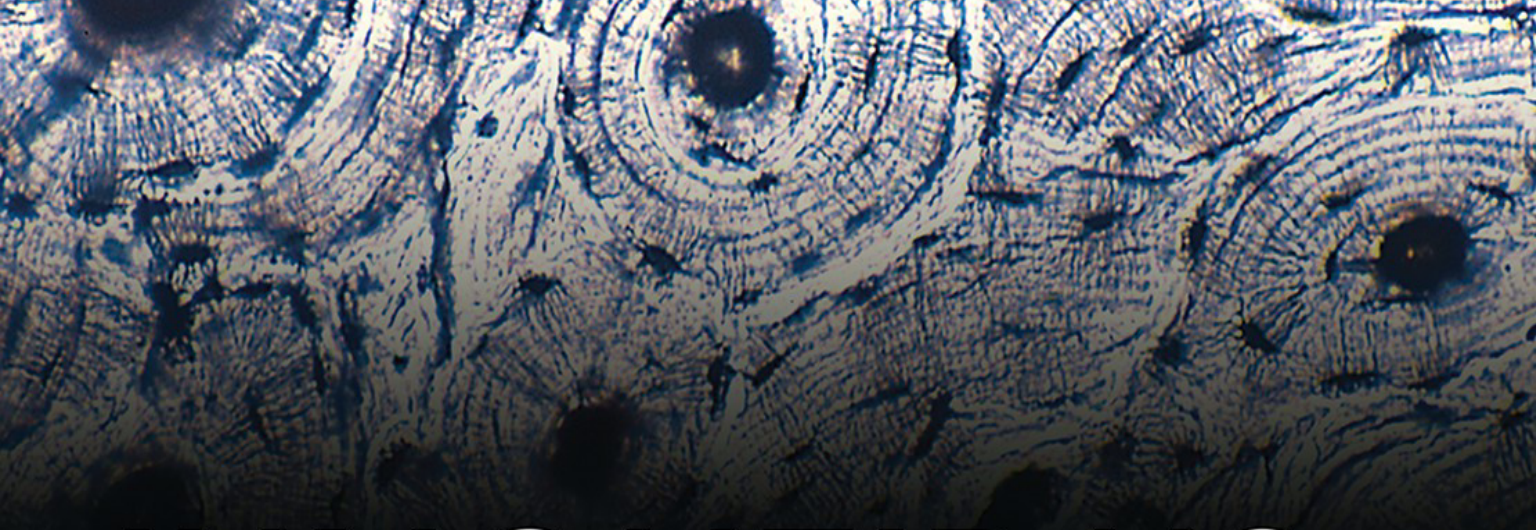
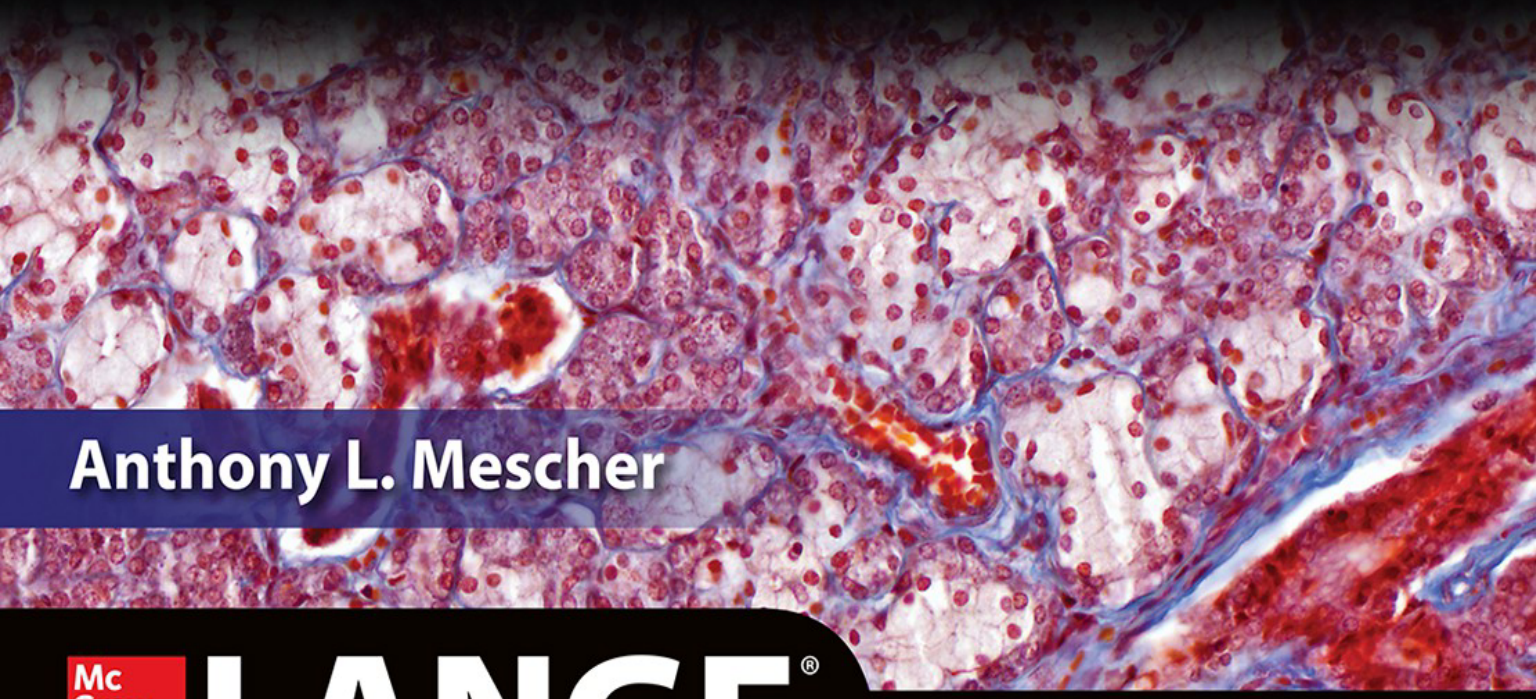


JUNQUEIRA'S Basic Histology

Text and Atlas

Fourteenth Edition



Anthony L. Mescher

Mc Graw Hill Education

LANGE®

FOURTEENTH EDITION

Junqueira's Basic Histology

TEXT AND ATLAS

Anthony L. Mescher, PhD

Professor of Anatomy and Cell Biology
Indiana University School of Medicine
Bloomington, Indiana

Mc Graw Hill Education

New York Chicago San Francisco Athens London Madrid Mexico City
Milan New Delhi Singapore Sydney Toronto

ISBN: 978-0-07-184268-6

MHID: 0-07-184268-3

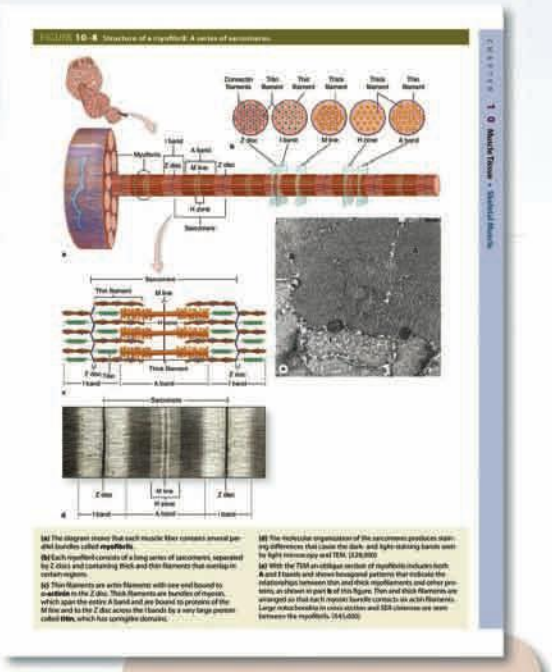
The material in this eBook also appears in the print version of this title: ISBN: 978-0-07-184270-9,
MHID: 0-07-184270-5.

eBook conversion by codeMantra
Version 1.0

Contents

Key Features of Junqueira's Basic Histology, Fourteenth Edition:

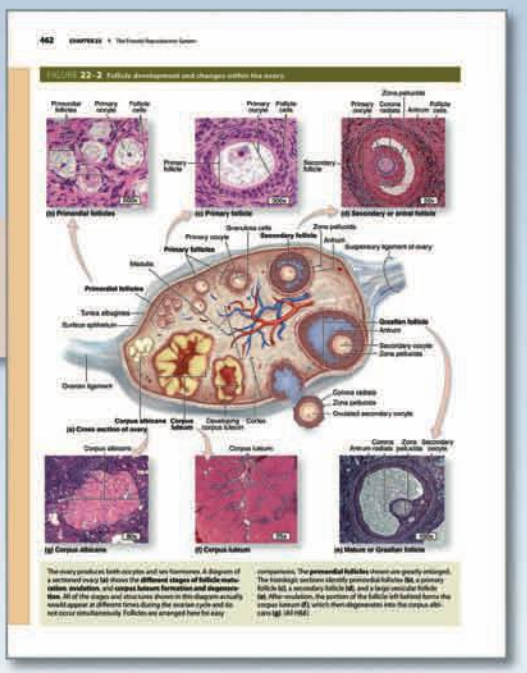
- **Recognized for more than three decades** as the most authoritative, comprehensive, and effective approach to understanding medical histology
- **Unmatched** in its ability to explain the relationship between cell and tissue structure and their function in the human body
- **Updated** to the latest research and developments on each topic
- **New and additional Medical Applications** throughout each chapter provide clinical relevance for every subject



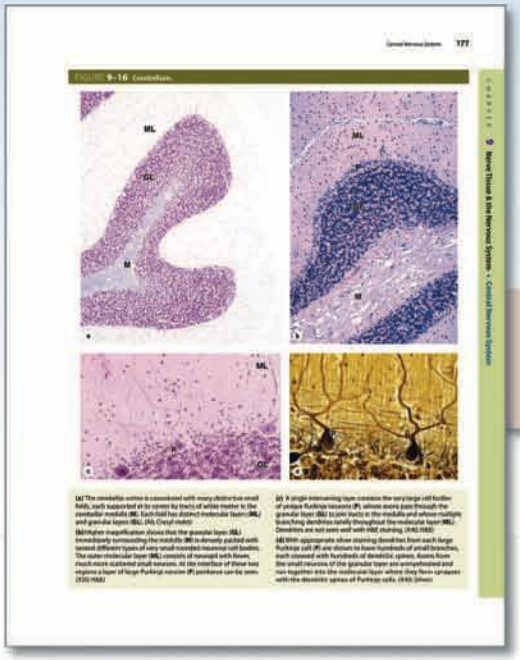
1,500+ illustrations bring important concepts to life

- **New design** incorporates full color for each figure and table in addition to color micrographs of each cell and tissue
- **Figure legends** summarize and provide easy access to key points of each topic
- **Author Anthony L. Mescher, Ph.D.** has over 30 years' experience teaching cell biology and histology to medical students
- **Summaries of Key Points** at the end of each chapter list main points concisely for very easy review
- **Many new tables** help integrate each histological topic with relevant physiological aspects of the system
- **New, detailed tables of contents** within each chapter.

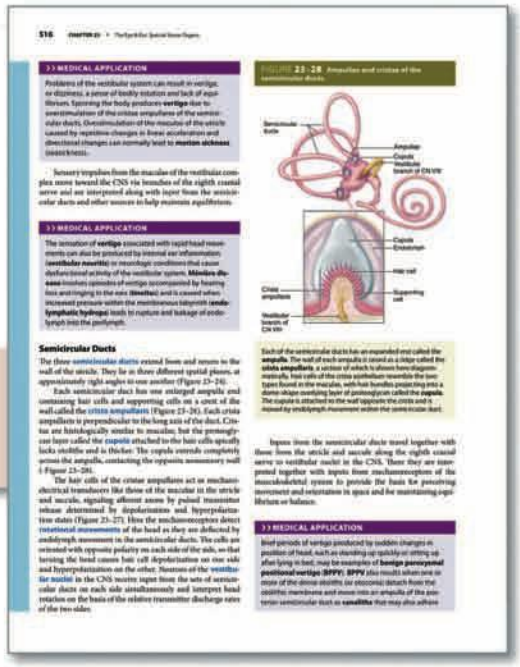
Student-suggested detailed legends



State-of-the art micrographs are the core of an all-new art program



Medical Applications are incorporated into every chapter



Preface

With this 14th edition, *Junqueira's Basic Histology* continues as the preeminent source of **concise yet thorough** information on human tissue structure and function. For nearly 45 years this educational resource has met the needs of learners for a well-organized and concise presentation of **cell biology and histology** that integrates the material with that of **biochemistry, immunology, endocrinology, and physiology** and provides an excellent foundation for subsequent studies in **pathology**. The text is prepared specifically for students of medicine and other health-related professions, as well as for advanced undergraduate courses in tissue biology. As a result of its value and appeal to students and instructors alike, *Junqueira's Basic Histology* has been translated into a dozen different languages and is used by medical students throughout the world.

