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Histology & Its Methods of Study

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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

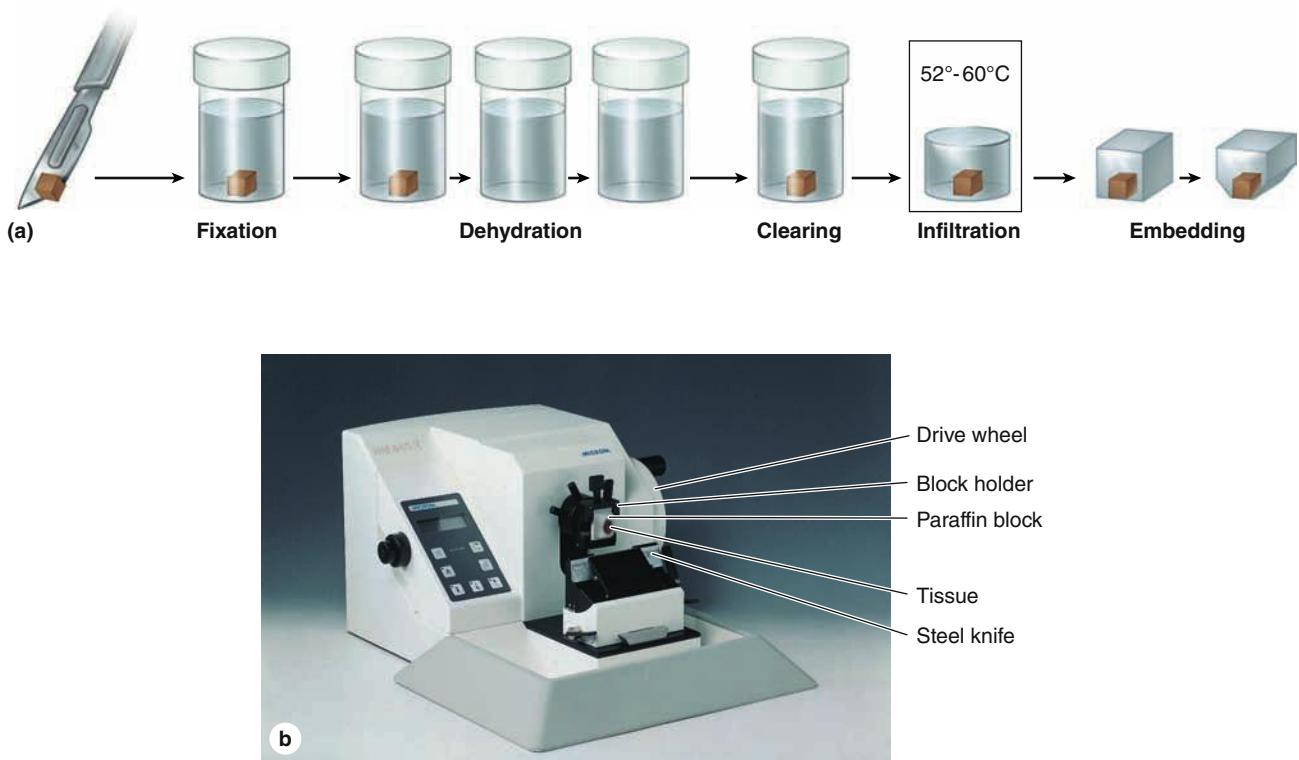
The most common procedure used in histologic research is the preparation of tissue slices or "sections" that can be examined visually with transmitted light. Because most tissues and organs are too thick for light to pass through, thin translucent sections are cut from them and placed on glass slides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

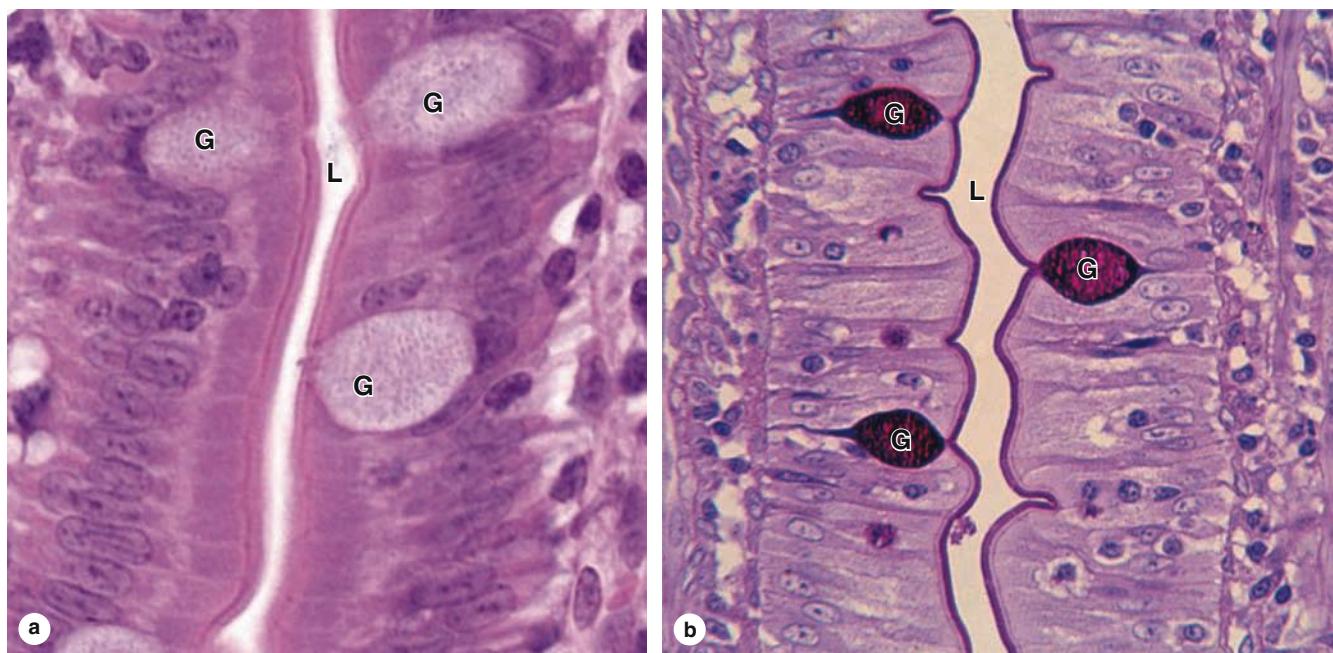
Of all staining methods, the simple combination of **hematoxylin and eosin (H&E)** is used most commonly. Hematoxylin stains DNA in the cell nucleus, RNA-rich portions of the cytoplasm, and the matrix of cartilage, producing a dark blue or purple color. In contrast, eosin stains other cytoplasmic structures 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 visualize 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

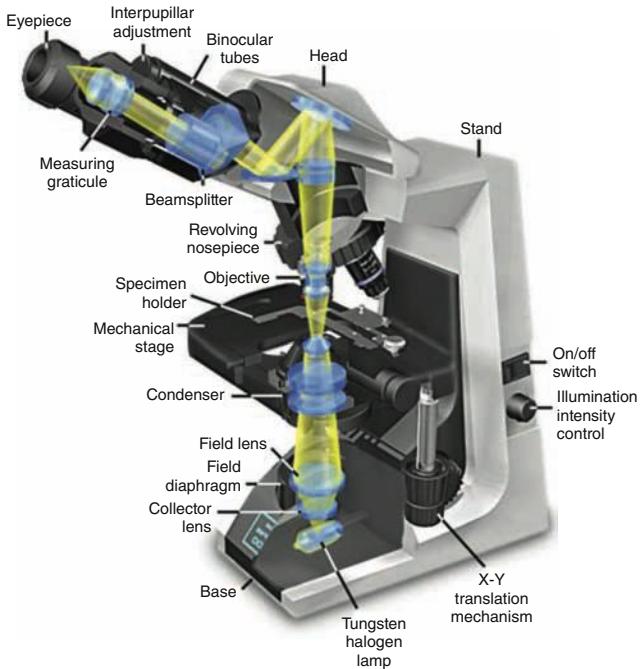
With the **bright-field microscope** stained tissue is examined with ordinary light passing through the preparation. As shown in Figure 1–3, the microscope includes an optical system and mechanisms to move and focus the specimen. The optical components are the **condenser** focusing light on the object 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

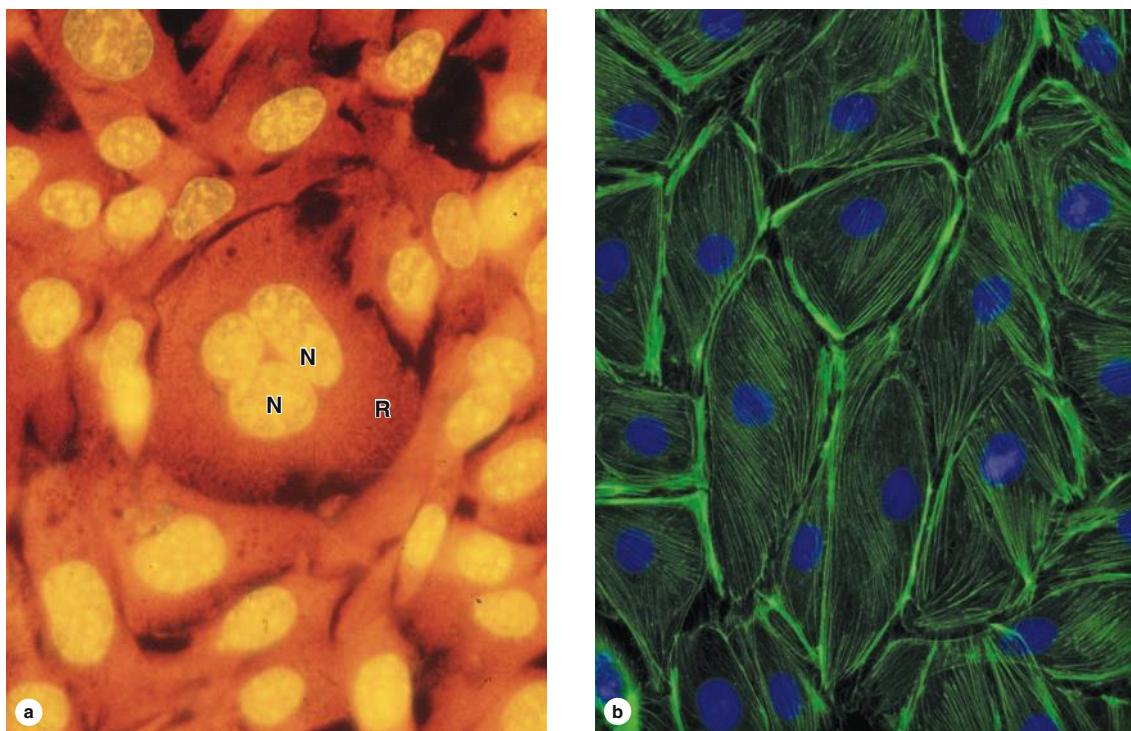
Unstained cells and tissue sections, which are usually transparent and colorless, can be studied with these modified light microscopes. Cellular detail is normally difficult to see in unstained tissues because all parts of the specimen have roughly similar optical densities. **Phase-contrast microscopy**, 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.

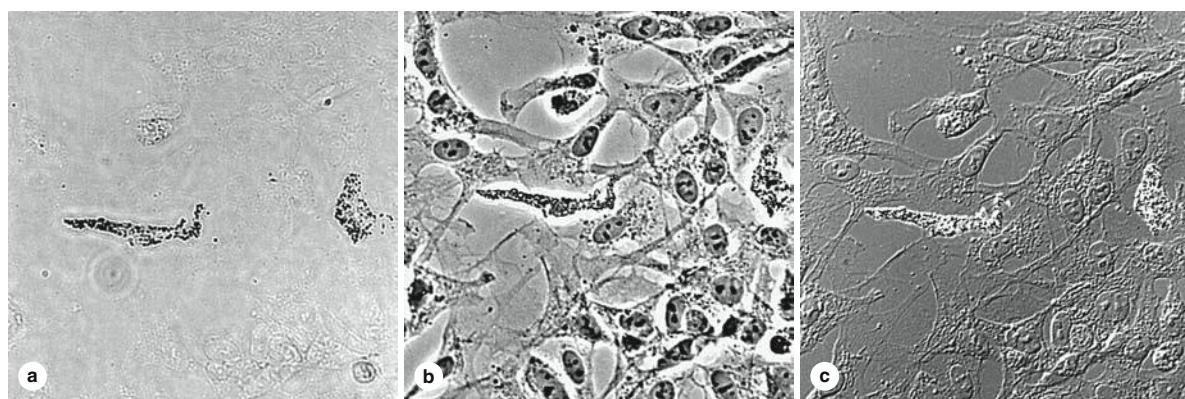
(a) Acridine orange binds nucleic acids and causes DNA in cell nuclei (**N**) to emit yellow light and the RNA-rich cytoplasm (**R**) to appear orange in these cells of a kidney tubule.

(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):

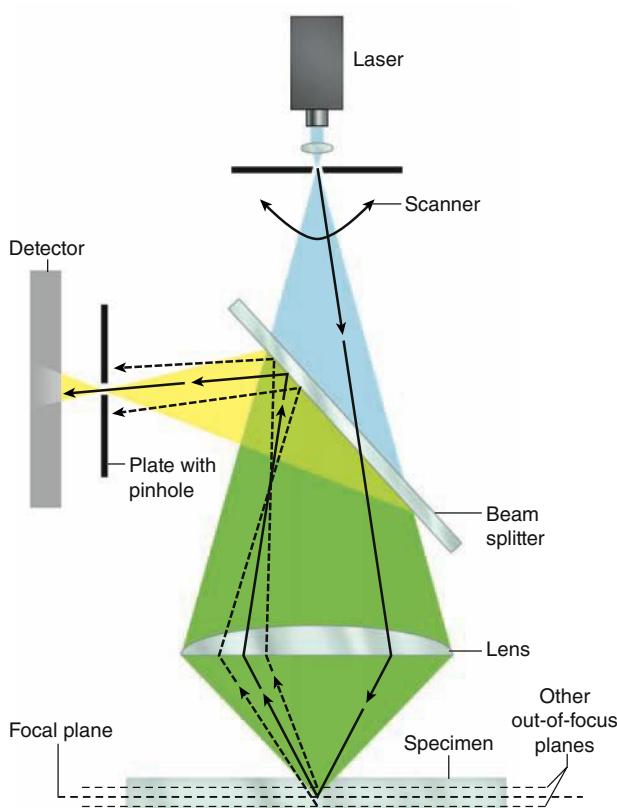
(a) **Bright-field microscopy:** Without fixation and staining, only the two pigment cells can be seen.

(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.

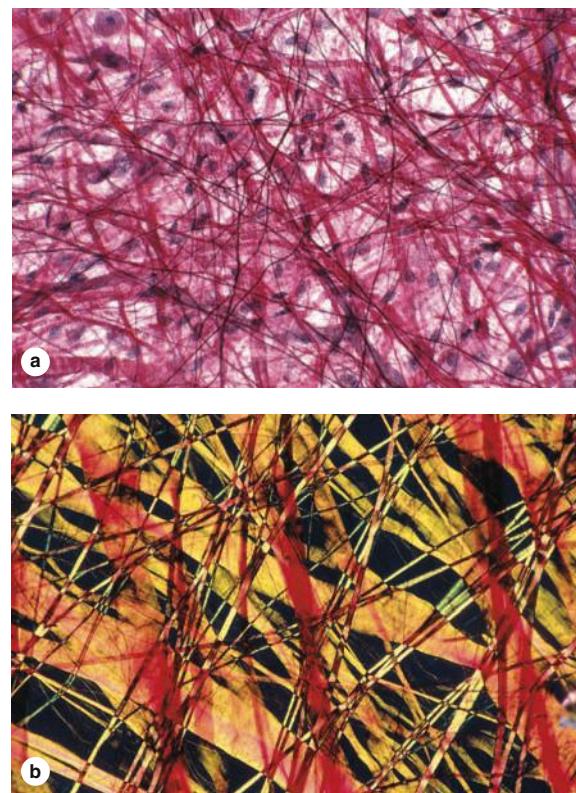
power of the objective lens. Confocal microscopy (Figure 1–6) avoids these problems and achieves high resolution and sharp focus by using (1) a small point of high-intensity light, often from a laser, and (2) a plate with a pinhole aperture in front of the image detector. The point light source, the 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.

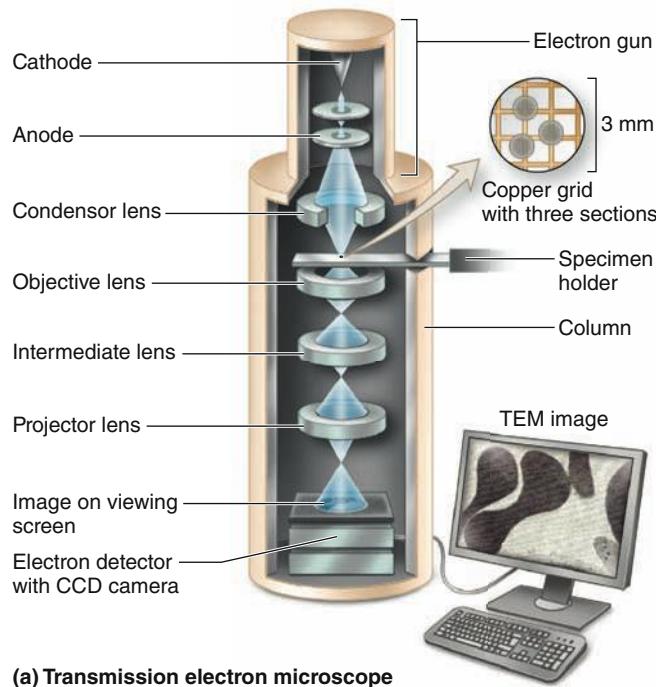
The wavelength in an electron beam is much shorter than that of light, allowing a 1000-fold increase in resolution.

Transmission Electron Microscopy

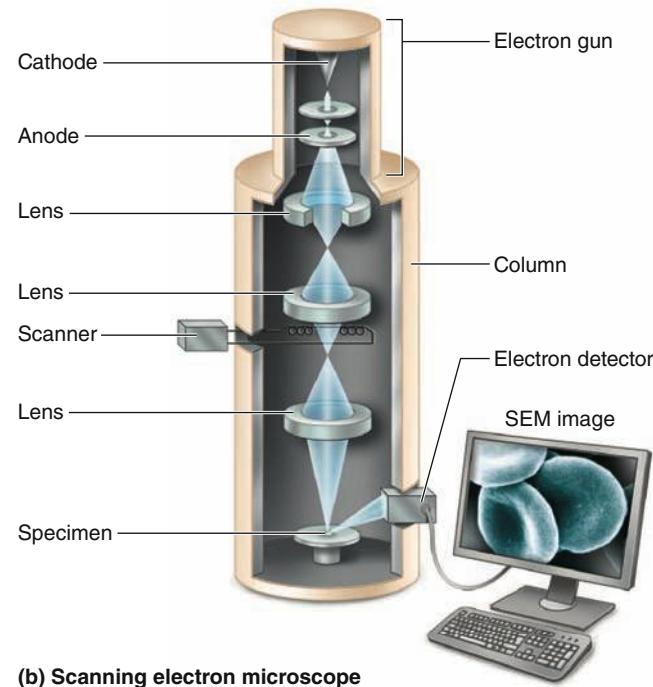
The **transmission electron microscope (TEM)** is an imaging system that permits resolution around 3 nm. This high resolution allows isolated particles magnified as much as 400,000 times to be viewed in detail. Very thin (40–90 nm), resin-embedded tissue sections are typically studied by TEM at magnifications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

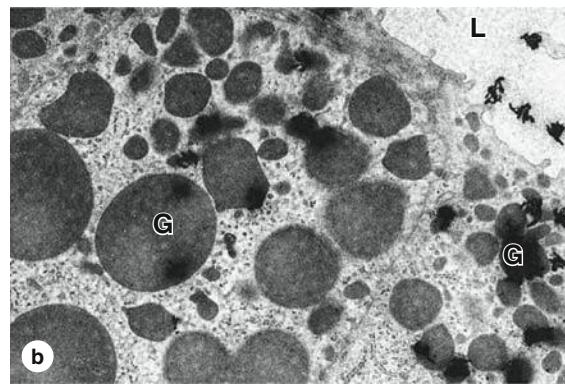
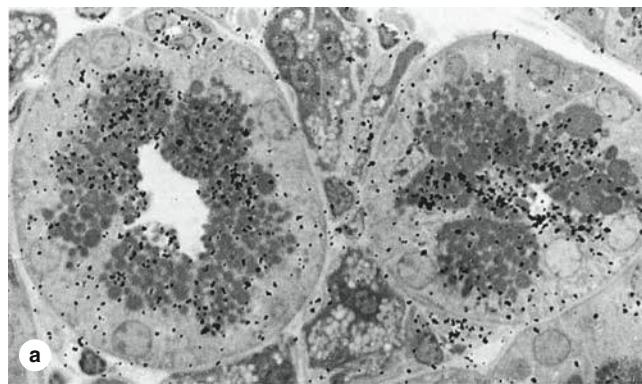
layer of heavy metal (often gold) which reflects electrons in a beam scanning the specimen. The reflected electrons are captured by a detector, producing signals that are processed to produce a black-and-white image. SEM images are usually easy to interpret because they present a three-dimensional view 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 ^3H -fucose 8 hours before tissue fixation. Fucose was incorporated into oligosaccharides, and the free ^3H -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:

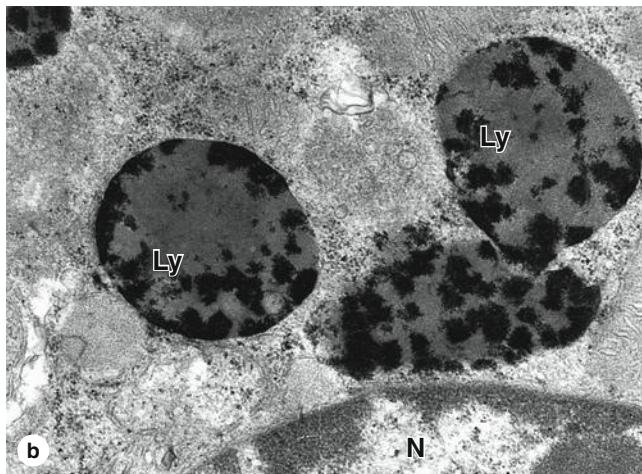
- **Phosphatases**, which remove phosphate groups from macromolecules (Figure 1–10).
- **Dehydrogenases**, which transfer hydrogen ions from one substrate to another, such as many enzymes of the citric acid (Krebs) cycle, allowing histochemical identification of such enzymes in mitochondria.
- **Peroxidase**,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

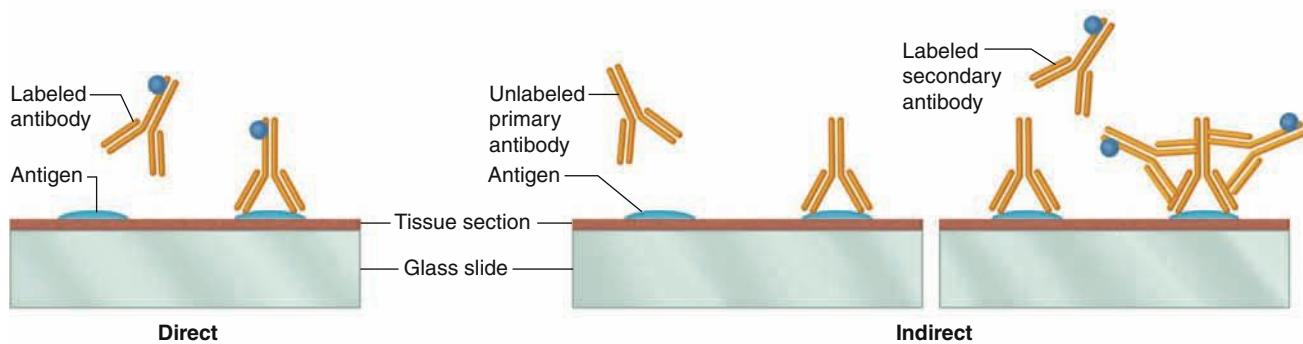
» MEDICAL APPLICATION

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

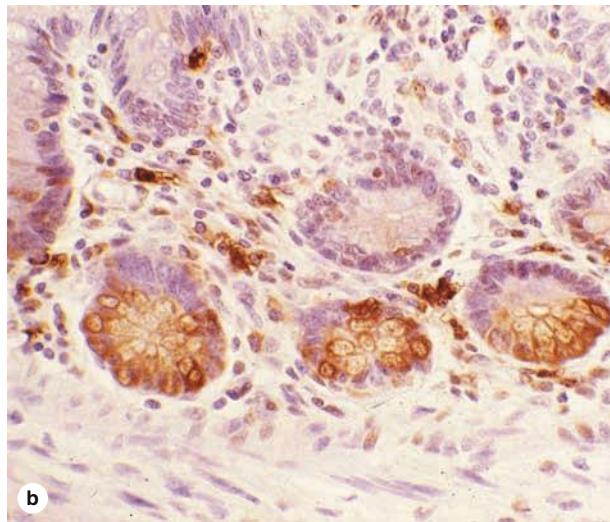
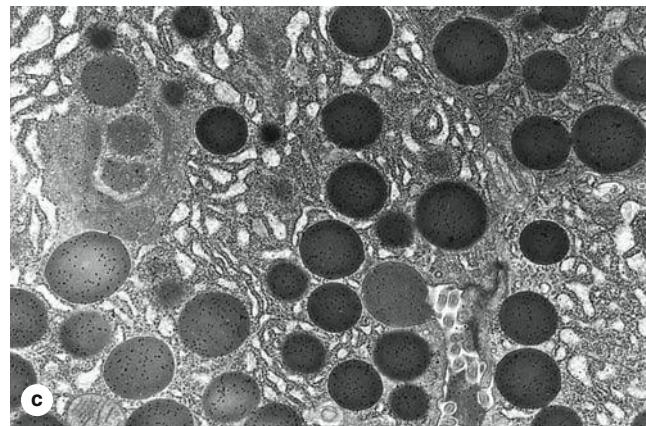
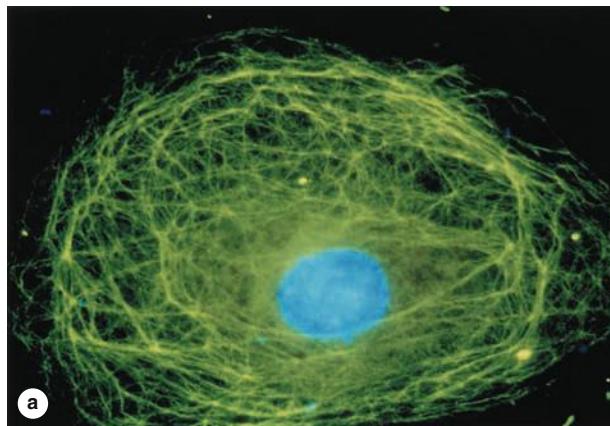
Hybridization usually implies the specific binding between two single strands of nucleic acid, which occurs under appropriate conditions if the strands are complementary. The greater the similarities of their nucleotide sequences, the more readily the complementary strands form “hybrid” double-strand 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.)

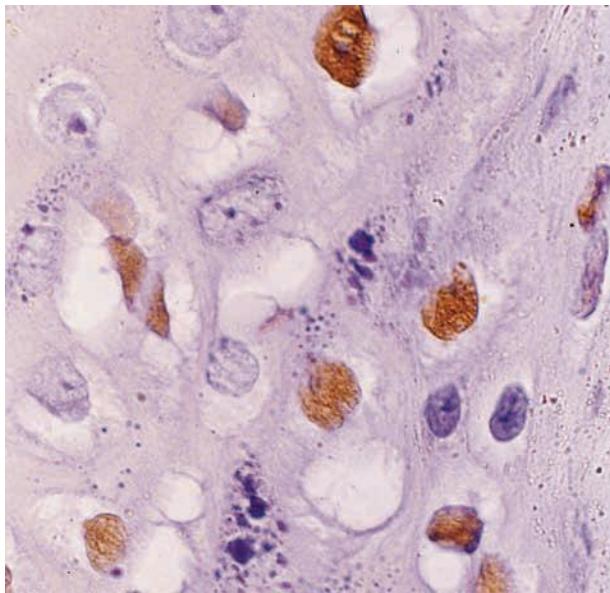
TABLE 1-1**Examples of specific antigens with diagnostic importance.**

| 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

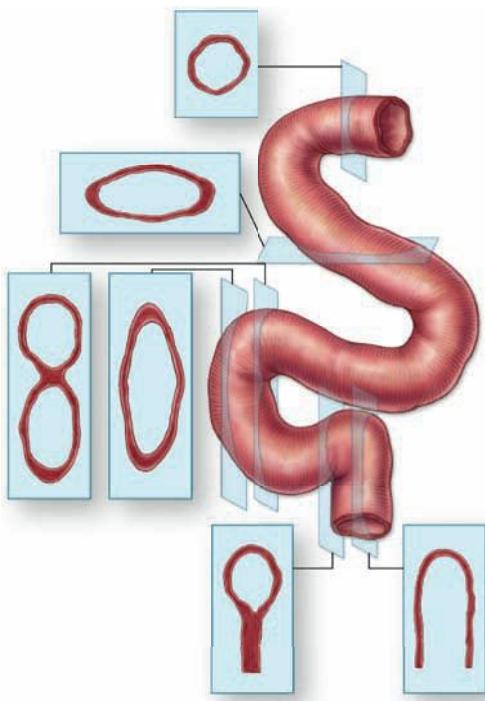
In studying and interpreting stained tissue sections, it is important to remember that microscopic preparations are the end result of a series of processes that began with collecting the tissue and ended with mounting a coverslip on the slide. Certain steps in this procedure may distort the tissues slightly, 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

| | | | |
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| Endoplasmic Reticulum | 28 | Microtubules | 43 |
| Golgi Apparatus | 31 | Microfilaments (Actin Filaments) | 44 |
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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. Planted 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.

TABLE 2–1

Differentiated cells typically specialize in one activity.

| General Cellular Activity | Specialized Cell(s) |
|---|---|
| Movement | Muscle and other contractile cells |
| Form adhesive and tight junctions between cells | Epithelial cells |
| Synthesize and secrete components of the extracellular matrix | Fibroblasts, cells of bone and cartilage |
| Convert physical and chemical stimuli into action potentials | Neurons and sensory cells |
| Synthesis and secretion of degradative enzymes | Cells of digestive glands |
| Synthesis and secretion of glycoproteins | Cells of mucous glands |
| Synthesis and secretion of steroids | Certain cells of the adrenal gland, testis, and ovary |
| Ion transport | Cells of the kidney and salivary gland ducts |
| Intracellular digestion | Macrophages and neutrophils |
| Lipid storage | Fat cells |
| Metabolite absorption | Cells lining the intestine |

Although the plasma membrane defines the outer limit of the cell, a continuum exists between the interior of the cell and extracellular macromolecules. Certain plasma membrane proteins, the **integrins**, are linked to both the cytoskeleton and ECM components and allow continuous exchange of influences, in both directions, between the cytoplasm and material in the ECM.

Membranes range from 7.5 to 10 nm in thickness and consequently are visible only in the electron microscope. The line between adjacent cells sometimes seen faintly with the light microscope consists of plasma membrane proteins plus extracellular material, which together can reach a dimension visible by light microscopy.

Membrane phospholipids are amphipathic, consisting of two nonpolar (hydrophobic or water-repelling) long-chain fatty acids linked to a charged polar (hydrophilic or water-attracting) head that bears a phosphate group (Figure 2–1a). Phospholipids are most stable when organized into a double layer (bilayer) with the hydrophobic fatty acid chains located in a middle region away from water and the hydrophilic polar head groups contacting the water (Figure 2–1b). Molecules of cholesterol, a sterol lipid, insert at varying densities among the closely-packed phospholipid fatty acids, restricting their movements and modulating the fluidity of all membrane components. The phospholipids in each half of the bilayer are different. For example, in the well-studied membranes of red blood cells phosphatidylcholine and sphingomyelin are more

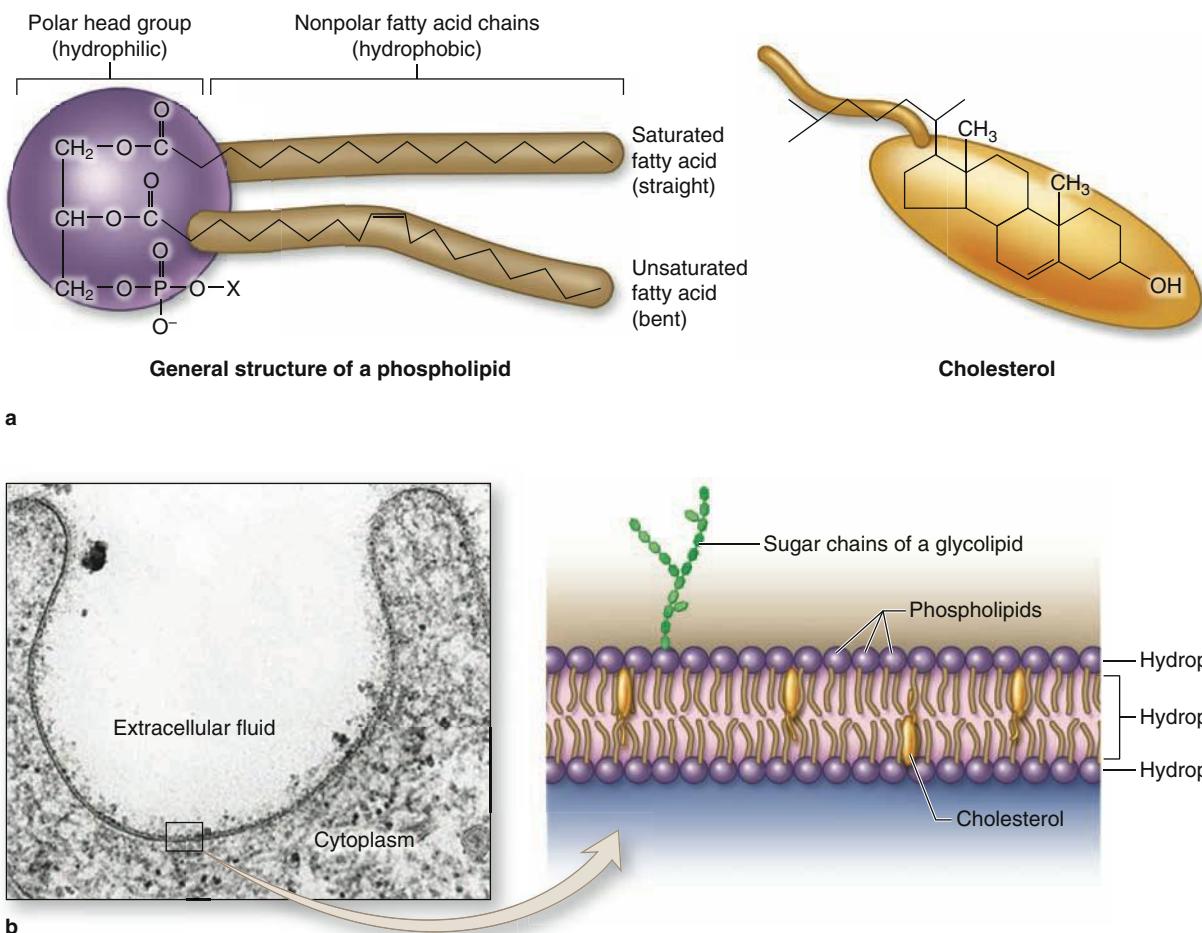
abundant in the outer half, while phosphatidylserine and phosphatidylethanolamine are more concentrated in the inner layer. Some of the outer layer's lipids, known as **glycolipids**, include oligosaccharide chains that extend outward from the cell surface and contribute to a delicate cell surface coating called the **glycocalyx** (Figures 2–1b and 2–2). With the transmission electron microscope (TEM) the cell membrane—as well as all cytoplasmic membranes—may exhibit a trilaminar appearance after fixation in osmium tetroxide; osmium binds the polar heads of the phospholipids and the oligosaccharide chains, producing the two dark outer lines that enclose the light band of osmium-free fatty acids (Figure 2–1b).

Proteins are major constituents of membranes (~50% by weight in the plasma membrane). **Integral proteins** are incorporated directly within the lipid bilayer, whereas **peripheral proteins** are bound to one of the two membrane surfaces, particularly on the cytoplasmic side (Figure 2–2). Peripheral proteins can be extracted from cell membranes with salt solutions, whereas integral proteins can be extracted only by using detergents to disrupt the lipids. The polypeptide chains of many integral proteins span the membrane, from one side to the other, several times and are accordingly called **multipass proteins**. Integration of the proteins within the lipid bilayer is mainly the result of hydrophobic interactions between the lipids and nonpolar amino acids of the proteins.

Freeze-fracture electron microscope studies of membranes show that parts of many integral proteins protrude from both the outer or inner membrane surface (Figure 2–2b). Like those of glycolipids, the carbohydrate moieties of glycoproteins project from the external surface of the plasma membrane and contribute to the glycocalyx (Figure 2–3). They are important components of proteins acting as **receptors**, which participate in important interactions such as cell adhesion, cell recognition, and the response to protein hormones. As with lipids, the distribution of membrane polypeptides is different in the two surfaces of the cell membranes. Therefore, all membranes in the cell are asymmetric.

Studies with labeled membrane proteins of cultured cells reveal that many such proteins are not bound rigidly in place and are able to move laterally (Figure 2–4). Such observations as well as data from biochemical, electron microscopic, and other studies showed that membrane proteins comprise a moveable mosaic within the fluid lipid bilayer, the well-established **fluid mosaic model** for membrane structure (Figure 2–2a). Unlike the lipids, however, lateral diffusion of many membrane proteins is often restricted by their cytoskeletal attachments. Moreover, in most epithelial cells tight junctions between the cells (see Chapter 4) also restrict lateral diffusion of unattached transmembrane proteins and outer layer lipids, producing different domains within the cell membranes.

Membrane proteins that are components of large enzyme complexes are also usually less mobile, especially those involved in the transduction of signals from outside the cell. Such protein complexes are located in specialized membrane patches termed **lipid rafts** with higher concentrations of

FIGURE 2–1 Lipids in membrane structure.

(a) Membranes of animal cells have as their major lipid components **phospholipids** and **cholesterol**. A phospholipid is amphipathic, with a phosphate group charge on the polar head and two long, nonpolar fatty acid chains, which can be straight (saturated) or kinked (at an unsaturated bond). Membrane cholesterol is present in about the same amount as phospholipid.

(b) The amphipathic nature of phospholipids produces the bilayer structure of membranes as the charged (hydrophilic) polar heads spontaneously form each membrane surface, in direct contact with water, and the hydrophobic nonpolar fatty acid chains are buried in the membrane's middle, away from water. Cholesterol molecules are also amphipathic and are interspersed less evenly

throughout the lipid bilayer; cholesterol affects the packing of the fatty acid chains, with a major effect on membrane fluidity. The outer layer of the cell membrane also contains **glycolipids** with extended carbohydrate chains.

Sectioned, osmium-fixed cell membrane may have a faint trilaminar appearance with the transmission electron microscope (TEM), showing two dark (electron-dense) lines enclosing a clear (electron-lucent) band. Reduced osmium is deposited on the hydrophilic phosphate groups present on each side of the internal region of fatty acid chains where osmium is not deposited. The "fuzzy" material on the outer surface of the membrane represents the **glycocalyx** of oligosaccharides of glycolipids and glycoproteins. (X100,000)

cholesterol and saturated fatty acids which reduce lipid fluidity. This together with the presence of scaffold proteins that maintain spatial relationships between enzymes and signaling proteins allows the proteins assembled within lipid rafts to remain in close proximity and interact more efficiently.

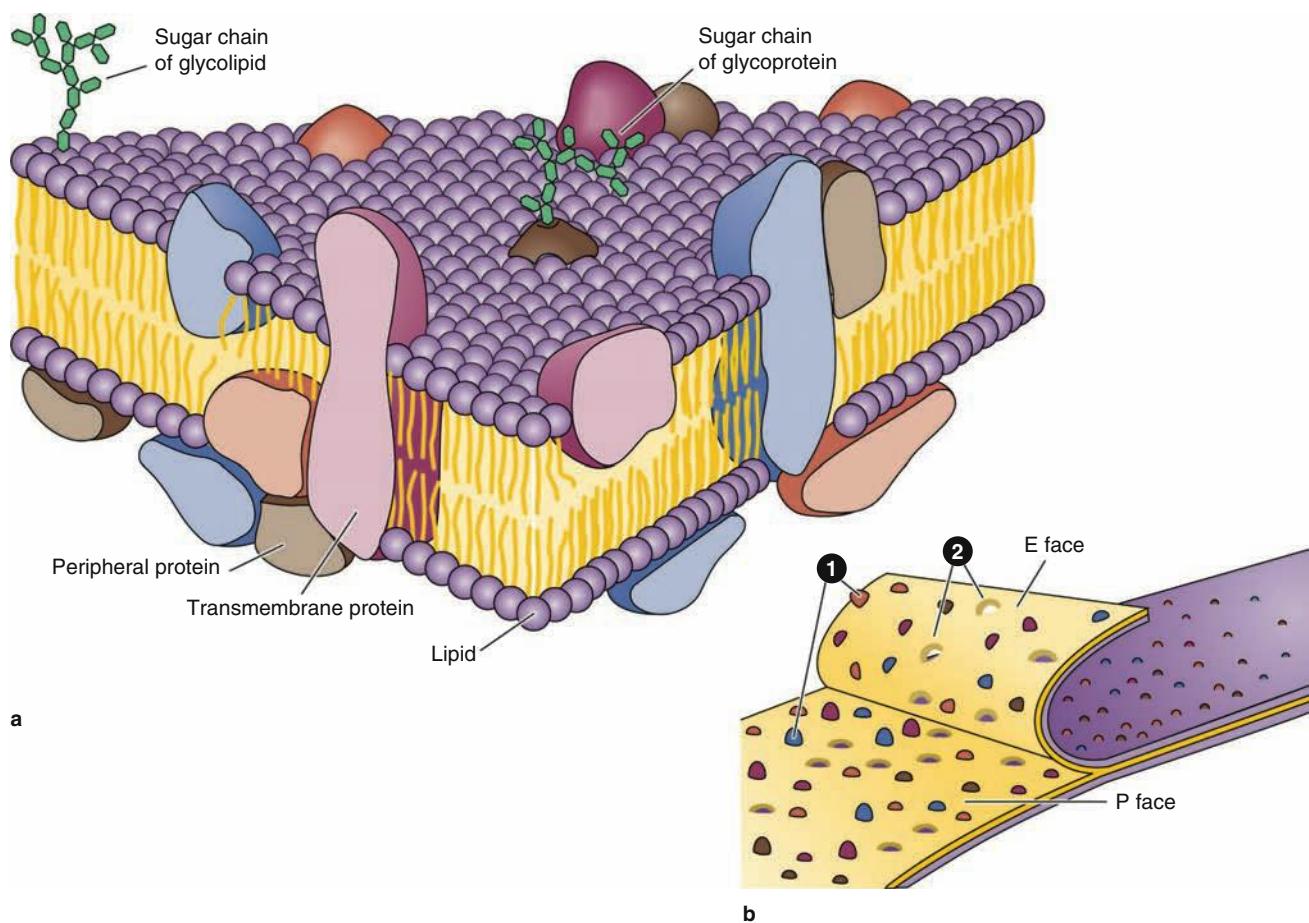
Transmembrane Proteins & Membrane Transport

The plasma membrane is the site where materials are exchanged between the cell and its environment. Most small

molecules cross the membrane by the general mechanisms shown schematically in Figure 2–5 and explained as follows:

- **Diffusion** transports small, nonpolar molecules directly through the lipid bilayer. Lipophilic (fat-soluble) molecules diffuse through membranes readily, water very slowly.
- **Channels** are multipass proteins forming transmembrane pores through which ions or small molecules pass selectively. Cells open and close specific channels for Na^+ ,

FIGURE 2–2 Proteins associated with the membrane lipid bilayer.



(a) The fluid mosaic model of membrane structure emphasizes that the phospholipid bilayer of a membrane also contains proteins inserted in it or associated with its surface (peripheral proteins) and that many of these proteins move within the fluid lipid phase. **Integral proteins** are firmly embedded in the lipid layers; those that completely span the bilayer are called **transmembrane proteins**. Hydrophobic amino acids of these proteins interact with the hydrophobic fatty acid portions of the membrane lipids. Both the proteins and lipids may have externally exposed oligosaccharide chains.

(b) When cells are frozen and fractured (**cryofracture**), the lipid bilayer of membranes is often cleaved along the hydrophobic

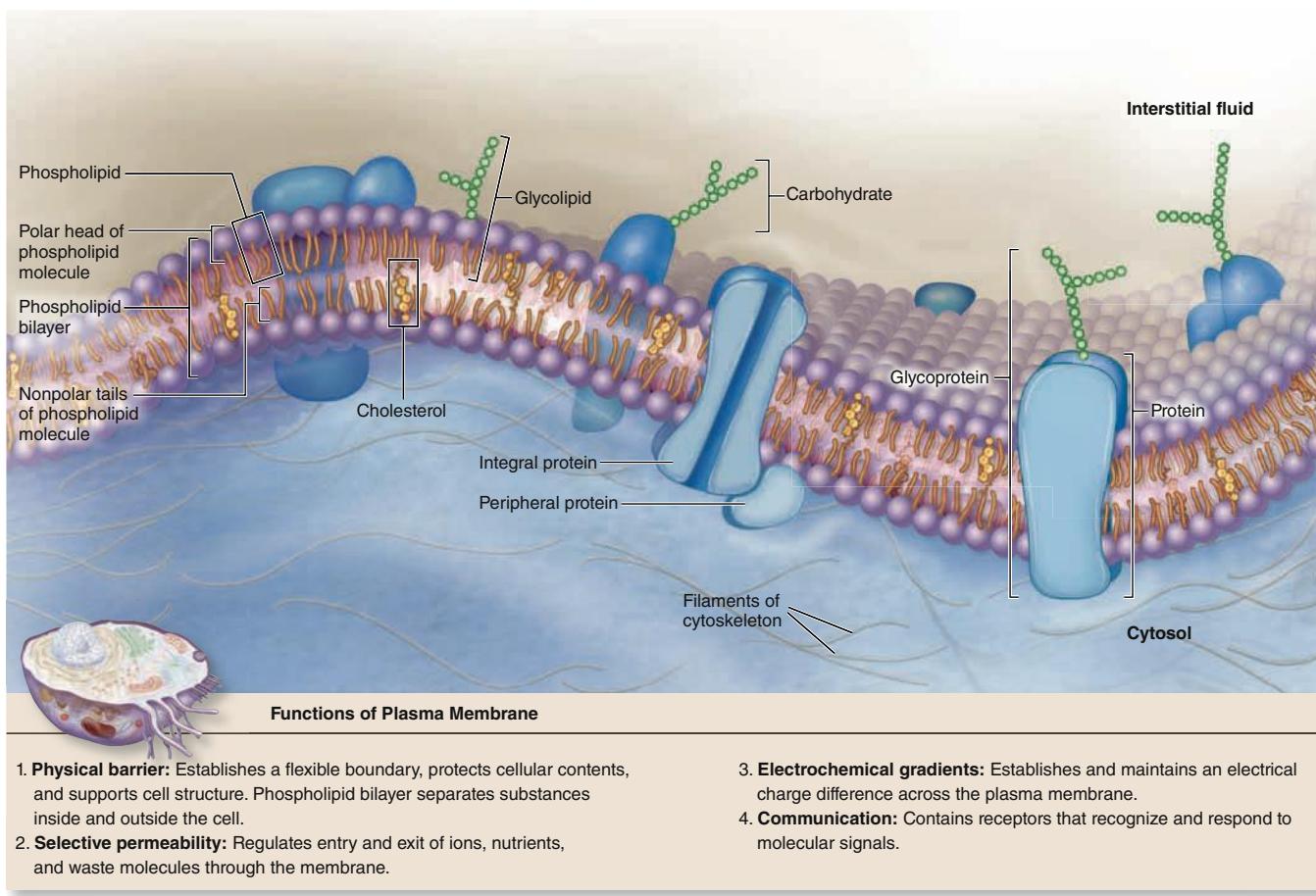
center. Splitting occurs along the line of weakness formed by the fatty acid tails of phospholipids. Electron microscopy of such cryofracture preparation replicas provides a useful method for studying membrane structures. Most of the protruding membrane particles seen (1) are proteins or aggregates of proteins that remain attached to the half of the membrane adjacent to the cytoplasm (P or protoplasmic face). Fewer particles are found attached to the outer half of the membrane (E or extracellular face). Each protein bulging on one surface has a corresponding depression (2) on the opposite surface.

K^+ , Ca^{2+} and other ions in response to various physiological stimuli. Water molecules usually cross the plasma membrane through channel proteins called **aquaporins**. ■ **Carriers** are transmembrane proteins that bind small molecules and translocate them across the membrane via conformational changes.

Diffusion, channels, and carrier proteins operate passively, allowing movement of substances across membranes

down a concentration gradient due to its kinetic energy. In contrast, membrane **pumps** are enzymes engaged in **active transport**, utilizing energy from the hydrolysis of adenosine triphosphate (ATP) to move ions and other solutes across membranes, against often steep concentration gradients. Because they consume ATP pumps they are often referred to as **ATPases**.

These transport mechanisms are summarized with additional detail in Table 2–2.

FIGURE 2–3 Membrane proteins.

Both protein and lipid components often have covalently attached oligosaccharide chains exposed at the external membrane surface. These contribute to the cell's **glycocalyx**, which provides important antigenic and functional properties to the cell surface. Membrane proteins serve as receptors for various signals coming from outside cells, as parts of intercellular

connections, and as selective gateways for molecules entering the cell.

Transmembrane proteins often have multiple hydrophobic regions buried within the lipid bilayer to produce a channel or other active site for specific transfer of substances through the membrane.

Vesicular Transport: Endocytosis & Exocytosis

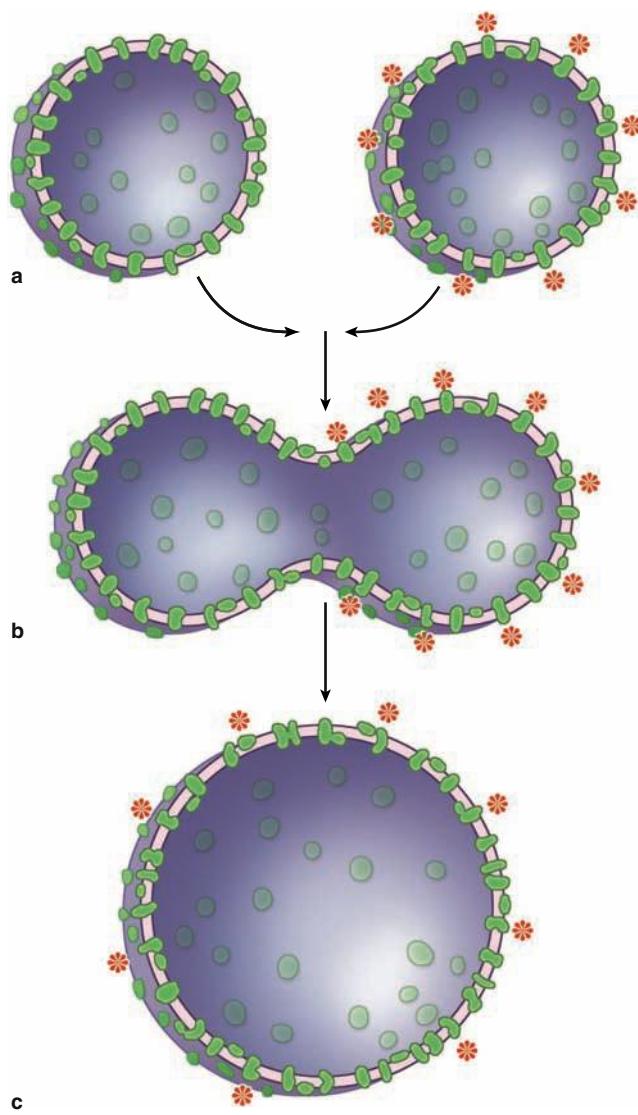
Macromolecules normally enter cells by being enclosed within folds of plasma membrane (often after binding specific membrane receptors) which fuse and pinch off internally as cytoplasmic **vesicles** (or vacuoles) in a general process known as **endocytosis**. Three major types of endocytosis are recognized, as summarized in Table 2–2 and Figure 2–6.

- Phagocytosis** ("cell eating") is the ingestion of particles such as bacteria or dead cell remnants. Certain blood-derived cells, such as macrophages and neutrophils, are specialized for this activity. When a bacterium becomes bound to the surface of a neutrophil, it becomes surrounded by extensions of plasmalemma and cytoplasm

which project from the cell in a process dependent on cytoskeletal changes. Fusion of the membranous folds encloses the bacterium in an intracellular vacuole called a **phagosome**, which then merges with a lysosome for degradation of its contents as discussed later in this chapter.

- Pinocytosis** ("cell drinking") involves smaller invaginations of the cell membrane which fuse and entrap extracellular fluid and its dissolved contents. The resulting **pinocytotic vesicles** (~80 nm in diameter) then pinch off inwardly from the cell surface and either fuse with lysosomes or move to the opposite cell surface where they fuse with the membrane and release their contents outside the cell. The latter process, called **transcytosis**,

FIGURE 2–4 Experiment demonstrating the fluidity of membrane proteins.



(a) Two types of cells were grown in tissue cultures, one with fluorescently labeled transmembrane proteins in the plasma-membrane (right) and one without.

(b) Cells of each type were fused together experimentally into hybrid cells.

(c) Minutes after the fusion of the cell membranes, the fluorescent proteins of the labeled cell spread to the entire surface of the hybrid cells. Such experiments provide important data supporting the fluid mosaic model. However, many membrane proteins show more restricted lateral movements, being anchored in place by links to the cytoskeleton.

accomplishes bulk transfer of dissolved substances across the cell.

3. **Receptor-mediated endocytosis:** Receptors for many substances, such as low-density lipoproteins and protein

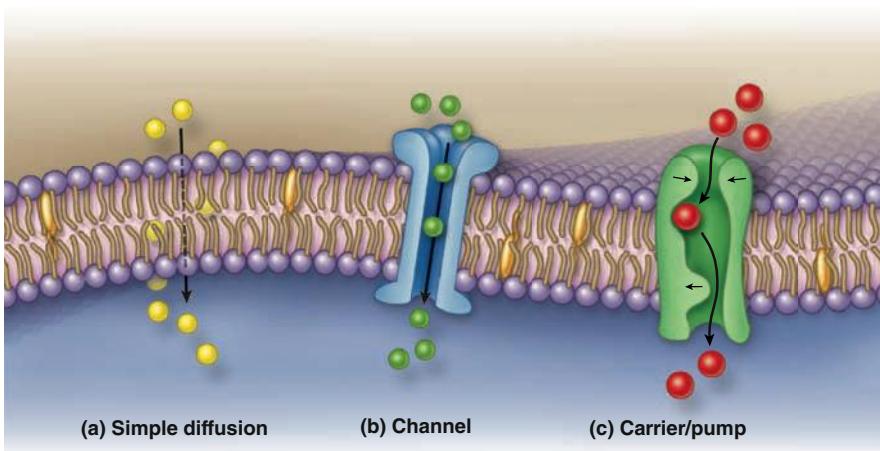
hormones, are integral membrane proteins at the cell surface. High-affinity binding of such **ligands** to their receptors causes these proteins to aggregate in special membrane regions that then invaginate and pinch off internally as vesicles.

The formation and fate of vesicles in receptor-mediated endocytosis also often depend on specific peripheral proteins on the cytoplasmic side of the membrane (Figure 2–7). The occupied cell-surface receptors associate with these cytoplasmic proteins and begin invagination as **coated pits**. The electron-dense coating on the cytoplasmic surface of such pits contains several polypeptides, the major one being **clathrin**. Clathrin molecules interact like the struts of a geodesic dome, forming that region of cell membrane into a cage-like invagination that is pinched off in the cytoplasm as a **coated vesicle** (Figure 2–7b) with the receptor-bound ligands inside. Another type of receptor-mediated endocytosis very prominent in endothelial cells produces invaginations called **caveolae** (L. *caveolae*, little caves) that involve the membrane protein **caveolin**.

In all these endocytotic processes, the vesicles or vacuoles produced quickly enter and fuse with the **endosomal compartment**, a dynamic collection in the peripheral cytoplasm of membranous tubules and vacuoles (Figure 2–7). The clathrin molecules separate from the coated vesicles and recycle back to the cell membrane for the formation of new coated pits. Vesicle trafficking through the endosomal compartment is directed largely through peripheral membrane G proteins called **Rab proteins**, small GTPases that bind guanine nucleotides and associated proteins.

As shown in Figure 2–7, phagosomes and pinocytotic vesicles typically fuse with lysosomes within the endosomal compartment for digestion of their contents, while molecules entering by receptor-mediated endocytosis may be directed down other pathways. The membranes of many late endosomes have ATP-driven H⁺ pumps that acidify their interior, activating the hydrolytic enzymes of lysosomes and in other endosomes causing ligands to uncouple from their receptors, after which the two molecules are sorted into separate endosomes. The receptors may be sorted into recycling endosomes and returned to the cell surface for reuse. Low-density lipoprotein receptors, for example, are recycled several times within cells. Other endosomes may release their entire contents at a separate domain of the cell membrane (transcytosis), which occurs in many epithelial cells.

Movement of large molecules from inside to outside the cell usually involves vesicular transport in the process of **exocytosis**. In exocytosis a cytoplasmic vesicle containing the molecules to be secreted fuses with the plasma membrane, resulting in the release of its contents into the extracellular space without compromising the integrity of the plasma membrane (see “Transcytosis” in Figure 2–7a). Exocytosis is triggered in many cells by transient increase in cytosolic Ca²⁺. Membrane fusion during exocytosis is highly regulated, with selective interactions between several specific membrane proteins.

FIGURE 2–5 Major mechanisms by which molecules cross membranes.

Lipophilic and some small, uncharged molecules can cross membranes by simple diffusion (a).

Most ions cross membranes in multipass proteins called channels (b) whose structures include transmembrane ion-specific pores.

Many other larger, water-soluble molecules require binding to sites on selective carrier proteins (c), which then change their

conformations and release the molecule to the other side of the membrane.

Diffusion, channels and most carrier proteins translocate substances across membranes using only kinetic energy. In contrast, **pumps** are carrier proteins for active transport of ions or other solutes and require energy derived from ATP.

Exocytosis of macromolecules made by cells occurs via either of two pathways:

- **Constitutive secretion** is used for products that are released from cells continuously, as soon as synthesis is complete, such as collagen subunits for the ECM.
- **Regulated secretion** occurs in response to signals coming to the cells, such as the release of digestive enzymes from pancreatic cells in response to specific stimuli. Regulated exocytosis of stored products from epithelial cells usually occurs specifically at the apical domains of cells, constituting a major mechanism of glandular secretion (see Chapter 4).

Portions of the cell membrane become part of the endocytic vesicles or vacuoles during endocytosis; during exocytosis, membrane is returned to the cell surface. This process of membrane movement and recycling is called **membrane trafficking** (Figure 2–7a). Trafficking of membrane components occurs continuously in most cells and is not only crucial for maintaining the cell but also for physiologically important processes such as reducing blood lipid levels.

In many cells subpopulations of vacuoles and tubules within the endosomal compartment accumulate small vesicles *within* their lumens by further invaginations of their limiting membranes, becoming **multivesicular bodies**. While multivesicular bodies may merge with lysosomes for selective degradation of their content, this organelle may also fuse with the plasma membrane and release the intraluminal vesicles outside the cell. The small (<120 nm diameter) vesicles released

are called **exosomes**, which can fuse with other cells transferring their contents and membranes.

Signal Reception & Transduction

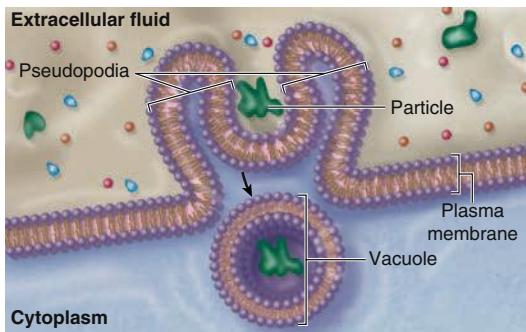
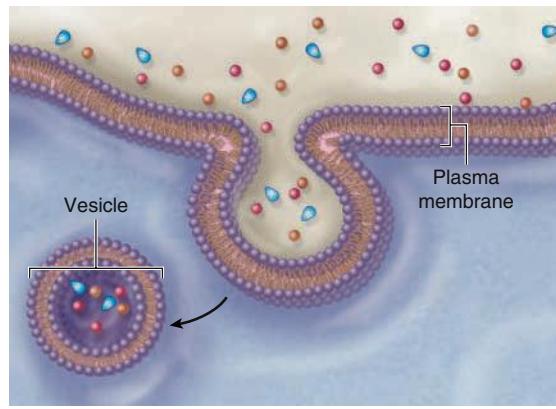
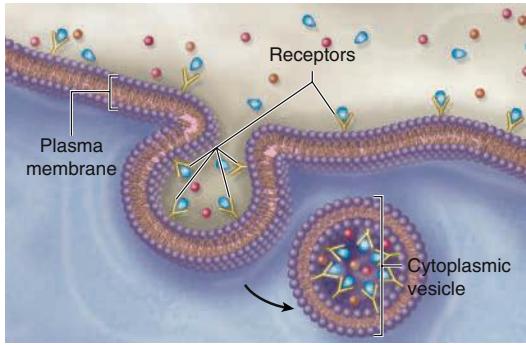
Cells in a multicellular organism communicate with one another to regulate tissue and organ development, to control their growth and division, and to coordinate their functions. Many adjacent cells form communicating **gap junctions** that couple the cells and allow exchange of ions and small molecules (see Chapter 4).

Cells also use about 25 families of **receptors** to detect and respond to various extracellular molecules and physical stimuli. Each cell type in the body contains a distinctive set of cell surface and cytoplasmic receptor proteins that enable it to respond to a complementary set of signaling molecules in a specific, programmed way. Cells bearing receptors for a specific ligand are referred to as **target cells** for that molecule. The routes of signal molecules from source to target provide one way to categorize the signaling process:

- In **endocrine signaling**, the signal molecules (here called **hormones**) are carried in the blood from their sources to target cells throughout the body.
- In **paracrine signaling**, the chemical ligand diffuses in extracellular fluid but is rapidly metabolized so that its effect is only local on target cells near its source.
- In **synaptic signaling**, a special kind of paracrine interaction, neurotransmitters act on adjacent cells through special contact areas called **synapses** (see Chapter 9).

TABLE 2–2**Mechanisms of transport across the plasma membrane.**

| Process | Type of Movement | Example |
|-------------------------------|---|---|
| PASSIVE PROCESSES | Movement of substances down a concentration gradient due to the kinetic energy of the substance; no expenditure of cellular energy is required; continues until equilibrium is reached (if unopposed) | |
| Simple diffusion | Unassisted net movement of small, nonpolar substances down their concentration gradient across a selectively permeable membrane | Exchange of oxygen and carbon dioxide between blood and body tissues |
| Facilitated diffusion | Movement of ions and small, polar molecules down their concentration gradient; assisted across a selectively permeable membrane by a transport protein | |
| Channel-mediated | Movement of ion down its concentration gradient through a protein channel | Na^+ moves through Na^+ channel into cell |
| Carrier-mediated | Movement of small, polar molecule down its concentration gradient by a carrier protein | Transport of glucose into cells by glucose carrier |
| Osmosis | Diffusion of water across a selectively permeable membrane; direction is determined by relative solute concentrations; continues until equilibrium is reached | Solutes in blood in systemic capillaries "pulls" fluid from interstitial space back into the blood |
| ACTIVE PROCESSES | Movement of substances requires expenditure of cellular energy | |
| Active transport | Transport of ions or small molecules across the membrane against a concentration gradient by transmembrane protein pumps | |
| Primary | Movement of substance up its concentration gradient; powered directly by ATP | Ca^{2+} pumps transport Ca^{2+} out of the cell Na^+/K^+ pump moves Na^+ out of cell and K^+ into cell |
| Secondary | Movement of a substance up its concentration gradient is powered by harnessing the movement of a second substance (eg, Na^+) down its concentration gradient | |
| Symport | Movement of substance up its concentration gradient in the same direction as Na^+ | $\text{Na}^+/\text{glucose}$ transport |
| Antiport | Movement of substance up its concentration gradient in the opposite direction from Na^+ | Na^+/H^+ transport |
| Vesicular transport | Vesicle formed or lost as material is brought into a cell or released from a cell | |
| Exocytosis | Bulk movement of substance out of the cell by fusion of secretory vesicles with the plasma membrane | Release of neurotransmitter by nerve cells |
| Endocytosis | Bulk movement of substances into the cell by vesicles forming at the plasma membrane | |
| Phagocytosis | Type of endocytosis in which vesicles are formed as particulate materials external to the cell are engulfed by pseudopodia | White blood cell engulfing a bacterium |
| Pinocytosis | Type of endocytosis in which vesicles are formed as interstitial fluid is taken up by the cell | Formation of small vesicles in capillary wall to move substances |
| Receptor-mediated endocytosis | Type of endocytosis in which plasma membrane receptors first bind specific substances; receptor and bound substance then taken up by the cell | Uptake of cholesterol into cells |

FIGURE 2–6 Three major forms of endocytosis.**a Phagocytosis****b Pinocytosis****c Receptor-mediated endocytosis**

There are three general types of endocytosis:

(a) Phagocytosis involves the extension from the cell of surface folds or pseudopodia which engulf particles such as bacteria,

and then internalize this material into a cytoplasmic vacuole or **phagosome**.

(b) In pinocytosis the cell membrane forms similar folds or invaginates (dimples inward) to create a pit containing a drop of extracellular fluid. The pit pinches off inside the cell when the cell membrane fuses and forms a pinocytotic vesicle containing the fluid.

(c) Receptor-mediated endocytosis includes membrane proteins called **receptors** that bind specific molecules (ligands). When many such receptors are bound by their ligands, they aggregate in one membrane region, which then invaginates and pinches off to create a vesicle or **endosome** containing both the receptors and the bound ligands.

- In **autocrine signaling**, signals bind receptors on the same cells that produced the messenger molecule.
- In **juxtacrine signaling**, important in early embryonic tissue interactions, the signaling molecules are cell membrane-bound proteins which bind surface receptors of the target cell when the two cells make direct physical contact.

Receptors for hydrophilic signaling molecules, including polypeptide hormones and neurotransmitters, are usually transmembrane proteins in the plasmalemma of target cells. Three important functional classes of such receptors are shown in Figure 2–8:

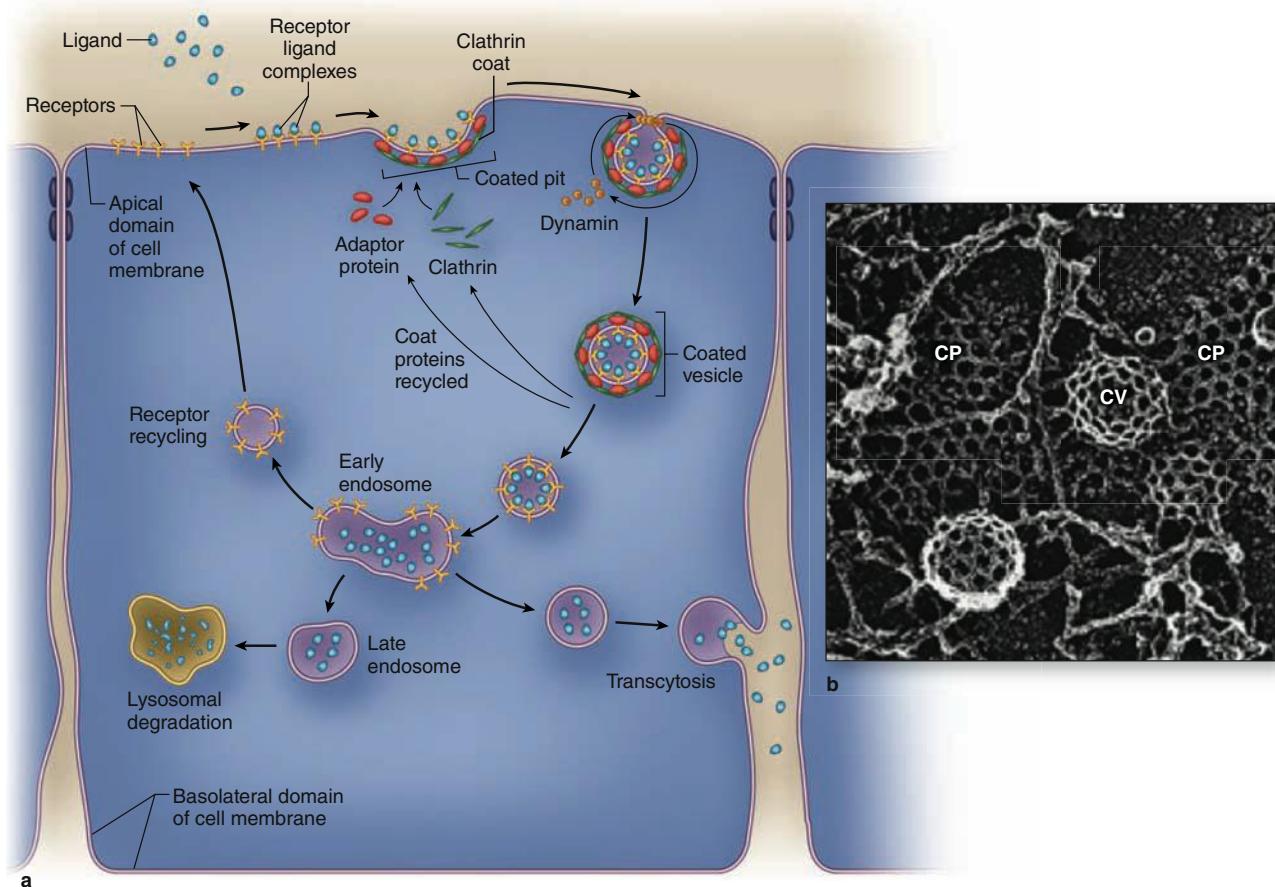
- **Channel-linked receptors** open associated channels upon ligand binding to promote transfer of molecules or ions across the membrane.
- **Enzymatic receptors**, in which ligand binding induces catalytic activity in associated peripheral proteins.
- **G protein-coupled receptors** upon ligand binding stimulate associated G proteins which then bind the guanine nucleotide GTP and are released to activate other cytoplasmic proteins.

» MEDICAL APPLICATION

Many diseases are caused by defective receptors. For example, **pseudohypoparathyroidism** and one type of **dwarfism** are caused by nonfunctioning parathyroid and growth hormone receptors, respectively. In these two conditions the glands produce the respective hormones, but the target cells cannot respond because they lack normal receptors.

Ligands binding such receptors in a cell membrane can be considered first messengers, beginning a process of **signal transduction** by activating a series of intermediary enzymes downstream to produce changes in the cytoplasm, the nucleus, or both. Channel-mediated ion influx or activation of kinases can activate various cytoplasmic proteins, amplifying the signal. Activated G proteins target ion channels or other membrane-bound effectors that also propagate the signal further into the cell (Figure 2–8). One such effector protein is the enzyme adenyl cyclase which generates large quantities of second messenger molecules, such as cyclic adenosine

FIGURE 2–7 Receptor-mediated endocytosis involves regulated membrane trafficking.



Major steps during and after endocytosis are indicated diagrammatically in part a. Ligands bind with high affinity to specific surface receptors, which then associate with specific cytoplasmic proteins, including clathrin and adaptor proteins, and aggregate in membrane regions to form **coated pits**. Clathrin facilitates invagination of the pits, and another peripheral membrane protein, **dynamin**, forms constricting loops around the developing neck of the pit, causing the invagination to pinch off as a **coated vesicle**. The clathrin lattice of coated pits (**CP**) and vesicles (**CV**) is shown ultrastructurally in part b.

Internalized vesicles lose their clathrin coats, which are recycled, and fuse with other endosomes that comprise the **endosomal compartment**. Ligands may have different fates within the endosomal compartment:

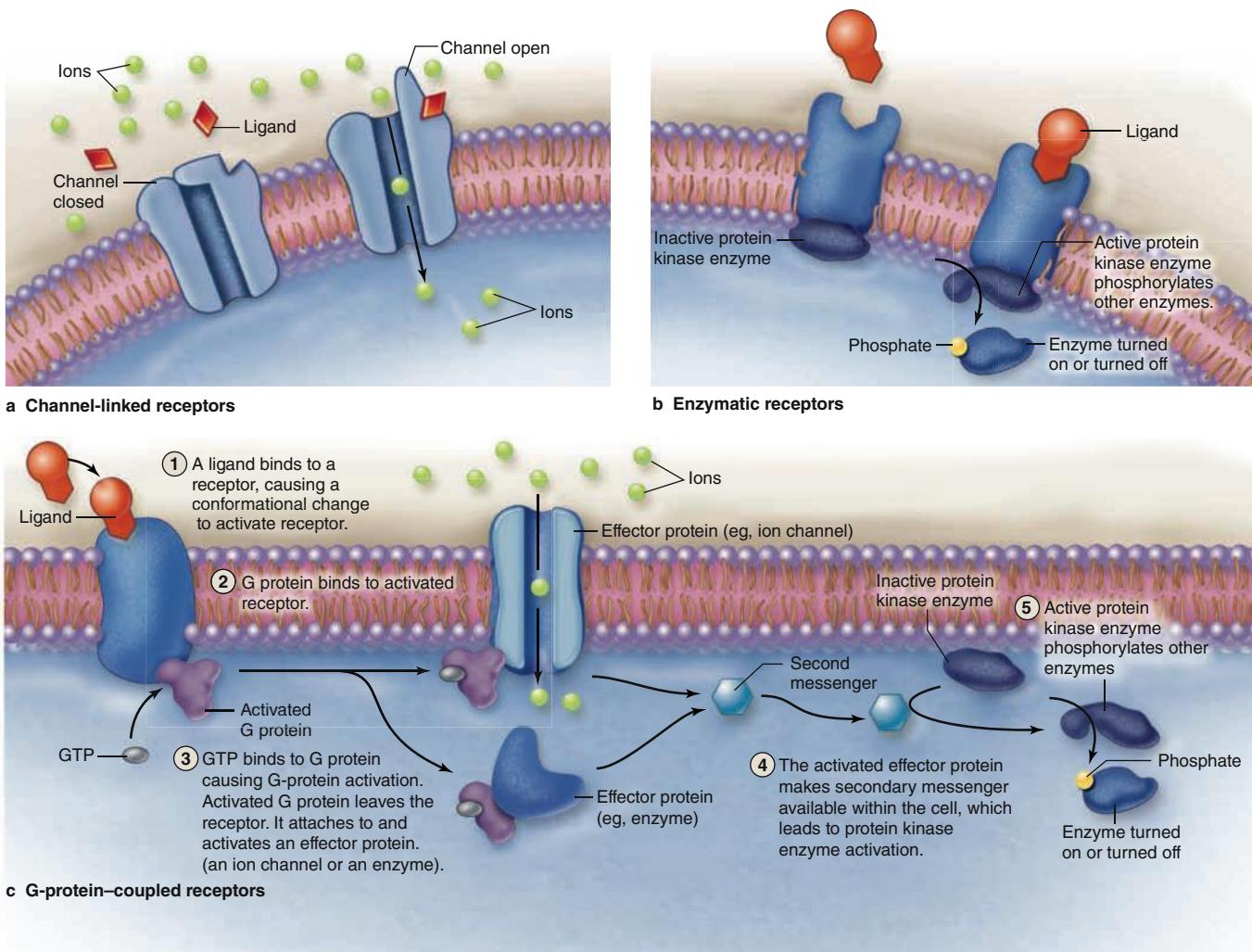
- Receptors and ligands may be carried to late endosomes and then to lysosomes for **degradation**.
- Ligands may be released from the receptors and the empty receptors sequestered into **recycling endosomes** and returned to the cell surface for reuse.
- Other endosomal vesicles containing ligands may move to and fuse with another cell surface, where the ligands are released again outside the cell in the process of **transcytosis**.

(Figure 2–7b, used with permission from Dr John Heuser, Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO.)

monophosphate (cAMP). Other second messengers include 1,2-diacylglycerol (DAG), and inositol 1,4,5-triphosphate (IP_3). The ionic changes or second messengers amplify the first signal and trigger a cascade of enzymatic activity, usually including kinases, leading to changes in gene expression or cell behavior. Second messengers may diffuse through the cytoplasm or be retained locally by scaffold proteins for more focused amplification of activity.

Low molecular weight **hydrophobic** signaling molecules, such as steroids and thyroid hormones, bind reversibly to

carrier proteins in the plasma for transport through the body. Such hormones are lipophilic and pass by diffusion through cell membranes, binding to specific cytoplasmic receptor proteins in target cells. With many steroid hormones, receptor binding activates that receptor, enabling the complex to move into the nucleus and bind with high affinity to specific DNA sequences. This generally increases the level of transcription of those genes. Each steroid hormone is recognized by a different member of a family of homologous receptor proteins.

FIGURE 2–8 Major types of membrane receptors.

Protein and most small ligands are hydrophilic molecules that bind transmembrane protein receptors to initiate changes in the target cell.

(a) Channel-linked receptors bind ligands such as neurotransmitters and open to allow influx of specific ions. **(b) Enzymatic receptors** are usually protein kinases that are activated to

phosphorylate (and usually activate) other proteins upon ligand binding. **(c) G-protein-coupled receptors** bind ligand, changing the conformation of its G-protein subunit, allowing it to bind GTP, and activating and releasing this protein to in turn activate other proteins such as ion channels and adenyl cyclase.

CYTOPLASMIC ORGANELLES

Inside the cell membrane the fluid cytoplasm (or cytosol) bathes metabolically active structures called **organelles**, which may be membranous (such as mitochondria) or non-membranous protein complexes (such as ribosomes and proteasomes). Most organelles are positioned in the cytoplasm by movements along the polymers of the **cytoskeleton**, which also determines a cell's shape and motility.

Cytosol also contains hundreds of enzymes, such as those of the glycolytic pathway, which produce building blocks for larger molecules and break down small molecules to liberate

energy. Oxygen, CO₂, electrolytic ions, low-molecular-weight substrates, metabolites, and waste products all diffuse through cytoplasm, either freely or bound to proteins, entering or leaving organelles where they are used or produced.

Ribosomes

Ribosomes are macromolecular machines, about 20 × 30 nm in size, which assemble polypeptides from amino acids on molecules of transfer RNA (tRNA) in a sequence specified by mRNA. A functional ribosome has two subunits of different sizes bound to a strand of mRNA. The core of the small

ribosomal subunit is a highly folded ribosomal RNA (rRNA) chain associated with more than 30 unique proteins; the core of the large subunit has three other rRNA molecules and nearly 50 other basic proteins.

The rRNA molecules in the ribosomal subunits not only provide structural support but also position transfer RNAs (tRNA) molecules bearing amino acids in the correct “reading frame” and catalyze the formation of the peptide bonds. The more peripheral proteins of the ribosome seem to function primarily to stabilize the catalytic RNA core.

These ribosomal proteins are themselves synthesized in cytoplasmic ribosomes, but are then imported to the nucleus where they associate with newly synthesized rRNA. The ribosomal subunits thus formed then move from the nucleus to the cytoplasm where they are reused many times, for translation of any mRNA strand.

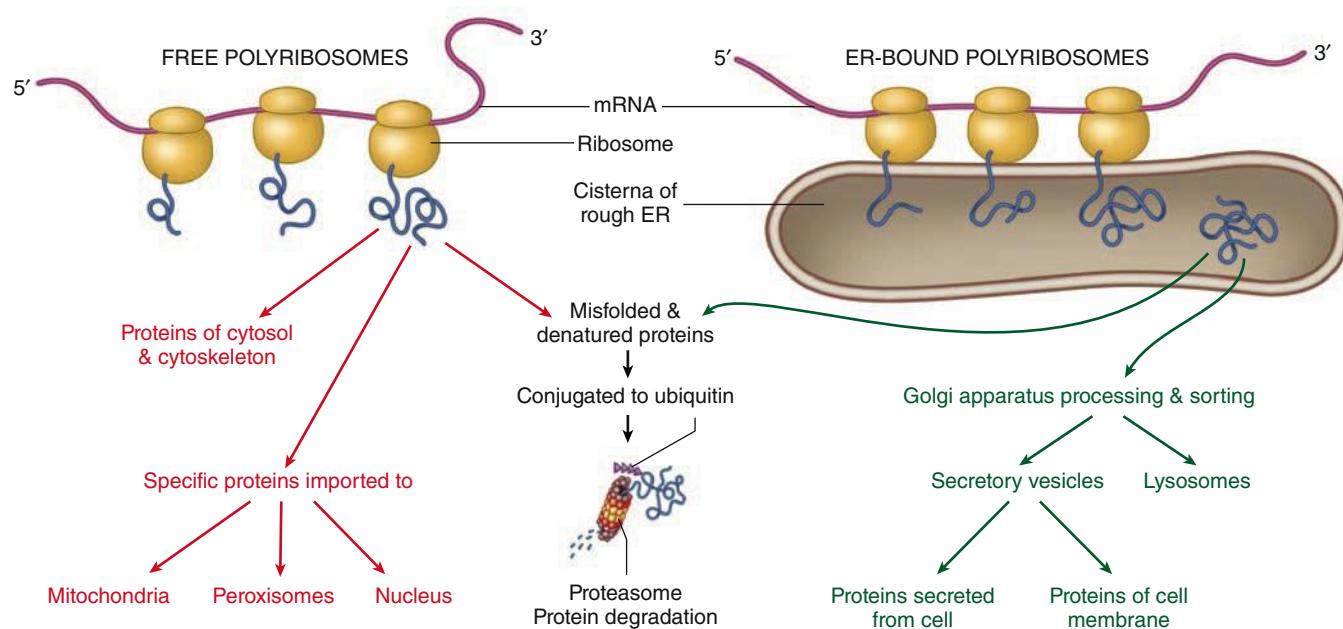
During protein synthesis many ribosomes typically bind the same strand of mRNA to form larger complexes called **polyribosomes**, or **polysomes** (Figure 2–9). In stained preparations of cells polyribosomes are intensely basophilic because of the numerous phosphate groups of the constituent RNA molecules that act as polyanions. Thus, cytoplasmic regions that stain intensely with hematoxylin and basic dyes, such as methylene and toluidine blue, indicate sites of active protein synthesis.

Proper folding of new proteins is guided by protein chaperones. Denatured proteins or those that cannot be refolded properly are conjugated to the protein ubiquitin that targets them for breakdown by proteasomes (discussed below). As indicated in Figure 2–9, proteins synthesized for use within the cytosol (eg, glycolytic enzymes) or for import into the nucleus and certain other organelles are synthesized on polyribosomes existing as isolated cytoplasmic clusters. Polyribosomes attached to membranes of the endoplasmic reticulum (ER) translate mRNAs coding for membrane proteins of the ER, the Golgi apparatus, or the cell membrane; enzymes to be stored in lysosomes; and proteins to undergo exocytosis from secretory vesicles.

Endoplasmic Reticulum

The cytoplasm of most cells contains a convoluted membranous network called the **endoplasmic reticulum (ER)**. As shown in Figure 2–10 this network (reticulum) extends from the surface of the nucleus throughout most of the cytoplasm and encloses a series of intercommunicating channels called **cisternae** (L. *cisterna*, reservoirs). With a membrane surface up to 30 times that of the plasma membrane, the ER is a major site for vital cellular activities, including biosynthesis of proteins and lipids. Numerous polyribosomes attached to the membrane in some regions of ER allow two types of ER to be distinguished.

FIGURE 2–9 Polyribosomes: free or bound to the endoplasmic reticulum.

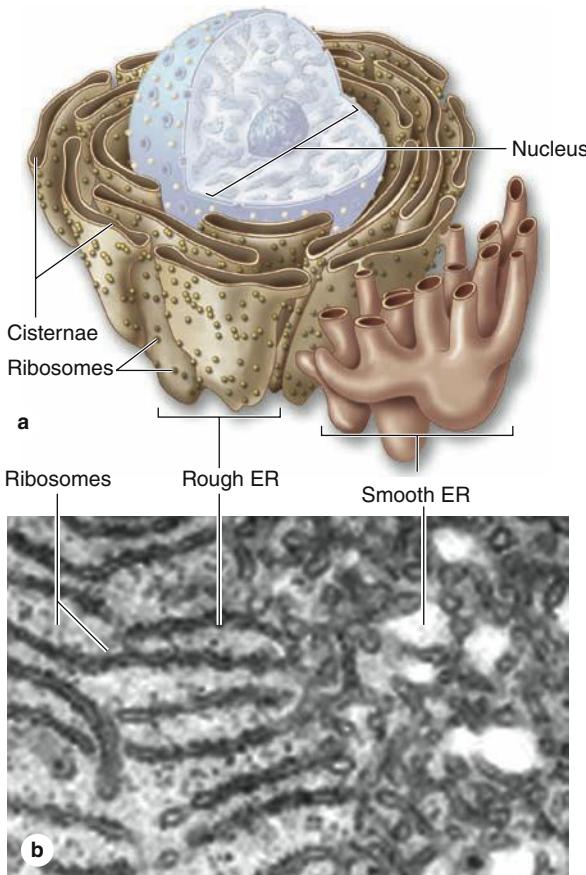


Free polyribosomes (not attached to the endoplasmic reticulum, or ER) synthesize cytosolic and cytoskeletal proteins and proteins for import into the nucleus, mitochondria, and peroxisomes.

Proteins that are to be incorporated into membranes, stored in lysosomes, or eventually secreted from the cell are made on

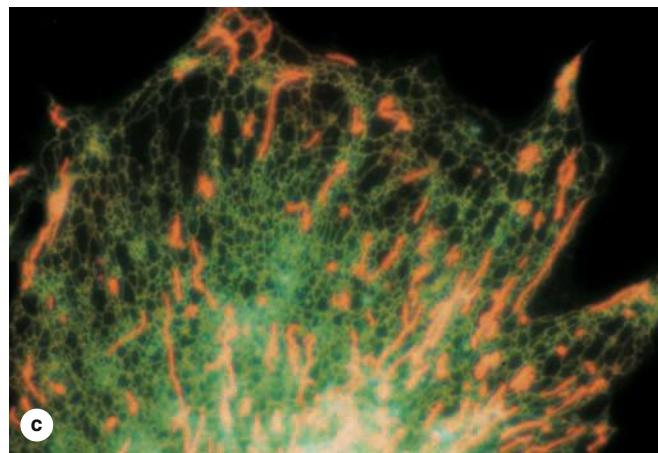
polyribosomes attached to the membranes of ER. The proteins produced by these ribosomes are segregated during translation into the interior of the ER's membrane cisternae.

In both pathways misfolded proteins are conjugated to ubiquitin and targeted for proteasomal degradation.

FIGURE 2–10 Rough and smooth endoplasmic reticulum.

(a) The **endoplasmic reticulum** is an anastomosing network of intercommunicating channels or **cisternae** formed by a continuous membrane, with some regions that bear polysomes appearing rough and other regions appearing smooth. While RER is the site for synthesis of most membrane-bound proteins, three diverse activities are associated with smooth ER: (1) lipid biosynthesis, (2) detoxification of potentially harmful compounds, and (3) sequestration of Ca^{++} ions. Specific cell types with well-developed SER are usually specialized for one of these functions.

(b) By TEM cisternae of RER appear separated, but they actually form a continuous channel or compartment in the cytoplasm.



Functions of Endoplasmic Reticulum

- Synthesis:** Provides a place for chemical reactions
 - Smooth ER is the site of lipid synthesis and carbohydrate metabolism
 - Rough ER synthesizes proteins for secretion, incorporation into the plasma, membrane, and as enzymes within lysosomes
- Transport:** Moves molecules through cisternal space from one part of the cell to another, sequestered away from the cytoplasm
- Storage:** Stores newly synthesized molecules
- Detoxification:** Smooth ER detoxifies both drugs and alcohol

The interconnected membranous cisternae of RER are flattened, while those of SER are frequently tubular. (14,000X)

(c) In a very thin cultured endothelial cell, both ER (green) and mitochondria (orange) can be visualized with vital fluorescent dyes that are sequestered specifically into those organelles. This staining method with intact cells clearly reveals the continuous, lace-like ER present in all regions of the cytoplasm.

(Figure 2–10c, © 2015 Thermo Fisher Scientific, Inc. Used under permission.)

Rough Endoplasmic Reticulum

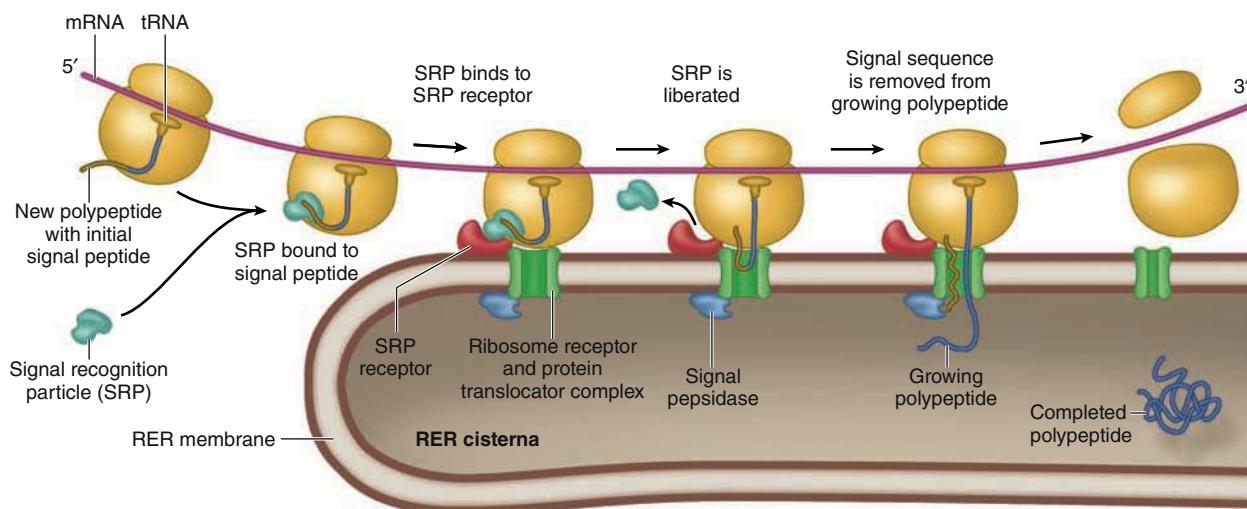
Rough endoplasmic reticulum (RER) is prominent in cells specialized for protein secretion, such as pancreatic acinar cells (making digestive enzymes), fibroblasts (collagen), and plasma cells (immunoglobulins). The RER consists of saclike as well as parallel stacks of flattened cisternae (Figure 2–10), each limited by membranes that are continuous with the outer membrane of the nuclear envelope. The presence of polyribosomes on the cytosolic surface of the RER confers basophilic staining properties on this organelle when viewed with the light microscope.

The major function of RER is production of membrane-associated proteins, proteins of many membranous organelles,

and proteins to be secreted by exocytosis. Production here includes the initial (core) glycosylation of glycoproteins, certain other posttranslational modifications of newly formed polypeptides, and the assembly of multichain proteins. These activities are mediated by resident enzymes of the RER and by protein complexes that act as chaperones guiding the folding of nascent proteins, inhibiting aggregation, and generally monitoring protein quality within the ER.

Protein synthesis begins on polyribosomes in the cytosol. The 5' ends of mRNAs for proteins destined to be segregated in the ER encode an N-terminal **signal sequence** of 15–40 amino acids that includes a series of six or more hydrophobic residues. As shown in Figure 2–11, the newly translated

FIGURE 2–11 Movement of polypeptides into the RER.



The newly translated amino terminus of a protein to be incorporated into membranes or sequestered into vesicles contains 15–40 amino acids that include a specific sequence of hydrophobic residues comprising the **signal sequence** or signal peptide. This sequence is bound by a signal-recognition particle (SRP), which then recognizes and binds a receptor on the ER. Another receptor in the ER membrane binds a structural protein of the large

ribosomal subunit, more firmly attaching the ribosome to the ER. The hydrophobic signal peptide is translocated through a protein pore (translocon) in the ER membrane, and the SRP is freed for reuse. The signal peptide is removed from the growing protein by a peptidase and translocation of the growing polypeptide continues until it is completely segregated into the ER cisterna.

signal sequence is bound by a protein complex called the **signal-recognition particle (SRP)**, which inhibits further polypeptide elongation. The SRP-ribosome-nascent peptide complex binds to SRP receptors on the ER membrane. SRP then releases the signal sequence, allowing translation to continue with the nascent polypeptide chain transferred to a **translocator complex** (also called a translocon) through the ER membrane (Figure 2–11). Inside the lumen of the RER, the signal sequence is removed by an enzyme, signal peptidase. With the ribosome docked at the ER surface, translation continues with the growing polypeptide pushing itself while chaperones and other proteins serve to “pull” the nascent polypeptide through the translocator complex. Upon release from the ribosome, posttranslational modifications and proper folding of the polypeptide continue.

RER has a highly regulated system to prevent nonfunctional proteins being forwarded to the pathway for secretion or to other organelles. New proteins that cannot be folded or assembled properly by chaperones undergo ER-associated degradation (ERAD), in which unsalvageable proteins are translocated back into the cytosol, conjugated to ubiquitin, and then degraded by proteasomes.

As mentioned, proteins synthesized in the RER can have several destinations: intracellular storage (eg, in lysosomes and specific granules of leukocytes), provisional storage in cytoplasmic vesicles prior to exocytosis (eg, in the pancreas, some endocrine cells), and as integral membrane proteins. Diagrams in Figure 2–12 show a few cell types with distinct

differences in the destinations of their major protein products and how these differences determine a cell's histologic features.

» MEDICAL APPLICATION

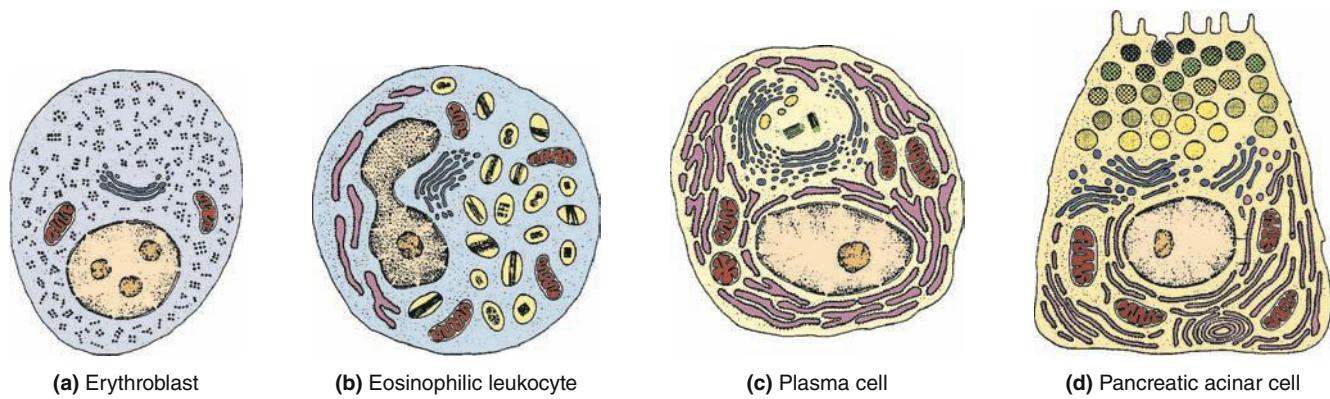
Quality control during protein production in the RER and properly functioning ERAD to dispose of defective proteins are extremely important and several inherited diseases result from malfunctions in this system. For example, in some forms of osteogenesis imperfecta bone cells synthesize and secrete defective procollagen molecules which cannot assemble properly and produce very weak bone tissue.

Smooth Endoplasmic Reticulum

Regions of ER that lack bound polyribosomes make up the smooth endoplasmic reticulum (SER), which is continuous with RER but frequently less abundant (Figure 2–10). Lacking polyribosomes, SER is not basophilic and is best seen with the TEM. Unlike the cisternae of RER, SER cisternae are more tubular or saclike, with interconnected channels of various shapes and sizes rather than stacks of flattened cisternae.

SER has three main functions, which vary in importance in different cell types.

- Enzymes in the SER perform *synthesis of phospholipids and steroids*, major constituents of cellular membranes. These lipids are then transferred from the SER to other membranes by lateral diffusion into adjacent

FIGURE 2–12 Protein localization and cell morphology.

The ultrastructure and general histologic appearance of a cell are determined by the nature of the most prominent proteins the cell is making.

- (a) Cells that make few or no proteins for secretion have very little RER, with essentially all polyribosomes free in the cytoplasm.
- (b) Cells that synthesize, segregate, and store various proteins in specific secretory granules or vesicles always have RER, a Golgi apparatus, and a supply of granules containing the proteins ready to be secreted.

(c) Cells with extensive RER and a well-developed Golgi apparatus show few secretory granules because the proteins undergo exocytosis immediately after Golgi processing is complete. Many cells, especially those of epithelia, are *polarized*, meaning that the distribution of RER and secretory vesicles is different in various regions or poles of the cell.

(d) Epithelial cells specialized for secretion have distinct polarity, with RER abundant at their basal ends and mature secretory granules at the apical poles undergoing exocytosis into an enclosed extracellular compartment, the lumen of a gland.

membranes, by phospholipid transfer proteins, or by vesicles which detach from the SER for movement along the cytoskeleton and fusion with other membranous organelles. In cells that secrete steroid hormones (eg, cells of the adrenal cortex), SER occupies a large portion of the cytoplasm.

- Other SER enzymes, including those of the cytochrome P450 family, allow *detoxification of potentially harmful exogenous molecules* such as alcohol, barbiturates, and other drugs. In liver cells these enzymes also process endogenous molecules such as the components of bile.
- SER vesicles are also responsible for *sequestration and controlled release of Ca²⁺*, which is part of the rapid response of cells to various stimuli. This function is particularly well developed in striated muscle cells, where the SER has an important role in the contraction process and assumes a specialized form called the **sarcoplasmic reticulum** (see Chapter 10).

