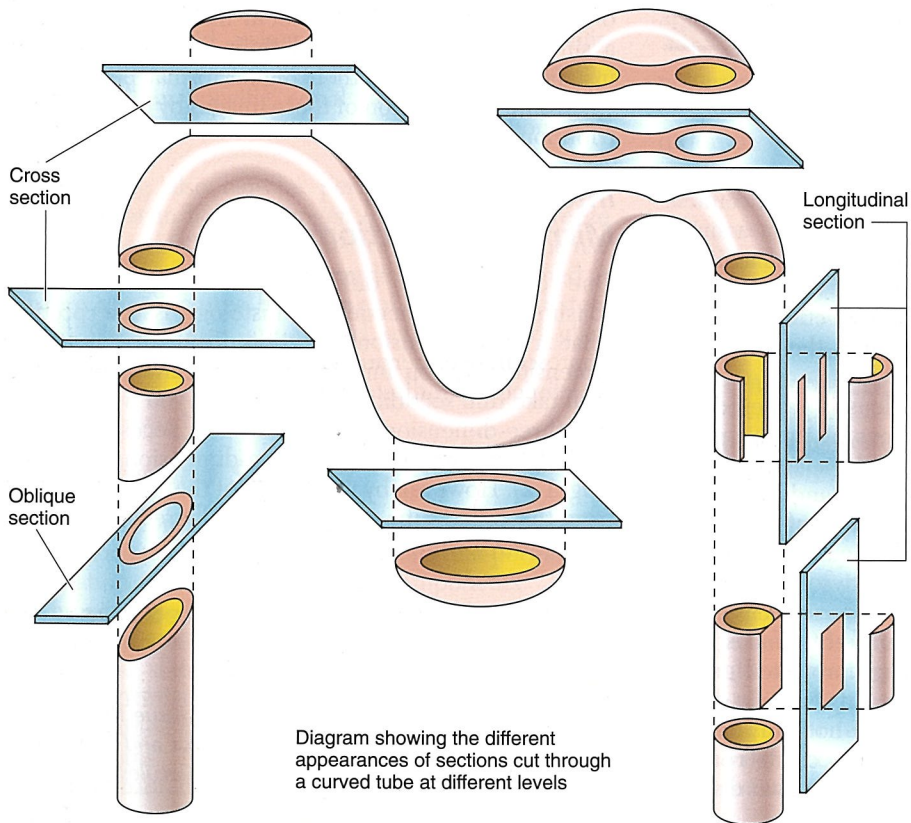
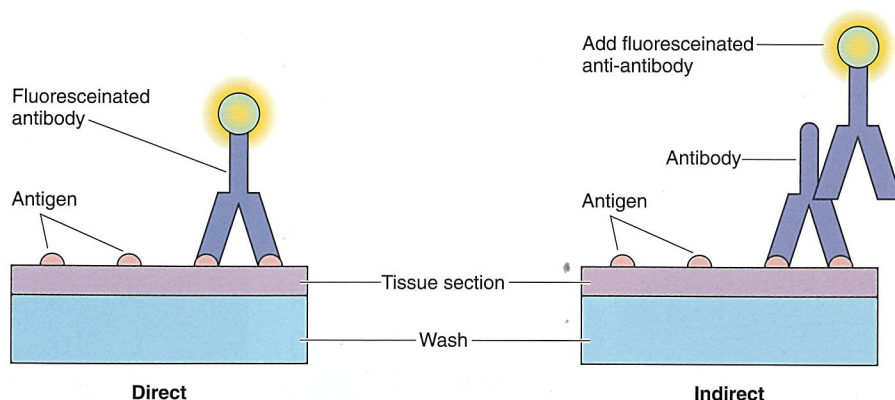


Figure 1-2 Histology requires a mental reconstruction of two-dimensional images into the three-dimensional solid from which they were sectioned. Here, a curved tube is sectioned in various planes to illustrate the relationship between a series of two-dimensional sections and their three-dimensional structure.