This edition now includes with each chapter a set of multiple-choice **Self-Test Questions** that allow readers to assess their comprehension and knowledge of important material in that chapter. At least a few questions in each set utilize clinical vignettes or cases to provide context for framing the medical relevance of concepts in basic science, as recommended by the US National Board of Medical Examiners. As with the last edition, each chapter also includes a **Summary of Key Points** designed to guide the students concerning what is clearly important and what is less so. **Summary Tables** in each chapter organize and condense important information, further facilitating efficient learning.

Each chapter has been revised and shortened, while coverage of specific topics has been expanded as needed. Study is facilitated by modern page design. Inserted throughout each chapter are more numerous, short paragraphs that indicate how the information presented can be used medically and which emphasize the foundational relevance of the material learned.

The art and other figures are presented in each chapter, with the goal to simplify learning and integration with related material. The McGraw-Hill medical illustrations, now used throughout the text and supplemented by numerous animations in the electronic version of the text, are the most useful, thorough, and attractive of any similar medical textbook. Electron and light micrographs have been replaced throughout the book as needed, and again make up a complete atlas of cell, tissue, and organ structures fully compatible with the students' own collection of glass or digital slides. A virtual microscope with over 150 slides of all human tissues and organs is available: http://medsci.indiana.edu/junqueira/virtual/junqueira.htm.

As with the previous edition, the book facilitates learning by its **organization**:

- An opening chapter reviews the **histological techniques** that allow understanding of cell and tissue structure.
- Two chapters then summarize the structural and functional organization of **human cell biology**, presenting the cytoplasm and nucleus separately.
- The next seven chapters cover the **four basic tissues** that make up our organs: epithelia, connective tissue (and its major sub-types), nervous tissue, and muscle.
- Remaining chapters explain the organization and functional significance of these tissues in **each of the body's organ systems**, closing with up-to-date consideration of cells in the eye and ear.

For additional review of what's been learned or to assist rapid assimilation of the material in *Junqueira's Basic Histology*, McGraw-Hill has published a set of 200 full-color ***Basic Histology Flash Cards***, Anthony Mescher author. Each card includes images of key structures to identify, a summary of important facts about those structures, and a clinical comment. This valuable learning aid is available as a set of actual cards from Amazon.com, or as an app for smart phones or tablets from the online App Store.

With its proven strengths and the addition of new features, I am confident that *Junqueira's Basic Histology* will continue as one of the most valuable and most widely read educational resources in histology. Users are invited to provide feedback to the author with regard to any aspect of the book's features.

Anthony L. Mescher
Indiana University School of Medicine
mescher@indiana.edu

Acknowledgments

I wish to thank the students at Indiana University School of Medicine and the undergraduates at Indiana University with whom I have studied histology and cell biology for over 30 years and from whom I have learned much about presenting basic concepts most effectively. Their input has greatly helped in the task of maintaining and updating the presentations in this classic textbook. As with the last edition the help of Sue Childress and Dr. Mark Braun was invaluable in slide preparation and the virtual microscope for human histology respectively.

A major change in this edition is the inclusion of self-assessment questions with each topic/chapter. Many of these questions were used in my courses, but others are taken or modified from a few of the many excellent review books published by McGraw-Hill/Lange for students preparing to take the U.S. Medical Licensing Examination. These include *Histology and Cell Biology: Examination and Board Review*, by Douglas Paulsen; *USMLE Road Map: Histology*, by Harold Sheedlo; and *Anatomy, Histology, & Cell Biology: PreTest Self-Assessment & Review*, by Robert Klein and George Enders. The use here of questions from these valuable resources is gratefully acknowledged. Students are referred to those review books for hundreds of additional self-assessment questions.

I am also grateful to my colleagues and reviewers from throughout the world who provided specialized expertise or original photographs, which are also acknowledged in figure captions. I thank those professors and students in the United States, as well as Argentina, Canada, Iran, Ireland, Italy, Pakistan, and Syria, who provided useful suggestions that have improved the new edition of *Junqueira's Basic Histology*. Finally, I am pleased to acknowledge the help and collegiality provided by the staff of McGraw-Hill, especially editors Michael Weitz and Brian Kearns, whose work made possible publication of this 14th edition of *Junqueira's Basic Histology*.

Anthony L. Mescher
Indiana University School of Medicine
mescher@indiana.edu

CHAPTER 1

Histology & Its Methods of Study

Histology is the study of the tissues of the body and how these tissues are arranged to constitute organs. This subject involves all aspects of tissue biology, with the focus on how cells' structure and arrangement optimize functions specific to each organ.

Tissues have two interacting components: cells and extracellular matrix (ECM). The ECM consists of many kinds of macromolecules, most of which form complex structures, such as collagen fibrils. The ECM supports the cells and contains the fluid transporting nutrients to the cells, and carrying away their wastes and secretory products. Cells produce the ECM locally and are in turn strongly influenced by matrix molecules. Many matrix components bind to specific cell surface receptors that span the cell membranes and connect to structural components inside the cells, forming a continuum in which cells and the ECM function together in a well-coordinated manner.

During development, cells and their associated matrix become functionally specialized and give rise to fundamental types of tissues with characteristic structural features. Organs are formed by an orderly combination of these tissues, and their precise arrangement allows the functioning of each organ and of the organism as a whole.

The small size of cells and matrix components makes histology dependent on the use of microscopes and molecular methods of study. Advances in biochemistry, molecular biology, physiology, immunology, and pathology are essential for a better knowledge of tissue biology. Familiarity with the tools and methods of any branch of science is essential for a proper understanding of the subject. This chapter reviews common methods used to study cells and tissues, focusing on microscopic approaches.

PREPARATION OF TISSUES FOR STUDY

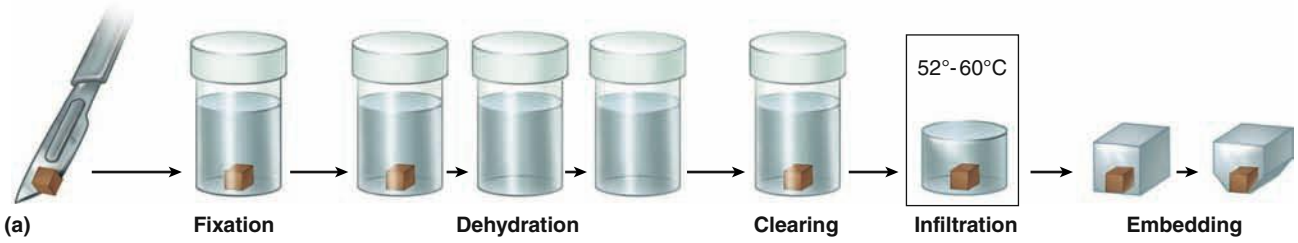
The most common procedure used in histologic research is the preparation of tissue slices or "sections" that can be examined visually with transmitted light. Because most tissues and organs are too thick for light to pass through, thin translucent sections are cut from them and placed on glass slides for microscopic examination of the internal structures.

The ideal microscopic preparation is preserved so that the tissue on the slide has the same structural features it had in the body. However, this is often not feasible because the preparation process can remove cellular lipid, with slight distortions of cell structure. The basic steps used in tissue preparation for light microscopy are shown in Figure 1–1.

Fixation

To preserve tissue structure and prevent degradation by enzymes released from the cells or microorganisms, pieces of

FIGURE 1–1 Sectioning fixed and embedded tissue.



Most tissues studied histologically are prepared as shown, with this sequence of steps **(a)**:

- **Fixation:** Small pieces of tissue are placed in solutions of chemicals that cross-link proteins and inactivate degradative enzymes, which preserves cell and tissue structure.
- **Dehydration:** The tissue is transferred through a series of increasingly concentrated alcohol solutions, ending in 100%, which removes all water.
- **Clearing:** Alcohol is removed in organic solvents in which both alcohol and paraffin are miscible.
- **Infiltration:** The tissue is then placed in melted paraffin until it becomes completely infiltrated with this substance.
- **Embedding:** The paraffin-infiltrated tissue is placed in a small mold with melted paraffin and allowed to harden.
- **Trimming:** The resulting paraffin block is trimmed to expose the tissue for sectioning (slicing) on a microtome.

Similar steps are used in preparing tissue for transmission electron microscopy (TEM), except special fixatives and dehydrating solutions are used with smaller tissue samples and embedding involves epoxy resins which become harder than paraffin to allow very thin sectioning.

(b) A **microtome** is used for sectioning paraffin-embedded tissues for light microscopy. The trimmed tissue specimen is mounted in the paraffin block holder, and each turn of the drive wheel by the histologist advances the holder a controlled distance, generally from 1 to 10 µm. After each forward move, the tissue block passes over the steel knife edge and a section is cut at a thickness equal to the distance the block advanced. The paraffin sections are placed on glass slides and allowed to adhere, deparaffinized, and stained for light microscope study. For TEM, sections less than 1 µm thick are prepared from resin-embedded cells using an ultramicrotome with a glass or diamond knife.

organs are placed as soon as possible after removal from the body in solutions of stabilizing or cross-linking compounds called **fixatives**. Because a fixative must fully diffuse through the tissues to preserve all cells, tissues are usually cut into small fragments before fixation to facilitate penetration. To improve cell preservation in large organs fixatives are often introduced via blood vessels, with vascular perfusion allowing fixation rapidly throughout the tissues.

One widely used fixative for light microscopy is formalin, a buffered isotonic solution of 37% formaldehyde. Both this compound and glutaraldehyde, a fixative used for electron microscopy, react with the amine groups (NH_2) of proteins, preventing their degradation by common proteases. Glutaraldehyde also cross-links adjacent proteins, reinforcing cell and ECM structures.

Electron microscopy provides much greater magnification and resolution of very small cellular structures and fixation must be done very carefully to preserve additional "ultrastructural" detail. Typically in such studies glutaraldehyde-treated tissue is then immersed in buffered osmium tetroxide, which preserves (and stains) cellular lipids as well as proteins.

Embedding & Sectioning

To permit thin sectioning fixed tissues are infiltrated and embedded in a material that imparts a firm consistency. Embedding materials include paraffin, used routinely for light microscopy, and plastic resins, which are adapted for both light and electron microscopy.

Before infiltration with such media the fixed tissue must undergo **dehydration** by having its water extracted gradually by transfers through a series of increasing ethanol solutions, ending in 100% ethanol. The ethanol is then replaced by an organic solvent miscible with both alcohol and the embedding medium, a step referred to as **clearing** because infiltration with the reagents used here gives the tissue a translucent appearance.

The fully cleared tissue is then placed in melted paraffin in an oven at 52°-60°C, which evaporates the clearing solvent and promotes **infiltration** of the tissue with paraffin, and then **embedded** by allowing it to harden in a small container of paraffin at room temperature. Tissues to be embedded with plastic resin are also dehydrated in ethanol and then infiltrated with plastic solvents that harden when cross-linking polymerizers are added. Plastic embedding avoids the higher temperatures needed with paraffin, which helps avoid tissue distortion.

The hardened block with tissue and surrounding embedding medium is trimmed and placed for sectioning in an instrument called a **microtome** (Figure 1–1). Paraffin sections are typically cut at 3-10 µm thickness for light microscopy, but electron microscopy requires sections less than 1 µm thick. One micrometer (1 µm) equals 1/1000 of a millimeter (mm) or 10^{-6} m. Other spatial units commonly used in microscopy are the nanometer (1 nm = 0.001 µm = 10^{-6} mm = 10^{-9} m) and angstrom (1 Å = 0.1 nm or 10^{-4} µm). The sections are placed on glass slides and stained for light microscopy or on metal grids for electron microscopic staining and examination.