► MEDICAL APPLICATION

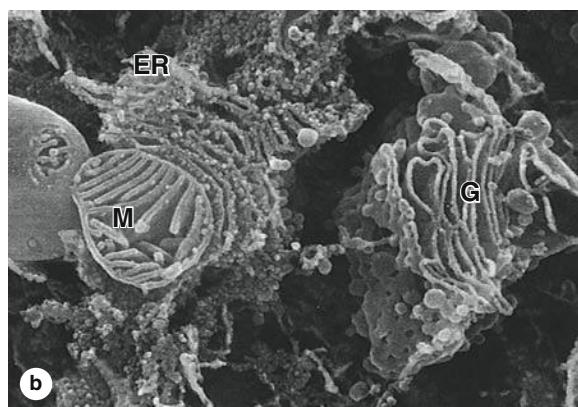
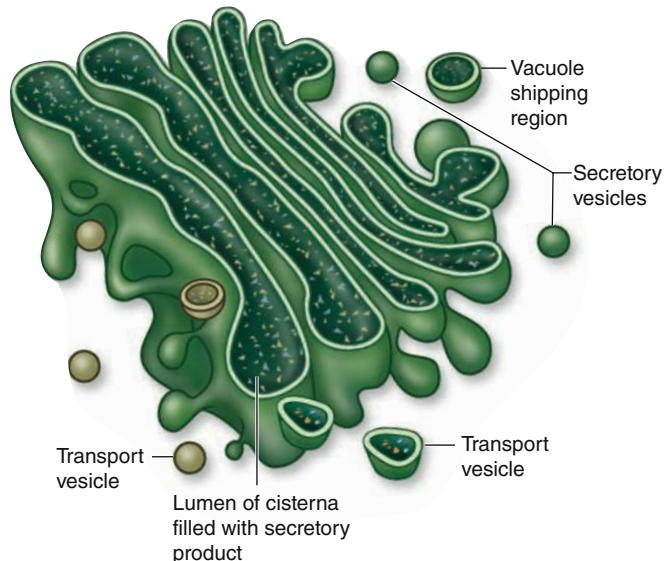
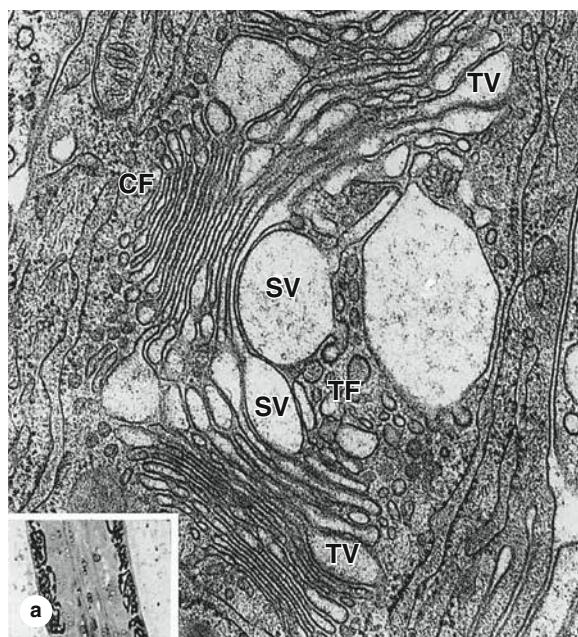
Jaundice denotes a yellowish discoloration of the skin and is caused by accumulation in extracellular fluid of bilirubin and other pigmented compounds, which are normally metabolized by SER enzymes in cells of the liver and excreted as bile. A frequent cause of jaundice in newborn infants is an underdeveloped state of SER in liver cells, with failure of bilirubin to be converted to a form that can be readily excreted.

Golgi Apparatus

The dynamic organelle called the **Golgi apparatus**, or Golgi complex, completes posttranslational modifications of proteins produced in the RER and then packages and addresses these proteins to their proper destinations. The organelle was named after histologist Camillo Golgi who discovered it in 1898. The Golgi apparatus consists of many smooth membranous saccules, some vesicular, others flattened, but all containing enzymes and proteins being processed (Figure 2–13). In most cells the small Golgi complexes are located near the nucleus.

As shown in Figure 2–13, the Golgi apparatus has two distinct functional sides or faces, formed by the complex traffic of vesicles within cells. Material moves from the RER cisternae to the Golgi apparatus in small, membrane-enclosed carriers called **transport vesicles** that are transported along cytoskeletal polymers by motor proteins. The transport vesicles merge with the Golgi-receiving region, or *cis face*. On the opposite side of the Golgi network, at its shipping or *trans face*, larger saccules or vacuoles accumulate, condense, and generate other vesicles that carry completed protein products to organelles away from the Golgi (Figure 2–13).

Formation of transport vesicles and secretory vesicles is driven by assembly of various coat proteins (including clathrin), which also regulate vesicular traffic to, through, and beyond the Golgi apparatus. Forward movement of vesicles in the *cis* Golgi network of saccules is promoted by the **coat protein COP-II**, while retrograde movements in that region involve **COP-I**. Other membrane proteins important

FIGURE 2–13 Golgi apparatus.

The **Golgi apparatus** is a highly plastic, morphologically complex system of membrane vesicles and cisternae in which proteins and other molecules made in the RER undergo further modification and sorting into specific vesicles destined for different roles in the cell.

(a) TEM of the Golgi apparatus provided early evidence about how this organelle functions. To the left is a cisterna of RER and close to it are many small vesicles at the *cis* face (**CF**), or receiving face, of the Golgi apparatus, merging with the first of several flattened Golgi cisternae. In the center are the characteristic flattened, curved, and stacked medial cisternae of the complex. Cytological and molecular data suggest that other transport vesicles (**TV**) move proteins serially through the cisternae until at the *trans* face (**TF**), or shipping region, larger condensing secretory vesicles (**SV**) and other vacuoles emerge to carry the modified proteins elsewhere in the cell. Formation and fusion of the vesicles through the Golgi apparatus is controlled by specific membrane proteins. (X30,000) **Inset:** A small region of a Golgi apparatus in a 1-μm section from a silver-stained cell, demonstrating abundant glycoproteins within cisternae.

(b) Morphological aspects of the Golgi apparatus are revealed more clearly by SEM, which shows a three-dimensional snapshot of the region between RER and the Golgi membrane compartments. Cells may have multiple Golgi apparatuses, each with the general organization shown here and typically situated near the cell nucleus. (X30,000)

(c) The Golgi apparatus location can be clearly seen in intact cultured cells processed by immunocytochemistry using an antibody against golgin-97 to show the many complexes of Golgi vesicles (green), all near the nucleus, against a background of microfilaments organized as stress fibers and stained with fluorescent phalloidin (violet). Because of the abundance of lipids in its many membranes, the Golgi apparatus is difficult to visualize in typical paraffin-embedded, H&E-stained sections. In developing white blood cells with active Golgi complexes, the organelle can sometimes be seen as a faint unstained juxtanuclear region (sometimes called a “Golgi ghost”) surrounded by basophilic cytoplasm.

(Figure 2–13b reproduced, with permission from Naguro T, Iino A. Prog Clin Biol Res. 1989;295:250; Figure 2–13c, © 2015 Thermo Fisher Scientific, Inc. Used under permission.)

for directed vesicle fusion include various Rab proteins and other enzymes, receptors and specific binding proteins, and fusion-promoting proteins that organize and shape membranes. Depending on the activity of these proteins, vesicles are directed toward different Golgi regions and give rise to lysosomes or secretory vesicles for exocytosis.

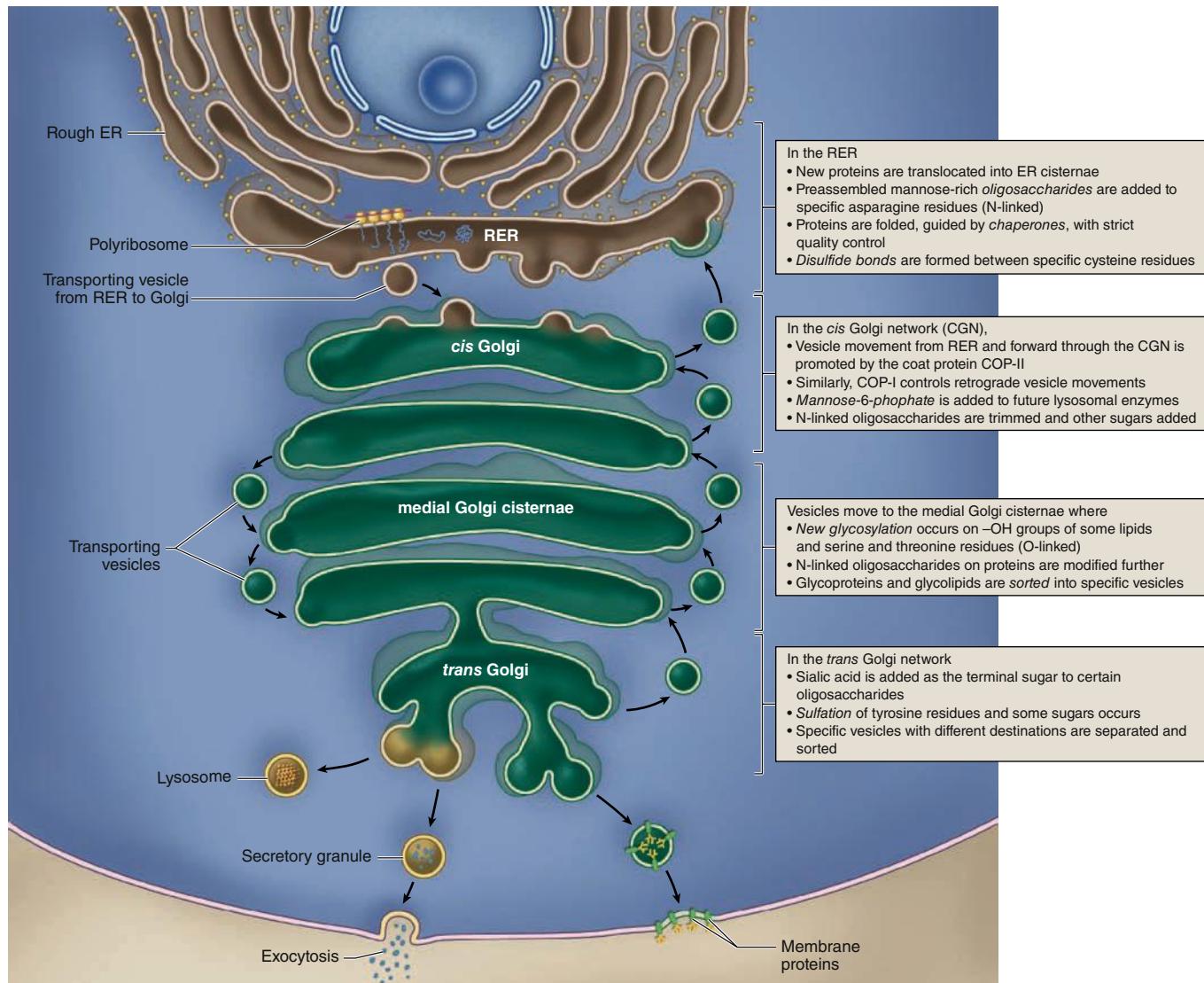
As indicated in Figure 2–14, Golgi saccules at sequential locations contain different enzymes at different *cis*, *medial*, and *trans* levels. Enzymes of the Golgi apparatus are important for glycosylation, sulfation, phosphorylation, and limited proteolysis of proteins. Along with these activities, the Golgi apparatus initiates packing, concentration, and storage of secretory

products. Protein movements through the Golgi and the control of protein processing are subjects of active research.

Secretory Granules

Originating as condensing vesicles in the Golgi apparatus, **secretory granules** are found in cells that store a product until its release by exocytosis is signaled by a metabolic, hormonal, or neural message (regulated secretion). The granules are surrounded by membrane and contain a concentrated form of the secretory product (Figure 2–15). The contents of some secretory granules may be up to 200 times more

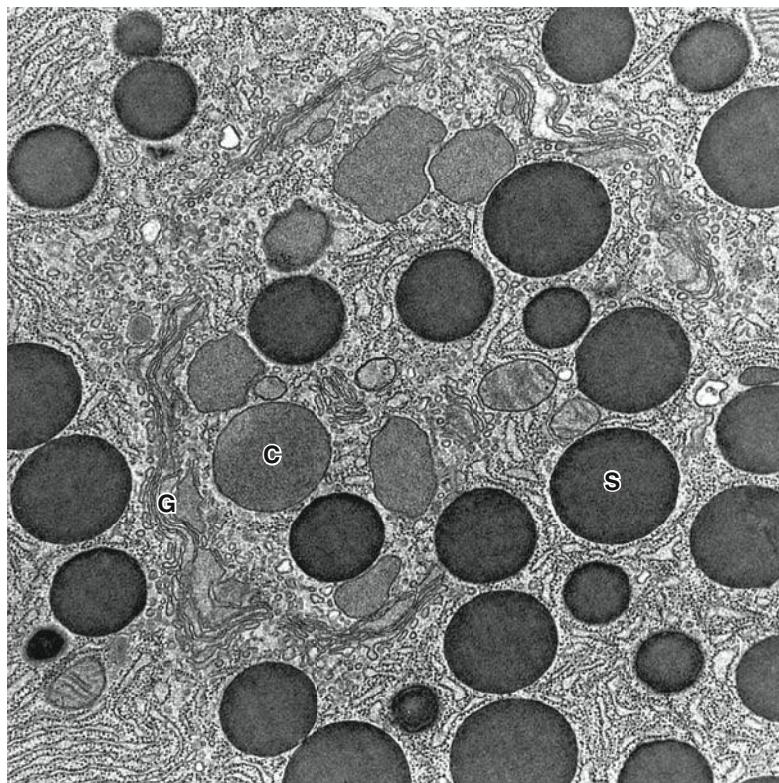
FIGURE 2–14 Summary of functions within the Golgi apparatus.



The main molecular processes are listed at the right, with the major compartments where they occur. In the *trans* Golgi network, the proteins and glycoproteins combine with specific

receptors that guide them to the next stages toward their destinations.

FIGURE 2–15 Secretory granules.



TEM of one area of a pancreatic acinar cell shows numerous mature, electron-dense **secretory granules (S)** in association with condensing vacuoles (**C**) of the Golgi apparatus (**G**). Such granules form as the contents of the Golgi vacuoles become more

condensed. In H&E-stained sections secretory granules are often shown as intensely eosinophilic structures, which in polarized epithelial cells are concentrated at the apical region prior to exocytosis. (X18,900)

concentrated than those in the cisternae of the RER. Secretory granules with dense contents of digestive enzymes are also referred to as **zymogen granules**.

Lysosomes

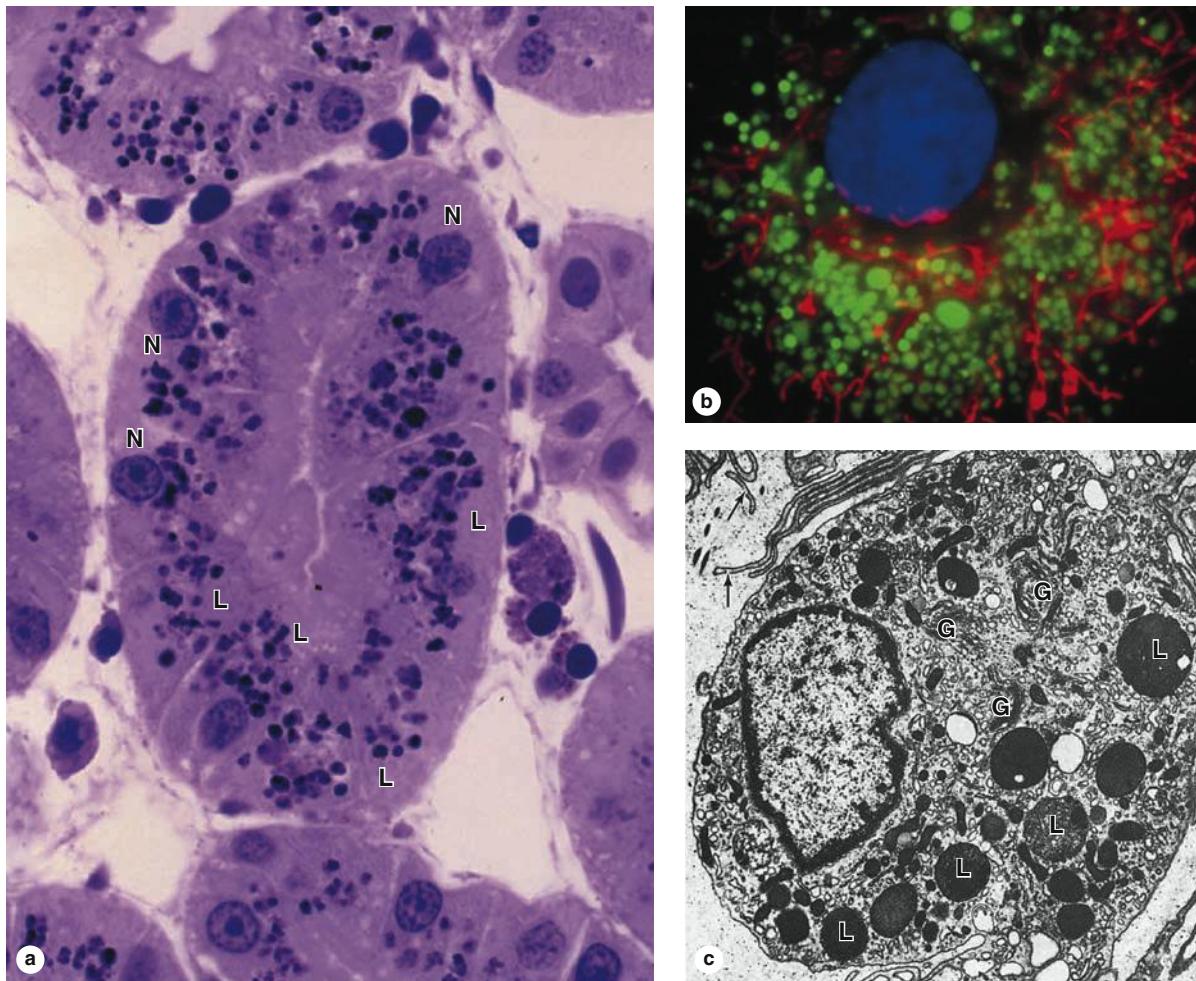
Lysosomes are sites of intracellular digestion and turnover of cellular components. Lysosomes (Gr. *lysis*, solution, + *soma*, body) are membrane-limited vesicles that contain about 40 different hydrolytic enzymes and are particularly abundant in cells with great phagocytic activity (eg, macrophages, neutrophils). Although the nature and activity of lysosomal enzymes vary depending on the cell type, the most common are acid hydrolyases such as proteases, nucleases, phosphatase, phospholipases, sulfatases, and β -glucuronidase. As can be seen from this list, lysosomal enzymes are capable of breaking down most macromolecules.

Lysosomes, which are usually spherical, range in diameter from 0.05 to 0.5 μm and present a uniformly granular, electron-dense appearance in the TEM (Figure 2–16). In macrophages and neutrophils, lysosomes are slightly larger and visible with the light microscope, especially after histochemical staining.

Cytosolic components are protected from these enzymes by the membrane surrounding lysosomes and because the enzymes have optimal activity at an acidic pH (~5.0). Any leaked lysosomal enzymes are practically inactive at the pH of cytosol (~7.2) and harmless to the cell.

Lysosomal hydrolases are synthesized and segregated in the RER and then transferred to the Golgi apparatus, where the enzymes are further modified and packaged in vacuoles that form lysosomes. The marker mannose-6-phosphate (M6P) is added by a phosphotransferase in the *cis* Golgi only to the N-linked oligosaccharides of the hydrolases destined for lysosomes. Membrane receptors for M6P-containing proteins in the *trans* Golgi network then bind these proteins and divert them from the secretory pathway for segregation into lysosomes.

Material taken from outside the cell by endocytosis is digested when the membrane of the phagosome or pinocytotic vesicle fuses with a lysosome. This mixes the endocytosed material with the lysosomal enzymes and activates proton pumps in the lysosomal membrane that acidify the contents, allowing digestion. The composite, active organelle is now termed a secondary or

FIGURE 2–16 Lysosomes.

Lysosomes are spherical membrane-enclosed vesicles that function as sites of intracellular digestion and are particularly numerous in cells active after the various types of endocytosis. Lysosomes are not well shown on H&E-stained cells but can be visualized by light microscopy after staining with toluidine blue.

(a) Cells in a kidney tubule show numerous purple lysosomes (**L**) in the cytoplasmic area between the basally located nuclei (**N**) and apical ends of the cells at the center of the tubule. Using endocytosis, these cells actively take up small proteins in the lumen of the tubule, degrade the proteins in lysosomes, and then release the resulting amino acids for reuse. (X300)

(b) Lysosomes in cultured vascular endothelial cells can be specifically stained using fluorescent dyes sequestered into these organelles (green), which are abundant around the blue Hoechst-stained nucleus. Mitochondria (red) are scattered among the lysosomes.

(c) In the TEM lysosomes (**L**) have a characteristic very electron-dense appearance and are shown here near groups of Golgi cisternae (**G**). The less electron-dense lysosomes represent heterolysosomes in which digestion of the contents is under way. The cell is a macrophage with numerous fine cytoplasmic extensions (arrows). (X15,000)

(Figure 2–16b, © 2015 Thermo Fisher Scientific, Inc. Used under permission.)

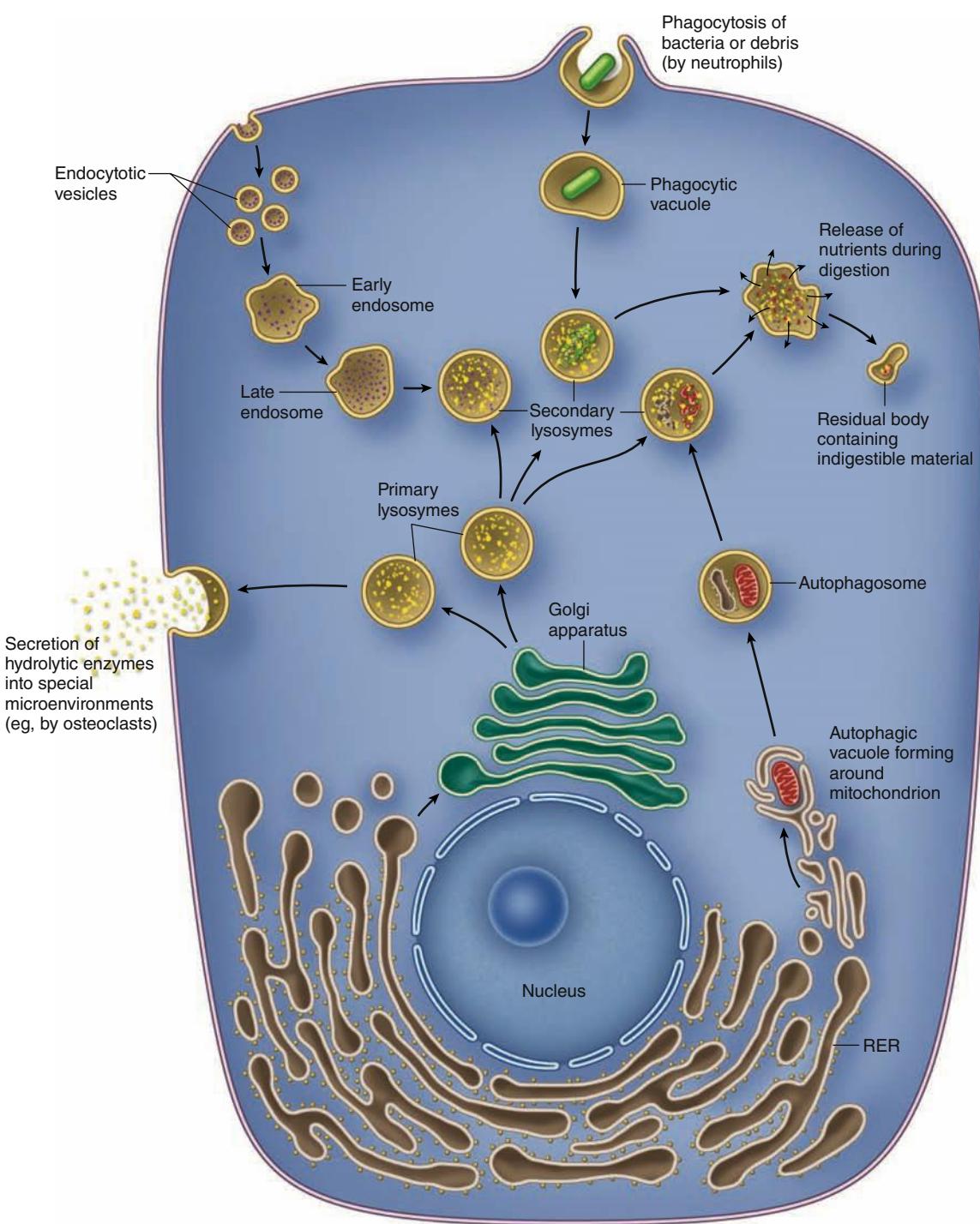
heterolysosome. Heterolysosomes are generally somewhat larger and have a more heterogeneous appearance in the TEM because of the wide variety of materials they may be digesting (Figure 2–16c).

During this digestion of macromolecules, released nutrients diffuse into the cytosol through the lysosomal membrane. Indigestible material is retained within a small vacuolar remnant called a **residual body** (Figure 2–17). In some long-lived

cells (eg, neurons, heart muscle), residual bodies can accumulate over time as granules of **lipofuscin**.

Besides degrading exogenous macromolecules, lysosomes also function in the removal of excess or nonfunctional organelles in a process called **autophagy** (Figures 2–17 and 2–18). A membrane from SER forms around the organelle or cytoplasmic portion to be removed, producing an **autophagosome** (Gr. *autos*, self, + *phagein*, to eat, + *soma*). These then

FIGURE 2–17 Lysosomal functions.



Synthesis of lysosomal enzymes occurs in the RER, with packaging in the Golgi apparatus. Endocytosis produces vesicles that fuse with **endosomes** before merging with **lysosomes**. Phagocytic vacuoles (or phagosomes) fuse with primary lysosomes to become **secondary lysosomes** (or heterolysosomes), in which ingested material is degraded. **Autophagosomes**, such as those depicted here with a mitochondrion in the process of digestion,

are formed after nonfunctional or surplus organelles become enclosed with membrane and the resulting structure fuses with a lysosome. The products of lysosomal digestion are recycled to the cytoplasm, but indigestible molecules remain in a membrane-enclosed **residual body**, which may accumulate in long-lived cells as lipofuscin. In some cells, such as osteoclasts, the lysosomal enzymes are secreted into a restricted extracellular compartment.

FIGURE 2–18 Autophagy.

Autophagy is a process in which the cell uses lysosomes to dispose of excess or nonfunctioning organelles or membranes. Membrane that appears to emerge from the SER encloses the organelles to be destroyed, forming an autophagosome that then fuses with a lysosome for digestion of the contents. In this TEM the two autophagosomes at the **upper left** contain portions of RER more electron dense than the neighboring normal RER and one near the **center** contains what may be mitochondrial membranes plus RER. Also shown is a vesicle with features of a residual body (**RB**). (30,000X)

fuse with lysosomes for digestion of the enclosed material. Autophagy is enhanced in secretory cells that have accumulated excess secretory granules and in times of nutrient stress, such as starvation. Digested products from autophagosomes are reused in the cytoplasm.

» MEDICAL APPLICATION

Diseases categorized as *lysosomal storage disorders* stem from defects in one or more of the digestive enzymes present in lysosomes, usually due to a mutation leading to a deficiency of one of the enzymes, or defects due to faulty posttranslational processing. In cells that must digest the substrate of the missing or defective enzyme following autophagy, the lysosomes cannot function properly. Such cells accumulate large secondary lysosomes or residual bodies filled with the indigestible macromolecule. The accumulation of these vacuoles may eventually interfere with normal cell or tissue function, producing symptoms of the disease. A few lysosomal storage diseases are listed in Table 2–3, with the enzyme involved for each and the tissue affected.

Proteasomes

Proteasomes are very small abundant protein complexes not associated with membrane, each approximately the size of the small ribosomal subunit. They function to degrade denatured or otherwise nonfunctional polypeptides. Proteasomes also remove proteins no longer needed by the cell and provide an important mechanism for restricting activity of a specific protein to a certain window of time. Whereas lysosomes digest organelles or membranes by autophagy, proteasomes deal primarily with free proteins as individual molecules.

As shown in Figure 2–9, the proteasome is a cylindrical structure made of four stacked rings, each composed of seven proteins including proteases. At each end of the cylinder is a regulatory particle that contains ATPase and recognizes proteins with attached molecules of **ubiquitin**, an abundant cytosolic 76-amino acid protein found in all cells. Misfolded or denatured proteins, or short-lived proteins with oxidized amino acids, are recognized by chaperones and targeted for destruction by other enzyme complexes that conjugate ubiquitin to lysine residues of the protein, followed by formation of a polyubiquitin chain. Ubiquinated proteins are recognized by the regulatory particles of proteasomes, unfolded by the ATPase using energy from ATP, and then translocated into the core of the cylindrical structure and degraded by endopeptidases. The ubiquitin molecules are released for reuse and the peptides produced may be broken down further to

TABLE 2–3 Examples of lysosomal storage diseases caused by defective lysosomal enzymes.

| Disease | Faulty Enzyme | Main Organs Affected |
|-------------------------|--------------------------------------|-----------------------------|
| Hurler syndrome (MPS I) | α -L-Iduronidase | Skeleton and nervous system |
| McArdle syndrome | Muscle phosphorylase | Skeletal muscles |
| Tay-Sachs | GM ₂ -gangliosidase | Nervous system |
| Gaucher | Glucocerebrosidase | Liver and spleen |
| I-cell disease | Phosphotransferase for M6P formation | Skeleton and nervous system |

amino acids or they may have other specialized destinations, such as the antigen-presenting complexes of cells activating an immune response.

» MEDICAL APPLICATION

Failure of proteasomes or other aspects of a cell's protein quality control can allow large aggregates of protein to accumulate in affected cells. Such aggregates may adsorb other macromolecules to them and damage or kill cells. Aggregates released from dead cells can accumulate in the extracellular matrix of the tissue. In the brain this can interfere directly with cell function and lead to neurodegeneration.

Alzheimer disease and **Huntington disease** are two neurologic disorders caused initially by such protein aggregates.

Mitochondria

Mitochondria (Gr. *mitos*, thread, + *chondros*, granule) are membrane-enclosed organelles with arrays of enzymes specialized for aerobic respiration and production of **adenosine triphosphate (ATP)**, with high-energy phosphate bonds, which supplies energy for most cellular activities. Glycolysis converts glucose anaerobically to pyruvate in the cytoplasm, releasing some energy. The rest of the energy is captured when pyruvate is imported into mitochondria and oxidized to CO_2 and H_2O . Mitochondrial enzymes yield 15 times more ATP than is produced by glycolysis alone. Some of the energy released in mitochondria is not stored in ATP but is dissipated as heat that maintains body temperature.

Mitochondria are usually elongated structures with diameters of 0.5–1 μm and lengths up to 10 times greater. They are highly plastic, rapidly changing shape, fusing with one another and dividing, and are moved through the cytoplasm along microtubules. The number of mitochondria is related to the cell's energy needs: cells with a high-energy metabolism (eg, cardiac muscle, cells of some kidney tubules) have abundant mitochondria, whereas cells with a low-energy metabolism have few mitochondria. Similarly, mitochondria of differentiated cells are concentrated in cytoplasmic regions where energy utilization is more intense.

» MEDICAL APPLICATION

Myoclonic epilepsy with ragged red fibers (MERRF) is a rare disease occurring in individuals in whom cells of specific tissues, notably regions of skeletal muscle, inherit mitochondrial DNA with a mutated gene for lysine-tRNA, leading to defective synthesis of respiratory chain proteins which can produce structural abnormality in muscle fibers and other cells.

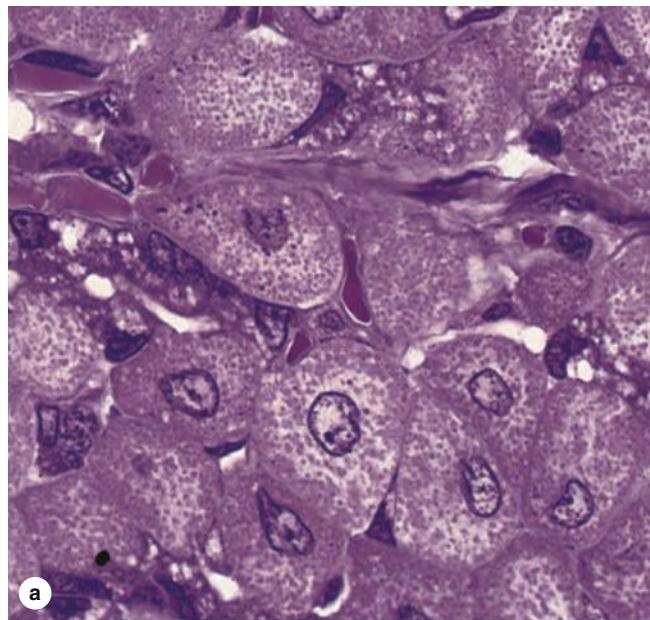
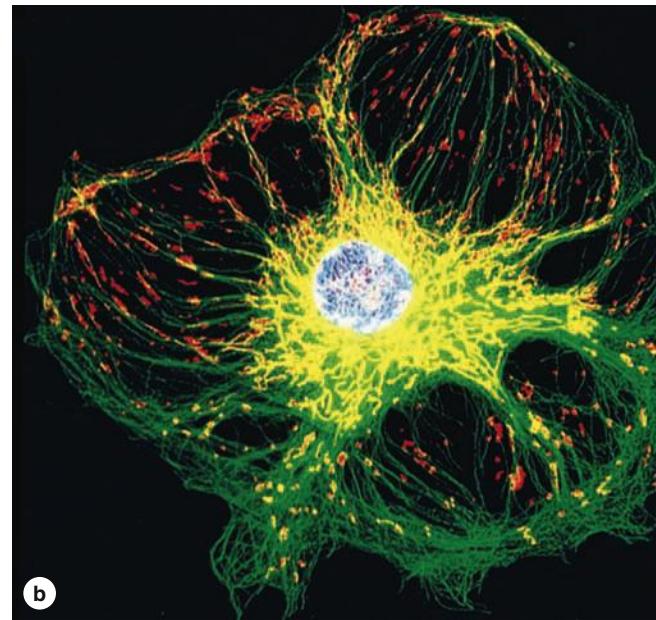
Mitochondria are often large enough to be visible with the light microscope as numerous discrete organelles (Figure 2–19).

Under the TEM each mitochondrion is seen to have two separated and very different membranes that together create two compartments: the innermost **matrix** and a narrow **intermembrane space** (Figure 2–20a). Both mitochondrial membranes contain a higher density of protein molecules than other membranes in the cell and have reduced fluidity. The **outer membrane** is sieve-like, containing many transmembrane proteins called **porins** that form channels through which small molecules such as pyruvate and other metabolites readily pass from the cytoplasm to the intermembrane space.

The **inner membrane** has many long folds called **cristae**, which project into the matrix and greatly increase the membrane's surface area (Figure 2–20). The number of cristae in mitochondria also corresponds to the energy needs of the cell. The lipid bilayer of the inner membrane contains unusual phospholipids and is highly impermeable to ions (Figure 2–20). Integral proteins include various transport proteins that make the inner membrane selectively permeable to the small molecules required by enzymes in the matrix. Mitochondrial matrix enzymes include those that oxidize pyruvate and fatty acids to form acetyl coenzyme A (CoA) and those of the citric acid cycle that oxidize acetyl CoA, releasing CO_2 as waste and small energy-rich molecules that provide electrons for transport along the **electron-transport chain** (or respiratory chain). Enzymes and other components of this chain are embedded in the inner membrane and allow oxidative phosphorylation, which produces most of the ATP in animal cells.

Formation of ATP by oxidative phosphorylation enzymes occurs by a **chemiosmotic process**. Membrane proteins guide the small electron carrier molecules through closely packed enzyme complexes so that the electrons move sequentially along the chain. Electron transfer is coupled with oriented proton uptake and release, with protons accumulating in the intermembrane space (Figure 2–20) and producing an **electrochemical gradient** across the inner membrane. Membrane-associated proteins of the **ATP synthase** system form large (10-nm), multisubunit, globular complexes on stalk-like structures that project from the matrix side of the inner membrane (Figure 2–20). This enzyme complex contains a channel through which protons flow down the electrochemical gradient, crossing the membrane back into the matrix. Passage of protons through this channel causes rotation of specific polypeptides in the globular ATP synthase complex, converting the energy of proton flow into the mechanical energy of protein movement. Mechanical energy is stored in the new phosphate bond of ATP by other subunit polypeptides binding adenosine diphosphate (ADP) and inorganic phosphate (Figure 2–20). A steady torrent of protons along the gradient allows each of these remarkable synthase complexes to produce more than 100 molecules of ATP per second.

Another role for mitochondria occurs at times of cell stress, when the protein cytochrome c is released from the inner membrane's electron transport chain. In the cytoplasm

FIGURE 2–19 Mitochondria in the light microscope.**a****b**

(a) In certain sectioned cells stained with H&E, mitochondria appear throughout the cytoplasm as numerous eosinophilic structures. The mitochondria usually appear round or slightly elongated and are more numerous in cytoplasmic regions with higher energy demands, such as near the cell membrane in cells undergoing much active transport. The central nuclei are also clearly seen in these cells.

(b) Entire mitochondria can be shown in cultured cells, such as the endothelial cells shown here, and often appear as the elongated structures (shown in yellow or orange here), usually arrayed in

parallel along microtubules. Such preparations also show that mitochondrial shape can be quite plastic and variable. Specific mitochondrial staining such as that shown here involves incubating living cells with specific fluorescent compounds that are specifically sequestered into these organelles, followed by fixation and immunocytochemical staining of the microtubules. In this preparation, microtubules are stained green and mitochondria appear yellow or orange, depending on their association with the green microtubules. The cell nucleus was stained with DAPI (*4',6-diamidino-2-phenylindole*).

this protein activates sets of proteases that degrade all cellular components in a regulated process called **apoptosis** which results in rapid cell death (see Chapter 3).

New mitochondria originate by growth and division (fission) of preexisting mitochondria. During cell mitosis each daughter cell receives approximately half the mitochondria in the parent cell.

Unlike most organelles mitochondria are partly autonomous of nuclear genes and activities. The mitochondrial matrix contains a small circular chromosome of DNA, ribosomes, mRNA, and tRNA, all with similarities to the corresponding bacterial components. Protein synthesis occurs in mitochondria, but because of the reduced amount of mitochondrial DNA, only a small subset of mitochondrial proteins is produced locally. Most are encoded by nuclear DNA and synthesized on free polyribosomes of the cytosol. These proteins have short terminal amino acid sequences that serve as signals for their uptake across the mitochondrial membranes. The observation that mitochondria have

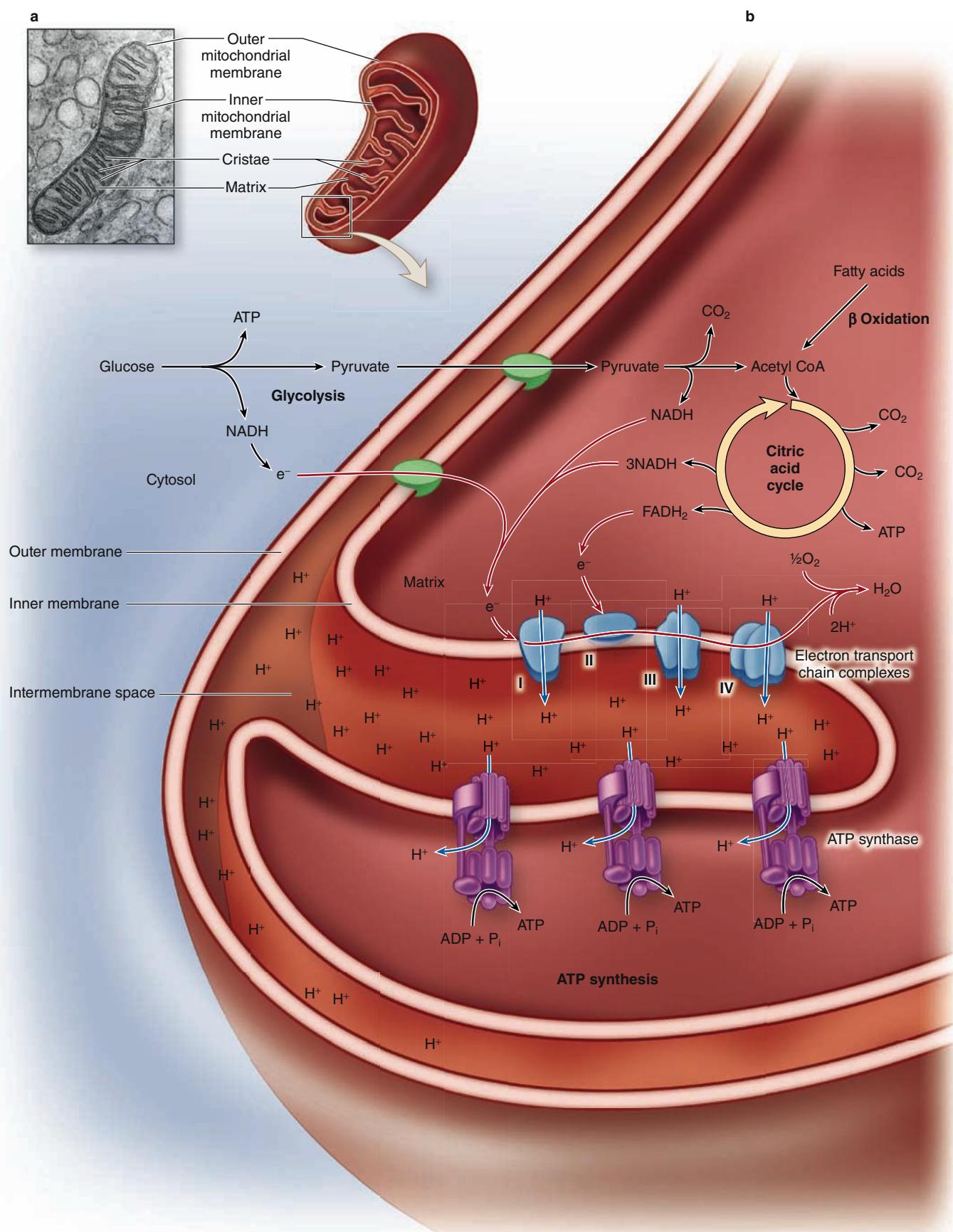
certain bacterial characteristics led with later work to the understanding that mitochondria evolved from an ancestral aerobic prokaryote that lived symbiotically within an ancestral eukaryotic host cell.

Peroxisomes

Peroxisomes are spherical organelles enclosed by a single membrane and named for their enzymes producing and degrading hydrogen peroxide, H_2O_2 (Figure 2–21). **Oxidases** located here oxidize substrates by removing hydrogen atoms that are transferred to molecular oxygen (O_2), producing H_2O_2 . Peroxidases such as **catalase** immediately break down H_2O_2 , which is potentially damaging to the cell. These enzymes also inactivate various potentially toxic molecules, including some prescription drugs, particularly in the large and abundant peroxisomes of liver and kidney cells.

Other diverse enzymes in peroxisomes complement certain functions of the SER and mitochondria in the metabolism

FIGURE 2–20 Mitochondrial structure and ATP formation (Legend Opposite).



(a) The two mitochondrial membranes and the innermost matrix can be seen in the TEM and diagram. The **outer membrane** is smooth and the **inner membrane** has many sharp folds called **cristae** that increase its surface area greatly. The **matrix** is a gel with a high concentration of enzymes.

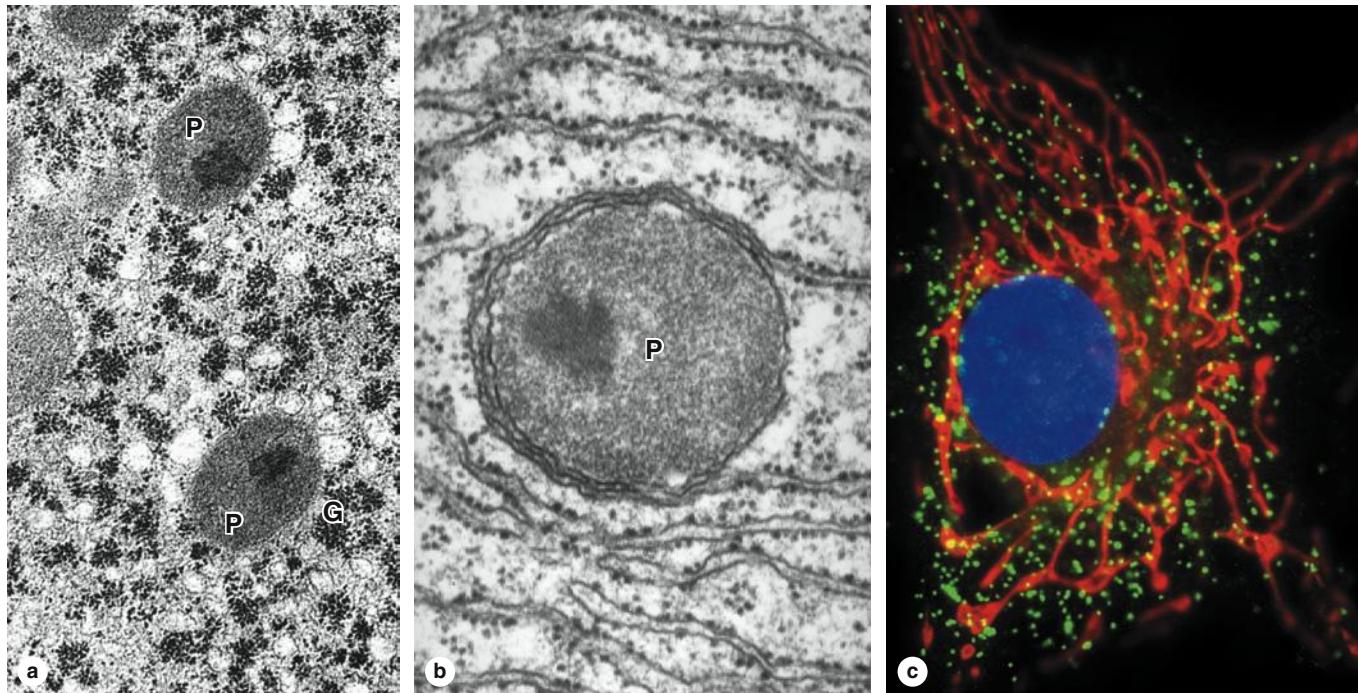
(b) Metabolites such as pyruvate and fatty acids enter mitochondria via membrane porins and are converted to acetyl CoA by matrix enzymes of the **citric acid cycle** (or Krebs cycle), yielding some ATP and NADH (nicotinamide adenine dinucleotide), a major source of electrons for the **electron-transport chain**. The movement of electrons through the protein complexes of the inner membrane's electron-transport system is accompanied by

the directed movement of protons (H^+) from the matrix into the intermembranous space, producing an electrochemical gradient across the membrane. Other membrane-associated proteins make up the **ATP synthase** systems, each of which forms a globular complex on a stalk-like structure projecting from the matrix side of the inner membrane. A channel in this enzyme complex allows proton flow down the electrochemical gradient and across the membrane back into the matrix. The flow of protons causes rapid spinning of specific polypeptides in the globular ATP synthase complex, converting the energy of proton flow into mechanical energy, which other subunit proteins store in the new phosphate bond of ATP.

of lipids and other molecules. Thus, the β -oxidation of long-chain fatty acids (18 carbons and longer) is preferentially accomplished by peroxisomal enzymes that differ from their mitochondrial counterparts. Certain reactions leading to the formation of bile acids and cholesterol also occur in peroxisomes.

Peroxisomes form in two ways: budding of precursor vesicles from the ER or growth and division of preexisting peroxisomes. Peroxisomal proteins are synthesized on free polyribosomes and have targeting sequences of amino acids at either terminus recognized by receptors located in the peroxisomal membrane for import into the organelle.

FIGURE 2–21 Peroxisomes.



Peroxisomes are small spherical, membranous organelles, containing enzymes that use O_2 to remove hydrogen atoms from fatty acids, in a reaction that produces hydrogen peroxide (H_2O_2) that must be broken down to water and O_2 by another enzyme, **catalase**.

(a) By TEM peroxisomes (P) generally show a matrix of moderate electron density. Aggregated electron-dense particles represent glycogen (G). (X30,000)

(b) Peroxisomes (P) in most species are characterized by a central, more electron-dense crystalloid aggregate of constituent enzymes, as shown here. (X60,000)

(c) A cultured endothelial cell processed by immunocytochemistry shows many peroxisomes (green) distributed throughout the cytoplasm among the vitally stained elongate mitochondria (red) around the DAPI-stained nucleus (blue). Peroxisomes shown here were specifically stained using an antibody against the membrane protein PMP70.

(Figure 2–21c, © 2015 Thermo Fisher Scientific, Inc. Used under permission.)

» MEDICAL APPLICATION

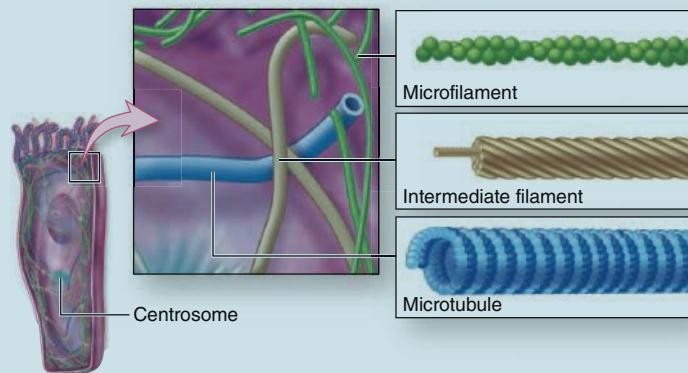
Several fairly rare disorders arise from defective peroxisomal proteins. **Neonatal adrenoleukodystrophy** is caused by a defective integral membrane protein needed for transport of very-long-chain fatty acids into the peroxisome for β -oxidation. Accumulation of these fatty acids in body fluids can disrupt the myelin sheaths in nerve tissue, causing severe neurologic symptoms. Deficiencies of peroxisomal enzymes cause **Zellweger syndrome** that affects the structure and functions of several organ systems.

THE CYTOSKELETON

The cytoplasmic **cytoskeleton** is a complex array of (1) microtubules, (2) microfilaments (also called actin filaments), and (3) intermediate filaments. These protein polymers determine the shapes of cells, play an important role in the movements of organelles and cytoplasmic vesicles, and also allow the movement of entire cells. Important properties, functions, and locations of the cytoskeletal components are summarized in Table 2–4.

TABLE 2–4

Properties of cytoskeletal components (microtubules, microfilaments, and intermediate filaments).



General Function of Cytoskeleton

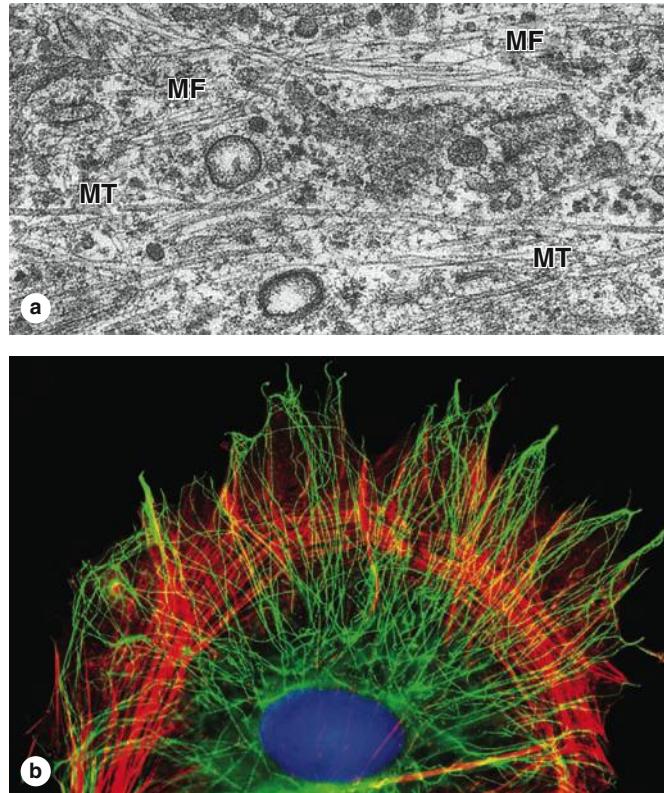
- Structural:** Provides structural support to cell; stabilizes junctions between cells
- Movement:** Assists with cytosol streaming and cell motility; helps move organelles and materials throughout cell; helps move chromosomes during cell division

| | Microtubules | Microfilaments | Intermediate filaments |
|--------------------|---|--|---|
| Polymer | | | |
| Subunit | Heterodimers of $\alpha\beta$ -tubulin | G-actin monomers | Antiparallel tetramers of 2 rod-like dimers |
| Overall structure | Hollow tube with a wall of 13 parallel protofilaments | 2 intertwined filaments of F-actin | Cable of 4 intertwined protofibrils, each consisting of bundled tetramers associated end to end |
| Diameter | 25 nm | 5-7 nm | 8-10 nm |
| Monomeric proteins | α and β tubulin (54 kDa) | Globular G-actin (42 kDa) | Various α -helical rod-like proteins (~55 kDa, Table 2–5) |
| Polarity | + and – ends | + and – ends | No apparent polarity |
| Relative stability | Dynamic in cytoplasm; stable in axonemes | Dynamic | Stable |
| General locations | Radiating through cytoplasm from concentration at centrosomes; axonemes | Concentrated beneath cell membrane; in cell extensions like microvilli | Arrayed throughout cytoplasm; at desmosomes; inside nuclear envelope |
| Key functions | Maintain cell's shape and polarity; provide tracks for organelle and chromosome movement; move cilia and flagella | Contract and move cells; change cell shape; cytokinesis; cytoplasmic transport and streaming | Strengthen cell and tissue structure; maintain cell shape; maintain nuclear shape (lamins) |

Microtubules

Within the cytoplasm of all eukaryotic cells are fine tubular structures known as **microtubules** (Table 2–4; Figure 2–22), most of which are highly dynamic in length. Microtubules

FIGURE 2–22 Microtubules and actin filaments in cytoplasm.



(a) Microtubules (MT) and actin microfilaments (MF) can both be clearly distinguished in this TEM of fibroblast cytoplasm, which provides a good comparison of the relative diameters of these two cytoskeletal components. (X60,000)

(b) Arrays of microfilaments and microtubules are easily demonstrated by immunocytochemistry using antibodies against their subunit proteins, as in this cultured cell. Actin filaments (red) are most concentrated at the cell periphery, forming prominent circumferential bundles from which finer filaments project into cellular extensions and push against the cell membrane. Actin filaments form a dynamic network important for cell shape changes such as those during cell division, locomotion, and formation of cellular processes, folds, pseudopodia, lamellipodia, microvilli, etc., which serve to change a cell's surface area or give direction to a cell's crawling movements.

Microtubules (green/yellow) are oriented in arrays that generally extend from the centrosome area near the nucleus into the most peripheral extensions. Besides serving to stabilize cell shape, microtubules form the tracks for kinesin-based transport of vesicles and organelles into the cell periphery and dynein-based transport toward the cell nucleus.

(Figure 2–22b, used with permission from Dr Albert Tousson, University of Alabama—Birmingham High Resolution Imaging Facility, Birmingham.)

are also organized into larger, more stable arrays called **axonemes** in the cytoplasmic extensions called cilia (discussed in Chapter 4) and flagella. Each microtubule is hollow, with an outer diameter of 25 nm and a wall 5-nm thick, a structure that confers significant rigidity to help maintain cell shape. Microtubules vary in length, but can become many micrometers long. Two or more microtubules are often linked side by side by protein arms or bridges, which are particularly important in the axonemes of cilia and flagella.

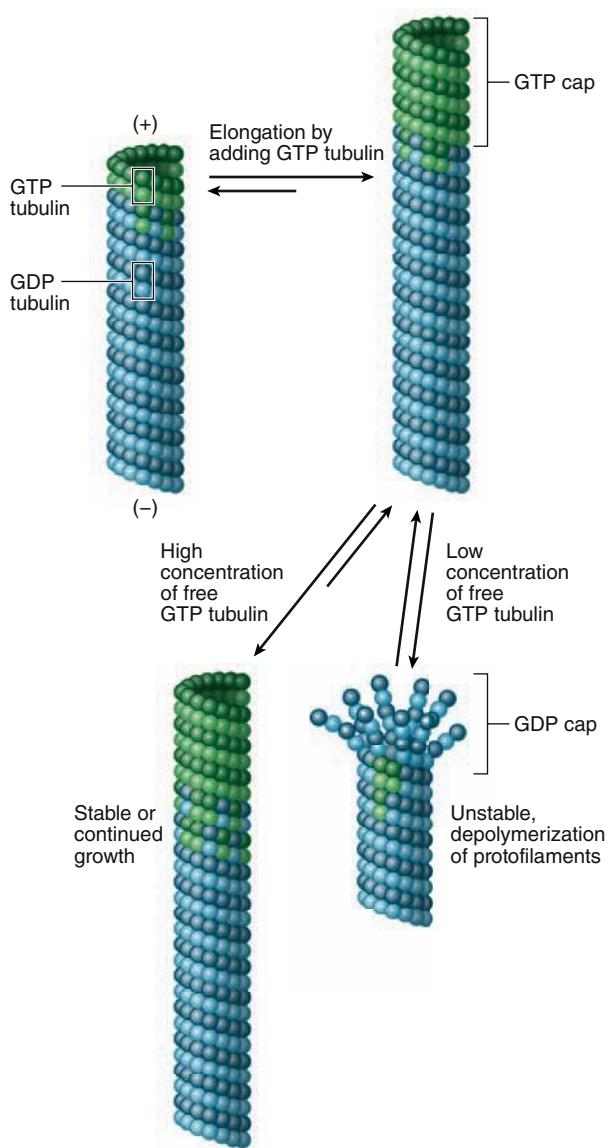
As indicated in Table 2–4, the protein subunit of a microtubule is a heterodimer of α and β **tubulin**, each with a molecular mass of about 50 kDa. Under appropriate conditions the tubulin heterodimers polymerize to form the microtubules, which have a slightly spiral organization. The tubulin subunits align lengthwise as protofilaments, with 13 parallel protofilaments forming the circumference of each microtubule wall.

Polymerization of tubulins is directed by **microtubule organizing centers (MTOCs)**, which contain short assemblies of tubulin that act as nucleating sites for further polymerization. Microtubules are polarized structures, with growth (polymerization) occurring more rapidly at the (+) end (Figure 2–23). Microtubules show **dynamic instability**, with continuous cycles of polymerization and depolymerization at steady-state conditions, which depend on concentrations of tubulin, Ca^{2+} , Mg^{2+} , and the presence of various **microtubule-associated proteins (MAPs)**. Energy for assembly is derived from GTP bound to incoming tubulin subunits. Individual microtubules shorten as depolymerization exceeds growth. Microtubule stability varies greatly with cellular location and function; microtubules of cilia are very stable, while those of the mitotic spindle are short-lived.

The dominant MTOC in most cells is the **centrosome**, which is organized around two cylindrical **centrioles**, each about 0.2 μm in diameter and 0.3–0.5 μm in length. Each centriole is composed of nine highly organized microtubule triplets (Figure 2–24). With their long axes at right angles, the paired centrioles organize nearby tubulin complexes and other proteins as a pericentriolar matrix found close to the nucleus of nondividing cells. Before cell division, more specifically during the period of DNA replication, each centrosome duplicates itself so that now each centrosome has two pairs of centrioles. During mitosis, the centrosome divides into halves, which move to opposite poles of the cell, and become organizing centers for the microtubules of the mitotic spindle.

Microtubules also form part of the system for intracellular transport of membranous vesicles, macromolecular complexes, and organelles. Well-studied examples include axoplasmic transport in neurons, melanin transport in pigment cells, chromosome movements by the mitotic spindle, and vesicle movements among different cell compartments. In each of these examples, movement is suspended if microtubules are disrupted. Transport along microtubules is under the control of proteins called **motor proteins**, which use ATP in moving the larger structures. **Kinesins** carry material away from the MTOC near the nucleus toward the plus end of

FIGURE 2–23 Dynamic instability of microtubules.



At stable tubulin concentrations some microtubules grow while others shrink, each existing in a condition called dynamic instability. In cytoplasmic areas where the tubulin concentration is high, tubulin GTP is added at a microtubule's (+) end faster than the incorporated GTP can be hydrolyzed. The resulting "GTP cap" stabilizes that end of the microtubule and promotes further rapid growth. As free tubulin concentrations decrease, the rate of growth also decreases, thereby allowing GTP hydrolysis to catch up. The resulting "GDP cap" at the microtubule end is unstable and favors rapid depolymerization (termed "catastrophe"). This increases the local concentration of free, monomeric tubulin that "rescues" the microtubule before it completely disappears and produces another short period of microtubule elongation.

Dynamic instability allows the growing ends of microtubules to explore the cytoplasm and become stabilized when they contact stabilizing structures, such as kinetochores on chromosomes early in mitosis (see Chapter 3).

microtubules (anterograde transport); **cytoplasmic dyneins** carry material along microtubules in the opposite direction (retrograde transport), generally toward the nucleus. Important roles for this system include extending the ER from the nuclear envelope to the plasmalemma and moving vesicles to and through the Golgi apparatus.

» MEDICAL APPLICATION

Several inhibitory compounds used by cell biologists to study details of microtubule dynamics are also widely used in cancer chemotherapy to block activity of the mitotic spindle in rapidly growing neoplastic cells. Such drugs include vinblastine, vincristine, and paclitaxel, all of which were originally discovered as plant derivatives.

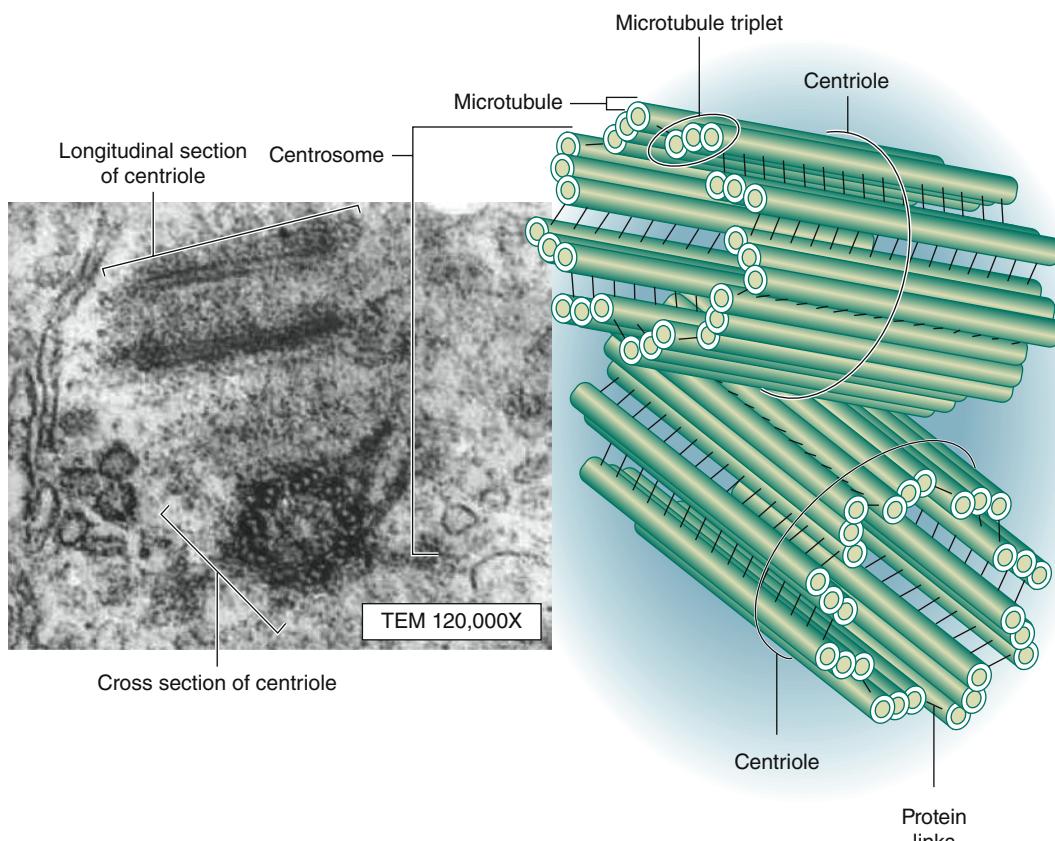
Microfilaments (Actin Filaments)

Microfilaments are composed of **actin** subunits and allow motility and most contractile activity in cells, using reversible assembly of the actin filaments and interactions between these filaments and associated **myosin** family proteins. Actin filaments are thin (5–7 nm diameter), polarized polymers, shorter and more flexible than microtubules (Figure 2–22). They are composed of globular G-actin monomers that assemble in the presence of K⁺ and Mg²⁺ into a double-stranded helix of filamentous F-actin (Table 2–4). G-actin is generally added to preexisting filaments, but new filaments can be formed from a pool of G-actin by the action of nucleating proteins, such as formin. Another nucleating factor, a complex of polypeptides called Arp2/3, also binds to the side of preexisting actin filaments and induces a new F-actin branch, a process which can lead to formation of a microfilament network.

Like microtubules actin filaments are highly dynamic. Monomers are added rapidly at the (+) or barbed end, with ATP hydrolysis at each addition; at the same time monomers dissociate at the (−) or pointed end. This leads to migration of subunits through the polymer, which occurs rapidly in a process called *treadmilling* (Figure 2–25). In cells both the assembly and disassembly of subunits from F-actin are promoted by other proteins, such as *profilin* and *cofilin*, respectively.

Actin is very abundant in all cells and is concentrated in networks of actin filaments and as free G-actin subunits near the cell membrane, a cytoplasmic region often called the **cell cortex**. Arp2/3 activity produces an important branched network of microfilaments in this region. Microfilament-rich cellular extensions, including surface folds or ruffles, are important for endocytosis and microfilaments of the cortex underlie endosomal trafficking. Cell movements on firm substrates involve sheet-like protrusions, or lamellipodia, in which the concentrated actin filaments are continuous with deeper parallel F-actin bundles called **stress fibers** (Figure 2–13c).

Actin-binding proteins, such as formin and others just mentioned, change the dynamic physical properties of microfilaments, particularly their lengths and interactions with

FIGURE 2–24 Centrosome.

The **centrosome** is the microtubule-organizing center for the mitotic spindle and consists of paired centrioles. The TEM reveals that the two centrioles in a centrosome exist at right angles to each other in a dense matrix of free tubulin subunits and other proteins. Each centriole consists of **nine microtubular triplets**. In a poorly

understood process, the centrosome duplicates itself and is divided equally during a cell's interphase, each half having a duplicated centriole pair. At the onset of mitosis, the two daughter centrosomes move to opposite sides of the nucleus and become the two poles of the mitotic spindle of microtubules attaching to chromosomes.

other structures, and this determines the viscosity and other mechanical properties of the local cytoplasm. Cross-linking within networks of F-actin increases cytoplasmic viscosity, while severing (and capping) the filaments tends to decrease viscosity. The lengths and other physical properties of actin filaments are controlled by various other types of actin-binding proteins, including those indicated in Figure 2–26.

Just as the molecular motors kinesin and dynein move structures along microtubules, various **myosin motors** use ATP to transport cargo along F-actin. Movement is usually toward the barbed (+) ends of actin filaments; myosin VI is the only known myosin that moves in the other direction. Interactions between F-actin and myosins form the basis for various cell movements:

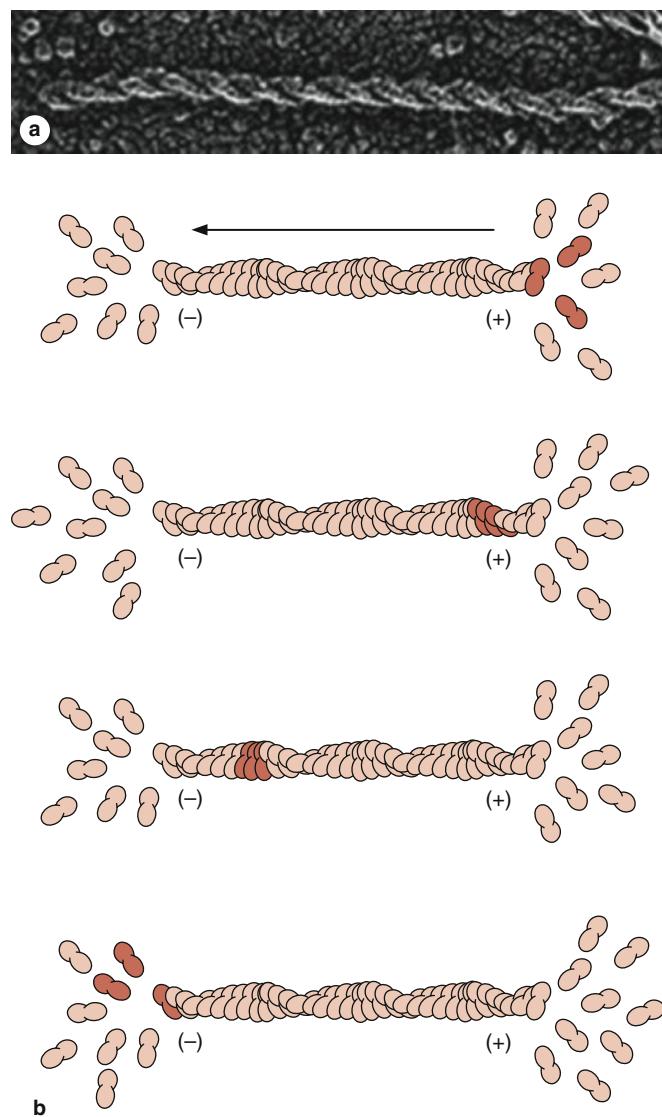
- Transport of organelles, vesicles, and granules in the process of *cytoplasmic streaming*
- Contractile rings of microfilaments with myosin II constricting to produce two cells by *cytokinesis* during mitosis

- Membrane-associated molecules of myosin I whose movements along microfilaments produce the cell surface changes during *endocytosis*

Stabilized arrays of actin filaments integrated with arrays of thicker (16-nm) myosin filaments permit very forceful contractions in specialized cells such as those of muscle (see Chapter 10).

Intermediate Filaments

The third class of cytoskeletal components includes filaments intermediate in size between the other two, with a diameter averaging 10 nm (Table 2–4). Unlike microtubules and actin filaments these **intermediate filaments** are stable, confer increased mechanical stability to cell structure, and are made up of different protein subunits in different cell types. More than a dozen proteins, ranging in size from 40 to 230 kDa, serve as subunits of various intermediate filaments and can be localized by immunohistochemistry in various cells.

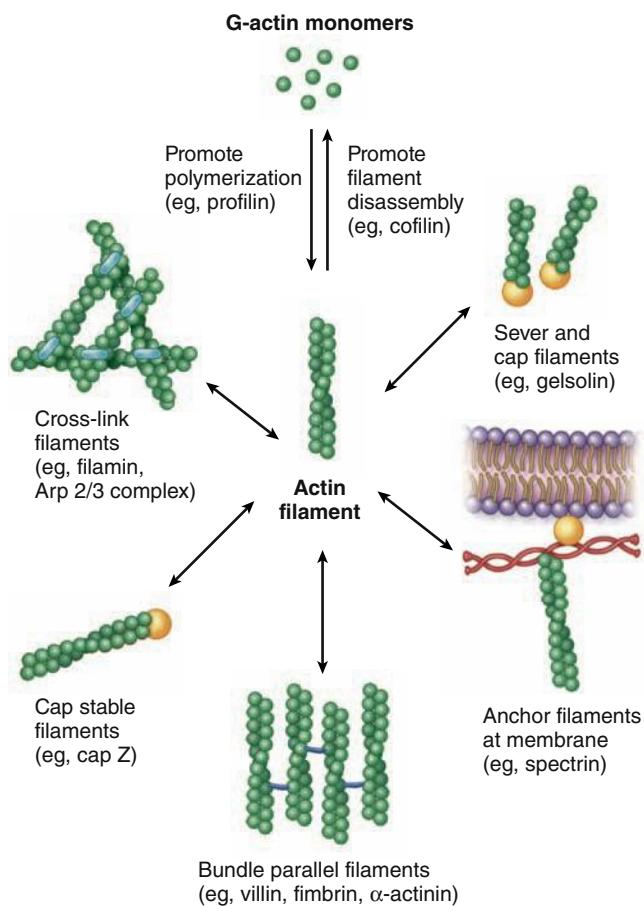
FIGURE 2–25 Actin filament treadmilling.

(a) Actin filaments or microfilaments are helical two-stranded polymers assembled from **globular actin subunits**.

(b) Assembly of actin filaments (F-actin) is polarized, with G-actin subunits added to the plus (+) end and removed at the minus (-) end. Even actin filaments of a constant length are highly dynamic structures, balancing G-actin assembly and disassembly at the opposite ends, with a net movement or flow along the polymer known as **treadmilling**.

(Figure 2-25a, used with permission from John Heuser, Washington University School of Medicine, St. Louis, MO.)

As indicated in Table 2-4, nearly all these subunits are coiled, rod-like dimers that form antiparallel tetramers, which self-assemble into large cable-like bundles or protofibrils stabilized by further lateral interactions. Table 2-5 lists six classes of intermediate filament proteins forming rod-like subunits, their sizes and cell distributions, and diseases that result from their disruption.

FIGURE 2–26 Roles of major actin-binding proteins in regulating the organization of microfilaments.

A large number of proteins regulate the assembly of microfilaments and the interactions of these filaments with one another. By altering microfilament length and cross-linking, such proteins greatly influence physical properties of the local cytoplasm.

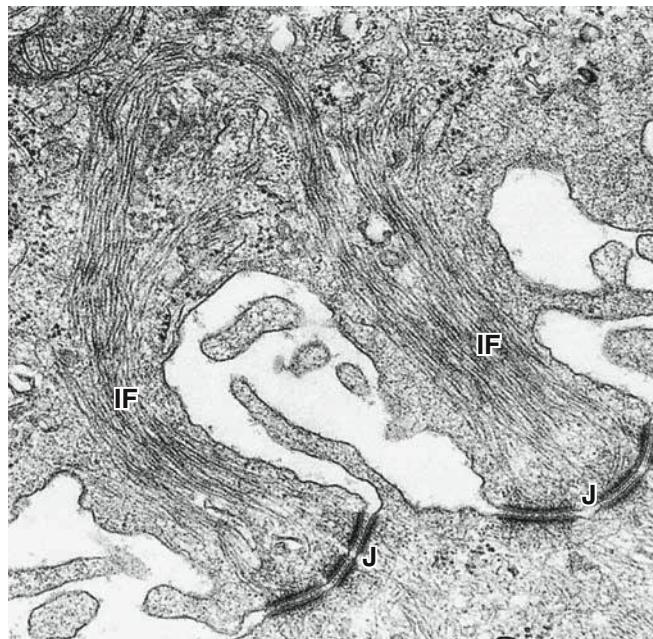
Intermediate filament proteins with particular biological, histological, or pathological importance include the following:

- **Keratins** (Gr. *keras*, horn) or **cytokeratins** are a diverse family of acidic and basic isoforms that compose heterodimer subunits of intermediate filaments in all epithelial cells (see Chapter 4). They are encoded by over 30 related genes and produce filaments with different chemical and immunologic properties for various functions. Intermediate filaments of keratins form large bundles (tonofibrils) that attach to certain junctions between epithelial cells (Figure 2-27). In skin epidermal cells, cytokeratins accumulate during differentiation in the process of *keratinization*, producing an outer layer of nonliving cells that reduces dehydration. Keratinization of skin made terrestrial life possible in the course of evolution. Keratinization also provides some protection from minor abrasions and produces various hard

TABLE 2–5

Major classes and representatives of intermediate filament proteins, their sizes and locations.

| Class | Protein | Size (kDa) | Cell Distribution | Disease Involvement (If Known) |
|-------|--------------------|------------|--|--|
| I | Acidic cytokeratin | 40-65 | Epithelial cells | Certain skin-blistering disorders |
| II | Basic cytokeratin | 51-68 | Epithelial cells | Keratoderma; corneal dystrophy |
| III | Desmin | 53 | Muscle cells | Myopathies |
| | Synemin | 190 | Muscle cells | |
| | GFAP | 50 | Astrocytes (less in other glial cells) | Alexander disease |
| | Peripherin | 57 | Neurons | |
| | Vimentin | 54 | Mesenchymal cells | |
| IV | NF-L | 68 | Neurons | |
| | NF-M | 110 | Neurons | |
| | NF-H | 130 | Neurons | |
| | α-internexin | 55 | Embryonic neurons | |
| V | Lamins | 62-72 | Nuclei of all cells | Cardiomyopathy; muscular dystrophies; progeria |
| VI | Nestin | 230 | Some stem and embryonic cells | |

FIGURE 2–27 Intermediate filaments of keratin.

Intermediate filaments (IF) display an average diameter of 8-10 nm, between that of actin filaments and microtubules, and serve to provide mechanical strength or stability to cells. A large and important class of intermediate filaments is composed of **keratin** subunits, which are prominent in epithelial cells. Bundles of keratin filaments called **tonofibrils** associate with certain classes of intercellular junctions (J) common in epithelial cells and are easily seen with the TEM, as shown here in two extensions in an epidermal cell bound to a neighboring cell. (60,000X)

protective structures of skin, such as nails (as well as feathers, beaks, horns, and the scales of reptiles).

- **Vimentin** is the most common class III intermediate filament protein and is found in most cells derived from embryonic mesenchyme. Important vimentin-like proteins include **desmin** found in almost all muscle cells and **glial fibrillar acidic protein (GFAP)** found especially in astrocytes, supporting cells of central nervous system tissue. Desmin filaments of a cultured cell are shown immunocytochemically in Figure 1–12a.
- **Neurofilament** proteins of three distinct sizes make heterodimers that form the subunits of the major intermediate filaments of neurons.
- **Lamins** are a family of seven isoforms present in the cell nucleus, where they form a structural framework called the **nuclear lamina** just inside the nuclear envelope (see Chapter 3).

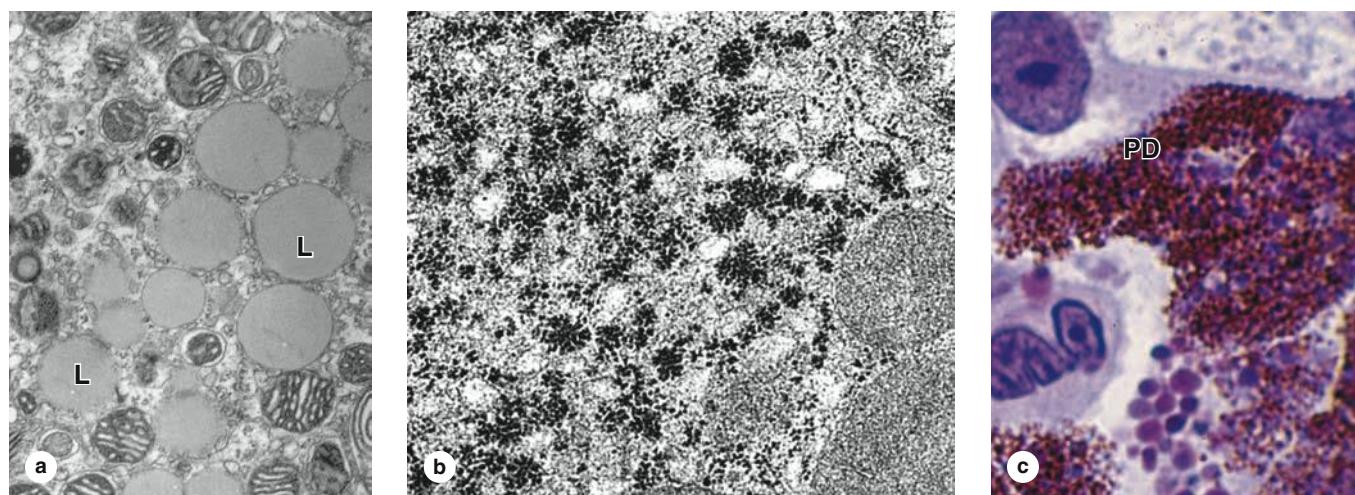
» MEDICAL APPLICATION

The presence of a specific type of intermediate filament in tumors can often reveal the cellular origin of the tumor, information important for diagnosis and treatment of the cancer. Identification of intermediate filament proteins by means of immunocytochemical methods is a routine procedure. One example is the use of GFAP to identify **astrocytomas**, the most common type of brain tumor.

» INCLUSIONS

Cytoplasmic **inclusions** contain accumulated metabolites or other substances, but unlike organelles have little or no metabolic activity themselves. Most inclusions are transitory

FIGURE 2–28 Cellular inclusions.



Inclusions are cytoplasmic structures or deposits filled with stored macromolecules and are not present in all cells.

(a) **Lipid droplets** are abundant in cells of the adrenal cortex and appear with the TEM as small spherical structures with homogenous matrices (L). Mitochondria are also seen here. As aggregates of hydrophobic lipid molecules these inclusions are enclosed by a single monolayer of phospholipids with various peripheral proteins, including enzymes for lipid metabolism. In routine processing of tissue for paraffin sections, fat droplets are generally removed, leaving empty spaces in the cells. Common fat cells have cytoplasm essentially filled with one large lipid droplet. (X19,000)

(b) TEM of a liver cell cytoplasm shows numerous individual or clustered electron-dense particles representing **glycogen granules**, although these granules lack membrane. Glycogen granules

usually form characteristic aggregates such as those shown. Glycogen is a ready source of energy, and such granules are often abundant in cells with high metabolic activity. (X30,000)

(c) **Pigment deposits (PD)** occur in many cell types and may contain various complex substances, such as **lipofuscin** or melanin. Lipofuscin granules represent an accumulating by-product of lysosomal digestion in long-lived cells, but melanin granules serve to protect cell nuclei from damage to DNA caused by light. Many cells contain pigmented deposits of **hemosiderin granules** containing the protein ferritin, which forms a storage complex for iron. Hemosiderin granules are very electron dense, but with the light microscope they appear brownish and resemble lipofuscin. The liver cells shown have large cytoplasmic regions filled with pigment deposits, which probably represent iron-containing hemosiderin. (X400; Giemsa)

structures not enclosed by membrane. Features of important and commonly seen inclusions are shown Figure 2–28 and include:

- **Lipid droplets**, accumulations of lipid filling adipocytes (fat cells) and present in various other cells,
- **Glycogen granules**, aggregates of the carbohydrate polymer in which glucose is stored, visible as irregular clumps of periodic acid-Schiff (PAS)—positive or electron-dense material in several cell types, notably liver cells, and
- Pigmented deposits of naturally colored material, including **melanin**, dark brown granules which in skin serve to protect cells from ultraviolet radiation; **lipofuscin**, a pale brown granule found in many cells, especially in stable nondividing cells (eg, neurons, cardiac muscle), containing a complex mix of material partly derived from residual bodies after lysosomal digestion; and **hemosiderin**, a dense brown aggregate of denatured ferritin proteins

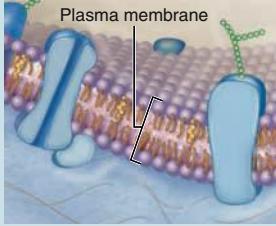
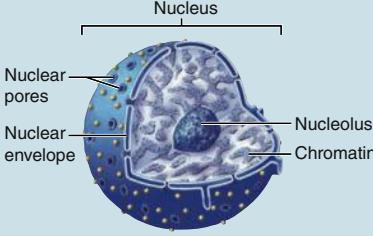
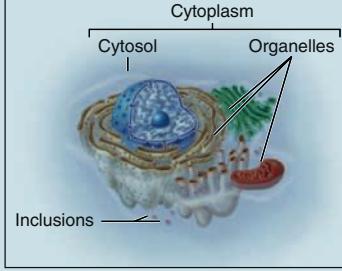
with many atoms of bound iron, prominent in phagocytic cells of the liver and spleen, where it results from phagocytosis of red blood cells

» MEDICAL APPLICATION

A condition termed **hemochromatosis**, in which the iron-containing inclusion **hemosiderin** occurs in cells of organs throughout the body, may be seen with increased uptake of dietary iron, impaired iron utilization, or with excessive lysis of red blood cells. Hemochromatosis itself does not damage cell or organ function. However, extreme accumulations of iron in cellular hemosiderin can lead to disorders such as hemochromatosis and iron overload syndrome, in which tissues of the liver and other organs are damaged.

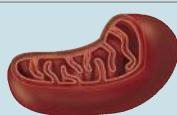
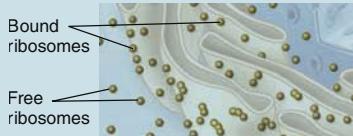
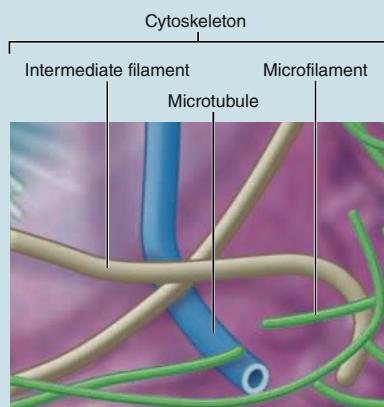
A summary of the major structural and functional features of all cytoplasmic components is presented in Table 2–6.

TABLE 2–6 Summary of cellular structural components.

| Component | Structure | Major Function | Appearance |
|---|--|--|---|
| Plasma membrane | Phospholipid bilayer containing cholesterol and proteins (integral and peripheral) and some carbohydrates (externally); forms a selectively permeable boundary of the cell | Acts as a physical barrier to enclose cell contents; regulates material movement into and out of the cell; establishes and maintains an electrical charge difference across the plasma membrane; functions in cell communication |  |
| Cilia | Short, numerous membrane extensions supported by microtubules, which occur on exposed membrane surfaces of some cells | Move substances (eg, mucus, and dissolved materials) over the cell surface |  |
| Flagellum | Long, singular membrane extension supported by microtubules; present on sperm cells | Propels sperm |  |
| Microvilli | Numerous thin membrane folds projecting from the free cell surface; supported by microfilaments | Increase membrane surface area for greater absorption |  |
| Nucleus | Large structure enclosed within a double membrane; contains chromatin, nucleolus, and nucleoplasm | Houses the DNA that serves as the genetic material for directing protein synthesis | |
| Nuclear envelope | Double membrane boundary between cytoplasm and nuclear contents; continuous with rough endoplasmic reticulum | Separates nucleus from cytoplasm | |
| Nuclear pores | Openings through the nuclear envelope | Allow passage of materials between the cytoplasm and nucleoplasm, including ribonucleic acid (RNA), protein, ions, and small water-soluble molecules |  |
| Nucleolus | Large, prominent structure within the nucleus | Functions in synthesis of ribosomes | |
| Cytoplasm | Contents of cells between the plasma membrane and nuclear envelope | Responsible for many cellular processes | |
| Cytosol | Viscous fluid medium with dissolved solutes (eg, ions, proteins, carbohydrates, lipids) | Provides support for organelles; serves as the viscous fluid medium through which diffusion occurs |  |
| Organelles | Membrane-bound and non-membrane-bound structures | Carry out specific metabolic activities of the cell | |
| Rough endoplasmic reticulum (rough ER) | Extensive interconnected membrane network that varies in shape (eg, cisternae, tubules); ribosomes attached on cytoplasmic surface | Modifies, transports, and stores proteins produced by attached ribosomes; these proteins are secreted, become components of the plasma membrane, or serve as enzymes of lysosomes |  |
| Smooth endoplasmic reticulum (smooth ER) | Extensive interconnected membrane network lacking ribosomes | Synthesizes, transports, and stores lipids (eg, steroids); metabolizes carbohydrates; detoxifies drugs, alcohol, and poisons; forms vesicles and peroxisomes |  |

(Continued)

TABLE 2–6 Summary of cellular structural components. (Continued)

| Component | Structure | Major Function | Appearance |
|-------------------------------|--|--|--|
| Golgi apparatus | Series of several elongated, flattened saclike membranous structures | Modifies, packages, and sorts materials that arrive from the ER in transport vesicles; forms secretory vesicles and lysosomes |  |
| Vesicles | Spherical-shaped membrane-bound sacs; contain various types of materials to be transported through the cell | Transport cellular material |  |
| Lysosomes | Spherical-shaped membrane-bound organelles formed from the Golgi apparatus; contain digestive enzymes | Digest microbes or materials (eg, ingested by the cell, worn-out cellular components, or the entire cell) |  |
| Peroxisomes | Smaller, spherical-shaped membrane-bound organelles formed from the ER or through fission; contain oxidative enzymes | Detoxify specific harmful substances either produced by the cell or taken into the cell; engage in beta oxidation of fatty acids to acetyl CoA |  |
| Mitochondria | Double membrane-bound organelles containing a circular strand of DNA (genes for producing mitochondrial proteins) | Synthesize most ATP during aerobic cellular respiration by digestion of fuel molecules (eg, glucose) in the presence of oxygen |  |
| Ribosomes | Organelles composed of both protein and ribosomal RNA (rRNA) that are organized into both a large and small subunit; may be bound to a membrane or free in cytosol | Engage in protein synthesis: Bound ribosomes produce proteins that are secreted, incorporated into plasma membrane, and within lysosomes; free ribosomes produce proteins used within the cell |  Bound ribosomes Free ribosomes |
| Cytoskeleton | Organized network of protein filaments and hollow tubules, including microfilaments, intermediate filaments, and microtubules | Maintains intracellular structural support and organization of cells; participates in cell division; facilitates movement |  Cytoskeleton Intermediate filament Microfilament Microtubule |
| Microfilaments | Actin protein monomers organized into two thin, intertwined protein filaments (actin filaments) | Maintain cell shape; support microvilli; separate two cells during cytokinesis (a process of cell division); facilitate change in cell shape; participate in muscle contraction |  |
| Intermediate filaments | Various protein components | Provide structural support; stabilize junctions between cells | |
| Microtubules | Hollow cylinders composed of tubulin protein | Maintain cell shape and rigidity; organize and move organelles; support cilia and flagella; participate in vesicular transport; separate chromosomes during the process of cell division |  |
| Centrosome | Amorphous region adjacent to nucleus; contains a pair of centrioles | Organizes microtubules; participates in mitotic spindle formation during cell division |  Centrosome Centriole |
| Proteasomes | Large, barrel-shaped protein complexes located in both the cytosol and nucleus | Degradate and digest damaged or unneeded proteins; ensure quality of exported proteins |  |
| Inclusions | Aggregates of specific types of molecules (eg, melanin protein, glycogen, or lipid) | Serve as temporary storage for these molecules | Variable appearance |

The Cytoplasm SUMMARY OF KEY POINTS

- **Cell differentiation** is the process by which cells of an embryo become specialized structurally to augment specific cytoplasmic activities for functions at the level of tissues and organs.
- **Organelles** are metabolically active structures or complexes, with or without membranes, in the cytoplasm of eukaryotic cells.

Plasma Membrane

- The **plasma membrane** (**cell membrane** or **plasmalemma**) is the lipid bilayer with embedded proteins that surrounds a cell and is seen only with the TEM.
- The **lipid bilayer** forms from amphipathic **phospholipids**, stabilized by **cholesterol**, and contains many **embedded (integral) proteins** and many **peripheral proteins** on its cytoplasmic surface.
- Membrane proteins move laterally within the lipid bilayer, with less movement in areas referred to as **lipid rafts**, which have higher concentrations of cholesterol and saturated fatty acids.
- Integral membrane proteins include **receptors** for external ligands, **channels** for passive or active movement of molecules across the membrane, and **pumps** for active membrane transport.
- **Endocytosis** is cellular uptake of macromolecules or fluid by plasma membrane engulfment or invagination, followed by the “pinching off” of a filled membranous vesicle in the cytoplasm.
- Major types of endocytosis include **phagocytosis** (uptake of particulate material), **pinocytosis** (uptake of dissolved substances), and **receptor-mediated endocytosis** (uptake of specific molecules bound to integral membrane receptor proteins).
- **Exocytosis** is a type of cellular secretion in which cytoplasmic membrane vesicles fuse with the plasma membrane and release their contents to the extracellular space.
- All types of **cell** signaling use membrane receptor proteins that are often linked to enzymes such as kinases or adenylyl cyclase whose activities initiate intracellular signaling pathways.

Ribosomes

- The two **ribosomal subunits**, each a complex of rRNA and many proteins, attach to mRNA and translate that message into protein.
- Multiple ribosomes on the same mRNA make up a **polyribosome** (**polysome**), and an abundance of these produces basophilic cytoplasm after H&E staining.

Endoplasmic Reticulum

- The ER is a convoluted network of membrane enclosing continuous spaces called **cisternae** and extending from the nucleus to the plasma membrane.
- **Rough ER** has a **granular, basophilic cytoplasmic surface** due to the presence of polysomes making most membrane proteins, proteins in certain other organelles, or for exocytosis; RER is always well developed in cells actively secreting proteins.
- Proteins to be processed through the RER contain initial **signal peptides** which bind receptors in the ER membrane, localizing them to that organelle.
- After **translocation** across the membrane into the cisterna, the proteins undergo **posttranslational modification and folding** in a process monitored by RER molecular chaperones and enzymes.
- **Smooth ER (SER)** lacks ribosomes, but includes enzymes for **lipid** and **glycogen metabolism**, for **detoxification reactions**, and for temporary Ca^{2+} sequestration.

Golgi Apparatus

- The **Golgi apparatus** is a dynamic organelle consisting of stacked membranous cisternae in which proteins made in RER are **processed** further and **packaged** for secretion or other roles.
- Proteins in **transport vesicles** enter the *cis* or receiving face of the Golgi, move through medial cisternae of the Golgi network for enzymatic modifications, and are released in other vesicles at the *trans* face.

- Vesicle movement through the Golgi apparatus is guided by specific **coat proteins** such as COPII and COPI.
- Important protein modifications in the Golgi apparatus include **sulfation** and many **glycosylation** reactions.
- Modified proteins leave the Golgi apparatus after packaging in vesicles with coat proteins that direct movement to lysosomes, the plasma membrane, or secretion by exocytosis.

Lysosomes

- **Primary lysosomes** emerge from the Golgi apparatus containing inactive acid hydrolases specific for degrading a wide variety of cellular macromolecules.
- **Secondary lysosomes** are more heterogeneous, having fused with vesicles produced by endocytosis that contain material to be digested by the hydrolytic enzymes.
- During **autophagy**, lysosomes digest unneeded or nonfunctional organelles after these are surrounded by membrane that then fuses with a lysosome.
- Products of digestion in secondary lysosomes are released to the cytoplasm for reuse; final condensed vesicles containing any indigestible molecules are called **residual bodies**.

Proteasomes

- Proteasomes are small cytoplasmic protein complexes which degrade improperly folded proteins after they are tagged with the polypeptide **ubiquitin**.

Mitochondria

- **Mitochondria** are the major sites of **ATP synthesis** and are abundant in cells or cytoplasmic regions where large amounts of energy are expended.
- Mitochondria are usually **elongated organelles** and form by fission of preexisting mitochondria.
- Mitochondria have two membranes: a **porous outer membrane** encloses the intermembrane space and an **inner membrane with many folds (cristae)** enclosing a gel-like matrix.
- The **mitochondrial matrix** contains enzymes for β -oxidation of fatty acids and the citric acid (Krebs) cycle.
- The inner membrane includes enzyme assemblies of the **electron-transport system and ATP synthase**.
- Mitochondria of stressed cells may release **cytochrome c** from the inner membrane, triggering a regulated series of events culminating in cell death (**apoptosis**).

Peroxisomes

- Peroxisomes are small spherical organelles containing enzymes for various metabolic reactions, notably **for oxidation and detoxification**, and **catalase** that breaks down the H_2O_2 resulting from those reactions.

Cytoskeleton

- The cytoskeleton contains three types of polymers: (1) **microtubules** 25 nm in diameter; (2) actin filaments or **microfilaments** (5-7 nm); and (3) **intermediate filaments** (8-10 nm).
- Microtubules are semirigid tubular structures with walls composed of **polymerized tubulin** heterodimers; their structure is often very dynamic, with steady addition and dissociation of tubulin.
- Microtubules are important in **maintaining cell shape** and as **tracks for transport** of vesicles and organelles by the **motor proteins kinesin and dynein**.
- Microfilaments are short, flexible, highly dynamic filaments of **actin subunits**, in which changes in length and interactions with binding proteins regulate cytoplasmic viscosity and movement.
- **Myosins** are **motor proteins** that bind and move along actin filaments, carrying vesicles or producing cytoplasmic movement.

- Movements of cytoplasm produced by actin filaments and myosins are important for endocytosis, cell cleavage after mitosis, and cell locomotion on substrates.
- Intermediate filaments are the **most stable** cytoskeletal component, conferring strong mechanical stability to cells.
- Intermediate filaments are composed of various protein subunits in different cells; they include **vimentin**; **nuclear lamins**; **neurofilament**

The Cytoplasm ASSESS YOUR KNOWLEDGE

1. In transmission EM preparations of cells the cell membrane often appears as a trilaminar structure having two parallel dark-staining components on either side of an unstained middle layer. This central poorly stained region of the membrane is primarily responsible for which of the following functions?
 - a. Creation of a barrier to water-soluble molecules
 - b. Binding by cellular receptors to specific ligands
 - c. Catalyzing membrane-associated activities
 - d. Transport of ions
 - e. Connections to the cytoskeleton
2. Chaperonins are cytoplasmic proteins most likely to be found in which of the following organelles?
 - a. Lysosomes
 - b. Golgi complexes
 - c. Rough endoplasmic reticulum
 - d. Smooth endoplasmic reticulum
 - e. Mitochondria
3. Which of the following best defines the term "exocytosis"?
 - a. The discharge of ions or small molecules from a cell by protein pumps in the cell membrane
 - b. The uptake of material at one domain of a cell's surface and its discharge from the opposite side of the cell
 - c. The process by which proteins move from one cytoplasmic compartment to another
 - d. The discharge of proteins in cytoplasmic vesicles from a cell following fusion of the vesicles with the plasmalemma
 - e. Diffusion of lipid-soluble molecules from a cell across the cell membrane
4. Cytoplasm often stains poorly because its lipid content is removed by the organic solvents used in the clearing step in routine histological preparations. This problem is most likely to occur with cytoplasmic regions rich in which of the following organelles?
 - a. Free polysomes
 - b. Mitochondria
 - c. Lysosomes
 - d. Smooth endoplasmic reticulum
 - e. Rough endoplasmic reticulum
5. Polarity in microtubules is important in determining which of the following?
 - a. The strength of vinblastine binding to microtubules
 - b. The velocity of transport along microtubules with myosin motors
 - c. The overall dynamic instability of the microtubules
 - d. The linkage of microtubules to intermediate filaments
 - e. The direction of vesicular transport along microtubules
6. Which of the following proteins is/are most likely to have initially contained a "signal peptide" that bound a "signal recognition particle" during its translation?
 - a. An enzyme of the respiratory chain
 - b. Lamins
 - c. Proteins in secretory granules
 - d. F-actin
 - e. Proteins in the large ribosomal subunit
7. Vesicles of a Golgi apparatus that are destined to become part of other organelles most likely have which of the following on their membranes?
 - a. Channel proteins
 - b. Clathrin
 - c. COP II
 - d. Actin
 - e. GTP
8. About 3 years ago, a 39-year-old construction worker became increasingly uncoordinated. His wife describes bouts of depression and apathy beginning about a decade ago. Laboratory tests are normal. MRI and CT reveal striatal and caudate atrophy with "boxcar ventricles." His mini-mental status examination score is 24/30. The cranial nerve examination shows dysarthria, saccadic extraocular eye movements, and a hyperactive gag reflex. There is increased tone in all extremities. Polymerase chain reaction reveals one normal band with 20 CAG (trinucleotide) repeats and the other with 49 CAG repeats. Modulation of respiration and mitochondrial membrane potential, and bioenergetic failure are associated with the abnormal gene in this disease. Which of the following mechanisms used to establish the mitochondrial electrochemical gradient may be altered in this disease?
 - a. The action of ATP synthase
 - b. Transfer of electrons from NADH to O₂ in the intermembrane space
 - c. Pumping of protons into the mitochondrial matrix by respiratory chain activity
 - d. Proton-translocating activity in the inner membrane
 - e. Transport of ATP out of the matrix compartment by a specific transporter
9. A 56-year-old man has been taking atorvastatin because of a poor lipid profile and a family history of cardiovascular disease. The statin family of drugs enhances endocytosis of low density lipoprotein (LDL) from the blood. Endocytosis of LDL differs from phagocytosis of bacterial cells in which of the following ways?
 - a. Use of membrane-enclosed vesicles in the uptake process
 - b. Coupling with the lysosomal system
 - c. Dependence on acidification
 - d. Use of clathrin-coated pits
 - e. Use of hydrolases
10. A 14-year-old boy is diagnosed with epidermolysis bullosa simplex (EBS). His skin blisters easily with rubbing or scratching. Blisters occur primarily on his hands and feet and heal without leaving scars. Genetic analysis shows mutations in the *KRT5* and *KRT14* genes, which code keratin 5 and keratin 14. What is the primary function of those proteins?
 - a. Generate movement
 - b. Provide mechanical stability
 - c. Carry out nucleation of microtubules
 - d. Stabilize microtubules against disassembly
 - e. Transport organelles within the cell

proteins; and **keratins**, which are especially important in epithelial cells.