Figure 1-3 Direct and indirect methods of immunocytochemistry. *Left*, An antibody against the antigen was labeled with a fluorescent dye and viewed with a fluorescent microscope. The fluorescence occurs only over the location of the antibody. *Right*, Fluorescent-labeled antibodies are prepared against an antibody that reacts with a particular antigen. When viewed with fluorescent microscopy, the region of fluorescence represents the location of the antibody.



Specific chemical constituents of tissues and cells can be localized by the methods of histochemistry and cytochemistry. These methods capitalize on the enzyme activity, chemical reactivity, and other physicochemical phenomena associated with the constituent of interest. Reactions of interest are monitored by the formation of an insoluble precipitate that takes on a certain color. Frequently, histochemistry is performed on frozen tissues and can be applied to both light and electron microscopy.

A common histochemical reaction uses the periodic acid-Schiff (PAS) reagent, which forms a magenta precipitate with molecules rich in glycogen and carbohydrate-rich molecules. To ensure that the reaction is specific for glycogen, consecutive sections are treated with amylase. Thus, sections not treated with amylase display a magenta deposit, whereas amylase-treated sections display a lack of staining in the same region.

Although enzymes can be localized by histochemical procedures, the product of enzymatic reaction rather than the enzyme itself is visualized. The reagent is designed so that the product precipitates at the site of the reaction and is visible either as a metallic or a colored deposit.

Immunocytochemistry

Immunocytochemistry uses fluoresceinated antibodies and anti-antibodies to provide more precise intracellular and extracellular localization of macromolecules than is possible with histochemistry.

Although histochemical procedures permit relatively good localization of some enzymes and macromolecules in cells and tissues, more precise localization can be achieved by the use of immunocytochemistry. This procedure requires developing an antibody against the particular macromolecule to be localized and labeling the antibody with a fluorescent dye such as fluorescein or rhodamine.

There are two methods of antibody labeling: direct and indirect. In the **direct method** (Fig. 1-3) the antibody against the macromolecule is labeled with a fluorescent dye. The antibody is then permitted to react with the macromolecule, and the resultant complex may be viewed with a fluorescent microscope (Fig. 1-4).

In the **indirect method** (see Fig. 1-3) a fluorescent-labeled antibody is prepared against the primary antibody specific for the macromolecule of interest. Once the primary antibody has reacted with the antigen, the preparation is washed to remove unbound primary antibody; the labeled antibody is then added and reacts with the original antigen-antibody complex, forming a secondary complex visible by fluorescent microscopy (Fig. 1-5). The indirect method is more sensitive than the direct method because numerous labeled anti-antibodies bind to the primary antibody, making them easier to visualize. In addition, the indirect method does not require labeling of the primary antibody, which often is available only in limited quantities.

Immunocytochemistry can be used with specimens for electron microscopy by labeling the antibody with ferritin, an electron-dense molecule, instead of with a fluorescent dye. Ferritin labeling can be applied in both the direct and indirect methods.

Autoradiography

Autoradiography is a method that uses the incorporation of radioactive isotopes into macromolecules, which are then visualized by the use of an overlay of film emulsion.

Autoradiography (or radioautography) is a particularly useful method for localizing and investigating a specific temporal sequence of events. The method requires incorporation of a radioactive isotope—most commonly tritium (^3H)—into the compound being studied (Fig. 1-6). An example is the use of tritiated amino acid to