›› MEDICAL APPLICATION

Biopsies are tissue samples removed during surgery or routine medical procedures. In the operating room, biopsies are fixed in vials of formalin for processing and microscopic analysis in a pathology laboratory. If results of such analyses are required before the medical procedure is completed, for example to know whether a growth is malignant before the patient is closed, a much more rapid processing method is used. The biopsy is rapidly frozen in liquid nitrogen, preserving cell structures and making the tissue hard and ready for sectioning. A microtome called a **cryostat** in a cabinet at subfreezing temperature is used to section the block with tissue, and the frozen sections are placed on slides for rapid staining and microscopic examination by a pathologist.

Freezing of tissues is also effective in histochemical studies of very sensitive enzymes or small molecules because freezing, unlike fixation, does not inactivate most enzymes. Finally, because clearing solvents often dissolve cell lipids in fixed tissues, frozen sections are also useful when structures containing lipids are to be studied histologically.

Staining

Most cells and extracellular material are completely colorless, and to be studied microscopically tissue sections must be stained (dyed). Methods of staining have been devised that make various tissue components not only conspicuous but also distinguishable from one another. Dyes stain material more or less selectively, often behaving like acidic or basic compounds and forming electrostatic (salt) linkages with ionizable radicals of macromolecules in tissues. Cell components such as nucleic acids with a net negative charge (anionic) have an affinity for basic dyes and are termed **basophilic**; cationic components, such as proteins with many ionized amino groups, stain more readily with acidic dyes and are termed **acidophilic**.

Examples of basic dyes include toluidine blue, alcian blue, and methylene blue. Hematoxylin behaves like a basic dye, staining basophilic tissue components. The main tissue components that ionize and react with basic dyes do so because of acids in their composition (DNA, RNA, and glycosaminoglycans). Acid dyes (eg, eosin, orange G, and acid fuchsin) stain the acidophilic components of tissues such as mitochondria, secretory granules, and collagen.

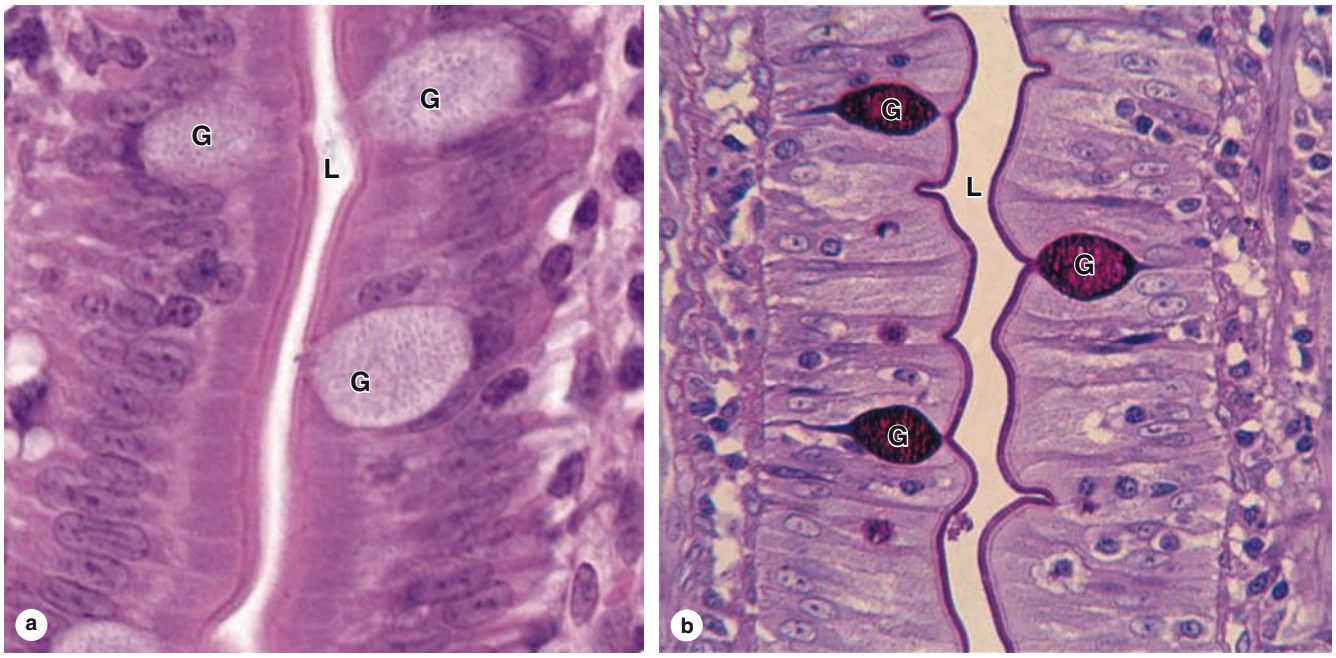
Of all staining methods, the simple combination of **hematoxylin and eosin (H&E)** is used most commonly. Hematoxylin stains DNA in the cell nucleus, RNA-rich portions of the cytoplasm, and the matrix of cartilage, producing a dark blue or purple color. In contrast, eosin stains other cytoplasmic structures and collagen pink (Figure 1–2a). Here eosin is considered a **counterstain**, which is usually a single dye applied separately to distinguish additional features of a tissue. More complex procedures, such as trichrome stains (eg, Masson trichrome), allow greater distinctions among various extracellular tissue components.

The **periodic acid-Schiff (PAS) reaction** utilizes the hexose rings of polysaccharides and other carbohydrate-rich tissue structures and stains such macromolecules distinctly purple or magenta. Figure 1–2b shows an example of cells with carbohydrate-rich areas well-stained by the PAS reaction. The DNA of cell nuclei can be specifically stained using a modification of the PAS procedure called the Feulgen reaction.

Basophilic or PAS-positive material can be further identified by enzyme digestion**,** pretreatment of a tissue section with an enzyme that specifically digests one substrate. For example, pretreatment with ribonuclease will greatly reduce cytoplasmic basophilia with little overall effect on the nucleus, indicating the importance of RNA for the cytoplasmic staining.

Lipid-rich structures of cells are revealed by avoiding the processing steps that remove lipids, such as treatment with heat and organic solvents, and staining with **lipid-soluble dyes** such as **Sudan black,** which can be useful in diagnosis of metabolic diseases that involve intracellular accumulations of cholesterol, phospholipids, or glycolipids. Less common methods of staining can employ **metal impregnation** techniques, typically using solutions of silver salts to visual certain ECM fibers and specific cellular elements in nervous tissue. The Appendix lists important staining procedures used for most of the light micrographs in this book.

FIGURE **1–2** **Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining.**



Micrographs of epithelium lining the small intestine, **(a)** stained with H&E, and **(b)** stained with the PAS reaction for glycoproteins. With H&E, basophilic cell nuclei are stained purple while cytoplasm stains pink. Cell regions with abundant oligosaccharides on glycoproteins, such as the ends of the cells at the lumen **(L)** or the scattered mucus-secreting goblet cells **(G)**, are poorly stained. With PAS, however, cell staining is most intense at the lumen, where projecting microvilli have a prominent layer of glycoproteins at the lumen (L) and in the mucin-rich secretory granules of goblet cells. Cell surface glycoproteins and mucin are PAS-positive because of their high content of oligosaccharides and polysaccharides respectively. The PAS-stained tissue was counterstained with hematoxylin to show the cell nuclei. (a. X400; b. X300)

Slide preparation, from tissue fixation to observation with a light microscope, may take from 12 hours to 2½ days, depending on the size of the tissue, the embedding medium, and the method of staining. The final step before microscopic observation is mounting a protective glass coverslip on the slide with clear adhesive.

LIGHT MICROSCOPY

Conventional bright-field microscopy, as well as more specialized applications like fluorescence, phase-contrast, confocal, and polarizing microscopy, are all based on the interaction of light with tissue components and are used to reveal and study tissue features.

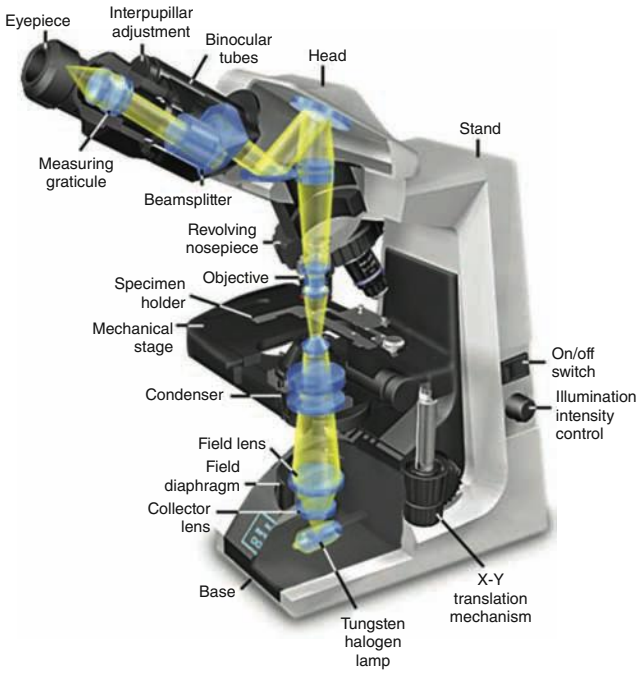
Bright-Field Microscopy

With the **bright-field microscope** stained tissue is examined with ordinary light passing through the preparation. As shown in Figure 1–3, the microscope includes an optical system and mechanisms to move and focus the specimen. The optical components are the **condenser** focusing light on the object to be studied; the **objective** lens enlarging and projecting the image of the object toward the observer; and the **eyepiece** (or ocular lens) further magnifying this image and projecting it onto the viewer's retina or a charge-coupled device (CCD) highly sensitive to low light levels with a camera and monitor. The total magnification is obtained by multiplying the magnifying power of the objective and ocular lenses.

The critical factor in obtaining a crisp, detailed image with a light microscope is its **resolving power**, defined as the smallest distance between two structures at which they can be seen as separate objects. The maximal resolving power of the light microscope is approximately 0.2 µm, which can permit clear images magnified 1000-1500 times. Objects smaller or thinner than 0.2 µm (such as a single ribosome or cytoplasmic microfilament) cannot be distinguished with this instrument. Likewise, two structures such as mitochondria will be seen as only one object if they are separated by less than 0.2 µm. The microscope's resolving power determines the quality of the image, its clarity and richness of detail, and depends mainly on the quality of its objective lens. Magnification is of value only when accompanied by high resolution. Objective lenses providing higher magnification are designed to also have higher resolving power. The eyepiece lens only enlarges the image obtained by the objective and does not improve resolution.

Virtual microscopy, typically used for study of bright-field microscopic preparations, involves the conversion of a

FIGURE 1–3 Components and light path of a bright-field microscope.



Photograph of a bright-field light microscope showing its mechanical components and the pathway of light from the substage lamp to the eye of the observer. The optical system has three sets of lenses:

- The **condenser** collects and focuses a cone of light that illuminates the tissue slide on the stage.
- **Objective** lenses enlarge and project the illuminated image of the object toward the eyepiece. Interchangeable objectives with different magnifications routinely used in histology include X4 for observing a large area (field) of the tissue at low magnification; X10 for medium magnification of a smaller field; and X40 for high magnification of more detailed areas.
- The two **eyepieces** or oculars magnify this image another X10 and project it to the viewer, yielding a total magnification of X40, X100, or X400.

(*Used with permission from Nikon Instruments.*)

stained tissue preparation to high-resolution digital images and permits study of tissues using a computer or other digital device, without an actual stained slide or a microscope. In this technique regions of a glass-mounted specimen are captured digitally in a grid-like pattern at multiple magnifications using a specialized slide-scanning microscope and saved as thousands of consecutive image files. Software then converts this dataset for storage on a server using a format that allows access, visualization, and navigation of the original slide with common web browsers or other devices. With advantages in cost and ease of use, virtual microscopy is rapidly replacing light microscopes and collections of glass slides in histology laboratories for students.