Inclusions

- Unlike organelles, inclusions are **not metabolically active** and are primarily **storage sites**, such as lipid droplets, glycogen granules, pigment granules, or residual bodies (also called **lipofuscin**).

3

The Nucleus

| | | | |
|----------------------------------|-----------|--|-----------|
| COMPONENTS OF THE NUCLEUS | 53 | STEM CELLS & TISSUE RENEWAL | 65 |
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| Nucleolus | 56 | | |
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| MITOSIS | 61 | APOPTOSIS | 67 |
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Containing the code for all of a cell's enzymes and other proteins, the **nucleus** is the command center of the cell. The nucleus also contains the molecular machinery to replicate the DNA and to synthesize and process all types of RNA. During interphase, pore complexes in the membrane enclosing the nucleus regulate macromolecular transfer between the nuclear and cytoplasmic compartments. Mature RNA molecules pass into the cytoplasm for their roles in protein synthesis, while proteins needed for nuclear activities are imported there from the cytoplasm. Restricting protein synthesis to the cytoplasm helps ensure that newly made RNA molecules do not become involved in translation before processing is complete.

» COMPONENTS OF THE NUCLEUS

The nucleus usually appears as a large rounded or oval structure, often near the cell's center (Figure 3–1). Typically the largest structure within a cell, it consists of a **nuclear envelope** containing **chromatin**, the mass of DNA and its associated proteins, with one or more specialized regions of chromatin called **nucleoli**. In specific tissues the size and shape of nuclei normally tend to be uniform.

Nuclear Envelope

The **nuclear envelope** forms a selectively permeable barrier between the nuclear and cytoplasmic compartments. Electron microscopy reveals that the envelope has two concentric membranes separated by a narrow (30–50 nm) **perinuclear space** (Figures 3–2 and 3–3). This space and the outer nuclear membrane are continuous with the extensive cytoplasmic network of the rough endoplasmic reticulum (RER). Closely associated with the inner nuclear membrane is a highly organized meshwork of proteins called the **nuclear lamina** (Figure 3–4),

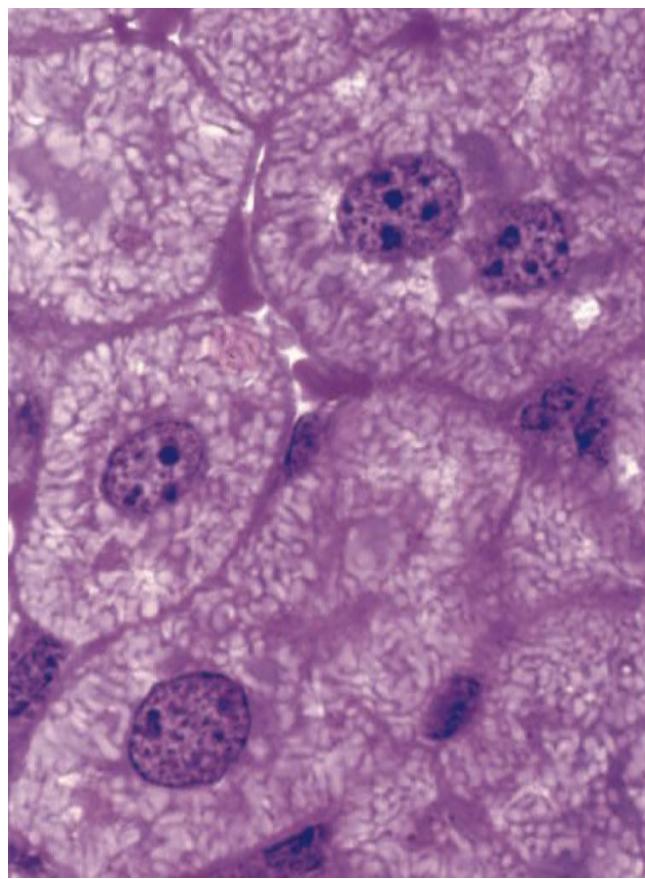
which stabilizes the nuclear envelope. Major components of this layer are the class of intermediate filament proteins called **lamins** that bind to membrane proteins and associate with chromatin in nondividing cells.

The inner and outer nuclear membranes are bridged at **nuclear pore complexes** (Figures 3–2 through 3–6). Various core proteins of a nuclear pore complex, called **nucleoporins**, display 8-fold symmetry around the lumen. Although ions and small solutes pass through the channels by simple diffusion, the pore complexes regulate movement of macromolecules between the nucleus and cytoplasm. A growing cell has 3000–4000 such channels, each providing passage for up to 1000 macromolecules per second. Individual pores permit molecular transfer in both directions simultaneously. Macromolecules shipped out of the nucleus include ribosomal subunits and other RNAs associated with proteins, while inbound traffic consists of chromatin proteins, ribosomal proteins, transcription factors, and enzymes. Using mechanisms similar to that by which specific proteins are recognized and translocated across the RER membrane, proteins of complexes destined for the cytoplasm have specific nuclear export sequences and proteins to be imported have nuclear localization sequences. Such sequences bind specifically to transport proteins (importins, exportins, etc) that in turn interact with proteins of the pore complexes for transfer across the nuclear envelope. Energy for the transport is derived from guanosine 5'-triphosphate (GTP), with specific GTPases helping provide directionality to the transfer.

Chromatin

Chromatin consists of DNA and all of the associated proteins involved in the organization and function of DNA. In humans each cell's chromatin (except that of eggs and sperm) is divided among 46 **chromosomes** (23 pairs). After DNA replication but before cell division, each chromosome consists of two

FIGURE 3-1 Nuclei of large, active cells.



Liver cells have large, central nuclei. One or more highly basophilic nucleoli are visible within each nucleus, indicating intense protein synthesis by these cells. Most of the chromatin is light staining or euchromatic, with small areas of more darkly stained heterochromatin scattered throughout the nucleus and just inside the nuclear envelope. This superficial heterochromatin allows the boundary of the organelle to be seen more easily by light microscopy. One cell here has two nuclei, which is fairly common in the liver. (X500; Pararosaniline-toluidine blue)

identical chromatin units called **chromatids** held together by complexes of cohesin proteins.

DNA of each human cell is approximately 2 m long, with 3.2 billion base pairs (bp), and therefore must be extensively packaged within the nucleus. This occurs initially by the DNA associating with sets of small basic proteins called **histones**. The structural unit of DNA and histones is called the **nucleosome** (Figures 3-7 and 3-8), which has a core of eight histones (two copies each of histones H2A, H2B, H3, and H4), around which is wrapped about 150 bp of DNA. Each nucleosome also has a larger histone (H1) associated with both the wrapped DNA and the surface of the core. In the EM the series of nucleosomes on DNA resembles “beads

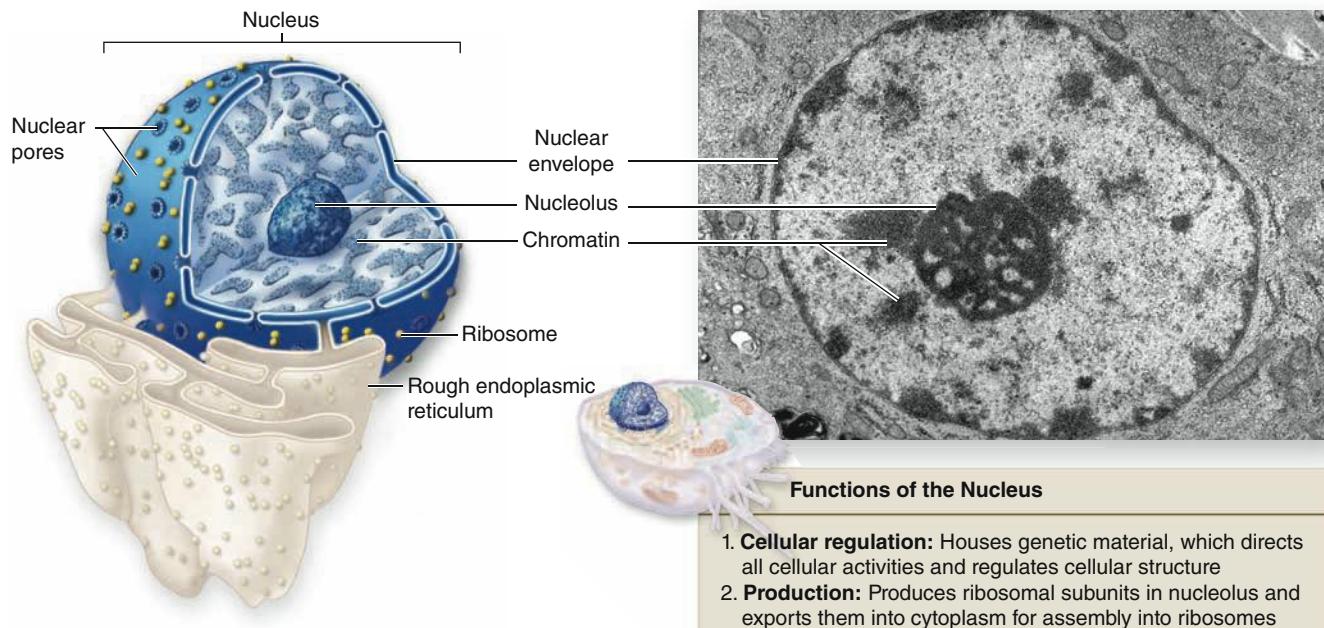
on a string”, with 50-80 bp of linker DNA separating each bead. Nucleosomes are structurally dynamic; modification and rearrangement of the histones allows temporary unwrapping of the DNA and arrival of enzymes and other proteins required for replication and gene transcription.

DNA wrapped around nucleosomes is coiled further for greater compaction within the nucleus and for general regulation of gene activity. The 10-nm fiber of nucleosomes and DNA undergoes helical folding to yield a fiber with a diameter of 30 nm (Figure 3-8). Beyond the 30-nm fiber chromatin structure is much less well understood, but it does form nearly ubiquitous kilobase-sized and larger loops of coiled DNA, some of which are unstable and may reflect gene activity rather than a distinct hierarchical level of chromatin structure. Many such loops are tethered to a central scaffold containing various proteins, including condensins, which promote compaction of chromatin. Further packaging during the first phase of cell division causes chromosomes to become visible as discrete structures by light microscopy (Figure 3-8).

Microscopically two categories of chromatin can be distinguished in nuclei of most nondividing cells (Figure 3-3). **Euchromatin** is visible as finely dispersed granular material in the electron microscope and as lightly stained basophilic areas in the light microscope. **Heterochromatin** (Gr. *heteros*, other + *chroma*, color) appears as coarse, electron-dense material in the electron microscope and as intensely basophilic clumps in the light microscope.

DNA in the more open structure of euchromatin is rich with genes, although not all of the genes are transcribed in all cells. Heterochromatin is always more compact than euchromatin, shows little or no transcriptional activity, and includes at least two types of genomic material called constitutive and facultative heterochromatin. *Constitutive heterochromatin* is generally similar in all cell types and contains mainly repetitive, gene-poor DNA sequences, including the large chromosomal regions called **centromeres** and **telomeres**, which are located near the middle (most often) and at the ends of chromosomes respectively. *Facultative heterochromatin* contains other regions of DNA with genes where transcription is variably inactivated in different cells by epigenetic mechanisms and can undergo reversible transitions from compact, transcriptionally silent states to more open, transcriptionally active conformations.

The ratio of heterochromatin to euchromatin seen with nuclear staining can provide a rough indicator of a cell's metabolic and biosynthetic activity (Figure 3-3). Euchromatin predominates in active cells such as large neurons, while heterochromatin is more abundant in cells with little synthetic activity such as circulating lymphocytes. Facultative heterochromatin also occurs in the small, dense “sex chromatin” or **Barr body** which is one of the two large X chromosomes present in human females but not males. The Barr body remains tightly coiled, while the other X chromosome is uncoiled, transcriptionally active, and not visible. Cells of males have one X chromosome and one Y chromosome; like the other chromosomes, the single X chromosome remains largely euchromatic.

FIGURE 3–2 Relationship of nuclear envelope to the rough ER (RER).

Three-dimensional representation of a cell nucleus shows a single large nucleolus and the distribution of the nuclear pores in the

nuclear envelope. The outer membrane of the nuclear envelope is continuous with the RER. (TEM X20,000)

► MEDICAL APPLICATION

Barr bodies or gender chromatin permit gender to be determined microscopically in patients whose external sex organs do not permit that determination, as in hermaphroditism and pseudohermaphroditism. Sex chromatin analysis also helps reveal other anomalies involving the sex chromosomes, such as the presence of XXY chromosomes (Klinefelter syndrome), which causes testicular abnormalities and azoospermia (absence of sperm).

Although much heterochromatin tends to be concentrated near the nuclear lamina, evidence for spatial organization of chromatin is not normally seen. Recent *in situ* hybridization studies of cultured human fibroblast nuclei, using differently labeled fluorescent probes for sequences on each individual chromosome, have revealed that these structures occupy discrete **chromosomal territories** within dispersed chromatin (Figure 3–9). Such studies show further that chromosomal domains with few genes form a layer beneath the nuclear envelope, while domains with many active genes are located deeper in the nucleus.

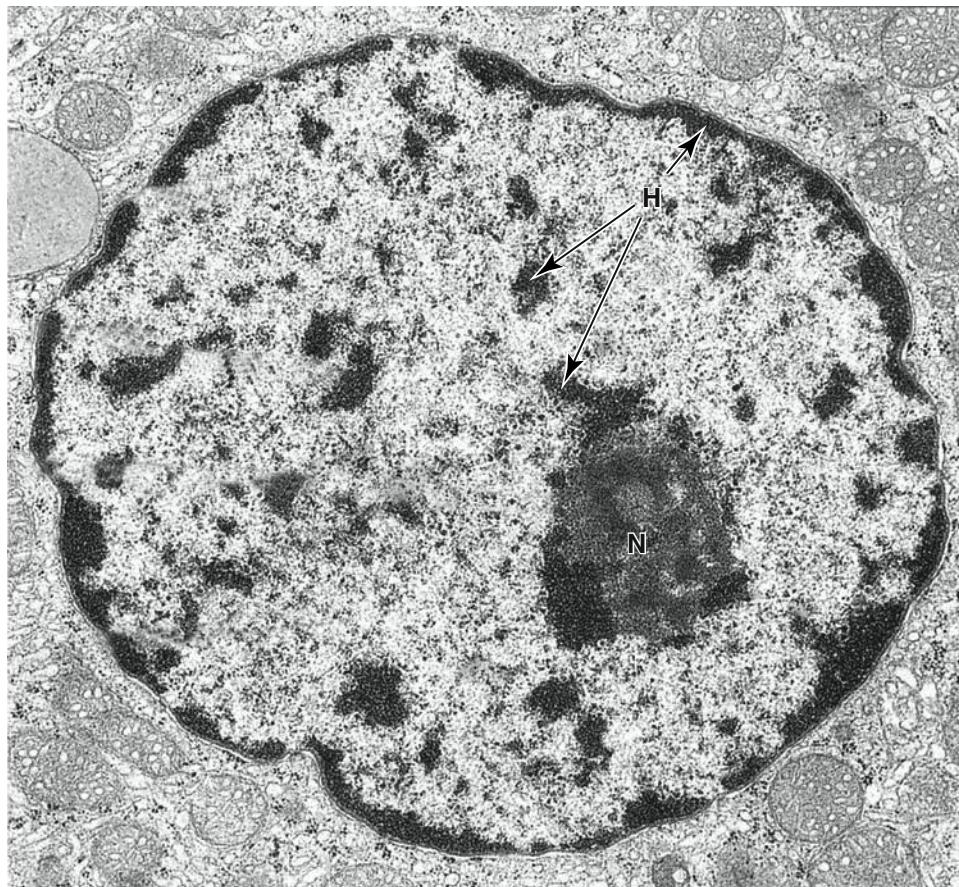
The X and Y sex chromosomes contain genes determining whether an individual will develop as a female or a male. In addition to the pair of sex chromosomes, cells contain 22 pairs of autosomes. Each pair contains one chromosome originally derived from the mother and one from the father. The members

of each chromosomal pair are called **homologous** because, although from different parents, they contain forms (alleles) of the same genes. Cells of most tissues (somatic cells) are considered **diploid** because they contain these pairs of chromosomes. Geneticists refer to diploid cells as $2n$, where n is the number of unique chromosomes in a species, 23 in humans. Sperm cells and mature oocytes (germ cells) are **haploid**, with half the diploid number of chromosomes, each pair having been separated during meiosis (described later in the chapter).

Microscopic analysis of chromosomes usually begins with cultured cells arrested in mitotic metaphase by colchicine or other compounds that disrupt microtubules. After processing and staining the cells, the condensed chromosomes of one nucleus are photographed by light microscopy and rearranged digitally to produce a **karyotype** in which stained chromosomes can be analyzed (Figure 3–10).

► MEDICAL APPLICATION

Karyotyping is important for many prenatal diagnoses, in which chromosomal analysis of cultured cells from the fetus or amniotic fluid can detect certain genetic anomalies. As with karyotypes of adults, missing or extra chromosomes and chromosomal deletions or translocations are readily seen. New methods of chromosomal staining and molecular techniques such as fluorescence *in situ* hybridization (FISH) are continuously being developed and used for cytogenetic diagnosis.

FIGURE 3–3 Ultrastructure of a nucleus.

Regions of euchromatin and heterochromatin display variable electron densities with the transmission electron microscope (TEM). An active nucleus typically has much diffuse, light-staining euchromatin and smaller subdomains of electron-dense heterochromatin (**H**), with many of these associated at the periphery

associated with the nuclear lamina. The more heterogeneous electron-dense subdomain is the nucleolus (**N**), the site of rRNA synthesis, and ribosomal subunit assembly. (X25,000)

Nucleolus

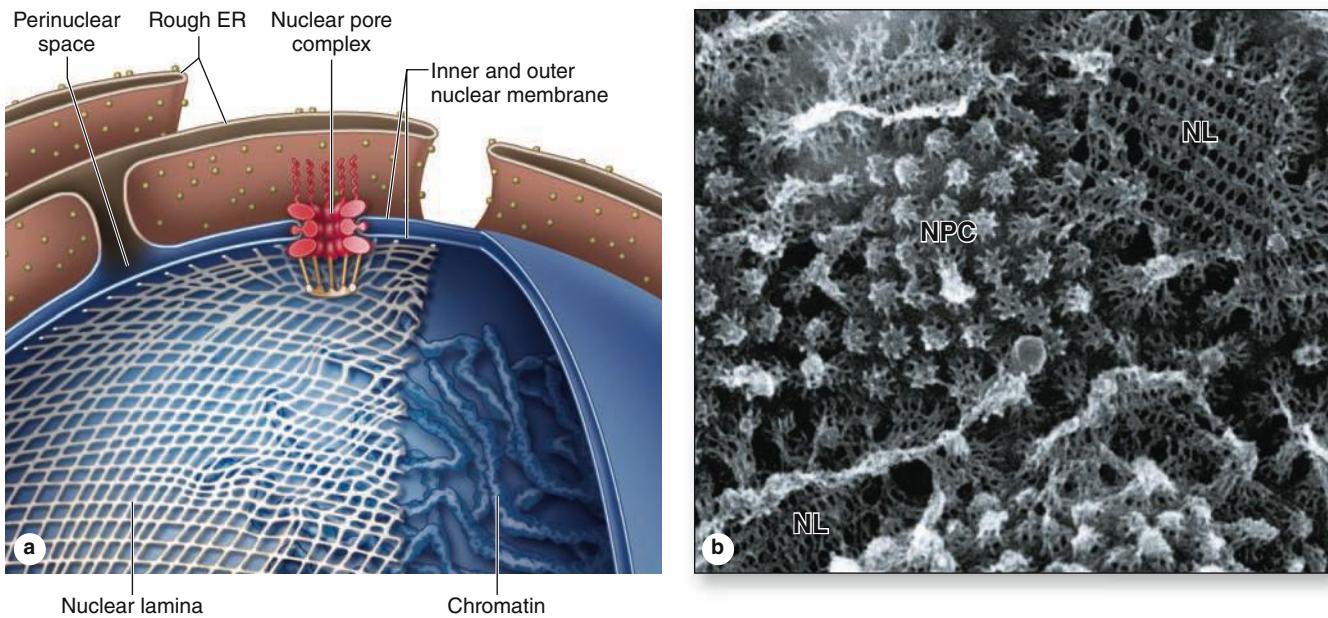
The **nucleolus** is a generally spherical, highly basophilic subdomain of nuclei in cells actively engaged in protein synthesis (Figures 3–1 through 3–3). The intense basophilia of nucleoli is due not to heterochromatin but to the presence of densely concentrated ribosomal RNA (rRNA) that is transcribed, processed, and assembled into ribosomal subunits. Chromosomal regions with the genes for rRNA organize one or more nucleoli in cells requiring intense ribosome production for protein synthesis during growth or secretion. Ultrastructural analysis of an active nucleolus reveals fibrillar and granular subregions with different staining characteristics that reflect stages of rRNA maturation, as described in Figure 3–11. Molecules of rRNA are processed in the nucleolus and very quickly associate with the ribosomal proteins imported from the cytoplasm via nuclear pores. The newly organized small and large

ribosomal subunits are then exported back to the cytoplasm through those same nuclear pores.

» MEDICAL APPLICATION

Tissues with either stable or rapidly renewing cell populations can include cells that become transformed to grow at a higher rate and in an uncoordinated manner. Such **neoplastic proliferation** typically follows damage to the DNA of proto-oncogenes and failure of the cells to be eliminated. Neoplastic growth can be either benign (with slow growth and no invasiveness to neighboring organs) or malignant (with rapid growth and great capacity to invade other organs). **Cancer** is the common term for all malignant tumors.

FIGURE 3–4 The nuclear envelope, nuclear lamina, and nuclear pore complexes.



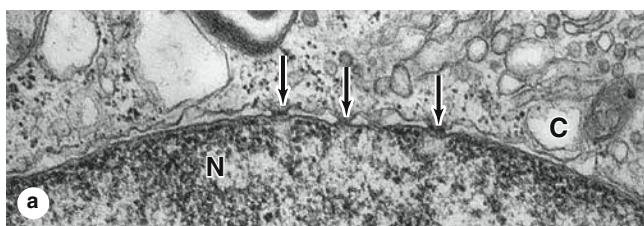
(a) Bound to the inner membrane of the nuclear envelope is the **nuclear lamina**, a meshwork assembled from lamins (class V intermediate filament proteins). **Nuclear pore complexes** contain more than 30 core proteins (nucleoporins), span both membranes of the nuclear envelope, and regulate the bidirectional transfer of macromolecular complexes between the nucleus and cytoplasm.

(b) Scanning EM of the inner nuclear membrane (nucleoplasmic face) showing portions of the nuclear lamina (**NL**) meshwork with

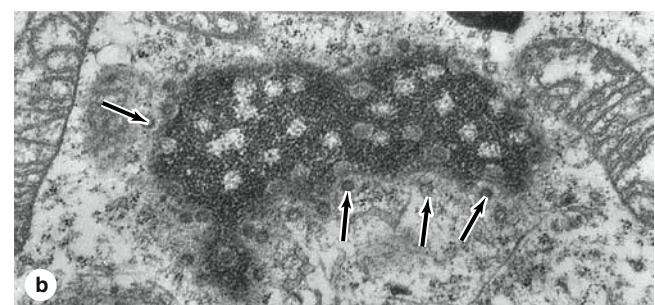
many embedded nuclear pore complexes (**NPC**). The preparation is from an actively growing amphibian oocyte. Nuclei of these very large cells can be isolated manually, facilitating ultrastructural studies of the nuclear envelope. (X100,000)

(Used with permission from Dr M.W. Goldberg, Department of Biological and Biomedical Sciences, Durham University, UK.)

FIGURE 3–5 Nuclear pores.

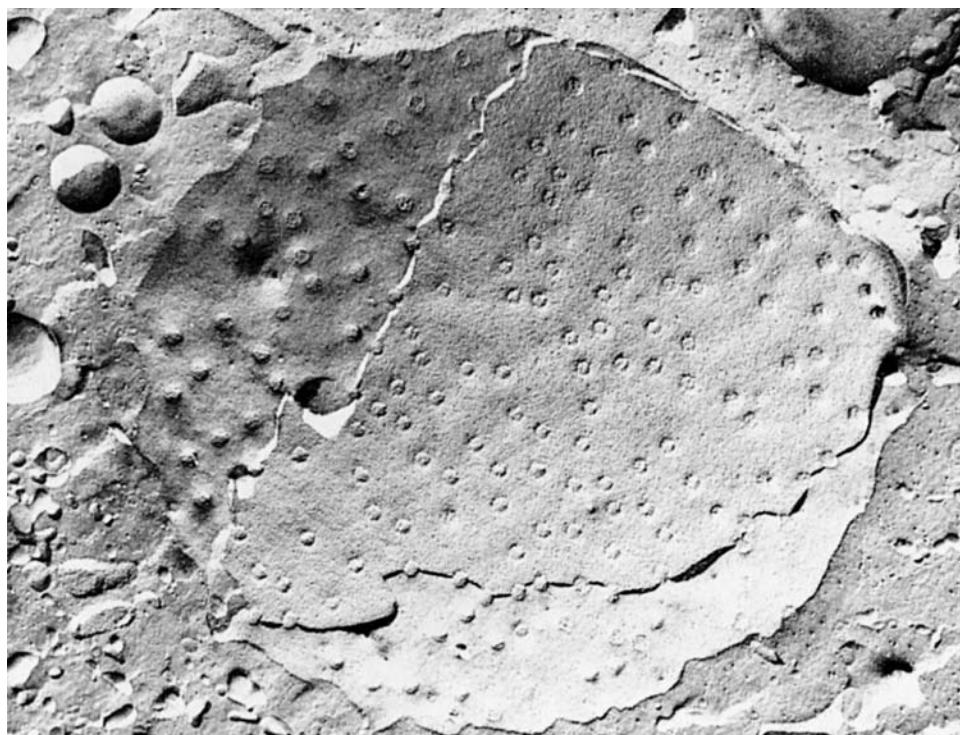


(a) A TEM section through the nuclear envelope between nucleus (**N**) and cytoplasm (**C**) shows its two-membrane structure. The electron-dense nuclear pore complexes bridging the nuclear envelope can also be seen (arrows). Electron-dense heterochromatin is adjacent to the envelope, except at the nuclear pores.



(b) A tangential section through a nuclear envelope shows the nuclear pore complexes (arrows) and the electron-lucent patches in the peripheral heterochromatin, which represent the areas just inside pores. (X80,000)

FIGURE 3–6 Cryofracture of nuclear envelope showing nuclear pores.



An electron micrograph obtained by freeze-fractured cell shows the two layers of the nuclear envelope and nuclear pores. The fracture plane occurs partly between the two nuclear envelope

membranes (left) but mostly just *inside* the envelope with the chromatin removed. The size and distribution of the nuclear pore complexes are clearly seen. (X60,000)

» MEDICAL APPLICATION

Certain mutations in the gene coding for lamin A are associated with a subtype of the disorder progeria, which causes premature aging. In this and other rare "laminopathies," the nuclear envelope is abnormal, but how this is linked to the disorder is unclear. Laminopathies affect some tissues much more than others, although the lamins involved are in all the body's cells.

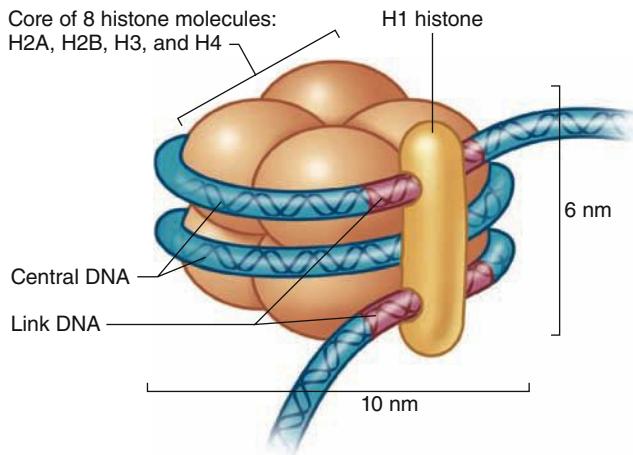
G₁ (the gap between DNA duplication and the next mitosis). The approximate durations of these phases in rapidly dividing human cells are illustrated in Figure 3–12. The G₁ phase, usually the longest and most variable part of the cycle, is a period of active RNA and protein synthesis, including proteins controlling progress through the cell cycle. Also in G₁, the cell volume, reduced by half during mitosis, returns to its previous size. The S phase is characterized by DNA replication, histone synthesis, and the beginning of centrosome duplication. In the relatively short G₂ phase, proteins required for mitosis accumulate. As new postmitotic cells specialize and differentiate, cell cycle activities may be temporarily or permanently suspended, with the cells sometimes referred to as being in the G₀ phase. Some differentiated cells, such as those of the liver, renew cycling under certain conditions; others, including most muscle and nerve cells, are *terminally differentiated*.

Cycling is activated in postmitotic G₀ cells by protein signals from the extracellular environment called **mitogens** or **growth factors** that bind to cell surface receptors and trigger a cascade of kinase signaling in the cells. The cells are then maintained at the *restriction point* at the G₁/S "boundary" until sufficient nutrients and enzymes required for DNA synthesis have accumulated, and when all is ready DNA replication (S phase) begins.

» THE CELL CYCLE

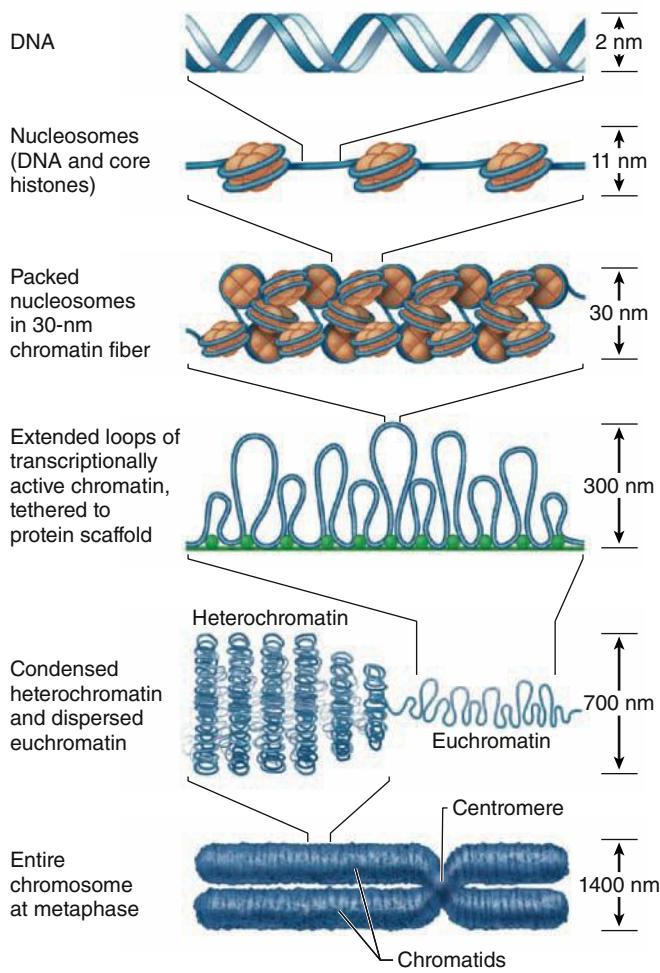
Before differentiation, most cells undergo repeated cycles of macromolecular synthesis (growth) and division (mitosis). The regular sequence of events that produce new cells is termed the **cell cycle**. Improved knowledge about how each phase of the cell cycle is controlled and how the quality of molecular synthesis, particularly DNA replication, is monitored has led to understanding the causes of many types of cancer, in which cells proliferate without those controls.

The cell cycle has four distinct phases: **mitosis** and periods termed **G₁** (the time gap between mitosis and the beginning of DNA replication), **S** (the period of DNA synthesis), and

FIGURE 3–7 Components of a nucleosome.

Nucleosome is a structure that produces the initial organization of free double-stranded DNA into chromatin. Each nucleosome has an octomeric core complex made up of four types of **histones**, two copies each of H2A, H2B, H3, and H4. Around this core is wound DNA approximately 150 base pairs in length. One H1 histone is located outside the DNA on the surface of each nucleosome. DNA associated with nucleosomes *in vivo* thus resembles a long string of beads. Nucleosomes are very dynamic structures, with H1 loosening and DNA unwrapping at least once every second to allow other proteins, including transcription factors and enzymes, access to the DNA.

As shown in Figure 3–13, entry or progression through other phases of the cycle is also monitored at other specific *checkpoints*, where certain conditions must be met before the cell continues cycling. Overall cycling is regulated by a family of cytoplasmic proteins called **cyclins**. With different cyclins present during different cell cycle phases, each activates one or more specific **cyclin-dependent kinases (CDKs)**. Each activated CDK then phosphorylates specific proteins, including enzymes, transcription factors for specific sets of genes, and cytoskeletal subunits, triggering the activities that characterize the next phase of the cycle. When each successive set of activities is complete, the cyclin controlling that cell cycle phase is ubiquitinated and removed rapidly by proteasomes and a new cyclin that promotes activities for the next phase takes over. In this way diverse cellular activities are coordinated with specific phases of the cell cycle. The major cyclins, CDKs, and important target proteins are summarized in Table 3–1.

FIGURE 3–8 From DNA to chromatin.

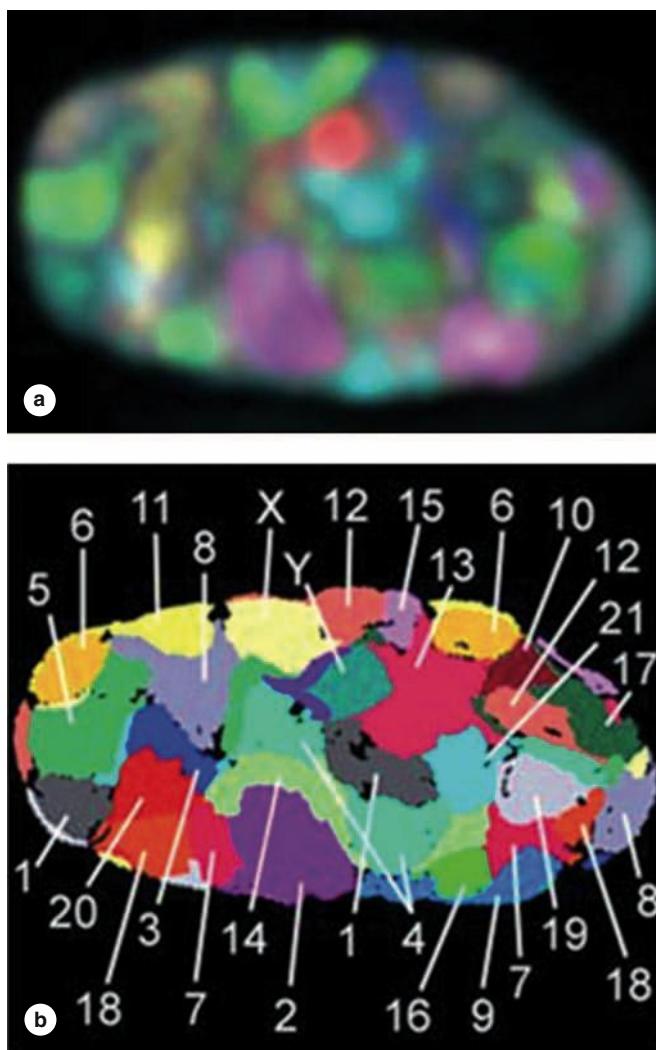
Several orders of DNA packing occur in chromatin and during chromatin condensation of mitotic prophase. The top drawing shows the 2-nm DNA double helix, followed by the association of DNA with histones to form 11-nm filaments of **nucleosomes** connected by the DNA ("beads on a string"). Nucleosomes on the DNA then interact in a manner not well understood to form a more compact **30-nm** fiber. DNA in such fibers forms various loops, some of which in **euchromatin** involve gene transcription. Loops remain tethered to and stabilized by interactions with **protein scaffolds** that eventually make up a central framework at the long axis of each chromosome. **Heterochromatin** is not transcribed and remains more highly condensed. The bottom drawing shows a metaphase chromosome, with maximum packing of DNA. The **chromosome** consists of two **chromatids** held together at a constriction called the **centromere**.

► MEDICAL APPLICATION

Many mitogenic growth factors for research are produced commercially from microorganisms or cells with recombinant DNA, and some have important medical uses. Important examples include analogs of granulocyte colony-stimulating

factor (G-CSF), which stimulates neutrophil production in immunocompromised patients, and erythropoietin, which can stimulate red blood cell formation in patients with anemia.

FIGURE 3–9 Chromosome territories of a human fibroblast nucleus.

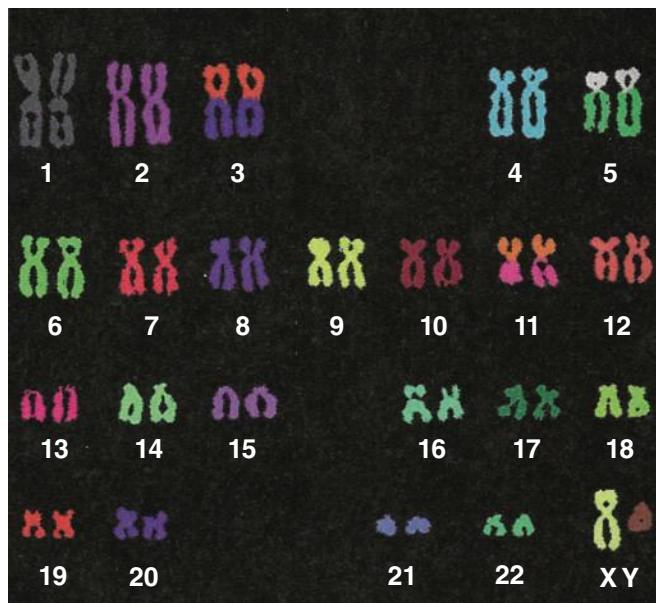


Fluorescence *in situ* hybridization (FISH) can be used with a combination of labeled probes, each specific for sequences on different chromosomes. A nucleus of a cultured human fibroblast was processed by 24-color FISH, photographed by confocal microscopy in appropriate channels, and the results superimposed to form an RGB (red-green-blue) image (**a**) of the 24 differently labeled chromosome types (1-22, X, and Y). Individual chromosome territories in the image were identified and false-colored after classification by software developed for such analyses (**b**).

(Used with permission from Dr Thomas Cremer, Department of Biology II, Anthropology and Human Genetics, Ludwig Maximilian University, Munich, Germany.)

Progression through the cell cycle is halted by adverse conditions such as inadequate nutrition (nutrient stress), inappropriate cellular microenvironments, or DNA damage. Nuclear DNA is monitored very closely, and damage here can arrest the cell cycle not only at the **G₁ restriction point** but also during S or at a checkpoint in G₂ (Figure 3-13). G₁ arrest may permit

FIGURE 3–10 Human karyotype.



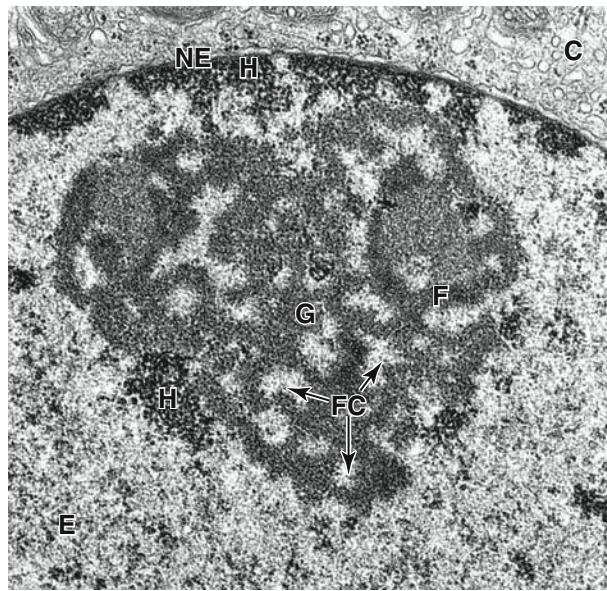
Karyotypes provide light microscopic information regarding the number and morphology of chromosomes in an organism. Such preparations are made by staining and photographing the chromosomes of a cultured cell arrested with colchicine during mitosis, when chromosomes are maximally condensed. From the image individual chromosomes are typically placed together in pairs. With certain stains each chromosome has a particular pattern of banding that facilitates its identification and shows the relationship of the banding pattern to genetic anomalies. Hybridization with fluorescent probes specific for each chromosome (FISH) followed by karyotyping yields an image like that shown here. Note that the 22 pairs of autosomes, as well as the X and Y chromosomes, differ in size, morphology, and location of the centromere.

repair of the damage before the cell enters S phase, so that the damaged DNA does not reproduce gene defects during replication. If the problem encountered at any checkpoint cannot be corrected fairly quickly while cycling is halted, proteins encoded by tumor suppressor genes are activated and that cell's activity is redirected toward cell suicide or apoptosis.

» MEDICAL APPLICATION

Many genes coding for proteins important in the control of cell proliferation and differentiation are often called **proto-oncogenes**; changes in the structure or expression of these can convert them to **oncogenes** causing uncontrolled cell growth and a potential for cancer. Altered proto-oncogenes are associated with many types of tumors and hematologic cancers. Proto-oncogenes can encode almost any protein involved in the control of mitotic activity, including various specific growth factors, the receptors for growth factors, and various kinases and other proteins involved in intracellular signaling of growth factors.

FIGURE 3–11 Regions within a nucleolus.



TEM reveals morphologically distinct regions within a nucleolus. Small, light-staining areas are fibrillar centers (**FC**), containing the DNA sequences for the rRNA genes (the nucleolar organizers). The darker fibrillar material (**F**) surrounding the fibrillar centers consists of accumulating rRNA transcripts. More granular material (**G**) of the nucleolus contains mainly the large and small ribosomal subunits being assembled from rRNA and ribosomal proteins synthesized in the cytoplasm. Various amounts of heterochromatin (**H**) are also typically found near the nucleolus, scattered in the euchromatin (**E**), and adjacent to the nuclear envelope (**NE**) that separates chromatin from cytoplasm (**C**). (X35,000)

» MEDICAL APPLICATION

Retinoblastoma is a type of cancer occurring in the eyes, usually in young children. One form of the disease is inherited or familial. Research on the genetic basis of this disease led to the discovery of Rb, a gene coding for a key protein active at the G_1 restriction point that blocks cell cycle progression until a mitogenic stimulus arrives. A kinase activated by a growth factor receptor phosphorylates the Rb protein, causing it to release the E2F transcription factor. This factor then activates genes needed for DNA replication.

DNA changes (mutations) resulting from damage are not always detected and corrected (or eliminated). If such a change occurs in a gene important for cell cycle activities, such as genes for certain growth factors, their receptors or signaling kinases, normal controls on the cell cycle may be affected and growth may occur in a less-regulated manner usually detected by the tumor suppressor proteins such as p53. Failure to detect unregulated cell cycling can lead to additional defects and the

cellular changes found in the various types of cancer. In many forms of human cancer, the gene for the key tumor suppressor p53 is itself mutated, thus reducing the ability to eliminate cells with damaged DNA and facilitating proliferation of cells with new genetic defects.

➤ MITOSIS

The period of cell division, or **mitosis** (Gr. *mitos*, a thread), is the only cell cycle phase that can be routinely distinguished with the light microscope. During mitosis, a parent cell divides and each of the two daughter cells receives a chromosomal set identical to that of the parent cell. The chromosomes replicated during the preceding S phase are distributed to the daughter cells. The long period between mitoses (the G_1 , S, and G_2 phases) is also commonly called **interphase**. The events of mitosis are subdivided into four major stages (Figure 3–12). Important details of each mitotic phase are included in Figure 3–14 and summarized here. During the relatively long **prophase**, several changes occur:

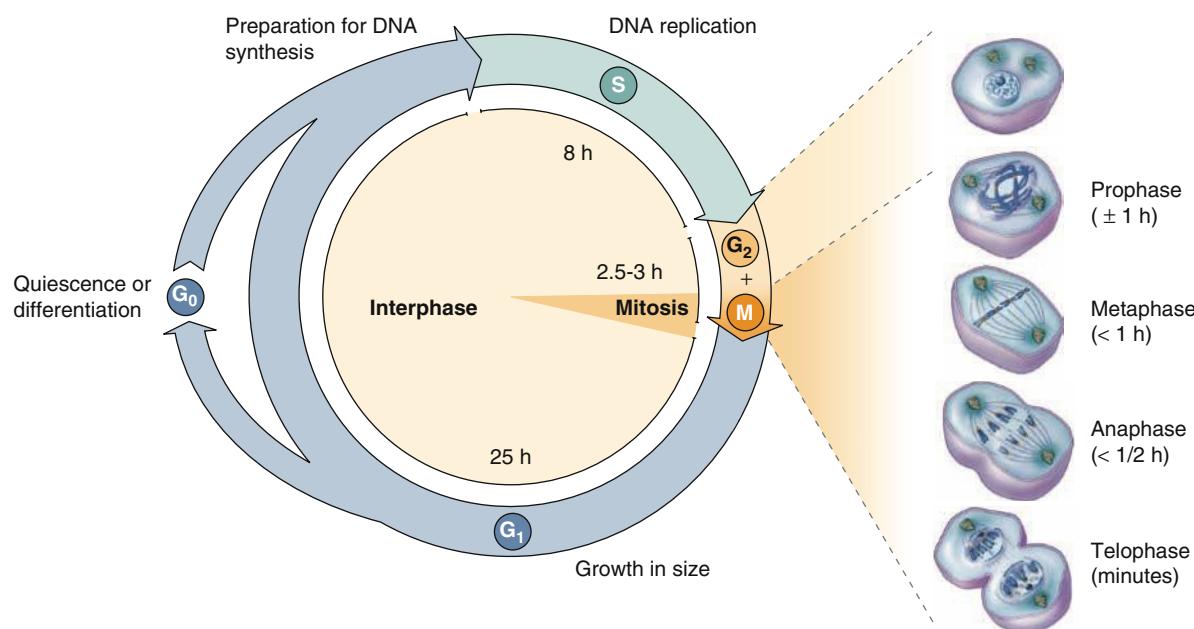
- The nucleolus disappears and the replicated chromatin condenses into discrete threadlike chromosomes, each consisting of duplicate sister chromatids joined at the centromere.
- The two centrosomes with their now-duplicated centrioles separate and migrate to opposite poles of the cell and organize the microtubules of the **mitotic spindle**.
- Late in prophase, lamins and inner nuclear membrane are phosphorylated, causing the nuclear lamina and nuclear pore complexes to disassemble and disperse in cytoplasmic membrane vesicles.

During **metaphase**, chromosomes condense further and protein complexes called **kinetochores** (Gr. *kinetos*, moving) at each centromere attach to the mitotic spindle (Figure 3–15). The cell is now more spherical and the chromosomes are moved into alignment at the equatorial plate.

In **anaphase** sister chromatids (now called chromosomes themselves) separate and move toward opposite spindle poles by a combination of microtubule motor proteins and dynamic changes in the lengths of the microtubules as the spindle poles move farther apart.

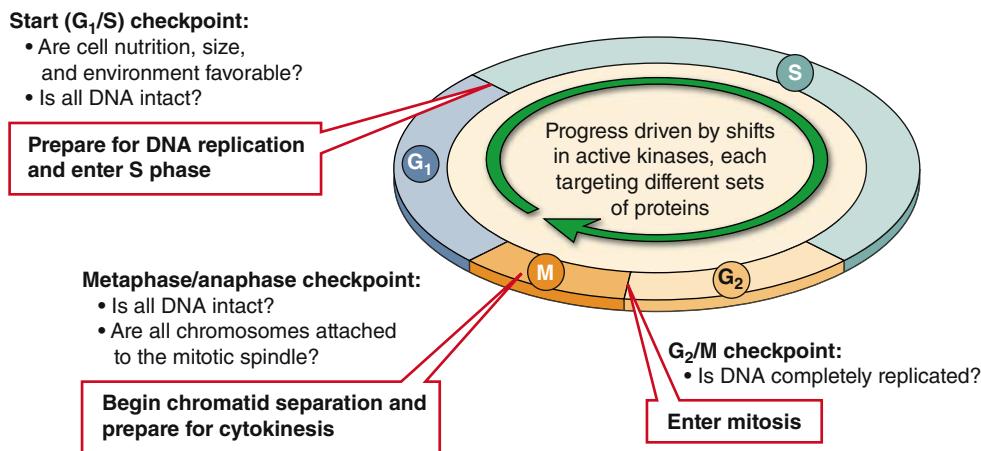
At **telophase** the following occur:

- The two sets of chromosomes are at the spindle poles and begin reverting to their decondensed state.
- The spindle depolymerizes and the nuclear envelope begins to reassemble around each set of daughter chromosomes.
- A belt-like contractile ring of actin filaments associated with myosins develops in the cortical cytoplasm at the cell's equator. During **cytokinesis** at the end of telophase, constriction of this ring produces a cleavage furrow and progresses until the cytoplasm and its organelles are divided into two daughter cells, each with one nucleus.

FIGURE 3–12 The cell cycle.

The ability to recognize microscopically cells during both mitosis and DNA replication (by autoradiography after administering radiolabeled thymidine) led to the concept of the cell “cycle” with the phases occurring as shown here. In rapidly dividing cells, **G₁** is a period in which cells accumulate the enzymes and nucleotides required for DNA replication, **S** is the period devoted primarily to DNA replication, **G₂** is a usually short period of preparation for

mitosis, and **M** includes all phases of mitosis itself. In rapidly growing human tissues the cell cycle varies from 24 to 36 hours. The length of **G₁** depends on many factors and is usually the longest and most variable period; the length of **S** is largely a function of the genome size. **G₂** and mitosis together normally last only 2-3 hours. Differentiating cells in growing tissues may have very long **G₁** periods and such cells are often said to be in the **G₀** phase of the cell cycle.

FIGURE 3–13 Controls at cell cycle checkpoints.

Each phase of the cell cycle has one or more checkpoints where the quality of specific cell activities is checked. Progression to the next phase of the cycle does not occur until all activities of the preceding phase are completed satisfactorily. Three important checkpoints are shown here, including

- The start or restriction checkpoint just before the start of S
- The G₂/M checkpoint that ensures that DNA replication is complete

■ The metaphase spindle checkpoint that ensures that all chromosomes will be segregated

Overall progression in the cycle is regulated by proteins called **cyclins** and **cyclin-dependent kinases (CDKs)** that phosphorylate/activate enzymes and other proteins needed for phase-specific functions. Major cyclins, their CDKs, and important protein targets are summarized in Table 3–1.

Table 3–1

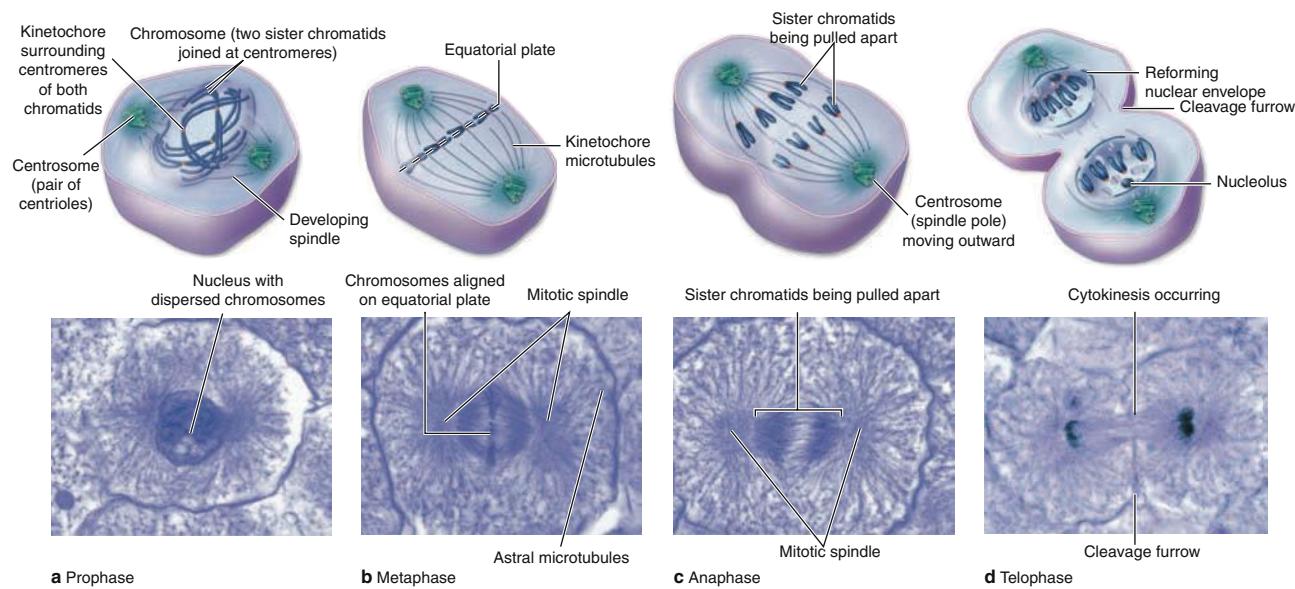
Major cyclin and cyclin-dependent kinase complexes regulating the human cell cycle and important target proteins.

| Cycle Phase or Checkpoint | Active Cyclin-CDK Complex | Examples of Target Proteins |
|---------------------------------|---------------------------|---|
| Early G ₁ | Cyclin D-CDK4 or 6 | Phosphorylates Rb protein, releasing E2F, a transcription factor that activates genes for many G ₁ activities and for cyclin A |
| Late G ₁ /entry of S | Cyclin E-CDK2 | Further activation E2F-mediated gene transcription; protein p53; other kinases |
| Progression through S | Cyclin A-CDK2 | DNA polymerase and other proteins for DNA replication |
| G ₂ /entry of M | Cyclin A-CDK1 | Specific phosphatases and cyclin B |
| Progression through M | Cyclin B-CDK1 | Nuclear lamins; histone H1; chromatin- and centrosome-associated proteins |

Most tissues undergo cell turnover with slow cell division and cell death. Nerve tissue and cardiac muscle are exceptions because their differentiated cells cannot undergo mitosis. As discussed in later chapters, a capacity for mitosis within a tissue, either by differentiated cells or by a reserve of stem cells, largely determines the tissue's potential to

regeneration. The cell turnover rate is rapid in the epithelium lining the digestive tract and uterus or covering the skin. Mitotic cells are usually difficult to identify conclusively in sections of adult organs but may often be detected in rapidly growing tissues by their condensed chromatin (Figure 3–16).

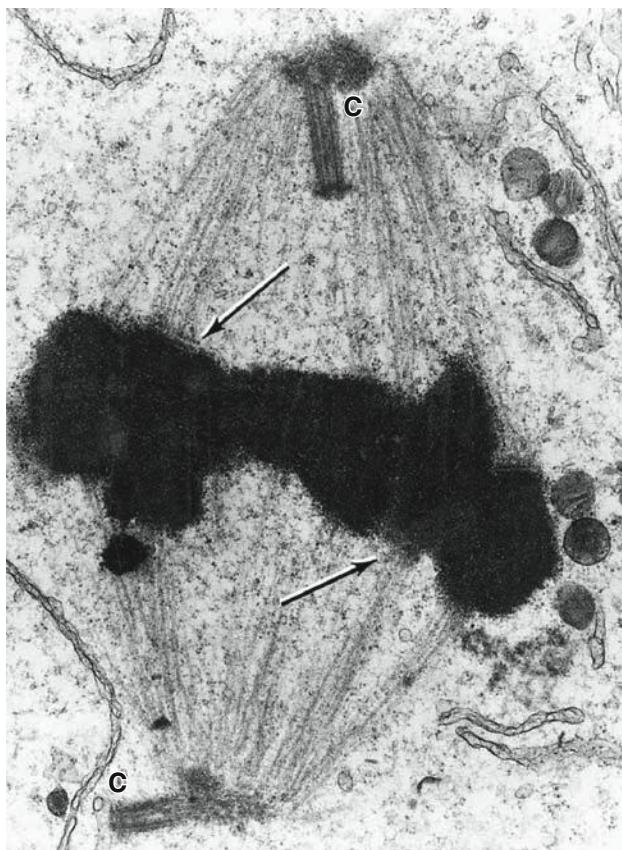
FIGURE 3–14 Phases of mitosis.



The chromosomal changes that occur during mitosis are easily and commonly studied in the large cells of very early embryos of fish after sectioning, such as the mitotic cells shown here. (a) During the relatively long **prophase** the **centrosomes** move to opposite poles, the nuclear envelope disappears by fragmentation, and the chromosomes condense and become visible. Having undergone DNA replication, each chromosome consists of two chromatids joined at their centromere regions by **kinetochore** protein complexes. (b) At the short **metaphase** the chromosomes have become aligned at the **equatorial plate** as a result of their attachments to the dynamic microtubules of the **mitotic spindle**

organized by the centrosomes. The spindle consists of **kinetochore microtubules**, polar microtubules which interdigitate near the equatorial plate, and shorter astral microtubules anchoring the spindle to the cell membrane. (c) During **anaphase** the kinetochores separate and the chromatids (now called chromosomes themselves) are pulled on their microtubules toward each centrosome. (d) In **telophase** the cell pinches itself in two by contraction of the F-actin bundle in the cell cortex, after which the chromosomes decondense, transcription resumes, nucleoli reappear, and the nuclear lamina and nuclear envelope reassemble. (All X500; H&E)

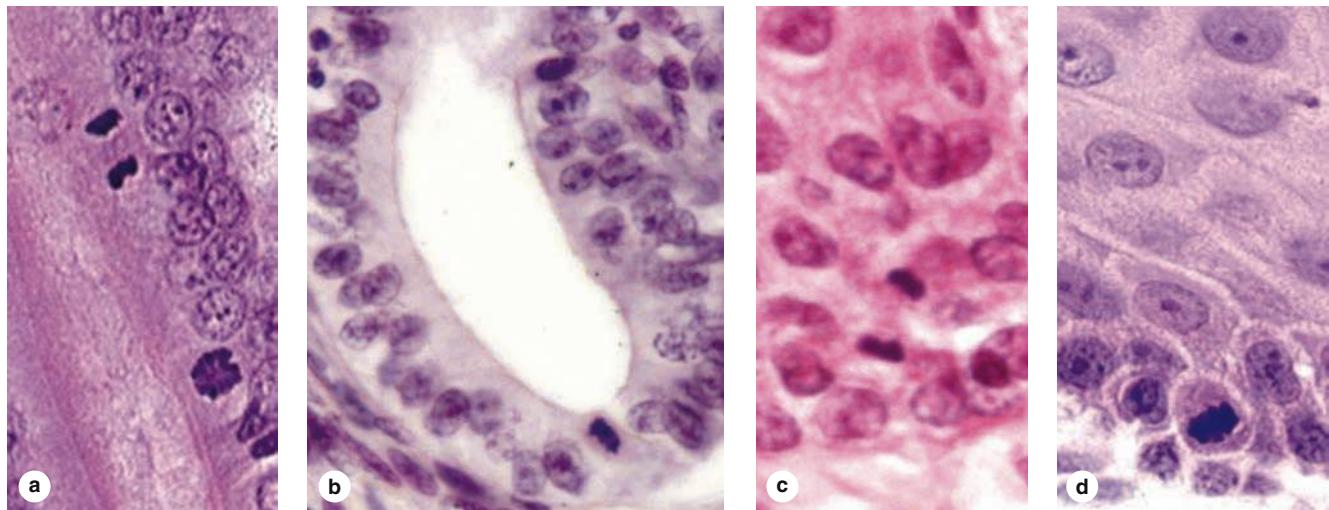
FIGURE 3–15 Mitotic spindle and metaphase chromosomes.



TEM of a sectioned metaphase cell shows several features of the mitotic apparatus, including the very electron-dense chromosomes bound at kinetochores (arrows) to the microtubules of the spindle. The microtubules converge on the centrosomes (**C**), each containing a pair of centrioles. The flattened membrane vesicles near the mitotic spindle may represent fragments of the nuclear envelope, which begin to reassemble during late telophase. (X19,000)

(Used with permission from Richard McIntosh, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder.)

FIGURE 3–16 Mitotic cells in adult tissues.



Dividing cells in recognizable stages of mitosis are rarely observed in adult tissues because they are rare and the cells are small, with variable shapes and orientations. However, *mitotic figures*, nuclei with clumped, darkly stained chromatin, can sometimes be identified, as shown here in various rapidly renewing tissues.

(a) In the lining of the small intestine, many mitotic *transit amplifying cells* can be found in the area above the most basal region

of the intestinal crypts. Condensed chromosomes of cells in late anaphase and prophase phase can be distinguished here.

(b) Metaphase cell in a gland of proliferating uterine endometrium. (c) Telophase cell in the esophagus lining. (d) Metaphase in the basal layer of epidermis. (X400; H&E)

› STEM CELLS & TISSUE RENEWAL

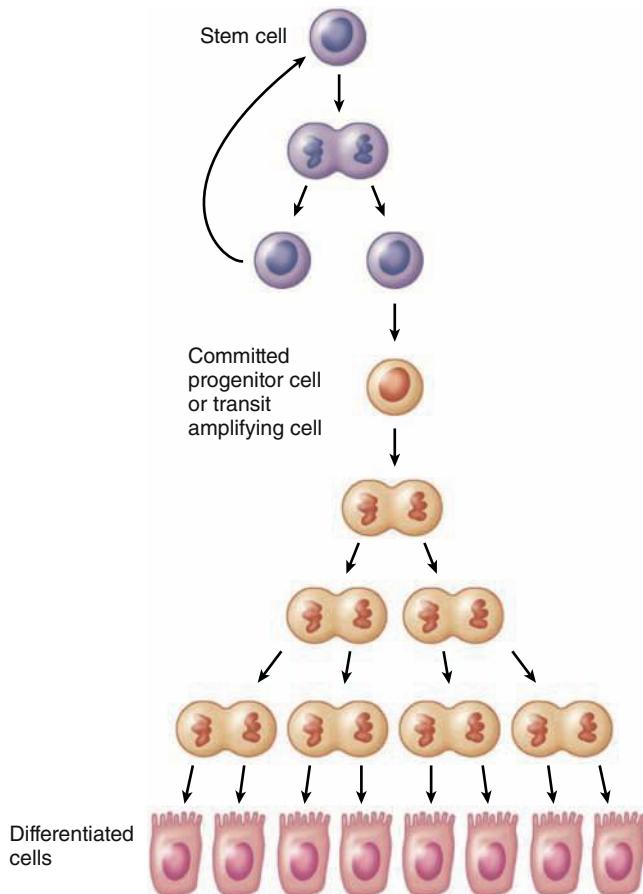
Throughout an individual's lifetime, many tissues and organs contain a small population of undifferentiated **stem cells** whose cycling serves to renew the differentiated cells of tissues as needed. Many stem cells divide infrequently and the divisions are asymmetric; that is, one daughter cell remains as a stem cell while the other becomes committed to a path that leads to differentiation (Figure 3–17). Stem cells of many tissues are found in specific locations or niches where the microenvironment helps maintain their uniquely undifferentiated properties; they are often rare and inconspicuous by routine histologic methods.

Stem cells are best studied in tissues with *rapidly renewing cell populations*, including blood cells, skin cells, and cells

lining the digestive tract. Most mitotic cells here are not stem cells but the more rapidly dividing progeny of the cells committed to differentiation (Figure 3–17). They are commonly called **progenitor cells** or transit amplifying cells because they are in transit along the path from the stem cell niche to a differentiated state, while still amplifying by mitosis the number of new cells available for the differentiated tissue. Cells formed by progenitor cells may become terminally differentiated, meaning that renewed cycling cannot occur and the specialized cells exist for only a short time.

In tissues with *stable cell populations*, such as most connective tissues, smooth muscle, and the cells lining blood vessels, stem cells are not readily apparent and differentiated cells appear to undergo slow and episodic division to maintain tissue integrity.

FIGURE 3–17 Stem cells.



In rapidly growing adult tissues and perhaps in other tissues there are slowly dividing populations of **stem cells**. Stem cells divide asymmetrically, producing one cell that remains as a stem cell and another that becomes committed to a differentiative pathway but divides a few more times at a more rapid rate. Such cells have been termed **progenitor cells**, or "transit amplifying cells," each of which eventually stops dividing and becomes fully differentiated.

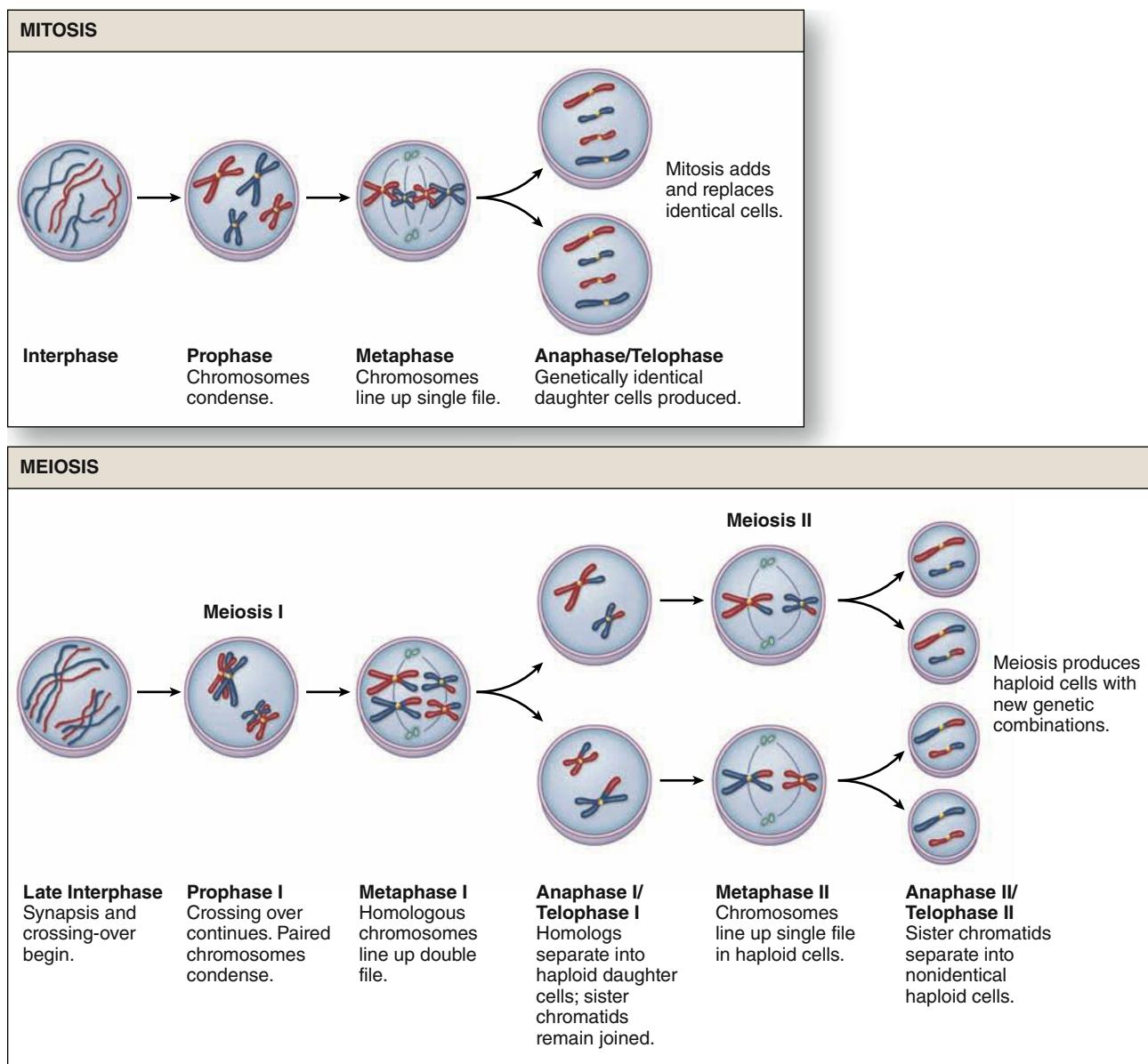
› MEIOSIS

Meiosis is a specialized process involving *two* unique and closely associated cell divisions that occurs only in the cells that will form sperm and egg cells. Differentiation of these two "germ cells" or **gametes** is discussed fully in Chapters 21 and 22, but the chromosomal aspects of meiosis are described here for comparison with the events of mitosis (Figure 3–18).

Two key features characterize meiosis. (1) Early in the process the homologous chromosomes of each pair (one from the mother, one from the father) come together in an activity termed **synapsis**. During synapsis double-stranded breaks and repairs occur in the DNA, some of which result in reciprocal DNA exchanges called **crossovers** between the aligned homologous chromosomes. This produces new combinations of genes in the chromosomes so that few if any chromosomes in the germ cells are exactly the same as those in the mother and father. (2) The cells produced are **haploid**, having just one chromosome from each pair present in the body's somatic cells. The union of haploid eggs and sperm at fertilization forms a new diploid cell (the zygote) that can develop into a new individual.

As shown in Figure 3–18, the important events of meiosis unfold as follows:

- A cell approaching the first meiotic division has just completed a typical S phase and replicated its DNA; each chromosome contains the two identical DNA molecules called *sister chromatids*.
- During a greatly elongated prophase of the first meiotic division (prophase I), the partially condensed chromatin of homologous chromosomes begins to come together and physically associate along their lengths during synapsis. Because each of the paired chromosomes has two chromatids, geneticists refer to synaptic chromosomes as *tetrads* to emphasize that four

FIGURE 3–18 Mitosis and meiosis.

Mitosis and meiosis share many aspects of chromatin condensation and separation but differ in key ways. Mitosis produces two **diploid cells** that are the same genetically. In meiosis, the two homologous maternal and paternal chromosomes physically align

in **synapsis** and regions are exchanged during *crossing over* or genetic recombination. This is followed by **two meiotic divisions** with no intervening S phase, producing **four haploid cells**.

copies of each genetic sequence are present. During synapsis recombination or crossing over occurs among the four chromatids, which mixes up the genes inherited from each parent and yields a new and different set of genes to be passed on to the next generation.

In human spermatogenesis prophase I normally lasts for 3 weeks; oocytes arrest in this meiotic phase from the time of their formation in the fetal ovary through the woman's reproductive maturity, that is, for about 12 years to nearly five decades!

- When synapsis and crossing over are completed, the chromosomes become fully condensed and undergo metaphase, anaphase, and telophase as the cell divides. This first meiotic division separates the homologous chromosomes that paired during synapsis; each of the separated chromosomes still contains two chromatids held together at the centromere.
- Each of the two new cells now divides again, much more rapidly and without an intervening S phase. In the second meiotic division the chromatids separate to opposite poles as individual chromosomes. In each new cell a nuclear envelope forms around this new haploid set of chromosomes.

In summary, meiosis and mitosis share many aspects of chromatin condensation and separation (Figure 3–18), but differ in key ways:

- Mitosis is a cell division that produces *two diploid cells*. Meiosis involves two cell divisions and produces *four haploid cells*.
- During meiotic crossing over, new combinations of genes are produced and every haploid cell is genetically unique. Lacking synapsis and the opportunity for DNA recombination, mitosis yields two cells that are the same genetically.

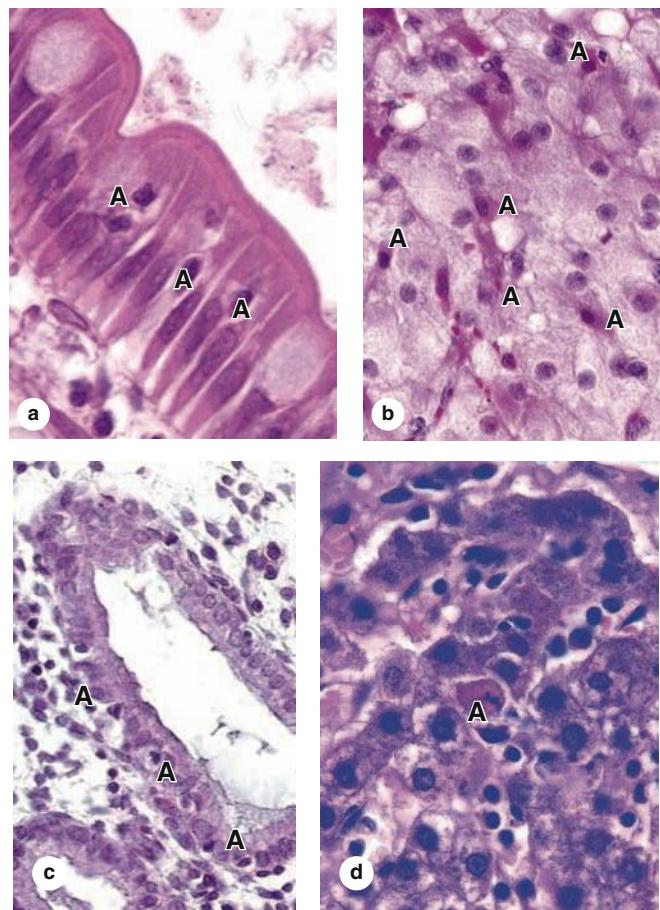
► MEDICAL APPLICATION

In humans chromosome 21 is a very small chromosome and the one most likely to be “overlooked” at the metaphase/anaphase checkpoint. Failure of these homologous chromosomes to separate (nondisjunction) in the first meiotic division also occurs with greater frequency in older oocytes (or sperm progenitor cells). A gamete retaining this chromosome pair forms a viable zygote after fertilization, but the developing **trisomy 21** individual has morphologic and cognitive impairments associated with Down syndrome.

APOPTOSIS

Less evident, but no less important than cell proliferation for body functions, is the process of cell suicide called **apoptosis** (Gr. *apo*, off + *ptosis*, a falling). Apoptosis is a rapid, highly regulated cellular activity that shrinks and eliminates defective and unneeded cells (Figure 3–19). It results in small membrane-enclosed *apoptotic bodies*, which quickly undergo phagocytosis by neighboring cells or cells specialized for debris removal. Apoptotic cells do not rupture and release none of their contents, unlike cells that die as a result of injury and undergo *necrosis*. This difference is highly significant because release of cellular components triggers a local inflammatory reaction and immigration of leukocytes. Such a response is avoided when cells are rapidly eliminated by apoptosis following cell cycle arrest or as part of normal organ development.

FIGURE 3–19 Apoptotic cells.



Apoptotic cells in adult tissues are rare because the process is completed very rapidly. Moreover, with their highly condensed chromatin in pyknotic nuclei and rounded shape, cells early in apoptosis may superficially resemble some mitotic cells. Shown here are apoptotic cells (**A**) in the epithelium covering an intestinal villus (**a**), in a corpus luteum beginning to undergo involution (**b**), in the epithelium of a uterine endometrial gland at the onset of menstruation (**c**), and in the liver (**d**). (X400; H&E)

► MEDICAL APPLICATION

Cancer cells often deactivate the genes that control the apoptotic process, thus preventing their elimination in this type of cell death and allowing progression toward a more malignant state. The Bcl-2 family of proteins that controls the onset of apoptosis was first identified by a genetic mutation in a specific B-cell lymphoma, which provided the name for the original protein.

Apoptosis is controlled by cytoplasmic proteins in the **Bcl-2 family**, which regulate the release of death-promoting factors from mitochondria. Activated by either external signals or irreversible internal damage, specific Bcl-2 proteins induce a process with the following features:

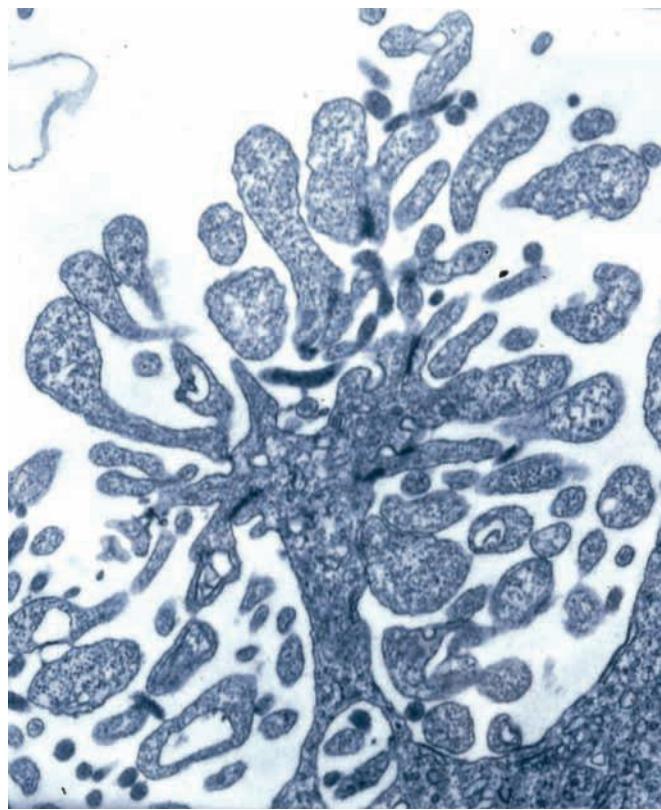
- **Loss of mitochondrial function and caspase activation:** Bcl-2 proteins associated with the outer mitochondrial membrane compromise membrane integrity, stopping normal activity and releasing cytochrome c into the cytoplasm where it activates proteolytic enzymes called **caspases**. The initial caspases activate a cascade of other caspases, resulting in protein degradation throughout the cell.
- **Fragmentation of DNA:** **Endonucleases** are activated, which cleave DNA between nucleosomes into small fragments. (The new ends produced in the fragmented DNA allow apoptotic cells to be stained histochemically using an appropriate enzyme that adds labeled nucleotides at these sites.)
- **Shrinkage of nuclear and cell volumes:** Destruction of the cytoskeleton and chromatin causes the cell to shrink quickly, producing small structures with dense, darkly stained **pyknotic nuclei** that may be identifiable with the light microscope (Figure 3–19).
- **Cell membrane changes:** The plasma membrane of the shrinking cell undergoes dramatic shape changes, such as “blebbing,” as membrane proteins are degraded and lipid mobility increases.
- **Formation and phagocytic removal:** Membrane-bound remnants of cytoplasm and nucleus separate as very small **apoptotic bodies** (Figure 3–20). Newly exposed phospholipids on these bodies induce their phagocytosis by neighboring cells or white blood cells.

» MEDICAL APPLICATION

Nuclei of cells in malignant tumors are often enlarged, abnormally shaped, and extremely dark staining, with abnormal nucleoli, in comparison with nuclei of normal cells. Such changes are useful to pathologists looking for evidence of cancer during microscopic examinations of biopsies.

A few examples of apoptosis emphasize its significance. In the ovary, apoptosis is the mechanism for both the monthly loss of luteal cells and the removal of excess oocytes and their follicles. Apoptosis was first discovered as programmed cell death in embryos, where it is important in shaping developing

FIGURE 3–20 Late apoptosis—apoptotic bodies.



TEM of a cell in late apoptosis shows radical changes in cell shape, with membrane blebbing and the formation of many membrane-bound cytoplasmic regions. These apoptotic bodies may separate from one another but remain enclosed by plasma membrane so that no contents are released into the extracellular space. The membrane changes are recognized by neighboring cells, and macrophages and apoptotic bodies are very rapidly phagocytosed. (X10,000)

organs or body regions, such as the free spaces between embryonic fingers and toes. Apoptosis of excess nerve cells plays an important role in the final development of the central nervous system.

Triggered by p53 and other tumor suppressor proteins, apoptosis is the method for eliminating cells whose survival is blocked by lack of nutrients or by damage caused by free radicals or radiation. In all these examples apoptosis occurs very rapidly, in less time than required for mitosis, and the affected cells are removed without a trace.

The Nucleus SUMMARY OF KEY POINTS

Nuclear Envelope

- Cytoplasm is separated from nucleoplasm by the **nuclear envelope**, a double set of membranes with a narrow perinuclear space; the outer membrane binds ribosomes and is continuous with the RER.
- The nuclear envelope is penetrated by **nuclear pore complexes**, large assemblies of nucleoporins with 8-fold symmetry through which proteins and protein-RNA complexes move in both directions.
- The nuclear envelope is supported internally by a meshwork, the nuclear lamina, composed of intermediate filament subunits called **lamins**.

Chromatin

- Chromatin is the combination of DNA and its associated proteins.
- Chromatin with DNA that is active in transcription stains lightly and is called **euchromatin**; inactive chromatin stains more darkly and is called **heterochromatin**.
- The DNA molecule initially wraps around complexes of basic proteins called **histones** to form **nucleosomes**, producing a structure resembling beads on a string.
- Additional levels of chromatin fiber condensation are less well understood and involve nonhistone proteins, including complexes of condensins.
- The extra X chromosome in cells of female mammals forms facultative heterochromatin and can be seen as the Barr body.

Nucleolus

- The **nucleolus** is a very basophilic or electron-dense area of chromatin localized where **rRNA transcription and ribosomal subunits assembly** occur.
- By TEM, an active nucleolus is seen to have **fibrous and granular parts** where rRNA forms and ribosomal subunits are assembled, respectively.

The Cell Cycle

- The **cell cycle** is the sequence of events that controls cell growth and division.
- The **G₁ phase**, the longest part of the cycle, begins immediately after mitosis and includes all preparations for DNA replication.
- The period of DNA (and histone) synthesis is the **S phase**.
- In a short **G₂ phase** the cell prepares for division during **mitosis (M)**.
- Cell cycling is controlled by the sequential appearance of key cytoplasmic proteins, the **cyclins**, which bind **cyclin-dependent kinases (CDKs)**.
- CDKs phosphorylate and activate the enzymes and transcription factors whose functions characterize each phase of the cell cycle.
- Progress through the cell cycle stages is monitored at checkpoints, including the **G₁ restriction point**; only when each phase's activities are completed are the cyclins changed to trigger those of the next phase.

Mitosis

- Stages of mitotic cell divisions include **prophase**, when chromosomes condense, the nuclear envelope disassembles, and the microtubular spindle forms; **metaphase**, when chromosomes are aligned; **anaphase**, when they begin to separate toward the two centrosomes; and **telophase**, when nuclear envelope re-forms around the separated chromosomes.
- Telophase ends with **cytokinesis** or cell cleavage into two daughter cells by a contractile ring of actin filaments and myosin.

Stem Cells & Tissue Renewal

- Stem cells** occur in all tissues with rapid cell turnover; they divide slowly in an **asymmetric** manner, with one daughter cell remaining a stem cell and one becoming committed toward differentiation.
- Cells committed to differentiate (**transit amplifying or progenitor cells**) typically divide more rapidly than stem cells before slowing or stopping division to differentiate.

Meiosis

- Meiosis** is the process by which two successive cell divisions produce cells called **gametes** containing half the number of chromosomes found in somatic cells.
- Prophase of the first meiotic division is a unique, extended period in which homologous chromosomes pair and undergo genetic recombination during the process called **synapsis**.
- Synaptic pairs separate toward two daughter cells at the first meiotic division.
- The second meiotic division occurs with no intervening S phase and separates the sister chromatids into two final cells that are **haploid**.

Apoptosis

- Apoptosis** is the process by which redundant or defective cells are rapidly eliminated in a manner that does not provoke a local inflammatory reaction in the tissue.
- Apoptosis involves a cascade of events controlled by the **Bcl-2 family of proteins** regulating the release of death-promoting factors from mitochondria.
- Cytochrome c from mitochondria activates cytoplasmic proteases called **caspases**, which degrade proteins of the cytosol, cytoskeleton, and cell membrane.
- Endonucleases** are activated, which degrade all nuclear DNA.
- Cell and nuclear volumes shrink rapidly, and the cell membrane changes produce extensive blebbing of the cell surface.
- Late in apoptosis, the cell breaks into many small **apoptotic bodies** that undergo **phagocytosis** by neighboring cells.
- Apoptosis occurs rapidly, with little or no release of proteins that would trigger inflammation, unlike the **death of injured cells by necrosis** that typically induces local inflammation.

The Nucleus ASSESS YOUR KNOWLEDGE

1. Which of the following facilitates breakdown of the nuclear envelope during the onset of mitosis?
 - a. Disassembly of nucleosomes in the associated constitutive heterochromatin
 - b. Increased export of material by the nuclear pore complexes into the perinuclear space
 - c. Phosphorylation of lamin subunits by a cyclin-dependent kinase (CDK)
 - d. Activities triggered at a restriction point late in G₁
 - e. The activity of proteasomes
2. Binding of histone H1 proteins to importins is important for which of the following?
 - a. Transport through the nuclear pores complexes
 - b. Properly directed vesicular transport through the Golgi apparatus
 - c. Transport from the granular part of the nucleolus
 - d. Further binding to the “linker DNA” and proper assembly of nucleosomes
 - e. Phosphorylation of cyclins
3. Which of the following is a region of chromatin that is well developed in large neurons active in protein synthesis?
 - a. Heterochromatin
 - b. The nucleolus
 - c. The Nissl substance (neuronal RER)
 - d. The Barr body
 - e. The nucleosome
4. Which of the following is found during meiosis but not mitosis?
 - a. Chromatids
 - b. Polar microtubules
 - c. Metaphase
 - d. Synapsis
 - e. Cytokinesis
5. Transitions in the cell cycle from one phase to the next are regulated by protein kinases whose activity depends on what other proteins?
 - a. Tumor suppressors
 - b. Cyclins
 - c. Actins
 - d. Lamins
 - e. Importins
6. Mitotic figures visible in a tissue section from the lining of the small intestine are most likely to belong to which of the following categories?
 - a. Terminally differentiated cells
 - b. Partially differentiated cells
 - c. Blood cells
 - d. Stem cells
 - e. Progenitor cells
7. Key differences between apoptotic and necrotic cell death include which of the following?
 - a. Apoptotic cells do not release factors that induce inflammation.
 - b. Necrosis does not trigger inflammation.
 - c. Apoptosis does not utilize intracellular proteases.
 - d. Apoptosis usually follows lethal physical damage to a cell.
 - e. Necrosis is involved in formation of some organs during embryonic development.
8. A 29-year-old woman presents with a 101°F fever, pericardial effusions and Libman-Sachs endocarditis, arthralgia, and facial rash across the malar region (“butterfly rash”) that is accentuated by sun exposure. Laboratory tests show creatine 1.7 mg/dL (normal 0.5–1.1 mg/dL), high titers of antinuclear autoantibodies (ANA), Smith antigen, and antinucleosome antibodies in the blood. Which one of the following is most likely to be directly affected by the disruption of nucleosomes in this patient?
 - a. Packaging of genetic material in a condensed form
 - b. Transcribing DNA
 - c. Forming pores for bidirectional nuclear-cytoplasmic transport
 - d. Forming the nuclear lamina
 - e. Holding together adjacent chromatids
9. A 32-year-old man and his 30-year-old wife are referred for a reproductive endocrinologist infertility (REI) consult after 2 years of “trying to get pregnant.” He is diagnosed with oligozoospermia. Ejaculated mature sperm are collected and undergo genetic analysis. Using gene linkage analysis, his REI specialist determines that he has aberrations in spermatogenic meiotic recombination, including both diminished frequency and suboptimal location, resulting in high frequency of aneuploid sperm. In explaining the diagnosis, she explains meiosis and recombination attributing the problem to a specific phase of the meiosis. Which part of meiosis is most closely associated with recombination?
 - a. Metaphase I
 - b. Anaphase I/Telophase I
 - c. Prophase I
 - d. Prophase II
 - e. Anaphase II/Telophase II
10. A newborn boy is diagnosed with Apert syndrome. He has craniosynostosis, hypoplasia of the middle part of the face with retrusion of the eyes, and syndactyly that includes fusion of the skin, connective tissue, and muscle of the first, middle, and ring fingers with moderate fusion of those digits. There is very limited joint mobility past the first joint. Which one of the following is most likely *decreased* in cells of the interdigital regions of the developing hand of this newborn child?
 - a. Random DNA degradation
 - b. Inflammation
 - c. Cell swelling
 - d. Bcl-2
 - e. DNA degradation by endonucleases

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Despite its complexity, the organs of the human body are composed of only **four basic tissue types**: epithelial, connective, muscular, and nervous tissues. Each tissue is an assemblage of similarly specialized cells united in performing a specific function. The basic tissues, each containing extracellular matrix (ECM) as well as cells, associate with one another in the variable proportions and morphologies characteristic of each organ. The main features of the basic tissue types are summarized in Table 4–1.

Connective tissue is characterized by cells producing very abundant ECM; muscle tissue is composed of elongated cells specialized for contraction and movement; and nervous tissue is composed of cells with long, fine processes specialized to receive, generate, and transmit nerve impulses. Most organs can be divided into the **parenchyma**, which is composed of the cells responsible for the organ's specialized functions, and the **stroma**, the cells of which have a supporting role in

the organ. Except in the brain and spinal cord, the stroma is always connective tissue.

Epithelial tissues are composed of closely aggregated polyhedral cells adhering strongly to one another and to a thin layer of ECM, forming cellular sheets that line the cavities of organs and cover the body surface. Epithelia (Gr. *epi*, upon + *thele*, nipple) line all external and internal surfaces of the body and all substances that enter or leave an organ must cross this type of tissue.

The principal functions of epithelial tissues include the following:

- Covering, lining, and protecting surfaces (eg, epidermis)
- Absorption (eg, the intestinal lining)
- Secretion (eg, parenchymal cells of glands)

Specific cells of certain epithelia may be contractile (myoepithelial cells) or specialized sensory cells, such as those of taste buds or the olfactory epithelium.

TABLE 4–1 Main characteristics of the four basic types of tissues.

| Tissue | Cells | Extracellular Matrix | Main Functions |
|------------|---|----------------------|---|
| Epithelial | Aggregated polyhedral cells | Small amount | Lining of surface or body cavities; glandular secretion |
| Connective | Several types of fixed and wandering cells | Abundant amount | Support and protection of tissues/organs |
| Muscle | Elongated contractile cells | Moderate amount | Strong contraction; body movements |
| Nervous | Elongated cells with extremely fine processes | Very small amount | Transmission of nerve impulses |

CHARACTERISTIC FEATURES OF EPITHELIAL CELLS

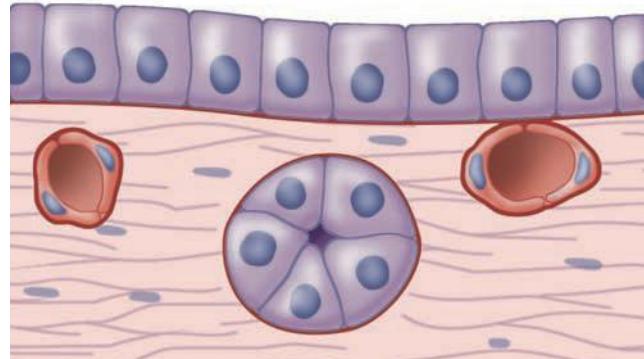
The shapes and dimensions of epithelial cells are quite variable, ranging from tall **columnar** to **cuboidal** to low **squamous** cells. The cells' size and morphology are generally dictated by their function. Epithelial cell nuclei vary in shape and may be elliptic (oval), spherical, or flattened, with nuclear shape corresponding roughly to cell shape. Columnar cells generally have elongated nuclei, squamous cells have flattened nuclei, and cuboidal or pyramidal cells have more spherical nuclei (Figure 4–1).

Because the lipid-rich membranes of epithelial cells are frequently indistinguishable by light microscopy, the number and shape of their stained nuclei are important indicators of cell shape and density. The nuclei also allow one to determine the number of cell layers in an epithelium, a primary morphologic criterion for classifying epithelia.

Most epithelia are adjacent to connective tissue containing blood vessels from which the epithelial cells receive nutrients and O₂. Even thick epithelia do not themselves normally contain blood vessels. The connective tissue that underlies the epithelia lining the organs of the digestive, respiratory, and urinary systems is called the **lamina propria**. The area of contact between the two tissues may be increased by small evaginations called **papillae** (*L. papula*, nipple) projecting from the connective tissue into the epithelium. Papillae occur most frequently in epithelial tissues subject to friction, such as the covering of the skin or tongue.

Epithelial cells generally show **polarity**, with organelles and membrane proteins distributed unevenly within the cell. The region of the cell contacting the ECM and connective tissue is called the **basal pole** and the opposite end, usually

FIGURE 4–1 Epithelia and adjacent connective tissue.



Cuboidal or pyramidal cells of epithelia generally have spherical nuclei, while nuclei of squamous epithelial cells are flattened. An extracellular **basement membrane** (red) always lies at the interface of epithelial cells and connective tissue. Nutrients for epithelial cells must diffuse across the basement membrane. Nerve fibers normally penetrate this structure, but small blood capillaries (being epithelial themselves) normally never enter epithelia.

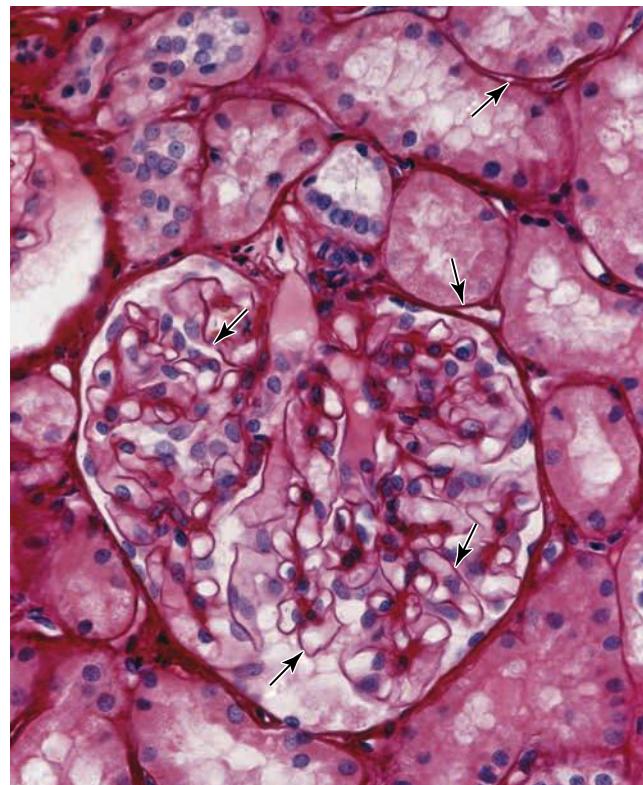
facing a space, is the **apical pole**, with the two poles differing significantly in both structure and function. Regions of cuboidal or columnar cells that adjoin neighboring cells comprise the cells' **lateral surfaces**; cell membranes here often have numerous folds which increase the area and functional capacity of that surface.

Basement Membranes

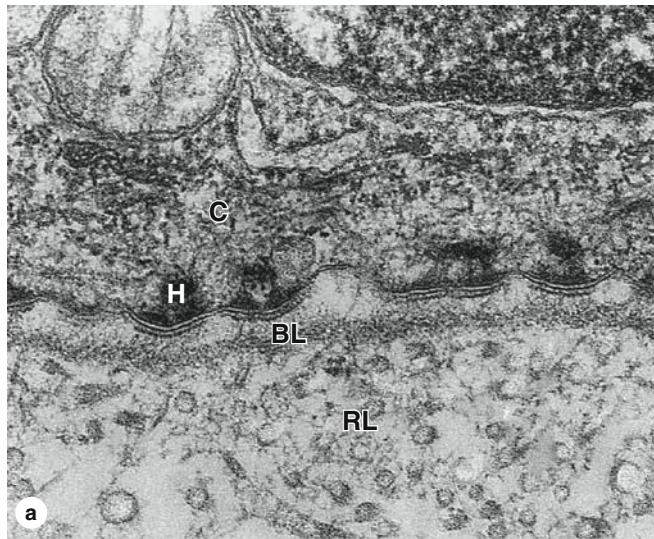
The basal surface of all epithelia rests on a thin extracellular, felt-like sheet of macromolecules referred to as the **basement membrane** (Figure 4–1), a semipermeable filter for substances reaching epithelial cells from below. Glycoproteins and other components in this structure can often be stained and visualized with the light microscope (Figure 4–2).

With the transmission electron microscope (TEM) two parts of the basement membrane may be resolved. Nearest the epithelial cells is an electron-dense layer, 20–100 nm thick, consisting of a network of fine fibrils that comprise the **basal lamina** and beneath this layer is a more diffuse and fibrous **reticular lamina** (Figure 4–3a). The terms

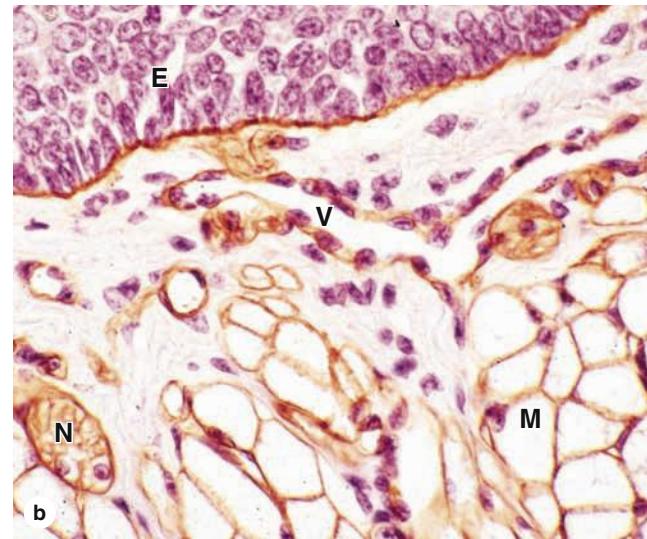
FIGURE 4–2 Basement membranes.



This section of kidney shows the well-stained basement membranes (arrows) of epithelia forming structures within the large, round renal glomerulus and its surrounding tubules. In kidney glomeruli the basement membrane, besides having a supporting function, has a highly developed role as a filter that is key to renal function. (X100; Picosirius-hematoxylin [PSH])

FIGURE 4–3 Basal and reticular laminae of basement membranes.

(a) The ultrastructural components of the basement membrane are revealed by TEM. The dense **basal lamina** (BL) may appear with thin clear zones on each side and is anchored to a thicker, more diffuse **reticular lamina** (RL) which contains collagen III reticular fibers. **Hemidesmosomes** (H) bind the basal surface of the epithelial cell (C) to the basal lamina. (X54,000)



(b) **Laminin**, a major glycoprotein within basal laminae, is shown here by immunohistochemistry and identifies the basement membranes of the stratified epithelium (E) and the simple epithelium lining a small blood vessel (V). Laminin also occurs in the **external laminae** surrounding nerves (N) and muscle (M) fibers, seen here in cross-section. (X200)

“basement membrane” and “basal lamina” are sometimes used interchangeably, but “basal lamina” usually denotes the fine extracellular layer seen ultrastructurally and “basement membrane” the entire structure beneath the epithelial cells visible with the light microscope.

The macromolecules of the basal lamina are secreted from the basal sides of the epithelial cells and form a sheet-like array. ECM components are described more fully in Chapter 5, but those of the basal lamina characteristically include the following:

- **Type IV collagen:** Monomers of type IV collagen self-assemble into a two-dimensional network of evenly spaced subunits.
- **Laminin:** These are large glycoproteins that attach to transmembrane proteins called **integrins** at the cells’ basal surface and project through the network of type IV collagen.
- **Nidogen and perlecan:** Respectively a short, rod-like protein and a proteoglycan, both of these cross-link laminin to the collagen network and help determine the porosity of the basal lamina and the size of molecules able to filter through it.