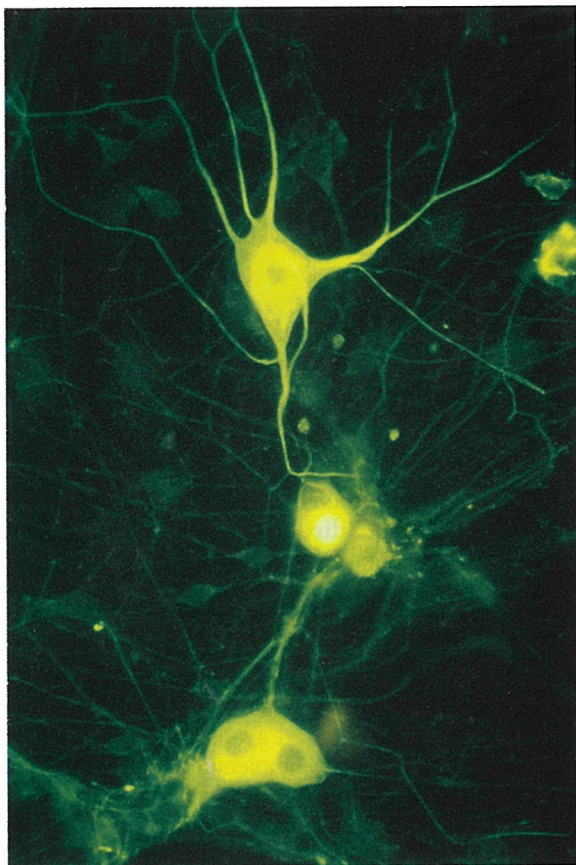


Figure 1-4 Example of direct immunocytochemistry. Cultured neurons from rat superior cervical ganglion were immunostained with fluorescent-labeled antibody specific for the insulin receptor. The bright areas correspond to sites where the antibody has bound to insulin receptors. The staining pattern indicates that receptors are located throughout the cytoplasm of the soma and processes but are missing from the nucleus. (From James S, Patel N, Thomas P, Burnstock G: Immunocytochemical localisation of insulin receptors on rat superior cervical ganglion neurons in dissociated cell culture. *J Anat* 182:95-100, 1993.)

track the synthesis and packaging of proteins. After the radiolabeled compound is injected into an animal, tissue specimens are taken at selected time intervals. The tissue is processed as usual and placed on a glass slide; however, instead of the tissue being sealed with a coverslip, a thin layer of photographic emulsion is placed over it. The tissue is placed in a dark box for a few days or weeks, during which time particles emitted from the radioactive isotope expose the emulsion over the cell sites where the isotope is located. The emulsion is developed and fixed by means of photographic techniques, and small silver grains are left over the exposed portions of the emulsion. The specimen then is sealed with a coverslip and viewed with a light micro-

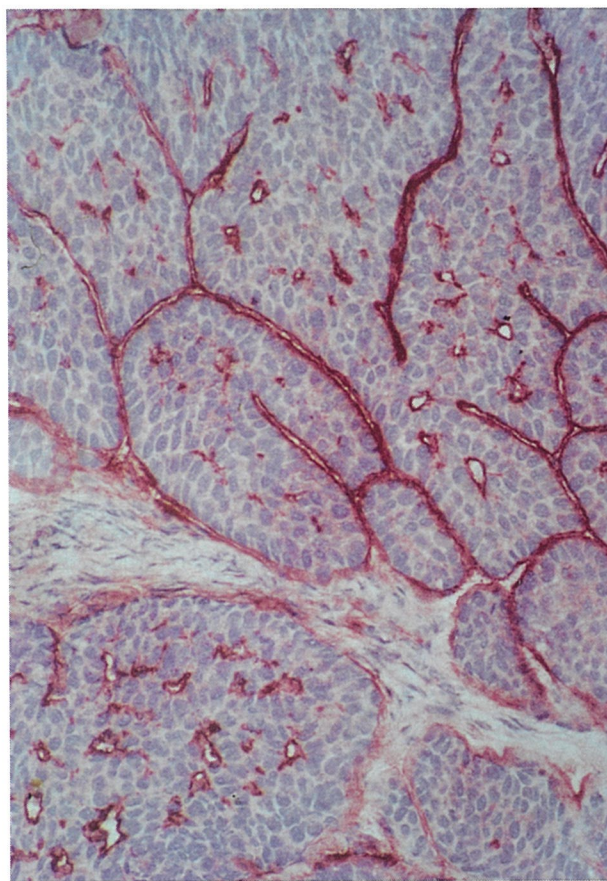


Figure 1-5 Indirect immunocytochemistry. Fluorescent antibodies were prepared against primary antibodies against type IV collagen, to demonstrate the presence of a continuous basal lamina at the interface between malignant clusters of cells and the surrounding connective tissue. (From Kopf-Maier P, Schroter-Kermani C: Distribution of type VII collagen in xenografted human carcinomas. *Cell Tissue Res* 272:395-405, 1993.)

scope. The silver grains are positioned over the regions of the specimen that incorporated the radioactive compound.