Fluorescence Microscopy

When certain cellular substances are irradiated by light of a proper wavelength, they emit light with a longer wavelength—a phenomenon called **fluorescence**. In **fluorescence microscopy**, tissue sections are usually irradiated with ultraviolet (UV) light and the emission is in the visible portion of the spectrum. The fluorescent substances appear bright on a dark background. For fluorescent microscopy the instrument has a source of UV or other light and filters that select rays of different wavelengths emitted by the substances to be visualized.

Fluorescent compounds with affinity for specific cell macromolecules may be used as fluorescent stains. Acridine orange, which binds both DNA and RNA, is an example. When observed in the fluorescence microscope, these nucleic acids emit slightly different fluorescence, allowing them to be localized separately in cells (Figure 1–4a). Other compounds such as DAPI and Hoechst stain specifically bind DNA and are used to stain cell nuclei, emitting a characteristic blue fluorescence under UV. Another important application of fluorescence microscopy is achieved by coupling compounds such as fluorescein to molecules that will specifically bind to certain cellular components and thus allow the identification of these structures under the microscope (Figure 1–4b). Antibodies labeled with fluorescent compounds are extremely important in immunohistologic staining. (See the section Visualizing Specific Molecules.)

Phase-Contrast Microscopy

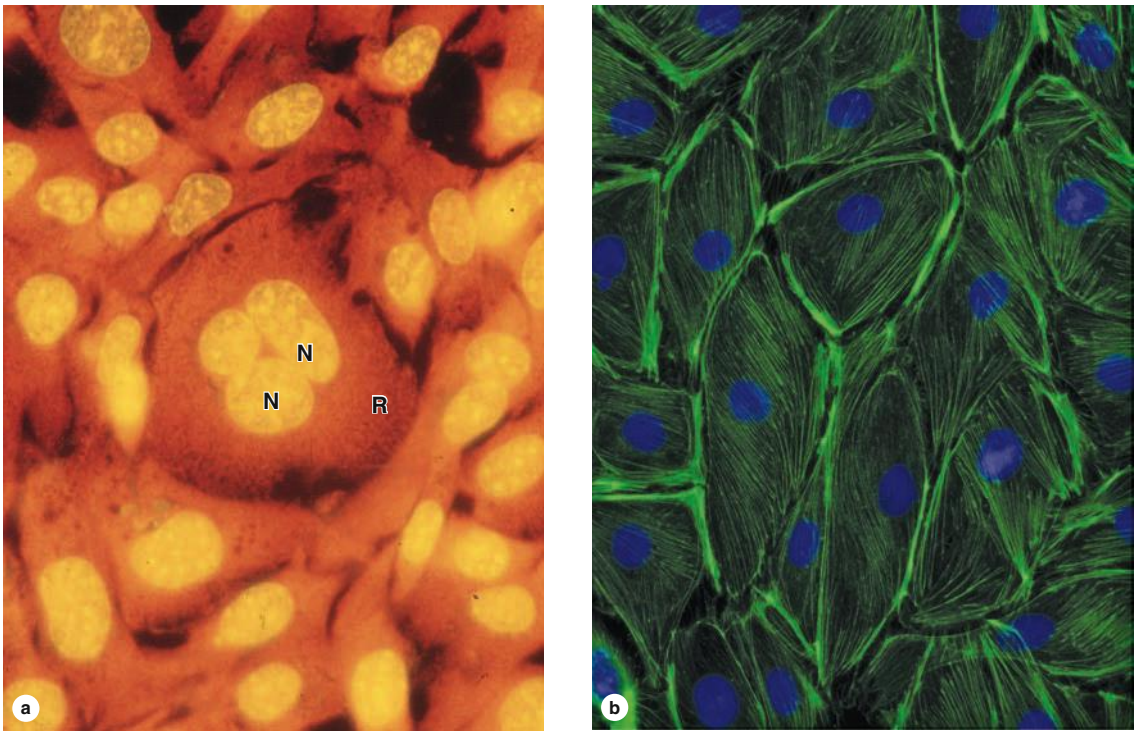
Unstained cells and tissue sections, which are usually transparent and colorless, can be studied with these modified light microscopes. Cellular detail is normally difficult to see in unstained tissues because all parts of the specimen have roughly similar optical densities. **Phase-contrast microscopy**, however, uses a lens system that produces visible images from transparent objects and, importantly, can be used with living, cultured cells (Figure 1–5).

Phase-contrast microscopy is based on the principle that light changes its speed when passing through cellular and extracellular structures with different refractive indices. These changes are used by the phase-contrast system to cause the structures to appear lighter or darker in relation to each other. Because they allow the examination of cells without fixation or staining, phase-contrast microscopes are prominent tools in all cell culture laboratories. A modification of phase-contrast microscopy is **differential interference microscopy** with Nomarski optics, which produces an image of living cells with a more apparent three-dimensional (3D) aspect (Figure 1–5c).

Confocal Microscopy

With a regular bright-field microscope, the beam of light is relatively large and fills the specimen. Stray (excess) light reduces contrast within the image and compromises the resolving

FIGURE 1–4 Appearance of cells with fluorescent microscopy.



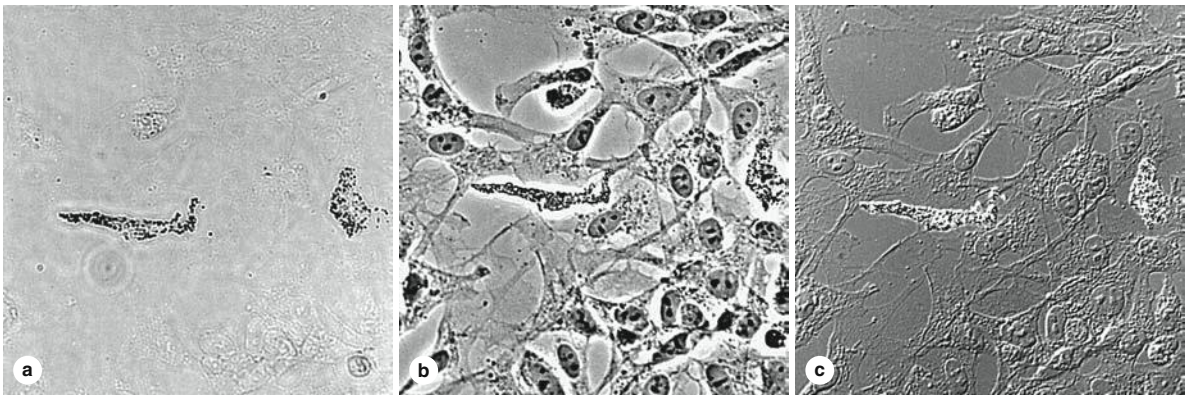
Components of cells are often stained with compounds visible by fluorescence microscopy.

(a) Acridine orange binds nucleic acids and causes DNA in cell nuclei **(N)** to emit yellow light and the RNA-rich cytoplasm **(R)** to appear orange in these cells of a kidney tubule.

(b) Cultured cells stained with DAPI (4′,6-diamino-2-phenylindole) that binds DNA and with fluorescein-phalloidin that binds actin filaments show nuclei with blue fluorescence and actin filaments stained green. Important information such as the greater density of microfilaments at the cell periphery is readily apparent. (Both X500)

(*Figure 1–4b, used with permission from Drs Claire E. Walczak and Rania Rizk, Indiana University School of Medicine, Bloomington.*)

FIGURE 1–5 Unstained cells' appearance in three types of light microscopy.



Living neural crest cells growing in culture appear differently with various techniques of light microscopy. Here the *same field* of unstained cells, including two differentiating pigment cells, is shown using three different methods (all X200):

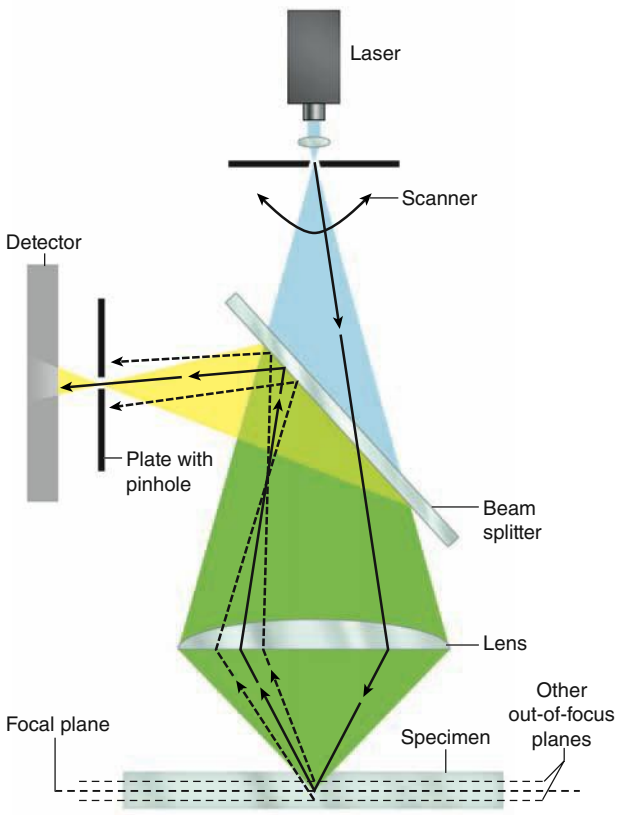
(a) Bright-field microscopy: Without fixation and staining, only the two pigment cells can be seen.

(b) Phase-contrast microscopy: Cell boundaries, nuclei, and cytoplasmic structures with different refractive indices affect in-phase light differently and produce an image of these features in *all* the cells.

(c) Differential interference microscopy: Cellular details are highlighted in a different manner using Nomarski optics. Phase-contrast microscopy, with or without differential interference, is widely used to observe live cells grown in tissue culture.

(*Used with permission from Dr Sherry Rogers, Department of Cell Biology and Physiology, University of New Mexico, Albuquerque, NM.*)

FIGURE 1–6 Principle of confocal microscopy.



Although a very small spot of light originating from one plane of the section crosses the pinhole and reaches the detector, rays originating from other planes are blocked by the blind. Thus, only one very thin plane of the specimen is focused at a time. The diagram shows the practical arrangement of a confocal microscope. Light from a laser source hits the specimen and is reflected. A beam splitter directs the reflected light to a pinhole and a detector. Light from components of the specimen that are above or below the focused plane is blocked by the blind. The laser scans the specimen so that a larger area of the specimen can be observed.

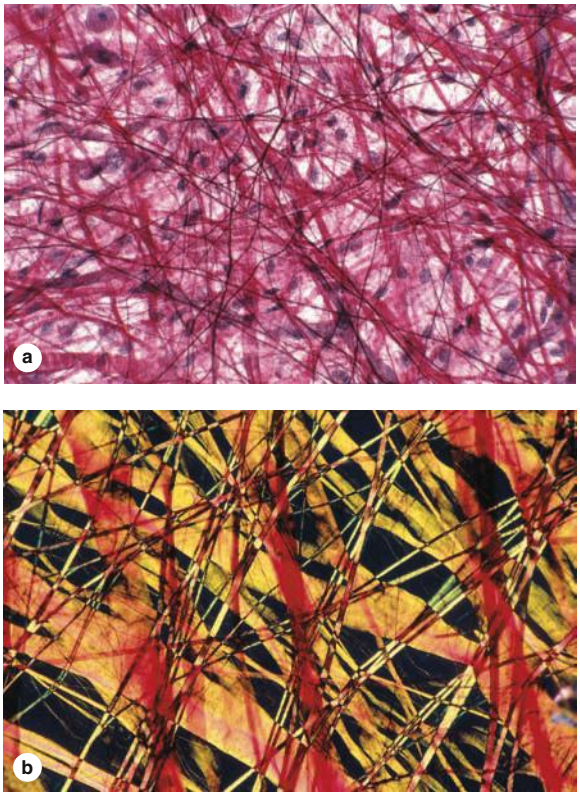
power of the objective lens. Confocal microscopy (Figure 1–6) avoids these problems and achieves high resolution and sharp focus by using (1) a small point of high-intensity light, often from a laser, and (2) a plate with a pinhole aperture in front of the image detector. The point light source, the focal point of the lens, and the detector's pinpoint aperture are all optically conjugated or aligned to each other in the focal plane (confocal), and unfocused light does not pass through the pinhole. This greatly improves resolution of the object in focus and allows the localization of specimen components with much greater precision than with the bright-field microscope.