Basal laminae often called *external laminae* but with similar composition also exist as sleeves surrounding muscle cells, nerves (Figure 4-3b), and fat-storing cells, where they serve as semipermeable barriers regulating macromolecular exchange between the enclosed cells and connective tissue.

The more diffuse meshwork of the reticular lamina contains **type III collagen** and is bound to the basal lamina by

anchoring fibrils of **type VII collagen**, both of which are produced by cells of the connective tissue (Figure 4-3).

Besides acting as filters, functions of basement membranes include helping to provide structural support for epithelial cells and attach epithelia to underlying connective tissue. Basal lamina components help organize integrins and other proteins in the plasma membrane of epithelial cells, maintaining cell polarity and helping to localize endocytosis, signal transduction, and other activities. Basement membrane proteins also mediate many cell-to-cell interactions involving epithelia and mark routes for certain cell migrations along epithelia. Finally, the basement membrane also serves as a scaffold that allows rapid epithelial repair and regeneration.

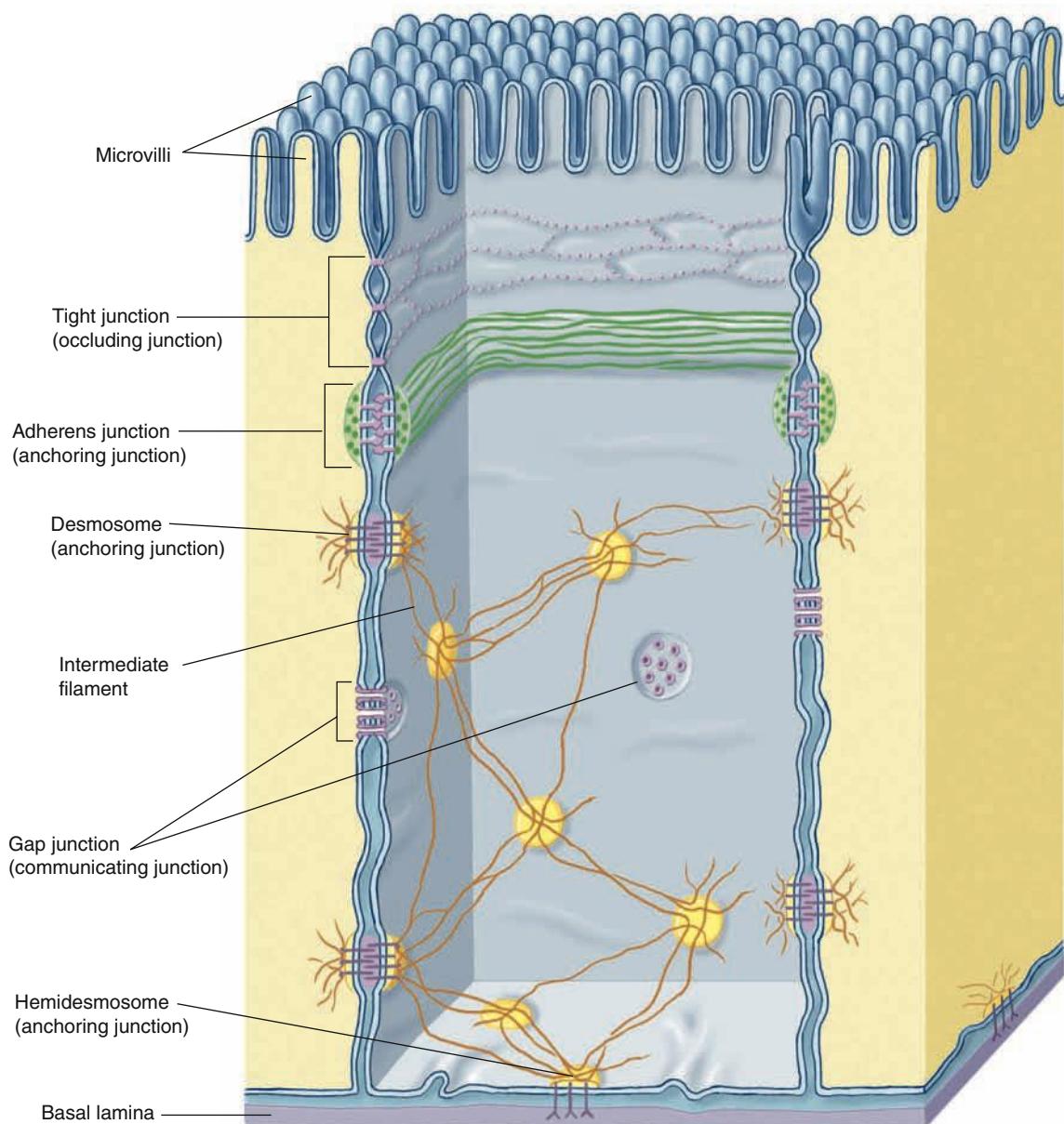
Intercellular Adhesion & Other Junctions

Several membrane-associated structures provide adhesion and communication between cells. Some are present in other tissues but all are particularly numerous and prominent in epithelia. Epithelial cells adhere strongly to neighboring cells and basal laminae, particularly in epithelia subject to friction or other mechanical forces.

As shown in Figure 4-4 and summarized in Table 4-2, lateral surfaces of epithelial cells have complexes of several specialized intercellular junctions with different functions:

- **Tight or occluding junctions** form a seal between adjacent cells.

FIGURE 4–4 Junctional complexes of epithelial cells.



Most cuboidal or columnar epithelial cells have intercellular junctional complexes with the different types of junctions shown schematically here. At the apical end, **tight junctions** (zonulae occludens) and **adherent junctions** (zonulae adherens) are typically close together and each forms a continuous band around the cell. Multiple ridges of the tight junction prevent passive flow of material between the cells but are not very strong; the adhering junctions immediately below them serve to stabilize and strengthen the circular occluding bands and help hold the cells together.

Both **desmosomes** and **gap junctions** are spot-like, not circular, structures between two cells. Bound to intermediate filaments inside the cells, desmosomes form very strong attachment points that supplement the zonulae adherens and play a major role to maintain the integrity of an epithelium. Gap junctions, each a patch of many **connexons** in the adjacent cell membranes, have little strength but serve as intercellular channels for flow of molecules. All of these junctional types are also found in certain other cell types besides epithelia. **Hemidesmosomes** bind epithelial cells to the underlying basal lamina.

TABLE 4–2**Epithelial cell junctions, their major structural features and functions, and medical significance.**

| Junction | Tight Junction (Zonula Occludens) | Adherent Junction (Zonula Adherens) | Desmosome (Macula Adherens) | Hemidesmosome | Gap Junction (Nexus) |
|-----------------------------------|---|--|--|---|---|
| Major transmembrane link proteins | Occludins, claudins, ZO proteins | E-cadherin, catenin complexes | Cadherin family proteins (desmogleins, desmocollin) | Integrins | Connexin |
| Cytoskeletal components | Actin filaments | Actin filaments | Intermediate filaments (keratins) | Intermediate filaments | None |
| Major functions | Seals adjacent cells to one another, controlling passage of molecules between them; separates apical and basolateral membrane domains | Provides points linking the cytoskeletons of adjacent cells; strengthens and stabilizes nearby tight junctions | Provides points of strong intermediate filament coupling between adjacent cells, strengthening the tissue | Anchors cytoskeleton to the basal lamina | Allows direct transfer of small molecules and ions from one cell to another |
| Medical significance | Defects in occludins may compromise the fetal blood-brain barrier, leading to severe neurologic disorders | Loss of E-cadherin in epithelial cell tumors (carcinomas) promotes tumor invasion and the shift to malignancy | Autoimmunity against desmoglein I leads to dyshesive skin disorders characterized by reduced cohesion of epidermal cells | Mutations in the integrin- $\beta 4$ gene are linked to some types of epidermolysis bullosa, a skin blistering disorder | Mutations in various connexin genes have been linked to certain types of deafness and peripheral neuropathy |

- **Adherent or anchoring junctions** are sites of strong cell adhesion.
- **Gap junctions** are channels for communication between adjacent cells.

In many epithelia these junctions are present in a definite order at the apical end of the cells. **Tight junctions**, also called zonulae occludens, are the most apical of the junctions. The term “zonula” indicates that the junction forms a band completely encircling each cell. In TEM the adjacent membranes at these junctions appear fused or very tightly apposed (Figures 4–5). The seal between the two cell membranes is due to tight interactions between the transmembrane proteins **claudin** and **occludin**. Tight junctions are clearly seen after cryofracture of epithelia (Figure 4–6), where they appear as a band of branching strands in the membrane around each cell’s apical end. The intercellular seal of tight junctions ensures that molecules crossing an epithelium in either direction do so by going *through* the cells (a transcellular path) rather than *between* them (the paracellular pathway). Epithelia with one or very few fused sealing strands (eg, proximal renal tubule) are more permeable to water and solutes than are epithelia with many fused strands (eg, the lining of the urinary bladder).

Epithelial tight junctions also serve a related purpose: these continuous zones within cell membranes serve as fences restricting movements of membrane lipids and proteins at the apical cell surface into the lateral and basal surfaces, and vice versa. The tight junctions thus maintain two distinct membrane domains (apical and basolateral) with different sets of

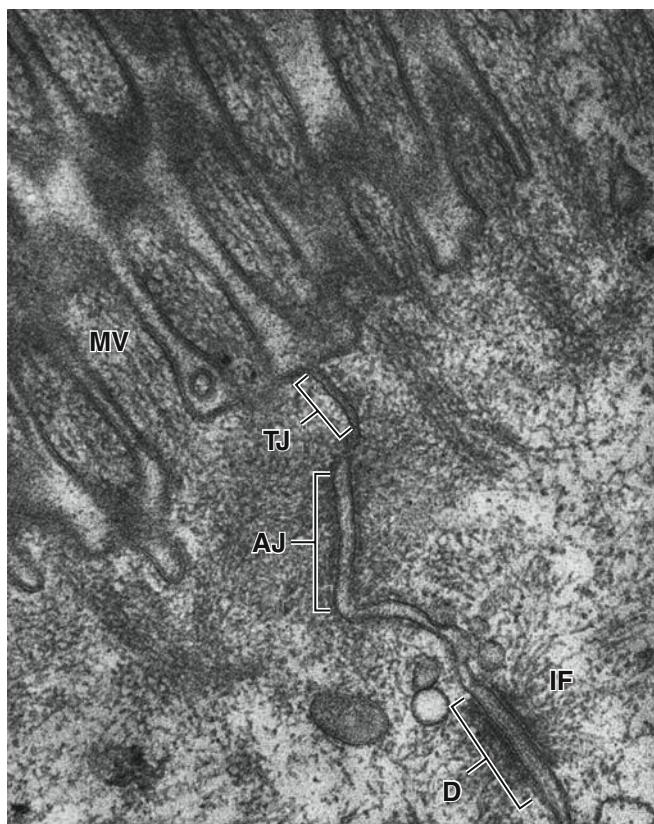
components, which allows these two sides of the epithelium to display different receptors and other proteins and to function differently. Apical cell membranes of epithelia are part of the luminal compartment of a tissue or organ, while the basolateral domains are part of a basal compartment that also encompasses the underlying connective tissue.

► MEDICAL APPLICATION

Proteins of **tight junctions** provide the targets for certain common bacteria of medical importance. The enterotoxin secreted by *Clostridium perfringens*, which causes “food poisoning,” binds claudin molecules of intestinal cells, prevents insertion of these proteins during maintenance of tight junctions, and causes loss of tissue fluid into the intestinal lumen via the paracellular pathway.

Similarly, *Helicobacter pylori*, which is important in the etiology of **gastric ulcers**, binds the extracellular domains of tight-junction proteins in cells of the stomach and inserts a protein into these cells, which targets ZO-1 and disrupts signaling from the junction.

The second type of junction is the **adherens junction** or zonula adherens (Figures 4–4 and 4–5), which also encircles the epithelial cell, usually immediately below the tight junction. This is an adherent junction, firmly anchoring a cell to its neighbors. Cell adhesion is mediated by **cadherins**, transmembrane glycoproteins of each cell that bind each other in

FIGURE 4–5 Epithelial cell junctional complex.

Ultrastructural view of the apical region near microvilli (**MV**) of two epithelial cells, revealing a junctional complex with a tight junction (**TJ**) or zonula occludens, an adherent junction (**AJ**) or zonula adherens, and a desmosome (**D**) associated with intermediate filaments (**IF**). The functions and major protein components of these junction types are summarized in Table 4–2. (X195,000)

the presence of Ca^{2+} . At their cytoplasmic ends, cadherins bind **catenins** that link to actin filaments with actin-binding proteins. The actin filaments linked to the adherens junctions form part of the “terminal web,” a cytoskeletal feature at the apical pole in many epithelial cells. Together, the tight and adherent junctions encircling the apical ends of epithelial cells function like the plastic bands that hold a six-pack of canned drinks together.

Another anchoring junction is the **desmosome** (Gr. *desmos*, binding and *soma*, body) or macula adherens (L. *macula*, spot). As the name implies this junction resembles a single “spot-weld” and does not form a belt around the cell. Desmosomes are disc-shaped structures at the surface of one cell that are matched with identical structures at an adjacent cell surface (Figures 4–4 and 4–5). Desmosomes contain larger members of the cadherin family called desmogleins and desmocollins. The cytoplasmic ends of these clustered transmembrane proteins bind plakoglobins, catenin-like proteins which link to larger

FIGURE 4–6 View of a tight junction after cryofracture.

Just below the apical microvilli (**MV**) of this epithelial cell, a cryofracture plane splitting fused cell membranes reveals the fused strands of transmembrane proteins forming the tight junction. (zonula occludens; X100,000)

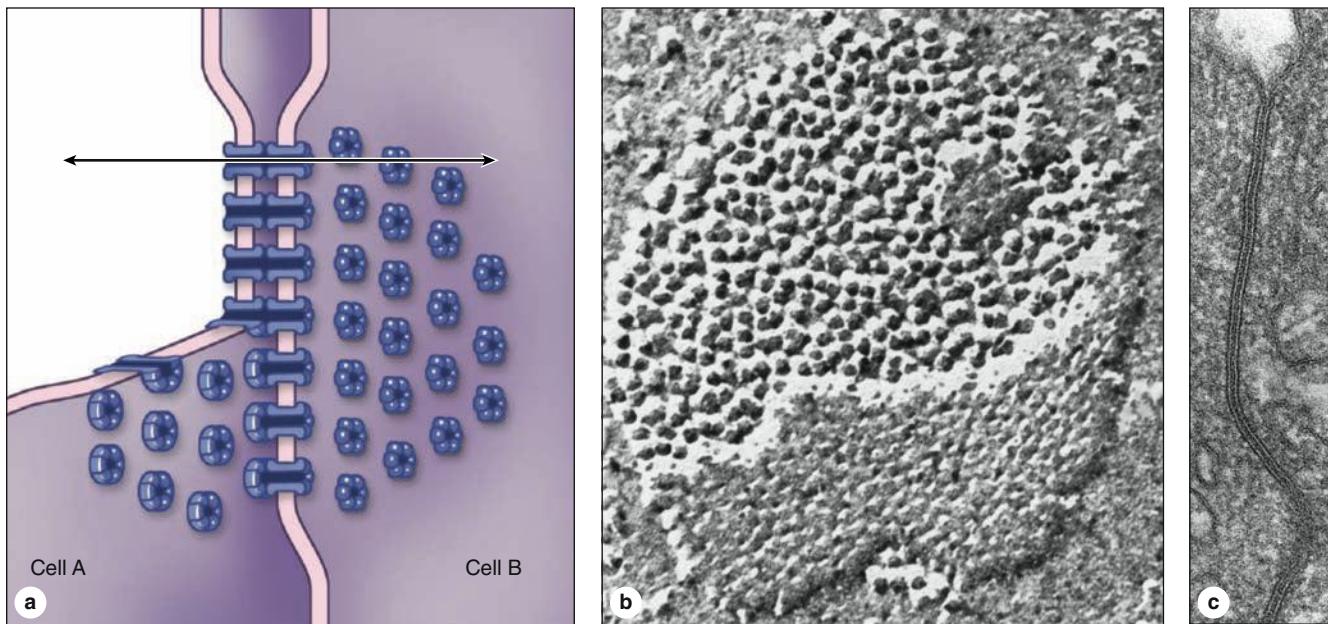
proteins called desmoplakins in an electron-dense plaque. Desmoplakins in turn bind intermediate filament proteins rather than actins. Epithelial desmosomes attach to cable-like filaments of cytokeratin, sometimes referred to tonofilaments. Such intermediate filaments are very strong and desmosomes provide firm cellular adhesion and strength throughout the epithelium.

» MEDICAL APPLICATION

Various **blistering (bullous) diseases**, such as pemphigus vulgaris, involving the epidermis or stratified squamous epithelia of the oral mucosa, are due to abnormal desmosome function caused by autoimmune reactions against specific desmogleins that reduce cell-to-cell adhesion. Similar disorders arise with genetic mutations for various junctional proteins.

Gap junctions, shown in Figure 4–7, mediate intercellular communication rather than adhesion or occlusion between cells. Abundant in many epithelia, gap junctions are also functionally important in nearly all mammalian tissues. Cryofracture preparations show that gap junctions consist of aggregated transmembrane protein complexes that form circular patches in the plasma membrane (Figure 4–7b).

The transmembrane gap junction proteins, **connexins**, form hexameric complexes called **connexons**, each of which has a central hydrophilic pore about 1.5 nm in diameter. When two cells attach, connexins in the adjacent cell membranes move laterally and align to produce connexons between the two cells (Figures 4–4 and 4–7a), with each junction having dozens or hundreds of aligned connexon pairs. Gap junctions permit

FIGURE 4–7 Gap junctions.

(a) A diagram of a gap junction shows the structural elements that allow the exchange of nutrients and signal molecules between cells without loss of material into the intercellular space. The communicating channels are formed by pairs of abutting particles (**connexons**), which are in turn each composed of six protein subunits (connexins) that span the lipid bilayer of each cell membrane. The channel formed by paired connexons (arrow) is about 1.5 nm in diameter, limiting the size of transmitted molecules.

(b) A cryofracture preparation of a gap junction, showing the patch of aggregated transmembrane protein complexes, the connexons. (X150,000) **(c)** A section perpendicular to a gap junction between two cells shows that their cell membranes are very closely apposed, separated only by a 2-nm-wide electron-dense space. Individual connexons are not resolved in sections prepared for TEM. (X150,000)

intercellular exchange of molecules with small (< 1.5 nm) diameters. Some molecules mediating signal transduction, such as cyclic nucleotides and ions, move rapidly through gap junctions, allowing cells in many tissues to act in a coordinated manner rather than as independent units. For example, in heart and visceral muscles gap junctions help produce rhythmic contractions.

On the basal epithelial surface, cells (Figure 4–4) attach to the basal lamina by anchoring junctions called **hemidesmosomes** (Gr. *hemi*, half + *desmos* + soma), which can be seen by TEM (Figure 4–3). These adhesive structures resemble a half-desmosome ultrastructurally, but unlike desmosomes the clustered transmembrane proteins that indirectly link to cyto-keratin intermediate filaments are integrins rather than cadherins. The integrins of hemidesmosomes bind primarily to laminin molecules in the basal lamina.

Another basal anchoring junction found in cells that are moving during epithelial repair or reorganization is the **focal adhesion**, or focal contact. Although resembling hemidesmosomes superficially, focal adhesions are smaller, more numerous, and consist of integrins linked indirectly to bundled actin filaments, not intermediate filaments. Importantly, integrins of focal adhesions are also linked via paxillin to **focal adhesion kinase**, a signaling protein which upon integrin binding to laminin or other specific ECM proteins initiates a cascade

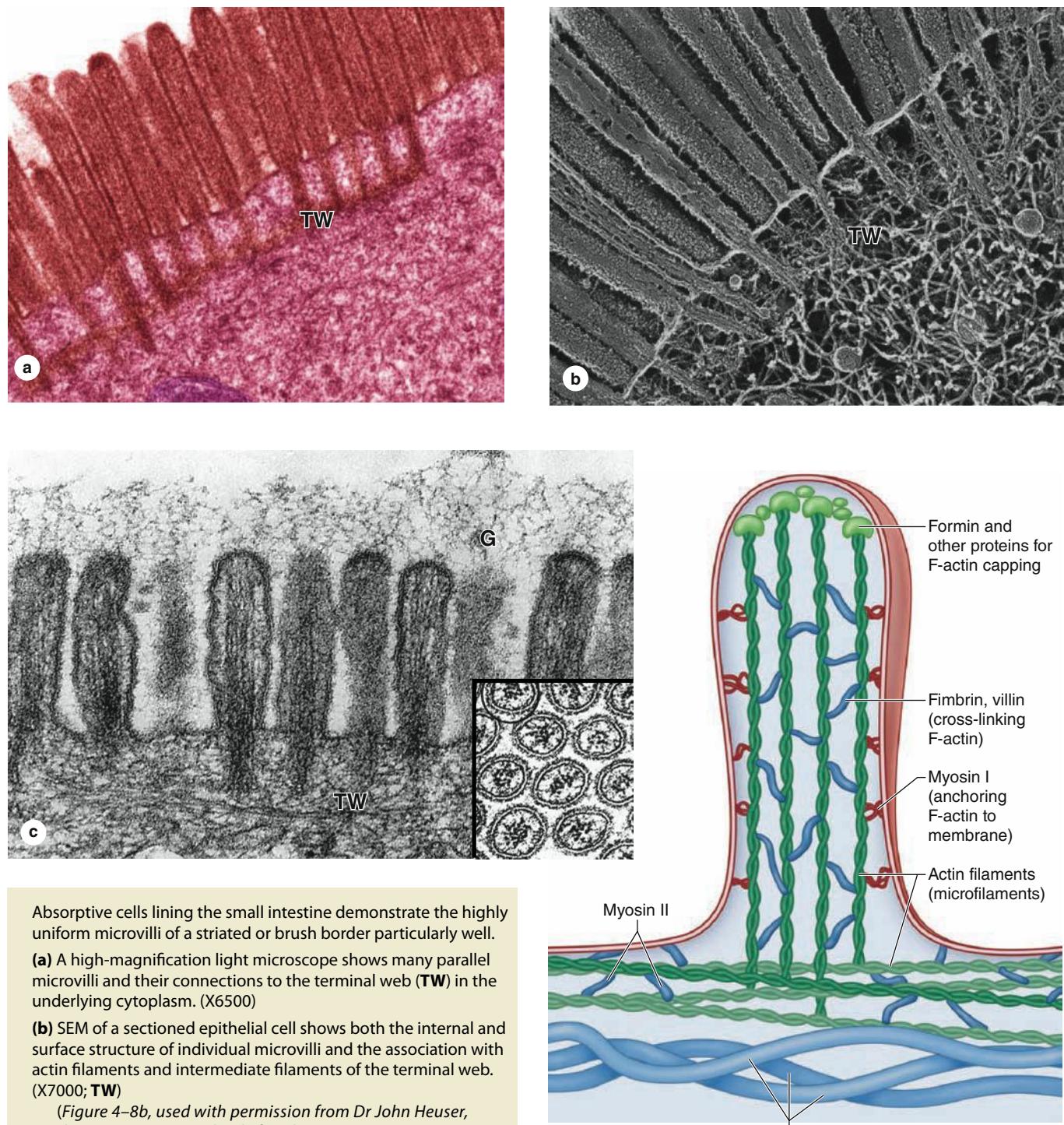
of intracellular protein phosphorylation affecting cell adhesion, mobility, and gene expression. Focal adhesions are also important in migrating nonepithelial cells such as fibroblasts.

➤ SPECIALIZATIONS OF THE APICAL CELL SURFACE

The apical ends of many columnar and cuboidal epithelial cells have specialized structures projecting from the cells. These function either to increase the apical surface area for better absorption or to move substances along the epithelial surface.

Microvilli

Many cells have cytoplasmic projections best seen with the electron microscope. Such extensions usually reflect the movements and activity of actin filaments and are both temporary and variable in their length, shape, and number. However, in epithelia specialized for absorption the apical cell surfaces are often filled with an array of projecting **microvilli** (L. *villus*, tuft), usually of uniform length. In cells such as those lining the small intestine, densely packed microvilli are visible as a **brush** or **striated border** projecting into the lumen (Figure 4–8). The average microvillus is about 1-μm long and 0.1-μm wide, but

FIGURE 4–8 Microvilli.

capping, cross-linking, and movement. Like microfilaments in other regions of the cytoskeleton, those of microvilli are highly dynamic, with treadmilling and various myosin-based interactions. Myosin motors import various microvilli components along the actin filaments.

with hundreds or thousands present on the end of each absorptive cell, the total surface area can be increased by 20- or 30-fold. The thick glycocalyx covering microvilli of the intestinal brush border includes membrane-bound proteins and enzymes for digestion of certain macromolecules.

Each microvillus contains bundled actin filaments capped and bound to the surrounding plasma membrane by actin-binding proteins (Figure 4–8d). Although microvilli are relatively stable, the microfilament arrays are dynamic and undergo various myosin-based movements, which help maintain optimal conditions for absorption via numerous channels, receptors, and other proteins in the plasmalemma. The actin filaments insert into the **terminal web** of cortical microfilaments at the base of the microvilli.

» MEDICAL APPLICATION

Celiac disease, also called **gluten-sensitive enteropathy** or **sprue**, is a disorder of the small intestine in which one of the first pathologic changes is loss of the microvilli brush border of the absorptive cells. This is caused by an immune reaction against the wheat protein gluten during its digestion, which produces diffuse enteritis (intestinal inflammation), changes to the epithelial cells leading to malabsorption, and eventually to pathologic changes in the intestinal wall. The malabsorption problems and structural changes are reversible when gluten is removed from the diet.

Stereocilia

Stereocilia are a much less common type of apical process, best seen on the absorptive epithelial cells lining the male reproductive system (Figure 4–9). Like microvilli, stereocilia increase the cells' surface area, facilitating absorption. More specialized stereocilia with a motion-detecting function are important components of inner ear sensory cells.

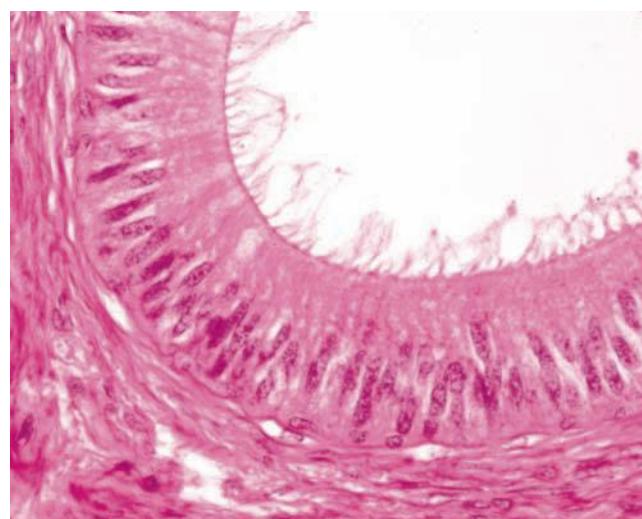
Stereocilia resemble microvilli in containing arrays of microfilaments and actin-binding proteins, with similar diameters, and with similar connections to the cell's terminal web. However, stereocilia are typically much longer and less motile than microvilli, and may show branching distally.

Cilia

Cilia are long, highly motile apical structures, larger than microvilli, containing internal arrays of microtubules not microfilaments (Figure 4–10). In addition to cilia on epithelial cells, most (if not all) other cell types have at least one short projection called a *primary cilium*, which is not motile but is enriched with receptors and signal transduction complexes for detection of light, odors, motion, and flow of liquid past the cells.

Motile cilia are abundant on cuboidal or columnar cells of many epithelia. Typical cilia are 5–10 µm long and 0.2 µm in diameter, which is much longer and two times wider than

FIGURE 4–9 Stereocilia.

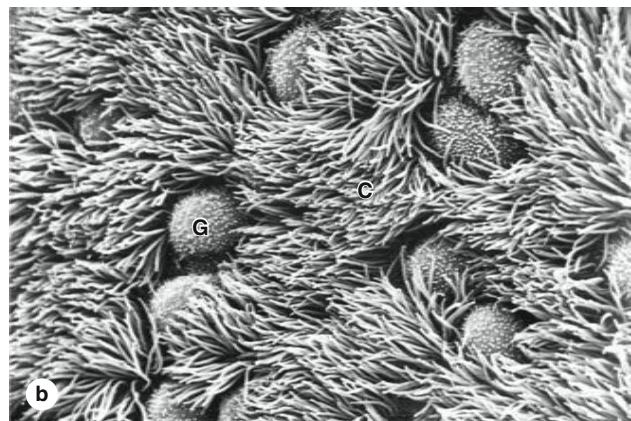
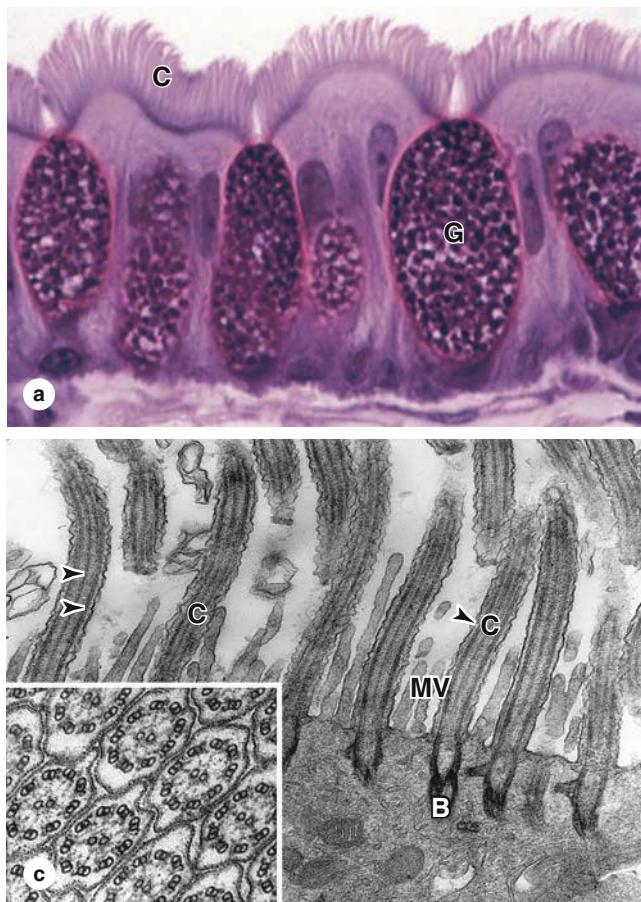


At the apical ends of the tall epithelial cells lining organs such as the epididymis (shown here) are numerous very long stereocilia, which increase the surface area available for absorption. Stereocilia are much longer than microvilli and often have distal branching. (X400; H&E)

a typical microvillus. As shown in Figure 4–11, each cilium has a core structure consisting of nine peripheral microtubule doublets (in which a few tubulin protofilaments are shared) arrayed around two central microtubules. This **9 + 2 assembly** of microtubules is called an **axoneme** (Gr. *axon*, axis + *nema*, thread). As with other microtubules, kinesin and cytoplasmic dynein motors move along the peripheral microtubules for the transport of molecular components into and out of these structures.

Microtubules of axonemes are continuous with those in **basal bodies**, which are apical cytoplasmic structures just below the cell membrane (Figures 4–10 and 4–11). Basal bodies have a structure similar to that of centrioles, with triplets of microtubules and dynamic tubulin protofilaments forming rootlets anchoring the entire structure to the cytoskeleton.

Cilia exhibit rapid beating patterns that move a current of fluid and suspended matter in one direction along the epithelium. Ciliary motion occurs through successive changes in the conformation of the axoneme, in which various accessory proteins make each cilium relatively stiff, but elastic. Complexes with **axonemal dynein** bound to one microtubule in each doublet extend as “arms” toward a microtubule of the next doublet. With energy from ATP dynein-powered sliding of adjacent doublets relative to each other bends the axoneme and a rapid series of these sliding movements produces the beating motion of the cilium. The long flagellum that extends from each fully differentiated sperm cell has an axonemal structure like that of a cilium and moves with a similar mechanism.

FIGURE 4–10 Cilia.

Epithelial cells lining the respiratory tract have many very well-developed cilia.

(a) By light microscopy cilia (**C**) on the columnar cells appear as a wave of long projections, interrupted by nonciliated, mucus-secreting goblet cells (**G**). (X400; Toluidine blue)

(b) SEM of the apical surfaces of this epithelium shows the density of the cilia (**C**) and the scattered goblet cells (**G**). (X600)

(c) TEM of cilia (**C**) sectioned longitudinally reveals central and peripheral microtubules (arrowheads) of the axonemes, with cross sections (inset) clearly showing the $9 + 2$ array of the microtubule doublets. At the base of each cilium is a basal body (**B**) anchoring the axoneme to the apical cytoplasm. Much shorter microvilli (**MV**) can be seen between the cilia. (X59,000; Inset: X80,000)

» MEDICAL APPLICATION

Several mutations have been described in the proteins of the cilia and flagella. They are responsible for the **immotile cilia syndrome** (Kartagener syndrome), whose symptoms are chronic respiratory infections caused by the lack of the cleansing action of cilia in the respiratory tract and immotile spermatozoa, causing male infertility.

» TYPES OF EPITHELIA

Epithelia can be divided into two main groups: **covering (or lining) epithelia** and **secretory (glandular) epithelia**. This is an arbitrary functional division for there are lining epithelia in which all the cells also secrete (eg, the lining of the stomach) or in which glandular cells are distributed among the lining cells (eg, mucous cells in the small intestine or trachea).

Covering or Lining Epithelia

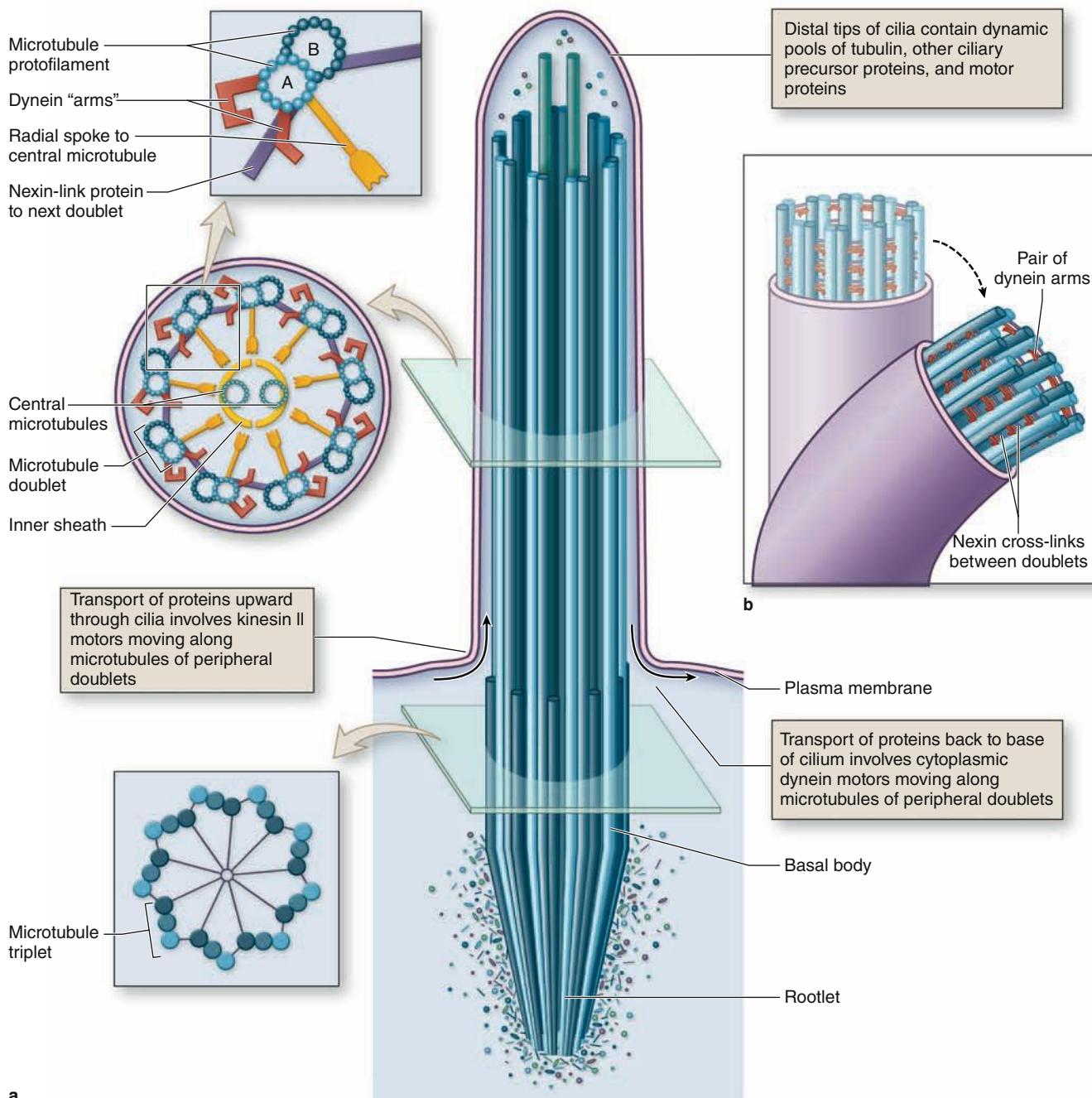
Cells of covering epithelia are organized into one or more layers that cover the surface or line the cavities of an organ. As summarized in Table 4–3, such epithelia are classified

according to the number of cell layers and the cell morphology in the outer layer. **Simple epithelia** contain one cell layer and **stratified epithelia** contain two or more layers.

Based on cell shape, simple epithelia are further classified as **squamous** (thin cells), **cuboidal** (cell width and thickness roughly similar) or **columnar** (cells taller than they are wide). Examples of these epithelial types are shown in Figures 4–12 through 4–14.

Most stratified epithelia (Figure 4–15) are classified according to the cell shape of the superficial outer layer(s): **squamous**, **cuboidal**, or **columnar**.

The very thin surface cells of stratified squamous epithelia can be “keratinized” (packed with keratin filaments) or “nonkeratinized” (with relatively sparse keratin). **Stratified squamous keratinized epithelium** is found mainly in the epidermis of skin, where it helps prevent dehydration from the tissue (Figure 4–15a). Its cells form many layers, with the less differentiated cuboidal cells near the basement membrane. These cells have many desmosomes and become more irregular in shape and then flatten as they accumulate keratin in the process of **keratinization** and are moved progressively toward the skin surface, where they become thin, metabolically inactive packets (squames) of keratin lacking nuclei. As discussed

FIGURE 4–11 Ciliary axoneme.

(a) A diagram of a cilium with the **axoneme** consisting of **two central microtubules** surrounded by **nine peripheral microtubular doublets** associated with other proteins. In the doublets, microtubule A is complete, consisting of 13 protofilaments, whereas microtubule B shares some of A's protofilament heterodimers. The axoneme is elastic but relatively stiff, with its structure maintained by nexins linking the peripheral doublets and other protein complexes forming a sheath and radial spokes between the doublets and the central microtubules.

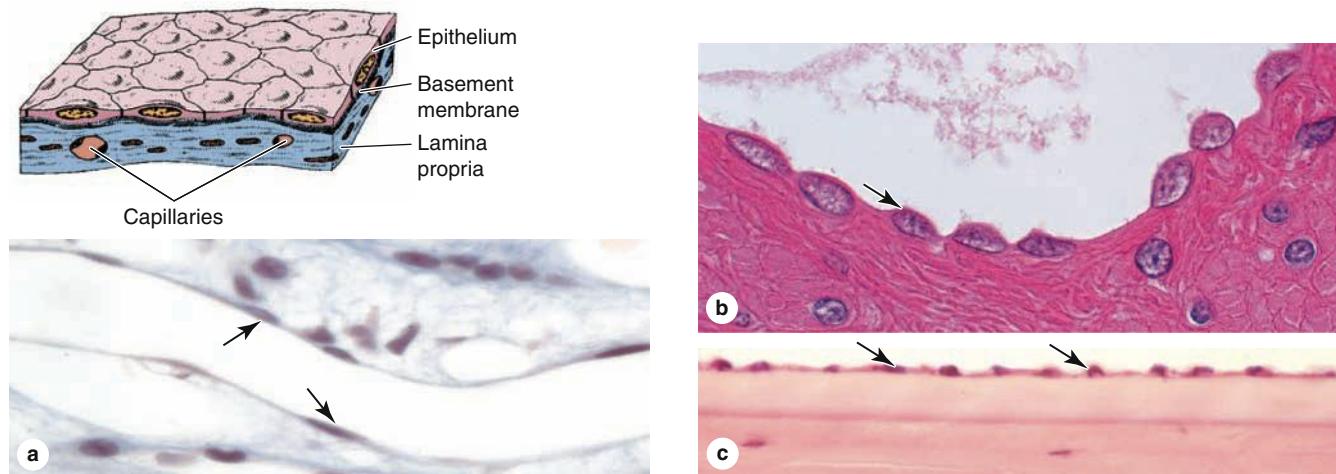
The axoneme is continuous with a **basal body** located in the apical cytoplasm. Basal bodies are structurally very similar to centrioles, consisting of nine relatively short **microtubular triplets** linked together in a pinwheel-like arrangement. A dynamic pool of tubulin

and other proteins exists distally in cilia, and proteins are transported into and out of the structure by **kinesin** and **cytoplasmic dynein** motors moving along the peripheral doublets of microtubules.

(b) Ciliary movement involves a rapid series of changes in the shape of the axoneme. Along the length of each doublet, a series of paired "arms" with **axonemal dynein** is bound to microtubule A, with each pair extended toward microtubule B of the next doublet. When activated by ATP, the dynein arms briefly bind the neighboring microtubule and the doublets slide past each other slightly. The sliding motion is restricted by nexin cross-links between the doublets, causing the axoneme to bend. A rapid succession of this movement along the axoneme produces ciliary motion.

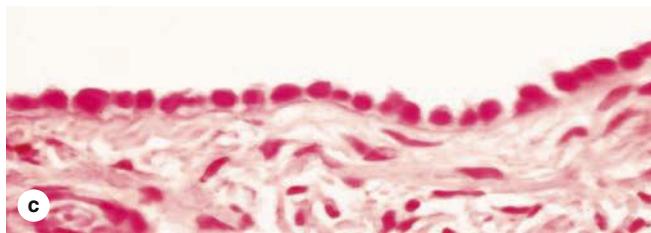
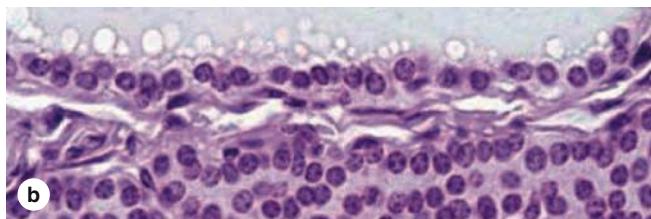
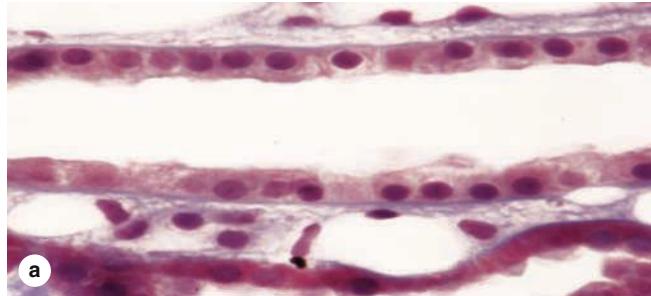
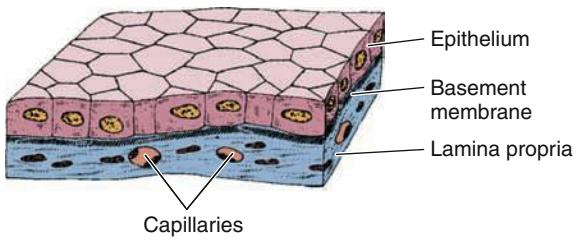
TABLE 4–3 Common types of covering epithelia.

| Major Feature | Cell Form | Examples of Distribution | Main Function |
|--|---------------------------------|---|--|
| Simple (one layer of cells) | Squamous | Lining of vessels (endothelium); Serous lining of cavities: pericardium, pleura, peritoneum (mesothelium) | Facilitates the movement of the viscera (mesothelium), active transport by pinocytosis (mesothelium and endothelium), secretion of biologically active molecules (mesothelium) |
| | Cuboidal | Covering the ovary, thyroid | Covering, secretion |
| | Columnar | Lining of intestine, gallbladder | Protection, lubrication, absorption, secretion |
| Stratified (two or more layers of cells) | Squamous keratinized (dry) | Epidermis | Protection; prevents water loss |
| | Squamous nonkeratinized (moist) | Mouth, esophagus, larynx, vagina, anal canal | Protection, secretion; prevents water loss |
| | Cuboidal | Sweat glands, developing ovarian follicles | Protection, secretion |
| | Transitional | Bladder, ureters, renal calyces | Protection, distensibility |
| | Columnar | Conjunctiva | Protection |
| Pseudostratified (layers of cells with nuclei at different levels; not all cells reach surface but all adhere to basal lamina) | | Lining of trachea, bronchi, nasal cavity | Protection, secretion; cilia-mediated transport of particles trapped in mucus out of the air passages |

FIGURE 4–12 Simple squamous epithelium.

This is a single layer of thin cells, in which the **cell nuclei** (arrows) are the thickest and most visible structures. Simple epithelia are typically specialized as lining of vessels and cavities, where they regulate passage of substances into the underlying tissue. The

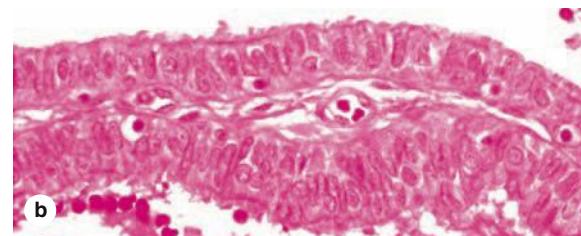
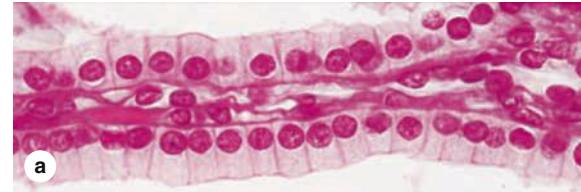
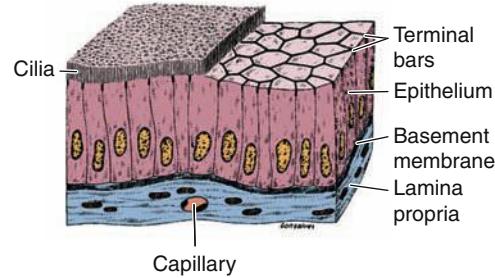
thin cells often exhibit transcytosis. Examples shown here are those lining the thin renal loops of Henle (**a**), covering the outer wall of the intestine (**b**), and lining the inner surface of the cornea (**c**). (a, c X400; b X600; H&E)

FIGURE 4–13 Simple cuboidal epithelium.

Cells here are roughly as tall as they are wide. Their greater thickness allows cytoplasm to be rich in mitochondria and other organelles for a high level of active transport across the epithelium and other functions. Examples shown here are from a renal collecting tubule (**a**), a large thyroid follicle (**b**), and the thick mesothelium covering an ovary (**c**). (All X400; H&E)

with skin, this surface layer of cells helps protect against water loss across this epithelium. **Stratified squamous nonkeratinized epithelium** (Figure 4–15b) lines moist internal cavities (eg, mouth, esophagus, and vagina) where water loss is not a problem. Here the flattened cells of the surface layer retain their nuclei and most metabolic functions.

Stratified cuboidal and **stratified columnar epithelia** are both relatively rare. Stratified cuboidal epithelium occurs in the excretory ducts of salivary and sweat glands (Figure 4–15d). Stratified columnar epithelium is seen in the conjunctiva lining the eyelids, where it is both protective and mucus secreting.

FIGURE 4–14 Simple columnar epithelium.

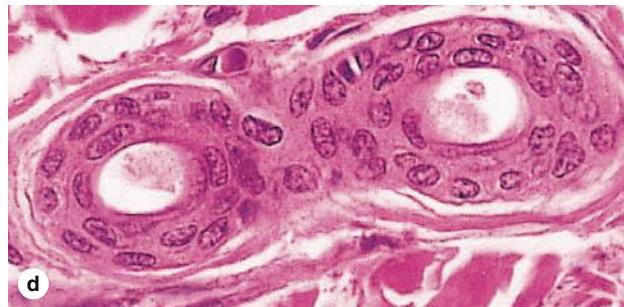
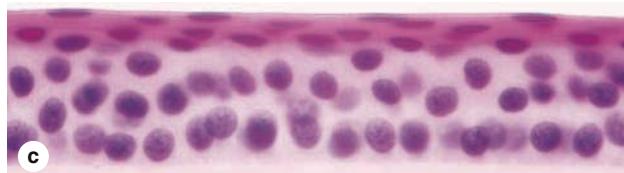
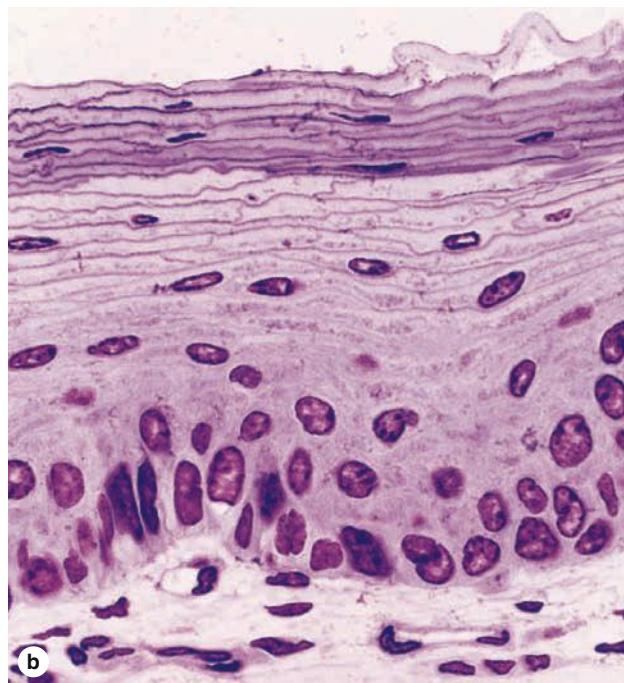
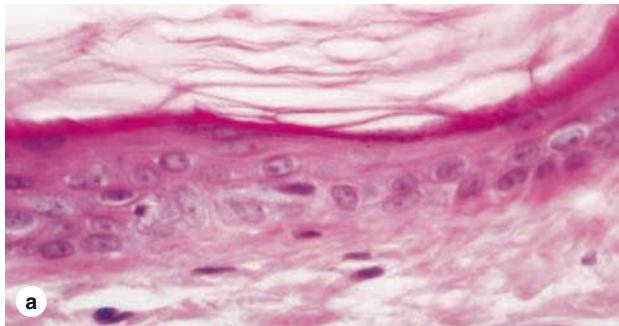
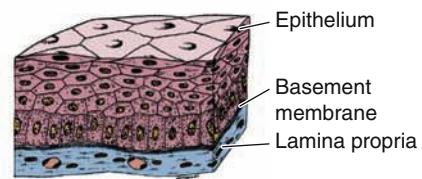
Cells here are always taller than they are wide, with apical cilia or microvilli, and are often specialized for absorption. Complexes of tight and adherent junctions, sometimes called "terminal bars" in light microscopic images, are present at the apical ends of cells. The examples shown here are from a renal collecting duct (**a**), the oviduct lining, with both secretory and ciliated cells (**b**), and the lining of the gall bladder (**c**). (All X400; H&E)

Unique **transitional epithelium** or **urothelium** lines much of the urinary tract, extending from the kidneys to the proximal part of the urethra, and is characterized by a superficial layer of large, dome-like cells sometimes called umbrella cells (Figure 4–16). As discussed further with the urinary system, these cells are specialized to protect underlying tissues from the hypertonic and potentially cytotoxic effects of urine. Importantly, unique morphological features of the cells allow *distension* of transitional epithelium as the urinary bladder fills.

» MEDICAL APPLICATION

In individuals with chronic vitamin A deficiency, epithelial tissues of the type found in the bronchi and urinary bladder may gradually be replaced by stratified squamous epithelium.

FIGURE 4–15 Stratified epithelium.



Stratified squamous epithelia usually have protective functions: protection against easy invasion of underlying tissue by microorganisms and protection against water loss. These functions are particularly important in the epidermis (**a**) in which differentiating cells become **keratinized**, that is, filled with keratin and other substances, eventually lose their nuclei and organelles, and form superficial layers flattened squames that impede water loss. Keratinized cells are sloughed off and replaced by new cells from more basal layers, which are discussed fully with the skin in Chapter 18.

Nonkeratinized epithelia occur in many organs, such as the esophageal lining (**b**) or outer covering of the cornea (**c**). Here cells accumulate much less keratin and retain their nuclei but still provide protection against microorganisms.

Stratified cuboidal or columnar epithelia are fairly rare but occur in excretory ducts of certain glands, such as sweat glands (**d**) where the double layer of cells allows additional functions. All X400; (b) PT, (a, c, and d) H&E.

A final morphological type of epithelium is called **pseudostratified columnar epithelium** (Figure 4–17). Here tall, irregular cells all are attached to the basement membrane but their nuclei are at different levels and not all cells extend to the free surface, giving a stratified appearance. A good example of pseudostratified columnar epithelium is that lining the upper respiratory tract, where the cells are also heavily ciliated.

Secretory Epithelia & Glands

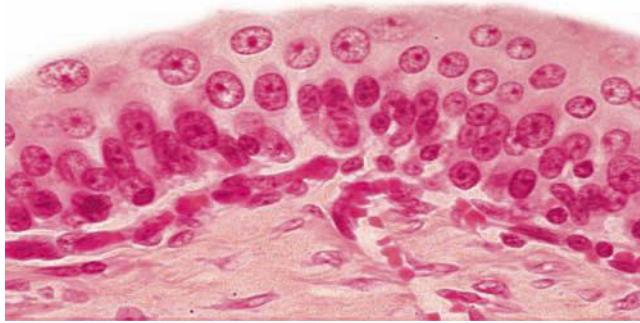
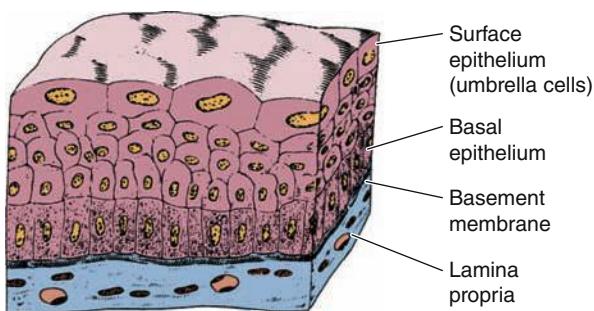
Epithelial cells that function mainly to produce and secrete various macromolecules may occur in epithelia with other major functions or comprise specialized organs called **glands**.

» MEDICAL APPLICATION

In **chronic bronchitis**, common among habitual smokers, the number of goblet cells in the lining of airways in the lungs often increases greatly. This leads to excessive mucus production in areas where there are too few ciliated cells for its rapid removal and contributes to obstruction of the airways. The ciliated pseudostratified epithelium lining the bronchi of smokers can also be transformed into stratified squamous epithelium by metaplasia.

Secretory cells may synthesize, store, and release proteins (eg, in the pancreas), lipids (eg, adrenal, sebaceous glands), or complexes of carbohydrates and proteins (eg, salivary glands). Epithelia of mammary glands secrete all three substances. The

FIGURE 4–16 Transitional epithelium or urothelium.



Urothelium is stratified and lines much of the urinary tract. The superficial cells are rounded or dome-shaped, and have specialized membrane features enabling them to withstand the hypertonic effects of urine and protect underlying cells from this toxic solution. Cells of this epithelium are also able to adjust their relationships with one another and undergo a transition in their appearance as the urinary bladder fills and the wall is distended. These unique features of transitional epithelium are discussed more extensively in Chapter 19. (X400; H&E)

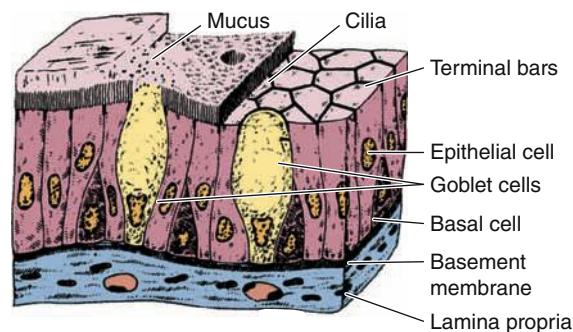
cells of some glands (eg, sweat glands) have little synthetic activity and secrete mostly water and electrolytes (ions) transferred from the blood.

Scattered secretory cells, sometimes called unicellular glands, are common in simple cuboidal, simple columnar, and pseudostratified epithelia. An important, easily seen example is the **goblet cell** abundant in the lining of the small intestine (Figure 4–18) and respiratory tract (Figure 4–17), which secretes lubricating mucus that aids the function of these organs.

Glands develop from covering epithelia in the fetus by cell proliferation and growth into the underlying connective tissue, followed by further differentiation (Figure 4–19).

Exocrine glands remain connected with the surface epithelium, the connection forming the tubular ducts lined with epithelium which deliver the secreted material where it is used. **Endocrine glands** lose the connection to their original epithelium and therefore lack ducts. Thin-walled blood vessels (capillaries) adjacent to endocrine cells absorb their

FIGURE 4–17 Pseudostratified epithelium.



Cells of pseudostratified epithelia appear to be in several layers, but their basal ends all rest on the basement membrane. The pseudostratified columnar epithelium of the upper respiratory tract shown here contains many ciliated cells, as well as other cells with their nuclei at different levels. (X400; H&E)

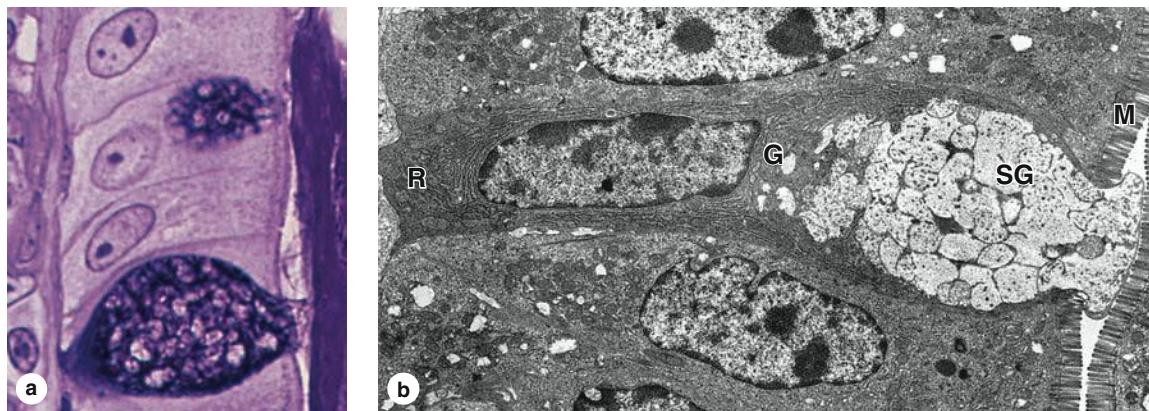
secreted hormone products for transport in blood to target cells throughout the body.

As shown in Figure 4–20, epithelia of exocrine glands are organized as a continuous system of many small **secretory portions** and **ducts** that transport the secretion out of the gland. In both exocrine and endocrine glands the secretory units are supported by a stroma of connective tissue. In larger glands layers of connective tissue also surround the larger ducts, form partitions or *septa* separating the gland into lobules, each containing secretory units connected to a small part of the duct system, and enclose the entire gland as its *capsule* (Figure 4–20).

The structures of their secretory portions and ducts allow exocrine glands to be classified as shown schematically in Table 4–4. Although the three-dimensional morphology is often not prominent in histologic sections, the key points are summarized as follows:

- Glands can be **simple** (ducts not branched) or **compound** (ducts with two or more branches).
- Secretory portions can be **tubular** (either short or long and **coiled**) or **acinar** (rounded and saclike); either

FIGURE 4–18 Goblet cells: unicellular glands.



The simple columnar epithelium lining the small intestine shows many isolated goblet cells secreting mucus into the lumen. (a) With a stain for the oligosaccharide components of mucin glycoproteins, the cytoplasmic secretory granules of two goblet cells and secreted mucus are stained purple. (X600; PAS-PT) (b) As shown ultrastructurally, goblet cells always have basal

nuclei surrounded by RER (R), a large Golgi complex (G), and abundant apical cytoplasm filled with large secretory granules (SG). After exocytosis mucin components are hydrated and become mucus. A brush border of microvilli (M) is seen on neighboring columnar cells. (X17,000)

type of secretory unit may be **branched**, even if the duct is not branched.

- **Compound** glands can have branching ducts and can have multiple tubular, acinar, or tubuloacinar secretory portions.

Three basic mechanisms for releasing the product are commonly used by cells specialized for secretion (Figure 4–21), and cells engaged in each type of secretion can be distinguished histologically:

1. **Merocrine secretion:** This is the most common method of protein or glycoprotein secretion and involves typical exocytosis from membrane-bound vesicles or secretory granules.
2. **Holocrine secretion:** Here cells accumulate product continuously as they enlarge and undergo terminal differentiation, culminating in complete cell disruption which releases the product and cell debris into the gland's lumen. This is best seen in the sebaceous glands producing lipid-rich material in skin (Figure 4–22).
3. **Apocrine secretion:** Here product accumulates at the cells' apical ends, portions of which are then extruded to release the product together with small amounts of cytoplasm and cell membrane. Lipid droplets are secreted in the mammary gland in this manner (Figure 4–23).

Exocrine glands with merocrine secretion can be further categorized as either **serous** or **mucous** according to the nature of their secretory products, which give distinct staining properties to the cells. Serous cells synthesize proteins that are mostly not glycosylated, such as digestive enzymes. The cells

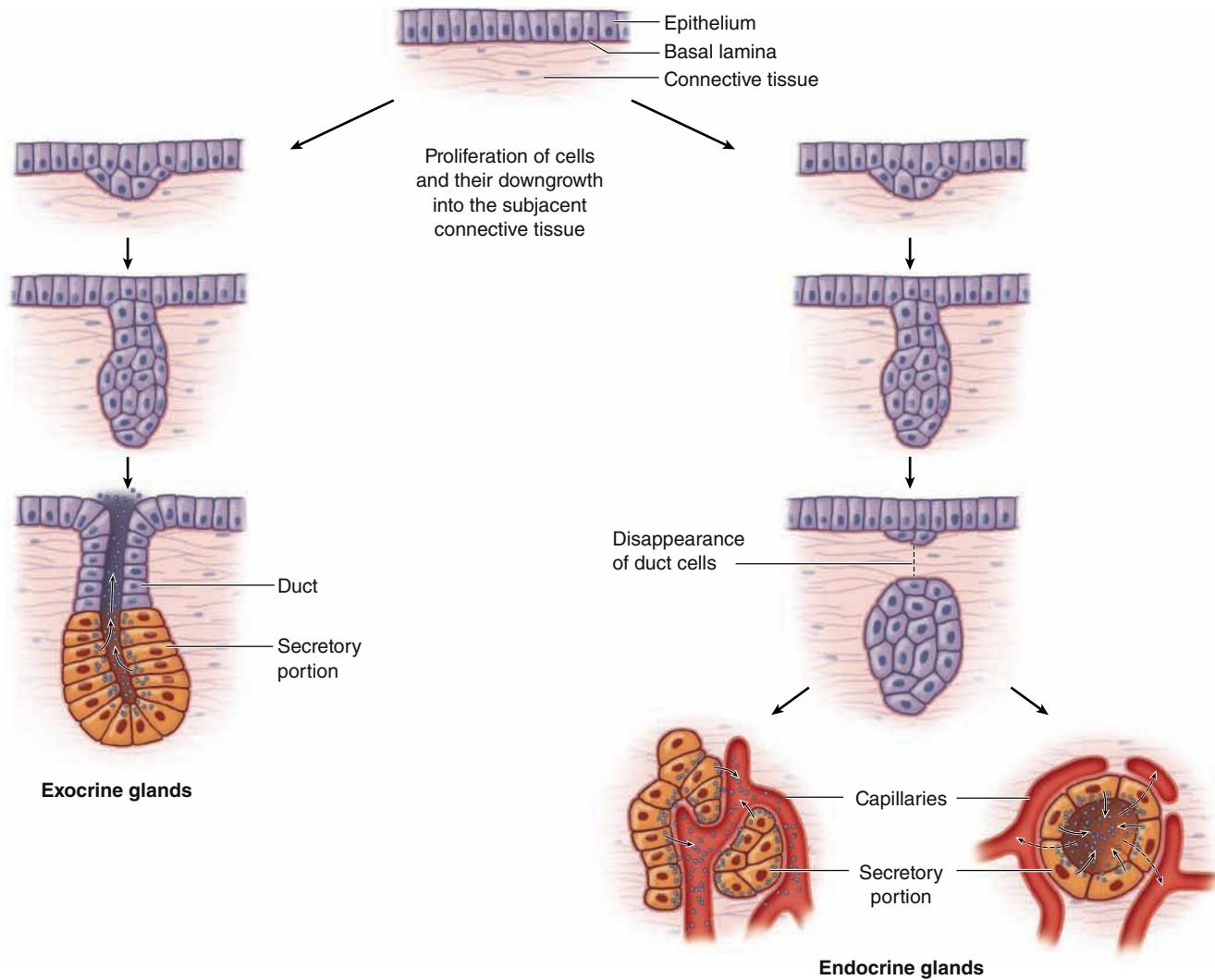
» MEDICAL APPLICATION

The holocrine sebaceous glands are the primary structure involved in the common form of **acne**, acne vulgaris. Excessive holocrine secretion of sebum and keratin triggered by the surge of the steroid hormone testosterone that occurs in both genders at puberty frequently leads to blocked ducts within the gland. Activity of the normal commensal skin bacterium *Propionibacterium acnes* within the blocked duct commonly produces localized inflammation.

have well-developed RER and Golgi complexes and are filled apically with secretory granules in different stages of maturation (Figure 4–24). Serous cells therefore stain intensely with basophilic or acidophilic stains. Acini of the pancreas and parotid salivary glands are composed of serous cells.

Mucous cells, such as goblet cells, also have RER and Golgi complexes and are filled apically with secretory granules, but these contain heavily glycosylated proteins called **mucins**. When mucins are released from the cell, they become hydrated and form a layer of **mucus**. The hydrophilic mucins are usually washed from cells during routine histological preparations, causing the secretory granules to stain poorly with eosin (Figure 4–25). Sufficient oligosaccharides remain in developing mucinogen granules, however, to allow mucous cells to be stained by the PAS method (Figure 4–18a).

Some salivary glands are mixed **seromucous glands**, having both serous acini and mucous tubules with clustered serous cells (see Figure 16–5). The product of such glands is a mixture of digestive enzymes and watery mucus.

FIGURE 4–19 Formation of glands from covering epithelia.

During fetal development epithelial cells proliferate and penetrate the underlying connective tissue. These cells may—or may not—maintain a connection with the surface epithelium. The connection is maintained to form a duct in exocrine glands; it is lost as endocrine glands develop. Exocrine glands secrete substances to specific organs via duct systems. Endocrine glands produce hormones

and are always rich in capillaries. Hormones are released outside the cells and picked up by these blood vessels for distribution throughout the body, where specific target cells are identified by receptors for the hormones. Endocrine glands can have secretory cells arranged as irregular cords (left) or as rounded follicles (right) with lumens for temporary storage of the secretory product.

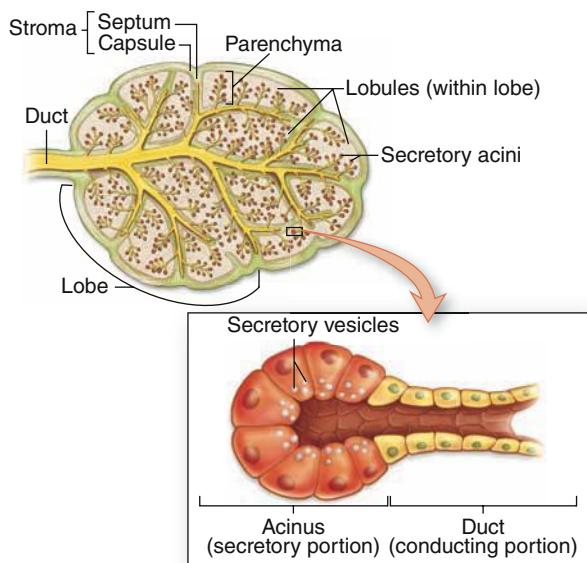
In addition to secretory cells, epithelia of many exocrine glands (eg, sweat, lachrymal, salivary, and mammary glands) contain contractile **myoepithelial cells** at the basal ends of the secretory cells (Figure 4–26). Long processes of these cells embrace an acinus as an octopus might embrace a rounded boulder. Bound to the basal lamina by hemidesmosomes and connected to the other epithelial cells by both gap junctions and desmosomes, myoepithelial cells are rich in actin filaments and myosins. Strong contractions in these cells serve to help propel secretory products from acini into the duct system.

Endocrine glands lack myoepithelial cells and are specialized for either protein or steroid **hormone** synthesis, with

cytoplasmic staining characteristic of RER or SER, respectively. The proteins are released by exocytosis and the lipophilic steroids by diffusion through the cell membrane for uptake by binding proteins outside the cell. As mentioned previously, endocrine signaling involves hormone transport in the blood to target cells throughout the body, often within other endocrine glands. The receptors may also be on cells very close to the hormone-secreting cell or on the secreting cell itself, signaling which is termed paracrine or autocrine, respectively.

Important but inconspicuous endocrine or paracrine cells also occur singly or in small groups in epithelia of the

FIGURE 4–20 General structure of exocrine glands.



Exocrine glands by definition have ducts that lead to another organ or the body surface. Inside the gland the duct runs through the connective tissue of septa and branches repeatedly, until its smallest branches end in the secretory portions of the gland.

digestive, respiratory, and other organ systems. Hormones are also secreted from some cells specialized for other functions, such as certain cardiac muscle cells or fat cells. The pancreas contains both endocrine and exocrine cells. Liver cells exert both functions in the same cells, secreting bile components into a duct system and releasing other products to the bloodstream.

► TRANSPORT ACROSS EPITHELIUM

Many cells have the ability to actively transport certain ions against concentration and electrical potential gradients. An important example is the extrusion of Na^+ from cells by the transmembrane protein Na^+/K^+ -ATPase, also called the Na^+/K^+ pump, which allows cells to maintain the required low intracellular sodium concentration (5–15 mmol/L vs ~140 mmol/L in extracellular fluid).

Some epithelial cells specialize in the transfer of ions (by ion pumps) and water (via the membrane channels called aquaporins) in either direction across the epithelium, the process known as **transcellular transport** (Figure 4–27). Apical tight junctions prevent paracellular diffusion or backflow between the cells.

Epithelia of kidney tubules are key sites for ion and water transport, maintaining the body's overall balance of

salts and water. Cells of the proximal renal tubules are specialized structurally for transcellular transport. The apical surface at the tubule lumen is freely permeable to Na^+ , and the basolateral cell membranes have sodium pumps for the active extrusion of Na^+ into the interstitial fluid outside the tubules. Osmotic and electrical balance is maintained by the passive transfer of chloride ions (Cl^-) and water into the cell. The basal membrane of these cells is elaborately folded, with mitochondria located between the folds to supply ATP for Na^+/K^+ pumps (Figure 4–28). Lateral membrane folds interdigitating between the cells further increase the surface area for transport. Regulated transfer of ions and water by various epithelial cells along the renal tubules maintains the ionic balance within the body and allows excretion of excess water and salts in the urine.

All cells can also internalize extracellular molecules and fluid using endocytosis and formation of cytoplasmic, membrane-bound vesicles. This activity is clearly observed in the simple squamous epithelial cells lining blood and lymphatic capillaries (endothelia) or body cavities (mesothelia). These thin cells have few organelles other than the abundant pinocytotic vesicles, which cross the thin cells in both directions and release their contents on the opposite side by exocytosis. This process of **transcytosis** also occurs between the apical and basolateral membranes domains in cells of simple cuboidal and columnar epithelia and is important in many physiologic processes.

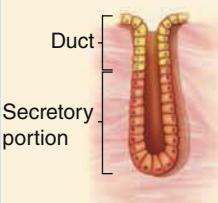
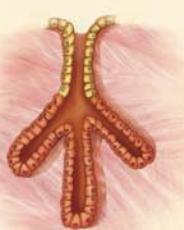
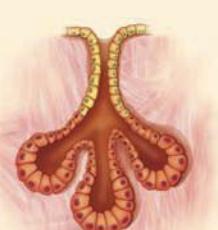
► RENEWAL OF EPITHELIAL CELLS

Epithelial tissues are relatively labile structures whose cells are renewed continuously by mitotic activity and stem cell populations. The rate of renewal varies widely; it can be fast in tissues such as the intestinal epithelium, which is replaced every week, or slow, as in the large glands. In stratified epithelial tissues, stem cells and mitosis occur only within the basal layer in contact with the basal lamina. In some functionally complex epithelia, stem cells are located only in restricted niches some distance from the transit amplifying cells and differentiating cells. For example, the epithelium lining the small intestine is derived completely from stem cells found in the simple glands between the intestinal villi. In the epidermis, many stem cells are located at a characteristic position along the wall of hair follicles.

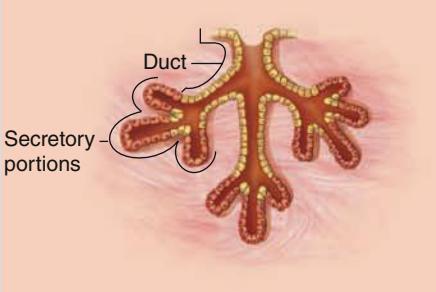
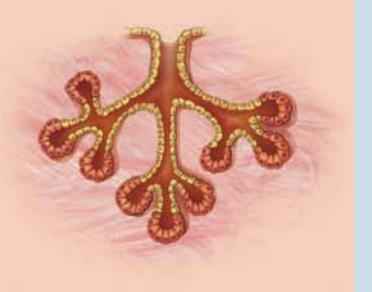
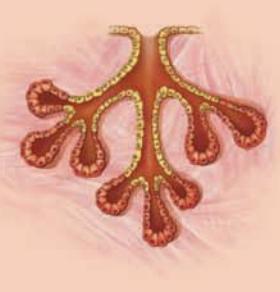
► MEDICAL APPLICATION

Both benign and malignant tumors can arise from most types of epithelial cells. Malignant tumors of epithelial origin are called **carcinomas** (Gr. *karkinos*, cancer + *oma*, tumor). Malignant tumors derived from glandular epithelial tissue are called **adenocarcinomas** (Gr. *adenos*, gland + *karkinos*). Adenocarcinomas are by far the most common tumors in adults after age 45.

TABLE 4-4**Structural classes of exocrine glands, features of each class, and examples.****SIMPLE Glands (Ducts Do Not Branch)**

| Class | Simple Tubular | Branched Tubular | Coiled Tubular | Acinar (or Alveolar) | Branched Acinar |
|----------|---|---|---|--|---|
| |  |  |  |  |  |
| Features | Elongated secretory portion; duct usually short or absent | Several long secretory parts joining to drain into 1 duct | Secretory portion is very long and coiled | Rounded, saclike secretory portion | Multiple saclike secretory parts entering the same duct |
| Examples | Mucous glands of colon; intestinal glands or crypts (of Lieberkühn) | Glands in the uterus and stomach | Sweat glands | Small mucous glands along the urethra | Sebaceous glands of the skin |

COMPOUND Glands (Ducts from Several Secretory Units Converge into Larger Ducts)

| Class | Tubular | Acinar (Alveolar) | Tubuloacinar |
|----------|--|---|--|
| |  |  |  |
| Features | Several elongated coiled secretory units and their ducts converge to form larger ducts | Several saclike secretory units with small ducts converge at a larger duct | Ducts of both tubular and acinar secretory units converge at larger ducts |
| Examples | Submucosal mucous glands (of Brunner) in the duodenum | Exocrine pancreas | Salivary glands |

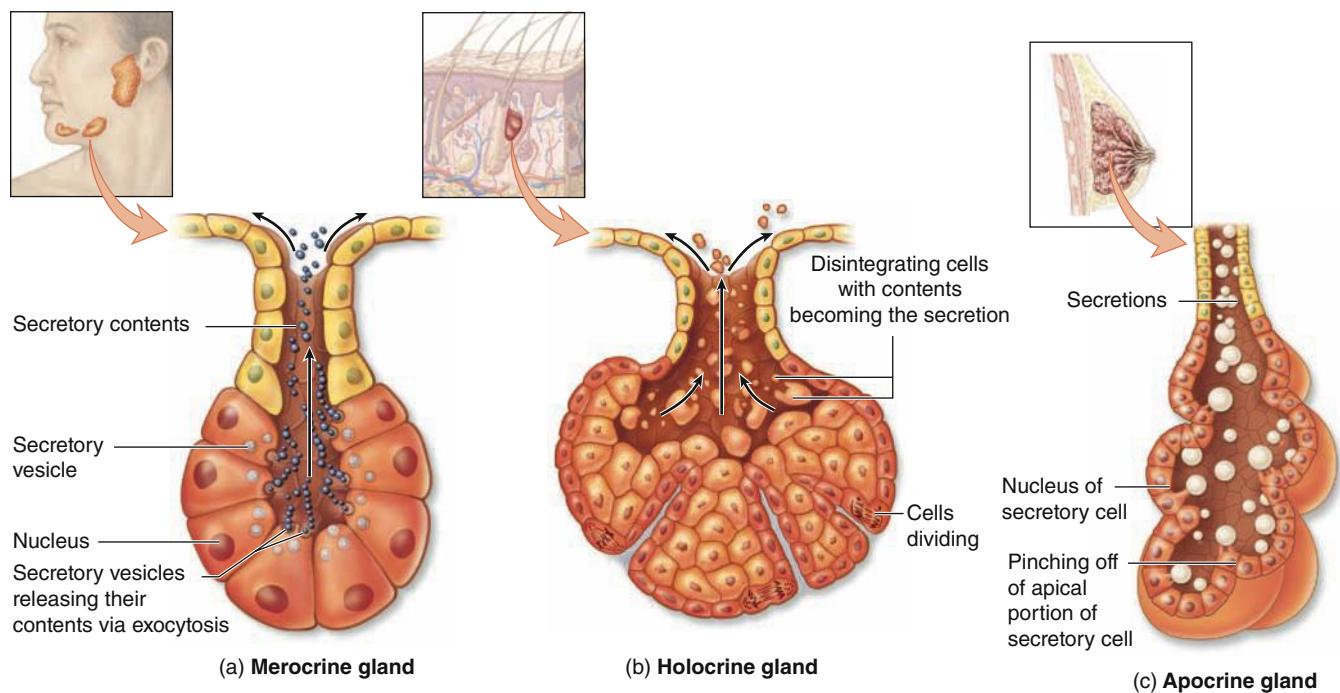
Epithelia are normally capable of rapid repair and replacement of apoptotic or damaged cells. In some large glands, most notably the liver, mitotic activity is normally rare but is actively renewed following major damage to the organ. When a portion of liver tissue is removed surgically or lost by the acute effects of toxic substances, cells of undamaged regions quickly begin active proliferation and a mass of liver tissue with normal function is regenerated.

>> MEDICAL APPLICATION

Some epithelial cells are prone to abnormal growth or dysplasia, which can progress to precancerous growth called **neoplasia**. Early neoplastic growth is often reversible and does not always result in cancer.

Under certain abnormal conditions, one type of epithelial tissue may undergo transformation into another type in another reversible process called **metaplasia**. In heavy cigarette smokers, the ciliated pseudostratified epithelium lining the bronchi can be transformed into stratified squamous epithelium.

FIGURE 4–21 Mechanisms of exocrine gland secretion.



Three basic types of secretion are used by cells of exocrine glands, depending on what substance is being secreted.

(a) **Merocrine** secretion releases products, usually containing proteins, by means of exocytosis at the apical end of the secretory cells. Most exocrine glands are merocrine.

(b) **Holocrine** secretion is produced by the disintegration of the secretory cells themselves as they complete their terminal

differentiation, which involves becoming filled with product. Sebaceous glands of hair follicles are the best examples of holocrine glands.

(c) **Apocrine** secretion involves loss of membrane-enclosed apical cytoplasm, usually containing one or more lipid droplets. Apocrine secretion, along with merocrine secretion, is seen in mammary glands.

Epithelial Tissue SUMMARY OF KEY POINTS

- An **epithelium** is a tissue in which cells are bound tightly together structurally and functionally to form a sheetlike or tubular structure with little extracellular material between the cells.
- Cells in epithelia each have an **apical side** facing the sheet's free surface and a **basal side** facing a basement membrane and underlying connective tissue.
- Epithelia are often specialized for absorption or **transcytosis**, pinocytosis of material at the apical side and exocytosis at the basolateral side (or vice versa).
- Cells of most epithelia exhibit **continuous renewal**, with the locations of stem cells and rates of cell turnover variable in various specialized epithelia.

Basement Membrane

- The **basement membrane** of all epithelia is a thin extracellular layer of specialized proteins, usually having two parts: a basal lamina and a more fibrous reticular lamina.
- The **basal lamina** is a thin meshwork of type IV collagen and laminin produced by the epithelial cells.
- The **reticular lamina** contains type III collagen and anchoring fibrils of VII collagen, all secreted by cells of the immediately adjacent connective tissue.

- Together, these components **attach** epithelia to connective tissue, regulate (**filter**) substances passing from connective tissue into epithelia, provide a guide or **scaffold** during tissue regeneration after injury, and **compartmentalize** epithelial cells from other tissues.

Intercellular Junctions

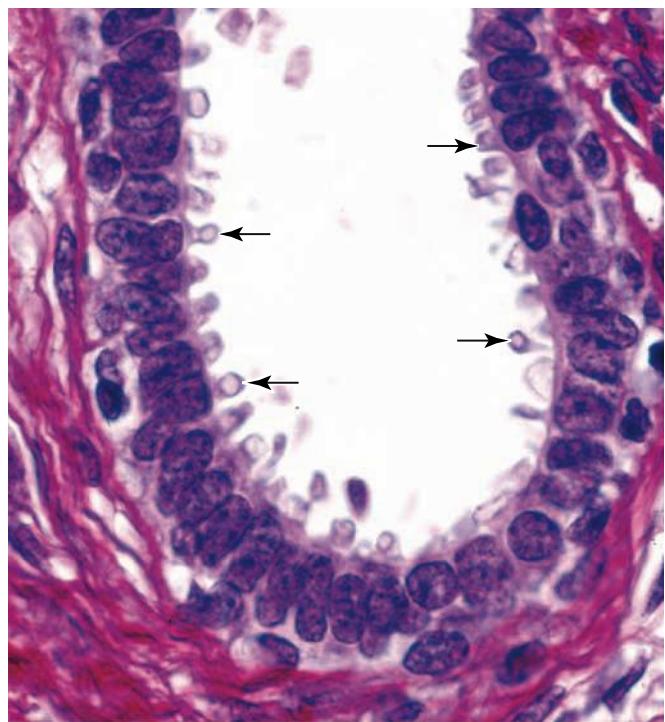
- Intercellular junctions are well developed in epithelia and consist of three major types, with different functions.
- Tight or occluding junctions** are formed by interacting transmembrane proteins such as **claudin** and **occludin**; linear arrangements of these linked proteins surround the apical ends of the cells and **prevent paracellular passage** of substances (between the cells).
- Adherent or anchoring junctions**, formed by interacting proteins of the **cadherin** family, are points of strong **attachment** holding together cells of the epithelium.
- Adherent junctions may form **zonula adherens** that encircle epithelial cells just below their tight junctions or scattered, spot-like attachment sites called **desmosomes** or **maculae adherens**, both of which are attached to cytoplasmic **keratins**.

FIGURE 4–22 Holocrine secretion in a sebaceous gland.



In holocrine secretion, best seen in the sebaceous gland adjacent to hair follicles, entire cells fill with a lipid-rich product as they differentiate. Mature (terminally differentiated) cells separate and completely disintegrate, releasing the lipid that serves to protect and lubricate adjacent skin and hair. Sebaceous glands lack myoepithelial cells; cell proliferation inside a dense, inelastic connective tissue capsule continuously forces product into the duct. (X200; H&E)

FIGURE 4–23 Apocrine secretion in the mammary gland.



The secretory portions of a mammary gland demonstrate apocrine secretion, characterized by extrusion of the secretion product along with a bit of apical cytoplasm (**arrows**). The released portion of cell contains lipid droplet(s). Merocrine secretion also occurs from the same and other cells of the gland. (X400; PSH)

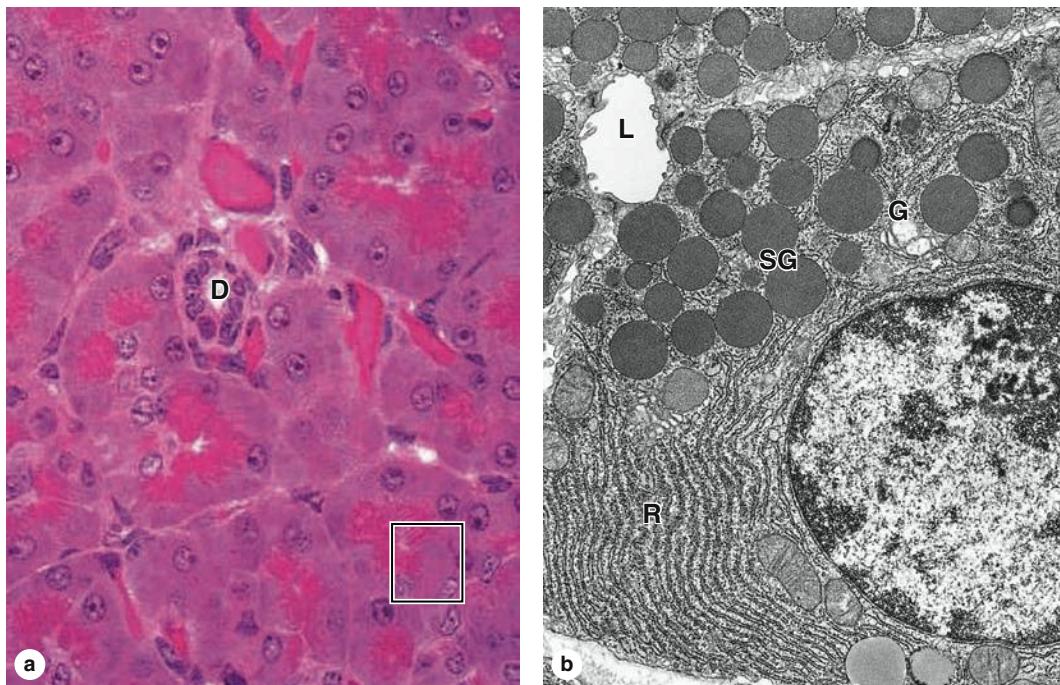
- **Hemidesmosomes** composed of transmembrane **integrins** attach cells to proteins of the basal lamina.
- **Gap or communicating junctions** are points of cell contact where both plasma membranes have numerous hexameric complexes of transmembrane **connexons**, each forming a channel allowing passage of small molecules from one cell to the other.

Apical Structures of Epithelial Cells

- **Microvilli** are small membrane projections with cores of **actin** filaments that generally function to increase epithelial cells' apical surface area for **absorption**.
- **Stereocilia** are long microvilli with specialized mechanosensory function in cells of the inner ear and for absorption in tissues of the male reproductive tract.
- **Cilia** are larger projecting structures with a well-organized core of **microtubules** (in a 9 + 2 arrangement called the **axoneme**) in which restricted, dynein-based sliding of microtubules causes ciliary movement that propel material along an epithelial surface.

Morphological Types of Epithelia

- An epithelium in which the basement membrane has one cell layer is **simple**; the cells of different simple epithelia range widely in height, from very thin or **squamous**, to roughly **cuboidal**, to very tall or **columnar**.
- Epithelia with two or more layers of cells are **stratified** and almost all such epithelia are stratified squamous, in which the outer cell layers are thin and flattened.
- Cells of stratified squamous epithelia move gradually from the basal to the surface layers, changing shape and becoming filled with **keratin** intermediate filaments.
- Stratified squamous epithelia such as the epidermis cover the body surface, **protecting** underlying tissues from excess water loss (dehydration) and microbial invasion.
- **Pseudostratified epithelia** are thick and appear to have several cell layers; all cells attach to the basal lamina but not all extend to the free epithelial surface.

FIGURE 4–24 Serous cells.

The small serous acini of the exocrine pancreas each have 5-10 cells facing a very small central lumen. Each acinar cell is roughly pyramidal, with its apex at the lumen. (a) As seen by light microscopy, the apical ends are very eosinophilic due to the abundant secretory granules present there. The cells' basal ends contain the nuclei and an abundance of RER, making this area basophilic. A small duct (D)

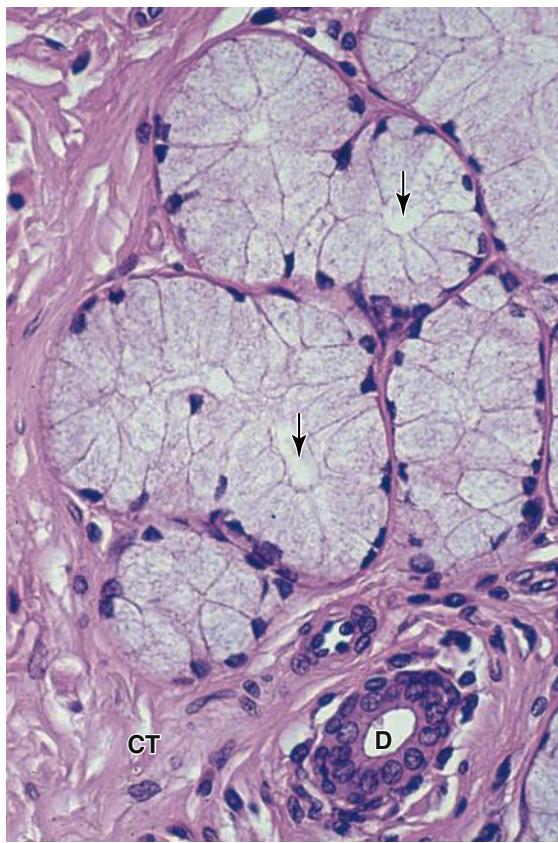
is seen, but lumens of acini are too small to be readily visible. The enclosed area is comparable to that shown in part b. (X300; H&E) (b) A portion of one acinar cell is shown ultrastructurally, indicating the abundant RER (R), a Golgi complex (G), apical secretory granules (SG) and the small acinar lumen (L). (X13,000)

- **Transitional epithelium or urothelium**, found only in the lining of the urinary system, is stratified, with large rounded surface cells protective against urine.

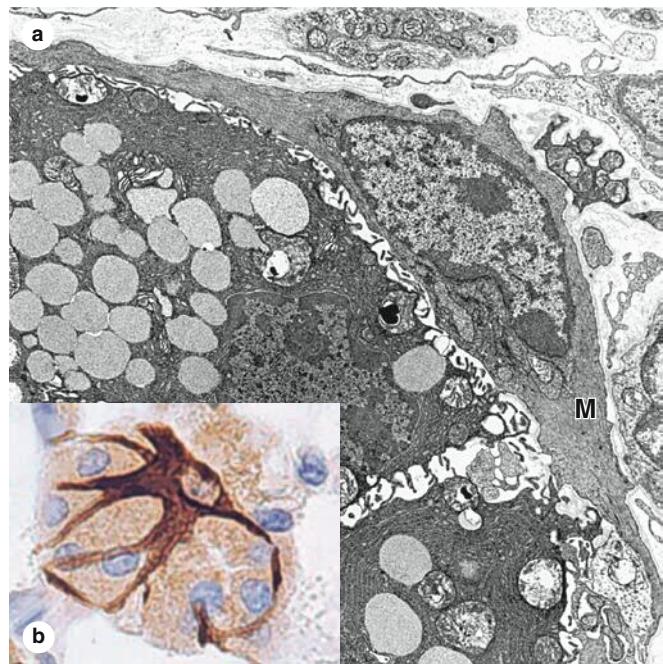
Epithelial Secretion/Glands

- The major function in many epithelial cells is synthesis and secretion of specialized products; organs composed primarily of such epithelia are called **glands**.
- **Exocrine glands** have epithelial ducts carrying secretions to specific sites; the ducts of **simple glands** are unbranched and those of **compound glands** are branched.
- The secretory portions of exocrine glands may form round, saclike **acini** (also called **alveoli**) or elongated **tubules**; both types of secretory units may themselves branch.

- **Endocrine glands** lack ducts; secreted substances are hormones carried throughout the body by the interstitial fluid and blood, with specificity produced by the hormone receptors of target cells.
- Glands have three basic secretory mechanisms: **merocrine**, which uses exocytosis; **holocrine**, in which terminally differentiated cells filled with lipid product are released; and **apocrine**, in which apical, product-filled areas of cells are extruded.
- Exocrine glands producing mucus, or similar individual cells called **goblet cells**, are called **mucous glands**; oligosaccharide components of mucus stain poorly with routine dyes but stain well with PAS stain.
- Exocrine glands producing largely enzymes (proteins) are called **serous glands** and stain darkly with H&E due to the cells' content of RER and secretory granules.

FIGURE 4–25 Mucous cells.

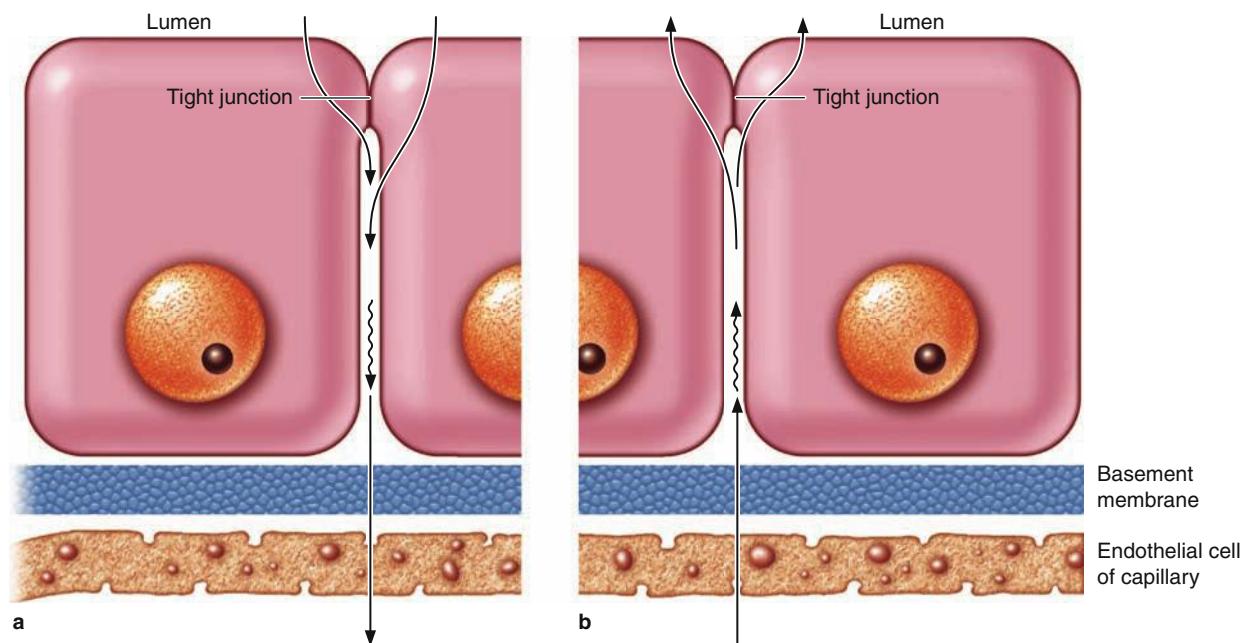
Mucous cells of salivary glands are typically larger than serous cells, with flattened basal nuclei. Most of the cytoplasm is filled with secretory granules containing mucinogen like that of goblet cells. The RER and Golgi complexes of mucous cells produce heavily glycosylated glycoproteins with water-binding properties. The lumens (arrows) of mucous tubules are larger than those of serous acini. Much connective tissue surrounds the mucous tubules and ducts (D). (X200; PT)

FIGURE 4–26 Myoepithelial cells.

(a) The TEM shows two salivary gland cells containing secretory granules, with an associated myoepithelial cell (M). (X20,000) (b) A myoepithelial cell immunostained brown with antibodies against actin shows its association with cells of an acinus stained by H&E. Contraction of the myoepithelial cell compresses the acinus and aids in the expulsion of secretory products into the duct. (X200)

Epithelial Tissue ASSESS YOUR KNOWLEDGE

1. Functions of the basement membrane include which of the following?
 - a. Contractility
 - b. Molecular filtering
 - c. Active ion transport
 - d. Excitability
 - e. Modification of secreted proteins
2. Using immunohistochemistry a population of cells is shown to be positive for the protein connexin. From this we can infer that the cells are connected by what type of junction?
 - a. Tight (occluding) junctions
 - b. Zonula adherens
 - c. Gap junctions
 - d. Hemidesmosomes
 - e. Desmosomes (macula adherens)
3. An individual genetically unable to synthesize normal occludin is likely to have epithelia with defective regulation in which of the following?
 - a. Material crossing the epithelium between the cells (paracellular movement)
 - b. Communication between the cells
 - c. Attachment to the basement membrane
 - d. Strong attachment to neighboring cells
 - e. Movement of membrane proteins in the apical domains of cells
4. An intermediate filament protein found in cytoplasm of most epithelial cells is which of the following?
 - a. Actin
 - b. Vimentin
 - c. Laminin
 - d. Myosin
 - e. Keratin

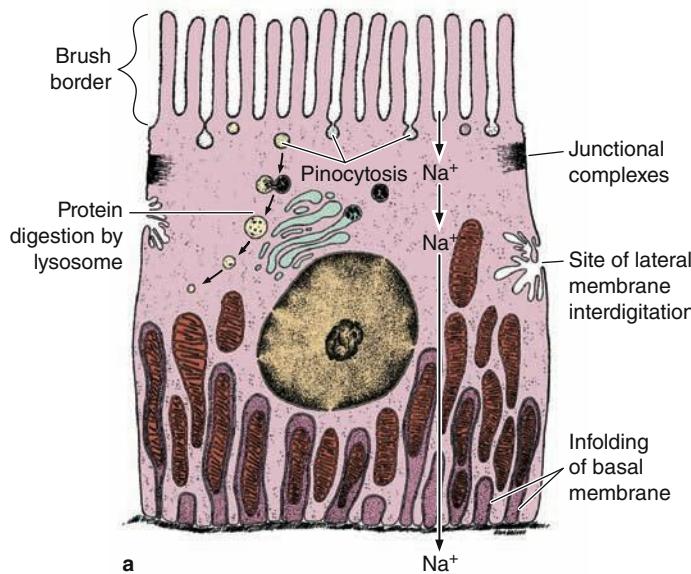
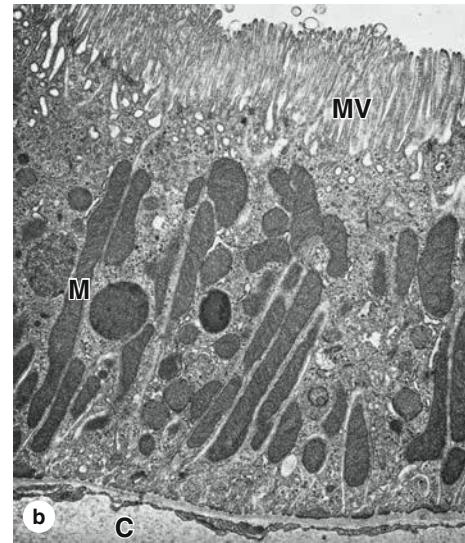
FIGURE 4–27 Ion and water absorption and secretion.