Autoradiography has been used to follow the time course of incorporation of tritiated proline into the basement membrane underlying endodermal cells of the yolk sac (see Fig. 1-6). An adaptation of the autoradiography method of electron microscopy has been used to show that the tritiated proline first appears in the cytosol of the endodermal cells, then travels to the rough endoplasmic reticulum, then to the Golgi apparatus, then into vesicles, and finally into the extracellular matrix (Fig. 1-7). In this manner, the sequence of events occurring in the synthesis of type IV collagen—the main protein in the lamina densa of the basal lamina—was visually demonstrated.

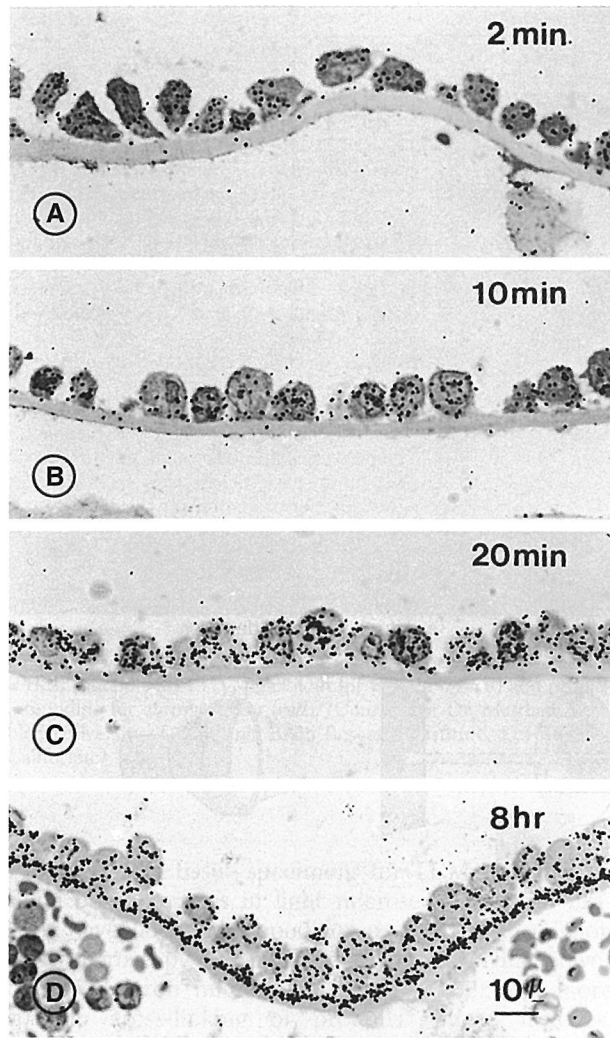


Figure 1-6 Autoradiography. Light microscopic examination of tritiated proline incorporation into the basement membrane as a function of time subsequent to tritiated proline injection (scale bar = 10 μ). In light micrographs A to C, the silver grains (black dots) are localized mostly in the endodermal cells; after 8 hours (light micrograph D), however, the silver grains are also localized in the basement membrane. The presence of silver grains indicates the location of tritiated proline. (From Mazariegos MR, Leblond CP, van der Rest M: Radioautographic tracing of ^3H -proline in endodermal cells of the parietal yolk sac as an indicator of the biogenesis of basement membrane components. *Am J Anat* 179:79-93, 1987.)

CONFOCAL MICROSCOPY

Confocal microscopy relies on a laser beam for the light source and a pinhole screen to eliminate undesirable reflected light from being observed. Thus, the only light that can be observed is that which is located at the focal point of the objective lens, making the pinhole conjugate of the focal point.