Confocal microscopes include a computer-driven mirror system (the beam splitter) to move the point of illumination across the specimen automatically and rapidly. Digital images captured at many individual spots in a very thin plane of focus are used to produce an "optical section" of that plane. Creating such optical sections at a series of focal planes through the specimen allows them to be digitally reconstructed into a 3D image.

Polarizing Microscopy

Polarizing microscopy allows the recognition of stained or unstained structures made of highly organized subunits. When normal light passes through a **polarizing** filter, it exits vibrating in only one direction. If a second filter is placed in the microscope above the first one, with its main axis perpendicular to the first filter, no light passes through. If, however, tissue structures containing oriented macromolecules are located between the two polarizing filters, their repetitive structure rotates the axis of the light emerging from the polarizer and they appear as bright structures against a dark background (Figure 1–7). The ability to rotate the direction of vibration of polarized light is called **birefringence** and is

FIGURE 1–7 Tissue appearance with bright-field and polarizing microscopy.



Polarizing light microscopy produces an image only of material having repetitive, periodic macromolecular structure; features without such structure are not seen. Pieces of thin, unsectioned mesentery were stained with red picrosirius, orcein, and hematoxylin, placed on slides and observed by bright-field **(a)** and polarizing **(b)** microscopy.

(a) With bright-field microscopy collagen fibers appear red, with thin elastic fibers and cell nuclei darker. (X40)

(b) With polarizing microscopy, only the collagen fibers are visible and these exhibit intense yellow or orange birefringence. (a: X40; b: X100)

a feature of crystalline substances or substances containing highly oriented molecules, such as cellulose, collagen, microtubules, and actin filaments.

The utility of all light microscopic methods is greatly extended through the use of digital cameras. Many features of digitized histological images can be analyzed quantitatively using appropriate software. Such images can also be enhanced to allow objects not directly visible through the eyepieces to be examined on a monitor.

ELECTRON MICROSCOPY

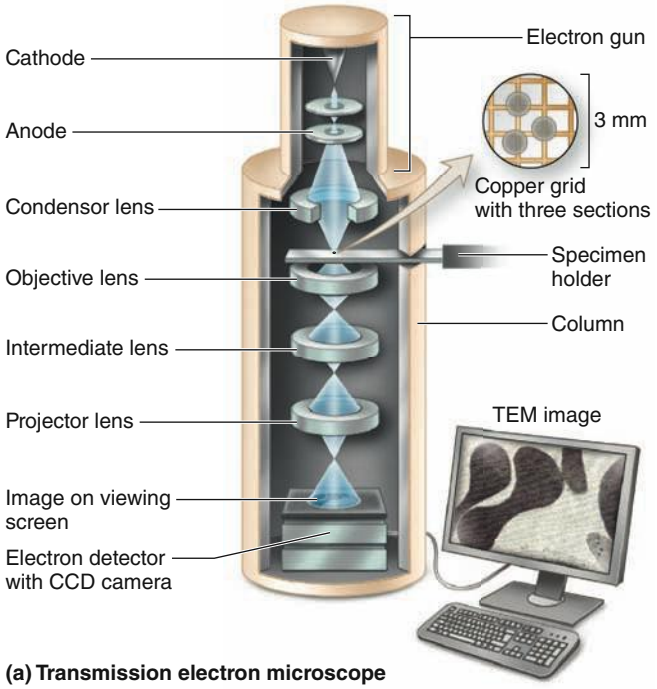
Transmission and scanning electron microscopes are based on the interaction of tissue components with beams of electrons. The wavelength in an electron beam is much shorter than that of light, allowing a 1000-fold increase in resolution.

Transmission Electron Microscopy

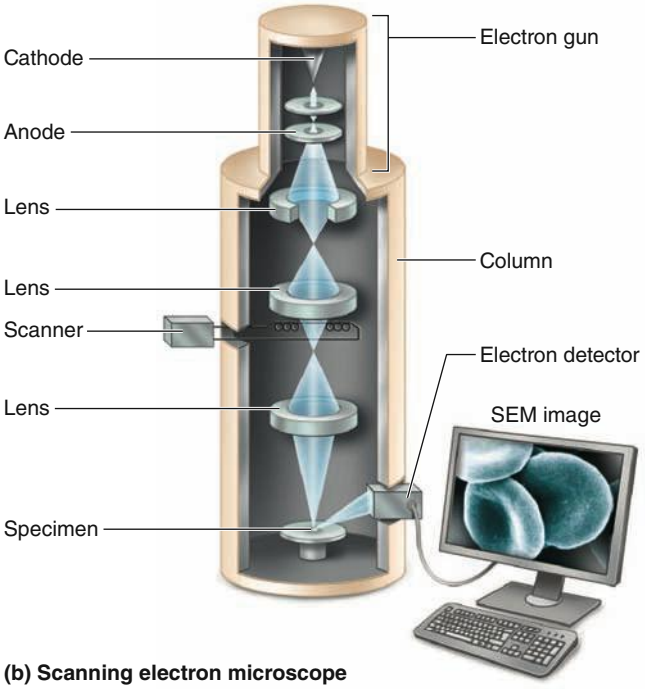
The **transmission electron microscope (TEM)** is an imaging system that permits resolution around 3 nm. This high resolution allows isolated particles magnified as much as 400,000 times to be viewed in detail. Very thin (40-90 nm), resin-embedded tissue sections are typically studied by TEM at magnifications up to approximately 120,000 times.

Figure 1–8a indicates the components of a TEM and the basic principles of its operation: a beam of electrons focused using electromagnetic "lenses" passes through the tissue section to produce an image with black, white, and intermediate

FIGURE **1–8** **Electron microscopes.**



(a) Transmission electron microscope



(b) Scanning electron microscope

Electron microscopes are large instruments generally housed in a specialized EM facility.

(a) Schematic view of the major components of a transmission electron microscope (TEM), which is configured rather like an upside-down light microscope. With the microscope column in a vacuum, a metallic (usually tungsten) filament (cathode) at the top emits electrons that travel to an anode with an accelerating voltage between 60 and 120 kV. Electrons passing through a hole in the anode form a beam that is **focused electromagnetically** by circular electric coils in a manner analogous to the effect of optical lenses on light.

The first lens is a condenser focusing the beam on the section. Some electrons interact with atoms in the section, being absorbed or scattered to different extents, while others are simply transmitted through the specimen with no interaction. Electrons reaching the objective lens form an image that is then magnified and finally projected on a fluorescent screen or a charge-coupled device (CCD) monitor and camera.

In a TEM image areas of the specimen through which electrons passed appear bright (electron lucent), while denser areas or those that bind heavy metal ions during specimen preparation absorb or deflect electrons and appear darker (electron dense). Such images are therefore always black, white, and shades of gray.

(b) The scanning electron microscope (SEM) has many similarities to a TEM. However, here the focused electron beam does not pass through the specimen, but rather is moved sequentially (scanned) from point to point across its surface similar to the way an electron beam is scanned across a television tube or screen. For SEM specimens are coated with metal atoms with which the electron beam interacts, producing reflected electrons and newly emitted secondary electrons. All of these are captured by a detector and transmitted to amplifiers and processed to produce a black-and-white image on the monitor. The SEM shows only surface views of the coated specimen but with a striking 3D, shadowed quality. The inside of organs or cells can be analyzed after sectioning to expose their internal surfaces.

shades of gray regions. These regions of an electron micrograph correspond to tissue areas through which electrons passed readily (appearing brighter or electron-lucent) and areas where electrons were absorbed or deflected (appearing darker or more electron-dense). To improve contrast and resolution in TEM, compounds with **heavy metal ions** are often added to the fixative or dehydrating solutions used for tissue preparation. These include osmium tetroxide, lead citrate, and uranyl compounds, which bind cellular macromolecules, increasing their electron density and visibility.

Cryofracture and **freeze etching** are techniques that allow TEM study of cells without fixation or embedding and have been particularly useful in the study of membrane structure. In these methods very small tissue specimens are rapidly frozen in liquid nitrogen and then cut or fractured with a knife. A replica of the frozen exposed surface is produced in a vacuum by applying thin coats of vaporized platinum or other metal atoms. After removal of the organic material, the replica of the cut surface can be examined by TEM. With membranes the random fracture planes often split the lipid bilayers, exposing protein components whose size, shape, and distribution are difficult to study by other methods.

Scanning Electron Microscopy

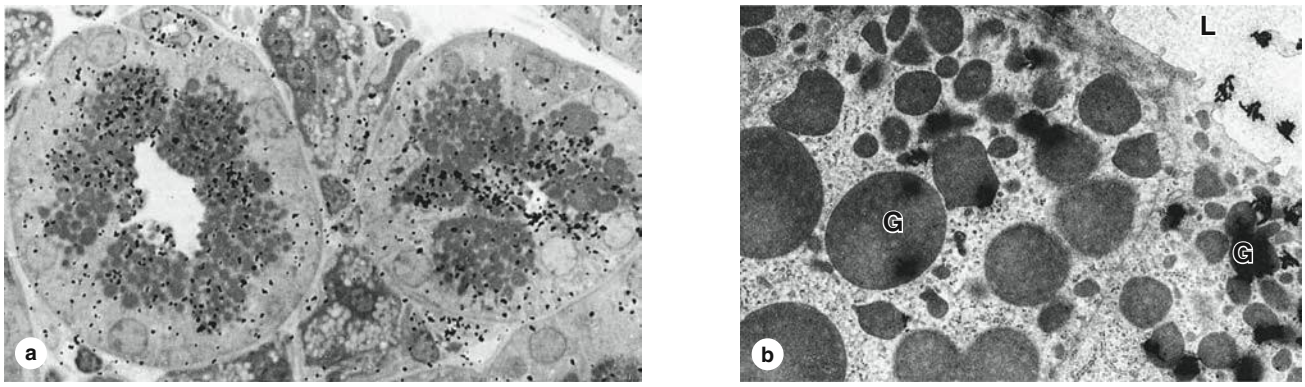
Scanning electron microscopy (SEM) provides a high-resolution view of the surfaces of cells, tissues, and organs. Like the TEM, this microscope produces and focuses a very narrow beam of electrons, but in this instrument the beam does not pass through the specimen (Figure 1–8b). Instead, the surface of the specimen is first dried and spray-coated with a very thin layer of heavy metal (often gold) which reflects electrons in a beam scanning the specimen. The reflected electrons are captured by a detector, producing signals that are processed to produce a black-and-white image. SEM images are usually easy to interpret because they present a three-dimensional view that appears to be illuminated in the same way that large objects are seen with highlights and shadows caused by light.

AUTORADIOGRAPHY

Microscopic **autoradiography** is a method of localizing newly synthesized macromolecules in cells or tissue sections. Radioactively labeled metabolites (nucleotides, amino acids, sugars) provided to the living cells are incorporated into specific macromolecules (DNA, RNA, protein, glycoproteins, and polysaccharides) and emit weak radiation that is restricted to those regions where the molecules are located. Slides with radiolabeled cells or tissue sections are coated in a darkroom with photographic emulsion in which silver bromide crystals act as microdetectors of the radiation in the same way that they respond to light in photographic film. After an adequate exposure time in lightproof boxes, the slides are developed photographically. Silver bromide crystals reduced by the radiation produce small black grains of metallic silver, which under either the light microscope or TEM indicate the locations of radiolabeled macromolecules in the tissue (Figure 1–9).

Much histological information becomes available by autoradiography. If a radioactive precursor of DNA (such as tritium-labeled thymidine) is used, it is possible to know which cells in a tissue (and how many) are replicating DNA

FIGURE **1–9** **Microscopic autoradiography.**



Autoradiographs are tissue preparations in which particles called **silver grains** indicate the cells or regions of cells in which specific macromolecules were synthesized just prior to fixation. Shown here are autoradiographs from the salivary gland of a mouse injected with ^{3}H-fucose 8 hours before tissue fixation. Fucose was incorporated into oligosaccharides, and the free ^{3}H-fucose was removed during fixation and sectioning of the gland. Autoradiographic processing and microscopy reveal locations of newly synthesized glycoproteins containing that sugar.