Ion and water transport across epithelia can occur in either direction, depending on the organ involved. (a) **Absorption** is the process of transport from an organ or duct's lumen to capillaries near the epithelial basement membrane and involves movement from the apical to the basolateral cell membrane domains. Absorption occurs for example in the epithelium of the gallbladder and intestine where it serves to concentrate bile or obtain water and ions from digested material.

(b) **Secretion** involves transport in the other direction from the capillaries into a lumen, as in many glands and the choroid plexus. Secretion by epithelial cells removes water from the neighboring interstitial fluid or plasma and releases it as part of the specialized aqueous fluids in such organs.

No matter whether an epithelium is involved in absorption or secretion, apical occluding junctions are necessary to maintain tight separation of the apical and basolateral compartments of either side of the epithelium.

5. Which of the following cellular features is used in naming types of epithelia?
 - a. Shape of cells in the basal layer
 - b. Number of cell layers
 - c. Presence of a basal lamina
 - d. Size of the nuclei
 - e. Nature of the cell junctions that are present
6. The release of lipid droplets from cells is which type of secretion?
 - a. Merocrine
 - b. Serous
 - c. Apocrine
 - d. Mucous
 - e. Holocrine
7. Exocrine glands in which the acini all produce a secretion of heavily glycosylated, hydrophilic proteins are an example of which type of gland?
 - a. Serous gland
 - b. Mixed gland
 - c. Mucous gland
 - d. Tubuloacinar gland
 - e. Simple gland
8. With a 5-year history of chronic respiratory infections, a 23-year-old, non-smoking man is referred to an otolaryngologist. A bronchial biopsy indicates altered structures in the epithelial cells. Which of the following, if altered to reduce function, is most likely involved in this patient's condition?
 - a. Hemidesmosomes
 - b. Cilia
 - c. Basolateral cell membrane folds
 - d. Microvilli
 - e. Tight junctions

FIGURE 4–28 Features of absorptive cells.**a****b****C**

A diagram and TEM photo showing the major ultrastructural features of a typical epithelial cell highly specialized for absorption, cells of proximal convoluted tubule of the kidney. The apical cell surface has a brush border consisting of uniform microvilli (MV) which increase the area of that surface to facilitate all types of membrane transport. Vesicles formed during pinocytosis may fuse with lysosomes as shown in (a) or mediate transcytosis by secreting their contents at the basolateral cell membrane. The basal cell surface is also enlarged, here by invaginations of the cell membrane which are associated with mitochondria (M) providing ATP for active transport. Basolateral membrane infoldings from

neighboring cells (the more heavily stippled structures) also with mitochondria interdigitate with those of this cell. Various ions entering through the apical membranes of renal epithelial cells undergo active transport out of the cells across the basolateral membrane. Immediately below the basal lamina shown in (b) is a capillary (C) that removes water and other substances absorbed across the epithelium. Junctional complexes between individual cells separate the apical and basolateral compartments on either side of the epithelium. Epithelial cells also show lateral membrane interdigitations with neighboring cells. (X9600)

9. An 11-month-old girl is referred to a pediatric gastroenterology clinic due to a history of generalized weakness, slow growth, and refractory diarrhea. For the past month she has been hospitalized regularly to receive parenteral nutrition. Examination of the epithelium lining her small intestine confirms that the failure to absorb nutrients is most likely due to a significant decrease in which of the following?
 - a. Microvilli
 - b. Gap junctions
 - c. Cilia
 - d. Cell layers
 - e. Basement membrane thickness
10. A 42-year-old woman of Mediterranean descent presents with multiple oral blisters and a few cutaneous blisters on her back and buttocks. The superficial bullae are fragile, some have unroofed to form ulcerated lesions, and there is a positive Nikolsky sign. Blood tests reveal antibodies to a subfamily of cadherins and immunohistochemical staining of a biopsy from the oral mucosa shows distribution of the antigen throughout the epithelium. In what structures is the defect that is causing this patient's condition?
 - a. Desmosomes
 - b. Tight junctions
 - c. Hemidesmosomes
 - d. Gap junctions
 - e. Reticular lamina

| | | | |
|--|------------|-----------------------------------|------------|
| CELLS OF CONNECTIVE TISSUE | 96 | | |
| Fibroblasts | 97 | Reticular Fibers | 106 |
| Adipocytes | 97 | Elastic Fibers | 109 |
| Macrophages & the Mononuclear Phagocyte System | 97 | GROUND SUBSTANCE | 111 |
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Connective tissue provides a matrix that supports and physically connects other tissues and cells together to form the organs of the body. The interstitial fluid of connective tissue gives metabolic support to cells as the medium for diffusion of nutrients and waste products.

Unlike the other tissue types (epithelium, muscle, and nerve), which consist mainly of cells, the major constituent of connective tissue is the **extracellular matrix (ECM)**. Extracellular matrices consist of different combinations of **protein fibers** (collagen and elastic fibers) and **ground substance**. Ground substance is a complex of anionic, hydrophilic proteoglycans, glycosaminoglycans (GAGs), and multiadhesive glycoproteins (laminin, fibronectin, and others). As described briefly in Chapter 4 with the basal lamina, such glycoproteins help stabilize the ECM by binding to other matrix components and to integrins in cell membranes. Water within this ground substance allows the exchange of nutrients and metabolic wastes between cells and the blood supply.

The variety of connective tissue types in the body reflects differences in composition and amount of the cells, fibers, and ground substance which together are responsible for the remarkable structural, functional, and pathologic diversity of connective tissue.

All connective tissues originate from embryonic **mesenchyme**, a tissue developing mainly from the middle layer of the embryo, the mesoderm. Mesenchyme consists largely of viscous ground substance with few collagen fibers (Figure 5–1). **Mesenchymal cells** are undifferentiated and have large nuclei, with prominent nucleoli and fine chromatin. They are often said to be “spindle-shaped,” with their scant cytoplasm extended as two or more thin cytoplasmic processes. Mesodermal cells

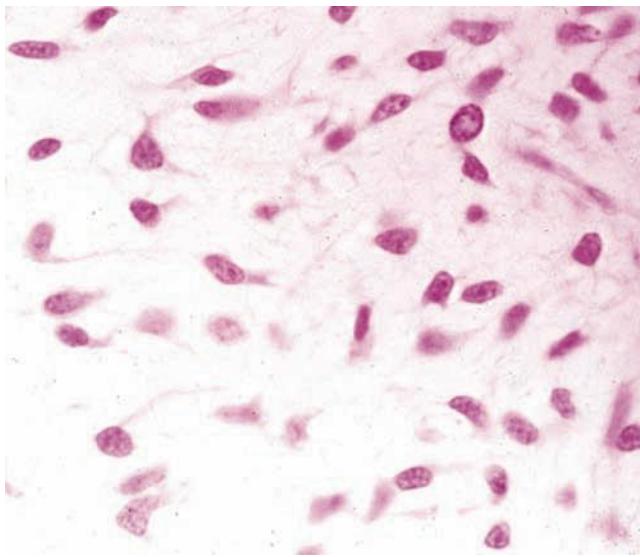
migrate from their site of origin in the embryo, surrounding and penetrating developing organs. In addition to producing all types of connective tissue proper and the specialized connective tissues bone and cartilage, the embryonic mesenchyme includes stem cells for other tissues such as blood, the vascular endothelium, and muscle. This chapter describes the features of soft, supportive connective tissue proper.

» MEDICAL APPLICATION

Some cells in mesenchyme are **multipotent stem cells** potentially useful in **regenerative medicine** after grafting to replace damaged tissue in certain patients. Mesenchyme-like cells remain present in some adult connective tissues, including that of tooth pulp and some adipose tissue, and are being investigated as possible sources of stem cells for therapeutic repair and organ regeneration.

» CELLS OF CONNECTIVE TISSUE

Fibroblasts are the key cells in connective tissue proper (Figure 5–2 and Table 5–1). Fibroblasts originate locally from mesenchymal cells and are permanent residents of connective tissue. Other cells found here, such as **macrophages**, **plasma cells**, and **mast cells**, originate from hematopoietic stem cells in bone marrow, circulate in the blood, and then move into connective tissue where they function. These and other white blood cells (leukocytes) are transient cells of most connective tissues, where they perform various functions for a short period as needed and then die by apoptosis.

FIGURE 5–1 Embryonic mesenchyme.

Mesenchyme consists of a population of undifferentiated cells, generally elongated but with many shapes, having large euchromatic nuclei and prominent nucleoli that indicate high levels of synthetic activity. These cells are called **mesenchymal cells**. Mesenchymal cells are surrounded by an ECM that they produced and that consists largely of a simple ground substance rich in hyaluronan (hyaluronic acid), but with very little collagen. (X200; Mallory trichrome)

Fibroblasts

Fibroblasts (Figure 5–3), the most common cells in connective tissue proper, produce and maintain most of the tissue's extracellular components. Fibroblasts synthesize and secrete collagen (the most abundant protein of the body) and elastin, which both form large fibers, as well as the GAGs, proteoglycans, and multiadhesive glycoproteins that comprise the ground substance. As described later, most of the secreted ECM components undergo further modification outside the cell before assembling as a matrix.

Distinct levels of fibroblast activity can be observed histologically (Figure 5–3b). Cells with intense synthetic activity are morphologically different from the quiescent fibroblasts that are scattered within the matrix they have already synthesized. Some histologists reserve the term "fibroblast" to denote the active cell and "fibrocyte" to denote the quiescent cell. The active fibroblast has more abundant and irregularly branched cytoplasm, containing much rough endoplasmic reticulum (RER) and a well-developed Golgi apparatus, with a large, ovoid, euchromatic nucleus and a prominent nucleolus. The quiescent cell is smaller than the active fibroblast, is usually spindle-shaped with fewer processes, much less RER, and a darker, more heterochromatic nucleus.

Fibroblasts are targets of many families of proteins called **growth factors** that influence cell growth and differentiation. In adults, connective tissue fibroblasts rarely undergo division. However, stimulated by locally released growth factors, cell cycling and mitotic activity resume when the tissue requires additional fibroblasts, for example, to repair a damaged organ. Fibroblasts involved in wound healing, sometimes called **myofibroblasts**, have a well-developed contractile function and are enriched with a form of actin also found in smooth muscle cells.

» MEDICAL APPLICATION

The regenerative capacity of connective tissue is clearly observed in organs damaged by ischemia, inflammation, or traumatic injury. Spaces left after such injuries, especially in tissues whose cells divide poorly or not at all (eg, cardiac muscle), are filled by connective tissue, forming dense irregular **scar tissue**. The healing of surgical incisions and other wounds depends on the reparative capacity of connective tissue, particularly on activity and growth of fibroblasts.

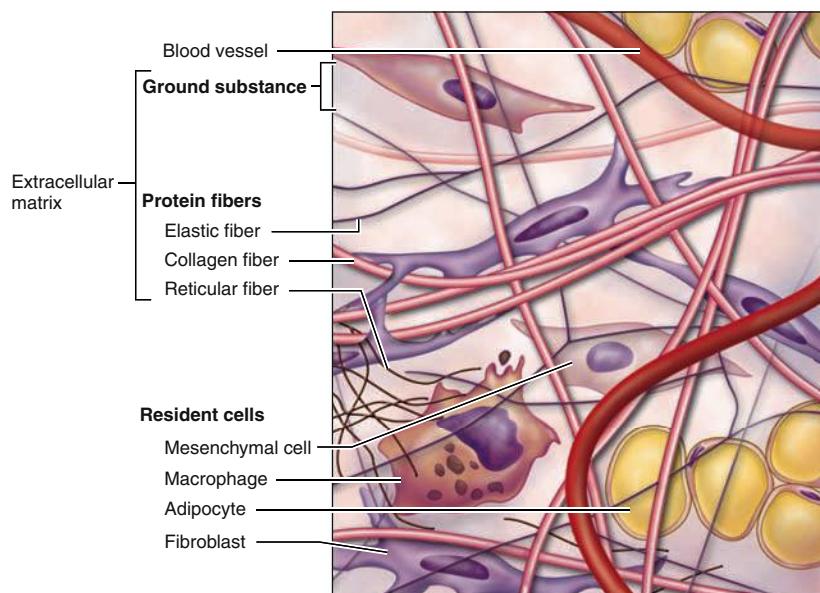
In some rapidly closing wounds, a cell called the myofibroblast, with features of both fibroblasts and smooth muscle cells, is also observed. These cells have most of the morphologic characteristics of fibroblasts but contain increased amounts of actin microfilaments and myosin and behave much like smooth muscle cells. Their activity is important for the phase of tissue repair called **wound contraction**.

Adipocytes

Adipocytes (L. *adeps*, fat + Gr. *kytos*, cell), or fat cells, are found in the connective tissue of many organs. These large, mesenchymally derived cells are specialized for cytoplasmic storage of lipid as neutral fats, or less commonly for the production of heat. Tissue with a large population of adipocytes, called adipose connective tissue, serves to cushion and insulate the skin and other organs. Adipocytes have major metabolic significance with considerable medical importance and are described and discussed separately in Chapter 6.

Macrophages & the Mononuclear Phagocyte System

Macrophages have highly developed phagocytic ability and specialize in turnover of protein fibers and removal of dead cells, tissue debris, or other particulate material, being especially abundant at sites of inflammation. Size and shape vary considerably, corresponding to their state of functional activity. A typical macrophage measures between 10 and 30 μm in diameter and has an eccentrically located, oval or kidney-shaped nucleus. Macrophages are present in the connective tissue of most organs and are sometimes referred to by pathologists as "histiocytes."

FIGURE 5–2 Cellular and extracellular components of connective tissue.

Connective tissue is composed of **fibroblasts** and other cells and an **extracellular matrix (ECM)** of various protein fibers, all of

which are surrounded by watery **ground substance**. In all types of connective tissue the extracellular volume exceeds that of the cells.

TABLE 5–1
Functions of cells in connective tissue proper.

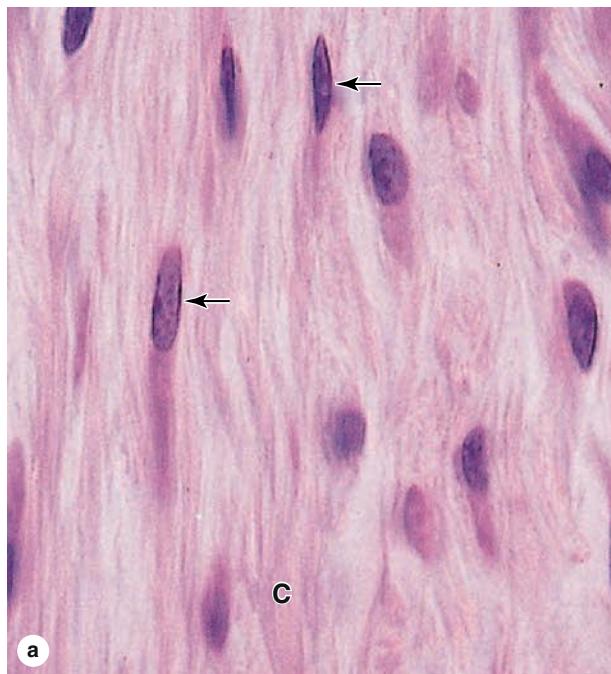
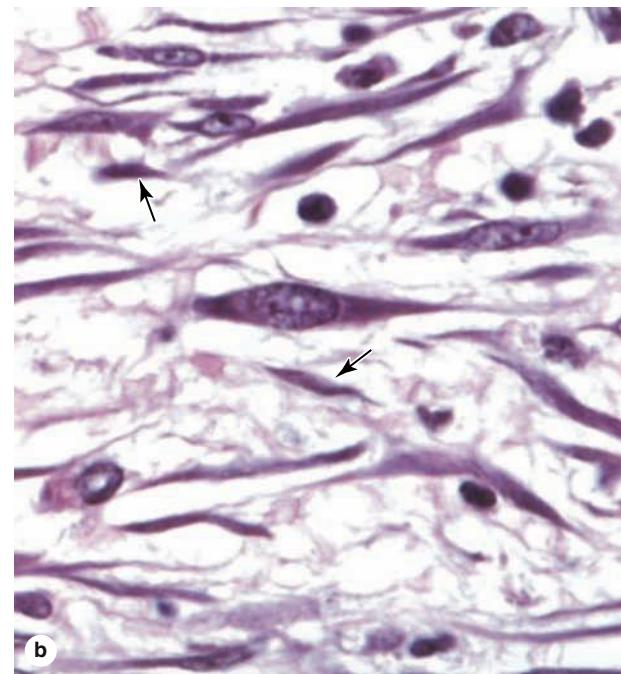
| Cell Type | Major Product or Activity |
|--------------------------------------|--|
| Fibroblasts (fibrocytes) | Extracellular fibers and ground substance |
| Plasma cells | Antibodies |
| Lymphocytes (several types) | Various immune/defense functions |
| Eosinophilic leukocytes | Modulate allergic/vasoactive reactions and defense against parasites |
| Neutrophilic leukocytes | Phagocytosis of bacteria |
| Macrophages | Phagocytosis of ECM components and debris; antigen processing and presentation to immune cells; secretion of growth factors, cytokines, and other agents |
| Mast cells and basophilic leukocytes | Pharmacologically active molecules (eg, histamine) |
| Adipocytes | Storage of neutral fats |

» MEDICAL APPLICATION

Besides their function in turnover of ECM fibers, macrophages are key components of an organism's innate immune defense system, removing cell debris, neoplastic cells, bacteria, and other invaders. Macrophages are also important antigen-presenting cells required for the activation and specification of lymphocytes.

When macrophages are stimulated (by injection of foreign substances or by infection), they change their morphologic characteristics and properties, becoming **activated macrophages**. In addition to showing an increase in their capacity for phagocytosis and intracellular digestion, activated macrophages exhibit enhanced metabolic and lysosomal enzyme activity. Macrophages are also secretory cells producing an array of substances, including various enzymes for ECM breakdown and various growth factors or cytokines that help regulate immune cells and reparative functions.

When adequately stimulated, macrophages may increase in size and fuse to form **multinuclear giant cells**, usually found only in pathologic conditions.

FIGURE 5-3 Fibroblasts.**a****b**

(a) Fibroblasts typically have large active nuclei and eosinophilic cytoplasm that tapers off in both directions along the axis of the nucleus, a morphology often referred to as “spindle-shaped.” Nuclei (**arrows**) are clearly seen, but the eosinophilic cytoplasmic processes resemble the collagen bundles (**C**) that fill the ECM and are difficult to distinguish in H&E-stained sections.

(b) Both active and quiescent fibroblasts may sometimes be distinguished, as in this section of dermis. Active fibroblasts have large, euchromatic nuclei and basophilic cytoplasm, while inactive fibroblasts (or fibrocytes) are smaller with more heterochromatic nuclei (**arrows**). The round, very basophilic round cells are in leukocytes. (Both X400; H&E)

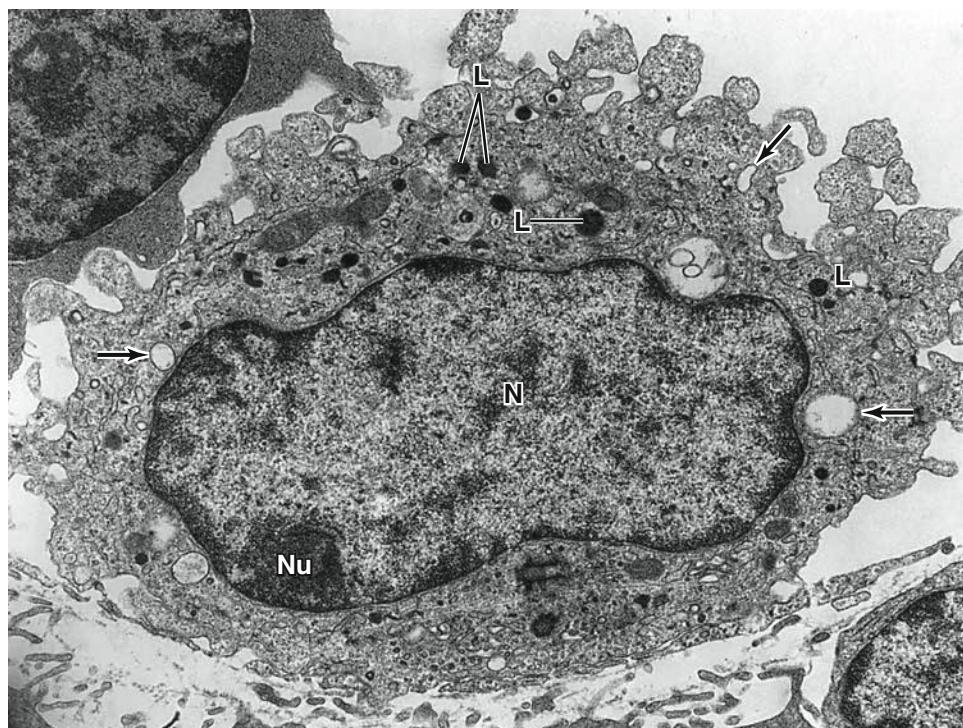
In the TEM, macrophages are shown to have a characteristic irregular surface with pleats, protrusions, and indentations, features related to their active pinocytotic and phagocytic activities (Figure 5-4). They generally have well-developed Golgi complexes and many lysosomes.

Macrophages derive from bone marrow precursor cells called **monocytes** that circulate in the blood. These cells cross the epithelial wall of small venules to enter connective tissue, where they differentiate, mature, and acquire the morphologic features of phagocytic cells. Therefore, monocytes and macrophages are the same cell at different stages of maturation. Macrophages play a very important role in the early stages of repair and inflammation after tissue damage. Under such conditions these cells accumulate in connective tissue by local proliferation of macrophages and recruitment of more monocytes from the blood. Macrophages are distributed throughout the body and are normally present in the stroma of most organs. Along with other monocyte-derived cells, they comprise a family of cells called the **mononuclear phagocyte system** (Table 5-2). All of these macrophage-like cells are derived from monocytes, but have different names in various organs, for example, Kupffer cells in the liver, microglial cells in the central nervous system,

Langerhans cells in the skin, and osteoclasts in bone. All are long-living cells and may survive in the tissues for months. In addition to debris removal, these cells are highly important for the uptake, processing, and presentation of antigens for lymphocyte activation, a function discussed later with the immune system. The transformation from monocytes to macrophages in connective tissue involves increases in cell size, increased protein synthesis, and increases in the number of Golgi complexes and lysosomes.

Mast Cells

Mast cells are oval or irregularly shaped cells of connective tissue, between 7 and 20 μm in diameter, filled with basophilic secretory granules which often obscure the central nucleus (Figure 5-5). These granules are electron-dense and of variable size, ranging from 0.3 to 2.0 μm in diameter. Because of the high content of acidic radicals in their sulfated GAGs, mast cell granules display **metachromasia**, which means that they can change the color of some basic dyes (eg, toluidine blue) from blue to purple or red. The granules are poorly preserved by common fixatives, so that mast cells may be difficult to identify in routinely prepared slides.

FIGURE 5–4 Macrophage ultrastructure.

Characteristic features of macrophages seen in this TEM of one such cell are the prominent nucleus (**N**) and the nucleolus (**Nu**) and the numerous secondary lysosomes (**L**). The arrows indicate

phagocytic vacuoles near the protrusions and indentations of the cell surface. (X10,000)

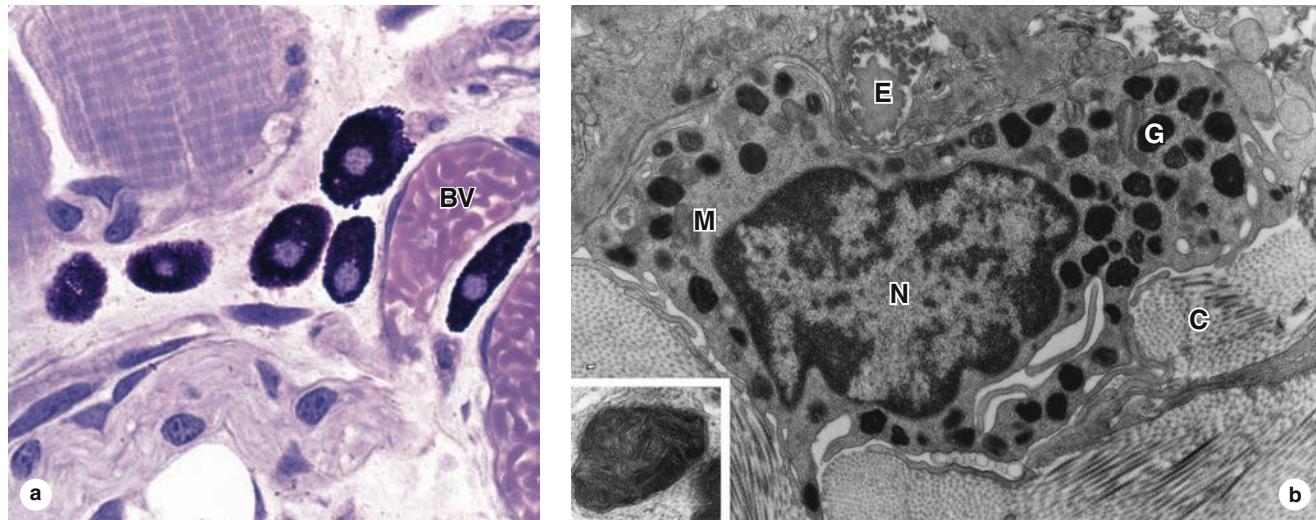
Mast cells function in the localized release of many bioactive substances important in the local inflammatory response, innate immunity, and tissue repair. A partial list of molecules released from these cells' secretory granules includes the following:

- **Heparin**, a sulfated GAG that acts locally as an anticoagulant

- **Histamine**, which promotes increased vascular permeability and smooth muscle contraction
- **Serine proteases**, which activate various mediators of inflammation
- **Eosinophil** and **neutrophil chemotactic factors**, which attract those leukocytes

TABLE 5–2 Distribution and main functions of the cells of the mononuclear phagocyte system.

| Cell Type | Major Location | Main Function |
|---|---|--|
| Monocyte | Blood | Precursor of macrophages |
| Macrophage | Connective tissue, lymphoid organs, lungs, bone marrow, pleural and peritoneal cavities | Production of cytokines, chemotactic factors, and several other molecules that participate in inflammation (defense), antigen processing, and presentation |
| Kupffer cell | Liver (perisinusoidal) | Same as macrophages |
| Microglial cell | Central nervous system | Same as macrophages |
| Langerhans cell | Epidermis of skin | Antigen processing and presentation |
| Dendritic cell | Lymph nodes, spleen | Antigen processing and presentation |
| Osteoclast (from fusion of several macrophages) | Bone | Localized digestion of bone matrix |
| Multinuclear giant cell (several fused macrophages) | In connective tissue under various pathological conditions | Segregation and digestion of foreign bodies |

FIGURE 5–5 Mast cells.

Mast cells are components of loose connective tissues, often located near small blood vessels (BV). (a) They are typically oval shaped, with cytoplasm filled with strongly basophilic granules. (X400; PT)

(b) Ultrastructurally mast cells show little else around the nucleus (N) besides these cytoplasmic granules (G), except for occasional

mitochondria (M). The granule staining in the TEM is heterogeneous and variable in mast cells from different tissues; at higher magnifications some granules may show a characteristic scroll-like substructure (inset) that contains preformed mediators such as histamine and proteoglycans. The ECM near this mast cell includes elastic fibers (E) and bundles of collagen fibers (C).

- **Cytokines**, polypeptides directing activities of leukocytes and other cells of the immune system
- **Phospholipid** precursors, which are converted to prostaglandins, leukotrienes, and other important lipid mediators of the inflammatory response.

Occurring in connective tissue of many organs, mast cells are especially numerous near small blood vessels in skin and mesenteries (*perivascular* mast cells) and in the tissue that lines digestive and respiratory tracts (*mucosal* mast cells); the granule content of the two populations differs somewhat. These major locations suggest that mast cells place themselves strategically to function as sentinels detecting invasion by microorganisms.

Release of certain chemical mediators stored in mast cells promotes the allergic reactions known as **immediate hypersensitivity reactions** because they occur within a few minutes after the appearance of an antigen in an individual previously sensitized to that antigen. There are many examples of immediate hypersensitivity reaction; a dramatic one is anaphylactic shock, a potentially fatal condition. Anaphylaxis consists of the following sequential events (Figure 5–6). The first exposure to an antigen (allergen), such as bee venom, causes antibody-producing cells to produce an immunoglobulin of the IgE class which binds avidly to receptors on the surface of mast cells. Upon a second exposure to

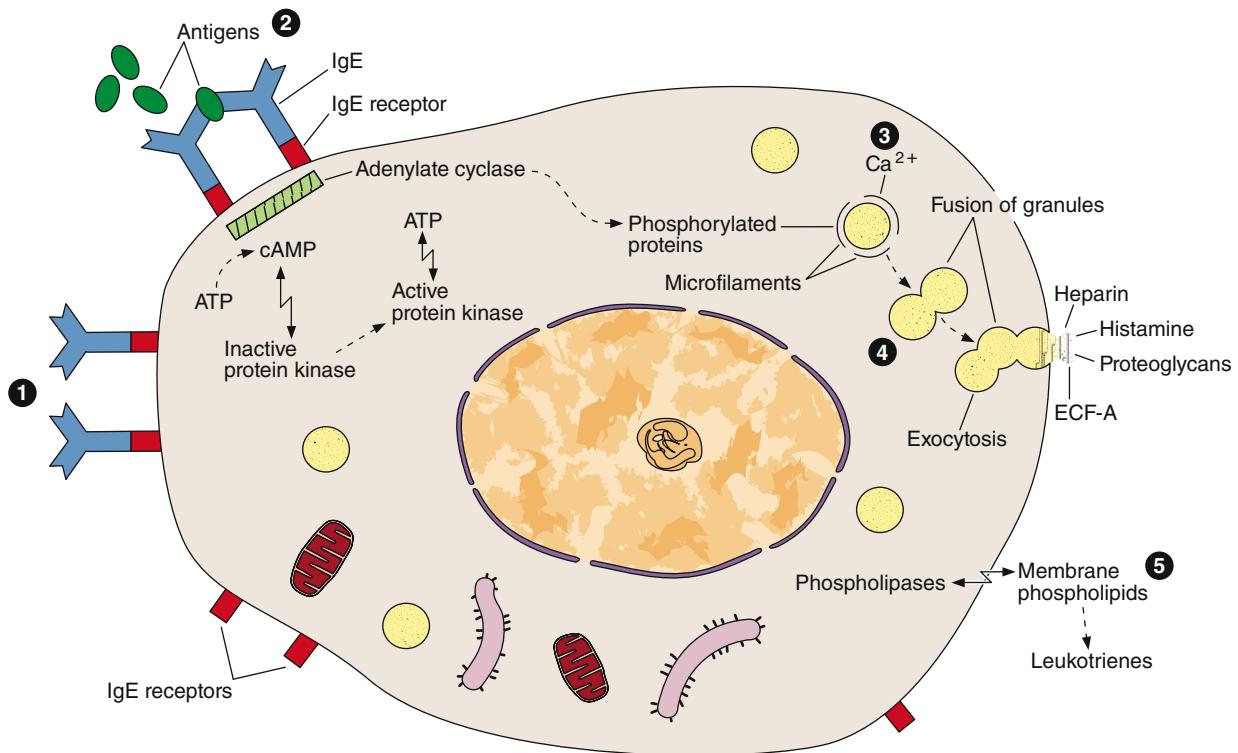
the antigen it reacts with the IgE on the mast cells, triggering rapid release of histamine, leukotrienes, chemokines, and heparin from the mast cell granules which can produce the sudden onset of the allergic reaction. Degranulation of mast cells also occurs as a result of the action of the complement molecules that participate in the immunologic reactions described in Chapter 14.

Like macrophages, mast cells originate from progenitor cells in the bone marrow, which circulate in the blood, cross the wall of small vessels called venules, and enter connective tissues, where they differentiate. Although mast cells are in many respects similar to basophilic leukocytes, they appear to have a different lineage at least in humans.

Plasma Cells

Plasma cells are lymphocyte-derived, antibody-producing cells. These relatively large, ovoid cells have basophilic cytoplasm rich in RER and a large Golgi apparatus near the nucleus that may appear pale in routine histologic preparations (Figure 5–7).

The nucleus of the plasma cell is generally spherical but eccentrically placed. Many of these nuclei contain compact, peripheral regions of heterochromatin alternating with lighter areas of euchromatin. At least a few plasma cells are present in most connective tissues. Their average lifespan is only 10–20 days.

FIGURE 5–6 Mast cell secretion.

Mast cell secretion is triggered by reexposure to certain antigens and allergens. Molecules of IgE antibody produced in an initial response to an allergen such as pollen or bee venom are bound to surface receptors for IgE (1), of which 300,000 are present per mast cell.

When a second exposure to the allergen occurs, IgE molecules bind this antigen and a few IgE receptors very rapidly become cross-linked (2). This activates adenylate cyclase, leading to phosphorylation of specific proteins (3), entry of Ca^{2+} and rapid

exocytosis of some granules (4). In addition, phospholipases act on specific membrane phospholipids, leading to production and release of leukotrienes (5).

The components released from granules, as well as the leukotrienes, are immediately active in the local microenvironment and promote a variety of controlled local reactions that together normally comprise part of the inflammatory process called the **immediate hypersensitivity reaction**. "ECF-A" is the eosinophil chemotactic factor of anaphylaxis.

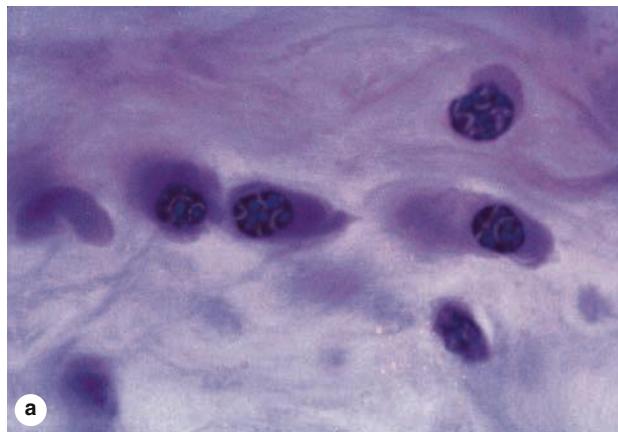
» MEDICAL APPLICATION

Plasma cells are derived from B lymphocytes and are responsible for the synthesis of immunoglobulin antibodies. Each antibody is specific for the one antigen that stimulated the clone of B cells and reacts only with that antigen or molecules resembling it (see Chapter 14). The results of the antibody-antigen reaction are variable, but they usually neutralize harmful effects caused by antigens. An antigen that is a toxin (eg, tetanus, diphtheria) may lose its capacity to do harm when it is bound by a specific antibody. Bound antigen-antibody complexes are quickly removed from tissues by phagocytosis.

Leukocytes

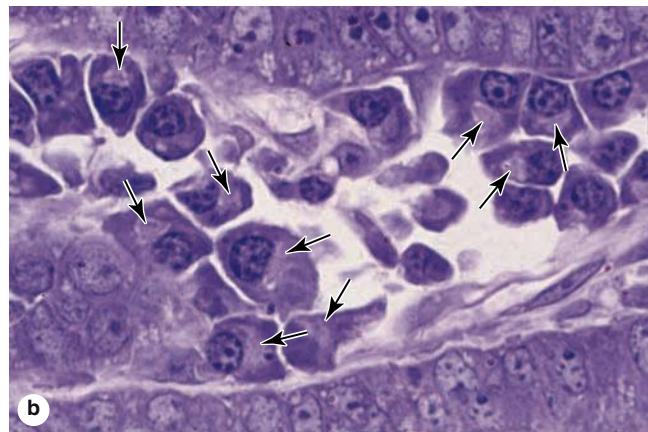
Other white blood cells, or **leukocytes**, besides macrophages and plasma cells normally comprise a population of wandering cells in connective tissue. Derived from circulating blood cells, they leave blood by migrating between the endothelial cells of venules to enter connective tissue. This process increases greatly during inflammation, which is a vascular and cellular defensive response to injury or foreign substances, including pathogenic bacteria or irritating chemical substances.

Inflammation begins with the local release of chemical mediators from various cells, the ECM, and blood plasma proteins. These substances act on local blood vessels, mast cells, macrophages, and other cells to induce events characteristic of

FIGURE 5–7 Plasma cells.

Antibody-secreting plasma cells are present in variable numbers in the connective tissue of many organs.

(a) Plasma cells are large, ovoid cells, with basophilic cytoplasm. The round nuclei frequently show peripheral clumps of heterochromatin, giving the structure a "clock-face" appearance. (X640; H&E)



(b) Plasma are often more abundant in infected tissues, as in the inflamed lamina propria shown here. A large pale Golgi apparatus (arrows) at a juxtanuclear site in each cell is actively involved in the terminal glycosylation of the antibodies (glycoproteins). Plasma cells leave their sites of origin in lymphoid tissues, move to connective tissue, and produce antibodies that mediate immunity. (X400 PT)

inflammation, for example, increased blood flow and vascular permeability, entry and migration of leukocytes, and activation of macrophages for phagocytosis.

Most leukocytes function in connective tissue only for a few hours or days and then undergo apoptosis. However, as discussed with the immune system, some lymphocytes and phagocytic antigen-presenting cells normally leave the interstitial fluid of connective tissue, enter blood or lymph, and move to selected lymphoid organs.

► MEDICAL APPLICATION

Increased vascular permeability is caused by the action of vasoactive substances such as histamine released from mast cells during **inflammation**. Classically, the major signs of inflamed tissues include "redness and swelling with heat and pain" (*rubor et tumor cum calore et dolore*). Increased blood flow and vascular permeability produce local tissue **swelling (edema)**, with increased redness and warmth. **Pain** is due mainly to the action of the chemical mediators on local sensory nerve endings. All these activities help protect and repair the inflamed tissue. **Chemotaxis** (Gr. *chemeia*, alchemy + *taxis*, orderly arrangement), the phenomenon by which specific cell types are attracted by specific molecules, draws much larger numbers of leukocytes into inflamed tissues.

► FIBERS

The fibrous components of connective tissue are elongated structures formed from proteins that polymerize after secretion from fibroblasts (Figure 5–2). The three main types of fibers include **collagen**, **reticular**, and **elastic fibers**. Collagen and reticular fibers are both formed by proteins of the collagen family, and elastic fibers are composed mainly of the protein **elastin**. These fibers are distributed unequally among the different types of connective tissue, with the predominant fiber type conferring most specific tissue properties.

Collagen

The **collagens** constitute a family of proteins selected during evolution for their ability to form various extracellular fibers, sheets, and networks, all of which extremely strong and resistant to normal shearing and tearing forces. Collagen is a key element of all connective tissues, as well as epithelial basement membranes and the external laminae of muscle and nerve cells.

Collagen is the most abundant protein in the human body, representing 30% of its dry weight. A major product of fibroblasts, collagens are also secreted by several other cell types and are distinguishable by their molecular compositions, morphologic characteristics, distribution,

functions, and pathologies. A family of 28 collagens exists in vertebrates, numbered in the order they were identified, and the most important are listed in Table 5–3. They can be categorized according to the structures formed by their interacting α -chains subunits:

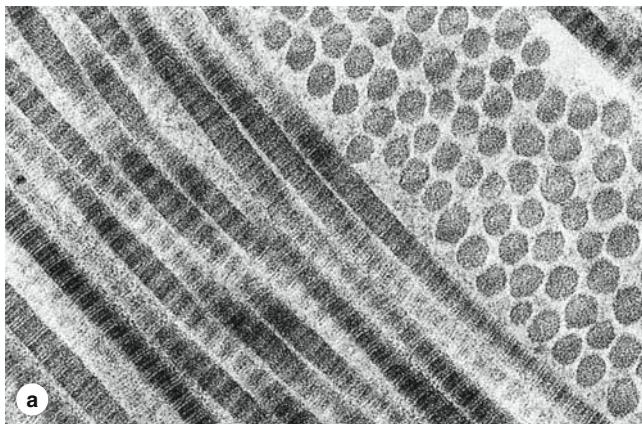
■ **Fibrillar collagens**, notably **collagen types I, II, and III**, have polypeptide subunits that aggregate to form large fibrils clearly visible in the electron or light microscope (Figure 5–8). Collagen type I, the most abundant and widely distributed collagen, forms large, eosinophilic bundles usually called **collagen fibers**. These often

densely fill the connective tissue, forming structures such as tendons, organ capsules, and dermis.

- **Network or sheet-forming collagens** such as **type IV collagen** have subunits produced by epithelial cells and are major structural proteins of external laminae and all epithelial basal laminae.
- **Linking/anchoring collagens** are short collagens that link fibrillar collagens to one another (forming larger fibers) and to other components of the ECM. **Type VII collagen** binds type IV collagen and anchors the basal lamina to the underlying reticular lamina in basement membranes (see Figure 4–3).

TABLE 5–3 Collagen types.

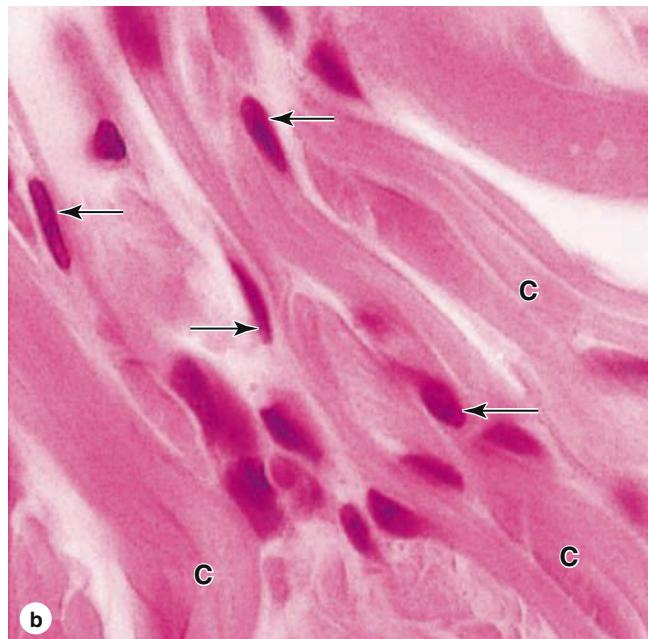
| Type | α -Chain Composition | Structure | Optical Microscopy | Major Location | Main Function |
|------------------------------------|--|--|---|--|--|
| Fibril-Forming Collagens | | | | | |
| I | $[\alpha 1(I)]_2 [\alpha 2(I)]$ | 300-nm molecule, 67-nm banded fibrils | Thick, highly picrosirius birefringent, fibers | Skin, tendon, bone, dentin | Resistance to tension |
| II | $[\alpha 1(II)]_3$ | 300-nm molecule, 67-nm banded fibrils | Loose aggregates of fibrils, birefringent | Cartilage, vitreous body | Resistance to pressure |
| III | $[\alpha 1(III)]_3$ | 67-nm banded fibrils | Thin, weakly birefringent, argyrophilic (silver-binding) fibers | Skin, muscle, blood vessels, frequently together with type I | Structural maintenance in expandable organs |
| V | $[\alpha 1(V)]_3$ | 390-nm molecule, N-terminal globular domain | Frequently forms fiber together with type I | Fetal tissues, skin, bone, placenta, most interstitial tissues | Participates in type I collagen function |
| XI | $[\alpha 1(XI)] [\alpha 2(XI)] [\alpha 3(XI)]$ | 300-nm molecule | Small fibers | Cartilage | Participates in type II collagen function |
| Network-Forming Collagens | | | | | |
| IV | $[\alpha 1(IV)]_2 [\alpha 2(IV)]$ | 2-dimensional cross-linked network | Detected by immunocytochemistry | All basal and external laminae | Support of epithelial cells; filtration |
| X | $[\alpha 1(X)]_3$ | Hexagonal lattices | Detected by immunocytochemistry | Hypertrophic cartilage involved in endochondral bone formation | Increases density of the matrix |
| Linking/Anchoring Collagens | | | | | |
| VII | $[\alpha 1(VII)]_3$ | 450 nm, globular domain at each end | Detected by immunocytochemistry | Epithelial basement membranes | Anchors basal laminae to underlying reticular lamina |
| IX | $[\alpha 1(IX)] [\alpha 2(IX)] [\alpha 3(IX)]$ | 200-nm molecule | Detected by immunocytochemistry | Cartilage, vitreous body | Binds various proteoglycans; associated with type II collagen |
| XII | $[\alpha 1(XII)]_3$ | Large N-terminal domain | Detected by immunocytochemistry | Placenta, skin, tendons | Interacts with type I collagen |
| XIV | $[\alpha 1(XIV)]_3$ | Large N-terminal domain; cross-shaped molecule | Detected by immunocytochemistry | Placenta, bone | Binds type I collagen fibrils, with types V and XII, strengthening fiber formation |

FIGURE 5–8 Type I collagen.

Subunits of type I collagen, the most abundant collagen, assemble to form extremely strong fibrils, which are then bundled together further by other collagens into much larger structures called **collagen fibers**.

(a) TEM shows fibrils cut longitudinally and transversely. In longitudinal sections fibrils display alternating dark and light bands; in cross section the cut ends of individual collagen molecules appear as dots. Ground substance completely surrounds the fibrils. (X100,000)

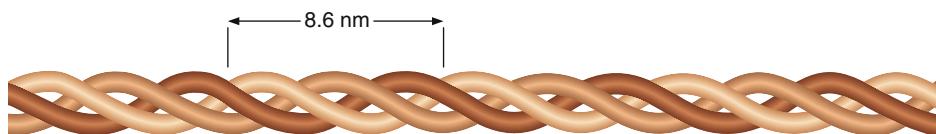
(b) The large bundles of type I collagen fibrils (C) appear as acidophilic collagen fibers in connective tissues, where they



may fill the extracellular space. Subunits for these fibers were secreted by the fibroblasts (arrows) associated with them. (X400; H&E)

Collagen synthesis occurs in many cell types but is a specialty of fibroblasts. The initial **procollagen α chains** are polypeptides made in the RER. Several different α chains of variable lengths and sequences can be synthesized from the related collagen genes. In the ER three α chains are selected, aligned, and stabilized by disulfide bonds at their carboxyl terminals, and folded as a **triple helix**, another defining feature of collagens. The triple helix

undergoes exocytosis and is cleaved to a rodlike **procollagen molecule** (Figure 5–9) that is the basic subunit from which the fibers or sheets are assembled. These subunits may be homotrimeric, with all three chains identical, or heterotrimeric, with two or all three chains having different sequences. Different combinations of procollagen α chains produce the various types of collagen with different structures and functional properties.

FIGURE 5–9 The collagen subunit.

In the most abundant form of collagen, type I, each procollagen molecule or subunit has two $\alpha 1$ - and one $\alpha 2$ -peptide chains, each with a molecular mass of approximately 100 kDa, intertwined in a right-handed helix and held together by hydrogen bonds and

hydrophobic interactions. The length of each molecule (sometimes called tropocollagen) is 300 nm, and its width is 1.5 nm. Each complete turn of the helix spans a distance of 8.6 nm.

» MEDICAL APPLICATION

A **keloid** is a local swelling caused by abnormally large amounts of collagen that form in scars of the skin. Keloids occur most often in individuals of African descent and can be a troublesome clinical problem to manage. Not only can they be disfiguring, but excision is almost always followed by recurrence.

An unusually large number of posttranslational processing steps are required to prepare collagen for its final assembly in the ECM. These steps have been studied most thoroughly for type I collagen, which accounts for 90% of all the body's collagen. The most important parts of this process are summarized in Figure 5–10 and described briefly here:

1. The procollagen α chains are produced on polyribosomes of the RER and translocated into the cisternae. These typically have long central domains rich in proline and lysine; in type I collagen every third amino acid is glycine.
2. **Hydroxylase** enzymes in the ER cisternae add hydroxyl (-OH) groups to some prolines and lysines in reactions that require O_2 , Fe^{2+} , and ascorbic acid (vitamin C) as cofactors.
3. Glycosylation of some hydroxylysine residues also occurs, to different degrees in various collagen types.
4. Both the amino- and carboxyl-terminal sequences of α chains have globular structures that lack the gly-X-Y repeats. In the RER the C-terminal regions of three selected α chains ($\alpha 1$, $\alpha 2$) are stabilized by cysteine disulfide bonds, which align the three polypeptides and facilitates their central domains folding as the triple helix. With its globular terminal sequences intact, the trimeric procollagen molecule is transported through the Golgi apparatus, packaged in vesicles and secreted.
5. Outside the cell, specific proteases called **procollagen peptidases** remove the terminal globular peptides, converting the procollagen molecules to collagen molecules. These now self-assemble (an entropy-driven process) into polymeric collagen fibrils, usually in specialized niches near the cell surface.
6. Certain proteoglycans and other collagens (eg, types V and XII) associate with the new collagen fibrils, stabilize these assemblies, and promote the formation of larger fibers from the fibrils.
7. Fibrillar structure is reinforced and disassembly is prevented by the formation of covalent cross-links between the collagen molecules, a process catalyzed by **lysyl oxidase**.

The other fibrillar and sheetlike collagens are formed in processes similar to that described for collagen type I and stabilized by linking or anchoring collagens. Because there are so

many steps in collagen biosynthesis, there are many points at which the process can be interrupted or changed by defective enzymes or by disease processes (Table 5–4).

Type I collagen fibrils have diameters ranging from 20 to 90 nm and can be several micrometers in length. Adjacent rod-like collagen subunits of the fibrils are staggered by 67 nm, with small gaps (lacunar regions) between their ends (Figure 5–11). This structure produces a characteristic feature of type I collagen visible by EM: transverse striations with a regular periodicity (Figure 5–11). Type I collagen fibrils assemble further to form large, extremely strong collagen fibers that may be further bundled by linking collagens and proteoglycans. Collagen type II (present in cartilage) occurs as fibrils but does not form fibers or bundles. Sheet-forming collagen type IV subunits assemble as a lattice-like network in epithelial basal laminae.

When they fill the ECM (eg, in tendons or the sclera of the eye), bundles of collagen appear white. The highly regular orientation of subunits makes collagen fibers birefringent with polarizing microscopy (see Figure 1–7). In routine light microscopy collagen fibers are acidophilic, staining pink with eosin, blue with Mallory trichrome stain, and red with Sirius red. Because collagen bundles are long and tortuous, their length and diameter are better studied in spread preparations rather than sections, as shown in Figure 1–7a. Mesentery is frequently used for this purpose; when spread on a slide, this structure is sufficiently thin to let the light pass through; it can be stained and examined directly under the microscope.

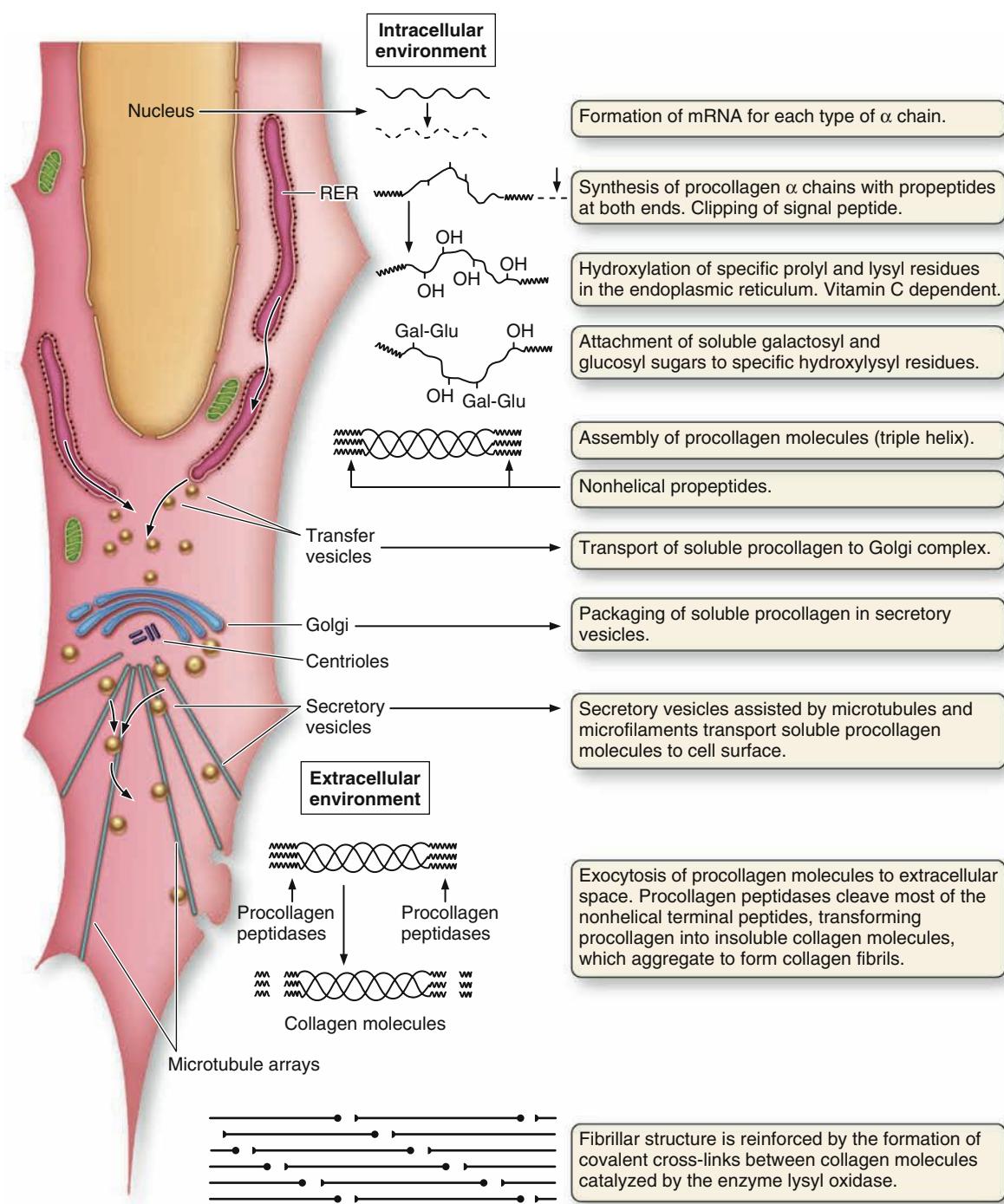
Collagen turnover and renewal in normal connective tissue is generally a very slow but ongoing process. In some organs, such as tendons and ligaments, the collagen is very stable, whereas in others, as in the periodontal ligament surrounding teeth, the collagen turnover rate is high. To be renewed, the collagen must first be degraded. Degradation is initiated by specific enzymes called **collagenases**, which are members of an enzyme class called **matrix metalloproteinases (MMPs)**, which clip collagen fibrils or sheets in such a way that they are then susceptible to further degradation by nonspecific proteases. Various MMPs are secreted by macrophages and play an important role in remodeling the ECM during tissue repair.

» MEDICAL APPLICATION

Normal collagen function depends on the expression of many different genes and adequate execution of several posttranslational events. It is not surprising, therefore, that many pathologic conditions are directly attributable to insufficient or abnormal collagen synthesis. A few such genetic disorders or conditions are listed in Table 5–4.

Reticular Fibers

Found in delicate connective tissue of many organs, notably in the immune system, **reticular fibers** consist mainly of collagen type III, which forms an extensive network (reticulum) of

FIGURE 5–10 Collagen synthesis.

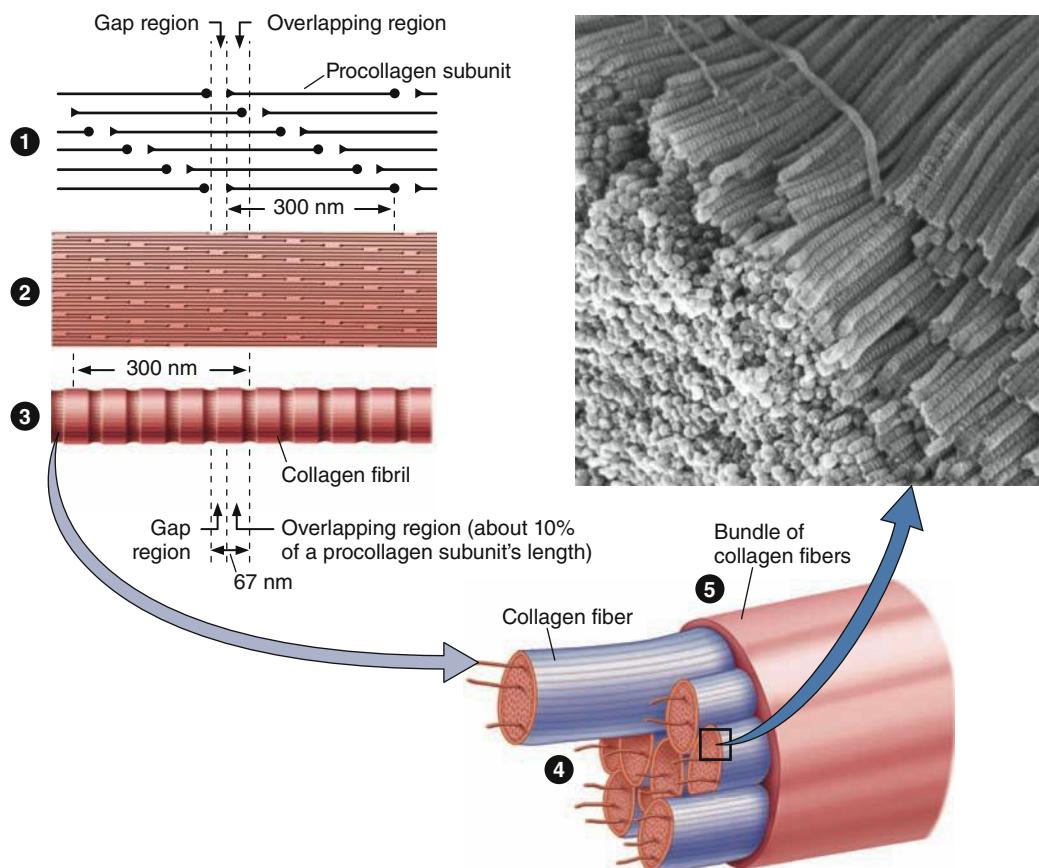
Hydroxylation and glycosylation of procollagen α chains and their assembly into triple helices occur in the RER, and further assembly into fibrils occurs in the ECM after secretion of procollagen. Because there are many slightly different genes for procollagen

α chains and collagen production depends on several posttranslational events involving several other enzymes, many diseases involving defective collagen synthesis have been described.

TABLE 5–4

Examples of clinical disorders resulting from defects in collagen synthesis.

| Disorder | Defect | Symptoms |
|-------------------------|---|---|
| Ehlers-Danlos type IV | Faulty transcription or translation of collagen type III | Aortic and/or intestinal rupture |
| Ehlers-Danlos type VI | Faulty lysine hydroxylation | Increased skin elasticity, rupture of eyeball |
| Ehlers-Danlos type VII | Decrease in procollagen peptidase activity | Increased articular mobility, frequent luxation |
| Scurvy | Lack of vitamin C, a required cofactor for prolyl hydroxylase | Ulceration of gums, hemorrhages |
| Osteogenesis imperfecta | Change of 1 nucleotide in genes for collagen type I | Spontaneous fractures, cardiac insufficiency |

FIGURE 5–11 Assembly of type I collagen.

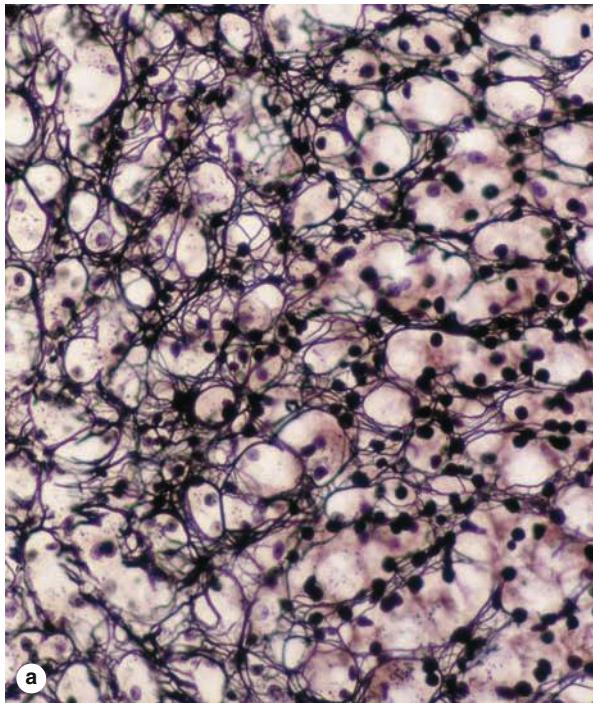
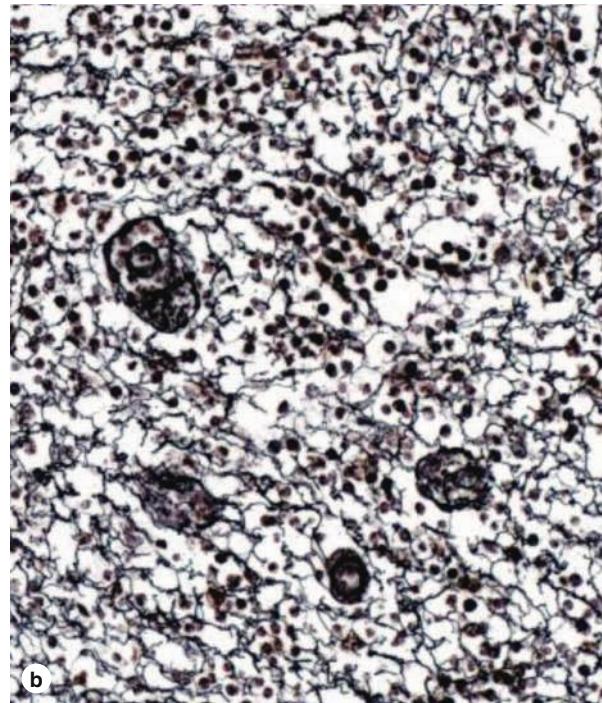
Shown here are the relationships among type I collagen molecules, fibrils, fibers, and bundles.

1. Rodlike triple-helix collagen molecules, each 300-nm long, self-assemble in a highly organized, lengthwise arrangement of overlapping regions.
2. The regular, overlapping arrangement of subunits continues as large collagen fibrils are assembled.
3. This structure causes fibrils to have characteristic cross striations with alternating dark and light bands when observed in the EM.

4. Fibrils assemble further and are linked together in larger collagen fibers visible by light microscopy.

5. Type I fibers often form into still larger aggregates bundled and linked together by other collagens.

The photo shows an SEM view of type I collagen fibrils closely aggregated as part of a collagen fiber. Striations are visible on the surface of the fibrils.

FIGURE 5–12 Reticular fibers.**a****b**

In these silver-stained sections of adrenal cortex (a) and lymph node (b), networks of delicate, black **reticular fibers** are prominent. These fibers serve as a supportive stroma in most lymphoid and hematopoietic organs and many endocrine glands. The fibers consist of

type III collagen that is heavily glycosylated, producing the black argyrophilia. Cell nuclei are also dark, but cytoplasm is unstained. (X100) Fibroblasts specialized for reticular fiber production in hematopoietic and lymphoid organs are often called reticular cells.

thin (diameter 0.5–2 µm) fibers for the support of many different cells. Reticular fibers are seldom visible in hematoxylin and eosin (H&E) preparations but are characteristically stained black after impregnation with silver salts (Figure 5–12) and are thus termed **argyrophilic** (Gr. *argyros*, silver). Reticular fibers are also periodic acid-Schiff (PAS) positive, which, like argyrophilia, is due to the high content of sugar chains bound to type III collagen α chains. Reticular fibers contain up to 10% carbohydrate as opposed to 1% in most other collagen fibers.

Reticular fibers produced by fibroblasts occur in the reticular lamina of basement membranes and typically also surround adipocytes, smooth muscle and nerve fibers, and small blood vessels. Delicate reticular networks serve as the supportive stroma for the parenchymal secretory cells and rich microvasculature of the liver and endocrine glands. Abundant reticular fibers also characterize the stroma of hematopoietic tissue (bone marrow), the spleen, and lymph nodes where they support rapidly changing populations of proliferating cells and phagocytic cells.

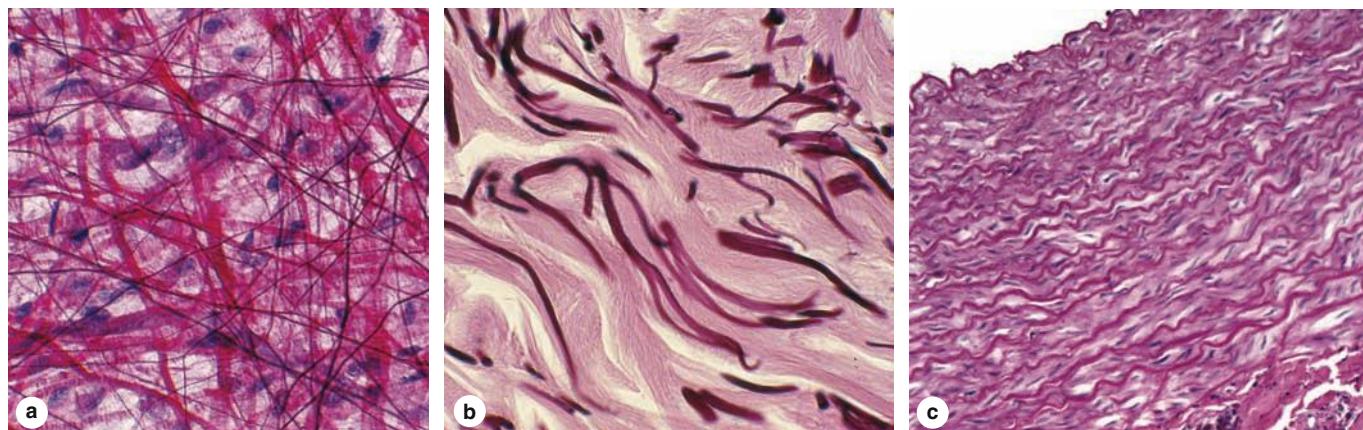
Elastic Fibers

Elastic fibers are also thinner than the type I collagen fibers and form sparse networks interspersed with collagen bundles

in many organs, particularly those subject to regular stretching or bending. As the name implies, elastic fibers have rubberlike properties that allow tissue containing these fibers, such as the stroma of the lungs, to be stretched or distended and return to their original shape. In the wall of large blood vessels, especially arteries, elastin also occurs as fenestrated sheets called **elastic lamellae**. Elastic fibers and lamellae are not strongly acidophilic and stain poorly with H&E; they are stained more darkly than collagen with other stains such as orcein and aldehyde fuchsin (Figure 5–13).

Elastic fibers (and lamellae) are a composite of **fibrillin** (350 kDa), which forms a network of **microfibrils**, embedded in a larger mass of cross-linked **elastin** (60 kDa). Both proteins are secreted from fibroblasts (and smooth muscle cells in vascular walls) and give rise to elastic fibers in a stepwise manner as shown in Figure 5–14. Initially, microfibrils with diameters of 10 nm form from fibrillin and various glycoproteins. The microfibrils act as scaffolding upon which elastin is then deposited. Elastin accumulates around the microfibrils, eventually making up most of the elastic fiber, and is responsible for the rubberlike property.

The elastic properties of these fibers and lamellae result from the structure of the elastin subunits and the unique

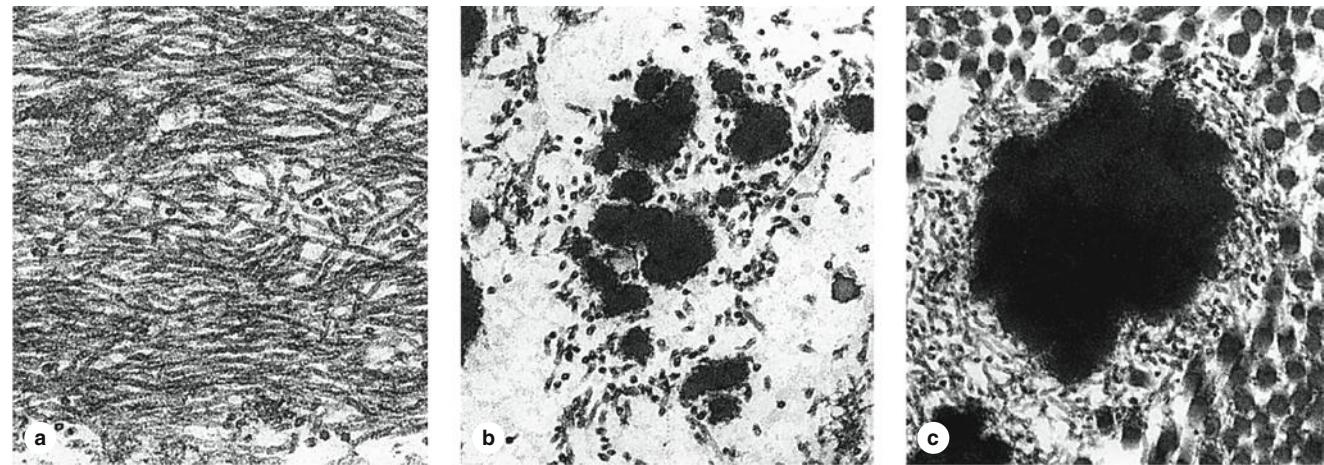
FIGURE 5–13 Elastic fibers.

Elastic fibers or lamellae (sheets) add resiliency to connective tissue. Such fibers may be difficult to discern in H&E-stained tissue, but elastin has a distinct, darker-staining appearance with other staining procedures.

(a) The length, diameter, distribution, and density of dark **elastic fibers** are easily seen in this spread preparation of nonstretched connective tissue in a mesentery. (X200; Hematoxylin and orcein)

(b) In sectioned tissue at higher magnification, **elastic fibers** can be seen among the acidophilic collagen bundles of dermis. (X400; Aldehyde fuchsin)

(c) **Elastic lamellae** in the wall of the aorta are more darkly stained, incomplete sheets of elastin between the layers of eosinophilic smooth muscle. (X80; H&E)

FIGURE 5–14 Formation of elastic fibers.

Stages in the formation of elastic fibers can be seen by TEM.

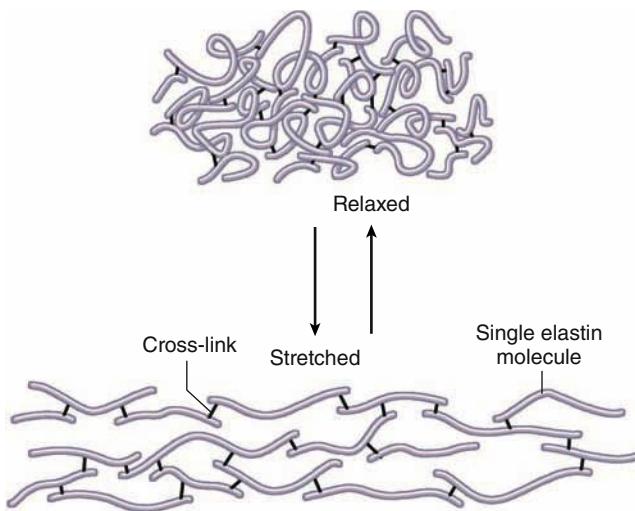
(a) Initially, a developing fiber consists of many 10-nm-diameter **microfibrils** composed of **fibrillin** subunits secreted by fibroblasts and smooth muscle cells.

(b) **Elastin** is deposited on the scaffold of microfibrils, forming growing, amorphous composite structures. The elastin molecules

are also secreted by the fibroblasts and quickly become covalently cross-linked into larger assemblies.

(c) Elastin accumulates and ultimately occupies most of the electron-dense center of the single elastic fiber shown here. Fibrillin microfibrils typically remain visible at the fiber surface. Collagen fibrils, seen in cross section, are also present surrounding the elastic fiber. (All X50,000)

FIGURE 5–15 Molecular basis of elastic fiber elasticity.



The diagram shows a small piece of an elastic fiber, in two conformations. Elastin polypeptides, the major components of elastic fibers, have multiple random-coil domains that straighten or stretch under force, and then relax. Most of the cross-links between elastin subunits consist of the covalent, cyclic structure **desmosine**, each of which involves four converted lysines in two elastin molecules. This unusual type of protein cross-link holds the aggregate together with little steric hindrance to elastin movements. These properties give the entire network its elastic quality.

cross-links holding them together. Elastin molecules have many lysine-rich regions interspersed with hydrophobic domains rich in lysine and proline which are thought to form extensible, random-coil conformations (like natural rubber). During deposition on the fibrillin microfibrils, lysyl oxidase converts the lysines' amino groups to aldehydes and four oxidized lysines on neighboring elastin molecules then condense covalently as a **desmosine** ring, cross-linking the polypeptides. Bound firmly by many desmosine rings, but maintaining the rubberlike properties of their hydrophobic domains, elastic fibers stretch reversibly when force is applied (Figure 5–15). Elastin resists digestion by most proteases, but it is hydrolyzed by pancreatic **elastase**.

» MEDICAL APPLICATION

Fibrillins comprise a family of proteins involved in making the scaffolding necessary for the deposition of elastin. Mutations in the fibrillin genes result in **Marfan syndrome**, a disease characterized by a lack of resistance in tissues rich in elastic fibers. Because the walls of large arteries are rich in elastic components and because the blood pressure is high in the aorta, patients with this disease often experience aortic swellings called **aneurysms**, which are life-threatening conditions.

» GROUND SUBSTANCE

The **ground substance** of the ECM is a highly hydrated (with much bound water), transparent, complex mixture of three major kinds of macromolecules: **glycosaminoglycans (GAGs)**, **proteoglycans**, and **multiadhesive glycoproteins**. Filling the space between cells and fibers in connective tissue, ground substance allows diffusion of small molecules and, because it is viscous, acts as both a lubricant and a barrier to the penetration of invaders. Physical properties of ground substance also profoundly influence various cellular activities. When adequately fixed for histologic analysis, its components aggregate as fine, poorly resolved material that appears in TEM preparations as electron-dense filaments or granules (Figure 5–16a).

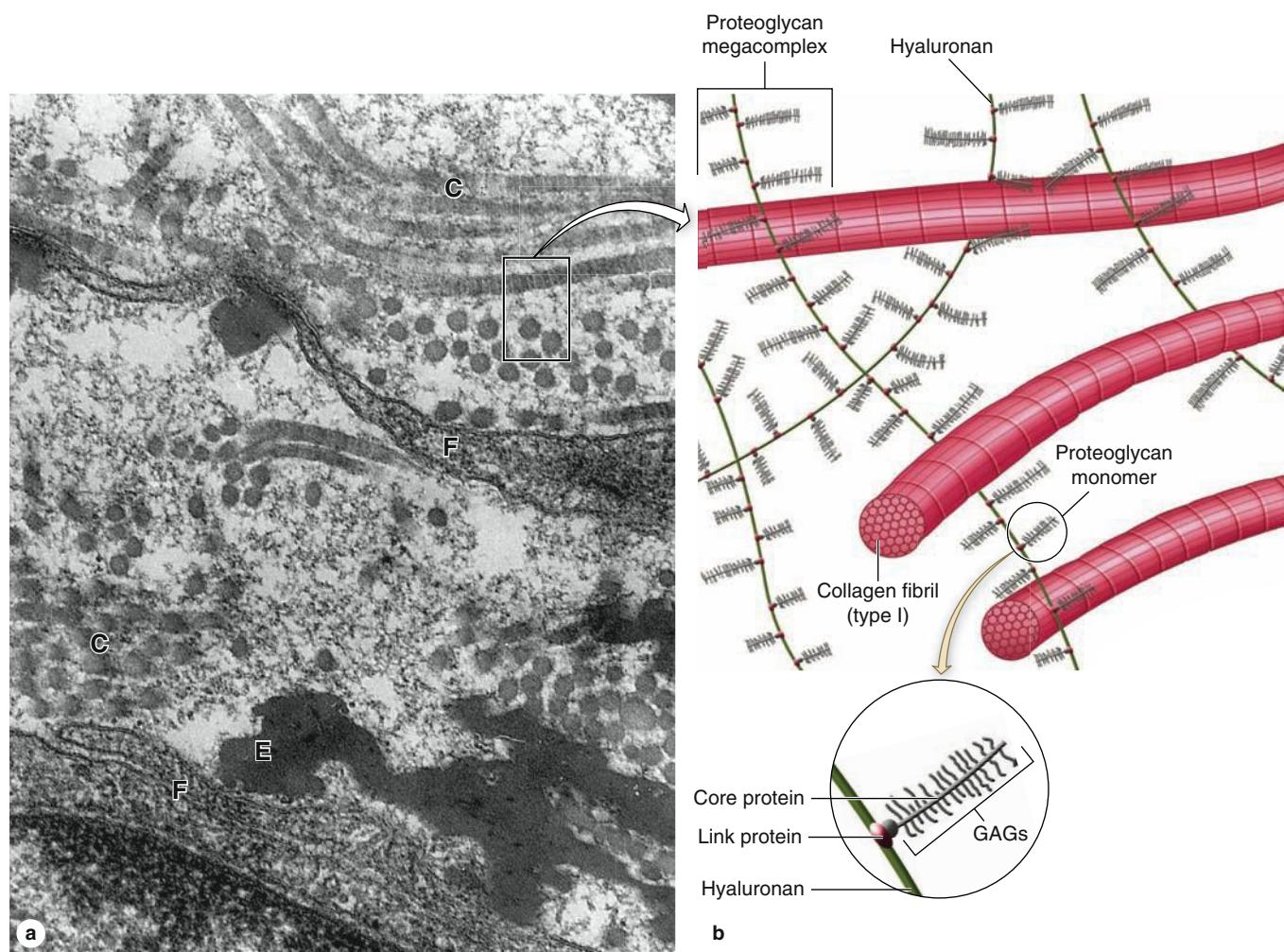
GAGs (also called mucopolysaccharides) are long polymers of repeating disaccharide units, usually a hexosamine and uronic acid. The hexosamine can be glucosamine or galactosamine, and the uronic acid can be glucuronate or iduronate. The largest and most ubiquitous GAG is **hyaluronan** (also called hyaluronate or hyaluronic acid). With a molecular weight from 100s to 1000s of kDa, hyaluronan is a very long polymer of the disaccharide glucosamine-glucuronate. Uniquely among GAGs, hyaluronan is synthesized directly into the ECM by an enzyme complex, **hyaluronan synthase**, located in the cell membrane of many cells. Hyaluronan forms a viscous, pericellular network which binds a considerable amount of water, giving it an important role in allowing molecular diffusion through connective tissue and in lubricating various organs and joints.

All other GAGs are much smaller (10–40 kDa), sulfated, bound to proteins (as parts of proteoglycans), and are synthesized in Golgi complexes. The four major GAGs found in proteoglycans are **dermatan sulfate**, **chondroitin sulfates**, **keratan sulfate**, and **heparan sulfate**, all of which have different disaccharide units modified further with carboxyl and sulfate groups and different tissue distributions (Table 5–5). Their high negative charge forces GAGs to an extended conformation and causes them to sequester cations as well as water. These features provide GAGs with space-filling, cushioning, and lubricant functions.

Proteoglycans consist of a core protein to which are covalently attached various numbers and combinations of the sulfated GAGs. Like glycoproteins, they are synthesized on RER, mature in the Golgi apparatus, where the GAG side-chains are added, and secreted from cells by exocytosis. Unlike glycoproteins, proteoglycans have attached GAGs which often comprise a greater mass than the polypeptide core. As shown in Figure 5–16b, after secretion proteoglycans become bound to the hyaluronan by link proteins and their GAG side-chains associate further with collagen fibers and other ECM components.

Proteoglycans are distinguished by their diversity, which is generated in part by enzymatic differences in the Golgi complexes. A region of ECM may contain several different core proteins, each with one or many sulfated GAGs of different lengths and composition. As mentioned with epithelia, **perlecan** is the key proteoglycan in all basal laminae. One of the

FIGURE 5–16 Ground substance of the extracellular matrix (ECM).



(a) TEM of connective tissue ECM reveals **ground substance** as areas containing only fine granular material among the collagen (C) fibers, elastic (E) fibers and fibroblast processes (F). X100,000.

(b) As shown here schematically, connective tissue ground substance contains a vast complex of **proteoglycans** linked to very long **hyaluronan** molecules. Each proteoglycan monomer has a **core protein** with a few or many side chains of the sulfated glycosaminoglycans (GAGs) listed in Table 5–5. Synthesized in

the RER and Golgi apparatus like glycoproteins, proteoglycan monomers are distinguished by often being more heavily glycosylated and by the addition and sulfation of GAGs, which vary significantly among proteoglycans in their number, length, and the degree to which the sugar polymers are modified. The large proteoglycan **aggrecan** (25,000 kDa) typically has about 50 chains of keratan sulfate chains and twice that number of chondroitin sulfate.

best studied proteoglycans, **aggrecan**, is very large (250 kDa), having a core protein heavily bound with chondroitin and keratan sulfate chains. A link protein joins aggrecan to hyaluronan (Figure 5–16b). Abundant in cartilage, aggrecan-hyaluronan complexes fill the space between collagen fibers and cells and contribute greatly to the physical properties of this tissue. Other proteoglycans include decorin, with very few GAG side chains that binds the surface of type I collagen fibrils, and syndecan, with an integral membrane core protein providing an additional attachment of ECM to cell membranes.

Embryonic mesenchyme (Figure 5–1) is very rich in hyaluronan and water, producing the characteristic wide spacing of cells and a matrix ideal for cell migrations and growth. In both developing and mature connective tissues, core proteins and GAGs (especially heparan sulfate) of many proteoglycans bind and sequester various growth factors and other signaling proteins. Degradation of such proteoglycans during the early phase of tissue repair releases these stored growth factors which then help stimulate new cell growth and ECM synthesis.

TABLE 5-5

Composition and distribution of glycosaminoglycans in connective tissue and their interactions with collagen fibers.

| Glycosaminoglycan | Repeating Disaccharides | | Distribution | Electrostatic Interaction with Collagen |
|-----------------------|--------------------------------------|-----------------|---|---|
| | Hexuronic Acid | Hexosamine | | |
| Hyaluronic acid | D-glucuronic acid | D-glucosamine | Umbilical cord, synovial fluid, vitreous humor, cartilage | |
| Chondroitin 4-sulfate | D-glucuronic acid | D-galactosamine | Cartilage, bone, cornea, skin, notochord, aorta | High levels of interaction, mainly with collagen type II |
| Chondroitin 6-sulfate | D-glucuronic acid | D-galactosamine | Cartilage, umbilical cord, skin, aorta (media) | High levels of interaction, mainly with collagen type II |
| Dermatan sulfate | L-iduronic acid or D-glucuronic acid | D-galactosamine | Skin, tendon, aorta (adventitia) | Low levels of interaction, mainly with collagen type I |
| Heparan sulfate | D-glucuronic acid or L-iduronic acid | D-galactosamine | Aorta, lung, liver, basal laminae | Intermediate levels of interaction, mainly with collagen types III and IV |
| Keratan sulfate | D-galactose | D-glucosamine | Cartilage, nucleus pulposus, annulus fibrosus | None |

» MEDICAL APPLICATION

The degradation of proteoglycans is carried out by several cell types and depends in part on the presence of several lysosomal enzymes. Several disorders have been described, including a deficiency in certain lysosomal enzymes that degrade specific GAGs, with the subsequent accumulation of these macromolecules in tissues. The lack of specific hydrolases in the lysosomes has been found to be the cause of several disorders, including the **Hurler**, **Hunter**, **Sanfilippo**, and **Morquio syndromes**.

Because of their high viscosity, hyaluronan and proteoglycans tend to form a barrier against bacterial penetration of tissues. Bacteria that produce hyaluronidase, an enzyme that hydrolyzes hyaluronan and disassembles proteoglycans complexes, reduce the viscosity of the connective tissue ground substance and have greater invasive power.

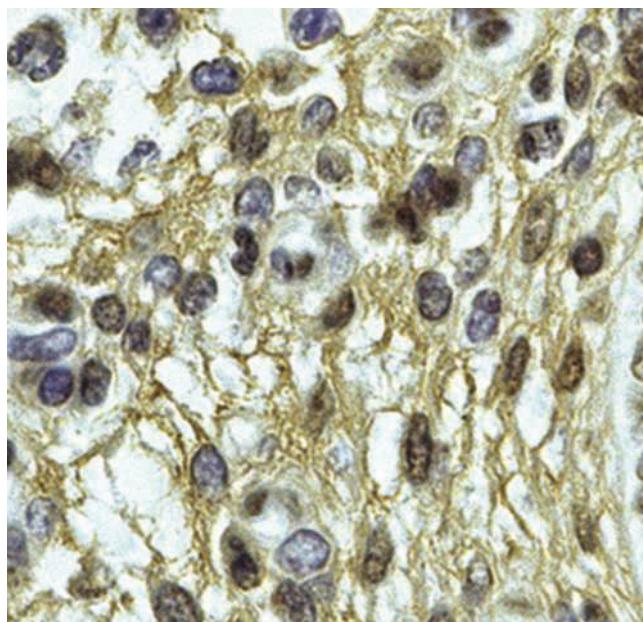
Making up the third major class of ground substance macromolecules, **multiadhesive glycoproteins** all have multiple binding sites for cell surface integrins and for other matrix macromolecules. The adhesive glycoproteins are large molecules with branched oligosaccharide chains and allow adhesion of cells to their substrate. An example is the large (200–400 kDa), trimeric glycoprotein **laminin** with binding sites for integrins, type IV collagen, and specific proteoglycans, providing adhesion for epithelial and other cells. As described in the previous chapter, all basal and external laminae are rich in laminin, which is essential for the assembly and maintenance of these structures.

Another glycoprotein, **fibronectin** (L. *fibra*, fiber + *nexus*, interconnection), is a 235–270 kDa dimer synthesized largely by fibroblasts, with binding sites for collagens and certain GAGs, and forms insoluble fibrillar networks throughout connective tissue (Figure 5–17). The fibronectin substrate provides specific binding sites for integrins and is important both for cell adhesion and cellular migration through the ECM.

As briefly described in Chapter 2 integrins are integral membrane proteins that act as matrix receptors for specific sequences on laminin, fibronectin, some collagens, and certain other ECM proteins. Integrins bind their ECM ligands with relatively low affinity, allowing cells to explore their environment without losing attachment to it or becoming glued to it. All are heterodimers with two transmembrane polypeptides: the α and β chains. Great diversity in the subsets of integrin α and β chains which cells express allows cells to have different specific ECM ligands.

Integrin-microfilament complexes are clustered in fibroblasts and other mesenchymal cells to form structures called **focal adhesions** that can be seen by TEM or immunocytochemistry. As mentioned in Chapter 4 this type of adhesive junction is typically present at the ends of actin filaments bundled by α -actinin as cytoplasmic stress fibers and focal adhesion kinases provide a mechanism by which pulling forces or other physical properties of the ECM can change various cellular activities.

Water in the ground substance of connective tissue is referred to as **interstitial fluid** and has an ion composition similar to that of blood plasma. Interstitial fluid also contains plasma proteins of low molecular weight that pass through

FIGURE 5–17 Fibronectin localization.

Like laminin of basement membranes, **fibronectin** is a multiahesive glycoprotein, with binding sites for ECM components and for integrins at cell surfaces, and has important roles in cell migration and the maintenance of tissue structure. As shown here by immunohistochemistry, fibronectin forms a fine network throughout the ECM of connective tissue. (X400)

the thin walls of the smallest blood vessels, the capillaries. Although only a small proportion of connective tissue proteins are plasma proteins, it is estimated that as much as one-third of the body's plasma proteins are normally found in the interstitial fluid of connective tissue because of its large volume and wide distribution.

» MEDICAL APPLICATION

Edema is the excessive accumulation of interstitial fluid in connective tissue. This water comes from the blood, passing through the capillary walls that become more permeable during inflammation and normally produces at least slight swelling.

Capillaries in connective tissue also bring the various nutrients required by cells and carry away their metabolic waste products to the detoxifying and excretory organs, the liver and kidneys. Interstitial fluid is the solvent for these substances.

As shown in Figure 5–18, two main forces act on the water in capillaries:

- The **hydrostatic pressure** of the blood caused by the pumping action of the heart, which forces water out across the capillary wall
- The colloid **osmotic pressure** produced by plasma proteins such as albumin, which draws water back into the capillaries

The colloid osmotic pressure exerted by the blood proteins—which are unable to pass through the capillary walls—tends to pull back into the capillary the water forced out by hydrostatic pressure (Figure 5–18). (Because the ions and low-molecular-weight compounds that pass easily through the capillary walls have similar concentrations inside and outside these blood vessels, the osmotic pressures they exert are approximately equal on either side of the capillaries and cancel each other.)

The quantity of water drawn back into capillaries is often less than that which was forced out. This excess fluid does not normally accumulate in connective tissue but drains continuously into lymphatic capillaries that eventually return it to the blood. Discussed later with the lymphoid system, lymphatic capillaries originate in connective tissue as delicate endothelial tubes (Figure 5–18).

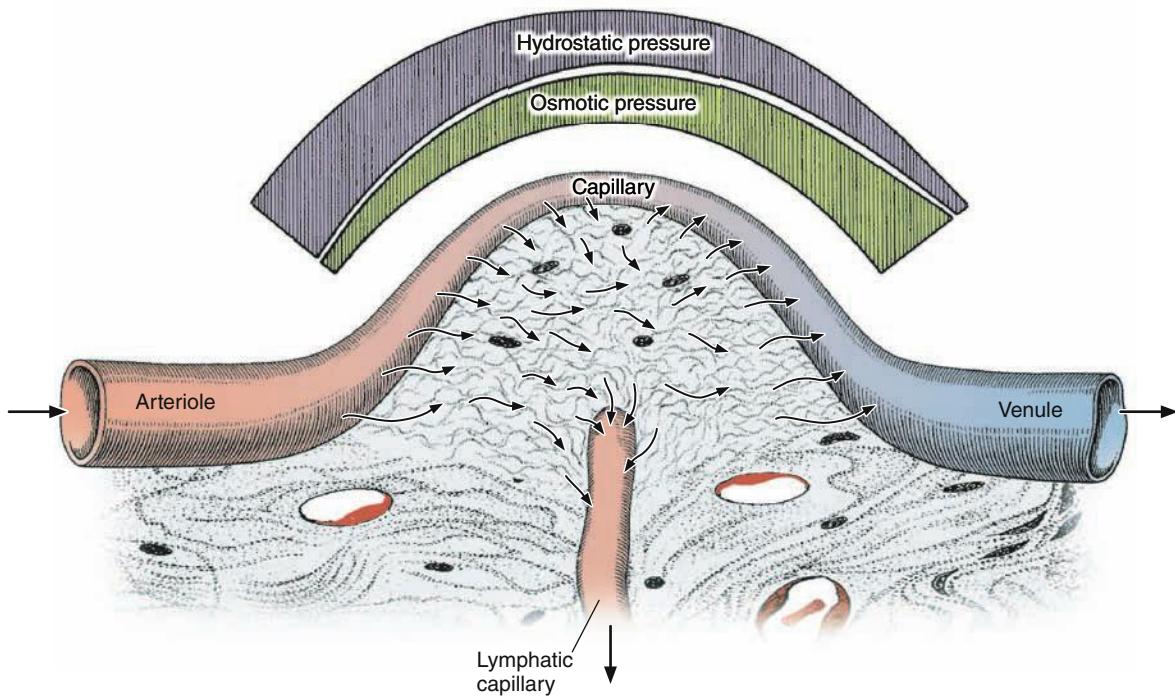
➤ TYPES OF CONNECTIVE TISSUE

Different combinations and densities of the cells, fibers, and other ECM components produce graded variations in histological structure within connective tissue. Descriptive names or classifications used for the various types of connective tissue typically denote either a structural characteristic or a major component. Table 5–6 gives a classification commonly used for the main types of connective tissue. Adipose tissue, an important specialized connective tissue, and two other supporting tissues, cartilage and bone, are covered in Chapters 6, 7, and 8.

Connective Tissue Proper

Connective tissue proper is broadly classified as “loose” or “dense,” terms which refer to the amount of collagen present (Figure 5–19). **Loose connective tissue** is common, forming a layer beneath the epithelial lining of many organs and filling the spaces between fibers of muscle and nerve (Figure 5–19).

Also called **areolar tissue**, the loose connective tissue typically contains cells, fibers, and ground substance in roughly equal parts. The most numerous cells are fibroblasts, but the other types of connective tissue cells are also normally found, along with nerves and small blood vessels. Collagen fibers predominate, but elastic and reticular fibers are also present. With at least a moderate amount of ground substance,

FIGURE 5–18 Movement of fluid in connective tissue.

Water normally passes through capillary walls into the ECM of surrounding connective tissues primarily at the arterial end of a capillary, because the **hydrostatic pressure** is greater than the colloid **osmotic pressure**. However, hydrostatic pressure decreases toward the venous end of the capillary, as indicated at the top of the figure. The fall in hydrostatic pressure parallels a rise in osmotic pressure of the capillary blood because the plasma protein concentration increases as water is pushed out across the capillary wall.

As a result of the increased protein concentration and decreased hydrostatic pressure, osmotic pressure at the venous

end is greater than hydrostatic pressure and water is drawn back into the capillary. In this way plasma and interstitial fluid constantly mix, nutrients in blood circulate to cells in connective tissue, and cellular wastes are removed.

Not all water that leaves capillaries by hydrostatic pressure is drawn back in by osmotic pressure. This excess tissue fluid is normally drained by the lymphatic capillaries, open-ended vessels that arise in connective tissue and enter the one-way lymphatic system that eventually delivers the fluid (now called **lymph**) back to veins.

loose connective tissue has a delicate consistency; it is flexible and not very resistant to stress.

Dense connective tissue has similar components as loose connective tissue, but with fewer cells, mostly fibroblasts, and a clear predominance of bundled type I collagen fibers over ground substance. The abundance of collagen here protects organs and strengthens them structurally. In **dense irregular connective tissue** bundles of collagen fibers appear randomly interwoven, with no definite orientation. The tough three-dimensional collagen network provides resistance to stress from all directions. Examples of dense irregular connective tissue include the deep dermis layer of skin and capsules surrounding most organs. Dense irregular and loose connective tissues are often closely associated, with the two types grading

into each other and making distinctions between them somewhat arbitrary (Figure 5–19).

Dense regular connective tissue consists mostly of type I collagen bundles and fibroblasts aligned in parallel for great resistance to prolonged or repeated stresses from the same direction (Figure 5–20).

The best examples of dense regular connective tissue are the very strong and flexible **tendons** (Figure 5–20), cords connecting muscles to bones; **aponeuroses**, which are sheet-like tendons; and **ligaments**, bands or sheets that hold together components of the skeletal system. Consisting almost entirely of densely packed parallel collagen fibers separated by very little ground substance and having very few blood vessels, these inextensible structures are white in

TABLE 5–6**Classification of connective or supporting tissues.**

| | General Organization | Major Functions | Examples |
|--|--|---|---|
| Connective Tissue Proper | | | |
| Loose (areolar) connective tissue | Much ground substance; many cells and little collagen, randomly distributed | Supports microvasculature, nerves, and immune defense cells | Lamina propria beneath epithelial lining of digestive tract |
| Dense irregular connective tissue | Little ground substance; few cells (mostly fibroblasts); much collagen in randomly arranged fibers | Protects and supports organs; resists tearing | Dermis of skin, organ capsules, submucosa layer of digestive tract |
| Dense regular connective tissue | Almost completely filled with parallel bundles of collagen; few fibroblasts, aligned with collagen | Provide strong connections within musculoskeletal system; strong resistance to force | Ligaments, tendons, aponeuroses, corneal stroma |
| Embryonic Connective Tissues | | | |
| Mesenchyme | Sparse, undifferentiated cells, uniformly distributed in matrix with sparse collagen fibers | Contains stem/progenitor cells for all adult connective tissue cells | Mesodermal layer of early embryo |
| Mucoid (mucous) connective tissue | Random fibroblasts and collagen fibers in viscous matrix | Supports and cushions large blood vessels | Matrix of the fetal umbilical cord |
| Specialized Connective Tissues | | | |
| Reticular connective tissue (see Chapter 14) | Delicate network of reticulin/collagen III with attached fibroblasts (reticular cells) | Supports blood-forming cells, many secretory cells, and lymphocytes in most lymphoid organs | Bone marrow, liver, pancreas, adrenal glands, all lymphoid organs except the thymus |
| Adipose Tissue (Chapter 6) | | | |
| Cartilage (Chapter 7) | | | |
| Bone (Chapter 8) | | | |
| Blood (Chapter 12) | | | |

the fresh state. Fibrocytes with elongated nuclei lie parallel to the collagen fibers of dense regular connective tissue, with cytoplasmic folds enveloping portions of the collagen bundles (Figure 5–20b). Cytoplasm in these “**tendinocytes**” is difficult to distinguish in H&E-stained preparations because it is very sparse and has acidophilia like that of the collagen. In aponeuroses the parallel bundles of collagen exist as multiple layers alternating at 90° angles to one another. Some ligaments, such as the elastic ligaments along the vertebral column, contain besides collagen many parallel bundles of elastic fibers.