In confocal microscopy, a laser beam passes through a dichroic mirror to be focused on the specimen by two motorized mirrors whose movements are computer-controlled to scan the beam along the sample. Because the sample is treated by fluorescent dyes, the impinging laser beam causes the emission of light from the dyes. The emitted light follows the same path taken by the laser beam, but in the opposite direction, and the dichroic mirror focuses this emitted light on a pinhole in a plate. A photomultiplier tube collects the emitted light passing through the pinhole while the plate containing the pinhole blocks all the extraneous light that would create a fuzzy image. It must be remembered that the light emerging from the pinhole at any particular moment in time represents a single point in the sample, and as the laser beam scans across the sample additional individual points are collected by the photomultiplier tube. All of these points gathered by the photomultiplier tube are then compiled by a computer, forming a composite image one pixel at a time (Fig. 1-8). Since the depth of field is very small (only a thin layer of the sample is observed at any one scan), the scanning may be repeated at deeper and deeper levels in the sample, allowing the compilation of a very good three-dimensional image (Fig. 1-9).

ELECTRON MICROSCOPY

The use of electrons as a light source in electron microscopy permits the achievement of much greater magnification and resolution than that realized by light microscopy.

In light microscopes, optical lenses focus visible light (a beam of photons). In electron microscopes, electromagnets serve the function of focusing a beam of electrons. Because the wavelength of an electron beam is much shorter than that of visible light, electron microscopes theoretically are capable of resolving two objects separated by 0.005 nm. In practice, however, the resolution of the **transmission electron microscope** is about 0.2 nm, which is still more than a thousand-fold greater than the resolution of the compound light microscope. The resolution of the **scanning electron microscope** is about 10 nm, considerably less than that of the transmission electron microscope. Moreover, modern electron microscopes can magnify an object as much as 150,000 times; this magnification is powerful enough to see individual macromolecules such as DNA and myosin.

Transmission Electron Microscopy

Transmission electron microscopy (TEM) uses much thinner sections compared with light microscopy and requires heavy metal precipitation techniques rather than water-soluble stains to stain tissues.

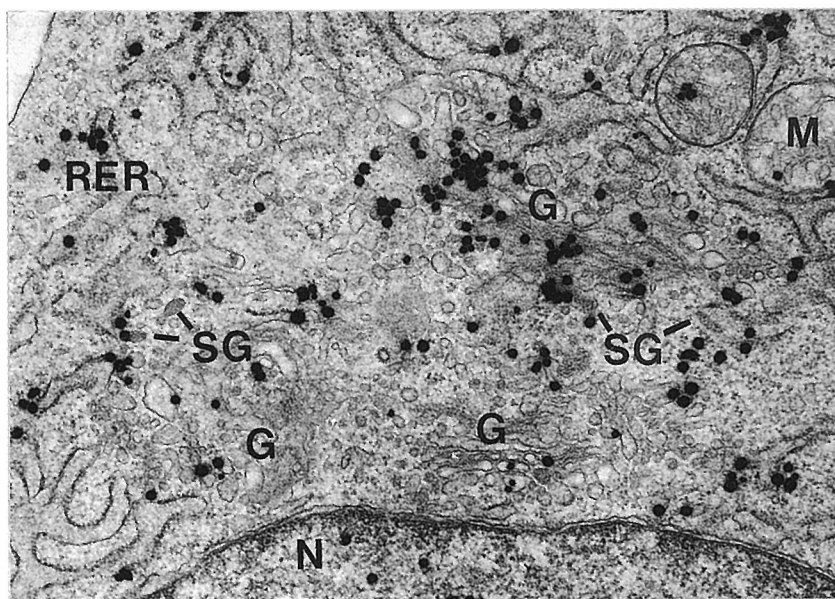


Figure 1-7 Autoradiography. In this electron micrograph of a yolk sac endodermal cell, silver grains (similar to those in Figure 1-6), representing the presence of tritiated proline, are evident overlying the rough endoplasmic reticulum (RER), Golgi apparatus (G), and secretory granules (SG). Type IV collagen, which is rich in proline, is synthesized in endodermal cells and released into the basement membrane. The tritiated proline is most concentrated in organelles involved in protein synthesis. M, mitochondria; N, nucleus. (From Mazariegos MR, Leblond CP, van der Rest M: Radioautographic tracing of ^3H -proline in endodermal cells of the parietal yolk sac as an indicator of the biogenesis of basement membrane components. *Am J Anat* 179:79-93, 1987.)