(a) Black grains of silver from the light-sensitive material coating the specimen are visible over cell regions with secretory granules and the duct indicating glycoprotein locations. (X1500)

(b) The same tissue prepared for TEM autoradiography shows silver grains with a coiled or amorphous appearance again localized mainly over the granules **(G)** and in the gland lumen **(L)**. (X7500)

(*Figure 1–9b, used with permission from Drs Ticiano G. Lima and A. Antonio Haddad, School of Medicine, Ribeirão Preto, Brazil.*)

and preparing to divide. Dynamic events may also be analyzed. For example, if one wishes to know where in the cell protein is produced, if it is secreted, and its path in the cell before being secreted, several animals are injected with a radioactive amino acid and tissues collected at different times after the injections. Autoradiography of the tissues from the sequential times will indicate the migration of the radioactive proteins.

CELL & TISSUE CULTURE

Live cells and tissues can be maintained and studied outside the body in culture (in vitro). In the organism (in vivo), cells are bathed in fluid derived from blood plasma and containing many different molecules required for survival and growth. Cell culture allows the direct observation of cellular behavior under a phase-contrast microscope and many experiments technically impossible to perform in the intact animal can be accomplished in vitro.

The cells and tissues are grown in complex solutions of known composition (salts, amino acids, vitamins) to which serum or specific growth factors are added. Cells to be cultured are dispersed mechanically or enzymatically from a tissue or organ and placed with sterile procedures in a clear dish to which they adhere, usually as a single layer (Figure 1–5). Such preparations are called **primary cell cultures**. Some cells can be maintained in vitro for long periods because they become immortalized and constitute a permanent **cell line**. Most cells obtained from normal tissues have a finite, genetically programmed life span. However certain changes (some related to oncogenes; see Chapter 3) can promote cell immortality, a process called **transformation**, and are similar to the initial changes in a normal cell's becoming a cancer cell. Improvements in culture technology and use of specific growth factors now allow most cell types to be maintained in vitro.

As shown in Chapter 2, incubation of living cells in vitro with a variety of new fluorescent compounds that are sequestered and metabolized in specific compartments of the cell provides a new approach to understanding these compartments both structurally and physiologically. Other histologic techniques applied to cultured cells have been particularly important for understanding the locations and functions of microtubules, microfilaments, and other components of the cytoskeleton.

MEDICAL APPLICATION

Cell culture is very widely used to study molecular changes that occur in cancer; to analyze infectious viruses, mycoplasma, and some protozoa; and for many routine genetic or chromosomal analyses. Cervical cancer cells from a patient later identified as Henrietta Lacks, who died from the disease in 1951, were used to establish one of the first cell lines, called **HeLa cells**, which are still used in research on cellular structure and function throughout the world.

ENZYME HISTOCHEMISTRY

Enzyme histochemistry (or cytochemistry) is a method for localizing cellular structures using a specific enzymatic activity present in those structures. To preserve the endogenous enzymes histochemical procedures usually use unfixed or mildly fixed tissue, which is sectioned on a cryostat to avoid adverse effects of heat and organic solvents on enzymatic activity. For enzyme histochemistry (1) tissue sections are immersed in a solution containing the substrate of the enzyme to be localized; (2) the enzyme is allowed to act on its substrate; (3) the section is then put in contact with a marker compound that reacts with a product of the enzymatic action on the substrate; and (4) the final product from the marker, which must be insoluble and visible by light or electron microscopy, precipitates over the site of the enzymes, identifying their location.

Examples of enzymes that can be detected histochemically include the following:

- **Phosphatases**, which remove phosphate groups from macromolecules (Figure 1–10).
- **Dehydrogenases**, which transfer hydrogen ions from one substrate to another, such as many enzymes of the citric acid (Krebs) cycle, allowing histochemical identification of such enzymes in mitochondria.
- **Peroxidase**, which promotes the oxidation of substrates with the transfer of hydrogen ions to hydrogen peroxide.

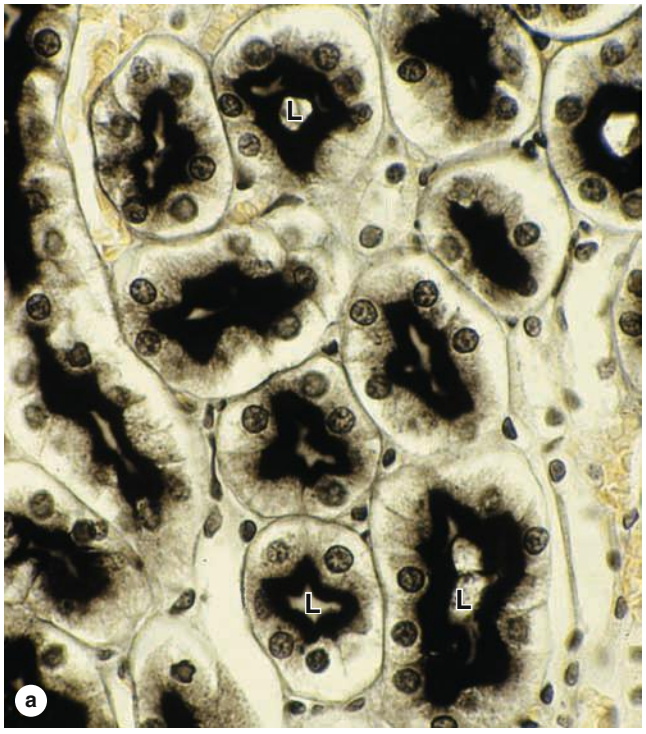
MEDICAL APPLICATION

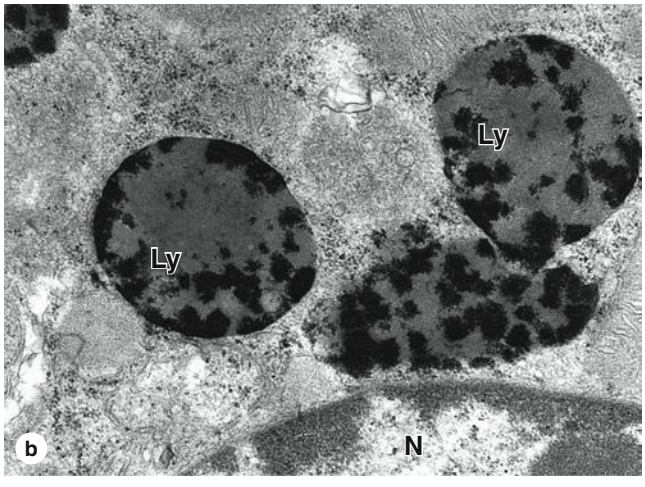
Many enzyme histochemical procedures are used in the medical laboratory, including Perls' Prussian blue reaction for iron (used to diagnose the iron storage diseases, hemochromatosis and hemosiderosis), the PAS-amylase and alcian blue reactions for polysaccharides (to detect glycogenosis and mucopolysaccharidosis), and reactions for lipids and sphingolipids (to detect sphingolipidosis).

VISUALIZING SPECIFIC MOLECULES

A specific macromolecule present in a tissue section may also be identified by using tagged compounds or macromolecules that bind *specifically* with the molecule of interest. The compounds that interact with the molecule must be visible with the light or electron microscope, often by being tagged with a detectible label. The most commonly used labels are fluorescent compounds, radioactive atoms that can be detected with autoradiography, molecules of peroxidase or other enzymes that can be detected with histochemistry, and metal (usually gold) particles that can be seen with light and electron microscopy. These methods can be used to detect and localize specific sugars, proteins, and nucleic acids.

FIGURE **1–10** **Enzyme histochemistry.**





(a) Micrograph of cross sections of kidney tubules treated histochemically to demonstrate alkaline phosphatases (with maximum activity at an alkaline pH) showing strong activity of this enzyme at the apical surfaces of the cells at the lumens **(L)** of the tubules. (X200)

(b) TEM image of a kidney cell in which acid phosphatase has been localized histochemically in three lysosomes **(Ly)** near the nucleus **(N)**. The dark material within these structures is lead phosphate that precipitated in places with acid phosphatase activity. (X25,000)

(*Figure 1–10b, used with permission from Dr Eduardo Katchburian, Department of Morphology, Federal University of São Paulo, Brazil.*)

Examples of molecules that interact specifically with other molecules include the following:

- **Phalloidin**, a compound extracted from mushroom, *Amanita phalloides*, interacts strongly with the actin protein of microfilaments.
- **Protein A**, purified from *Staphylococcus aureus* bacteria, binds to the Fc region of antibody molecules, and can therefore be used to localize naturally occurring or applied antibodies bound to cell structures.
- **Lectins**, glycoproteins derived mainly from plant seeds, bind to carbohydrates with high affinity and specificity. Different lectins bind to specific sugars or sequences of sugar residues, allowing fluorescently labeled lectins to be used to stain specific glycoproteins or other macromolecules bearing specific sequences of sugar residues.

Immunohistochemistry

A highly specific interaction between macromolecules is that between an antigen and its antibody. For this reason labeled antibodies are routinely used in **immunohistochemistry** to identify and localize many specific proteins, not just those with enzymatic activity that can be demonstrated by histochemistry.

The body's immune cells interact with and produce **antibodies** against other macromolecules—called antigens—that are recognized as "foreign," not a normal part of the organism, and potentially dangerous. Antibodies belong to the **immunoglobulin** family of glycoproteins and are secreted by lymphocytes. These molecules normally bind specifically to their provoking antigens and help eliminate them.

Widely applied for both research and diagnostic purposes, every immunohistochemical technique requires an antibody against the protein that is to be detected. This means that the protein must have been previously purified using biochemical or molecular methods so that antibodies against it can be produced. To produce antibodies against protein *x* of a certain animal species (eg, a human or rat), the isolated protein is injected into an animal of another species (eg, a rabbit or a goat). If the protein's amino acid sequence is sufficiently different for this animal to recognize it as foreign—that is, as an antigen—the animal will produce antibodies against the protein.

Different groups (clones) of lymphocytes in the injected animal recognize different parts of protein *x* and each clone produces an antibody against that part. These antibodies are collected from the animal's plasma and constitute a mixture of **polyclonal antibodies**, each capable of binding a different region of protein *x*.

It is also possible, however, to inject protein *x* into a mouse and a few days later isolate the activated lymphocytes and place them into culture. Growth and activity of these cells can be prolonged indefinitely by fusing them with lymphocytic tumor cells to produce hybridoma cells. Different hybridoma clones produce different antibodies against the several parts

of protein *x* and each clone can be isolated and cultured separately so that the different antibodies against protein *x* can be collected separately. Each of these antibodies is a **monoclonal antibody**. An advantage to using a monoclonal antibody rather than polyclonal antibodies is that it can be selected to be highly specific and to bind strongly to the protein to be detected, with less nonspecific binding to other proteins that are similar to the one of interest.

In immunohistochemistry a tissue section that one believes contains the protein of interest is incubated in a solution containing antibody (either monoclonal or polyclonal) against this protein. The antibody binds specifically to the protein and after a rinse the protein's location in the tissue or cells can be seen with either the light or electron microscope by visualizing the antibody. Antibodies are commonly tagged with fluorescent compounds, with peroxidase or alkaline phosphatase for histochemical detection, or with electron-dense gold particles for TEM.

As Figure 1–11 indicates, there are **direct and indirect methods of immunocytochemistry**. The direct method just involves a labeled antibody that binds the protein of interest.

Indirect immunohistochemistry involves sequential application of two antibodies and additional washing steps. The (primary) antibody specifically binding the protein of interest is not labeled. The detectible tag is conjugated to a **secondary antibody** made in an animal species different ("foreign") from that which made the primary antibody. For example, primary antibodies made by mouse lymphocytes (such as most monoclonal antibodies) are specifically recognized and bound by antibodies made in a rabbit or goat injected with mouse antibody immunoglobulin.

The indirect method is used more widely in research and pathologic tests because it is more sensitive, with the extra level of antibody binding serving to amplify the visible signal. Moreover, the same preparation of labeled secondary antibody can be used in studies with different primary antibodies (specific for different antigens) as long as all these are made in the same species. There are other indirect methods that involve the use of other intermediate molecules, such as the biotin-avidin technique, which are also used to amplify detection signals.