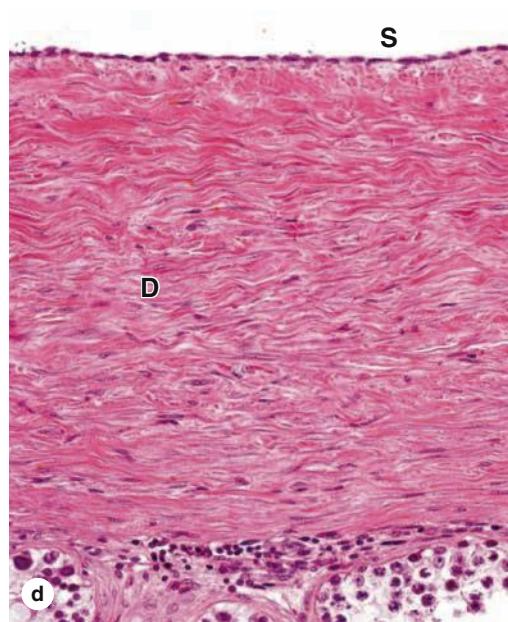
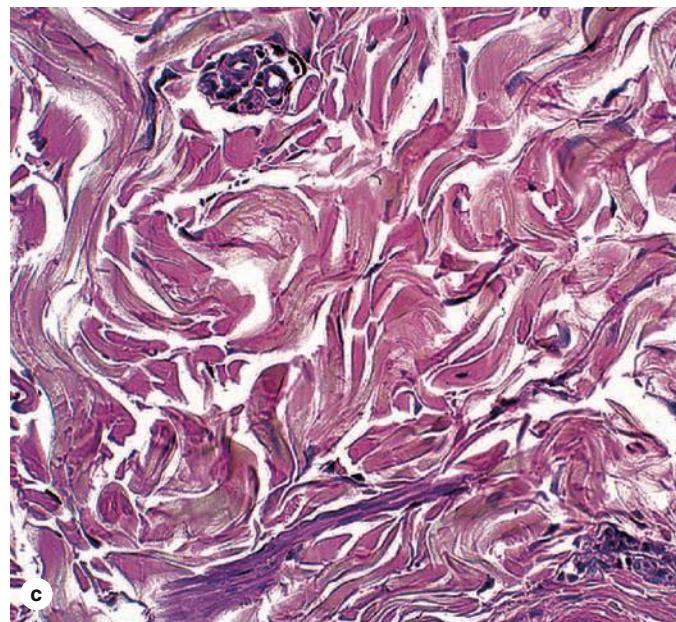
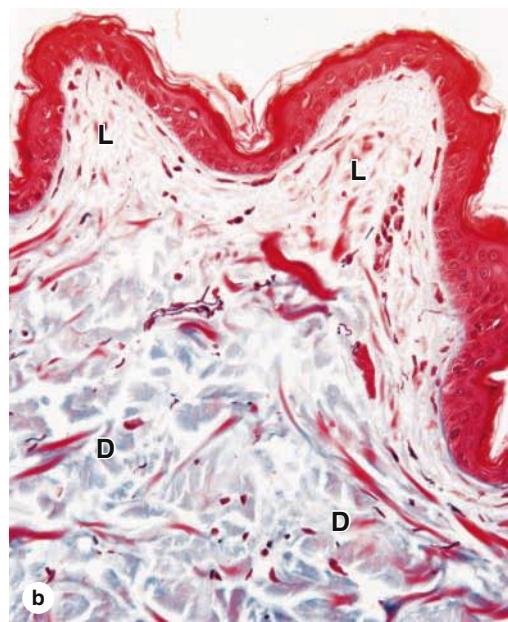
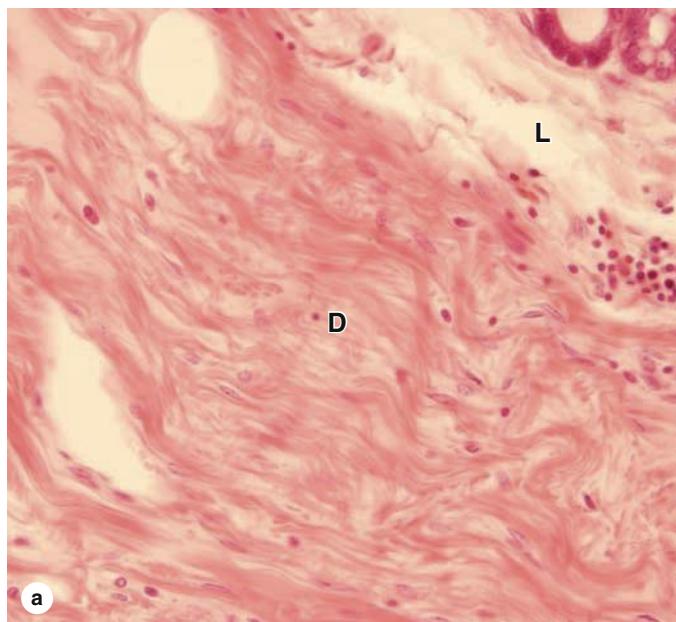
On their outer surface tendons and ligaments have a layer of dense irregular connective tissue that is continuous with the outermost layers of the adjacent muscles and bones. Collagen bundles vary in size in different tendons and ligaments, but all regular connective tissue structures are poorly vascularized and repair of damage in this tissue is usually slow. Ligaments and tendons will be discussed again in Chapters 8 and 10, respectively, with bone, joints, and muscle.

» MEDICAL APPLICATION

Overuse of tendon-muscle units can result in **tendonitis**, characterized by inflammation of the tendons and their attachments to muscle. Common locations are the elbow, the Achilles tendon of the heel, and the shoulder rotator cuff. The swelling and pain produced by the localized inflammation restricts the affected area’s normal range of motion and can be relieved by injections of anti-inflammatory agents such as cortisone. Fibroblasts eventually repair damaged collagen bundles of the area.

Reticular Tissue

Reticular tissue is characterized by abundant fibers of type III collagen (Figure 5–12) forming a delicate network that supports various types of cells. This collagen is also known as

FIGURE 5–19 Loose connective tissue and dense irregular connective tissue.

Examples of these connective tissue types shown here indicate the close association that often occurs between these two types.

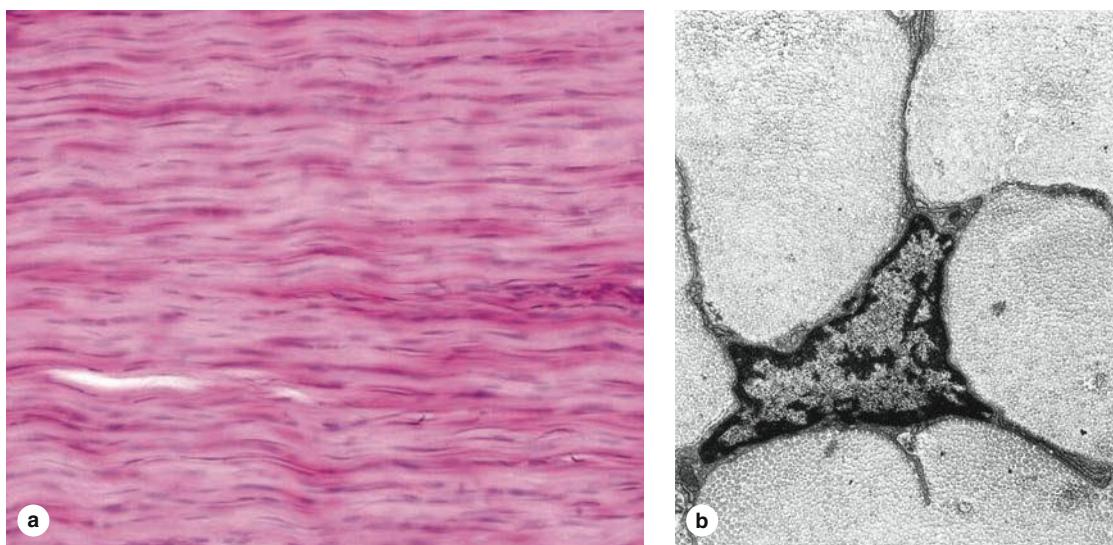
(a) Loose connective tissue (**L**) of a gland contains faintly stained ground substance with fine fibers of collagen and frequently forms a thin layer near epithelia, while dense irregular connective tissue (**D**) forms a thicker layer and is invariably much richer in larger bundles of collagen. Scattered leukocytes can be seen in both connective tissues, along with the large irregular spaces of lymphatic vessels (left). (X100; H&E)

(b) Trichrome staining of a section from skin demonstrates the blue staining of collagen with this method and its relative density in

loose (**L**) and dense irregular (**D**) connective tissue. (X100; Mallory trichrome)

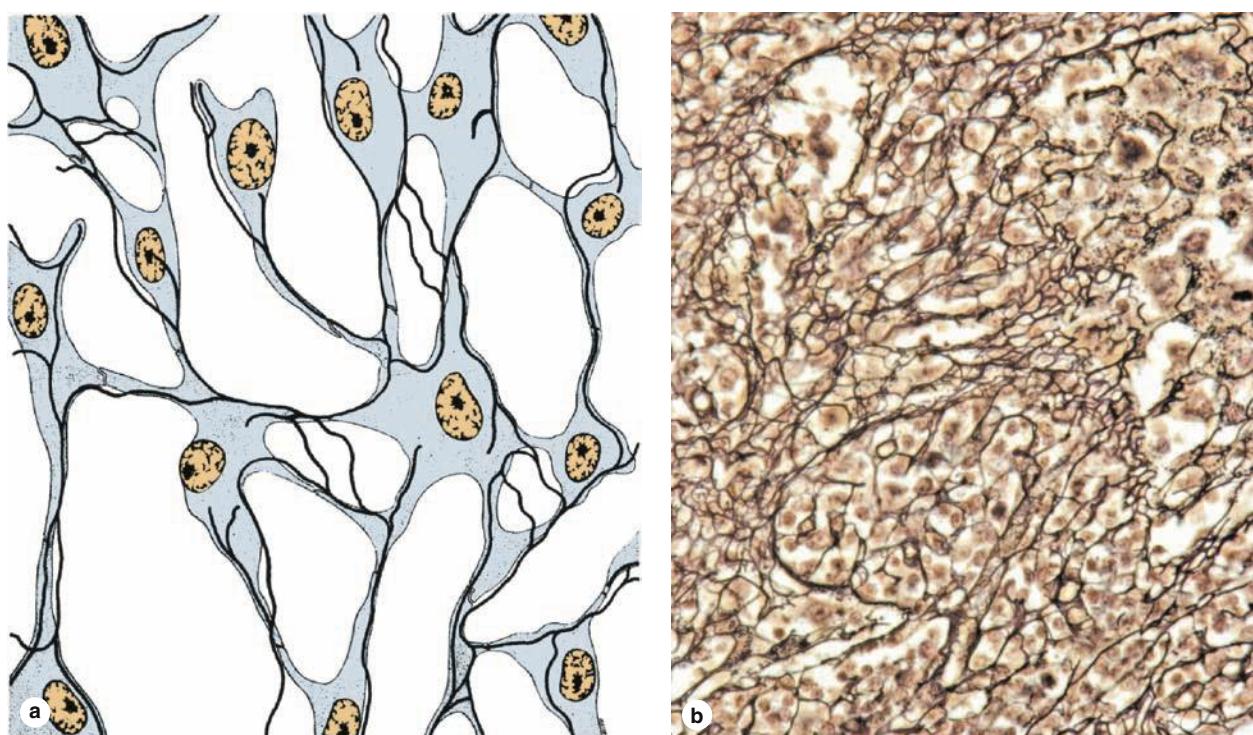
(c) Another example of dense irregular connective tissue, showing the randomly arranged large collagen bundles. The arrangement of collagen strengthens the tissues and resists tearing from all directions. (X150; H&E)

(d) Dense irregular connective tissue (**D**) forms a thick, protective capsule around many organs such as the testis shown here. Here the capsule is covered by a simple squamous epithelium of serous mesothelial cells (**S**), which produce a hyaluronate-rich lubricant around such organs. (X150; H&E)

FIGURE 5–20 Dense regular connective tissue.

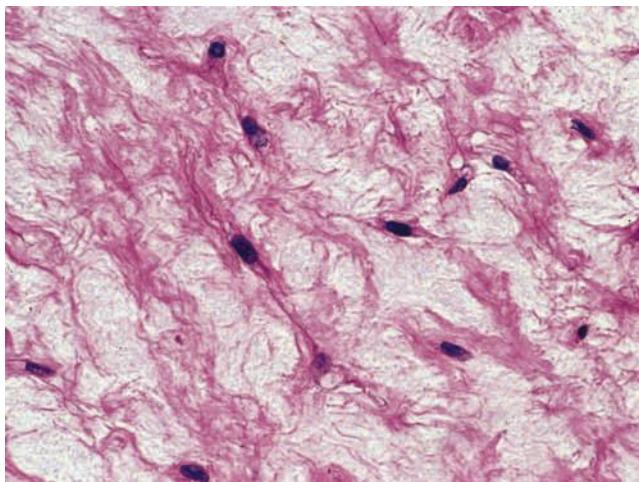
(a) Micrograph shows a longitudinal section of dense regular connective tissue in a tendon. Long, parallel bundles of collagen fibers fill the spaces between the elongated nuclei of fibrocytes. (X100; H&E stain)

(b) The electron micrograph shows one fibrocyte in a cross section of tendon, revealing that the sparse cytoplasm of the fibrocytes is divided into numerous thin cytoplasmic processes extending among adjacent collagen fibers. (X25,000)

FIGURE 5–21 Reticular tissue.

(a) The diagram shows only the fibers and attached reticular cells (free, transient cells are not represented). Reticular fibers of type III collagen (also referred to as "reticulin") are produced and enveloped by the reticular cells, forming an elaborate network through which interstitial fluid or lymph and wandering cells from blood pass continuously.

(b) The micrograph shows a silver-stained section of lymph node in which reticular fibers are seen as irregular black lines. Reticular cells are also heavily stained and dark. Most of the smaller, more lightly stained cells are lymphocytes passing through the lymph node. (X200; Silver)

FIGURE 5–22 Mucoid tissue.

A section of umbilical cord shows large fibroblasts surrounded by a large amount of very loose ECM containing mainly ground substances very rich in hyaluronan, with wisps of collagen. Histologically mucoid (or mucous) connective tissue resembles embryonic mesenchyme in many respects and is rarely found in adult organs. (X200; H&E)

reticulin and is produced by modified fibroblasts often called **reticular cells** that remain associated with and partially cover the fibers (Figure 5–21). The loose disposition of glycosylated reticular fibers provides a framework with specialized microenvironments for cells in hemopoietic tissue and some lymphoid organs (bone marrow, lymph nodes, and spleen). The resulting cell-lined system creates a meshwork for the passage of leukocytes and lymph. Macrophages and dendritic cells (also in the mononuclear phagocyte family) are also dispersed within these reticular tissues to monitor cells formed there or passing through and to remove debris.

Mucoid Tissue

Mucoid (or mucous) connective tissue is the principal component of the fetal umbilical cord, where it is referred to as **Wharton's jelly**. With abundant ground substance composed chiefly of hyaluronan, mucoid tissue is gelatinous, with sparse collagen fibers and scattered fibroblasts (Figure 5–22). Included among the fibroblastic cells are many mesenchymal stem cells, which are being studied for their potential in regenerative medicine. Mucoid connective tissue is similar to the tissue found in the vitreous chambers of eyes and pulp cavities of young teeth.

Connective Tissue SUMMARY OF KEY POINTS

- Connective tissue is specialized to physically **support** and **connect** other tissues and maintain the water required for metabolite diffusion to and from cells.
- Connective tissues all consist primarily of **extracellular** material rather than cells.
- Within most organs connective tissue proper forms the supportive **stroma**, which supports the organ's unique functional components or **parenchyma**.
- The **extracellular matrix (ECM)** of connective tissue proper usually consists of both large protein **fibers** and nonfibrous areas of unstained **ground substance** rich in various GAGs and water.
- All adult connective tissues are derived from an embryonic form of connective tissue called **mesenchyme**, which contains uniformly undifferentiated cells scattered in a gel-like matrix.

Cells of Connective Tissue

- **Fibroblasts** (fibrocytes), the major cells of connective tissue proper, are elongated, irregularly shaped cells with oval nuclei that synthesize and secrete most components of the ECM.
- **Adipocytes** (fat cells) are very large cells specialized for storage of triglycerides; they predominate in a specialized form of connective tissue called **adipose tissue**.
- **Macrophages** are short-lived cells that differentiate in connective tissue from precursor cells called **monocytes** circulating in the blood; they function in ECM turnover, phagocytosis of dead cells and debris, and antigen presentation to lymphocytes.
- **Mast cells** also originate from blood cell precursors and are filled with granules for the release of various vasoactive agents and other substances during inflammatory and allergic reactions.
- **Plasma cells** are short-lived cells that differentiate from B lymphocytes and are specialized for the abundant secretion of specific antibodies (immunoglobulins).

- Besides macrophages and plasma cells, other **leukocytes** normally wander through all types of connective tissue proper, providing surveillance against bacterial invaders and stimulating tissue repair.

Fibers of Connective Tissue

- The most important and abundant fibers of connective tissue are composed of the protein **collagen**, of which there are some 20 related types.
- Synthesis of collagen by fibroblasts and certain other cells involves posttranslational modifications in the RER, notably **hydroxylation** of the numerous prolines and lysines, and formation of helical trimeric subunits of **procollagen**.
- Upon exocytosis, the nonhelical ends of the procollagen subunits are removed, forming trimeric **collagen molecules** that aggregate and become covalently bound together in large **collagen fibrils**.
- The highly regular assembly of collagens in the fibrils produces a characteristic pattern of **crossbanding** visible ultrastructurally along the fibrils of some collagen types.
- Fibrils of type I collagen are bundled together by other forms of non-fibrillar, linking collagens to produce large **collagen bundles**.
- Collagen fibrils are degraded by collagenase enzymes classified as **matrix metalloproteinases (MMPs)**, produced primarily by macrophages.
- Type III collagen produces a network of delicate **reticular fibers**, which stain very dark with silver stains and are abundant in immune and lymphoid tissues.
- **Elastic fibers**, or sheets called **elastic lamellae**, are composed of the proteins **elastin** and **fibrillin**, which exist in a stretchable conformation that provides elastic properties to connective tissues rich in this material.

Ground Substance

- **Ground substance** is the watery, largely unstained extracellular material that is more abundant than fibers in some types of connective tissue proper.
- Ground substance is rich in **hydrated glycosaminoglycans (GAGs)**, **proteoglycans**, and **multiadhesive glycoproteins**.
- The major types of GAGs are **hyaluronan** (hyaluronic acid), which is a very long polymer of the disaccharide glucosamine-glucuronate, and various shorter chains of **sulfated GAGs** composed of other disaccharide polymers.
- Sulfated GAGs such as **chondroitin sulfate** and **keratan sulfate** have various sizes and compositions, but they are all bound to the core proteins of **proteoglycans** and are produced in the Golgi apparatus before secretion.
- Proteoglycans attach to polymers of HA via **linker proteins** to form huge complexes in ground substance that bind water and other substances, including certain polypeptide growth factors that help regulate fibroblast proliferation.
- **Multiadhesive glycoproteins** such as fibronectin and laminin have binding sites for collagens and for integrin proteins in cell membranes, thus allowing temporary attachments between cells and the ECM required for cell migration and positioning.

Types of Connective Tissue

- **Connective tissue proper** is usually classified as loose or dense according to the amount of collagen and ground substance present.
- **Loose connective tissue** (or **areolar tissue**) has relatively more ground substance than collagen, and it typically surrounds small blood vessels and occupies areas adjacent to other types of epithelia.
- **Dense irregular connective tissue** is filled primarily with randomly distributed bundles of type I collagen, with some elastic fibers, providing resistance to tearing from all directions as well as some elasticity.
- **Dense regular connective tissue**, prominent in tendons and ligaments, features bundles of essentially parallel type I collagen, providing great strength (but little stretch) in binding together components of the musculoskeletal system.
- **Reticular tissue** consists of delicate networks of type III collagen and is most abundant in certain lymphoid organs where the fibers form attachment sites for lymphocytes and other immune cells.
- **Mucoid tissue** is a gel-like connective tissue with few cells found most abundantly around blood vessels in the umbilical cord.

Connective Tissue ASSESS YOUR KNOWLEDGE

1. Which of the following connective tissue components is located in the ECM but not in the ground substance?
 - a. Collagen bundles
 - b. Fibronectin
 - c. GAGs
 - d. Hyaluronan
 - e. Proteoglycans
2. What cells numerous in loose connective tissue are filled with secretory granules and stain with metachromasia?
 - a. Macrophages
 - b. Mast cells
 - c. Fibrocytes
 - d. Active fibroblasts
 - e. Leukocytes
3. What is the first step of collagen production that occurs after the protein undergoes exocytosis?
 - a. Cross-linking of collagen fibrils with a short linking collagen
 - b. Removal of the terminal nonhelical domains by peptidases
 - c. Hydroxylation of lysine and proline
 - d. Assembly of subunits to form a larger structure
 - e. Disulfide bond formation
4. What is an important part of the role played by macrophages during maintenance and renewal of strong extracellular fibers in connective tissue?
 - a. Storage for a major energy source needed for ECM maintenance
 - b. Production of specific collagen subunits
 - c. A sentinel function against invaders entering the ECM
 - d. Secretion of matrix metalloproteinases
 - e. Presentation of antigens important for assembly of collagen bundles
5. Sulfated GAGs are important constituents of what extracellular structures?
 - a. Hyaluronan
 - b. Elastic fibers
 - c. Type I collagen
 - d. Proteoglycans
 - e. Multiadhesive glycoproteins
6. Which of the following contains binding sites for integrins and is an important part of the ECM in both loose connective tissue and dense irregular connective tissue?
 - a. Aggrecan
 - b. Fibronectin
 - c. Perlecan
 - d. Fibrillin
 - e. Most types of collagen
7. Dense regular connective tissue typically involves which of the following features?
 - a. Contains mostly synthetically active fibroblasts
 - b. Contains much ground substance
 - c. Contains a similar cell population as areolar connective tissue
 - d. Predominant tissue type in the stroma of most organs
 - e. Predominantly located in tendons and ligaments
8. Research scientists at a small biotech firm are investigating new methods of controlling the growth and metastasis of malignant cells in patients diagnosed with breast cancer. They have developed a novel peptide-based drug, potentially deliverable therapeutically, that disrupts the tumor cells' ability to adhere to the ECM, which in turn triggers apoptosis. Which of the following is a most likely target of such drugs?
 - a. Cadherins
 - b. Adhesins
 - c. Integrins
 - d. Glycolipids of the cell membrane
 - e. Fibrillin

9. A 36-year-old man is referred by his family physician to the pulmonary clinic. He complains of shortness of breath following physical activity and decreased capacity for exercise. He says that strenuous exercise including yard work is impossible without sitting down and resting every few minutes. After taking several deep breaths during the physical examination, he begins to wheeze. He is not a smoker and works in an office not exposed to dust, fumes, or other irritants. He appears slightly jaundiced. Serum alpha-1-antitrypsin (AAT) concentration analysis is below normal and is followed up with AAT phenotype and DNA testing which indicates one copy of S and one of Z mutations with 40% abnormal AAT production. Urinalysis shows elevated levels of desmosine and isodesmosine. These excreted compounds normally contribute to efficient lung function by which of the following mechanisms?
- Post-translational modification of fibrillin
 - Cross-linking elastin
 - Activating elastase
 - Activating AAT
 - Binding type IV collagen to elastin
10. A 33-year-old homeless woman has been living in an abandoned building eating dried meat and bread from the dumpster behind a delicatessen. She smokes cigarettes “bummed” from others. She presents at a free clinic with bleeding under the skin, particularly around hair follicles, and bruises on her arms and legs. She is irritable, clinically depressed, and fatigued with general muscle weakness. Her gums are bleeding, swollen, purple, and spongy, with several loose teeth. She has an infected toe, which may be broken. She is afebrile, a glucose finger-stick is normal, and the urine dipstick shows no sugar, protein, or ketones. You suspect a vitamin deficiency. What might be the underlying mechanism for this patient’s symptoms?
- Decreased degradation of collagen
 - Stimulation of prolyl hydroxylase
 - Formation of unstable collagen helices
 - Excessive callus formation in healing fractures
 - Organ fibrosis

WHITE ADIPOSE TISSUE

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BROWN ADIPOSE TISSUE

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SUMMARY OF KEY POINTS 127
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Connective tissue in which fat-storing cells or **adipocytes** predominate is called **adipose tissue**. These large cells are typically found isolated or in small groups within loose or dense irregular connective tissue but occur in large aggregates in adipose tissue or “fat” in many organs and body regions. Adipose tissue normally represents 15%-20% of the body weight in men, somewhat more in women. Besides serving as storage depots for neutral fats, chiefly triglycerides (long-chain fatty acyl esters of glycerol), adipocytes function as key regulators of the body’s overall energy metabolism. With a growing epidemic of obesity and its associated health problems, including diabetes and heart disease, adipocytes and adipose tissue now constitute a major area of medical research.

Two properties of triglyceride lipids explain their selection as the preferred form of nutrient storage. Insoluble in water, lipids can be concentrated with no adverse osmotic effects on cells. Also, the caloric density of triglycerides (9.3 kcal/g) is twice that of proteins or carbohydrates, including glycogen, making these simple lipids the most efficient means of storing calories. Adipocytes specialize in concentrating triglycerides as lipid droplet(s), with other cells normally accumulating relatively little lipid.

Adipocytes are active cells metabolically, responding to both nervous and hormonal stimuli. They release hormones and various other important substances and adipose tissue is now recognized as an endocrine organ at the center of nutritional homeostasis. With its unique physical properties, tissue rich in fat conducts heat poorly and provides thermal insulation for the body. Adipose tissue also fills spaces between other tissues, helping to keep some organs in place. Subcutaneous layers of adipose tissue help shape the body surface, and cushion regions subject to repeated mechanical stress such as the palms, heels, and toe pads.

There are two major types of adipose tissue with different locations, structures, colors, and functions. **White adipose**

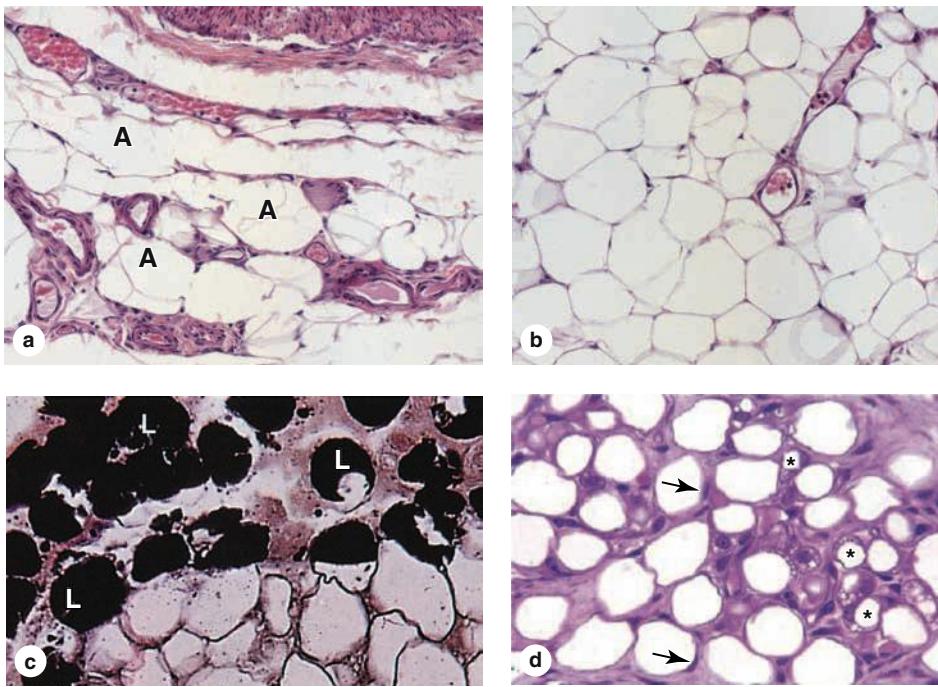
tissue, the more common type specialized for fat storage, consists of cells each containing one large cytoplasmic droplet of whitish-yellow fat. **Brown adipose tissue** contains cells with multiple lipid droplets interspersed among abundant mitochondria, which helps give this tissue a darker appearance. Brown adipocytes release heat and function to warm the blood. Both types of adipose tissue have a rich blood supply and the adipocytes, unlike other cells of connective tissue proper, are individually surrounded by a thin external lamina containing type IV collagen.

► WHITE ADIPOSE TISSUE

Specialized for relatively long-term energy storage, adipocytes of white adipose tissue are spherical when isolated but are polyhedral when closely packed in situ. When completely developed, a white adipocyte is very large, between 50 and 150 µm in diameter, and contains a single huge droplet of lipid filling almost the entire cell. With the single large droplets of triglycerides, white adipocytes are also called **unilocular** (Figure 6–1). Because lipid is removed from cells by xylene or other solvents used in routine histological techniques, unilocal adipocytes are often empty in standard light microscopy. The cells are sometimes said to have a signet-ring appearance, with the lipid droplet displacing and flattening the nucleus against the cell membrane (Figure 6–1d). This membrane and the thin rim of cytoplasm that remains after dissolution of the stored lipid may shrink, collapse, or rupture, distorting cell and tissue structure.

► MEDICAL APPLICATION

Unilocal adipocytes can generate benign tumors called **lipomas** that are relatively common, although malignant adipose tumors (**liposarcomas**) occur infrequently. Fetal lipomas of brown fat are sometimes called **hibernomas**.

FIGURE 6–1 White adipose tissue.

White or unilocular adipose tissue is commonly seen in sections of many human organs.

(a) Large white adipocytes (**A**) are seen in the connective tissue associated with small blood vessels. The fat cells are empty because lipid was dissolved away in slide preparation. Nuclei at the cell membranes are visible in some of the fat cells. (X100; H&E)

(b) Large (empty) adipocytes predominate in this typical white adipose tissue, which shows only a small portion of microvasculature. In a single histologic section, nuclei of most very large adipocytes are not included. (X100; H&E)

(c) Tissue was fixed here with osmium tetroxide, which preserves lipid (**L**) and stains it black. Many adipocytes in this slide retain at least part of their large lipid droplets. (X440; Osmium tetroxide)

(d) In this specimen from a young mammal the smaller adipocytes marked with asterisks are not unilocular, having many lipid droplets of various sizes. Such cells in white fat represent those in which differentiation is incomplete as well as a small subpopulation of beige cells with brown fat-forming potential. The eccentric nuclei of the unilocular cells are indicated by arrowheads. (X200; PT)

Most cytoplasmic organelles in a white adipocyte are near the peripheral nucleus, including mitochondria, a small Golgi apparatus, a few cisternae of RER, and free polyribosomes. The thin, submembranous layer of cytoplasm surrounding the lipid droplet contains cisternae of smooth ER (SER) and pinocytotic vesicles. TEM studies reveal that most adipocytes, especially immature cells, contain minute lipid droplets in addition to the large droplet. The lipid droplet-cytoplasm interface is reinforced only by intermediate filaments of vimentin.

As shown in Figure 6–1 white fat is subdivided into incomplete lobules by partitions of connective tissue containing a vascular bed and nerve network. Fibroblasts, macrophages, and other cells typically comprise about half the total cell number in white adipose tissue. Reticular fibers form a fine interwoven network that supports individual fat cells and binds them together, and the microvasculature between adipocytes may not always be apparent in tissue sections.

The distribution of white adipose tissue changes significantly through childhood and adult life and is partly regulated by sex hormones controlling adipose deposition in the breasts and thighs. The color of freshly dissected white adipose tissue depends on the diet, varying from white to yellow with the amount of carotenoids dissolved in the lipid.

Storage & Mobilization of Lipids

White adipocytes can store triglycerides derived from three sources:

- Dietary fats brought to the cells via the circulation as **chylomicrons**,
- Lipids synthesized in the liver and transported in blood with **very-low-density lipoproteins (VLDLs)**,
- Free fatty acids and glycerol synthesized by the adipocytes.

Chylomicrons (Gr. *chylōs*, juice + *micros*, small) are particles of variable size, up to 1200 nm in diameter, formed from ingested lipids in epithelial cells lining the small intestine and transported in the blood and lymph. They consist of a core containing mainly triglycerides, surrounded by a stabilizing monolayer of phospholipids, cholesterol, and several apolipoproteins.

VLDLs are smaller complexes (30–80 nm, providing a greater surface-to-volume ratio), of similar lipid and protein composition to chylomicrons, but are synthesized from lipids in liver cells. Levels of circulating lipoproteins are routinely measured in clinical tests for blood lipids, after fasting to allow depletion of chylomicrons. Varying levels of apoproteins and triglycerides in the complexes allow their categorization according to density, from VLDL to high-density lipoprotein (HDL).

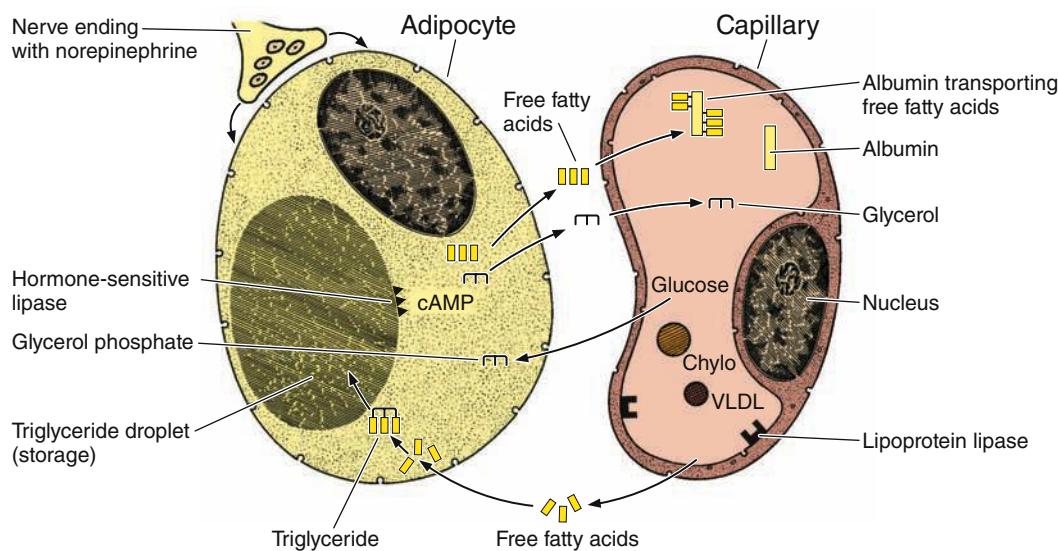
In adipose tissue both chylomicrons and VLDLs are hydrolyzed at the luminal surfaces of blood capillaries by **lipoprotein lipase**, an enzyme synthesized by the adipocytes and transferred to the capillary cell membrane (Figure 6–2). Free fatty acids then enter the adipocytes by both active transport and diffusion. Within the adipocytes, the fatty acids combine with glycerol phosphate, supplied by glucose metabolism, to again form triglycerides, which are then deposited in the growing lipid droplet. **Insulin** stimulates glucose uptake by

adipocytes and accelerates its conversion into triglycerides, and the production of lipoprotein lipase.

When adipocytes are stimulated by nerves or various hormones, stored lipids are mobilized and cells release fatty acids and glycerol. **Norepinephrine** released in the adrenal gland and by postganglionic sympathetic nerves in adipose tissue activates a **hormone-sensitive lipase** that breaks down triglycerides at the surface of the stored lipid droplets (Figure 6–2). This lipase activity is also stimulated by growth hormone (GH) from the pituitary gland. The free fatty acids diffuse across the membranes of the adipocyte and the capillary endothelium, and bind the protein albumin in blood for transport throughout the body. The more water-soluble glycerol remains free in blood and is taken up by the liver. Insulin inhibits the hormone-sensitive lipase, reducing fatty acid release, and also stimulates enzymes for lipid synthesis. Besides insulin and GH, other peptide hormones also cooperate in regulating lipid synthesis and mobilization in adipocytes.

Hormonal activity of white adipocytes themselves includes production of the 16-kDa polypeptide hormone **leptin** (Gr. *leptos*, thin), a “satiety factor” with target cells in the hypothalamus, other brain regions, and peripheral organs which helps regulate the appetite under normal conditions and participates in regulating the formation of new adipose tissue.

FIGURE 6–2 Lipid storage and mobilization from adipocytes.



Triglycerides are transported by blood and lymph from the intestine and liver in lipoprotein complexes known as **chylomicrons** (**Chylo**) and **VLDLs**. In the capillary endothelial cells of adipose tissue, these complexes are partly broken down by **lipoprotein lipase**, releasing free fatty acids and glycerol. The free fatty acids diffuse from the capillary into the adipocyte, where they are reesterified to glycerol phosphate, forming triglycerides that are stored in the lipid droplet until needed.

Norepinephrine from nerve endings stimulates the cyclic AMP (cAMP) system, which activates **hormone-sensitive lipase** to hydrolyze the stored triglycerides to free fatty acids and glycerol. These substances diffuse into the capillary, where the fatty acids bind albumin for transport throughout the body for use as an energy source.

» MEDICAL APPLICATION

Leptin was discovered and is well studied in genetically obese mice, but such studies have not yet led to new treatments for human obesity. In most obese humans adipocytes produce adequate or excess quantities of leptin, but target cells are not responsive due apparently to insufficient or defective receptors or post-receptor signal transduction.

Although white adipose tissue associated with different organs appears histologically similar, differences in gene expression have been noted between visceral deposits (in the abdomen) and subcutaneous deposits of white fat. Such differences may be important in the medical risks of obesity; it is well established that increased visceral adipose tissue raises the risk of diabetes and cardiovascular disease whereas increased subcutaneous fat does not. The release of visceral fat products directly to the portal circulation and liver may also influence the medical importance of this form of obesity.

In response to body needs, lipids are mobilized rather uniformly from white adipocytes in all parts of the body, although adipose tissue in the palms, soles, and fat pads behind the eyes resists even long periods of starvation. During starvation adipocytes can lose nearly all their fat and become polyhedral or spindle-shaped cells with only very small lipid droplets.

Histogenesis of White Adipose Tissue

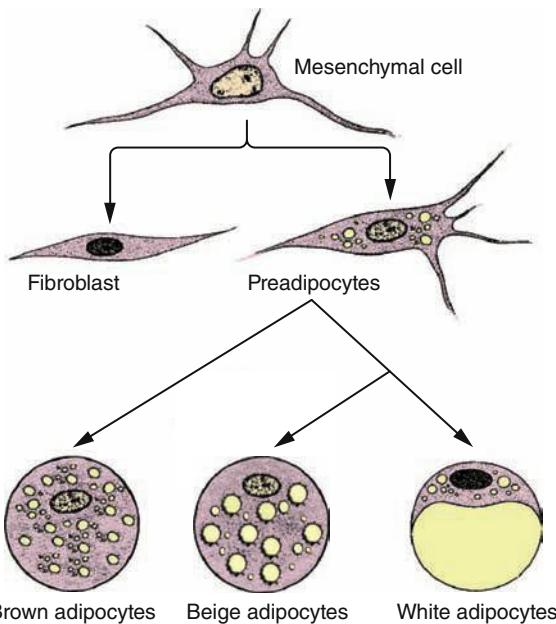
Like other connective tissue, skeletal and muscle cells, adipocytes develop from mesenchymal stem cells. Adipose development first produces **preadipocytes**, which look rather like larger fibroblasts with cytoplasmic lipid droplets (Figure 6–3). Initially the droplets of white adipocytes are isolated from one another but soon fuse to form the single large droplet (Figure 6–1).

As shown in Figure 6–3 white adipocytes develop together with a smaller population of cells termed beige adipocytes, which remain within white adipose tissue and have histological and metabolic features generally intermediate between white and brown adipocytes. With adaptation to cold temperatures beige adipocytes change reversibly, forming many more small lipid droplets, adopting a gene expression profile more like that of brown fat, and begin to release heat (see below).

» MEDICAL APPLICATION

In addition to leptin, white adipose tissue secretes numerous other cytokines and other factors with paracrine and auto-crime activity, including many proinflammatory cytokines. It is not clear whether these are produced by adipocytes or other cells of the tissue such as macrophages or fibroblasts. With its increased amounts of white adipose tissue, obesity is characterized by a state of chronic mild inflammation. Proinflammatory factors released from visceral fat are being investigated for links to the **inflammation-related disorders associated with obesity**, such as diabetes and heart disease.

FIGURE 6–3 Development of white and brown fat cells.



Mesenchymal stem cells differentiate as progenitor cells for all types of connective tissue, including **preadipocytes**. These are initially of at least two types. Preadipocytes developing within the lateral mesoderm of the embryo produce a large number of **white adipocytes** (forming white adipose tissue) and a smaller number of so-called beige adipocytes with cytological features and gene expression patterns of both white and brown adipocytes. White adipocytes are unilocular, with one large lipid droplet occupying most of the cytoplasm. The white adipocyte is usually much larger than that shown here in relation to the other cell types.

Brown adipocytes differentiate from another population of preadipocytes located in paraxial embryonic mesoderm and remain multilocular (having many small lipid droplets) with numerous mitochondria (not shown here). Mitochondrial metabolism of lipid in brown adipocytes releases heat rather than ATP. Cells functioning as brown adipocytes can also develop from beige adipocytes during adaptation to cold temperatures.

Humans are born with stores of white adipose tissue, which begin to accumulate by the 14th week of gestation. Both visceral and subcutaneous fat is well-developed before birth. Proliferation of progenitor cells diminishes by late gestation, and adipose tissue increases mainly by the filling of existing adipocytes until around age 10, followed by a period of new fat cell differentiation which lasts through adolescence. New adipocyte formation occurs around small blood vessels, where undifferentiated mesenchymal cells are most abundant.

Excessive adipose tissue accumulation, or obesity, occurs when nutritional intake exceeds energy expenditure, an increasingly common condition in modern, sedentary lifestyles. Although adipocytes can differentiate from mesenchymal stem cells throughout life, adult-onset obesity mainly involves

increasing the size of existing adipocytes (hypertrophy). Childhood obesity, in contrast, often involves increases in both adipocyte size and numbers due to the differentiation of more preadipocytes from mesenchymal cells (hyperplasia). Weight loss after dietary changes is due to reductions in adipocyte volume, but not their overall number.

» MEDICAL APPLICATION

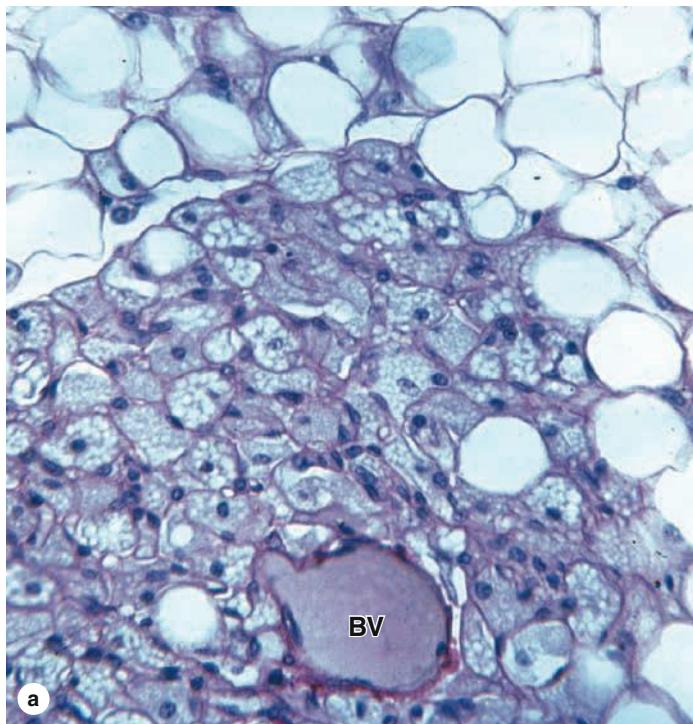
Adult-onset obesity is very often associated with age-related metabolic changes and may involve reduced activity of the hormone-sensitive lipases of adipocytes, causing less effective fat mobilization out of the cells. The increased number of adipocytes produced during **childhood obesity** predisposes an individual to obesity in later life. Despite claims of various fad diets, there is no evidence that any particular type of caloric restriction is more effective than others; rather, any intake of calories that is lower than the energy expenditure will result in loss of adipose tissue.

► BROWN ADIPOSE TISSUE

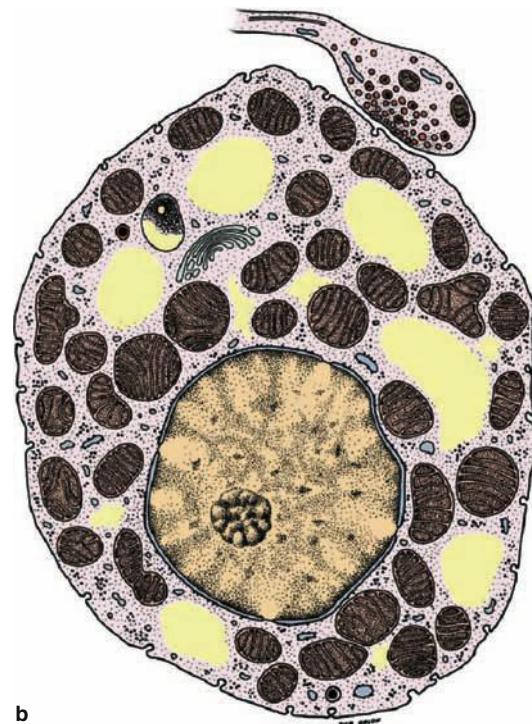
Brown adipose tissue constitutes 2%-5% of the newborn body weight, located mainly in the back, neck, and shoulders, but it is greatly reduced during childhood and adolescence. In adults it is found only in scattered areas, especially around the kidneys, adrenal glands, aorta, and mediastinum. The color of **brown fat** is due to both the very abundant mitochondria (containing cytochrome pigment) scattered among the lipid droplets of the fat cells and the large number of blood capillaries in this tissue. Brown adipocytes contain many small lipid inclusions and are therefore called **multilocular** (Figure 6-3). The small lipid droplets, abundant mitochondria, and rich vasculature all help mediate this tissue's principal function of **heat production** and warming the blood.

Cells of brown fat are polygonal and generally smaller than white adipocytes; their smaller lipid droplets allow the nucleus to be more centrally located (Figure 6-4). Brown adipocytes are often closely packed around large capillaries and the tissue is subdivided by connective tissue partitions into

FIGURE 6-4 Brown adipose tissue.



(a) Brown adipose tissue is shown here around a small blood vessel (**BV**) and adjacent white adipose tissue at the top of the photo. Brown adipocytes are slightly smaller and characteristically contain many small lipid droplets and central spherical nuclei. If the lipid has been dissolved from the cells, as shown here, the many mitochondria among the lipid spaces are retained and can be easily discerned. (X200; PT)



(b) A diagram of a single multilocular adipocyte showing the central nucleus, numerous small lipid droplets (yellow), and many mitochondria. Also shown is a sympathetic nerve ending that releases norepinephrine to stimulate mitochondrial production of heat.

lobules that are better delineated than the lobules of white adipose tissue. Cells of this tissue receive direct sympathetic innervation, which regulates their metabolic activity.

Function of Brown Adipocytes

The main function of these multilocular adipose cells is to produce heat by nonshivering **thermogenesis**. The physiology of brown fat is best understood from studies of the tissue in hibernating species. In animals ending their hibernation period, and in newborn humans, nerve impulses liberate norepinephrine into brown adipose tissue. As in white fat, this neurotransmitter activates the hormone-sensitive lipase of adipocytes, promoting hydrolysis of triglycerides to fatty acids and glycerol. However, unlike the process in white fat, liberated fatty acids of multilocular adipocytes are not released but are quickly metabolized, with a consequent increase in O₂ consumption and heat production. This raises the temperature within the tissue and warms the locally circulating blood, which then distributes the heat throughout the body.

Heat production in brown adipocytes is greater than that of other cells because their inner mitochondrial membranes have greatly upregulated levels of the transmembrane protein **uncoupling protein-1 (UCP1)** or **thermogenin**. In the presence of free fatty acids, UCP1 permits the flow of protons

from the intermembranous space to the matrix without passing through ATP synthetase complexes. Instead of producing ATP, the energy associated with this proton flow dissipates as heat.

Histogenesis of Brown Adipose Tissue

Brown adipose tissue also develops from mesenchyme, but involves preadipocytes in a different embryonic location (paraxial) from those producing white adipose tissue. Brown adipocytes also emerge earlier than white fat during fetal development. In humans the amount of brown fat is maximal relative to body weight at birth, when thermogenesis is most needed and partially disappears by involution and apoptosis during childhood. In adults the amount and activity of brown fat are higher in lean individuals.

The number of brown adipocytes increases during cold adaptation, usually appearing as clusters of multilocular cells in white adipose tissue. As indicated earlier this increase involves the reversible shift of beige cells to functional brown adipocytes, but may also include proliferation and differentiation of new adipocytes from preexisting progenitor cells. Besides stimulating thermogenic activity, autonomic nerves also promote brown adipocyte differentiation and prevent apoptosis in mature brown fat cells.

Adipose Tissue SUMMARY OF KEY POINTS

- The defining cells of adipose tissue (fat), **adipocytes**, are very large cells derived from mesenchyme and specialized for energy storage in lipid droplet(s) with **triglycerides**.
- Adipocytes store lipids from three sources: from dietary fats packaged as **chylomicrons** in the intestine; from triglycerides produced in the liver and circulating as **very-low-density lipoproteins (VLDLs)**; and from fatty acids synthesized locally.
- Lipids are mobilized from adipocytes by **hormone-sensitive lipase** activated by **norepinephrine** released from the adrenal gland and various peptide hormones.
- Cells of adipose tissue are supported by reticular fibers, with connective tissue septa dividing the tissue into lobules of various sizes.
- There are two types of adipose tissue: **white fat** and **brown fat**.

White Adipose Tissue

- **White adipose tissue** is found in many organs throughout the body, typically forming about 20% of the body weight in adults.
- Adipocytes of white fat are typically **very large cells**, ranging in diameter from 50 to 150 µm.

- These cells each contain primarily **one large lipid droplet** (they are **unilocular**), causing the nucleus and remaining cytoplasm to be pushed against the plasmalemma.
- Fatty acids are released from white adipocytes by **lipase** activity when nutrients are needed and carried throughout the body on plasma proteins such as albumin.
- **Leptin** is a polypeptide hormone with target cells in the hypothalamus that is released from white adipocytes and helps regulate eating behavior.

Brown Adipose Tissue

- **Brown fat** comprises up to 5% of the newborn body weight but smaller amounts in adults.
- Adipocytes of this tissue are typically smaller than those of white fat and contain primarily **many small lipid droplets** (they are **multilocular**) in cytoplasm containing many mitochondria and a central nucleus.
- Fatty acids released in adipocytes of brown fat are metabolized in mitochondria of these cells for **thermogenesis** rather than ATP synthesis, using **uncoupling protein-1**.

Adipose Tissue ASSESS YOUR KNOWLEDGE

1. White adipocytes are derived developmentally from what precursor cells?
 - a. Monocytes
 - b. Fibroblasts
 - c. Mesenchymal cells
 - d. Brown adipocytes
 - e. Mast cells
2. What are the relatively large particles formed in the intestinal epithelial cells and rich in ingested lipids?
 - a. Fatty acids
 - b. Chylomicrons
 - c. Glycerols
 - d. Very-low-density lipoproteins
 - e. Adipocytes
3. What substance, released from the adrenal gland and some autonomic neurons, increases lipolytic activity in white adipocytes?
 - a. Leptin
 - b. Insulin
 - c. Norepinephrine
 - d. Glycogen
 - e. Triglyceride
4. What is the most important form of lipid storage in both white and brown adipocytes?
 - a. Free fatty acids
 - b. Cholesterol
 - c. Chylomicrons
 - d. Glycerol
 - e. Triglycerides
5. Important target cells of leptin are found in which organ?
 - a. Small intestine
 - b. White adipose tissue
 - c. Large intestine
 - d. Hypothalamus
 - e. Brown adipose tissue
6. The hormone-sensitive lipase in the cells of adipose tissue acts primarily on what substrate?
 - a. Glucose
 - b. Free fatty acids
 - c. Glycerol
 - d. Triglycerides
 - e. Very-low-density lipoproteins
7. Applied to adipocytes, the term “multilocular” refers to which of the following?
 - a. The large number of small cytoplasmic lipid droplets
 - b. The proliferation of the cells in an obese individual
 - c. The large number of mitochondria in the cells
 - d. The high density of nerves supplying the tissue
 - e. The type of mesenchymal cells also present
8. Fully differentiated white adipocytes are large cells, typically having diameters of approximately what size?
 - a. 5 μm
 - b. 10 μm
 - c. 100 μm
 - d. 500 μm
 - e. 1000 μm
9. Ten days after birth a full-term newborn boy develops firm, erythematous nodules and plaques over his trunk, arms, buttocks, thighs, and cheeks. His mother’s pregnancy was complicated by placenta previa and his airway was cleared of aspirated meconium immediately after birth. A biopsy of subcutaneous tissue shows necrosis within the brown adipose tissue. What metabolic activity is liable to be affected in this patient?
 - a. Export of fatty acids from fat
 - b. Thermal insulation
 - c. Oxidation of fatty acids for thermogenesis
 - d. Activation of the adenylate cyclase system
 - e. Initiation of shivering
10. A 44-year-old African-American woman visits her family physician for a physical examination at the urging of her husband. She has no current complaints and is taking no medications. She is allergic to erythromycin. She works as a software developer and lives with her 52-year-old husband and 12-year-old daughter. She is a nonsmoker and drinks an occasional glass of wine when she and her husband go out to dinner. She is involved in no regular exercise. Her mother is 66 and suffers from type II diabetes, hyperlipidemia, and hypertension and had a myocardial infarction last year. The patient’s father died of a stroke last year at the age of 72. On examination, the patient’s blood pressure is 155/100 mm Hg, pulse 84, weight 215 lb (increased from 180 lb 3 years ago), and height 5 ft. 7 in. In this patient, during the period of weight gain which one of the following responses would be most likely in her white fat?
 - a. Increased synthesis of growth hormone
 - b. Decreased synthesis of leptin
 - c. Decreased release of leptin to the blood
 - d. Conversion of beige adipocytes to unilocular white adipocytes
 - e. Increased release of norepinephrine from nerve terminals near white adipocytes

HYALINE CARTILAGE

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ELASTIC CARTILAGE

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Cartilage is a tough, durable form of supporting connective tissue, characterized by an **extracellular matrix** (ECM) with high concentrations of GAGs and proteoglycans, interacting with collagen and elastic fibers. Structural features of its matrix make cartilage ideal for a variety of mechanical and protective roles within the adult skeleton and elsewhere (Figure 7–1).

Cartilage ECM has a firm consistency that allows the tissue to bear mechanical stresses without permanent distortion. In the respiratory tract, ears, and nose, cartilage forms the framework supporting softer tissues. Because of its resiliency and smooth, lubricated surface, cartilage provides cushioning and sliding regions within skeletal joints and facilitates bone movements. As described in Chapter 8, cartilage also guides development and growth of long bones, both before and after birth.

Cartilage consists of cells called **chondrocytes** (Gr. *chondros*, cartilage + *kytos*, cell) embedded in the ECM which unlike connective tissue proper contains no other cell types. Chondrocytes synthesize and maintain all ECM components and are located in matrix cavities called **lacunae**.

The physical properties of cartilage depend on electrostatic bonds between **type II collagen** fibrils, **hyaluronan**, and the sulfated GAGs on densely packed **proteoglycans**. Its semi-rigid consistency is attributable to water bound to the negatively charged hyaluronan and GAG chains extending from proteoglycan core proteins, which in turn are enclosed within a dense meshwork of thin type II collagen fibrils. The high content of bound water allows cartilage to serve as a shock absorber, an important functional role.

All types of cartilage lack vascular supplies and chondrocytes receive nutrients by diffusion from capillaries in surrounding connective tissue (the perichondrium). In some skeletal elements, large blood vessels do traverse cartilage to supply other tissues, but these vessels release few nutrients to the chondrocytes. As might be expected of cells in an avascular tissue, chondrocytes exhibit low metabolic activity. Cartilage also lacks nerves.

The **perichondrium** (Figure 7–2) is a sheath of dense connective tissue that surrounds cartilage in most places, forming an interface between the cartilage and the tissues supported by the cartilage. The perichondrium harbors the blood supply serving the cartilage and a small neural component. Articular cartilage, which covers the ends of bones in movable joints and which erodes in the course of arthritic degeneration, lacks perichondrium and is sustained by the diffusion of oxygen and nutrients from the synovial fluid.

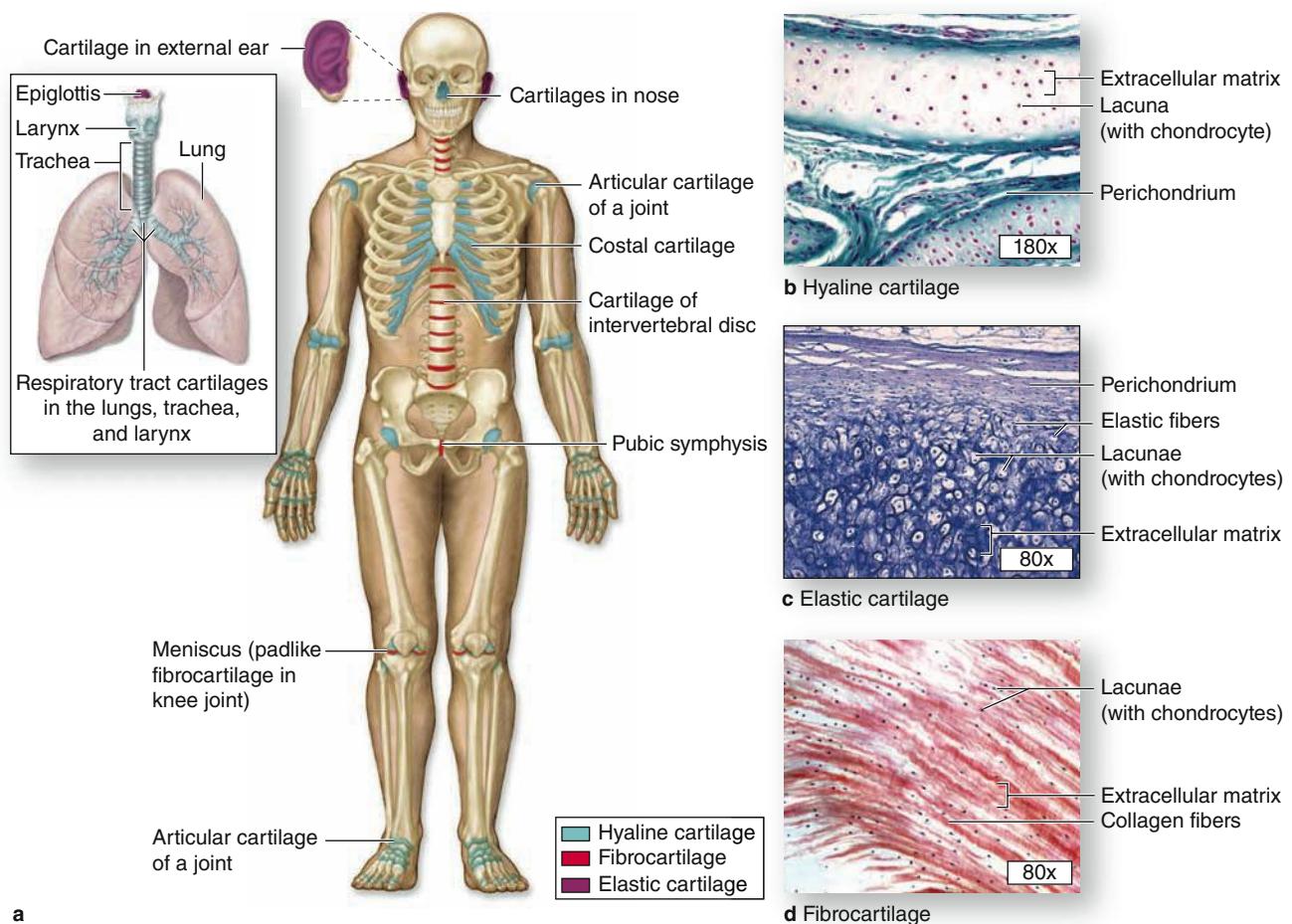
As shown in Figure 7–1, variations in the composition of the matrix characterize three main types of cartilage: hyaline cartilage, elastic cartilage, and fibrocartilage. Important features of these are summarized in Table 7–1.

>> MEDICAL APPLICATION

Many genetic conditions in humans or mice that cause defective cartilage, joint deformities, or short limbs are due to recessive mutations in genes for collagen type II, the aggrecan core protein, the sulfate transporter, and other proteins required for normal chondrocyte function.

> HYALINE CARTILAGE

Hyaline (Gr. *hyalos*, glass) cartilage, the most common of the three types, is homogeneous and semitransparent in the fresh state. In adults hyaline cartilage is located in the articular surfaces of movable joints, in the walls of larger respiratory passages (nose, larynx, trachea, bronchi), in the ventral ends of ribs, where they articulate with the sternum, and in the epiphyseal plates of long bones, where it makes possible longitudinal bone growth (Figure 7–1). In the embryo, hyaline cartilage forms the temporary skeleton that is gradually replaced by bone.

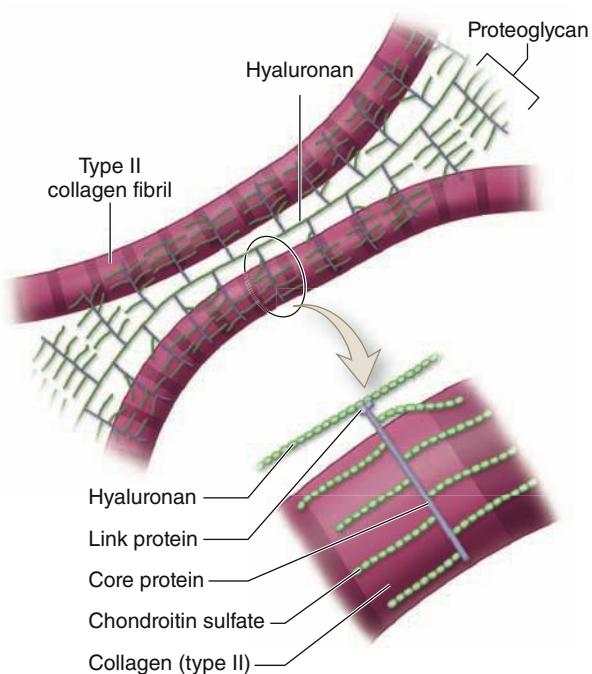
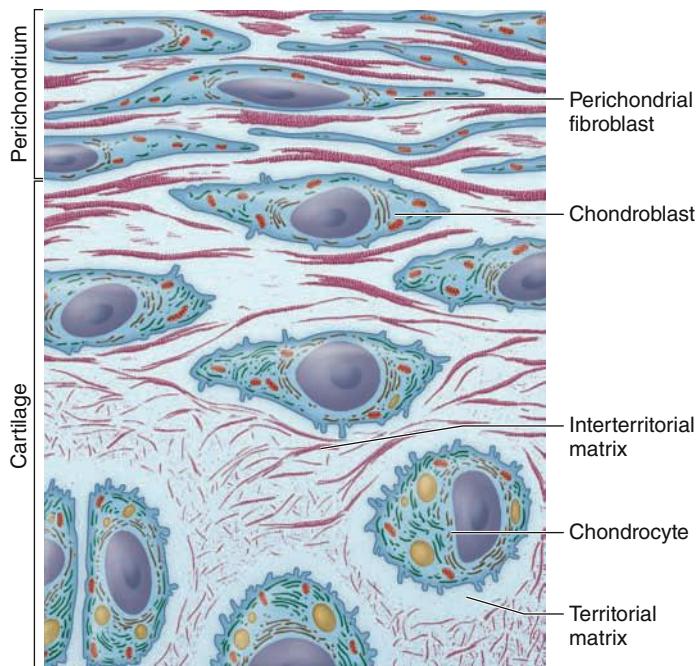
FIGURE 7–1 Distribution of cartilage in adults.

(a) There are three types of adult cartilage distributed in many areas of the skeleton, particularly in joints and where pliable support is useful, as in the ribs, ears, and nose. Cartilage support of other tissues throughout the respiratory tract is also prominent.

The photomicrographs show the main features of **(b)** hyaline cartilage, **(c)** elastic cartilage, and **(d)** fibrocartilage. Dense connective tissue of perichondrium is shown here with hyaline and elastic cartilage.

TABLE 7–1 Important features of the major cartilage types.

| | Hyaline Cartilage | Elastic Cartilage | Fibrocartilage |
|--|--|--|--|
| Main features of the extracellular matrix | Homogeneous, with type II collagen and aggrecan | Type II collagen, aggrecan, and darker elastic fibers | Type II collagen and large areas of dense connective tissue with type I collagen |
| Major cells | Chondrocytes, chondroblasts | Chondrocytes, chondroblasts | Chondrocytes, fibroblasts |
| Typical arrangement of chondrocytes | Isolated or in small isogenous groups | Usually in small isogenous groups | Isolated or in isogenous groups arranged axially |
| Presence of perichondrium | Yes (except at epiphyses and articular cartilage) | Yes | No |
| Main locations or examples | Many components of upper respiratory tract; articular ends and epiphyseal plates of long bones; fetal skeleton | External ear, external acoustic meatus, auditory tube; epiglottis and certain other laryngeal cartilages | Intervertebral discs, pubic symphysis, meniscus, and certain other joints; insertions of tendons |
| Main functions | Provides smooth, low-friction surfaces in joints; structural support for respiratory tract | Provides flexible shape and support of soft tissues | Provides cushioning, tensile strength, and resistance to tearing and compression |

FIGURE 7–2 The structure of cartilage matrix and cells.**a****b**

(a) A schematic representation of the most abundant molecules in cartilage matrix shows the interaction between type II collagen fibrils and proteoglycans linked to hyaluronan. Link proteins noncovalently bind the protein core of proteoglycans to the linear hyaluronan molecules. The chondroitin sulfate side chains of the proteoglycan electrostatically bind to the collagen fibrils, forming a cross-linked matrix. The circled area is shown larger in the lower part of the figure. Physical properties of these matrix components produce a highly hydrated, pliable material with great strength. Approximately 75% of the wet weight of hyaline cartilage is water.

(b) A diagram of the transitional area between the perichondrium and the cartilage matrix. Fibroblast-like progenitor cells in the perichondrium give rise to larger chondroblasts, which divide and differentiate as chondrocytes. These functional cells produce matrix components and exist in lacunae surrounded by the matrix. The ECM immediately around each lacuna, called the **territorial matrix**, contains mostly proteoglycans and sparse collagen; that more distant from lacunae, the **interterritorial matrix**, is richer in collagen and may be less basophilic.

» MEDICAL APPLICATION

Osteoarthritis, a chronic condition that commonly occurs during aging, involves the gradual loss or changed physical properties of the hyaline cartilage that lines the articular ends of bones in joints. Joints that are weight-bearing (knees, hips) or heavily used (wrist, fingers) are most prone to cartilage degeneration. Fragments released by wear-and-tear to the articular cartilage trigger secretion of matrix metalloproteinases and other factors from macrophages in adjacent tissues, which exacerbate damage and cause pain and inflammation within the joint.

Matrix

The dry weight of hyaline cartilage is nearly 40% collagen embedded in a firm, hydrated gel of proteoglycans and structural glycoproteins. In routine histology preparations, the proteoglycans

make the matrix generally basophilic and the thin collagen fibrils are barely discernible. Most of the collagen in hyaline cartilage is **type II**, although small amounts of minor collagens are also present.

Aggrecan (250 kDa), with approximately 150 GAG side chains of chondroitin sulfate and keratan sulfate, is the most abundant proteoglycan of hyaline cartilage. Hundreds of these proteoglycans are bound noncovalently by link proteins to long polymers of hyaluronan, as shown schematically in Figure 7–2a and discussed in Chapter 5. These proteoglycan complexes bind further to the surface of type II collagen fibrils (Figure 7–2a). Water bound to GAGs in the proteoglycans constitutes up to 60%–80% of the weight of fresh hyaline cartilage.

Another important component of cartilage matrix is the structural multiadhesive glycoprotein **chondronectin**. Like fibronectin in other connective tissues, chondronectin binds specifically to GAGs, collagen, and integrins, mediating the adherence of chondrocytes to the ECM.

Staining variations within the matrix reflect local differences in its molecular composition. Immediately surrounding each chondrocyte, the ECM is relatively richer in GAGs than collagen, often causing these areas of **territorial matrix** to stain differently from the intervening areas of interterritorial matrix (Figures 7–2b and 7–3).

Chondrocytes

Cells occupy relatively little of the hyaline cartilage mass. At the periphery of the cartilage, young chondrocytes or **chondroblasts** have an elliptic shape, with the long axes parallel to the surface (Figure 7–3). Deeper in the cartilage, they are round and may appear in groups of up to eight cells that originate from mitotic divisions of a single chondroblast and are called **isogenous aggregates**. As the chondrocytes become more active in secreting collagens and other ECM components, the aggregated cells are pushed apart and occupy separate lacunae.

Cartilage cells and matrix may shrink slightly during routine histologic preparation, resulting in both the irregular

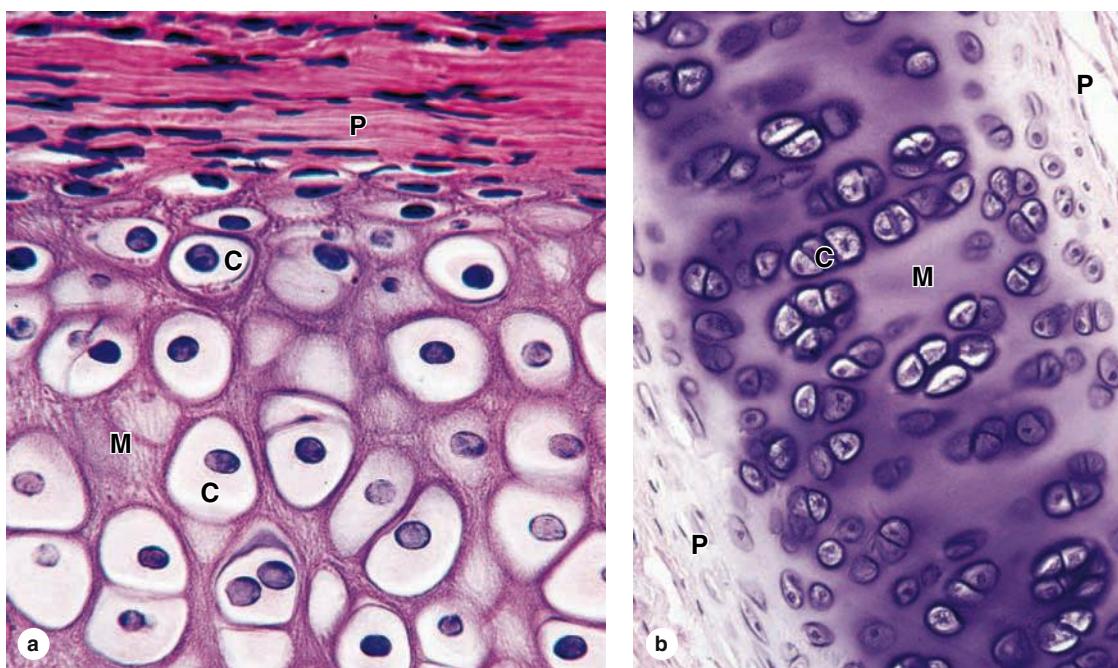
shape of the chondrocytes and their retraction from the matrix. In living tissue chondrocytes fill their lacunae completely.

Because cartilage matrix is avascular, chondrocytes respire under low-oxygen tension. Hyaline cartilage cells metabolize glucose mainly by anaerobic glycolysis. Nutrients from the blood diffuse to all the chondrocytes from the cartilage surface, with movements of water and solutes in the cartilage matrix promoted by intermittent tissue compression and decompression during body movements. The limits of such diffusion define the maximum thickness of hyaline cartilage, which usually exists as small, thin plates.

» MEDICAL APPLICATION

In contrast to other forms of cartilage and most other tissues, hyaline cartilage is susceptible to partial or isolated regions of **calcification** during aging, especially in the costal cartilage adjacent to the ribs. Calcification of the hyaline matrix, accompanied by degenerative changes in the chondrocytes, is a common part of the aging process and in many respects resembles endochondral ossification by which bone is formed.

FIGURE 7–3 Hyaline cartilage.



(a) The upper part of the photo shows the perichondrium (P), an example of dense connective tissue consisting largely of type I collagen. Among the fibroblastic cells of the perichondrium are indistinguishable mesenchymal stem cells. There is a gradual transition and differentiation of cells from the perichondrium to the cartilage, with some elongated fibroblast-like cells becoming larger and more rounded as chondroblasts and chondrocytes (C). These are located within lacunae surrounded by the matrix (M) which these cells secreted. (X200; H&E)

(b) The thin region of hyaline cartilage shown here has perichondrium (P) on both sides and shows larger lacunae containing isogenous groups of chondrocytes (C) within the matrix (M). Such groups of two, four, or more cells are produced by mitosis; the cells will separate into individual lacunae as they begin to secrete matrix. Territorial matrix immediately around the chondrocytes is more basophilic than interterritorial matrix farther from the cells. (X160; H&E)

Chondrocyte synthesis of sulfated GAGs and secretion of proteoglycans are accelerated by many hormones and growth factors. A major regulator of hyaline cartilage growth is the pituitary-derived protein called growth hormone or **somatotropin**. This hormone acts indirectly, promoting the endocrine release from the liver of insulin-like growth factors, or somatomedins, which directly stimulate the cells of hyaline cartilage.

» MEDICAL APPLICATION

Cells of cartilage can give rise to either benign (**chondroma**) or slow-growing, malignant (**chondrosarcoma**) tumors in which cells produce normal matrix components. Chondrosarcomas seldom metastasize and are generally removed surgically.

Perichondrium

Except in the articular cartilage of joints, all hyaline cartilage is covered by a layer of dense connective tissue, the

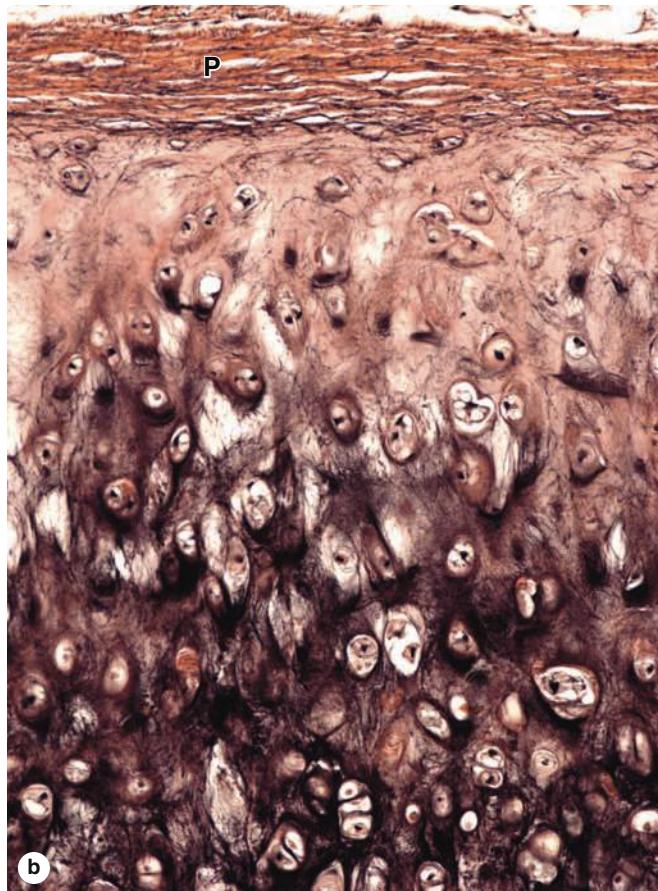
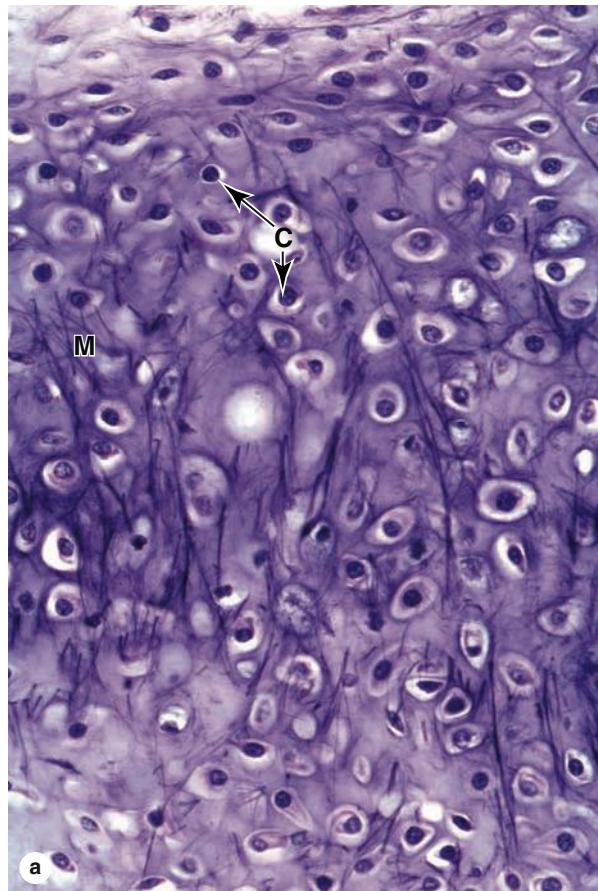
perichondrium, which is essential for the growth and maintenance of cartilage (Figures 7–2b and 7–3). The outer region of the perichondrium consists largely of collagen type I fibers and fibroblasts, but an inner layer adjoining the cartilage matrix also contains mesenchymal stem cells which provide a source for new chondroblasts that divide and differentiate into chondrocytes.

» ELASTIC CARTILAGE

Elastic cartilage is essentially similar to hyaline cartilage except that it contains an abundant network of elastic fibers in addition to a meshwork of collagen type II fibrils (Figures 7–4 and 7–1c), which give fresh elastic cartilage a yellowish color. With appropriate staining the elastic fibers usually appear as dark bundles distributed unevenly through the matrix.

More flexible than hyaline cartilage, elastic cartilage is found in the auricle of the ear, the walls of the external auditory

FIGURE 7–4 Elastic cartilage.



The chondrocytes (C) and overall organization of elastic cartilage are similar to those of hyaline cartilage, but the matrix (M) also contains elastic fibers that can be seen as darker components with proper staining. The abundant elastic fibers provide greater

flexibility to this type of cartilage. The section in part b includes perichondrium (P) that is also similar to that of hyaline cartilage. (a) X160; Hematoxylin and orcein. (b) X180; Weigert resorcin and van Gieson.

canals, the auditory (Eustachian) tubes, the epiglottis, and the upper respiratory tract. Elastic cartilage in these locations includes a perichondrium similar to that of most hyaline cartilage. Throughout elastic cartilage the cells resemble those of hyaline cartilage both physiologically and structurally.

► FIBROCARTILAGE

Fibrocartilage takes various forms in different structures but is essentially a mingling of hyaline cartilage and dense connective tissue (Figures 7–5 and 7–1d). It is found in intervertebral discs, in attachments of certain ligaments, and in the pubic symphysis—all places where it serves as very tough, yet cushioning support tissue for bone.

Chondrocytes of fibrocartilage occur singly and often in aligned isogenous aggregates, producing type II collagen and other ECM components, although the matrix around these chondrocytes is typically sparse. Areas with chondrocytes and hyaline matrix are separated by other regions with fibroblasts and dense bundles of type I collagen which confer extra tensile strength to this tissue (Figure 7–5). The relative scarcity of proteoglycans overall makes fibrocartilage matrix more acidophilic than that of hyaline or elastic cartilage. There is no distinct surrounding perichondrium in fibrocartilage.

Intervertebral discs of the spinal column are composed primarily of fibrocartilage and act as lubricated cushions and shock absorbers preventing damage to adjacent vertebrae from abrasive forces or impacts. Held in place by ligaments, intervertebral discs are discussed further with joints in Chapter 8.

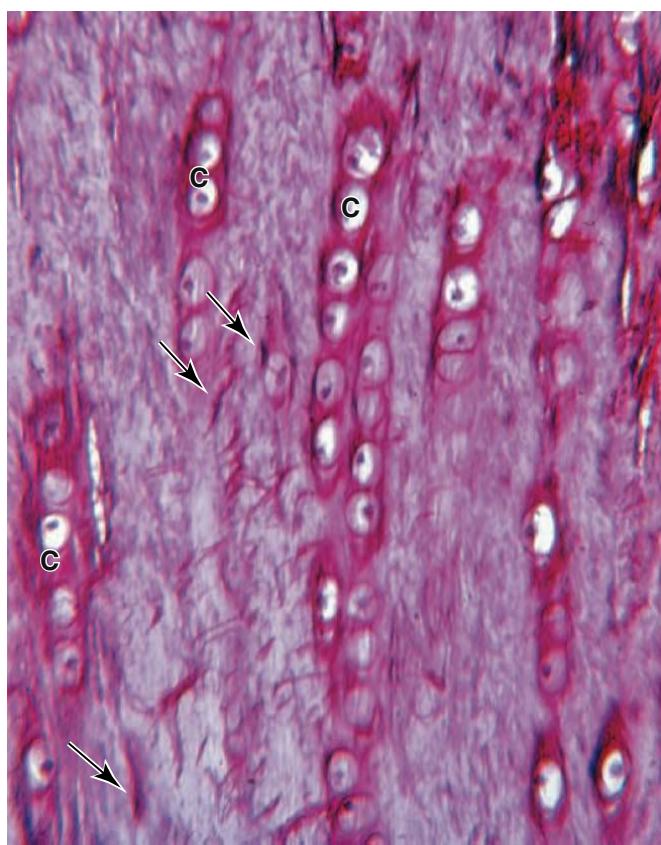
Important features of the three major types of cartilage are summarized in Table 7–1.

► CARTILAGE FORMATION, GROWTH, & REPAIR

All cartilage forms from embryonic mesenchyme in the process of **chondrogenesis** (Figure 7–6). The first indication of cell differentiation is the rounding up of the mesenchymal cells, which retract their extensions, multiply rapidly, and become more densely packed together. In general the terms “chondroblasts” and “chondrocytes” respectively refer to the cartilage cells during and after the period of rapid proliferation. At both stages the cells have basophilic cytoplasm rich in RER for collagen synthesis (Figure 7–7). Production of the ECM encloses the cells in their lacunae and then gradually separates chondroblasts from one another. During embryonic development, the cartilage differentiation takes place primarily from the center outward; therefore the more central cells have the characteristics of chondrocytes, whereas the peripheral cells are typical chondroblasts. The superficial mesenchyme develops as the perichondrium.

Once formed, the cartilage tissue enlarges both by **interstitial growth**, involving mitotic division of preexisting chondrocytes, and by **appositional growth**, which involves chondroblast differentiation from progenitor cells in the

FIGURE 7–5 Fibrocartilage.

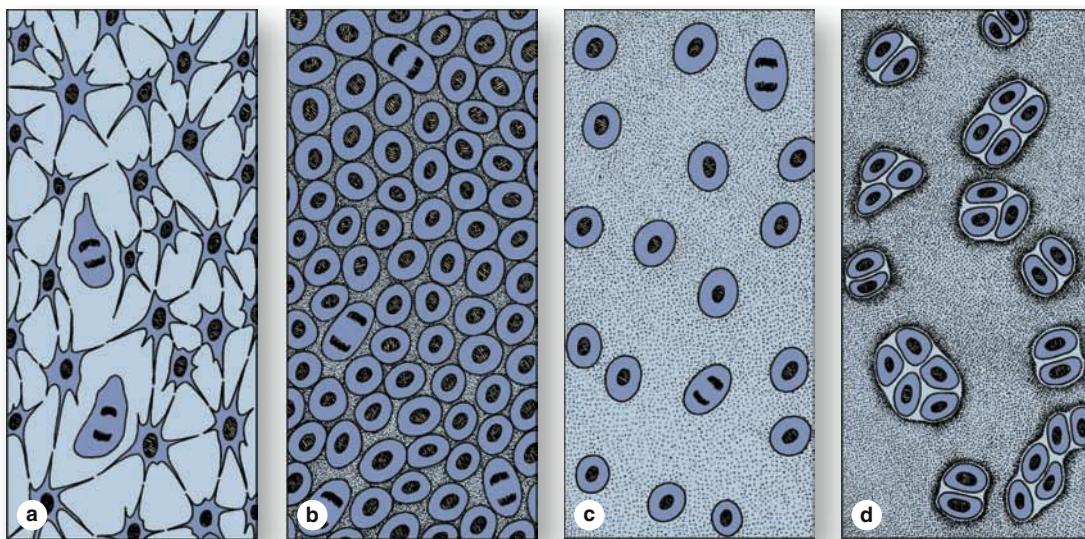


Fibrocartilage varies histologically in different structures, but is always essentially a mixture of hyaline cartilage and dense connective tissue.

In a small region of intervertebral disc, the axially arranged aggregates of chondrocytes (C) are seen to be surrounded by small amounts of matrix and separated by larger regions with dense collagen and scattered fibroblasts with elongated nuclei (arrows). (X250; Picosirius-hematoxylin)

perichondrium (Figure 7–2b). In both cases, the synthesis of matrix contributes greatly to the growth of the cartilage. Appositional growth of cartilage is more important during postnatal development, although as described in Chapter 8, interstitial growth in cartilaginous regions within long bones is important in increasing the length of these structures. In articular cartilage, cells and matrix near the articulating surface are gradually worn away and must be replaced from within, because there is no perichondrium to add cells by appositional growth.

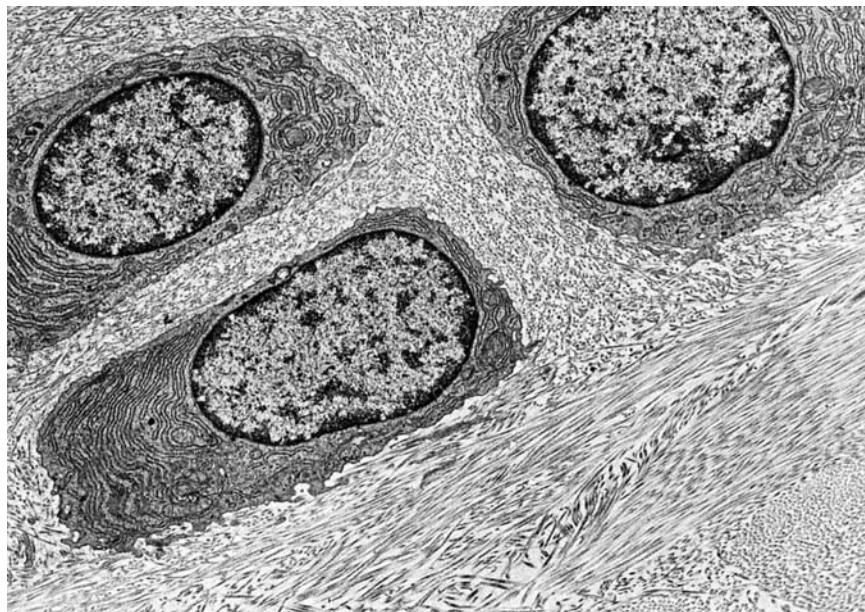
Except in young children, damaged cartilage undergoes slow and often incomplete **repair**, primarily dependent on cells in the perichondrium which invade the injured area and produce new cartilage. In damaged areas the perichondrium produces a scar of dense connective tissue instead of forming new cartilage. The poor capacity of cartilage for repair or regeneration is due in part to its avascularity and low metabolic rate.

FIGURE 7–6 Chondrogenesis.

The major stages of embryonic cartilage formation, or chondrogenesis, are shown here.

(a) Mesenchyme is the precursor for all types of cartilage. **(b)** Mitosis and initial cell differentiation produces a tissue with condensations of rounded cells called **chondroblasts**. **(c)** Chondroblasts are then separated from one another again by their production of the various matrix components, which collectively swell

with water and form the very extensive ECM. **(d)** Multiplication of chondroblasts within the matrix gives rise to isogenous cell aggregates surrounded by a condensation of territorial matrix. In mature cartilage, this interstitial mitotic activity ceases and all chondrocytes typically become more widely separated by their production of matrix.

FIGURE 7–7 Chondrocytes in growing cartilage.

This TEM of **fibrocartilage** shows **chondrocytes** with abundant RER actively secreting the collagen-rich matrix. Bundles of **collagen fibrils**, sectioned in several orientations, are very prominent around the chondrocytes of fibrocartilage. Collagen types I and II

are both present in fibrocartilage. Chondrocytes in growing hyaline and elastic cartilage have more prominent Golgi complexes and synthesize abundant proteoglycans in addition to collagens. (X3750)

Cartilage SUMMARY OF KEY POINTS

- Cartilage is a **tough, resilient** type of connective tissue that structurally supports certain soft tissues, notably in the respiratory tract, and provides cushioned, low-friction surfaces in joints.
- Cells of cartilage, **chondrocytes**, make up a small percentage of the tissue's mass, which is mainly a flexible mass of **extracellular matrix (ECM)**.
- Chondrocytes are embedded within **lacunae** surrounded by the ECM.
- Cartilage ECM typically includes **collagen** as well as abundant **proteoglycans**, notably **aggrecan**, which bind a large amount of water.
- Cartilage always **lacks blood vessels**, lymphatics, and nerves, but it is usually surrounded by a dense connective tissue **perichondrium** that is vascularized.
- There are three major forms of cartilage: (1) **hyaline cartilage**, (2) **elastic cartilage**, and (3) **fibrocartilage**.

Hyaline Cartilage

- The ECM of hyaline cartilage is **homogenous and glassy**, rich in fibrils of type II collagen and aggrecan complexes with bound water.
- The ECM has less collagen and more proteoglycan immediately around the lacunae, producing slight staining differences in this **territorial matrix**.
- Chondrocytes occur **singly** or in small, mitotically derived **isogenous groups**.
- **Perichondrium** is usually present, but not at the hyaline cartilage of articular surfaces or the epiphyses of growing long bones.

Elastic Cartilage

- Elastic cartilage generally resembles hyaline cartilage in its chondrocytes and major ECM components, but its matrix includes **abundant elastic fibers**, visible with special stains, which increase the tissue's **flexibility**.
- Elastic cartilage provides flexible support for the external ear as well as certain structures of the middle ear and larynx; it is always surrounded by **perichondrium**.

Fibrocartilage

- Fibrocartilage contains varying **combinations of hyaline cartilage** in small amounts of **dense connective tissue**.
- Histologically it consists of small **chondrocytes** in a hyaline matrix, usually layered with larger areas of bundled **type I collagen** with scattered **fibroblasts**.
- Fibrocartilage provides very **tough, strong support** at tendon insertions and in **intervertebral discs** and certain other joints.

Cartilage Formation, Growth, & Repair

- All forms of cartilage form from embryonic **mesenchyme**.
- Cartilaginous structures grow by mitosis of existing chondroblasts in lacunae (**interstitial growth**) or formation of new chondroblasts peripherally from progenitor cells in the perichondrium (**appositional growth**).
- Repair or replacement of injured cartilage is very slow and ineffective, due in part to the tissue's **avascularity** and **low metabolic rate**.

Cartilage ASSESS YOUR KNOWLEDGE

1. The molecular basis for the shock absorbing properties of cartilage involves which of the following?
 - a. Electrostatic interaction of proteoglycans with type IV collagen
 - b. Ability of glycosaminoglycans to bind anions
 - c. Noncovalent binding of glycosaminoglycans to protein cores
 - d. Sialic acid residues in the glycoproteins
 - e. Hydration of glycosaminoglycans
2. What distinguishes cartilage from most other connective tissues?
 - a. Its extracellular matrix is rich in collagen.
 - b. Its predominant cell type is a mesenchymal derivative.
 - c. Its predominant cell type secretes both fibers and proteoglycans.
 - d. It lacks blood vessels.
 - e. It functions in mechanical support.
3. Which feature is typical of elastic cartilage?
 - a. Primary skeletal tissue in the fetus
 - b. No identifiable perichondrium
 - c. Found in intervertebral discs
 - d. Most widely distributed cartilage type in the body
 - e. Collagen is mainly type II
4. Which area in cartilage is relatively collagen-poor and proteoglycan-rich?
 - a. Fibrocartilage
 - b. Territorial matrix
 - c. Epiphyseal plate
 - d. Interterritorial matrix
 - e. Perichondrium
5. What is the source of the mesenchymal progenitor cells activated for the repair of hyaline cartilage of accident-damaged costal cartilages?
 - a. Perichondrium
 - b. Adjacent loose connective tissue
 - c. Bone of the adjacent rib(s) and sternum
 - d. Chondrocytes of the injured cartilage
 - e. Stem cells circulating with blood
6. How does articular cartilage differ from most other hyaline cartilage?
 - a. It undergoes mainly appositional growth.
 - b. It contains isogenous groups of chondrocytes.
 - c. It lacks a perichondrium.
 - d. Its matrix contains aggrecan.
 - e. It is derived from embryonic mesenchyme.
7. Which step occurs first in chondrogenesis?
 - a. Appositional growth
 - b. Conversion of chondroblasts to chondrocytes
 - c. Formation of mesenchymal condensations
 - d. Interstitial growth
 - e. Secretion of collagen-rich and proteoglycan-rich matrix

8. Osteoarthritis is characterized by the progressive erosion of articular cartilage. The matrix metalloproteinases involved in this erosion primarily act on which matrix component?
- Aggrecan
 - Link proteins
 - Network-forming collagen
 - Fibril-forming collagen
 - Chondronectin
9. A 28-year-old woman visits the family medicine clinic complaining of loss of the sense of smell, nosebleeds, problems with swallowing, and hoarseness. She admits to “casual, social use” of cocaine on a regular basis since her sophomore year of college. A complete examination of her nose with a speculum and otoscope shows severe rhinitis (inflammation). There is also perforation and collapse of the nasal cartilage resulting in a “saddle nose” deformity. Erosions in the enamel of her front teeth are noted. The breakdown of the nasal cartilage releases collagen fibers primarily of which type?
- Type I
 - Type II
 - Type III
 - Type IV
 - Type VII
10. A 66-year-old man who suffered from severe osteoarthritis is referred to an orthopedic surgeon for replacement of his right knee. He had been actively involved in both high school and intercollegiate football and had continued running until about the age of 45 as a form of relaxation and exercise. With the patient’s permission the removed joint is used by investigators performing a proteomic analysis of different joint tissues. The meniscus was found to contain almost exclusively type I collagen and aggrecan was undetectable. What is the most likely explanation for this result?
- The meniscus normally consists of dense regular connective tissue, which contains primarily type I collagen.
 - The meniscus normally consists of fibrocartilage, which contains only type I collagen.
 - The meniscus had undergone repeated rounds of repair due to wear-and-tear during which its hyaline cartilage component was replaced by dense connective tissue.
 - Osteoarthritic injury in the knee resulted in the chondrocytes of the meniscus switching from expression of genes for type II collagen to type I collagen.
 - Elastic cartilage is normally replaced by fibrocartilage during aging and this process can be accelerated by exercise.

CHAPTER 8 Bone

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As the main constituent of the adult skeleton, bone tissue (Figure 8–1) provides solid support for the body, protects vital organs such as those in the cranial and thoracic cavities, and encloses internal (medullary) cavities containing bone marrow where blood cells are formed. Bone (or osseous) tissue also serves as a reservoir of calcium, phosphate, and other ions that can be released or stored in a controlled fashion to maintain constant concentrations in body fluids.

In addition, bones form a system of levers that multiply the forces generated during skeletal muscle contraction and transform them into bodily movements. This mineralized tissue therefore confers mechanical and metabolic functions to the skeleton.

Bone is a specialized connective tissue composed of calcified extracellular material, the **bone matrix**, and following three major cell types (Figure 8–2):

- **Osteocytes** (Gr. *osteon*, bone + *kytos*, cell), which are found in cavities (*lacunae*) between bone matrix layers (*lamellae*), with cytoplasmic processes in small **canalliculi** (L. *canalis*, canal) that extend into the matrix (Figure 8–1b)
- **Osteoblasts** (*osteon* + Gr. *blastos*, germ), growing cells which synthesize and secrete the organic components of the matrix
- **Osteoclasts** (*osteon* + Gr. *klastos*, broken), which are giant, multinucleated cells involved in removing calcified bone matrix and remodeling bone tissue

Because metabolites are unable to diffuse through the calcified matrix of bone, the exchanges between osteocytes and blood capillaries depend on communication through the very thin, cylindrical spaces of the canaliculari.

All bones are lined on their internal and external surfaces by layers of connective tissue containing osteogenic cells—**endosteum** on the internal surface surrounding the marrow cavity and **periosteum** on the external surface.

Because of its hardness, bone cannot be sectioned routinely. Bone matrix is usually softened by immersion in a decalcifying solution before paraffin embedding, or embedded in plastic after fixation and sectioned with a specialized microtome.

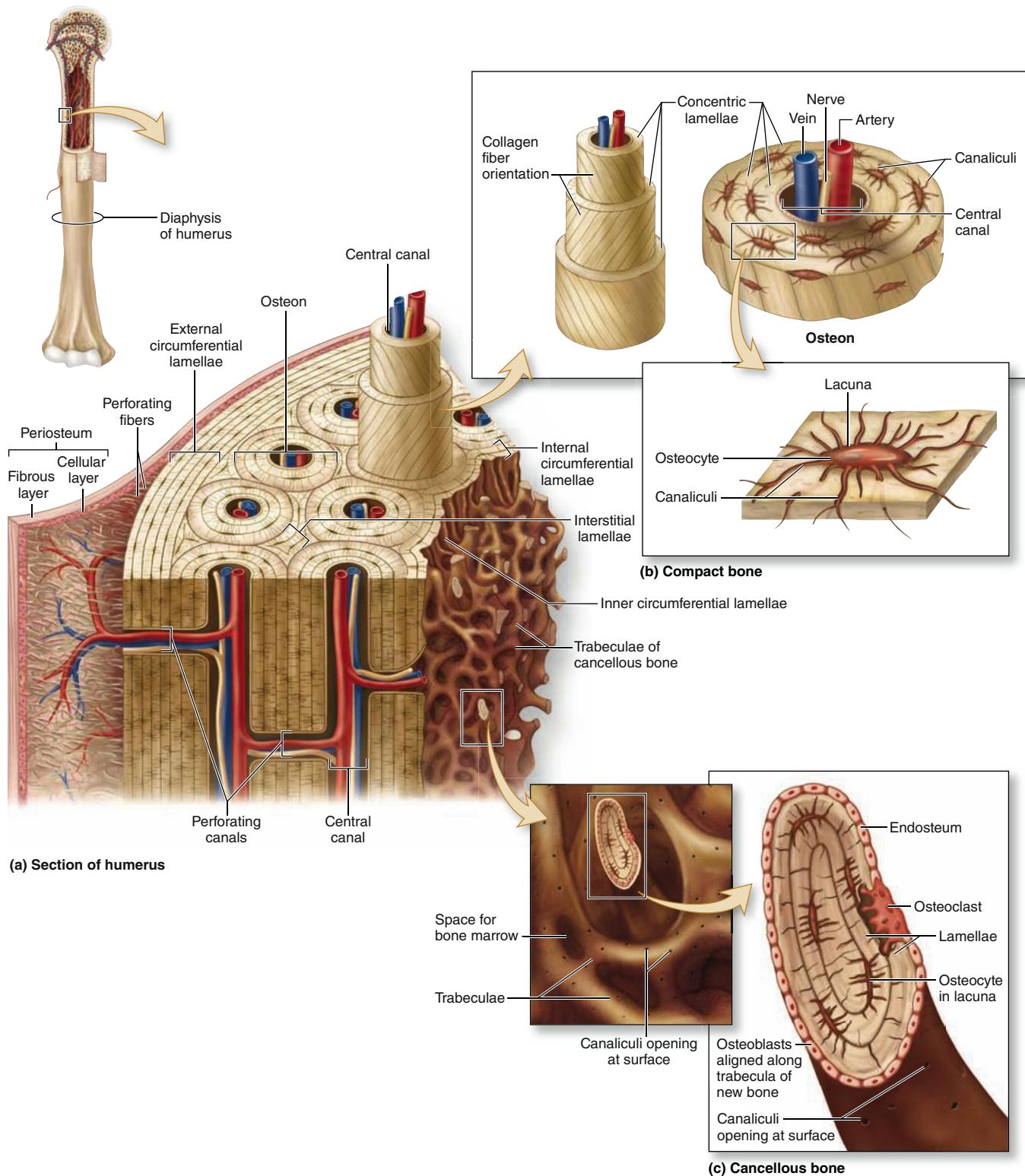
► BONE CELLS

Osteoblasts

Originating from mesenchymal stem cells, **osteoblasts** produce the organic components of bone matrix, including type I collagen fibers, proteoglycans, and matricellular glycoproteins such as osteonectin. Deposition of the inorganic components of bone also depends on osteoblast activity. Active osteoblasts are located exclusively at the surfaces of bone matrix, to which they are bound by integrins, typically forming a single layer of cuboidal cells joined by adherent and gap junctions (Figure 8–3). When their synthetic activity is completed, some osteoblasts differentiate as osteocytes entrapped in matrix-bound lacunae, some flatten and cover the matrix surface as **bone lining cells**, and the majority undergo apoptosis.