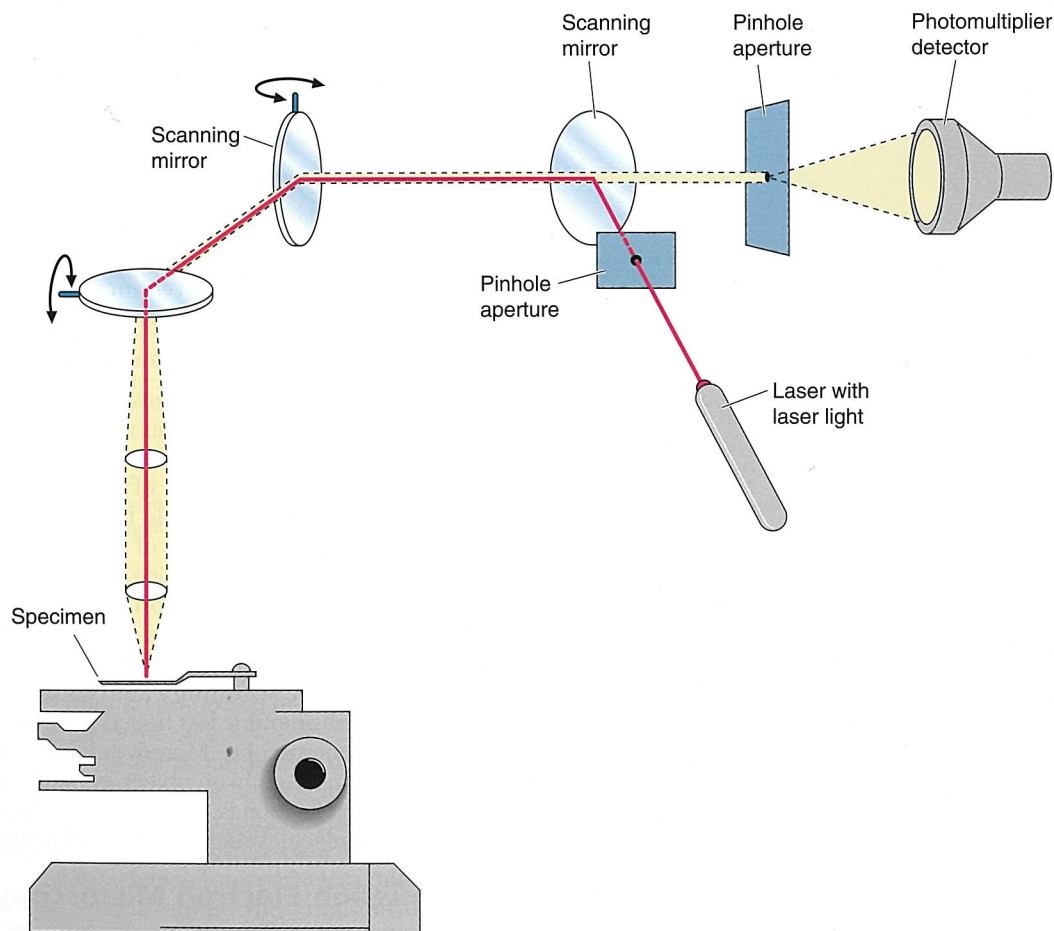


Figure 1-8 Confocal microscopy. A laser beam passes through a dichroic mirror to be focused on the specimen by two motorized mirrors whose movements are computer-controlled to scan the beam along the sample. The light emerging from the pinhole at any particular moment in time represents a single point in the sample, and as the laser beam scans across the sample additional individual points are collected by the photomultiplier tube. All the points are computer-assembled to produce the final confocal image.