Examples of indirect immunocytochemistry are shown in Figure 1–12, demonstrating the use of this method with cells in culture or after tissue sectioning for both light microscopy and TEM.

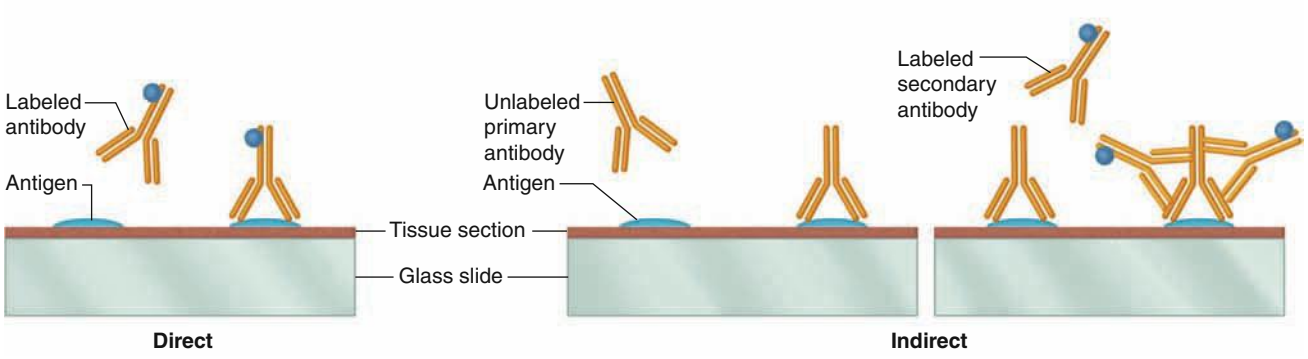
›› MEDICAL APPLICATION

Because cells in some diseases, including many cancer cells, often produce proteins unique to their pathologic condition, immunohistochemistry can be used by pathologists to diagnose many diseases, including certain types of tumors and some virus-infected cells. Table 1-1 shows some applications of immunocytochemistry routinely used in clinical practice.

Hybridization Techniques

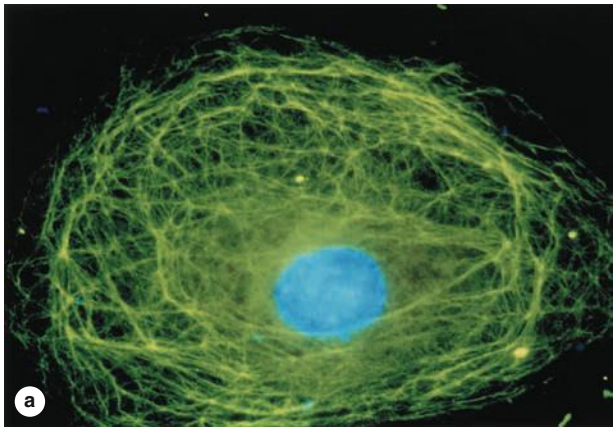
Hybridization usually implies the specific binding between two single strands of nucleic acid, which occurs under appropriate conditions if the strands are complementary. The greater the similarities of their nucleotide sequences, the more readily the complementary strands form "hybrid" double-strand molecules. Hybridization at stringent conditions allows the specific identification of sequences in genes or RNA. This can

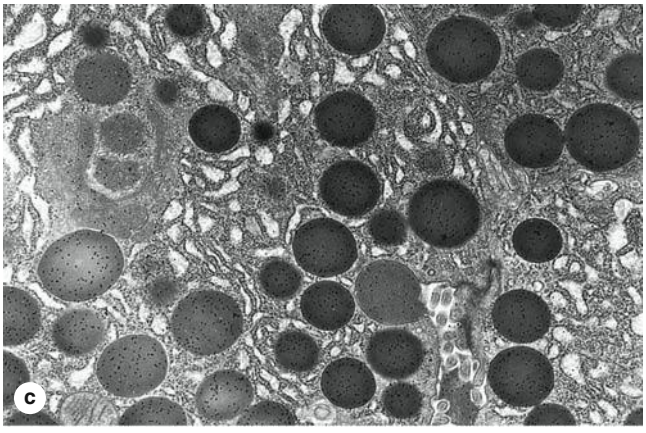
FIGURE **1–11** **Immunocytochemistry techniques.**

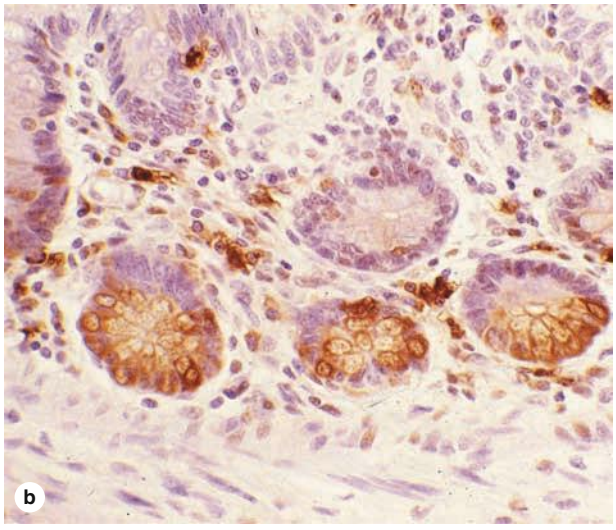


Immunocytochemistry (or immunohistochemistry) can be direct or indirect. **Direct immunocytochemistry** (left) uses an antibody made against the tissue protein of interest and tagged directly with a label such as a fluorescent compound or peroxidase. When placed with the tissue section on a slide, these labeled antibodies bind specifically to the protein (antigen) against which they were produced and can be visualized by the appropriate method. **Indirect immunocytochemistry** (right) uses first a **primary antibody** made against the protein (antigen) of interest and applied to the tissue section to bind its specific antigen. Then a **labeled secondary antibody** is obtained that was (1) made in another species against immunoglobulin proteins (antibodies) from the species in which the primary antibodies were made and (2) labeled with a fluorescent compound or peroxidase. When the labeled secondary antibody is applied to the tissue section, it specifically binds the primary antibodies, indirectly labeling the protein of interest on the slide. Because more than one labeled secondary antibody can bind each primary antibody molecule, labeling of the protein of interest is amplified by the indirect method.

FIGURE 1–12 Cells and tissues stained by immunohistochemistry.







Immunocytochemical methods to localize specific proteins can be applied to either light microscopic or TEM preparations using a variety of labels.

(a) A single cultured uterine cell stained fluorescently to reveal a meshwork of intermediate filaments (green) throughout the cytoplasm. Primary antibodies against the filament protein desmin and fluorescein isothiocyanate (FITC)–labeled secondary antibodies were used in the indirect staining technique, with the nucleus counterstained blue with DAPI. (X650)

(b) A section of small intestine treated with an antibody against the enzyme lysozyme. The secondary antibody labeled with peroxidase was then applied and the localized brown color produced histochemically with the peroxidase substrate 3,3′-diamino-azobenzidine (DAB). The method demonstrates lysozyme-containing structures in scattered macrophages and in the large clusters of cells. Nuclei were counterstained with hematoxylin. (X100)

(c) A section of pancreatic cells in a TEM preparation incubated with an antibody against the enzyme amylase and then with protein A coupled with gold particles. Protein A has high affinity toward antibody molecules and the resulting image reveals the presence of amylase with the gold particles localized as very small black dots over dense secretory granules and developing granules (left). With specificity for immunoglobulin molecules, labeled protein A can be used to localize any primary antibody. (X5000)

(*Figure 1–12c, used with permission from Dr Moise Bendayan, Departments of Pathology and Cell Biology, University of Montreal, Montreal, Canada.*)

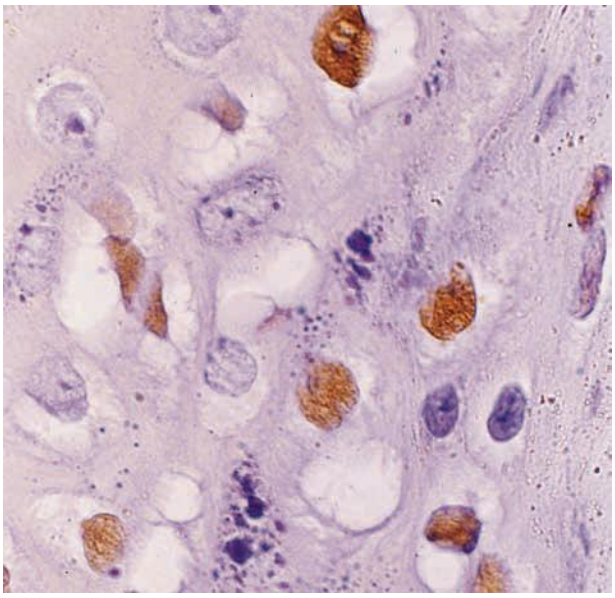
TABLE 1-1 Examples of specific antigens with diagnostic importance.

Antigens	Diagnosis
Specific cytokeratins	Tumors of epithelial origin
Protein and polypeptide hormones	Certain endocrine tumors
Carcinoembryonic antigen (CEA)	Glandular tumors, mainly of the digestive tract and breast
Steroid hormone receptors	Breast duct cell tumors
Antigens produced by viruses	Specific virus infections

occur with cellular DNA or RNA when nucleic acid sequences in solution are applied directly to prepared cells and tissue sections, a procedure called **in situ hybridization** (ISH).

This technique is ideal for (1) determining if a cell has a specific sequence of DNA, such as a gene or part of a gene (Figure 1–13), (2) identifying the cells containing specific messenger RNAs (mRNAs) (in which the corresponding gene is being transcribed), or (3) determining the localization of a gene in a specific chromosome. DNA and RNA of the cells must be initially denatured by heat or other agents to become completely single-stranded and the nucleotide sequences of interest are detected with **probes** consisting of single-stranded complementary DNA (cDNA). The probe may be obtained by cloning, by polymerase chain reaction (PCR) amplification of the target sequence, or by chemical synthesis if the desired sequence is short. The probe is tagged with nucleotides containing a radioactive isotope (localized by autoradiography) or modified with a small compound such as digoxigenin (identified by immunocytochemistry). A solution containing the probe is placed over the specimen under conditions allowing hybridization and after the excess unbound probe is washed off, the localization of the hybridized probe is revealed through its label.

FIGURE **1–13** **In situ hybridization (ISH).**



In situ hybridization of this tissue section with probes for the human papilloma virus (HPV) reveals the presence of many cells containing the virus. The section was incubated with a solution containing a digoxigenin-labeled complementary DNA (cDNA) probe for the HPV DNA. The probe was then visualized by direct immunohistochemistry using peroxidase-labeled antibodies against digoxigenin. This procedure stains brown only those cells containing HPV. (X400; H&E)

(*Used with permission from Dr Jose E. Levi, Virology Lab, Institute of Tropical Medicine, University of São Paulo, Brazil.*)

›› MEDICAL APPLICATION

Warts on the skin of the genitals and elsewhere are due to infection with the human papilloma virus (HPV) which causes the characteristic benign proliferative growth. As shown in Figure 1–12 such virus-infected cells can often be demonstrated by ISH. Certain cancer cells with unique or elevated expression of specific genes are also localized in tumors and studied microscopically by ISH.

› INTERPRETATION OF STRUCTURES IN TISSUE SECTIONS

In studying and interpreting stained tissue sections, it is important to remember that microscopic preparations are the end result of a series of processes that began with collecting the tissue and ended with mounting a coverslip on the slide. Certain steps in this procedure may distort the tissues slightly, producing minor structural abnormalities called **artifacts** not present in the living tissue.