During the processes of matrix synthesis and calcification, osteoblasts are polarized cells with ultrastructural features denoting active protein synthesis and secretion. Matrix components are secreted at the cell surface in contact with existing bone matrix, producing a layer of unique collagen-rich material

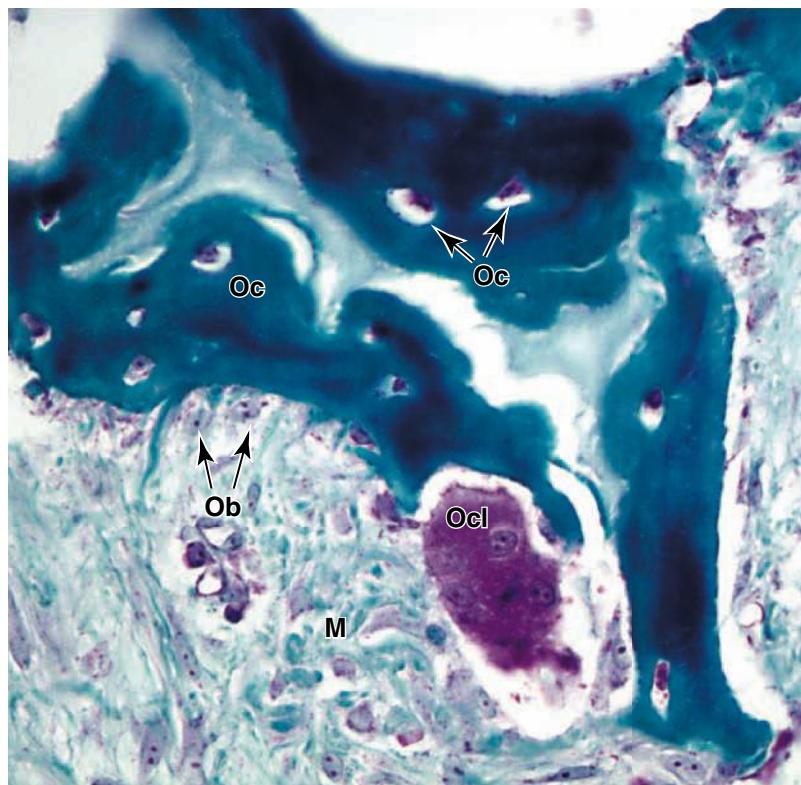
FIGURE 8–1 Components of bone.



A schematic overview of the basic features of bone, including the three key cell types: **osteocytes**, **osteoblasts**, and **osteoclasts**; their usual locations; and the typical **lamellar organization** of bone. Osteoblasts secrete the matrix that then hardens by calcification, trapping the differentiating cells now called **osteocytes** in individual **lacunae**. Osteocytes maintain the calcified matrix and receive nutrients from microvasculature in the central canals of the osteons via very small channels called **canaliculari** that interconnect

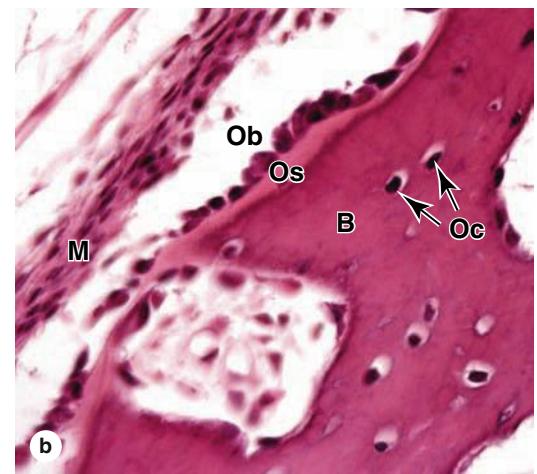
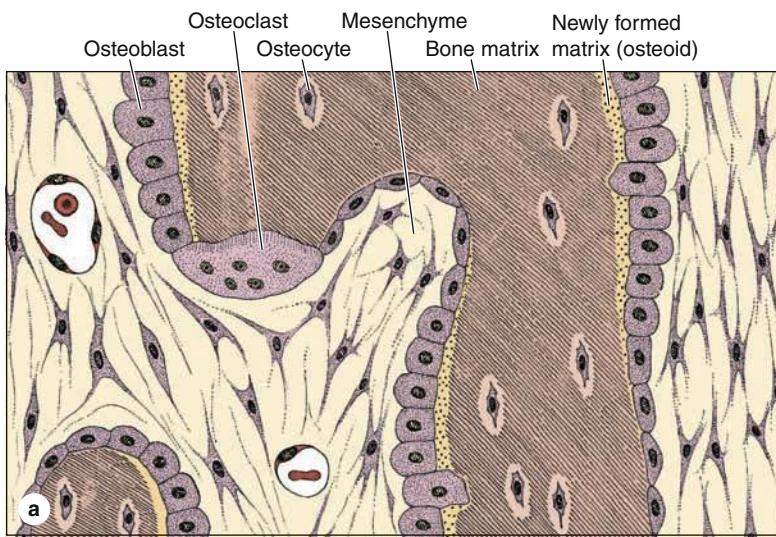
the lacunae. Osteoclasts are monocyte-derived cells in bone required for bone remodeling.

The **periosteum** consists of dense connective tissue, with a primarily fibrous layer covering a more cellular layer. Bone is vascularized by small vessels that penetrate the matrix from the periosteum. **Endosteum** covers all **trabeculae** around the marrow cavities.

FIGURE 8–2 Bone tissue.

Newly formed bone tissue decalcified for sectioning and stained with trichrome in which the collagen-rich ECM appears bright blue. The tissue is a combination of mesenchymal regions (**M**) containing capillaries, fibroblasts, and osteoprogenitor stem cells and regions of normally calcified matrix with varying amounts of collagen and the three major cell types found in all bone tissue.

Bone-forming osteoblasts (**Ob**) differentiate from osteoprogenitor cells in the periosteum and endosteum, and cover the surfaces of existing bone matrix. Osteoblasts secrete **osteoid** rich in collagen type I, but also containing proteoglycans and other molecules. As osteoid undergoes calcification and hardens, it entraps some osteoblasts which then differentiate further as osteocytes (**Oc**) occupying lacunae surrounded by bony matrix. The much less numerous large, multinuclear osteoclasts (**Ocl**), produced by the fusion of blood monocytes, reside on bony surfaces and erode the matrix during bone remodeling. (400X; Mallory trichrome)

FIGURE 8–3 Osteoblasts, osteocytes, and osteoclasts.

(a) Diagram showing the relationship of osteoblasts to the newly formed matrix called "osteoid," bone matrix, and osteocytes. Osteoblasts and most of the larger osteoclasts are part of the endosteum covering the bony trabeculae.

(b) The photomicrograph of developing bone shows the location and morphologic differences between active osteoblasts (**Ob**) and osteocytes (**Oc**). Rounded osteoblasts, derived from progenitor

cells in the adjacent mesenchyme (**M**), cover a thin layer of lightly stained osteoid (**Os**) on the surface of the more heavily stained bony matrix (**B**). Most osteoblasts that are no longer actively secreting osteoid will undergo apoptosis; others differentiate either as flattened bone lining cells on the trabeculae of bony matrix or as osteocytes located within lacunae surrounded by bony matrix. (X300; H&E)

called **osteoid** between the osteoblast layer and the preexisting bone surface (Figure 8–3). This process of bone appositional growth is completed by subsequent deposition of calcium salts into the newly formed matrix.

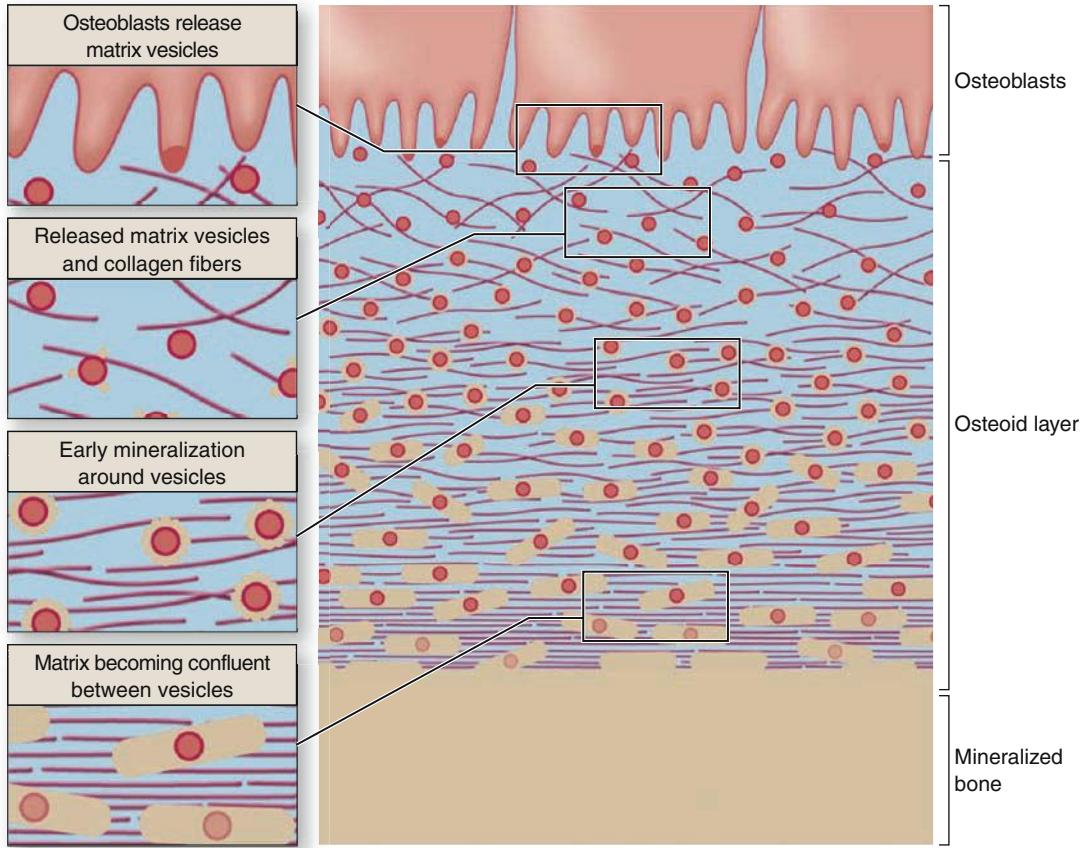
The process of matrix mineralization is not completely understood, but basic aspects of the process are shown in Figure 8–4. Prominent among the noncollagen proteins secreted by osteoblasts is the vitamin K-dependent polypeptide **osteocalcin**, which together with various glycoproteins binds Ca^{2+} ions and concentrates this mineral locally. Osteoblasts also release membrane-enclosed **matrix vesicles** rich in alkaline phosphatase and other enzymes whose activity raises the local concentration of PO_4^{3-} ions. In the microenvironment with high concentrations of both these ions, matrix vesicles serve as foci for the formation of hydroxyapatite

$[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ crystals, the first visible step in calcification. These crystals grow rapidly by accretion of more mineral and eventually produce a confluent mass of calcified material embedding the collagen fibers and proteoglycans (Figure 8–4).

» MEDICAL APPLICATION

Cancer originating directly from bone cells (a primary bone tumor) is fairly uncommon (0.5% of all cancer deaths), although a cancer called **osteosarcoma** can arise in osteoprogenitor cells. The skeleton is often the site of secondary, **metastatic tumors**, however, arising when cancer cells move into bones via small blood or lymphatic vessels from malignancies in other organs, most commonly the breast, lung, prostate gland, kidney, or thyroid gland.

FIGURE 8–4 Mineralization in bone matrix.



From their ends adjacent to the bone matrix, osteoblasts secrete type I collagen, several glycoproteins, and proteoglycans. Some of these factors, notably **osteocalcin** and certain glycoproteins, bind Ca^{2+} with high affinity, raising the local concentration of these ions. Osteoblasts also release very small membrane-enclosed **matrix vesicles** containing alkaline phosphatase and other enzymes. These enzymes hydrolyze PO_4^{3-} ions from various matrix macromolecules, creating a high concentration of these ions locally. The high Ca^{2+}

and PO_4^{3-} ion concentrations cause calcified nanocrystals to form in and around the matrix vesicles. The crystals grow and mineralize further with formation of small growing masses of calcium hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, which surround the collagen fibers and all other macromolecules. Eventually the masses of hydroxyapatite merge as a confluent solid bony matrix as calcification of the matrix is completed.

Osteocytes

As mentioned some osteoblasts become surrounded by the material they secrete and then differentiate as **osteocytes** enclosed singly within the **lacunae** spaced throughout the mineralized matrix. During the transition from osteoblasts to osteocytes, the cells extend many long dendritic processes, which also become surrounded by calcifying matrix. The processes thus come to occupy the many canaliculi, 250–300 nm in diameter, radiating from each lacuna (Figures 8–5 and 8–1b).

Diffusion of metabolites between osteocytes and blood vessels occurs through the small amount of interstitial fluid in the canaliculi between the bone matrix and the osteocytes and their processes. Osteocytes also communicate with one another and ultimately with nearby osteoblasts and bone lining cells via gap junctions at the ends of their processes.

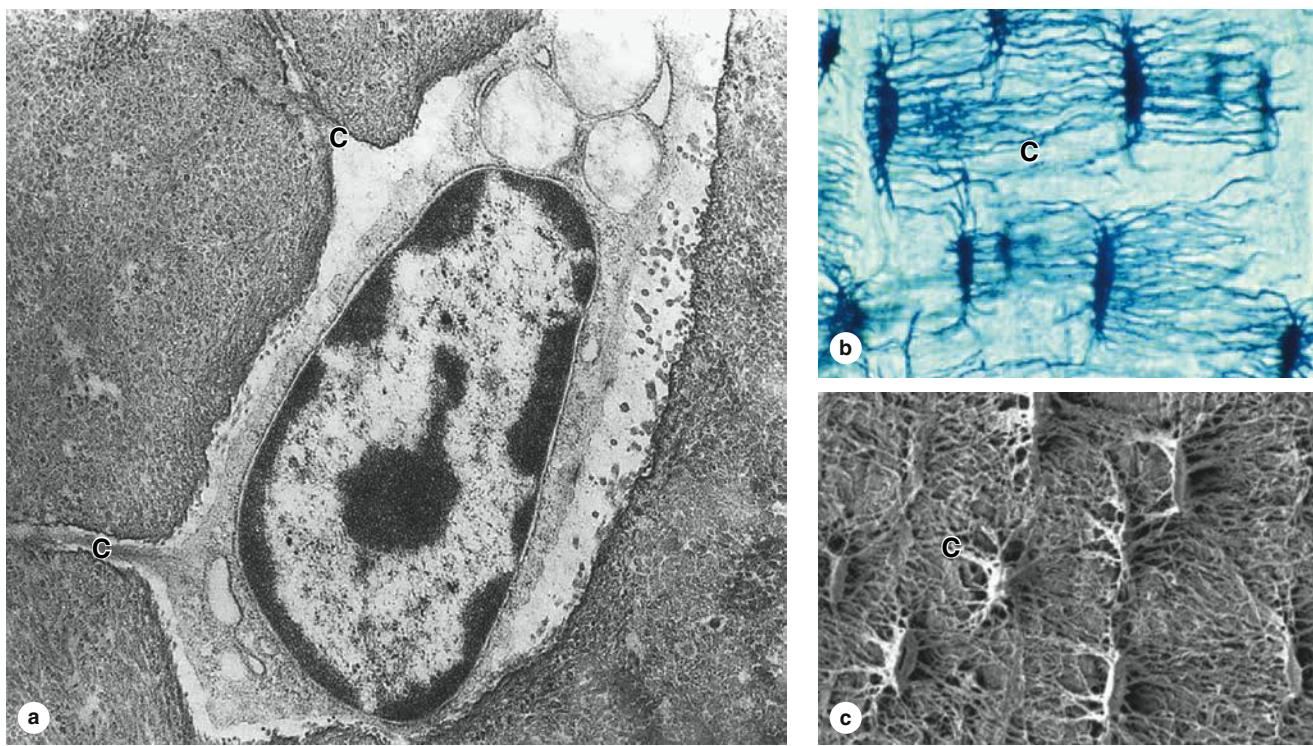
Normally the most abundant cells in bone, the almond-shaped osteocytes exhibit significantly less RER, smaller Golgi complexes, and more condensed nuclear chromatin than osteoblasts (Figure 8–5a). Osteocytes maintain the calcified

matrix, and their death is followed by rapid matrix resorption. While sharing most matrix-related activities with osteoblasts, osteocytes also express many different proteins, including factors with paracrine and endocrine effects that help regulate bone remodeling. The extensive lacunar-canalicular network of these cells and their communication with all other bone cells allow osteocytes to serve as sensitive detectors of stress- or fatigue-induced microdamage in bone and to trigger remedial activity in osteoblasts and osteoclasts.

» MEDICAL APPLICATION

The network of dendritic processes extending from osteocytes has been called a “mechanostat,” monitoring areas within bones where loading has been increased or decreased, and signaling cells to adjust ion levels and maintain the adjacent bone matrix accordingly. **Lack of exercise** (or the weightlessness experienced by astronauts) leads to **decreased bone density**, due in part to the lack of mechanical stimulation of these cells.

FIGURE 8–5 Osteocytes in lacunae.



(a) TEM showing an osteocyte in a lacuna and two dendritic processes in canaliculi (**C**) surrounded by bony matrix. Many such processes are extended from each cell as osteoid is being secreted; this material then undergoes calcification around the processes, giving rise to canaliculi. (X30,000)

(b) Photomicrograph of bone, not decalcified or sectioned, but ground very thin to demonstrate lacunae and canaliculi. The lacunae and canaliculi (**C**) appear dark and show the communication

between these structures through which nutrients derived from blood vessels diffuse and are passed from cell to cell in living bone. (X400; Ground bone)

(c) SEM of non-decalcified, sectioned, and acid-etched bone showing lacunae and canaliculi (**C**). (X400)

(Figure 8-5c, used with permission from Dr Matt Allen, Indiana University School of Medicine, Indianapolis.)

Osteoclasts

Osteoclasts are very large, motile cells with multiple nuclei (Figure 8–6) which are essential for matrix resorption during bone growth and remodeling. The large size and multinucleated condition of osteoclasts are due to their origin from the fusion of bone marrow–derived monocytes. Osteoclast development requires two polypeptides produced by osteoblasts: macrophage-colony-stimulating factor (M-CSF; discussed with hemopoiesis, Chapter 13) and the receptor activator of nuclear factor- κ B ligand (RANKL). In areas of bone undergoing resorption, osteoclasts on the bone surface lie within enzymatically etched depressions or cavities in the matrix known as **resorption lacunae** (or **Howship lacunae**).

In an active osteoclast the membrane domain that contacts the bone forms a circular **sealing zone** which binds the cell tightly to the bone matrix and surrounds an area with many surface projections, called the **ruffled border**. This circumferential sealing zone allows the formation of a specialized microenvironment between the osteoclast and the matrix in which bone resorption occurs (Figure 8–6b).

Into this subcellular pocket the osteoclast pumps protons to acidify and promote dissolution of the adjacent hydroxyapatite, and releases matrix metalloproteinases and other hydrolytic enzymes from lysosome-related secretory vesicles for the localized digestion of matrix proteins. Osteoclast activity is controlled by local signaling factors from other bone cells. Osteoblasts activated by parathyroid hormone produce M-CSF, RANKL, and other factors that regulate the formation and activity of osteoclasts.

» MEDICAL APPLICATION

In the genetic disease **osteopetrosis**, which is characterized by dense, heavy bones (“marble bones”), the osteoclasts lack ruffled borders and bone resorption is defective. This disorder results in overgrowth and thickening of bones, often with obliteration of the marrow cavities, depressing blood cell formation and causing anemia and the loss of white blood cells. The defective osteoclasts in most patients with osteopetrosis have mutations in genes for the cells’ proton-ATPase pumps or chloride channels.

» BONE MATRIX

About 50% of the dry weight of bone matrix is inorganic materials. Calcium hydroxyapatite is most abundant, but bicarbonate, citrate, magnesium, potassium, and sodium ions are also found. Significant quantities of noncrystalline calcium phosphate are also present. The surface of hydroxyapatite crystals are hydrated, facilitating the exchange of ions between the mineral and body fluids.

The organic matter embedded in the calcified matrix is 90% type I collagen, but also includes mostly small proteoglycans and multiadhesive glycoproteins such as **osteonectin**. Calcium-binding proteins, notably osteocalcin, and the phosphatases

released from cells in matrix vesicles promote calcification of the matrix. Other tissues rich in type I collagen lack osteocalcin and matrix vesicles and therefore do not normally become calcified.

The association of minerals with collagen fibers during calcification provides the hardness and resistance required for bone function. If a bone is decalcified by a histologist, its shape is preserved but it becomes soft and pliable like other connective tissues. Because of its high collagen content, decalcified bone matrix is usually acidophilic.

» PERIOSTEUM & ENDOSTEUM

External and internal surfaces of all bones are covered by connective tissue of the periosteum and endosteum respectively (Figures 8–1a and 8–1c). The **periosteum** is organized much like the perichondrium of cartilage, with an outer fibrous layer of dense connective tissue, containing mostly bundled type I collagen, but also fibroblasts and blood vessels. Bundles of periosteal collagen, called **perforating** (or **Sharpey**) **fibers**, penetrate the bone matrix and bind the periosteum to the bone. Periosteal blood vessels branch and penetrate the bone, carrying metabolites to and from bone cells.

The periosteum’s inner layer is more cellular and includes osteoblasts, bone lining cells, and mesenchymal stem cells referred to as **osteoprogenitor cells**. With the potential to proliferate extensively and produce many new osteoblasts, osteoprogenitor cells play a prominent role in bone growth and repair.

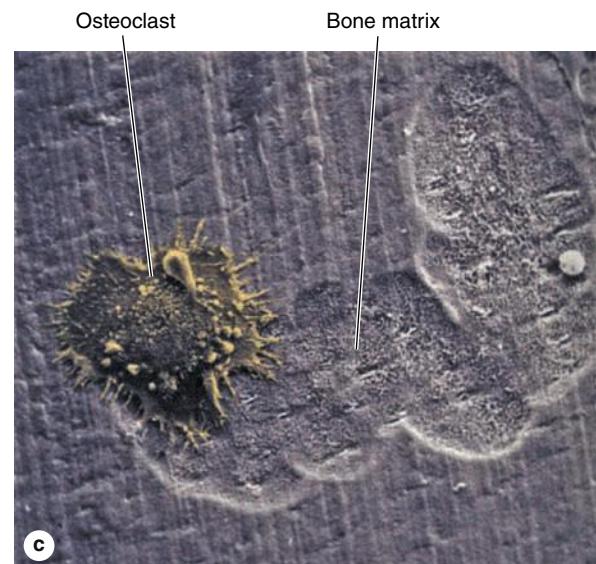
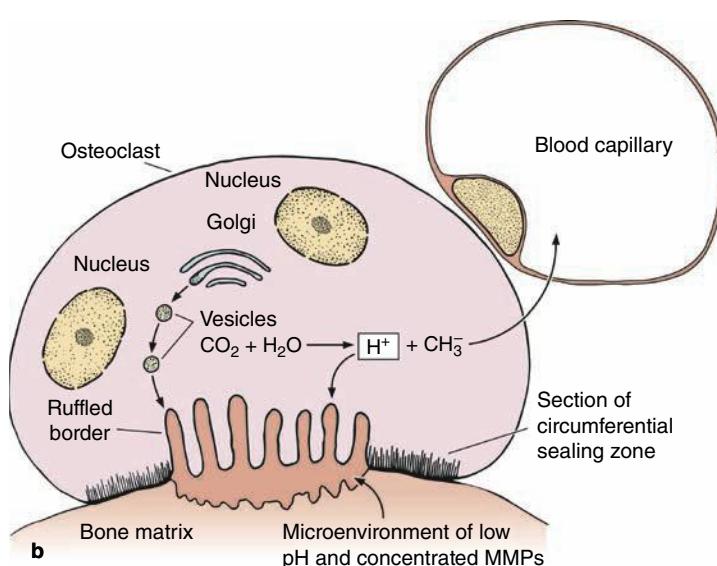
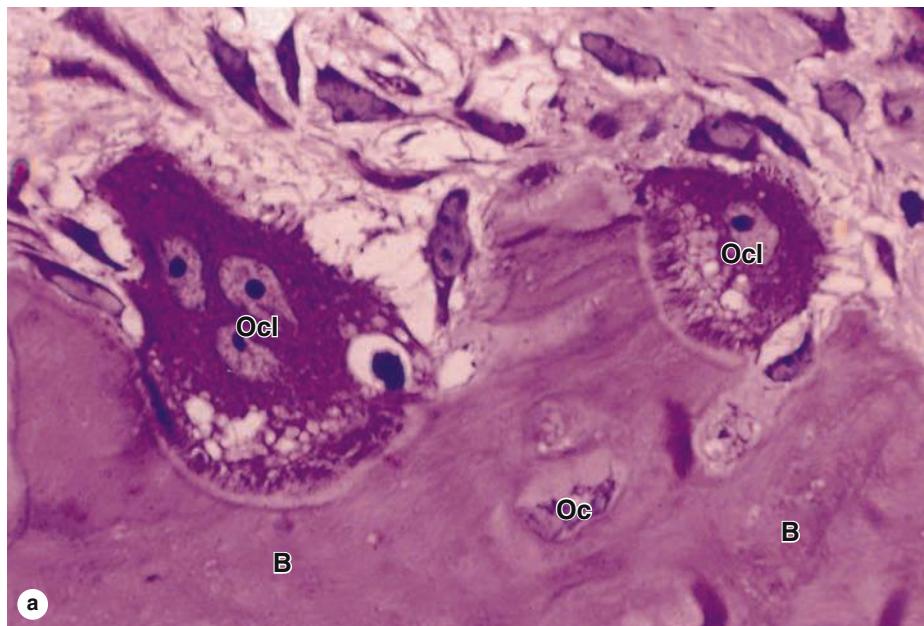
Internally the very thin **endosteum** covers small **trabeculae** of bony matrix that project into the marrow cavities (Figure 8–1). The endosteum also contains osteoprogenitor cells, osteoblasts, and bone lining cells, but within a sparse, delicate matrix of collagen fibers.

» MEDICAL APPLICATION

Osteoporosis, frequently found in immobilized patients and in postmenopausal women, is an imbalance in skeletal turnover so that bone resorption exceeds bone formation. This leads to calcium loss from bones and reduced **bone mineral density** (BMD). Individuals at risk for osteoporosis are routinely tested for BMD by **dual-energy x-ray absorptiometry** (DEXA scans).

» TYPES OF BONE

Gross observation of a bone in cross section (Figure 8–7) shows a dense area near the surface corresponding to **compact (cortical) bone**, which represents 80% of the total bone mass, and deeper areas with numerous interconnecting cavities, called **cancellous (trabecular) bone**, constituting about 20% of total bone mass. Histological features and important locations of the major types of bone are summarized in Table 8–1.

FIGURE 8–6 Osteoclasts and their activity.

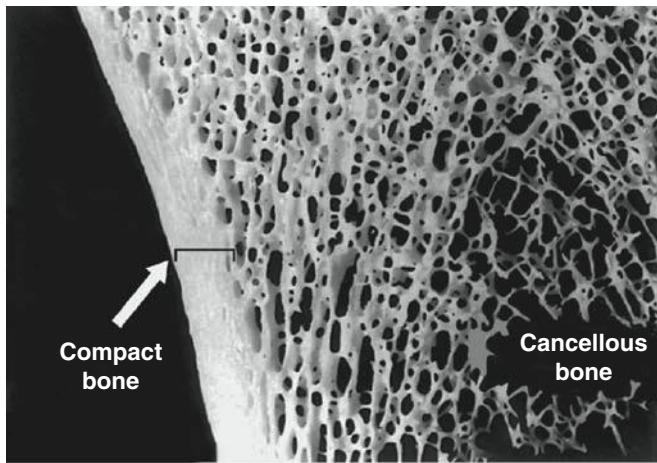
Osteoclasts are large multinucleated cells which are derived by the fusion in bone of several blood-derived monocytes. (a) Photo of bone showing two osteoclasts (**Ocl**) digesting and resorbing bone matrix (**B**) in relatively large resorption cavities (or Howship lacunae) on the matrix surface. An osteocyte (**Oc**) in its smaller lacuna is also shown. (X400; H&E)

(b) Diagram showing an osteoclast's circumferential **sealing zone** where integrins tightly bind the cell to the bone matrix. The sealing zone surrounds a **ruffled border** of microvilli and other cytoplasmic projections close to this matrix. The sealed space between the cell and the matrix is acidified to $\sim\text{pH } 4.5$ by proton pumps in the ruffled part of the cell membrane and receives secreted matrix

metalloproteases and other hydrolytic enzymes. Acidification of the sealed space promotes dissolution of hydroxyapatite from bone and stimulates activity of the protein hydrolases, producing localized matrix resorption. The breakdown products of collagen fibers and other polypeptides are endocytosed by the osteoclast and further degraded in lysosomes, while Ca^{2+} and other ions are released directly and taken up by the blood.

(c) SEM showing an active osteoclast cultured on a flat substrate of bone. A trench is formed on the bone surface by the slowly migrating osteoclast. (X5000)

(Figure 8–6c, used with permission from Alan Boyde, Centre for Oral Growth and Development, University of London.)

FIGURE 8–7 Compact and cancellous bone.

Macroscopic photo of a thick section of bone showing the cortical **compact bone** and the lattice of trabeculae in **cancellous bone** at the bone's interior. The small trabeculae that make up highly porous cancellous bone serve as supportive struts, collectively providing considerable strength, without greatly increasing the bone's weight. The compact bone is normally covered externally with periosteum and all trabecular surfaces of the cancellous bone are covered with endosteum. (X10)

In long bones, the bulbous ends—called **epiphyses** (Gr. *epiphysis*, an excrescence)—are composed of cancellous bone covered by a thin layer of compact cortical bone. The cylindrical part—the **diaphysis** (Gr. *diaphysis*, a growing between)—is almost totally dense compact bone, with a thin region of cancellous bone on the inner surface around the central **marrow cavity** (Figure 8–1). Short bones such as those of the wrist and ankle usually have cores of cancellous bone surrounded completely by compact bone. The flat bones that form the calvaria (skullcap) have two layers of compact bone called **plates**, separated by a thicker layer of cancellous bone called the **diploë**.

At the microscopic level both compact and cancellous bone typically show two types of organization: mature **lamellar bone**, with matrix existing as discrete sheets, and **woven bone**, newly formed with randomly arranged components.

Lamellar Bone

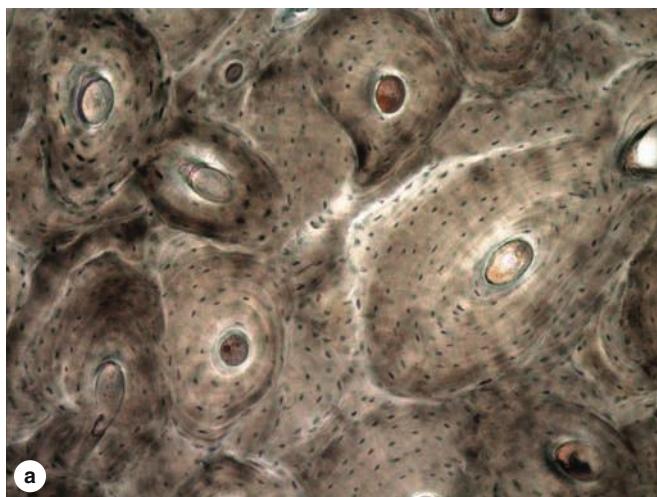
Most bone in adults, compact or cancellous, is organized as **lamellar bone**, characterized by multiple layers or **lamellae** of calcified matrix, each 3–7 µm thick. The lamellae are organized as parallel sheets or concentrically around a central canal. In each lamella, type I collagen fibers are aligned, with the pitch of the fibers' orientation shifted orthogonally (by about 90 degrees) in successive lamellae (Figure 8–1a). This highly ordered organization of collagen within lamellar bone causes birefringence with polarizing light microscopy; the alternating bright and dark layers are due to the changing orientation of collagen fibers in the lamellae (Figure 8–8). Like the orientation of wood fibers in plywood the highly ordered, alternating organization of collagen fibers in lamellae adds greatly to the strength of lamellar bone.

An **osteon** (or **Haversian system**) refers to the complex of concentric lamellae, typically 100–250 µm in diameter, surrounding a central canal that contains small blood vessels, nerves, and endosteum (Figures 8–1 and 8–9). Between successive lamellae are lacunae, each with one osteocyte, all interconnected by the canaliculi containing the cells' dendritic processes (Figure 8–9). Processes of adjacent cells are in contact via gap junctions, and all cells of an osteon receive nutrients and oxygen from vessels in the central canal (Figure 8–1). The outer boundary of each osteon is a layer called the cement line which includes many more noncollagen proteins in addition to mineral and collagen.

Each osteon is a long, sometimes bifurcated, cylinder generally parallel to the long axis of the diaphysis. Each has 5–20 concentric lamellae around the central canal which communicates with the marrow cavity and the periosteum. Canals also communicate with one another through transverse **perforating canals** (or **Volkmann canals**) which have few, if any, concentric lamellae (Figures 8–1 and 8–10). All central osteonic

Table 8–1**Summary of bone types and their organization.**

| Type of Bone | Histological Features | Major Locations | Synonyms |
|--|--|---|--|
| Woven bone , newly calcified | Irregular and random arrangement of cells and collagen; lightly calcified | Developing and growing bones; hard callus of bone fractures | Immature bone; primary bone; bundle bone |
| Lamellar bone , remodeled from woven bone | Parallel bundles of collagen in thin layers (lamellae), with regularly spaced cells between; heavily calcified | All normal regions of adult bone | Mature bone; secondary bone |
| Compact bone , ~80% of all lamellar bone | Parallel lamellae or densely packed osteons, with interstitial lamellae | Thick, outer region (beneath periosteum) of bones | Cortical bone |
| Cancellous bone , ~20% of all lamellar bone | Interconnected thin spicules or trabeculae covered by endosteum | Inner region of bones, adjacent to marrow cavities | Spongy bone; trabecular bone; medullary bone |

FIGURE 8–8 Lamellar bone.

Two photographs of the same area of an unstained section of compact bone, showing osteons with concentric lamellae around central canals. Lamellae are seen only faintly by bright-field microscopy (a), but they appear as alternating bright and dark bands under the polarizing light microscope (b). Bright bands are due to birefringence from the highly ordered collagen fibers in a lamella. Alternating bright and dark bands indicate that fibers in successive lamellae have different orientations, an organization that makes lamellar bone very strong. (Both X100)

(Used with permission from Dr Matt Allen, Indiana University School of Medicine, Indianapolis.)

canals and perforating canals form when matrix is laid down around areas with preexisting blood vessels.

Scattered among the intact osteons are numerous irregularly shaped groups of parallel lamellae called **interstitial lamellae**. These structures are lamellae remaining from osteons partially destroyed by osteoclasts during growth and remodeling of bone (Figure 8–10).

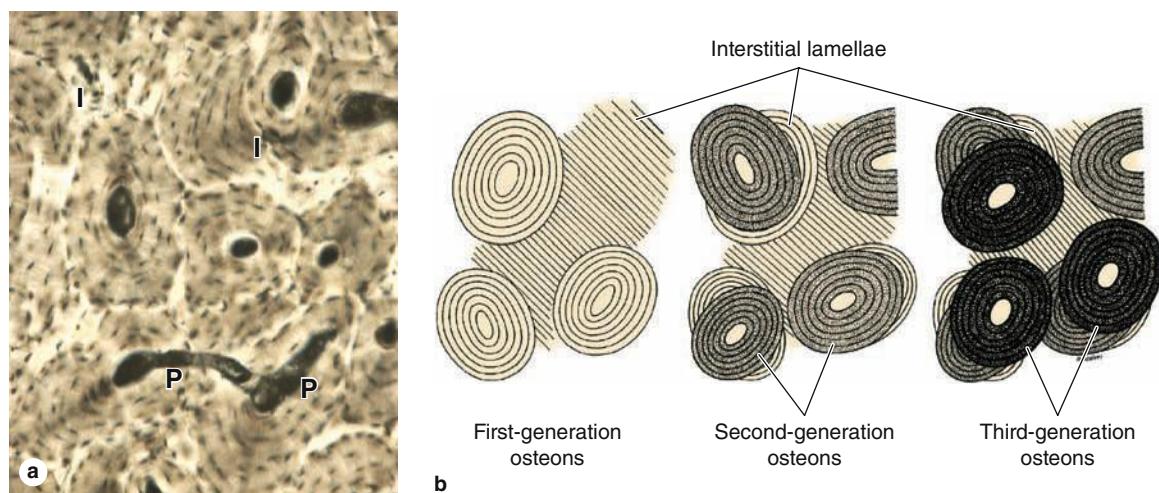
Compact bone (eg, in the diaphysis of long bones) also includes parallel lamellae organized as multiple **external**

FIGURE 8–9 An osteon.

Osteons (Haversian systems) constitute most of the compact bone. Shown here is an osteon with four to five concentric lamellae (L) surrounding the central canal (CC). Osteocytes (O) in lacunae are in communication with each other and with the central canal and periphery of the osteon via through hundreds of dendritic processes located within fine canaliculi (C). Also shown are the partial, interstitial lamellae (I) of an osteon that was eroded when the intact osteon was formed. (Ground bone; X500)

circumferential lamellae immediately beneath the periosteum and fewer **inner circumferential lamellae** around the marrow cavity (Figure 8–1a). The lamellae of these outer and innermost areas of compact bone enclose and strengthen the middle region containing vascularized osteons.

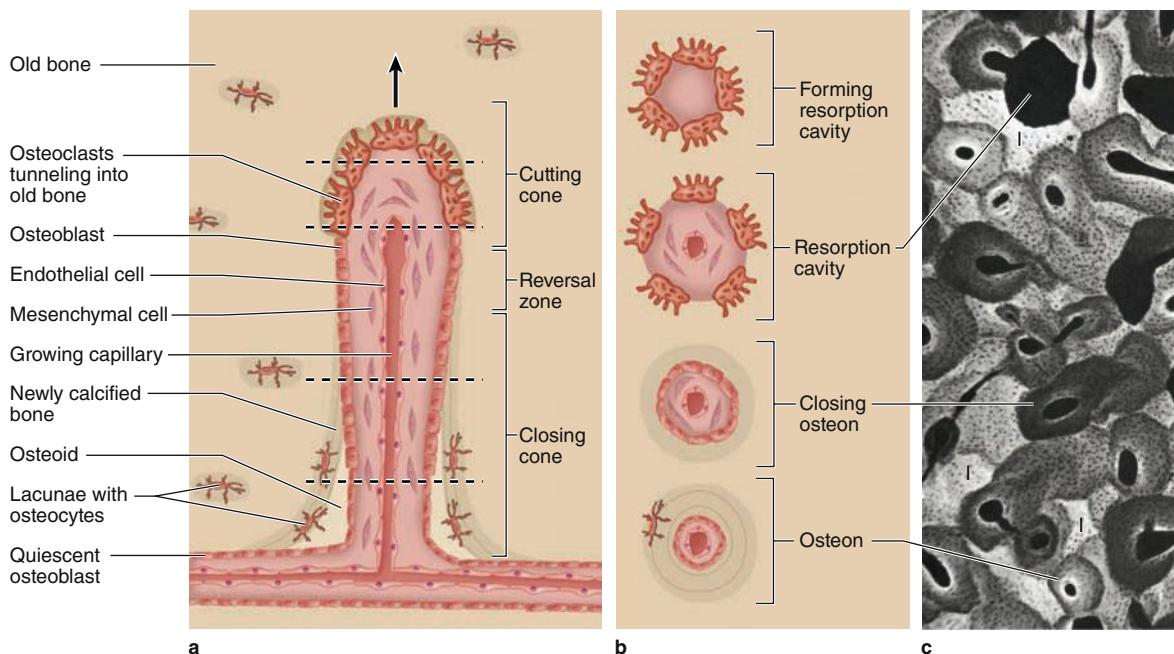
Bone remodeling occurs continuously throughout life. In compact bone, remodeling resorbs parts of old osteons and produces new ones. As shown in Figure 8–11 osteoclasts remove old bone and form small, tunnel-like cavities. Such tunnels are quickly invaded by osteoprogenitor cells from the endosteum or periosteum and sprouting loops of capillaries. Osteoblasts develop, line the wall of the tunnels, and begin to secrete osteoid in a cyclic manner, forming a new osteon with concentric

FIGURE 8–10 Lamellar bone: Perforating canals and interstitial lamellae.

(a) Transverse perforating (Volkmann) canals (**P**) connecting adjacent osteons are shown in this micrograph of compact lamellar bone. Such canals “perforate” lamellae and provide another source of microvasculature for the central canals of osteons. Among the intact osteons are also found remnants of eroded osteons, seen as irregular interstitial lamellae (**I**). (Ground bone; X100)

(b) Diagram showing the remodeling of compact lamellar bone with three generations of osteons and their successive

contributions to the formation of interstitial lamellae. The shading indicates that successive generations of osteons have different degrees of mineralization, with the most newly formed being the least mineralized. Remodeling is a continuous process that involves the coordinated activity of osteoblasts and osteoclasts, and is responsible for adaptation of bone to changes in stress, especially during the body’s growth.

FIGURE 8–11 Development of an osteon.

During remodeling of compact bone, osteoclasts act as a cutting cone that tunnels into existing bone matrix. Behind the osteoclasts, a population of osteoblast progenitors enters the newly formed tunnel and lines its walls. The osteoblasts secrete osteoid in a cyclic manner, producing layers of new matrix (lamellae) and trapping some cells (future osteocytes) in lacunae. The tunnel becomes

constricted with multiple concentric layers of new matrix, and its lumen finally exists as only a narrow central canal with small blood vessels. The dashed lines in (a) indicate the levels of the structures shown in cross section (b). An x-ray image (c) shows the different degrees of mineralization in osteons and in interstitial lamellae (**I**).

lamellae of bone and trapped osteocytes (Figures 8–11). In healthy adults 5%–10% of the bone turns over annually.

» MEDICAL APPLICATION

The antibiotic **tetracycline** is a fluorescent molecule that binds newly deposited osteoid matrix during mineralization with high affinity and specifically labels new bone under the UV microscope (Figure 8–12). This discovery led to methods for measuring the rate of bone growth, an important parameter in the diagnosis of certain bone disorders. In one technique tetracycline is administered twice to patients, with an intervening interval of 11–14 days. A bone biopsy is then performed, sectioned without decalcification, and examined. Bone formed while tetracycline was present appears as fluorescent lamellae and the distance between the labeled layers is proportional to the rate of bone appositional growth. This procedure is of diagnostic importance in such diseases as **osteomalacia**, in which mineralization is impaired, and **osteitis fibrosa cystica**, in which increased osteoclast activity results in removal of bone matrix and fibrous degeneration.

Woven Bone

Woven bone is nonlamellar and characterized by random disposition of type I collagen fibers and is the first bone tissue to appear in embryonic development and in fracture repair. Woven bone is usually temporary and is replaced in adults by lamellar bone, except in a very few places in the body, for example, near the sutures of the calvaria and in the insertions of some tendons.

In addition to the irregular, interwoven array of collagen fibers, woven bone typically has a lower mineral content (it is more easily penetrated by x-rays) and a higher proportion of osteocytes than mature lamellar bone. These features reflect the facts that immature woven bone forms more quickly but has less strength than lamellar bone.

» OSTEOGENESIS

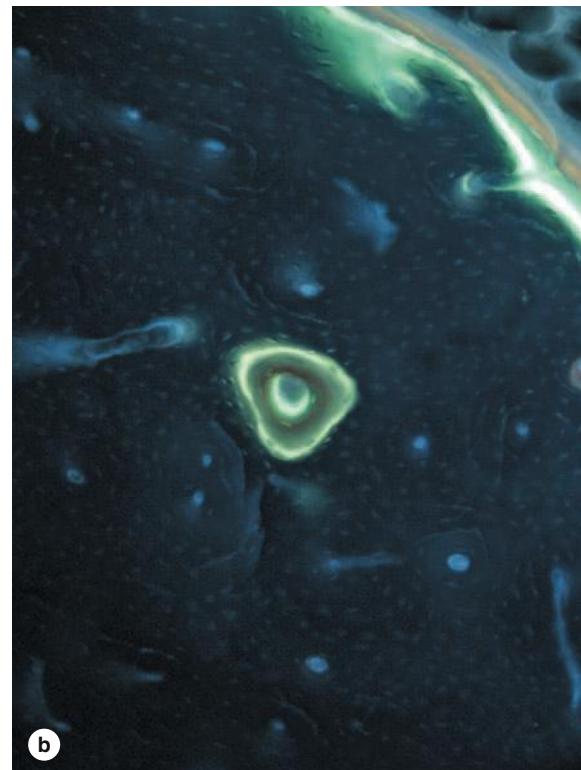
Bone development or **osteogenesis** occurs by one of two processes:

- **Intramembranous ossification**, in which osteoblasts differentiate directly from mesenchyme and begin secreting osteoid

FIGURE 8–12 Tetracycline localization of new bone matrix.



Newly formed bone can be labeled with tetracycline, which forms fluorescent complexes with calcium at ossification sites and provides an *in vivo* tracer by which newly formed bone can be localized. A group of osteons in bone after tetracycline incorporation *in vivo* seen with bright-field (**a**) and fluorescent microscopy



(**b**) reveals active ossification in one osteon (center) and in the external circumferential lamellae (upper right).

(Used with permission from Dr Matt Allen, Indiana University School of Medicine, Indianapolis.)

- **Endochondral ossification**, in which a preexisting matrix of *hyaline cartilage* is eroded and invaded by osteoblasts, which then begin osteoid production.

The names refer to the mechanisms by which the bone forms initially; in both processes woven bone is produced first and is soon replaced by stronger lamellar bone. During growth of all bones, areas of woven bone, areas of bone resorption, and areas of lamellar bone all exist contiguous to one another.

» MEDICAL APPLICATION

Osteogenesis imperfecta, or “brittle bone disease,” refers to a group of related congenital disorders in which the osteoblasts produce deficient amounts of type I collagen or defective type I collagen due to genetic mutations. Such defects lead to a spectrum of disorders, all characterized by significant fragility of the bones. The fragility reflects the deficit in normal collagen, which normally reinforces and adds a degree of resiliency to the mineralized bone matrix.

Intramembranous Ossification

Intramembranous ossification, by which most flat bones begin to form, takes place within condensed sheets (“membranes”) of embryonic mesenchymal tissue. Most bones of the skull and jaws, as well as the scapula and clavicle, are formed embryonically by intramembranous ossification.

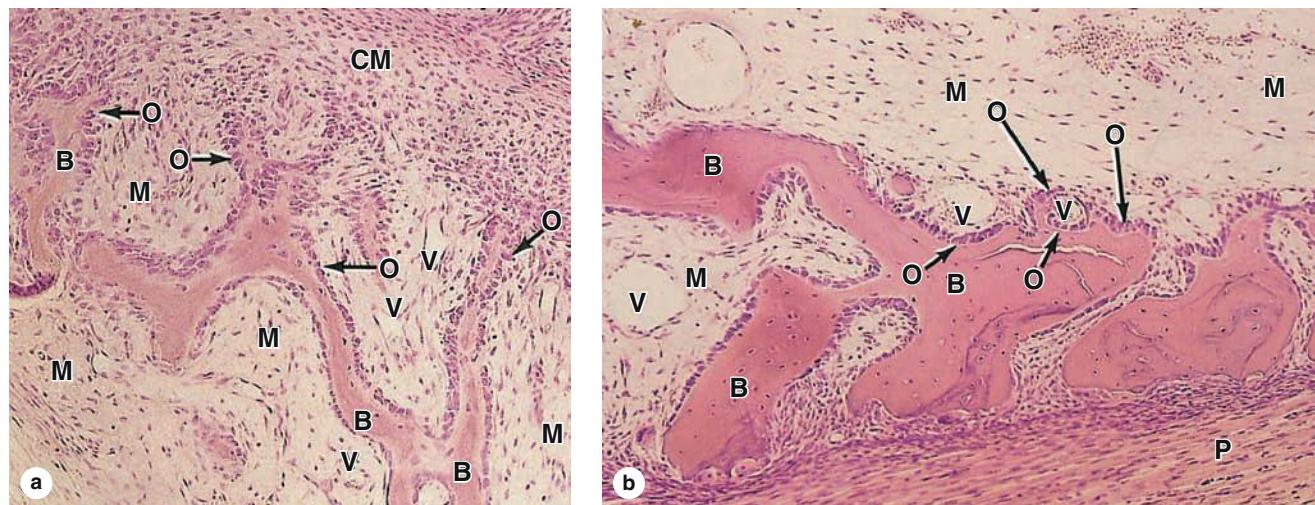
Within the condensed mesenchyme bone formation begins in **ossification centers**, areas in which osteoprogenitor cells arise, proliferate, and form incomplete layers of osteoblasts around a network of developing capillaries. Osteoid secreted by the osteoblasts calcifies as described earlier, forming small irregular areas of woven bone with osteocytes in lacunae and canaliculi (Figure 8–13). Continued matrix secretion and calcification enlarges these areas and leads to the fusion of neighboring ossification centers. The anatomical bone forms gradually as woven bone matrix is replaced by compact bone that encloses a region of cancellous bone with marrow and larger blood vessels. Mesenchymal regions that do not undergo ossification give rise to the endosteum and the periosteum of the new bone.

In cranial flat bones, lamellar bone formation predominates over bone resorption at both the internal and external surfaces. Internal and external plates of compact bone arise, while the central portion (diploë) maintains its cancellous nature. The fontanelles or “soft spots” on the heads of newborn infants are areas of the skull in which the membranous tissue is not yet ossified.

Endochondral Ossification

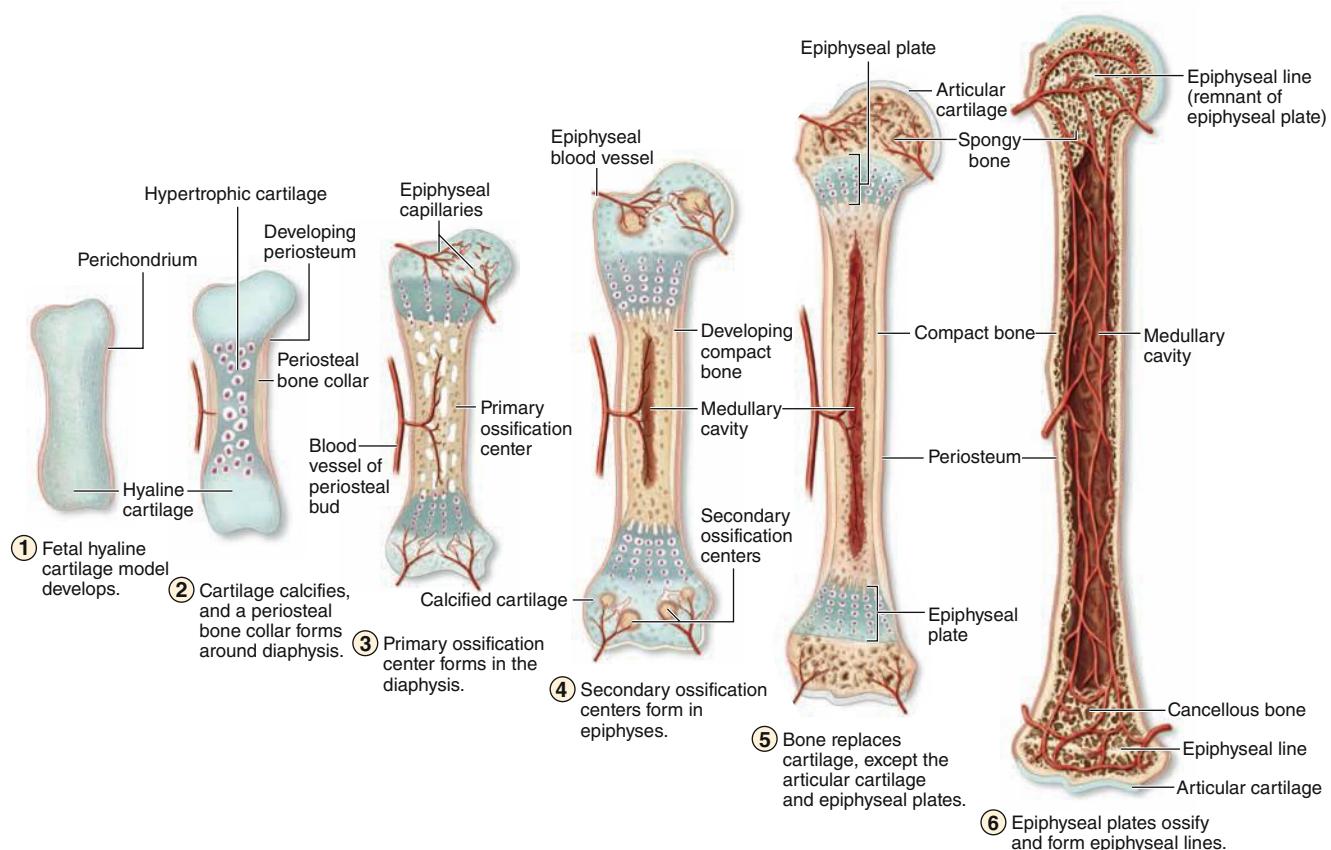
Endochondral (Gr. *endon*, within + *chondros*, cartilage) ossification takes place within hyaline cartilage shaped as a small version, or model, of the bone to be formed. This type of ossification forms most bones of the body and is especially well studied in developing long bones, where it consists of the sequence of events shown in Figure 8–14.

FIGURE 8–13 Intramembranous ossification.



A section of fetal pig mandible developing by intramembranous ossification. (a) Areas of typical mesenchyme (M) and condensed mesenchyme (CM) are adjacent to layers of new osteoblasts (O). Some osteoblasts have secreted matrices of bone (B), the surfaces of which remain covered by osteoblasts. Between these thin regions of new woven bone are areas with small blood vessels (V). (X40; H&E)

(b) At higher magnification another section shows these same structures, but also includes the developing periosteum (P) adjacent to the masses of woven bone that will soon merge to form a continuous plate of bone. The larger mesenchyme-filled region at the top is part of the developing marrow cavity. Osteocytes in lacunae can be seen within the bony matrix. (X100; H&E)

FIGURE 8–14 Osteogenesis of long bones by endochondral ossification.

This process, by which most bones form initially, begins with embryonic models of the skeletal elements made of hyaline cartilage (1). Late in the first trimester, a bone collar develops beneath the perichondrium around the middle of the cartilage model, causing chondrocyte hypertrophy in the underlying cartilage (2).

This is followed by invasion of that cartilage by capillaries and osteoprogenitor cells from what is now the periosteum to produce a **primary ossification center** in the diaphysis (3). Here osteoid is deposited by the new osteoblasts,

undergoes calcification into woven bone, and is then remodeled as compact bone.

(4) Around the time of birth **secondary ossification centers** begin to develop by a similar process in the bone's epiphyses. During childhood the primary and secondary ossification centers gradually come to be separated only by the **epiphyseal plate** (5) which provides for continued bone elongation. The two ossification centers do not merge until the epiphyseal plate disappears (6) when full stature is achieved.

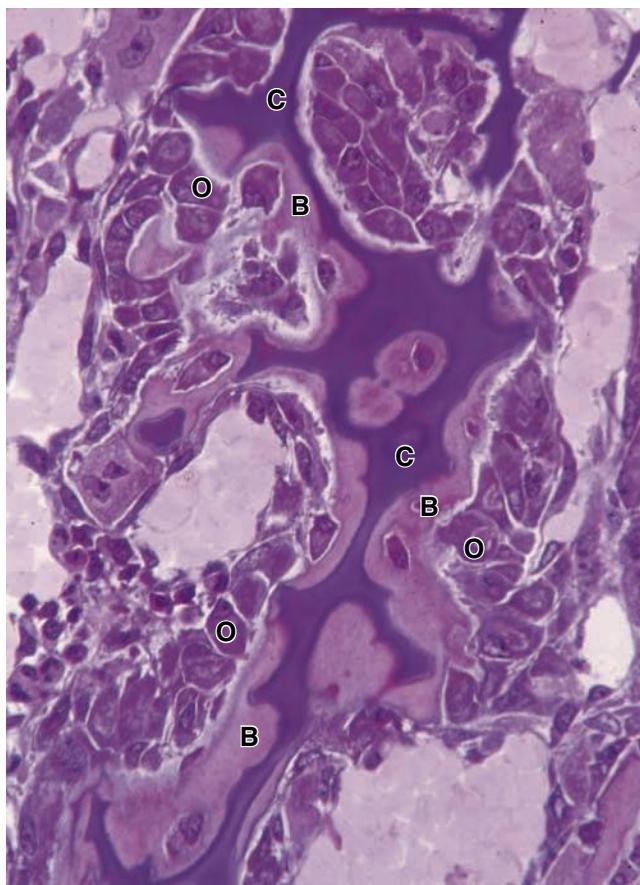
In this process ossification first occurs within a **bone collar** produced by osteoblasts that differentiate within the perichondrium (transitioning to periosteum) around the cartilage model diaphysis. The collar impedes diffusion of oxygen and nutrients into the underlying cartilage, causing local chondrocytes to swell up (hypertrophy), compress the surrounding matrix, and initiate its calcification by releasing osteocalcin and alkaline phosphatase. The hypertrophic chondrocytes eventually die, creating empty spaces within the calcified matrix. One or more blood vessels from the perichondrium (now the periosteum) penetrate the bone collar, bringing osteoprogenitor cells to the porous central region. Along with the vasculature newly formed osteoblasts move into all available spaces and produce woven bone. The remnants of calcified cartilage at this stage are basophilic and the new bone is more acidophilic (Figure 8–15).

This process in the diaphysis forms the **primary ossification center** (Figure 8–14), beginning in many embryonic bones as early as the first trimester. **Secondary ossification centers** appear later at the epiphyses of the cartilage model and develop in a similar manner. During their expansion and remodeling both the primary and secondary ossification centers produce cavities that are gradually filled with bone marrow and trabeculae of cancellous bone.

With the primary and secondary ossification centers, two regions of cartilage remain:

- **Articular cartilage** within the joints between long bones (Figure 8–14), which normally persists through adult life
- The specially organized **epiphyseal cartilage** (also called the **epiphyseal plate** or growth plate), which connects each epiphysis to the diaphysis and allows longitudinal bone growth (Figure 8–14).

FIGURE 8–15 Cells and matrices of a primary ossification center.



A small region of a primary ossification center showing key features of endochondral ossification. Compressed remnants of calcified cartilage matrix (**C**) are basophilic and devoid of chondrocytes. This material becomes enclosed by more lightly stained osteoid and woven bone (**B**) which contains osteocytes in lacunae. The new bone is produced by active osteoblasts (**O**) arranged as a layer on the remnants of old cartilage. (X200; Pararosaniline-toluidine blue)

The epiphyseal cartilage is responsible for the growth in length of the bone and disappears upon completion of bone development at adulthood. Elimination of these epiphyseal plates (“epiphyseal closure”) occurs at various times with different bones and by about age 20 is complete in all bones, making further growth in bone length no longer possible. In forensics or through x-ray examination of the growing skeleton, it is possible to determine the “bone age” of a young person, by noting which epiphyses have completed closure.

An epiphyseal growth plate shows distinct regions of cellular activity and is often discussed in terms of overlapping but histologically distinct zones (Figures 8–16 and 8–17), starting with the cartilage farthest from the ossification center in the diaphysis:

2. In the **proliferative zone**, the cartilage cells divide repeatedly, enlarge and secrete more type II collagen and proteoglycans, and become organized into columns parallel to the long axis of the bone.
3. The **zone of hypertrophy** contains swollen, terminally differentiated chondrocytes which compress the matrix into aligned spicules and stiffen it by secretion of type X collagen. Unique to the hypertrophic chondrocytes in developing (or fractured) bone, type X collagen limits diffusion in the matrix and with growth factors promotes vascularization from the adjacent primary ossification center.
4. In the **zone of calcified cartilage** chondrocytes about to undergo apoptosis release matrix vesicles and osteocalcin to begin matrix calcification by the formation of hydroxyapatite crystals.
5. In the **zone of ossification** bone tissue first appears. Capillaries and osteoprogenitor cells invade the now vacant chondrocytic lacunae, many of which merge to form the initial marrow cavity. Osteoblasts settle in a layer over the spicules of calcified cartilage matrix and secrete osteoid which becomes woven bone (Figures 8–16 and 8–17). This woven bone is then remodeled as lamellar bone.

In summary, longitudinal growth of a bone occurs by cell proliferation in the epiphyseal plate cartilage. At the same time, chondrocytes in the diaphysis side of the plate undergo hypertrophy, their matrix becomes calcified, and the cells die. Osteoblasts lay down a layer of new bone on the calcified cartilage matrix. Because the rates of these two opposing events (proliferation and destruction) are approximately equal, the epiphyseal plate does not change thickness, but is instead displaced away from the center of the diaphysis as the length of the bone increases.

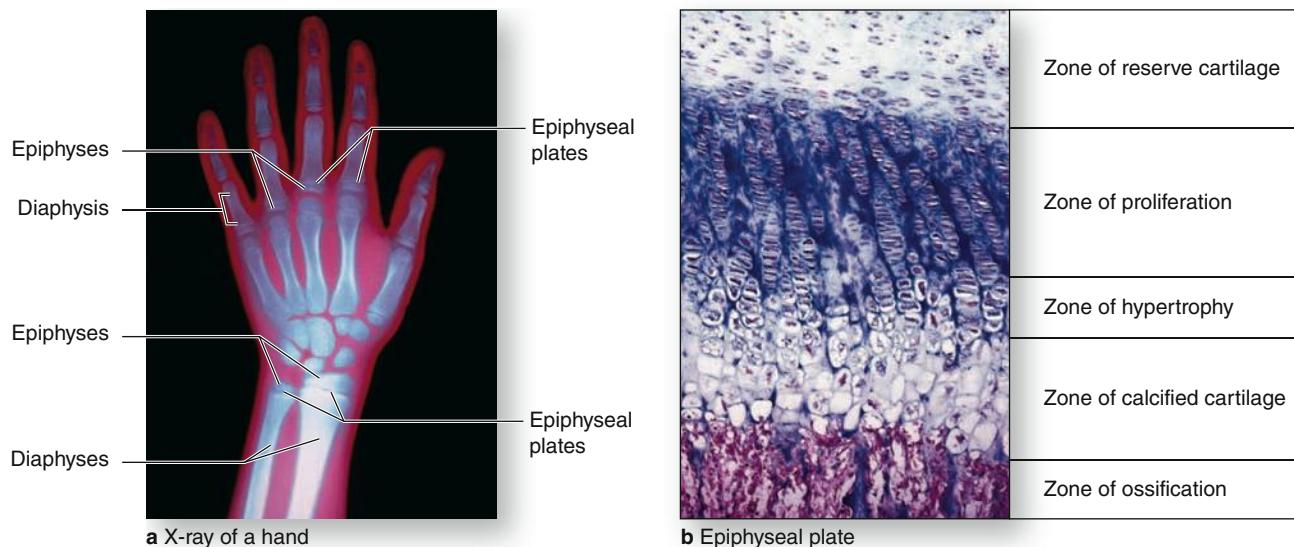
Growth in the circumference of long bones does not involve endochondral ossification but occurs through the activity of osteoblasts developing from osteoprogenitor cells in the periosteum by a process of **appositional growth** which begins with formation of the bone collar on the cartilaginous diaphysis. As shown in Figure 8–18 the increasing bone circumference is accompanied by enlargement of the central marrow cavity by the activity of osteoclasts in the endosteum.

► MEDICAL APPLICATION

Calcium deficiency in children can lead to **rickets**, a disease in which the bone matrix does not calcify normally and the epiphyseal plate can become distorted by the normal strains of body weight and muscular activity. Ossification processes are consequently impeded, which causes bones to grow more slowly and often become deformed. The deficiency can be due either to insufficient calcium in the diet or a failure to produce the steroid prohormone vitamin D, which is important for the absorption of Ca^{2+} by cells of the small intestine.

In adults calcium deficiency can give rise to **osteomalacia** (osteon + Gr. *malakia*, softness), characterized by deficient calcification of recently formed bone and partial decalcification of already calcified matrix.

1. The **zone of reserve (or resting) cartilage** is composed of typical hyaline cartilage.

FIGURE 8–16 Epiphyseal growth plate: Locations and zones of activity.

The large and growing primary ossification center in long bone diaphyses and the secondary ossification centers in epiphyses are separated in each developing bone by a plate of cartilage called the **epiphyseal plate**.

(a) Epiphyseal plates can be identified in an x-ray of a child's hand as marrow regions of lower density between the denser ossification centers. Cells in epiphyseal growth plates are responsible for continued elongation of bones until the body's full size is reached. Developmental activities in the epiphyseal growth plate occur in overlapping zones with distinct histological appearances.

(b) From the epiphysis to the diaphysis, five general zones have cells specialized for the following: (1) a reserve of normal hyaline cartilage, (2) cartilage with proliferating chondroblasts aligned as axial aggregates in lacunae, (3) cartilage in which the aligned cells are hypertrophic and the matrix condensed, (4) an area in which the chondrocytes have disappeared and the matrix is undergoing calcification, and (5) an ossification zone in which blood vessels and osteoblasts invade the lacunae of the old cartilage, producing marrow cavities and osteoid for new bone. (X100; H&E)

► BONE REMODELING & REPAIR

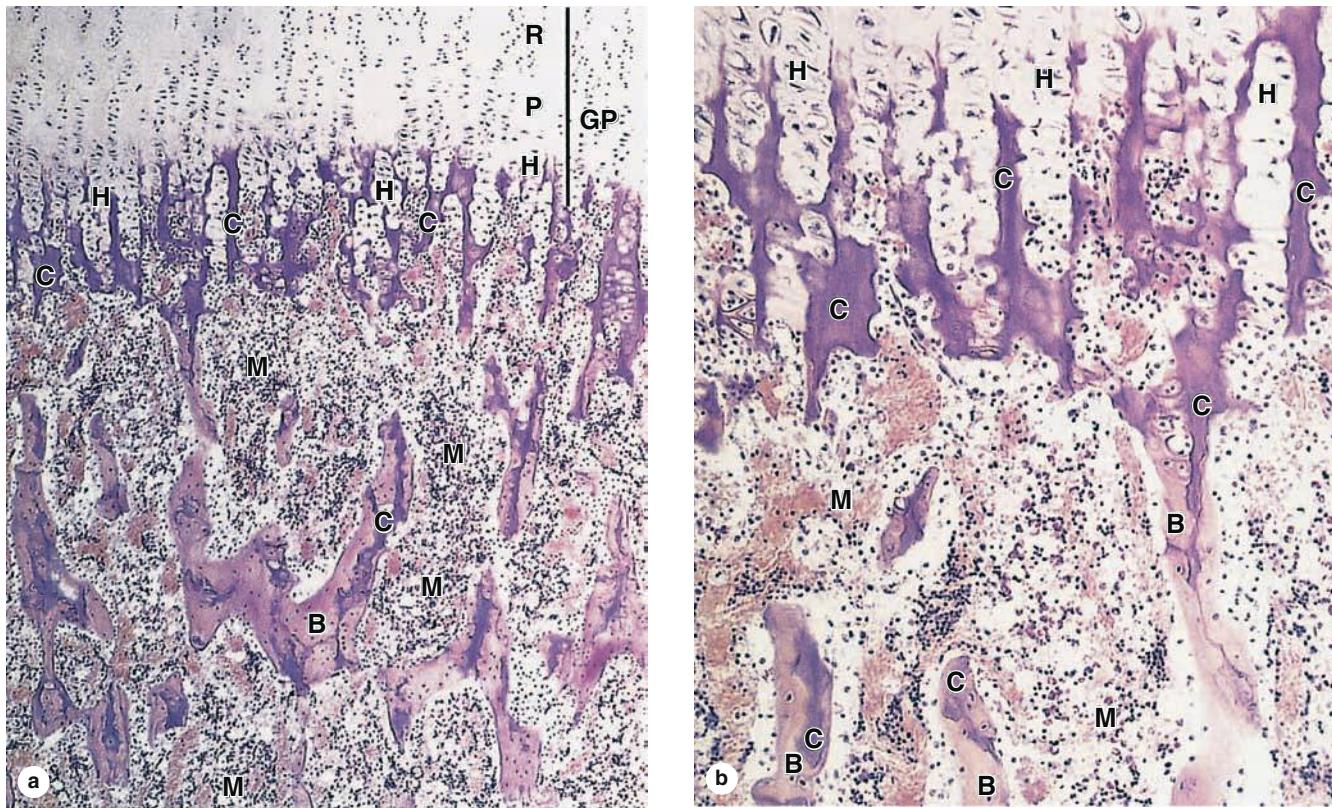
Bone growth involves both the continuous resorption of bone tissue formed earlier and the simultaneous laying down of new bone at a rate exceeding that of bone removal. The sum of osteoblast and osteoclast activities in a growing bone constitutes osteogenesis or the process of bone modeling, which maintains each bone's general shape while increasing its mass. The rate of **bone turnover** is very active in young children, where it can be 200 times faster than that of adults. In adults the skeleton is also renewed continuously in a process of **bone remodeling** which involves the coordinated, localized cellular activities for bone resorption and bone formation shown in the diagram of Figure 8–11.

The constant remodeling of bone ensures that, despite its hardness, this tissue remains plastic and capable of adapting its internal structure in the face of changing stresses. A well-known example of bone plasticity is the ability of the positions of teeth in the jawbone to be modified by the lateral pressures produced by orthodontic appliances. Bone forms on the side where traction is applied and is resorbed on the opposite side where pressure is exerted. In this way, teeth are moved within the jaw while the bone is being remodeled.

Because it contains osteoprogenitor stem cells in the periosteum, endosteum, and marrow and is very well vascularized, bone normally has an excellent capacity for repair. **Bone repair** after a fracture or other damage uses cells, signaling molecules, and processes already active in bone remodeling. Surgically created gaps in bone can be filled with new bone, especially when periosteum is left in place. The major phases that occur typically during bone fracture repair include initial formation of fibrocartilage and its replacement with a temporary **callus** of woven bone, as shown in Figure 8–19.

► MEDICAL APPLICATION

Bone fractures are repaired by a developmental process involving fibrocartilage formation and osteogenic activity of the major bone cells (Figure 8–19). Bone fractures disrupt blood vessels, causing bone cells near the break to die. The damaged blood vessels produce a localized hemorrhage or hematoma. Clotted blood is removed along with tissue debris by macrophages and the matrix of damaged, cell-free bone is resorbed by osteoclasts.

FIGURE 8–17 Details of the epiphyseal growth plate.

(a) At the top of the micrograph the growth plate (**GP**) shows its zones of hyaline cartilage with chondrocytes at rest (**R**), proliferating (**P**), and hypertrophying (**H**). As the chondrocytes swell they release alkaline phosphatase and type X collagen, which initiates hydroxyapatite formation and strengthens the adjacent calcifying spicules (**C**) of old cartilage matrix. The tunnel-like lacunae in which the chondrocytes have undergone apoptosis are invaded from the diaphysis by capillaries that begin to convert these spaces into marrow (**M**) cavities. Endosteum with osteoblasts also moves in from the diaphyseal primary ossification center, covering the spicules of calcified cartilage and laying down layers of osteoid to form a matrix of woven bone (**B**). (X40; H&E)

(b) Higher magnification shows more detail of the cells and matrix spicules in the zones undergoing hypertrophy (**H**) and ossification. Staining properties of the matrix clearly change as it is compressed and begins to calcify (**C**), and when osteoid and bone (**B**) are laid down. The large spaces between the ossifying matrix spicules become the marrow cavity (**M**), in which pooled masses of eosinophilic red blood cells and aggregates of basophilic white blood cell precursors can be distinguished. Still difficult to see at this magnification is the thin endosteum between the calcifying matrices and the marrow. (X100; H&E)

The periosteum and the endosteum at the fracture site respond with intense proliferation and produce a soft callus of fibrocartilage-like tissue that surrounds the fracture and covers the extremities of the fractured bone.

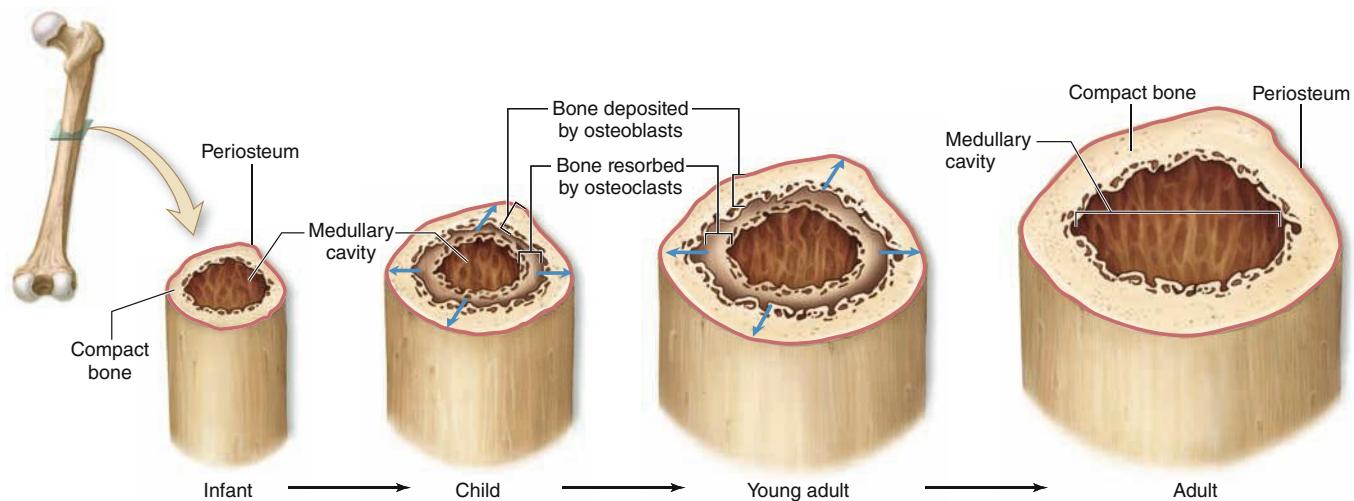
The fibrocartilaginous callus is gradually replaced in a process that resembles a combination of endochondral and intramembranous ossification. This produces a hard callus of woven bone around the fractured ends of bone.

Stresses imposed on the bone during repair and during the patient's gradual return to activity serve to remodel the bone callus. The immature, woven bone of the callus is gradually resorbed and replaced by lamellar bone, remodeling and restoring the original bone structure.

► METABOLIC ROLE OF BONE

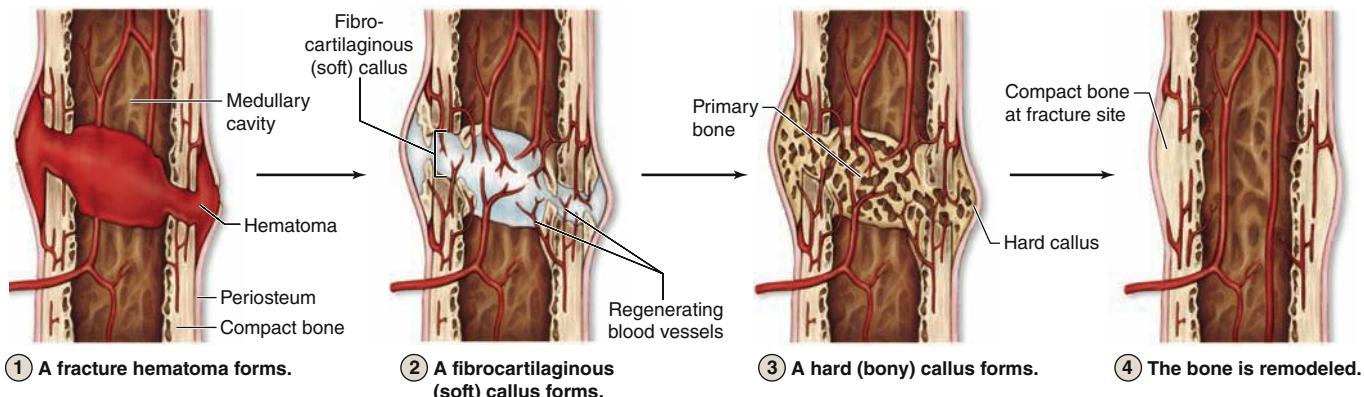
Calcium ions are required for the activity of many enzymes and many proteins mediating cell adhesion, cytoskeletal movements, exocytosis, membrane permeability, and other cellular functions. The skeleton serves as the calcium reservoir, containing 99% of the body's total calcium in hydroxyapatite crystals. The concentration of calcium in the blood (9–10 mg/dL) and tissues is generally quite stable because of a continuous interchange between blood calcium and bone calcium.

The principal mechanism for raising blood calcium levels is the mobilization of ions from hydroxyapatite to interstitial fluid, primarily in cancellous bone. Ca^{2+} mobilization is regulated mainly by paracrine interactions among bone cells, many of which are not well understood, but two

FIGURE 8–18 Appositional bone growth

Bones increase in diameter as new bone tissue is added beneath the periosteum in a process of appositional growth. Also called radial bone growth, such growth in long bones begins with formation of the bone collar early in endochondral ossification. During

radial bone growth formation of new bone at the periosteal surface occurs concurrently with bone removal at the endosteal surface around the large medullary, enlarging this marrow-filled region and not greatly increasing the bone's weight.

FIGURE 8–19 Main features of bone fracture repair.

Repair of a fractured bone occurs through several stages but utilizes the cells and mechanisms already in place for bone growth and remodeling. (1) Blood vessels torn within the fracture release blood that clots to produce a large fracture hematoma. (2) This is gradually removed by macrophages and replaced by a soft fibrocartilage-like mass called procallus tissue. If torn by the break the periosteum reestablishes its continuity over this tissue. (3) The

procallus is invaded by regenerating blood vessels and proliferating osteoblasts. In the next few weeks the fibrocartilage is gradually replaced by woven bone which forms a hard callus throughout the original area of fracture. (4) The woven bone is then remodeled as compact and cancellous bone in continuity with the adjacent uninjured areas and fully functional vasculature is reestablished.

polypeptide hormones also target bone cells to influence calcium homeostasis:

- **Parathyroid hormone (PTH)** from the parathyroid glands raises low blood calcium levels by stimulating osteoclasts and osteocytes to resorb bone matrix and release Ca^{2+} . The PTH effect on osteoclasts is indirect; PTH receptors occur on *osteoblasts*, which respond by secreting RANKL and other paracrine factors that stimulate osteoclast formation and activity.
- **Calcitonin**, produced within the thyroid gland, can reduce elevated blood calcium levels by opposing the effects of PTH in bone. This hormone directly targets osteoclasts to slow matrix resorption and bone turnover.

» MEDICAL APPLICATION

In addition to PTH and calcitonin, several other hormones act on bone. The anterior lobe of the pituitary synthesizes growth hormone (GH or somatotropin), which stimulates the liver to produce insulin-like growth factor-1 (IGF-1 or somatomedin). IGF has an overall growth-promoting effect, especially on the epiphyseal cartilage. Consequently, lack of growth hormone during the growing years causes **pituitary dwarfism**; an excess of growth hormone causes excessive growth of the long bones, resulting in **gigantism**. Adult bones cannot increase in length even with excess IGF because they lack epiphyseal cartilage, but they do increase in width by periosteal growth. In adults, an increase in GH causes **acromegaly**, a disease in which the bones—mainly the long ones—become very thick.

» MEDICAL APPLICATION

In **rheumatoid arthritis** chronic inflammation of the synovial membrane causes thickening of this connective tissue and stimulates the macrophages to release collagenases and other hydrolytic enzymes. Such enzymes eventually cause destruction of the articular cartilage, allowing direct contact of the bones projecting into the joint.

» JOINTS

Joints are regions where adjacent bones are capped and held together firmly by other connective tissues. The type of joint determines the degree of movement between the bones. Joints classified as **synarthroses** (Gr. *syn*, together + *arthrosis*, articulation) allow very limited or no movement and are subdivided into fibrous and cartilaginous joints, depending on the type of tissue joining the bones. Major subtypes of synarthroses include the following:

- **Synostoses** involve bones linked to other bones and allow essentially no movement. In older adults synostoses unite the skull bones, which in children and young adults are held together by **sutures**, or thin layers of dense connective tissue with osteogenic cells.

■ **Syndesmoses** join bones by dense connective tissue only. Examples include the interosseous ligament of the inferior tibiofibular joint and the posterior region of the sacroiliac joints.

■ **Symphyses** have a thick pad of fibrocartilage between the thin articular cartilage covering the ends of the bones. All symphyses, such as the intervertebral discs and pubic symphysis, occur in the midline of the body.

Intervertebral discs (Figure 8–20) are large symphyses between the articular surfaces of successive bony

FIGURE 8–20 Intervertebral disc.



Section of a rat tail showing an intervertebral disc and the two adjacent vertebrae with bone marrow (BM) cavities. The disc consists of concentric layers of fibrocartilage, comprising the annulus fibrosus (AF), which surrounds the nucleus pulposus (NP). The nucleus pulposus contains scattered residual cells of the embryonic notochord embedded in abundant gel-like matrix. The intervertebral discs function primarily as shock absorbers within the spinal column and allow greater mobility within the spinal column. (X40; PSH)

vertebral bodies. Held in place by ligaments these discoid components of the intervertebral joints cushion the bones and facilitate limited movements of the vertebral column. Each disc has an outer portion, the **annulus fibrosus**, consisting of concentric fibrocartilage laminae in which collagen bundles are arranged orthogonally in adjacent layers. The multiple lamellae of fibrocartilage produce a disc with unusual toughness able to withstand pressures and torsion generated within the vertebral column.

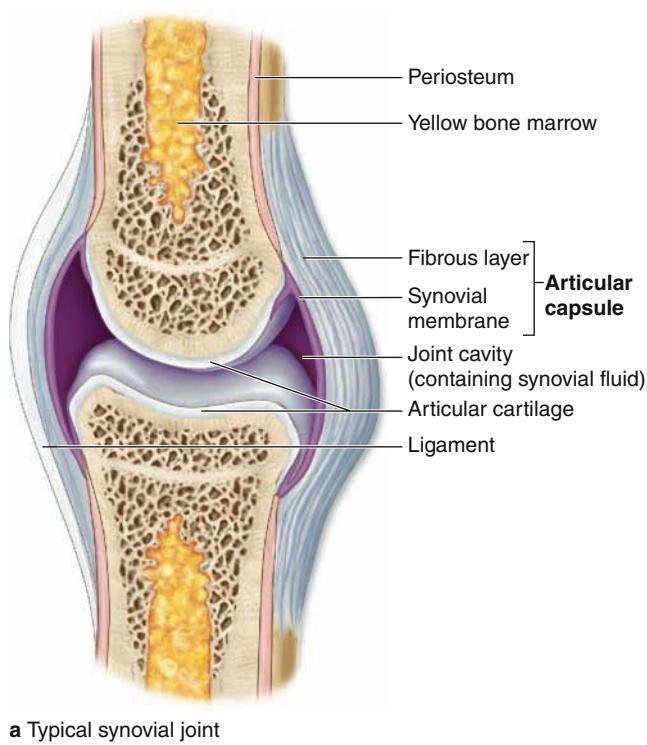
Situated in the center of the annulus fibrosus, a gel-like body called the **nucleus pulposus** allows each disc to function as a shock absorber (Figure 8–20). The nucleus pulposus consists of a viscous fluid matrix rich in hyaluronic acid and type II collagen fibers, but also contains scattered, vacuolated cells derived from the embryonic notochord, the only cells of that structure to persist postnatally. The nucleus pulposus is large in children, but these structures gradually become smaller with age and are partially replaced by fibrocartilage.

» MEDICAL APPLICATION

Within an intervertebral disc, collagen loss or other degenerative changes in the annulus fibrosus are often accompanied by displacement of the nucleus pulposus, a condition variously called a **slipped** or **herniated disc**. This occurs most frequently on the posterior region of the intervertebral disc where there are fewer collagen bundles. The affected disc frequently dislocates or shifts slightly from its normal position. If it moves toward nerve plexuses, it can compress the nerves and result in severe pain and other neurologic disturbances. The pain accompanying a slipped disc may be perceived in areas innervated by the compressed nerve fibers—usually the lower lumbar region.

Joints classified as **diarthroses** permit free bone movement. Diarthroses (Figure 8–21) such as the elbow and knee generally unite long bones and allow great mobility. In a diarthrosis ligaments and a capsule of dense connective tissue

FIGURE 8–21 Diarthroses or synovial joints.



a Typical synovial joint



b

Diarthroses are joints that allow free movement of the attached bones, such as knuckles, knees, and elbows. (a) Diagram showing major components of a diarthrosis, including the **articular capsule** continuous with a ligament inserting into the periosteum of both bones; the **joint cavity** containing synovial fluid lubricant; and the ends of epiphyses covered by **articular cartilage**. The **synovial membrane** lines the capsule and produces the synovial fluid.

(b) Longitudinal section through a diarthrosis with the growing bones of a mouse knee, showing the position near the boundaries of the capsule (C) of the epiphyseal growth plate (E) where endochondral ossification occurs. Also shown are the articular cartilage (A) and the folds of synovial membrane (SM), which extend prominently into the joint cavity from connective tissue of the capsule for production of synovial fluid. (X10; PSH stain)

maintain proper alignment of the bones. The capsule encloses a sealed **joint cavity** containing a clear, viscous liquid called **synovial fluid**. The joint cavity is lined, not by epithelium, but by a specialized connective tissue called the **synovial membrane** which extends folds and villi into the joint cavity and produces the lubricant synovial fluid.

In different diarthrotic joints the synovial membrane may have prominent regions with dense connective tissue or fat. The superficial regions of this tissue however are usually well vascularized, with many porous (fenestrated) capillaries. Besides having cells typical of connective tissue proper and a changing population of leukocytes, this area of a synovial membrane is characterized by two specialized cells with distinctly different functions and origins (Figure 8–22):

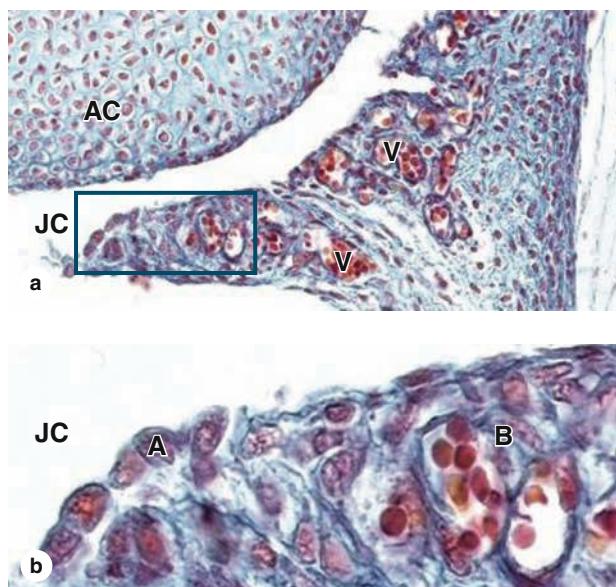
- **Macrophage-like synovial cells**, also called **type A cells**, are derived from blood monocytes and remove wear-and-tear debris from the synovial fluid. These

modified macrophages, which represent approximately 25% of the cells lining the synovium, are important in regulating inflammatory events within diarthrotic joints.

- **Fibroblastic synovial cells**, or **type B cells**, produce abundant hyaluronan and smaller amounts of proteoglycans. Much of this material is transported by water from the capillaries into the joint cavity to form the synovial fluid, which lubricates the joint, reducing friction on all internal surfaces, and supplies nutrients and oxygen to the articular cartilage.

The collagen fibers of the hyaline articular cartilage are disposed as arches with their tops near the exposed surface which, unlike most hyaline cartilage, is not covered by perichondrium (Figure 8–23). This arrangement of collagen helps distribute more evenly the forces generated by pressure on joints. The resilient articular cartilage efficiently absorbs the intermittent mechanical pressures to which many joints are subjected.

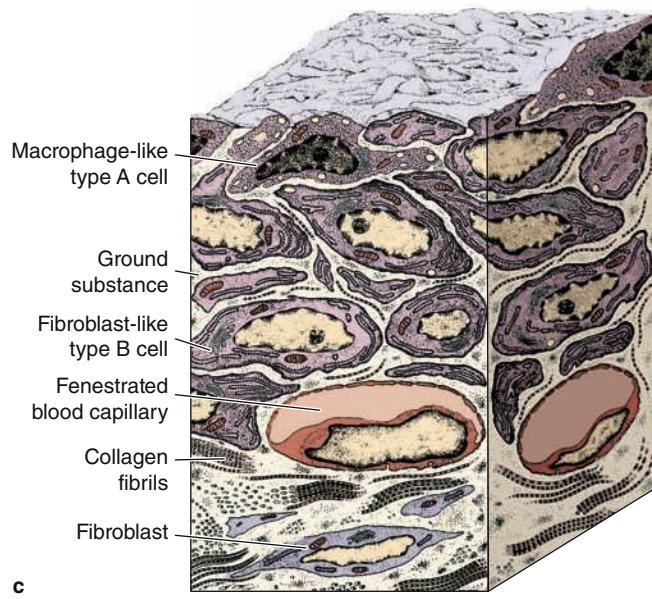
FIGURE 8–22 Synovial membrane.



The synovial membrane is a specialized connective tissue that lines capsules of synovial joints and contacts the synovial fluid lubricant, which it is primarily responsible for maintaining.

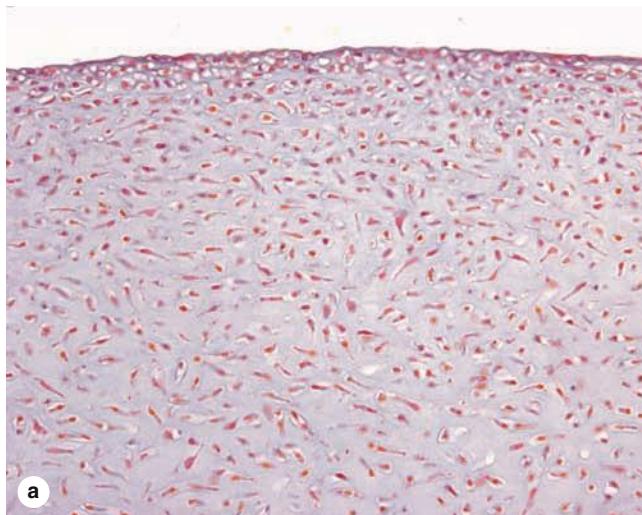
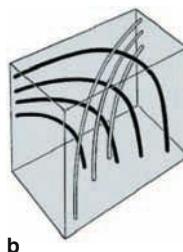
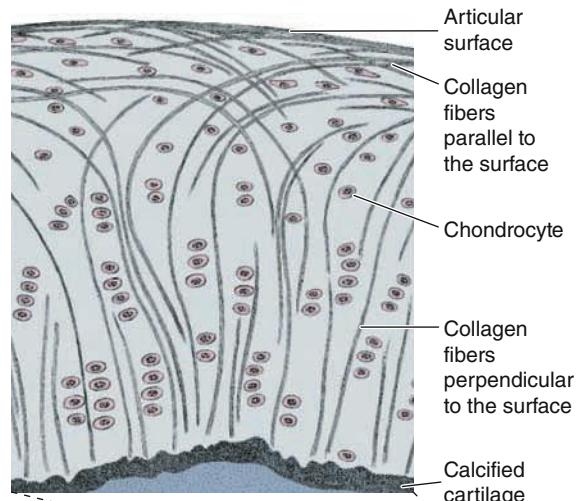
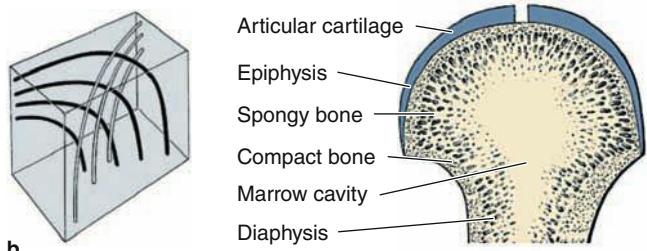
(a) The synovial membrane projects folds into the joint cavity (JC) and these contain many small blood vessels (V). The joint cavity surrounds the articular cartilage (AC). (X100; Mallory trichrome)

(b) Higher magnification of the fold showing a high density of capillaries and two specialized types of cells called **synoviocytes**. Contacting the synovial fluid at the tissue surface are many rounded **macrophage-like synovial cells (type A)** derived from blood monocytes. These cells bind, engulf, and remove tissue debris from synovial fluid. These cells often form a layer at the tissue surface



(A) and can superficially resemble an epithelium, but there is no basal lamina and the cells are not joined together by cell junctions. **Fibroblast-like (type B) synovial cells (B)** are mesenchymally derived and specialized for synthesis of hyaluronan that enters the synovial fluid, replenishing it. (X400)

(c) Schematic representation of synovial membrane histology. Among the macrophage-like and fibroblast-like synovial cells are collagen fibers and other typical components of connective tissue. Surface cells have no basement membrane or junctional complexes denoting an epithelium, despite the superficial resemblance. Blood capillaries are fenestrated, which facilitates exchange of substances between blood and synovial fluid.

FIGURE 8–23 Articular cartilage.**a****b**

(a) Articular surfaces of a diarthrosis are made of hyaline cartilage that lacks the usual perichondrium covering (X40; H&E). **(b)** The top diagram here shows a small region of articular cartilage in which type II collagen fibers run perpendicular to the tissue surface and then bend gradually in a broad arch. The lower left diagram shows a 3D view of arched collagen fibers in articular cartilage. Proteoglycan aggregates bound to hyaluronan fill the space among the collagen fibers and form a hydrated mega-complex that acts as a biomechanical spring. When pressure is applied a small amount of water is forced out of the cartilage matrix into the synovial fluid. When pressure is released water is attracted back into the interstices of the matrix. Such movements of water occur constantly with normal use of the joint and are essential for nutrition of the articular cartilage and for facilitating the interchange of O_2 , CO_2 , and metabolites between synovial fluid and chondrocytes.

Bone SUMMARY OF KEY POINTS

- Bone is a type of connective tissue with a **calcified** extracellular matrix (ECM), specialized to **support** the body, **protect** many internal organs, and act as the body's Ca^{2+} **reservoir**.

Major Cells & Matrix Components of Bone

- **Osteoblasts** differentiate from (stem) osteoprogenitor cells and secrete components of the initial matrix, called **osteoid**, that allow matrix mineralization to occur.
- Important components of osteoid include type I collagen, the protein **osteocalcin**, which binds Ca^{2+} and **matrix vesicles** with enzymes generating PO_4^- .
- High concentrations of Ca^{2+} and PO_4^- ions cause formation of **hydroxyapatite** crystals, whose growth gradually calcifies the entire matrix.
- **Osteocytes** differentiate further from osteoblasts when they become enclosed within matrix **lacunae** and act to maintain the matrix and detect mechanical stresses on bone.

- Osteocytes maintain communication with adjacent cells via a network of long **dendritic processes** that extend through the matrix via narrow **canaliculari** radiating from each lacuna.

- **Osteoclasts** are very large cells, formed by fusion of several blood monocytes, which locally erode bone matrix during osteogenesis and bone remodeling.

Periosteum & Endosteum

- **Periosteum** is a layer of dense connective tissue on the outer surface of bone, bound to bone matrix by bundles of type I collagen called **perforating (or Sharpey) fibers**.
- Regions of periosteum adjacent to bone are rich in **osteoprogenitor cells** and **osteoblasts** that mediate much bone growth and remodeling.
- The **endosteum** is a thin layer of active and inactive osteoblasts, which lines all the internal surfaces within bone; osteoblasts here are also required for bone growth.

Types & Organization of Bone (Table 8-1)

- Dense bone immediately beneath the periosteum is called **compact bone**; deep to the compact bone are small bony trabeculae or spicules of **cancellous (or spongy) bone**.
- In long bones of the limbs these two types of mature bone tissue occur in both the knobby, bulbous ends, called **epiphyses**, and in the intervening shaft or **diaphysis**.
- Immature bone, called **woven bone**, is formed during osteogenesis or repair and has a calcified matrix with randomly arranged collagen fibers.
- By the action of osteoclasts and osteoblasts, woven bone undergoes rapid turnover and is remodeled into **lamellar bone** with new matrix deposited in distinct layers with parallel collagen bundles; both compact and cancellous bone is lamellar bone.
- Most lamellar bone consists of lamellae organized concentrically around small **central canals** containing blood vessels and nerves; this organization is called an **osteon or Haversian system**.
- Within each osteon osteocytic lacunae occur between the lamellae, with **canaliculari** radiating through the lamellae, which allow all cells to communicate with the central canal.

Osteogenesis

- Bones of the skull and jaws form initially by **intramembranous ossification**, with osteoblasts differentiating directly from progenitor cells in condensed “**membranes**” of mesenchyme.
- All other bones form by **endochondral ossification**, in which osteoprogenitor cells surround and then invade hyaline **cartilage models** of the skeletal elements in the embryo.
- **Primary ossification centers** in diaphyses of fetal long bones form when chondrocytes die after enclosure of the cartilage within a collar of woven bone, creating an initial cavity that is entered by periosteal osteoblasts and vasculature.
- Later, **secondary ossification centers** develop similarly within the epiphyses, with cartilage of the **epiphyseal growth plate** between the primary and secondary ossification sites.
- The growth plates are the key to **bone elongation** during childhood and are organized as an interrelated series of developing zones.
- Most distally is a “**resting**” or **reserve zone** of typical hyaline cartilage.
- In an adjacent **zone of proliferation**, chondrocytes undergo mitosis and appear stacked within elongated lacunae.
- The most mature chondrocytes in these lacunae swell up, compress the matrix, and undergo apoptosis in a **zone of hypertrophy** closer to the large primary ossification center.
- Spaces created in the matrix by these events characterize the **zone of cartilage calcification** when they are invaded by osteoblasts, osteoclasts, and vasculature from the primary center.
- In the **zone of ossification** woven bone is laid down initially by osteoblasts and remodeled into lamellae bone.

- **Appositional bone growth** increases the circumference of a bone by osteoblast activity at the periosteum and is accompanied by enlargement of the medullary marrow cavity.

Bone Growth, Remodeling, & Repair

- **Growth** of bones occurs throughout life, with cells and matrix turning over continuously through activities of osteoblasts and osteoclasts.
- Lamellae and osteons are temporary structures and are replaced and rebuilt continuously in a process of **bone remodeling** by which bones change size and shape according to changes in mechanical stress.
- **Bone repair** after fracture or other injury involves the activation of periosteal fibroblasts to produce an initial **soft callus of fibrocartilage-like tissue**.
- The soft callus is gradually replaced by a **hard callus of woven bone** that is soon remodeled to produce stronger lamellar bone.

Metabolic Role of Bone

- Ca^{2+} , a key ion for all cells, is **stored** in bone when dietary calcium is adequate and **mobilized** from bone when dietary calcium is deficient.
- Maintenance of proper **blood calcium levels** involves activity of all three major bone cells and is largely regulated by subtle paracrine interaction among these and other cells.
- Hormones affecting calcium deposition and removal from bone include **parathyroid hormone (PTH)**, which indirectly stimulates osteoclasts to elevate levels of calcium in blood, and **calcitonin**, which can inhibit osteoclast activity, lowering blood calcium levels.

Joints

- **Joints** are places where bones meet, or articulate, allowing at least the potential for bending or movement in that portion of the skeleton.
- Joints with very limited or no movement are classified collectively as **synarthroses** and freely mobile joints are called **diarthroses**.
- **Intervertebral discs** are synarthroses in the vertebral column which cushion adjacent vertebrae.
- Each intervertebral disc consists of a thick outer layer of fibrocartilage forming a tough **annulus fibrosus** and a shock-absorbing inner, gel-like core, the **nucleus pulposus**.
- Diarthroses have a **joint cavity** filled with lubricant **synovial fluid**, enclosed within a tough, fibrous **articular capsule**; ends of the bones involved are covered with hyaline **articular cartilage**.
- Specialized connective tissue of the **synovial membrane** lines the capsule, with folds extended into some areas of the joint cavity.
- **Macrophage-like synovial cells** of the synovial membrane remove wear-and-tear debris from synovial fluid.
- **Fibroblast-like synovial cells** of the synovial membrane synthesize hyaluronan which moves into the synovial fluid with water from local capillaries to lubricate and nourish the articular cartilage.