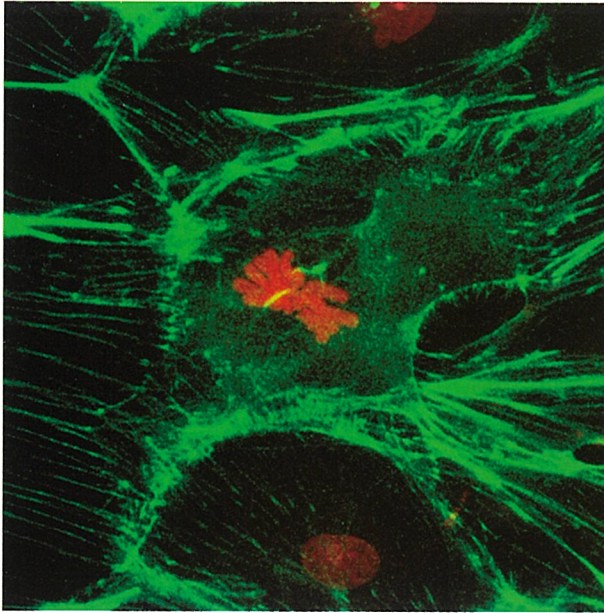


Figure 1-9 Confocal image of a metaphase Kangaroo rat cell (PtK2) stained with FITC-phalloidin for F-actin (*green*) and propidium iodide for chromosomes (*red*). (Courtesy of Dr. Matthew Schibler, University of California Brain Research Institute, Los Angeles, California.)

Preparation of tissue specimens for TEM involves the same basic steps as in light microscopy. Special fixatives have been developed for use with transmission light microscopy, because the greater resolving power of the electron microscope requires finer and more specific cross-linking of proteins. These fixatives, which include buffered solutions of **glutaraldehyde**, **paraformaldehyde**, **osmium tetroxide**, and **potassium permanganate**, not only preserve fine structural details but also act as electron-dense stains, which permit observation of the tissue with the electron beam.

Because these fixatives penetrate fresh tissues even less than fixatives for light microscopy, relatively small pieces of tissues are infiltrated in large volumes of fixatives. Tissue blocks for TEM are usually no larger than 1 mm³. Suitable embedding media have been developed, such as epoxy resin, so that plastic-embedded tissues may be cut into extremely thin (ultra-thin) sections (25 to 100 nm) that do not absorb the beam of electrons.

Electron beams are produced in an evacuated chamber by heating a tungsten filament, the **cathode**. The electrons then are attracted to the positively charged **anode**, a donut-shaped metal plate with a central hole. With a charge differential of about 60,000 volts placed between the cathode and the anode, the

electrons that pass through the hole in the anode have high kinetic energy.

The electron beam is focused on the specimen by the use of electromagnets, which are analogous to the condenser lens of a light microscope (see Fig. 1-1). Because the tissue is stained with heavy metals that precipitate preferentially on lipid membranes, the electrons lose some of their kinetic energy as they interact with the tissue. The heavier the metal encountered by an electron, the less energy the electron will retain.

The electrons leaving the specimen are subjected to the electromagnetic fields of several additional electromagnets, which focus the beam on a fluorescent plate. As the electrons hit the fluorescent plate, their kinetic energy is converted into points of light, whose intensity is a direct function of the electron's kinetic energy. You can make a permanent record of the resultant image by substituting an electron-sensitive film in place of the fluorescent plate and by producing a negative from which a black and white photomicrograph can be printed.

Freeze-Fracture Technique

The macromolecular structure of the internal aspects of membranes is revealed by the freeze-fracture technique (Fig. 1-10). Quick-frozen specimens that have been treated with cryopreservatives do not develop ice crystals during the freezing process; hence, the tissue does not suffer mechanical damage. As the frozen specimen is hit by a super-cooled razor blade, it fractures along cleavage planes, which are regions of least molecular bonding; in cells, fracture frequently occurs between the inner and outer leaflets of membranes.

The fracture face is coated at an angle by evaporated platinum and carbon, forming accumulations of platinum on one side of a projection and no accumulation on the opposite side next to the projection, thus generating a replica of the surface. The tissue is then digested away, and the replica is examined by TEM. This method allows display of the transmembrane proteins of cellular membranes.

Scanning Electron Microscopy

Scanning electron microscopy (SEM) provides a three-dimensional image of the specimen.

Unlike TEM, SEM is used to view the surface of a solid specimen. Using this technique, you can view a three-dimensional image of the object. Usually, the object to be viewed is prepared in a special manner that permits a thin layer of heavy metal, such as gold, to be deposited on its surface.