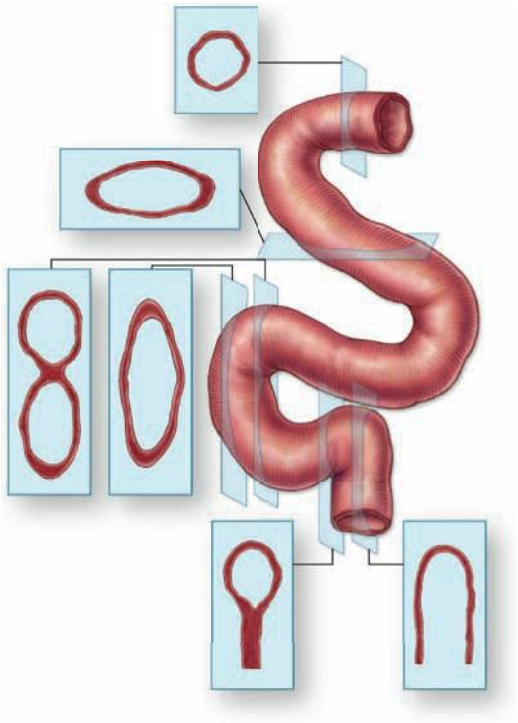
One such distortion is minor shrinkage of cells or tissue regions produced by the fixative, by the ethanol, or by the heat needed for paraffin embedding. Shrinkage can create artificial spaces between cells and other tissue components. Such spaces can also result from the loss of lipids or low-molecular-weight substances not preserved by the fixative or removed by the dehydrating and clearing fluids. Slight cracks in sections may also appear as large spaces in the tissue.

Other artifacts may include small wrinkles in the section (which the novice may confuse with linear structures in tissue) and precipitates from the stain (which may be confused with cellular structures such as cytoplasmic granules). Students must be aware of the existence of artifacts and able to recognize them.

Another difficulty in the study of histologic sections is the impossibility of differentially staining all tissue components on one slide. A single stain can seldom demonstrate well nuclei, mitochondria, lysosomes, basement membranes, elastic fibers, etc. With the light microscope, it is necessary to examine preparations stained by different methods before an idea of the whole composition and structure of a cell or tissue can be obtained. The TEM allows the observation of cells with all its internal structures and surrounding ECM components, but only a few cells in a tissue can be conveniently studied in these very small samples.

Finally, when a structure's **three-dimensional** volume is cut into very thin sections, the sections appear microscopically to have only two dimensions: length and width. When examining a section under the microscope, the viewer must always keep in mind that components are missing in front of and behind what is being seen because many tissue structures are thicker than the section. Round structures seen microscopically may actually be portions of spheres or tubes. Because structures in a tissue have different orientations, their two-dimensional (2D) appearance will also vary depending on the plane of section. A single convoluted tube will appear in a tissue section as many separate rounded or oval structures (Figure 1–14).

FIGURE 1–14 Interpretation of 3D structures in 2D sections.



In thin sections 3D structures appear to have only two dimensions. Such images must be interpreted correctly to understand the actual structure of tissue and organ components. For example, blood vessels and other tubular structures appear in sections as round or oval shapes whose size and shape depend on the transverse or oblique angle of the cut. A highly coiled tube will appear as several round and oval structures. In TEM sections of cells, round structures may represent spherical organelles or transverse cuts through tubular organelles such as mitochondria. It is important to develop such interpretive skill to understand tissue and cell morphology in microscopic preparations.

Histology & Its Methods of Study SUMMARY OF KEY POINTS

Preparation of Tissues for Study

- Chemical fixatives such as formalin are used to preserve tissue structure by cross-linking and denaturing proteins, inactivating enzymes, and preventing cell autolysis or self-digestion.
- Dehydration of the fixed tissue in alcohol and clearing in organic solvents prepare it for embedding and sectioning.
- Embedding in paraffin wax or epoxy resin allows the tissue to be cut into very thin sections (slices) with a microtome.
- Sections are mounted on glass slides for staining, which is required to reveal specific cellular and tissue components with the microscope.
- The most commonly used staining method is a combination of the stains hematoxylin and eosin (H&E), which act as basic and acidic dyes, respectively.
- Cell substances with a net negative (anionic) charge, such as DNA and RNA, react strongly with hematoxylin and basic stains; such material is said to be "basophilic."
- Cationic substances, such as collagen and many cytoplasmic proteins react with eosin and other acidic stains and are said to be "acidophilic."

Light Microscopy

- **Bright-field microscopy**, the method most commonly used by both students and pathologists, uses ordinary light and the colors are imparted by tissue staining.
- **Fluorescence microscopy** uses ultraviolet light, under which only fluorescent molecules are visible, allowing localization of fluorescent probes which can be much more specific than routine stains.
- **Phase-contrast microscopy** uses the differences in refractive index of various natural cell and tissue components to produce an image without staining, allowing observation of living cells.
- **Confocal microscopy** involves scanning the specimen at successive focal planes with a focused light beam, often from a laser, and produces a 3D reconstruction from the images.

Autoradiography

- This process localizes cell components synthesized using **radioactive precursors** by detecting silver grains produced by weakly emitted radiation in a photographic emulsion coating the tissue section or cells.
- With either light microscopy or TEM, autoradiography permits unique studies of processes such as tissue growth (using radioactive DNA precursors) or cellular pathways of macromolecular synthesis.

Cell & Tissue Culture

- Cells can be grown in vitro from newly **explanted** tissues (primary cultures) or as long-established cell lines and can be examined in the living state by phase-contrast light microscopy.

Enzyme Histochemistry

- **Histochemical** (or **cytochemical**) **techniques** use specific enzymatic activities in lightly fixed or unfixed tissue sections to produce visible products in the specific enzyme locations.
- Fixation and paraffin embedding denatures most enzymes, so histochemistry usually uses **frozen tissue** sectioned with a **cryostat**.
- Enzyme classes for which histochemical study is useful include phosphatases, dehydrogenases, and peroxidases, with peroxidase often conjugated to antibodies used in immunohistochemistry.

Visualizing Specific Molecules

- Some substances specifically bind certain targets in cells.
- **Immunohistochemistry** is based on specific reactions between an antigen and antibodies labeled with visible markers, often fluorescent compounds or peroxidase for light microscopy and gold particles for TEM.
- If the cell or tissue antigen of interest is detected by directly binding a labeled **primary antibody** specific for that antigen, the process is considered **direct immunohistochemistry**.

- **Indirect immunohistochemistry** uses an unlabeled primary antibody that is detected bound to its antigen with labeled **secondary antibodies**.
- The indirect immunohistochemical method is more commonly used because the added level of antibody binding amplifies the signal detected and provides greater technical flexibility.
- Specific gene sequences or mRNAs of cells can be detected microscopically using labeled complementary DNA (cDNA) probes in a technique called **in situ hybridization (ISH)**.

Interpretation of Structures in Tissue Sections

- Many steps in tissue processing, slide preparation, and staining can introduce minor **artifacts** such as spaces and precipitates that are not normally present in the living tissue and must be recognized.
- Sections of cells or tissues are essentially 2D planes through 3D structures, and understanding this fact is important for their correct interpretation and study.

Histology & Its Methods of Study ASSESS YOUR KNOWLEDGE

1. In preparing tissue for routine light microscopic study, which procedure immediately precedes clearing the specimen with an organic solvent?
   a. Dehydration
   b. Fixation
   c. Staining
   d. Clearing
   e. Embedding

2. Which of the following staining procedures relies on the cationic and anionic properties of the material to be stained?
   a. Enzyme histochemistry
   b. Periodic acid-Schiff reaction
   c. Hematoxylin & eosin staining
   d. Immunohistochemistry
   e. Metal impregnation techniques

3. In a light microscope used for histology, resolution and magnification of cells are largely dependent on which component?
   a. Condenser
   b. Objective lens
   c. Eyepieces or ocular lenses
   d. Specimen slide
   e. The control for illumination intensity

4. Cellular storage deposits of glycogen, a free polysaccharide, could best be detected histologically using what procedure?
   a. Autoradiography
   b. Electron microscopy
   c. Enzyme histochemistry
   d. Hematoxylin & eosin staining
   e. Periodic acid-Schiff reaction

5. Adding heavy metal compounds to the fixative and ultrathin sectioning of the embedded tissue with a glass knife are techniques used for which histological procedure?
   a. Scanning electron microscopy
   b. Fluorescent microscopy
   c. Enzyme histochemistry
   d. Confocal microscopy
   e. Transmission electron microscopy

6. Resolution in electron microscopy greatly exceeds that of light microscopy due to which of the following?
   a. The wavelength of the electrons in the microscope beam is shorter than that of a beam of light.
   b. The lenses of an electron microscope are of greatly improved quality.
   c. For electron microscopy the tissue specimen does not require staining.
   d. The electron microscope allows much greater magnification of a projected image than a light microscope provides.
   e. An electron microscope can be much more finely controlled than a light microscope.

7. Microscopic autoradiography uses radioactivity and can be employed to study what features in a tissue section?
   a. The types of enzymes found in various cell locations
   b. Cellular sites where various macromolecules are synthesized
   c. The sequences of mRNA made in the cells
   d. The dimensions of structures within the cells
   e. The locations of genes transcribed for specific mRNA

8. To identify and localize a specific protein within cells or the extracellular matrix one would best use what approach?
   a. Autoradiography
   b. Enzyme histochemistry
   c. Immunohistochemistry
   d. Transmission electron microscopy
   e. Polarizing microscopy

9. In situ hybridization is a histological technique used to visualize what type of macromolecule?
   a. Proteins
   b. Carbohydrates
   c. Certain enzymes
   d. Nucleic acids
   e. Lipids

10. Hospital laboratories frequently use unfixed, frozen tissue specimens sectioned with a cryostat for rapid staining, microscopic examination, and diagnosis of pathological conditions. Besides saving much time by avoiding fixation and procedures required for paraffin embedding, frozen sections retain and allow study of what macromolecules normally lost in the paraffin procedure?
    a. Carbohydrates
    b. Small mRNA
    c. Basic proteins
    d. Acidic proteins
    e. Lipids

Answers: 1a, 2c, 3b, 4e, 5e, 6a, 7b, 8c, 9d, 10e

CHAPTER 2

The Cytoplasm

Cells and extracellular material together comprise all the tissues that make up the organs of multicellular animals. In all tissues, cells themselves are the basic structural and functional units, the smallest living parts of the body. Animal cells are eukaryotic, with distinct membrane-limited nuclei surrounded by **cytoplasm** which contains various membrane-limited organelles and the cytoskeleton. In contrast, the smaller prokaryotic cells of bacteria typically have a cell wall around the plasmalemma and lack nuclei and membranous cytoplasmic structures.

CELL DIFFERENTIATION

The human organism consists of hundreds of different cell types, all derived from the zygote, the single cell formed by the merger of a spermatozoon with an oocyte at fertilization. The first zygotic cellular divisions produce cells called **blastomeres**, and as part of the early embryo's inner cell mass blastomeres give rise to all tissue types of the fetus. Explanted to tissue culture cells of the inner cell mass are called **embryonic stem cells**. Most cells of the fetus undergo a specialization process called **differentiation** in which they differentially express sets of genes that mediate specific cytoplasmic activities, becoming very efficient in specialized functions and usually changing their shape accordingly. For example, muscle cell precursors elongate into fiber-like cells containing large arrays of actin and myosin. All animal cells contain actin filaments and myosins, but muscle cells are specialized for using these proteins to convert chemical energy into forceful contractions.

Major cellular functions performed by specialized cells in the body are listed in Table 2–1. It is important to understand that the functions listed there can be performed by most cells of the body; specialized cells have greatly expanded their capacity for one or more of these functions during differentiation. Changes in cells' microenvironments under normal and pathologic conditions can cause the same cell type to have variable features and activities. Cells that appear similar structurally often have different families of receptors for signaling molecules such as hormones and extracellular matrix (ECM) components, causing them to behave differently. For example, because of their diverse arrays of receptors, breast fibroblasts and uterine smooth muscle cells are exceptionally sensitive to female sex hormones while most other fibroblasts and smooth muscle cells are insensitive.

THE PLASMA MEMBRANE

The **plasma membrane** (**cell membrane** or plasmalemma) that envelops every eukaryotic cell consists of phospholipids, cholesterol, and proteins, with oligosaccharide chains covalently linked to many of the phospholipid and protein molecules. This limiting membrane functions as a selective barrier regulating the passage of materials into and out of the cell and facilitating the transport of specific molecules. One important role of the cell membrane is to keep constant the ion content of cytoplasm, which differs from that of the extracellular fluid. Membranes also carry out a number of specific recognition and signaling functions, playing a key role in the interactions of the cell with its environment.