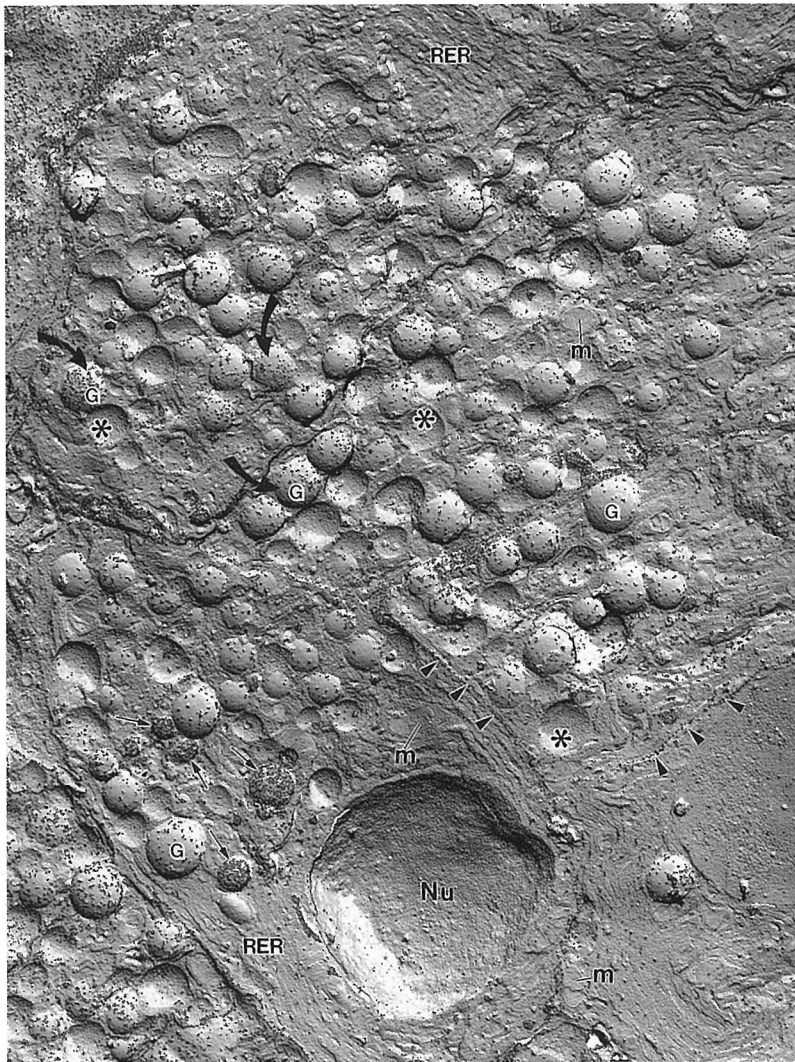
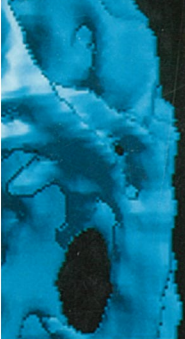


Figure 1-10 Cytochemistry and freeze-fracture. Fracture-label replica of an acinar cell of the rat pancreas. *N*-acetyl-D-galactosamine residues were localized by the use of *Helix pomatia* lectin-gold complex, which appears as black dots in the image. Arrowheads indicate cell membranes. The nucleus (Nu) appears as a depression, the rough endoplasmic reticulum (RER) as parallel lines, and secretory granules as small elevations or depressions. The elevations (G) represent the E-face half, and the depressions (asterisks) represent the P-face of the membrane of the secretory granule. m, mitochondria. (From Kan FWK, Bendayan M: Topographical and planar distribution of *Helix pomatia* lectin-binding glycoconjugates in secretory granules and plasma membrane of pancreatic acinar cells of the rat: Demonstration of membrane heterogeneity. *Am J Anat* 185:165-176, 1989.)

or palladium, to be deposited on the specimen's surface.

As a beam of electrons scans the surface of the object, some (backscatter electrons) are reflected and others (secondary electrons) are ejected from the heavy metal coat. The backscatter and secondary electrons are

captured by electron detectors that are interpreted, collated, and displayed on a monitor as a three-dimensional image (see Fig. 1-1). You can make the image permanent either by photographing it or digitizing it for storage in a computer.



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