Bone ASSESS YOUR KNOWLEDGE

1. Which component of bone impedes the distribution of nutrients and oxygen to osteocytes?
 - a. Extracellular matrix
 - b. Canaliculari
 - c. Periosteum
 - d. Cell processes
 - e. Haversian canals
2. Which if the following most accurately describes compact bone?
 - a. Predominant bone type in the epiphyses of adult long bones
 - b. Also known as cancellous bone
 - c. Characterized by the presence of osteons
 - d. Lines the medullary (marrow) cavity
 - e. Forms the diploë in cranial bones

3. In healthy bone canaliculi are likely to contain which one of the following?
 - a. Capillaries
 - b. Nerve axons
 - c. Osteocytic processes
 - d. Osteoid
 - e. Osteoclasts in resorption lacunae
4. Which of the following most accurately describes the endosteum?
 - a. Composed of two layers: osteogenic and fibrous
 - b. Continuous with the joint capsule
 - c. Attached to the bone surface by collagen bundles called Sharpey fibers
 - d. Lines the medullary cavity
 - e. Contains mature osteocytes
5. In the diaphysis of a typical long bone which of the following structures is in closest proximity to the trabeculae of cancellous bone?
 - a. Interstitial lamellae
 - b. Osteons
 - c. Sharpey fibers
 - d. Outer circumferential lamellae
 - e. Inner circumferential lamellae
6. Which “zone” of endochondral ossification in the growing femur of an adolescent is the farthest from that bone’s secondary ossification center?
 - a. Zone of hypertrophy
 - b. Zone of reserve cartilage
 - c. Zone of calcified cartilage
 - d. Zone of ossification
 - e. Zone of proliferation
7. The major lubricant for diarthrotic joints is synthesized by cells located in which joint structure?
 - a. Nucleus pulposus
 - b. Synovial membrane
 - c. Articular cartilage
 - d. Annulus fibrosus
 - e. Fibrous capsule
8. A 25-year-old man presents with persistent joint pain and a history of recurrent fractures of each humerus. His hematocrit and complete blood count (CBC) are normal, but blood calcium levels are high. Hormone levels are all within normal ranges except parathyroid hormone (PTH) which exceeds normal by 3-fold. Which of the following could be prescribed to offset the effects of the elevated PTH?
 - a. Vitamin D
 - b. Vitamin C
 - c. Recombinant RANK ligand
 - d. Somatotrophin (growth hormone)
 - e. Calcitonin
9. A 42-year-old woman, who has been a type I diabetic for 30 years, falls when she trips over the vacuum cleaner hose. She tried to break her fall by placing her hand out to save herself and in the process her wrist was forced backward, breaking her radius near the wrist. Which of the following is produced in the first step in healing this bone injury?
 - a. Osteoid
 - b. Hematoma
 - c. Bony callus
 - d. Fibrocartilage
 - e. Compact bone
10. A 46-year-old woman presents with pain in the left leg that worsens on weight bearing. An x-ray shows demineralization, and a bone biopsy decalcified with EDTA shows reduction in bone quantity. The patient had undergone menopause at age 45 without estrogen replacement. She reports long-standing diarrhea. In addition, laboratory tests show low levels of vitamin D, calcium and phosphorus, and elevated alkaline phosphatase. A second bone biopsy was taken but not decalcified, which showed extensive deposition of uncalcified osteoid on all the bone surfaces. On the basis of these data, the best diagnosis would be which of the following?
 - a. Osteoporosis
 - b. Scurvy
 - c. Osteomalacia
 - d. Rickets
 - e. Hypoparathyroidism

Nerve Tissue & the Nervous System

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The human nervous system, by far the most complex system in the body, is formed by a network of many billion nerve cells (**neurons**), all assisted by many more supporting cells called **glial cells**. Each neuron has hundreds of interconnections with other neurons, forming a very complex system for processing information and generating responses.

Nerve tissue is distributed throughout the body as an integrated communications network. Anatomically, the general organization of the nervous system (Figure 9–1) has two major divisions:

- **Central nervous system (CNS)**, consisting of the brain and spinal cord
- **Peripheral nervous system (PNS)**, composed of the cranial, spinal, and peripheral nerves conducting impulses to and from the CNS (sensory and motor nerves, respectively) and **ganglia** that are small aggregates of nerve cells outside the CNS.

Cells in both central and peripheral nerve tissue are of two kinds: **neurons**, which typically have numerous long processes, and various **glial cells** (Gr. *glia*, glue), which have short processes, support and protect neurons, and participate in many neural activities, neural nutrition, and defense of cells in the CNS.

Neurons respond to environmental changes (**stimuli**) by altering the ionic gradient that exists across their plasma membranes. All cells maintain such a gradient, also called an

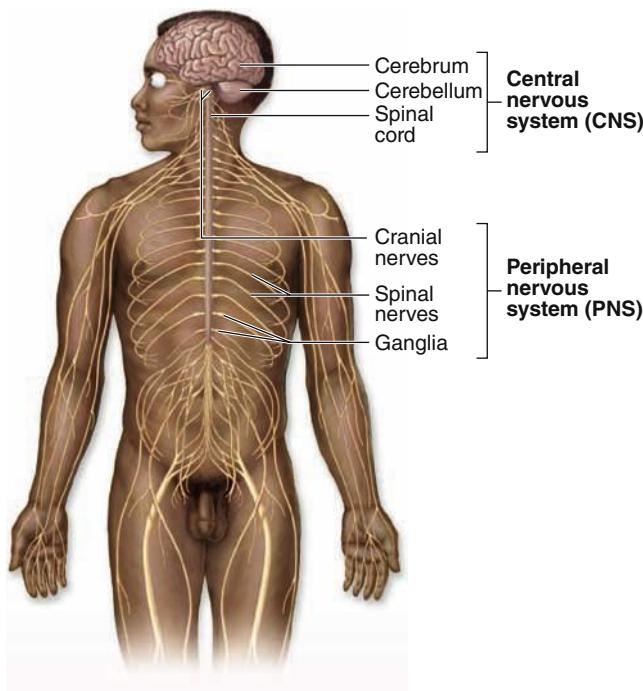
electrical potential, but cells that can rapidly change this potential in response to stimuli (eg, neurons, muscle cells, some gland cells) are said to be **excitable** or irritable. Neurons react promptly to stimuli with a reversal of the ionic gradient (**membrane depolarization**) that generally spreads from the place that received the stimulus and is propagated across the neuron's entire plasma membrane. This propagation, called the **action potential**, the **depolarization wave**, or the **nerve impulse**, is capable of traveling long distances along neuronal processes, transmitting such signals to other neurons, muscles, and glands.

By collecting, analyzing, and integrating information in such signals, the nervous system continuously stabilizes the intrinsic conditions of the body (eg, blood pressure, O₂ and CO₂ content, pH, blood glucose levels, and hormone levels) within normal ranges and maintains behavioral patterns (eg, feeding, reproduction, defense, interaction with other living creatures).

► DEVELOPMENT OF NERVE TISSUE

The nervous system develops from the outermost of the three early embryonic layers, the ectoderm, beginning in the third week of development (Figure 9–2). With signals from the underlying axial structure, the notochord, ectoderm on the mid-dorsal side of the embryo thickens to form the epithelial **neural plate**. The sides of this plate fold upward and grow toward each other medially, and within a few days fuse to form

FIGURE 9–1 The general organization of the nervous system.



Anatomically the nervous system is divided into the **CNS** and **PNS**, which have the major components shown in the diagram.

Functionally the nervous system consists of:

1. Sensory division (afferent)

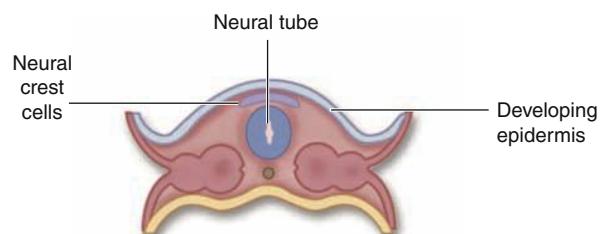
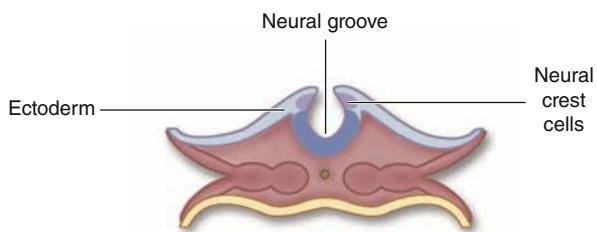
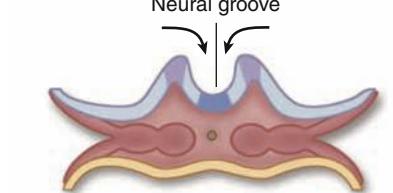
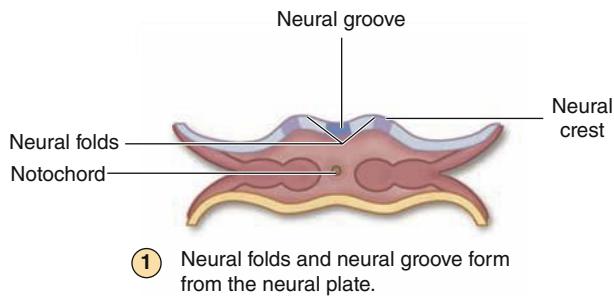
- A. **Somatic** – sensory input perceived consciously (eg, from eyes ears, skin, musculoskeletal structures)
- B. **Visceral** – sensory input not perceived consciously (eg, from internal organs and cardiovascular structures)

2. Motor division (efferent)

- A. **Somatic** – motor output controlled consciously or voluntarily (eg, by skeletal muscle effectors)
- B. **Autonomic** – motor output not controlled consciously (eg, by heart or gland effectors)

The autonomic motor nerves, comprising what is often called the **autonomic nervous system (ANS)**, all have pathways involving two neurons: a **preganglionic neuron** with the cell body in the CNS and a **postganglionic neuron** with the cell body in a ganglion. The ANS has two divisions: (1) The **parasympathetic division**, with its ganglia within or near the effector organs, maintains normal body homeostasis. (2) The **sympathetic division** has its ganglia close to the CNS and controls the body's responses during emergencies and excitement. ANS components located in the wall of the digestive tract are sometimes referred to as the enteric nervous system.

FIGURE 9–2 Neurulation in the early embryo.



Stages in the process of **neurulation**, by which cells of the CNS and PNS are produced, are shown in diagrammatic cross sections of a 3- and 4-week human embryo with the extraembryonic membranes removed. Under an inductive influence from the medial notochord, the overlying layer of ectodermal cells thickens as a bending **neural plate**, with a medial neural groove and lateral neural folds (1). All other ectoderm will become epidermis. The plate bends further, making the **neural folds** and **groove** more prominent (2). The neural folds rise and fuse at the midline (3), converting the groove into the **neural tube** (4), which is large at the cranial end of the embryo and much narrower caudally. The neural tube will give rise to the entire CNS.

As the neural tube detaches from the now overlying ectoderm, many cells separate from it and produce a mass of mesenchymal cells called the **neural crest**. Located initially above the neural tube, neural crest cells immediately begin migrating laterally. Cell derived from the neural crest will form all components of the PNS and also contribute to certain non-neuronal tissues.

the **neural tube**. Cells of this tube give rise to the entire CNS, including neurons and most glial cells.

As the folds fuse and the neural tube separates from the now overlying surface ectoderm that will form epidermis, a large population of developmentally important cells, the **neural crest**, separates from the neuroepithelium and becomes mesenchymal. Neural crest cells migrate extensively and differentiate as all the cells of the PNS, as well as a number of other non-neuronal cell types.

NEURONS

The functional unit in both the CNS and PNS is the **neuron**. Some neuronal components have special names, such as “neurolemma” for the cell membrane. Most neurons have three main parts (Figure 9–3):

- The **cell body** (also called the **perikaryon** or **soma**) which contains the nucleus and most of the cell's organelles and serves as the synthetic or trophic center for the entire neuron.
- The **dendrites**, which are the numerous elongated processes extending from the perikaryon and specialized to receive stimuli from other neurons at unique sites called **synapses**.
- The **axon** (Gr. *axon*, axis), which is a single long process ending at synapses specialized to generate and conduct nerve impulses to other cells (nerve, muscle, and gland cells). Axons may also receive information from other neurons, information that mainly modifies the transmission of action potentials to those neurons.

Neurons and their processes are extremely variable in size and shape. Cell bodies can be very large, measuring up to 150 µm in diameter. Other neurons, such as the cerebellar granule cells, are among the body's smallest cells.

Neurons can be classified according to the number of processes extending from the cell body (Figure 9–4):

- **Multipolar neurons**, each with one axon and two or more dendrites, are the most common.
- **Bipolar neurons**, with one dendrite and one axon, comprise the sensory neurons of the retina, the olfactory epithelium, and the inner ear.
- **Unipolar or pseudounipolar neurons**, which include all other sensory neurons, each have a single process that bifurcates close to the perikaryon, with the longer branch extending to a peripheral ending and the other toward the CNS.
- **Anaxonic neurons**, with many dendrites but no true axon, do not produce action potentials, but regulate electrical changes of adjacent CNS neurons.

Because the fine processes emerging from cell bodies are seldom seen in sections of nervous tissue, it is difficult to classify neurons structurally by microscopic inspection.

Nervous components can also be subdivided functionally (Figure 9–1). **Sensory neurons** are **afferent**, receiving

stimuli from receptors throughout the body. **Motor neurons** are **efferent**, sending impulses to effector organs such as muscle fibers and glands. **Somatic** motor nerves are under voluntary control and typically innervate skeletal muscle; **autonomic** motor nerves control the involuntary or unconscious activities of glands, cardiac muscle, and most smooth muscle.

Interneurons establish relationships among other neurons, forming complex functional networks or **circuits** in the CNS. Interneurons are either multipolar or anaxonic and comprise 99% of all neurons in adults.

In the CNS most neuronal perikarya occur in the **gray matter**, with their axons concentrated in the **white matter**. These terms refer to the general appearance of unstained CNS tissue caused in part by the different densities of nerve cell bodies. In the PNS cell bodies are found in ganglia and in some sensory regions, such as the olfactory mucosa, and axons are bundled in **nerves**.

» MEDICAL APPLICATION

Parkinson disease is a slowly progressing disorder affecting muscular activity characterized by tremors, reduced activity of the facial muscles, loss of balance, and postural stiffness. It is caused by gradual loss by apoptosis of dopamine-producing neurons whose cell bodies lie within the nuclei of the CNS substantia nigra. Parkinson disease is treated with **L-dopa** (L-3,4-dihydroxyphenylalanine), a precursor of dopamine which augments the declining production of this neurotransmitter.

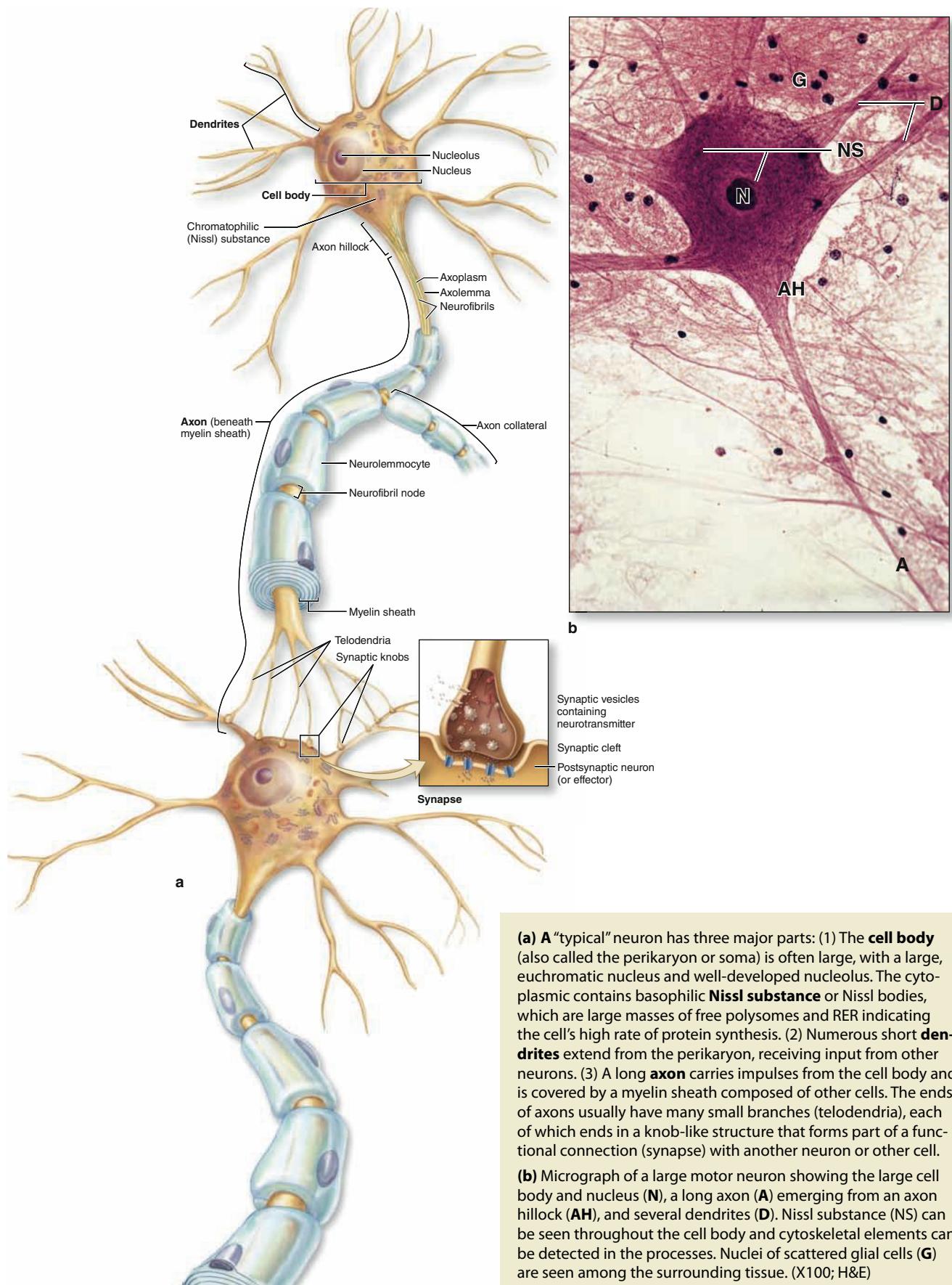
Cell Body (Perikaryon or Soma)

The neuronal **cell body** contains the nucleus and surrounding cytoplasm, exclusive of the cell processes (Figure 9–3). It acts as a trophic center, producing most cytoplasm for the processes. Most cell bodies are in contact with a great number of nerve endings conveying excitatory or inhibitory stimuli generated in other neurons. A typical neuron has an unusually large, euchromatic nucleus with a prominent nucleolus, indicating intense synthetic activity.

Cytoplasm of perikarya often contains numerous free polyribosomes and highly developed RER, indicating active production of both cytoskeletal proteins and proteins for transport and secretion. Histologically these regions with concentrated RER and other polysomes are basophilic and are distinguished as **chromatophilic substance** (or **Nissl substance**, **Nissl bodies**) (Figure 9–3). The amount of this material varies with the type and functional state of the neuron and is particularly abundant in large nerve cells such as motor neurons (Figure 9–3b). The Golgi apparatus is located only in the cell body, but mitochondria can be found throughout the cell and are usually abundant in the axon terminals.

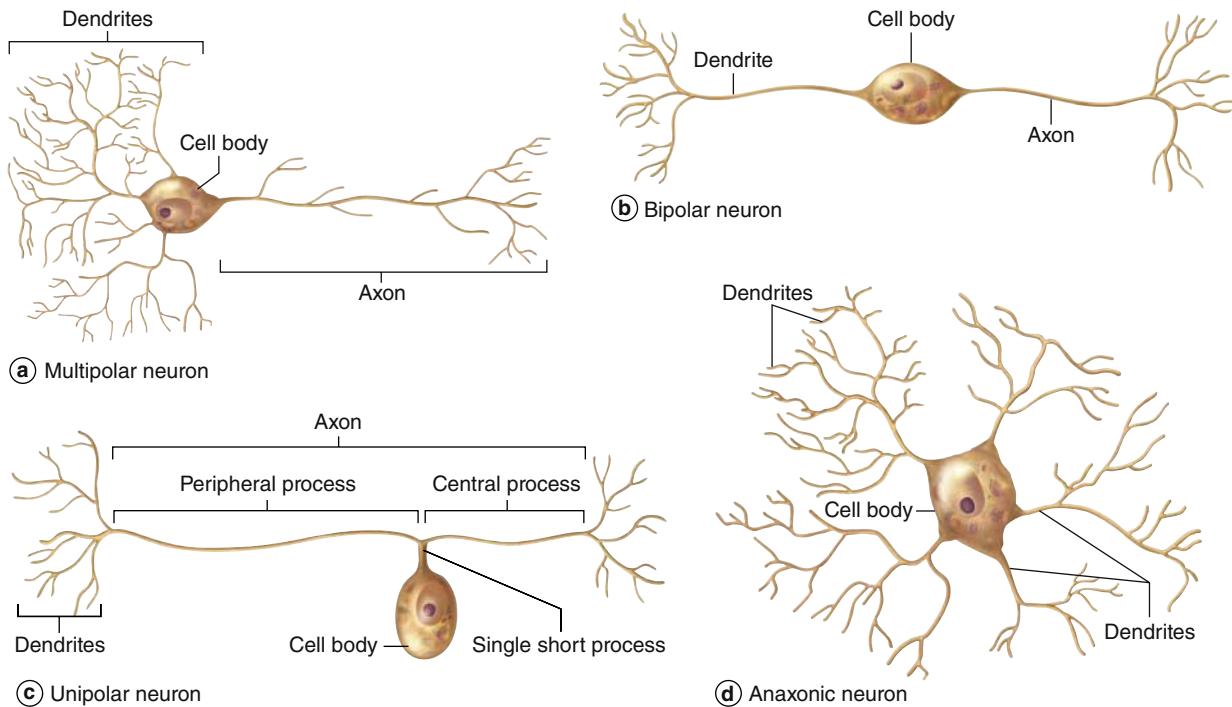
In both perikarya and processes microtubules, actin filaments, and intermediate filaments are abundant, with the

FIGURE 9–3 Structures of a typical neuron.



(a) A “typical” neuron has three major parts: (1) The **cell body** (also called the perikaryon or soma) is often large, with a large, euchromatic nucleus and well-developed nucleolus. The cytoplasmic contains basophilic **Nissl substance** or Nissl bodies, which are large masses of free polysomes and RER indicating the cell’s high rate of protein synthesis. (2) Numerous short **dendrites** extend from the perikaryon, receiving input from other neurons. (3) A long **axon** carries impulses from the cell body and is covered by a myelin sheath composed of other cells. The ends of axons usually have many small branches (telodendria), each of which ends in a knob-like structure that forms part of a functional connection (synapse) with another neuron or other cell.

(b) Micrograph of a large motor neuron showing the large cell body and nucleus (N), a long axon (A) emerging from an axon hillock (AH), and several dendrites (D). Nissl substance (NS) can be seen throughout the cell body and cytoskeletal elements can be detected in the processes. Nuclei of scattered glial cells (G) are seen among the surrounding tissue. (X100; H&E)

FIGURE 9–4 Structural classes of neurons.

Shown are the four main types of neurons, with short descriptions. (a) Most neurons, including all motor neurons and CNS interneurons, are **multipolar**. (b) **Bipolar neurons** include sensory neurons of the retina, olfactory mucosa, and inner ear. (c) All other sensory

neurons are **unipolar** or **pseudounipolar**. (d) **Anaxonic** neurons of the CNS lack true axons and do not produce action potentials, but regulate local electrical changes of adjacent neurons.

latter formed by unique protein subunits and called **neurofilaments** in this cell type. Cross-linked with certain fixatives and impregnated with silver stains, neurofilaments are also referred to as neurofibrils by light microscopists. Some nerve cell bodies also contain inclusions of pigmented material, such as lipofuscin, consisting of residual bodies left from lysosomal digestion.

Dendrites

Dendrites (Gr. *dendron*, tree) are typically short, small processes emerging and branching off the soma (Figure 9–3). Usually covered with many synapses, dendrites are the principal signal reception and processing sites on neurons. The large number and extensive arborization of dendrites allow a single neuron to receive and integrate signals from many other nerve cells. For example, up to 200,000 axonal endings can make functional contact with the dendrites of a single large Purkinje cell of the cerebellum.

Unlike axons, which maintain a nearly constant diameter, dendrites become much thinner as they branch, with cytoskeletal elements predominating in these distal regions. In the CNS most synapses on dendrites occur on **dendritic spines**, which are dynamic membrane protrusions along the small dendritic branches, visualized with silver staining (Figure 9–5).

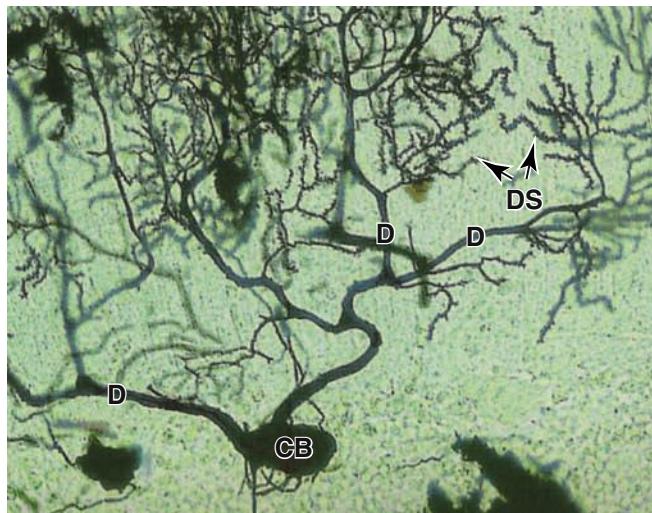
and studied by confocal or electron microscopy. Dendritic spines serve as the initial processing sites for synaptic signals and occur in vast numbers, estimated to be on the order of 10^{14} for cells of the human cerebral cortex. Dendritic spine morphology depends on actin filaments and changes continuously as synaptic connections on neurons are modified. Changes in dendritic spines are of key importance in the constant changes of the **neural plasticity** that occurs during embryonic brain development and underlies adaptation, learning, and memory postnatally.

Axons

Most neurons have only one **axon**, typically longer than its dendrites. Axonal processes vary in length and diameter according to the type of neuron. Axons of the motor neurons that innervate the foot muscles have lengths of nearly a meter; large cell bodies are required to maintain these axons, which contain most of such neurons' cytoplasm. The plasma membrane of the axon is often called the **axolemma** and its contents are known as **axoplasm**.

Axons originate from a pyramid-shaped region of the perikaryon called the **axon hillock** (Figure 9–3), just beyond which the axolemma has concentrated ion channels which

FIGURE 9–5 Dendrites and dendritic spines.



The large Purkinje neuron in this silver-impregnated section of cerebellum has many dendrites (**D**) emerging from its cell body (**CB**) and forming branches. The small dendritic branches each have many tiny projecting dendritic spines (**DS**) spaced closely along their length, each of which is a site of a synapse with another neuron. Dendritic spines are highly dynamic, the number of synapses changing constantly. (X650; Silver stain)

generate the action potential. At this initial segment of the axon the various excitatory and inhibitory stimuli impinging on the neuron are algebraically summed, resulting in the decision to propagate—or not to propagate—a nerve impulse.

Axons generally branch less profusely than dendrites, but do undergo **terminal arborization** (Figure 9–3). Axons of interneurons and some motor neurons also have major branches called **collaterals** that end at smaller branches with synapses influencing the activity of many other neurons. Each small axonal branch ends with a dilation called a **terminal bouton** (Fr. *bouton*, button) that contacts another neuron or non-nerve cell at a synapse to initiate an impulse in that cell.

Axoplasm contains mitochondria, microtubules, neurofilaments, and transport vesicles, but very few polyribosomes or cisternae of RER, features which emphasize the dependence of axoplasm on the perikaryon. If an axon is severed from its cell body its distal part quickly degenerates and undergoes phagocytosis.

Lively bidirectional transport of molecules large and small occurs within axons. Organelles and macromolecules synthesized in the cell body move by **anterograde transport** along axonal microtubules via **kinesin** from the perikaryon to the synaptic terminals. **Retrograde transport** in the opposite direction along microtubules via **dynein** carries certain other macromolecules, such as material taken up by endocytosis (including viruses and toxins), from the periphery to the cell body. Retrograde transport can be used to study the pathways of neurons: if peroxidase or another marker is

injected into regions with axon terminals, its later distribution throughout the neurons serving such regions can be determined histochemically.

Anterograde and retrograde transports both occur fairly rapidly, at rates of 50–400 mm/d. A much slower anterograde stream, moving only a few millimeters per day, involves movement of the axonal cytoskeleton itself. This slow axonal transport corresponds roughly to the rate of axon growth.

Nerve Impulses

A **nerve impulse**, or **action potential**, travels along an axon like a spark moves along an explosive's fuse. It is an electrochemical process initiated at the axon hillock when other impulses received at the cell body or dendrites meet a certain threshold. The action potential is propagated along the axon as a wave of membrane depolarization produced by **voltage-gated Na⁺ and K⁺ channels** in the axolemma that allow diffusion of these ions into and out of the axoplasm. The extracellular compartment around all regions of the neuron is a very thin zone immediately outside the cell that is formed by enclosing glial cells which also regulate its ionic contents.

In unstimulated neurons ATP-dependent Na-K pumps and other membrane proteins maintain an axoplasmic Na⁺ concentration only one-tenth of that outside the cell and a K⁺ level many times greater than the extracellular concentration. This produces a potential electrical difference across the axolemma of about –65 mV, with the inside negative to the outside. This difference is the axon's **resting potential**.

» MEDICAL APPLICATION

Most **local anesthetics** are low-molecular-weight molecules that bind to the voltage-gated sodium channels of the axolemma, interfering with sodium ion influx and, consequently, inhibiting the action potential responsible for the nerve impulse.

When the threshold for triggering an impulse is met, channels at the axon's initial segment open and allow a very rapid influx of extracellular Na⁺ that makes the axoplasm positive in relation to the extracellular environment and shifts (depolarizes) the resting potential from negative to positive, to +30 mV. Immediately after the membrane depolarization, the voltage-gated Na⁺ channels close and those for K⁺ open, which rapidly returns the membrane to its resting potential. This cycle of events occurs in less than 1 millisecond.

Depolarization stimulates adjacent portions of the axolemma to depolarize and return immediately to the resting potential, which causes a nerve impulse, or wave of depolarization, to move rapidly along the axon. After a refractory period also measured in milliseconds, the neuron is ready to repeat this process and generate another action potential. Impulses arriving at the synaptic nerve endings promote the discharge of stored neurotransmitter that stimulates or inhibits action potentials in another neuron or a non-neuronal cell.

Synaptic Communication

Synapses (Gr. *synapsis*, union) are sites where nerve impulses are transmitted from one neuron to another, or from neurons and other effector cells. The structure of a synapse (Figure 9–6) ensures that transmission is unidirectional. Synapses convert an electrical signal (nerve impulse) from the **presynaptic cell** into a chemical signal that affects the **postsynaptic cell**. Most synapses act by releasing **neurotransmitters**, which are usually small molecules that bind specific receptor proteins to either open or close ion channels or initiate second-messenger cascades. A synapse (Figure 9–6a) has the following components:

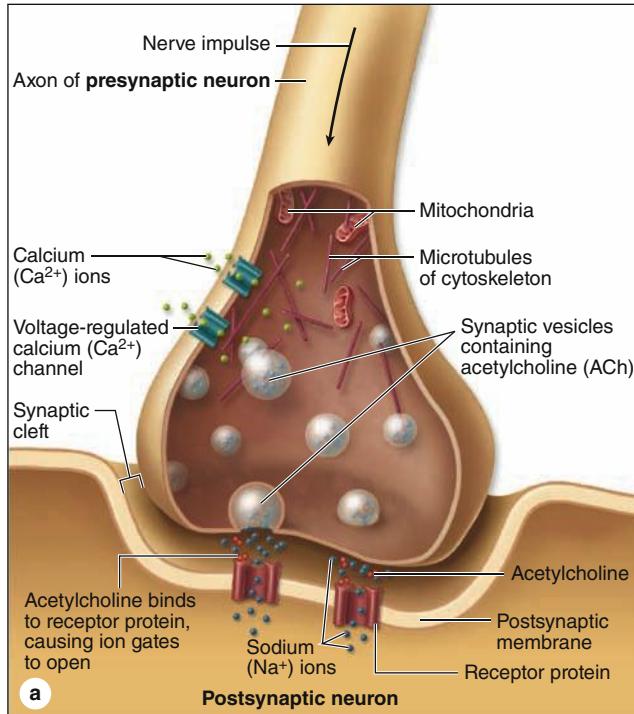
- The **presynaptic axon terminal (terminal bouton)** contains mitochondria and numerous **synaptic vesicles** from which neurotransmitter is released by exocytosis.

- The **postsynaptic cell membrane** contains receptors for the neurotransmitter, and ion channels or other mechanisms to initiate a new impulse.
- A 20- to 30-nm-wide intercellular space called the **synaptic cleft** separates these presynaptic and postsynaptic membranes.

At the presynaptic region the nerve impulse briefly opens calcium channels, promoting a Ca^{2+} influx that triggers neurotransmitter release by exocytosis or similar mechanisms. Immediately the released neurotransmitter molecules diffuse across the synaptic cleft and bind receptors at the postsynaptic region. This produces either an excitatory or an inhibitory effect at the postsynaptic membrane, as follows:

- Neurotransmitters from **excitatory synapses** cause postsynaptic Na^+ channels to open, and the resulting Na^+

FIGURE 9–6 Major components of a synapse.



(a) Diagram showing a synapse releasing neurotransmitters by exocytosis from the terminal bouton. Presynaptic terminals always contain a large number of **synaptic vesicles** containing neurotransmitters, numerous **mitochondria**, and smooth ER as a source of new membrane. Some neurotransmitters are synthesized in the cell body and then transported in vesicles to the presynaptic terminal. Upon arrival of a nerve impulse, voltage-regulated Ca^{2+} channels permit Ca^{2+} entry, which triggers neurotransmitter release into the synaptic cleft. Excess membrane accumulating at the presynaptic region as a result of exocytosis is recycled by clathrin-mediated endocytosis, which is not depicted here.

(b) The TEM shows a large presynaptic terminal (\mathbf{T}_1) filled with synaptic vesicles and asymmetric electron-dense regions around 20- to 30-nm-wide synaptic clefts (arrows). The postsynaptic membrane contains the neurotransmitter receptors and mechanisms to initiate an impulse at the postsynaptic neuron. The postsynaptic membrane on the right is part of a dendrite (\mathbf{D}), associated with fewer vesicles of any kind, showing this to be an axodendritic synapse. On the left is another presynaptic terminal (\mathbf{T}_2), suggesting an axoaxonic synapse with a role in modulating activity of the other terminal. (X35,000)

influx initiates a depolarization wave in the postsynaptic neuron or effector cell as just described.

- At **inhibitory synapses** neurotransmitters open Cl⁻ or other anion channels, causing influx of anions and **hyperpolarization** of the postsynaptic cell, making its membrane potential more negative and more resistant to depolarization.

Interplay between excitatory and inhibitory effects on postsynaptic cells allows synapses to process neuronal input and fine-tune the reaction of the effector cell. Impulses passing from presynaptic neurons to postsynaptic cells are usually modified at the synapse by similar connections there with other neurons (Figure 9–6b). The response in postsynaptic neurons is determined by the summation of activity at hundreds of synapses on that cell. Three common morphological types of synapses occur between neurons of the CNS and are shown in Figure 9–7.

The chemical transmitter used at neuromuscular junctions and some synapses of the CNS is **acetylcholine**. Within the CNS other major categories of neurotransmitters include:

- Certain **amino acids** (often modified), such as glutamate and γ-aminobutyrate (GABA)
- **Monoamines**, such as serotonin (5-hydroxytryptamine or 5-HT) and **catecholamines**, such as dopamine, all of which are synthesized from amino acids
- Small **polypeptides**, such as endorphins and substance P.

Important actions of these and other common neurotransmitters are summarized in Table 9–1. Different receptors and second messenger systems often occur for the same transmitter, greatly multiplying the possible effects of these molecules. After their release transmitters are removed quickly by enzymatic breakdown, by glial activity, or by endocytotic recycling involving presynaptic membrane receptors.

» MEDICAL APPLICATION

Levels of neurotransmitters in the synaptic cleft and available for binding postsynaptic receptors are normally regulated by several local mechanisms. **Selective serotonin reuptake inhibitors (SSRIs)**, a widely used class of drugs for treatment of depression and anxiety disorders, were designed to augment levels of this neurotransmitter at the postsynaptic membrane of serotonergic CNS synapses by specifically inhibiting its reuptake at the presynaptic membrane.

» GLIAL CELLS & NEURONAL ACTIVITY

Glial cells support neuronal survival and activities, and are ten times more abundant than neurons in the mammalian brain. Like neurons most glial cells develop from progenitor cells of the embryonic neural plate. In the CNS glial cells surround both the neuronal cell bodies, which are often larger than the glial cells, and the processes of axons and dendrites occupying the spaces between neurons. Except around the

larger blood vessels, the CNS has only a very small amount of connective tissue and collagen. Glial cells substitute for cells of connective tissue in some respects, supporting neurons and creating immediately around those cells microenvironments that are optimal for neuronal activity. The fibrous intercellular network of CNS tissue superficially resembles collagen by light microscopy, but is actually the network of fine cellular processes emerging from neurons and glial cells. Such processes are collectively called the **neuropil** (Figure 9–8).

There are six major kinds of glial cells, as shown schematically in Figure 9–9, four in the CNS, two in the PNS. Their main functions, locations, and origins are summarized in Table 9–2.

Oligodendrocytes

Oligodendrocytes (Gr. *oligos*, small, few + *dendron*, tree + *kertos*, cell) extend many processes, each of which becomes sheet-like and wraps repeatedly around a portion of a nearby CNS axon (Figure 9–9a). During this wrapping most cytoplasm gradually moves out of the growing extension, leaving multiple compacted layers of cell membrane collectively termed **myelin**. An axon's full length is covered by the action of many oligodendrocytes. The resulting **myelin sheath** electrically insulates the axon and facilitates rapid transmission of nerve impulses. Found only in the CNS oligodendrocytes are the predominant glial cells in white matter, which is white because of the lipid concentrated in the wrapped membrane sheaths. The processes and sheaths are not visible by routine light microscope staining, in which oligodendrocytes usually appear as small cells with rounded, condensed nuclei and unstained cytoplasm (Figure 9–8a).

Astrocytes

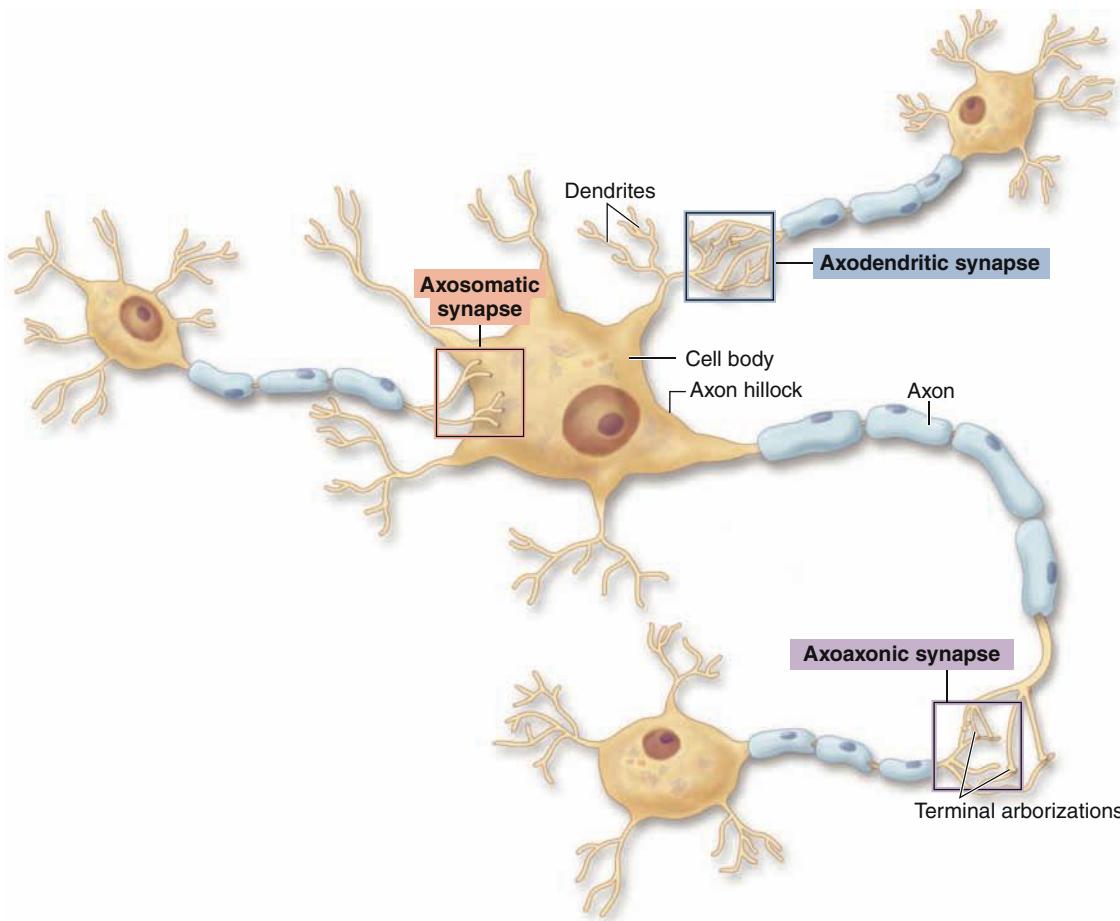
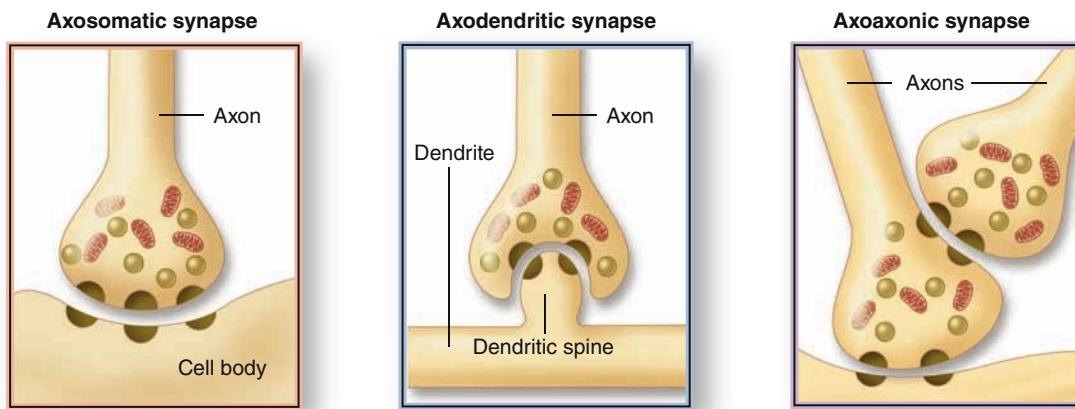
Also unique to the CNS **astrocytes** (Gr. *astro-*, star + *kertos*) have a large number of long radiating, branching processes (Figures 9–9a and 9–10). Proximal regions of the astrocytic processes are reinforced with bundles of intermediate filaments made of **glial fibrillary acid protein (GFAP)**, which serves as a unique marker for this glial cell. Distally the processes lack GFAP, are not readily seen by microscopy, and form a vast network of delicate terminals contacting synapses and other structures. Terminal processes of a single astrocyte typically occupy a large volume and associate with over a million synaptic sites.

Astrocytes originate from progenitor cells in the embryonic neural tube and are by far the most numerous glial cells of the brain, as well as the most diverse structurally and functionally. **Fibrous astrocytes**, with long delicate processes, are abundant in white matter; those with many shorter processes are called **protoplasmic astrocytes** and predominate in the gray matter. The highly variable and dynamic processes mediate most of these cells' many functions.

» MEDICAL APPLICATION

Most brain tumors are **astrocytomas** derived from fibrous astrocytes. These are distinguished pathologically by their expression of GFAP.

FIGURE 9–7 Types of synapses.



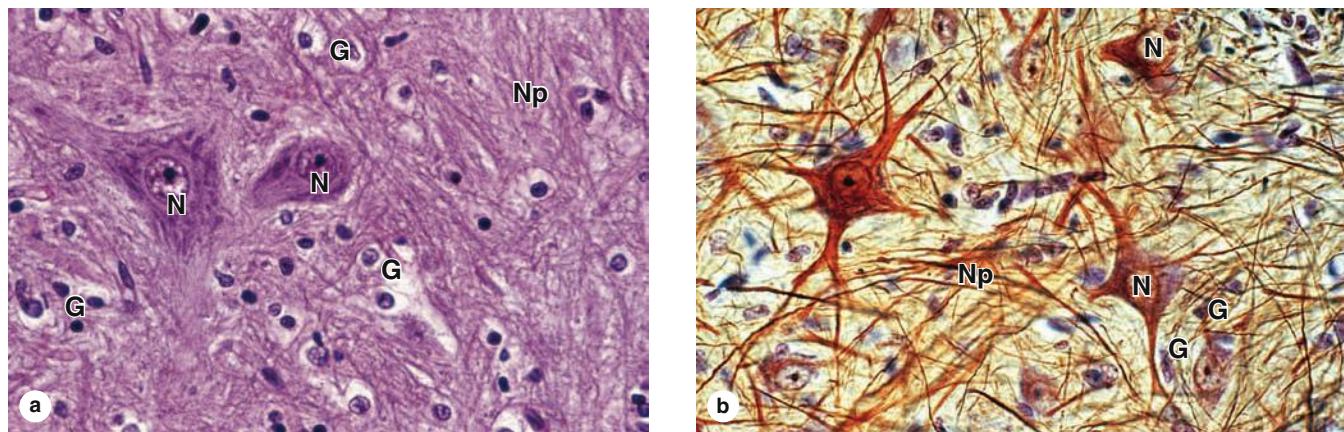
The diagrams show three common morphologic types of synapses. Branched axon terminals usually associate with and transmit a nerve impulse to another neuron's cell body (or soma) or a dendritic spine. These types of connections are termed an **axosomatic synapse** and an **axodendritic synapse**, respectively. Less frequently, an axon terminal forms a synapse with an axon terminal of another neuron; such an **axoaxonic synapse** functions to modulate synaptic activity in the other two types.

All three morphologic types of synapses have the features of all true synapses: a presynaptic axon terminal that releases a transmitter; a postsynaptic cell membrane with receptors for the transmitter; and an intervening synaptic cleft.

Synaptic structure usually cannot be resolved by light microscopy, although components such as dendritic spines may be shown with special techniques (Figure 9–5).

TABLE 9–1 Common neurotransmitters and their actions.

| Neurotransmitter | Description/Action |
|---|---|
| ACETYLCHOLINE (ACh) | |
| $\begin{array}{c} \text{CH}_3 \\ \\ \text{H}_3\text{C}-\text{N}^+-\text{CH}_2-\text{CH}_2-\text{O}-\text{C}(=\text{O})-\text{CH}_3 \\ \\ \text{CH}_3 \end{array}$ | Chemical structure significantly different from that of other neurotransmitters; active in CNS and in both somatic and autonomic parts of PNS; binds to ACh receptors (cholinergic receptors) in PNS to open ion channels in postsynaptic membrane and stimulate muscle contraction |
| AMINO ACIDS | |
| $\begin{array}{c} \text{NH}_2-\text{CH}_2-\overset{\text{O}}{\underset{\text{OH}}{\text{C}}}-\text{R} \end{array}$ | Molecules with both carboxyl ($-\text{COOH}$) and amine ($-\text{NH}_2$) groups and various R groups; act as important transmitters in the CNS |
| Glutamate | Excites activity in neurons to promote cognitive function in the brain (learning and memory); most common neurotransmitter in the brain; opens Na^+ channels |
| Gamma-aminobutyric acid (GABA) | Synthesized from glutamate; primary inhibitory neurotransmitter in the brain; also influences muscle tone; opens or closes various ion channels |
| Glycine | Inhibits activity between neurons in the CNS, including retina; opens Cl^- channels |
| MONOAMINES | |
| $\begin{array}{c} \text{OH} \\ \\ \text{NH}_2-\text{CH}_2-\text{CH}-\text{C}_6\text{H}_3(\text{OH})_2-\text{OH} \end{array}$ | Molecules synthesized from an amino acid by removing the carboxyl group and retaining the single amine group; also called biogenic amines |
| Serotonin or 5-hydroxytryptamine (5-HT) | Has various functions in the brain related to sleep, appetite, cognition (learning, memory), and mood; modulates actions of other neurotransmitters |
| Catecholamines | A distinct group of monoamines |
| Dopamine | Produces inhibitory activity in the brain; important roles in cognition (learning, memory), motivation, behavior, and mood; opens K^+ channels, closes Ca^{2+} channels |
| Norepinephrine (noradrenaline) | Neurotransmitter of PNS (sympathetic division of autonomic nervous system) and specific CNS regions |
| Epinephrine (adrenaline) | Has various effects in the CNS, especially the spinal cord, thalamus, and hypothalamus |
| NEUROPEPTIDES | |
| | Small polypeptides act as signals to assist in and modulate communication among neurons in the CNS |
| Enkephalin | Helps regulate response to noxious and potentially harmful stimuli |
| Neuropeptide Y | Involved in memory regulation and energy balance (increased food intake and decreased physical activity) |
| Somatostatin | Inhibits activities of neurons in specific brain areas |
| Substance P | Assists with pain information transmission into the brain |
| Cholecystokinin (CCK) | Stimulates neurons in the brain to help mediate satiation (fullness) and repress hunger |
| Beta-endorphin | Prevents release of pain signals from neurons and fosters a feeling of well-being |
| Neurotensin | Helps control and moderate the effects of dopamine |
| OTHERS | |
| Adenosine | Also part of a nucleotide, inhibits activities in certain CNS neurons |
| Nitric oxide | Involved in learning and memory; relaxes muscle in the digestive tract; important for relaxation of smooth muscle in blood vessels (vasodilation) |

FIGURE 9–8 Neurons, neuropil, and the common glial cells of the CNS.

(a) Most neuronal cell bodies (**N**) in the CNS are larger than the much more numerous glial cells (**G**) that surround them. The various types of glial cells and their relationships with neurons are difficult to distinguish by most routine light microscopic methods. However, oligodendrocytes have condensed, rounded nuclei and unstained cytoplasm due to very abundant Golgi complexes, which stain poorly and are very likely represented by the cells with

those properties seen here. The other glial cells seen here similar in overall size, but with very little cytoplasm and more elongated or oval nuclei, are mostly astrocytes. Routine H&E staining does not allow neuropil to stand out well. (X200; H&E)

(b) With the use of gold staining for neurofibrils, neuropil (**Np**) is more apparent. (X200; Gold chloride and hematoxylin)

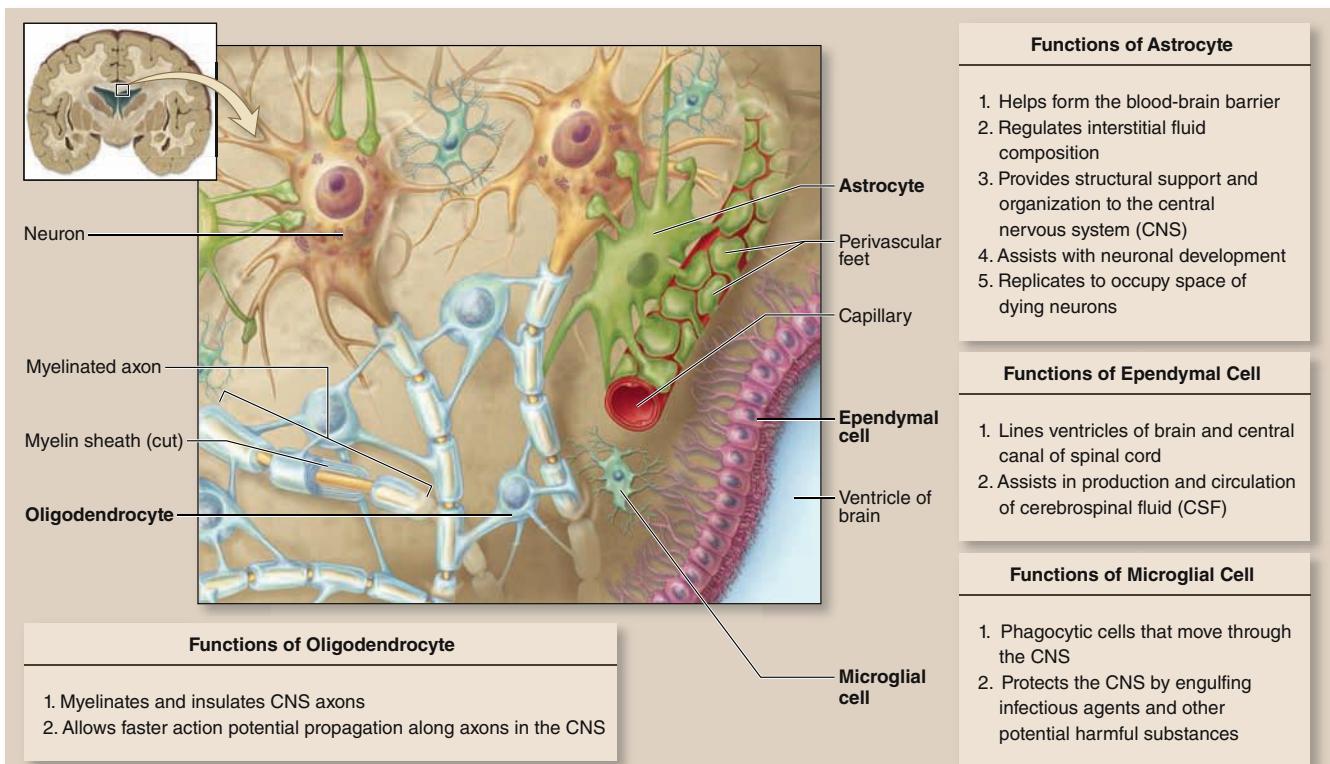
Functions attributed to astrocytes of various CNS regions include the following:

- Extending processes that associate with or cover synapses, affecting the formation, function, and plasticity of these structures
- Regulating the extracellular ionic concentrations around neurons, with particular importance in buffering extracellular K⁺ levels
- Guiding and physically supporting movements and locations of differentiating neurons during CNS development

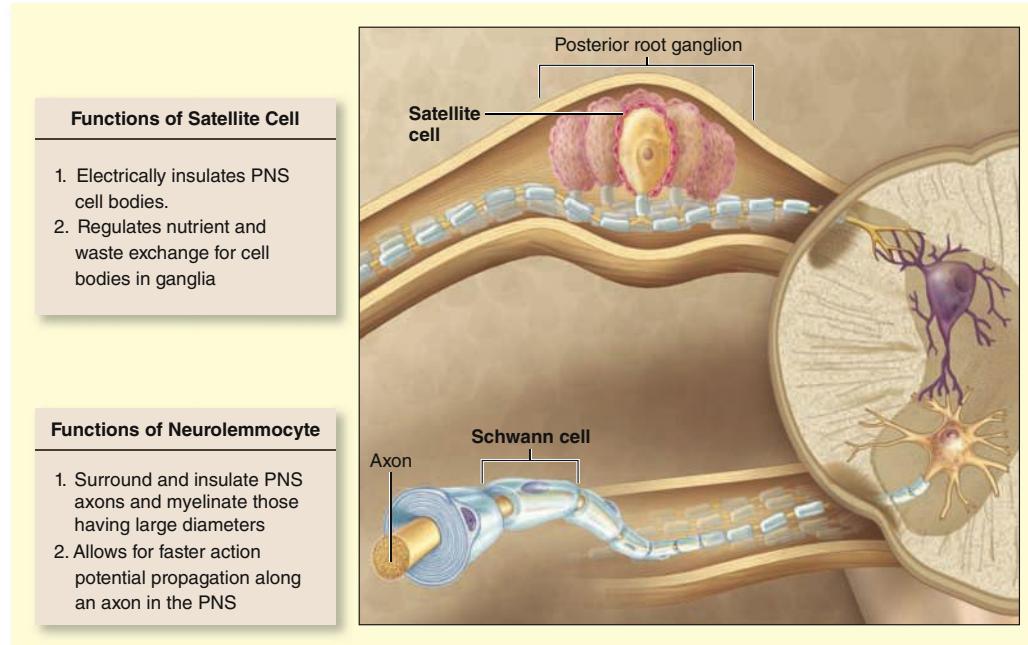
- Extending fibrous processes with expanded **perivascular feet** that cover capillary endothelial cells and modulate blood flow and help move nutrients, wastes, and other metabolites between neurons and capillaries (Figure 9–9a)
- Forming a barrier layer of expanded protoplasmic processes, called the **glial limiting membrane**, which lines the meninges at the external CNS surface
- Filling tissue defects after CNS injury by proliferation to form an **astrocytic scar**.

TABLE 9–2 Origin, location and principal functions of neuroglial cells.

| Glial Cell Type | Origin | Location | Main Functions |
|------------------------------|-------------------------|--|---|
| Oligodendrocyte | Neural tube | CNS | Myelin production, electrical insulation |
| Astrocyte | Neural tube | CNS | Structural and metabolic support of neurons, especially at synapses; repair processes |
| Ependymal cell | Neural tube | Line ventricles and central canal of CNS | Aid production and movement of CSF |
| Microglia | Bone marrow (monocytes) | CNS | Defense and immune-related activities |
| Schwann cell | Neural crest | Peripheral nerves | Myelin production, electrical insulation |
| Satellite cells (of ganglia) | Neural crest | Peripheral ganglia | Structural and metabolic support for neuronal cell bodies |

FIGURE 9–9 Glial cells of the CNS and PNS.

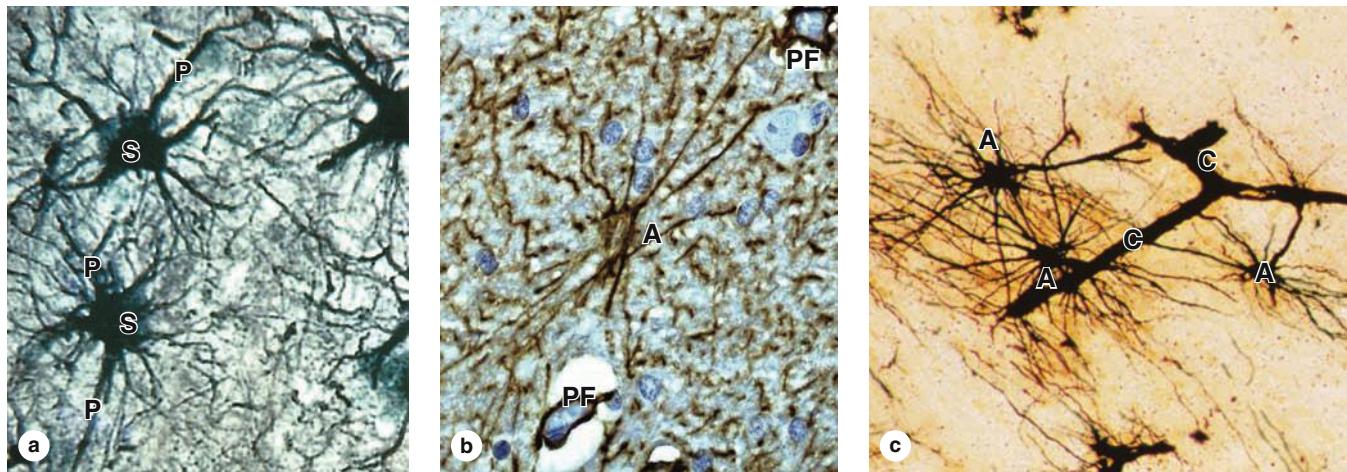
(a)



(b)

(a) There are four major kinds of glial cells in the CNS: **oligodendrocytes, astrocytes, ependymal cells, and microglial cells**. The interrelationships and major functions of these cells are shown diagrammatically here.

(b) Two glial cells occur in the PNS: **Schwann cells** (sometimes called neurolemmocytes), which surround peripheral nerve fibers, and **satellite cells**, which surround the nerve cell bodies and are thus found only in ganglia. Major functions of these cells are indicated.

FIGURE 9–10 Astrocytes.

(a) Astrocytes are the most abundant glial cells of the CNS and are characterized by numerous cytoplasmic processes (**P**) radiating from the glial cell body or soma (**S**). Astrocytic processes are not seen with routine light microscope staining but are easily seen after gold staining. Morphology of the processes allows astrocytes to be classified as fibrous (relatively few and straight processes) or protoplasmic (numerous branching processes), but functional differences between these types are not clear. (X500; Gold chloride)

(b) All astrocytic processes contain intermediate filaments of GFAP, and antibodies against this protein provide a simple method to stain these cells, as seen here in a fibrous astrocyte (**A**) and its

processes. The small pieces of other GFAP-positive processes in the neuropil around this cell give an idea of the density of this glial cell and its processes in the CNS. Astrocytes form part of the blood-brain barrier (BBB) and help regulate entry of molecules and ions from blood into CNS tissue. Capillaries at the extreme upper right and lower left corners are enclosed by GFAP-positive perivascular feet (**PF**) at the ends of numerous astrocytic processes. (X500; Anti-GFAP immunoperoxidase and hematoxylin counterstain)

(c) A length of capillary (**C**) is shown here completely covered by silver-stained terminal processes extending from astrocytes (**A**). (X400; Rio Hortega silver)

Finally, astrocytes communicate directly with one another via gap junctions, forming a very large cellular network for the coordinated regulation of their various activities in different brain regions.

» MEDICAL APPLICATION

Alzheimer disease, a common type of dementia in the elderly, affects both neuronal perikarya and synapses within the cerebrum. Functional defects are due to **neurofibrillary tangles**, which are accumulations of tau protein associated with microtubules of the neuronal perikaryon and axon hillock regions, and **neuritic plaques**, which are dense aggregates of β -amyloid protein that form around the outside of these neuronal regions.

Ependymal Cells

Ependymal cells are columnar or cuboidal cells that line the fluid-filled ventricles of the brain and the central canal of the spinal cord (Figures 9–9a and 9–11). In some CNS locations, the apical ends of ependymal cells have cilia, which facilitate the movement of cerebrospinal fluid (CSF), and long microvilli, which are likely involved in absorption.

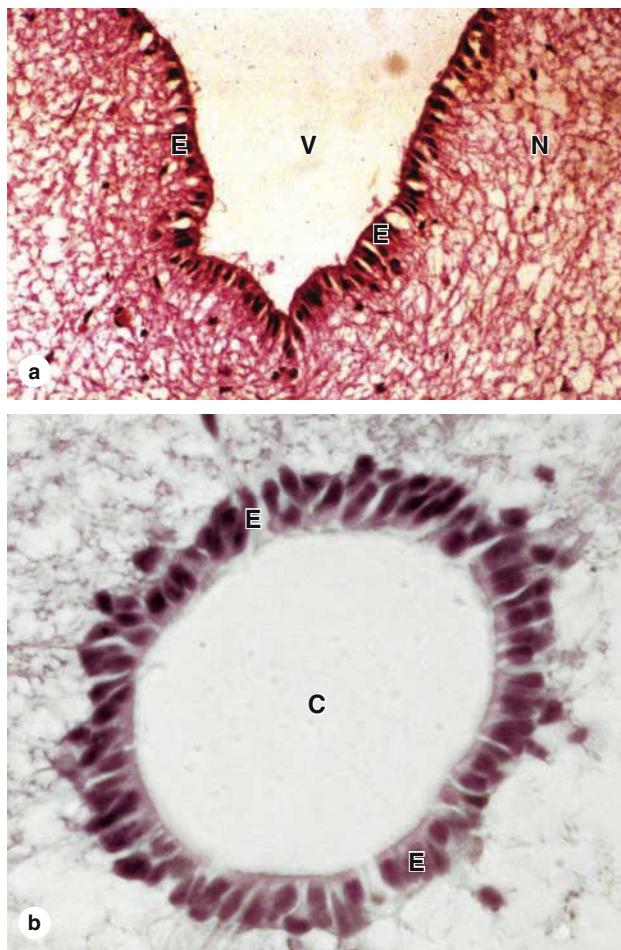
Ependymal cells are joined apically by apical junctional complexes similar to those of epithelial cells. However, unlike a true epithelium there is no basal lamina. Instead, the basal

ends of ependymal cells are elongated and extend branching processes into the adjacent neuropil.

Microglia

Less numerous than oligodendrocytes or astrocytes but nearly as common as neurons in some CNS regions, **microglia** are small cells with actively mobile processes evenly distributed throughout gray and white matter (Figures 9–9a and 9–12). Unlike other glial cells microglia migrate, with their processes scanning the neuropil and removing damaged or effete synapses or other fibrous components. Microglial cells also constitute the major mechanism of immune defense in the CNS, removing any microbial invaders and secreting a number of immunoregulatory cytokines. Microglia do not originate from neural progenitor cells like other glia, but from circulating blood monocytes, belonging to the same family as macrophages and other antigen-presenting cells.

Nuclei of microglial cells can often be recognized in routine hematoxylin and eosin (H&E) preparations by their small, dense, slightly elongated structure, which contrasts with the larger, spherical, more lightly stained nuclei of other glial cells. Immunohistochemistry using antibodies against cell surface antigens of immune cells demonstrates microglial processes. When activated by damage or microorganisms microglia retract their processes, proliferate, and assume the morphologic characteristics and functions of antigen-presenting cells (see Chapter 14).

FIGURE 9–11 Ependymal cells.

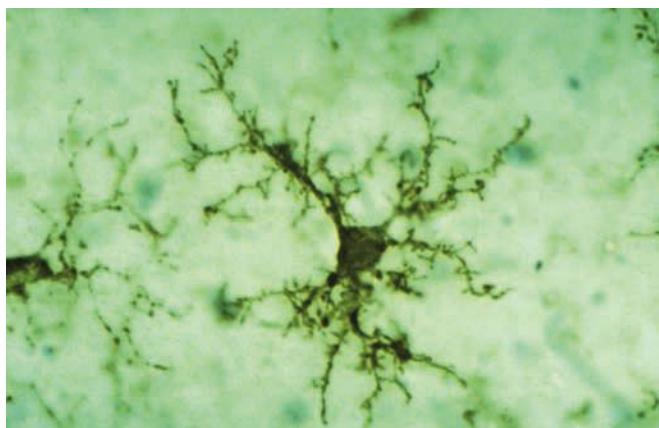
Ependymal cells are epithelial-like cells that form a single layer lining the fluid-filled ventricles and central canal of the CNS.

(a) Lining the ventricles of the cerebrum, columnar ependymal cells (E) extend cilia and microvilli from the apical surfaces into the ventricle (V). These modifications help circulate the CSF and monitor its contents. Ependymal cells have junctional complexes at their apical ends like those of epithelial cells but lack a basal lamina. The cells' basal ends are tapered, extending processes that branch and penetrate some distance into the adjacent neuropil (N). Other areas of ependyma are responsible for production of CSF. (X100; H&E)

(b) Ependymal cells (E) lining the central canal (C) of the spinal cord help move CSF in that CNS region. (X200; H&E)

» MEDICAL APPLICATION

In **multiple sclerosis** (MS) the myelin sheaths surrounding axons are damaged by an autoimmune mechanism that interferes with the activity of the affected neurons and produces various neurologic problems. T lymphocytes and microglia, which phagocytose and degrade myelin debris, play major roles in progression of this disease. In MS, destructive actions of these cells exceed the capacity of oligodendrocytes to produce myelin and repair the myelin sheaths.

FIGURE 9–12 Microglial cells.

Microglia are monocyte-derived, antigen-presenting cells of the CNS, less numerous than astrocytes but nearly as common as neurons and evenly distributed in both gray and white matter. By immunohistochemistry, here using a monoclonal antibody against **human leukocyte antigens (HLA)** of immune-related cells, the short branching processes of microglia can be seen. Routine staining demonstrates only the small dark nuclei of the cells. Unlike other glia of the CNS, microglia are not interconnected; they are motile cells, constantly used in immune surveillance of CNS tissues. When activated by products of cell damage or by invading microorganisms, the cells retract their processes, begin phagocytosing the damage- or danger-related material, and behave as antigen-presenting cells. (X500; Antibody against HLA-DR and peroxidase)

(Used with permission from Wolfgang Streit, Department of Neuroscience, University of Florida College of Medicine, Gainesville.)

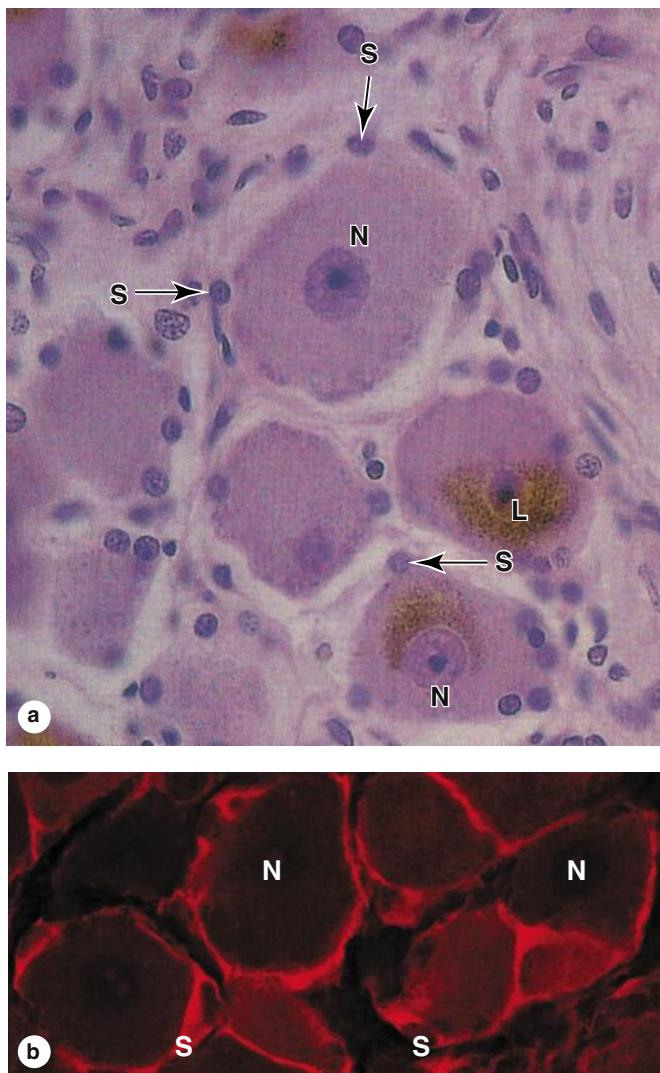
Schwann Cells

Schwann cells (named for 19th century German histologist **Theodor Schwann**), sometimes called **neurolemmocytes**, are found only in the PNS and differentiate from precursors in the neural crest. Schwann cells are the counterparts to oligodendrocytes of the CNS, having trophic interactions with axons and most importantly forming their **myelin sheathes**. However unlike an oligodendrocyte, a Schwann cell forms myelin around a portion of only one axon. Figure 9–9b shows a series of Schwann cells sheathing the full length of an axon, a process described more fully with peripheral nerves.

Satellite Cells of Ganglia

Also derived from the embryonic neural crest, small **satellite cells** form a thin, intimate glial layer around each large neuronal cell body in the ganglia of the PNS (Figures 9–9b and 9–13). Satellite cells exert a trophic or supportive effect on these neurons, insulating, nourishing, and regulating their microenvironments.

FIGURE 9–13 Satellite cells around neurons of ganglia in the PNS.



Satellite cells are very closely associated with neuronal cell bodies in sensory and autonomic ganglia of the PNS and support these cells in various ways.

(a) Nuclei of the many satellite cells (**S**) surrounding the perikarya of neurons (**N**) in an autonomic ganglion can be seen by light microscopy, but their cytoplasmic extensions are too thin to see with H&E staining. These long-lived neurons commonly accumulate brown lipofuscin (**L**). (X560; H&E)

(b) Immunofluorescent staining of satellite cells (**S**) reveals the cytoplasmic sheets extending from these cells and surrounding the neuronal cell bodies (**N**). The layer of satellite cells around each soma is continuous with the myelin sheath around the axon. Like the effect of Schwann cells on axons, satellite glial cells insulate, nourish, and regulate the microenvironment of the neuronal cell bodies. (X600; Rhodamine red-labeled antibody against glutamine synthetase)

(Used with permission from Menachem Hanani, Laboratory of Experimental Surgery, Hadassah University Hospital, Jerusalem, Israel.)

> CENTRAL NERVOUS SYSTEM

The major structures comprising the CNS are the **cerebrum**, **cerebellum**, and **spinal cord** (Figure 9–1). The CNS is completely covered by connective tissue layers, the meninges, but CNS tissue contains very little collagen or similar material, making it relatively soft and easily damaged by injuries affecting the protective skull or vertebral bones. Most CNS neurons and their functional organization are more appropriately covered in neuroscience rather than histology courses, but certain important cells and basic topics will be introduced here.

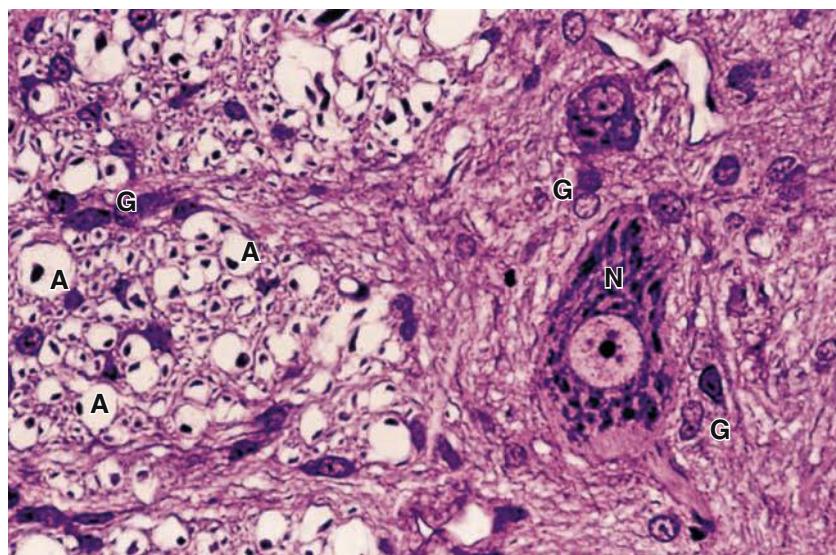
Many structural features of CNS tissues can be seen in unstained, freshly dissected specimens. Many regions show organized areas of **white matter** and **gray matter**, differences caused by the differential distribution of lipid-rich myelin. The main components of white matter are myelinated axons (Figure 9–14), often grouped together as **tracts**, and the myelin-producing oligodendrocytes. Astrocytes and microglia are also present, but very few neuronal cell bodies. Gray matter contains abundant neuronal cell bodies, dendrites, astrocytes, and microglial cells, and is where most synapses occur. Gray matter makes up the thick cortex or surface layer of both the cerebrum and the cerebellum; most white matter is found in deeper regions. Deep within the brain are localized, variously shaped darker areas called the **cerebral nuclei**, each containing large numbers of aggregated neuronal cell bodies.

In the folded **cerebral cortex** neuroscientists recognize six layers of neurons with different sizes and shapes. The most conspicuous of these cells are the efferent **pyramidal neurons** (Figure 9–15). Neurons of the cerebral cortex function in the integration of sensory information and the initiation of voluntary motor responses.

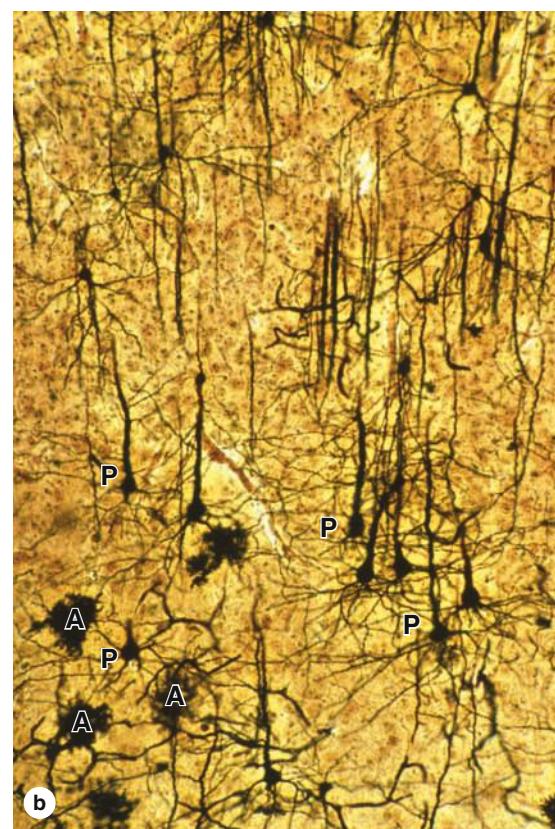
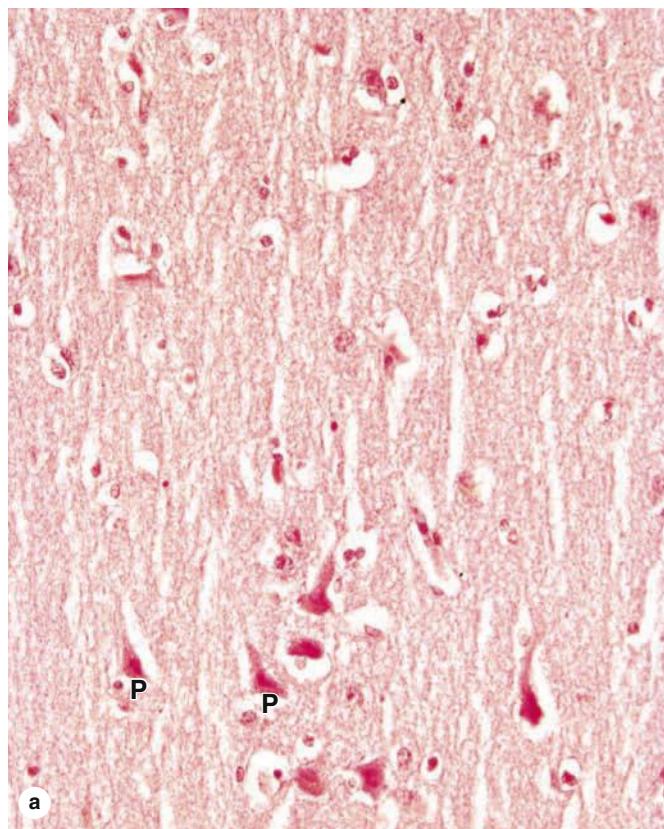
The sharply folded **cerebellar cortex** coordinates muscular activity throughout the body and is organized with three layers (Figure 9–16):

- A thick outer **molecular layer** has much neuropil and scattered neuronal cell bodies.
- A thin middle layer consists only of very large neurons called **Purkinje cells** (named for the 19th century Czech histologist Jan Purkinje). These are conspicuous even in H&E-stained sections, and their dendrites extend throughout the molecular layer as a branching basket of nerve fibers (Figures 9–16c and d).
- A thick inner **granular layer** contains various very small, densely packed neurons (including granule cells, with diameters of only 4–5 µm) and little neuropil.

In cross sections of the **spinal cord** the white matter is peripheral and the gray matter forms a deeper, H-shaped mass (Figure 9–17). The two anterior projections of this gray matter, the **anterior horns**, contain cell bodies of very large motor neurons whose axons make up the ventral roots of spinal nerves. The two **posterior horns** contain interneurons which receive sensory fibers from neurons in the spinal (dorsal root) ganglia. Near the middle of the cord the gray matter surrounds a small **central canal**, which develops from the lumen of the neural tube, is continuous with the ventricles of the brain, is lined by ependymal cells, and contains CSF.

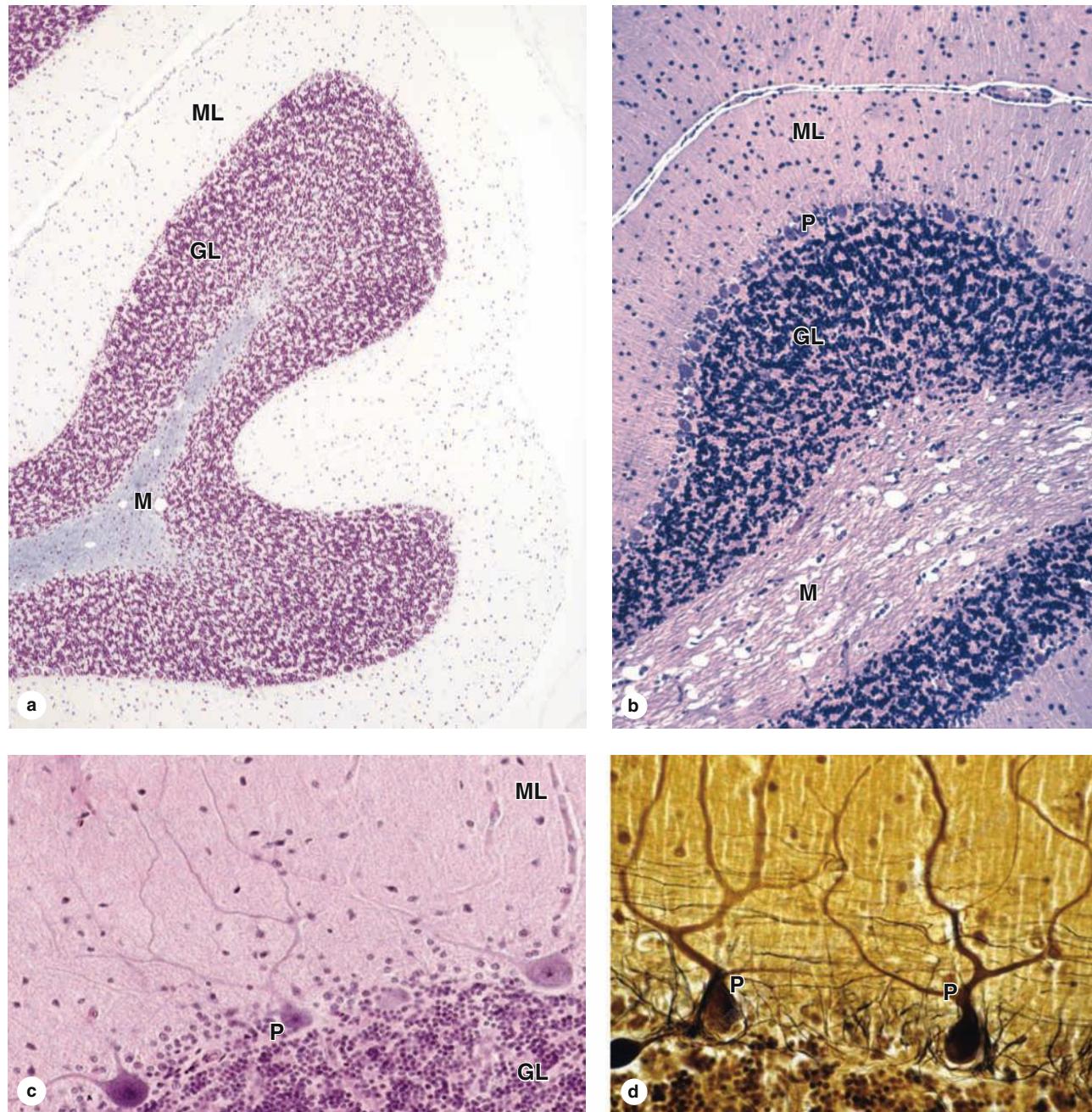
FIGURE 9–14 White versus gray matter.

A cross section of H&E-stained spinal cord shows the transition between white matter (left region) and gray matter (right). The gray matter has many glial cells (G), neuronal cell bodies (N), and neuropil; white matter also contains glia (G) but consists mainly of axons (A) whose myelin sheaths were lost during preparation, leaving the round empty spaces shown. Each such space surrounds a dark-stained spot that is a small section of the axon. (X400)

FIGURE 9–15 Cerebral cortex.

(a) Important neurons of the cerebrum are the pyramidal neurons (P), which are arranged vertically and interspersed with numerous smaller glial cells, mostly astrocytes, in the eosinophilic neuropil. (X200; H&E)

(b) From the apical ends of pyramidal neurons (P), long dendrites extend in the direction of the cortical surface, which can be best seen in thick silver-stained sections in which only a few other protoplasmic astrocytes (A) cells are seen. (X200; Silver)

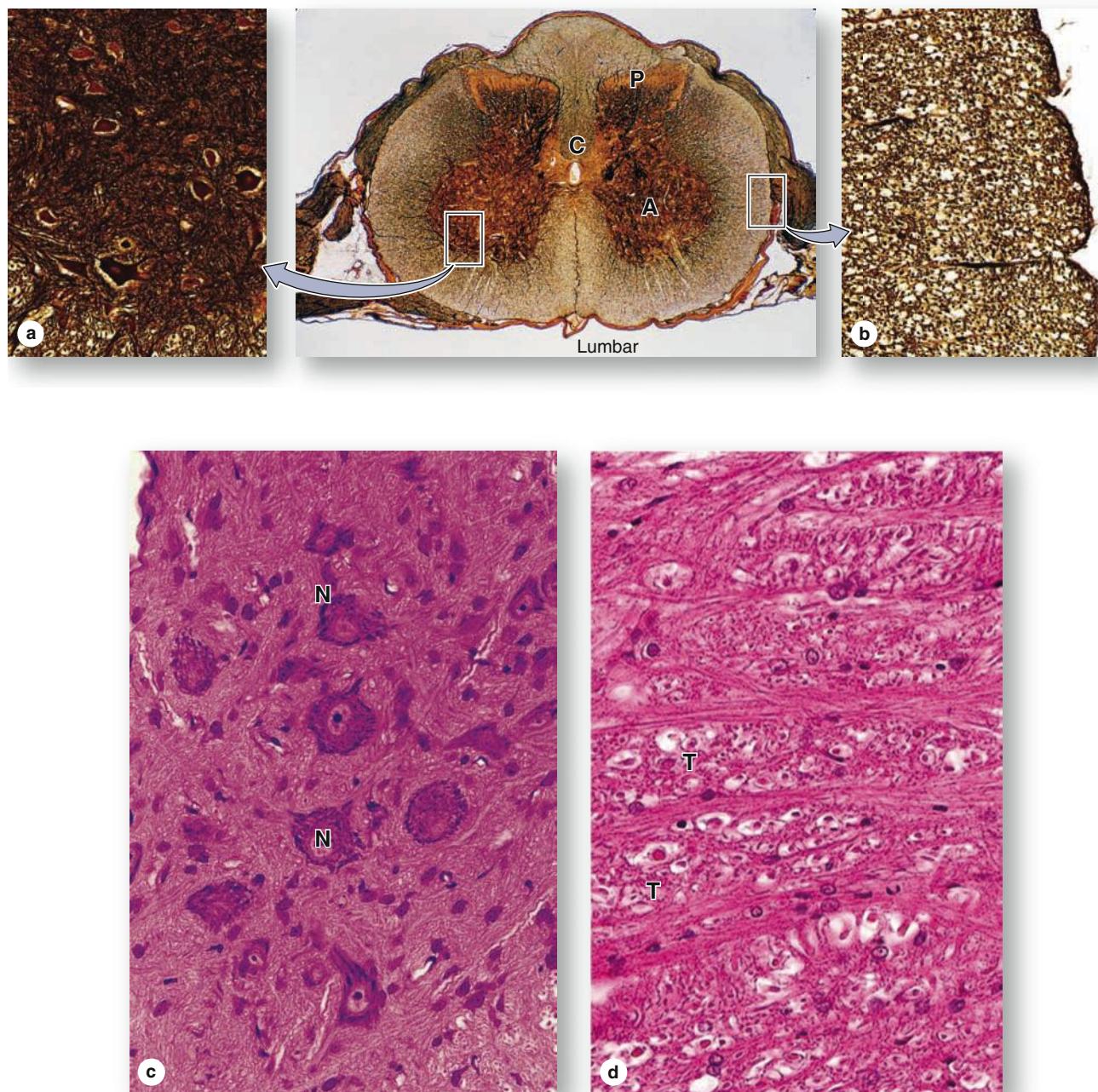
FIGURE 9–16 Cerebellum.

(a) The cerebellar cortex is convoluted with many distinctive small folds, each supported at its center by tracts of white matter in the cerebellar medulla (**M**). Each fold has distinct molecular layers (**ML**) and granular layers (**GL**). (X6; Cresyl violet)

(b) Higher magnification shows that the granular layer (**GL**) immediately surrounding the medulla (**M**) is densely packed with several different types of very small rounded neuronal cell bodies. The outer molecular layer (**ML**) consists of neuropil with fewer, much more scattered small neurons. At the interface of these two regions a layer of large Purkinje neuron (**P**) perikarya can be seen. (X20; H&E)

(c) A single intervening layer contains the very large cell bodies of unique Purkinje neurons (**P**), whose axons pass through the granular layer (**GL**) to join tracts in the medulla and whose multiple branching dendrites ramify throughout the molecular layer (**ML**). Dendrites are not seen well with H&E staining. (X40; H&E)

(d) With appropriate silver staining dendrites from each large Purkinje cell (**P**) are shown to have hundreds of small branches, each covered with hundreds of dendritic spines. Axons from the small neurons of the granular layer are unmyelinated and run together into the molecular layer where they form synapses with the dendritic spines of Purkinje cells. (X40; Silver)

FIGURE 9–17 Spinal cord.

The spinal cord varies slightly in diameter along its length but in cross section always shows bilateral symmetry around the small, CSF-filled central canal (**C**). Unlike the cerebrum and cerebellum, in the spinal cord the gray matter is internal, forming a roughly H-shaped structure that consists of two posterior (**P**) horns (sensory) and two anterior (**A**) (motor) horns, all joined by the gray commissure around the central canal.

(a) The gray matter contains abundant astrocytes and large neuronal cell bodies, especially those of motor neurons in the ventral horns.

(b) The white matter surrounds the gray matter and contains primarily oligodendrocytes and tracts of myelinated axons

running along the length of the cord. (Center X5, a, b X100; All silver-stained)

(c) With H&E staining the large motor neurons (**N**) of the ventral horns show large nuclei, prominent nucleoli, and cytoplasm rich in Nissl substance, all of which indicate extensive protein synthesis to maintain the axons of these cells that extend great distances.

(d) In the white commissure ventral to the central canal, tracts (**T**) run lengthwise along the cord, seen here in cross section with empty myelin sheaths surrounding axons, as well as small tracts running from one side of the cord to the other. (Both X200; H&E)

Meninges

The skull and the vertebral column protect the CNS, but between the bone and nervous tissue are membranes of connective tissue called the **meninges**. Three meningeal layers are distinguished: the dura, arachnoid, and pia maters (Figures 9–18 and 9–19).

Dura Mater

The thick external **dura mater** (L. *dura mater*, tough mother) consists of dense irregular connective tissue organized as an outer periosteal layer continuous with the periosteum of the skull, and an inner meningeal layer. These two layers are usually fused, but along the superior sagittal surface and other specific areas around the brain they separate to form the blood-filled **dural venous sinuses** (Figure 9–19). Around the spinal cord the dura mater is separated from the periosteum of the vertebrae by the **epidural space**, which contains a plexus of thin-walled veins and loose connective tissue (Figure 9–18). The dura mater may be separated from the arachnoid by formation of a thin subdural space.

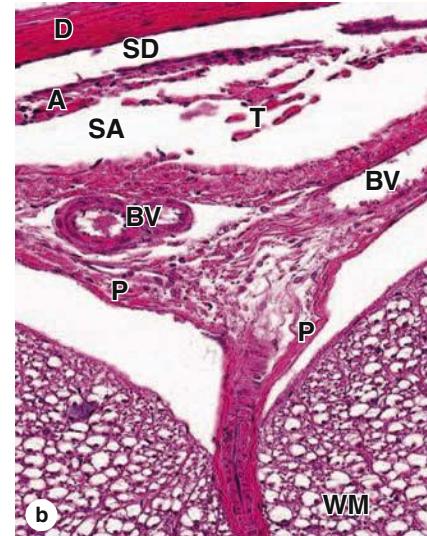
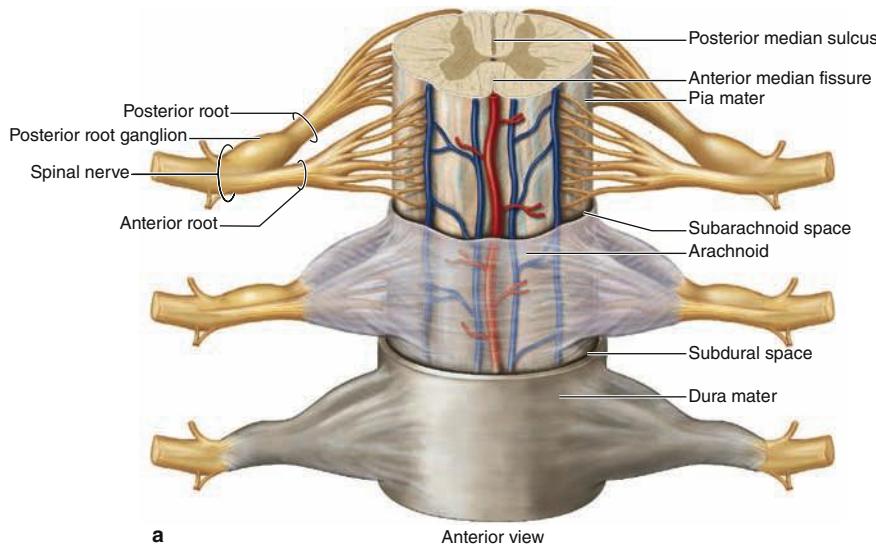
Arachnoid

The **arachnoid** (Gr. *arachnoeides*, spider web-like) has two components: (1) a sheet of connective tissue in contact with the dura mater and (2) a system of loosely arranged trabeculae composed of collagen and fibroblasts, continuous with the underlying pia mater layer. Surrounding these trabeculae is a large, sponge-like cavity, the **subarachnoid space**, filled with CSF. This fluid-filled space helps cushion and protect the CNS from minor trauma. The subarachnoid space communicates with the ventricles of the brain where the CSF is produced.

The connective tissue of the arachnoid is said to be avascular because it lacks nutritive capillaries, but larger blood vessels run through it (Figures 9–18 and 9–19). Because the arachnoid has fewer trabeculae in the spinal cord, it can be more clearly distinguished from the pia mater in that area. The arachnoid and the pia mater are intimately associated and are often considered a single membrane called the pia-arachnoid.

In some areas, the arachnoid penetrates the dura mater and protrudes into blood-filled dural venous sinuses located there (Figure 9–19). These CSF-filled protrusions, which are

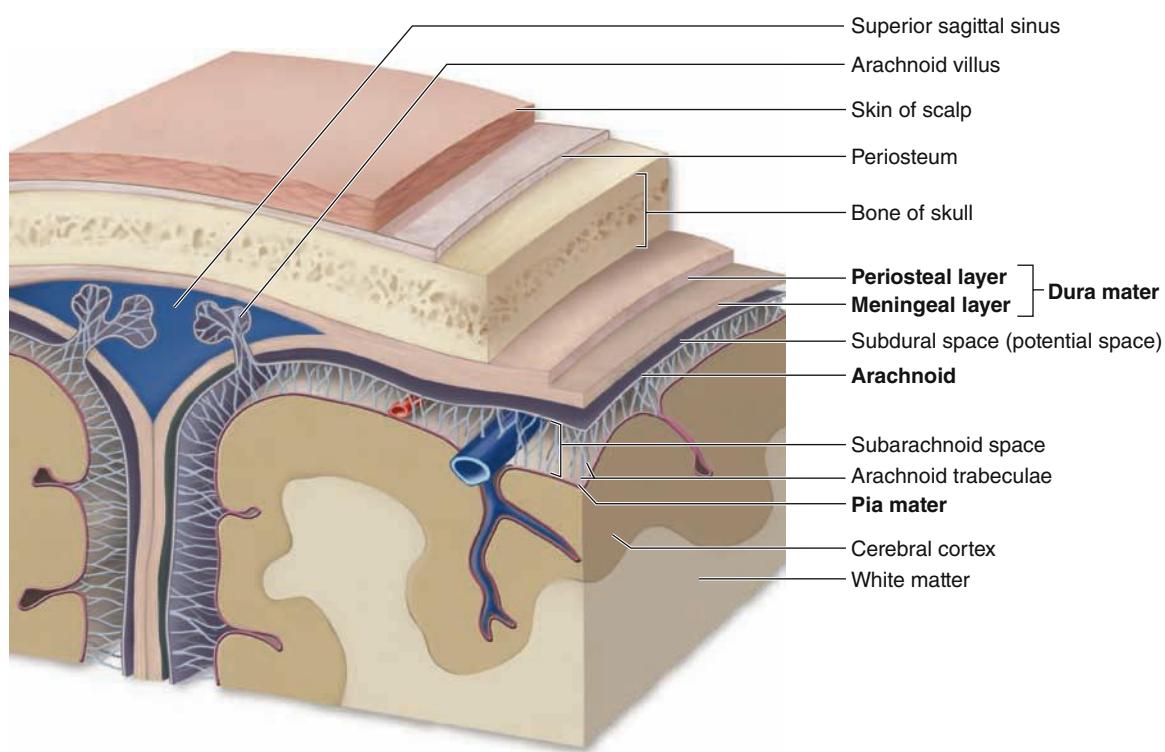
FIGURE 9–18 Spinal cord and meninges.



(a) A diagram of the spinal cord indicates the relationship of the three meningeal layers of connective tissue: the innermost **pia mater**, the **arachnoid**, and the **dura mater**. Also depicted are the blood vessels coursing through the subarachnoid space and the nerve rootlets that fuse to form the posterior and anterior roots of the spinal nerves. The posterior root ganglia contain the cell bodies of sensory nerve fibers and are located in intervertebral foramina.

(b) Section of an area near the anterior median fissure showing the tough dura mater (**D**). Surrounding the dura, the epidural space (not shown) contains cushioning adipose tissue and vascular plexuses. The subdural space (**SD**) is an artifact created by separation of the dura from underlying tissue. The middle meningeal layer

is the thicker weblike arachnoid mater (**A**) containing the large subarachnoid space (**SA**) and connective tissue trabeculae (**T**). The subarachnoid space is filled with CSF and the arachnoid acts as a shock-absorbing pad between the CNS and bone. Fairly large blood vessels (**BV**) course through the arachnoid. The innermost pia mater (**P**) is thin and is not clearly separate from the arachnoid; together, they are sometimes referred to as the pia-arachnoid or the leptomeninges. The space between the pia and the white matter (**WM**) of the spinal cord here is an artifact created during dissection; normally the pia is very closely applied to a layer of astrocytic processes at the surface of the CNS tissue. (X100; H&E)

FIGURE 9–19 Meninges around the brain.

The **dura, arachnoid, and pia maters** also surround the brain and as shown here the relationships among the cranial meninges are similar to those of the spinal cord. The diagram includes **arachnoid villi**, which are outpocketings of arachnoid away from the brain, which penetrate the dura mater and enter blood-filled **venous sinuses** located within that layer. The arachnoid villi function in

releasing excess CSF into the blood. Blood vessels from the arachnoid branch into smaller arteries and veins that enter brain tissue carrying oxygen and nutrients. These small vessels are initially covered with pia mater, but as capillaries they are covered only by the perivascular feet of astrocytes.

covered by the vascular endothelial cells lining the sinuses, are called **arachnoid villi** and function as sites for absorption of CSF into the blood of the venous sinuses.

Pia Mater

The innermost **pia mater** (L. *pia mater*, tender mother) consists of flattened, mesenchymally derived cells closely applied to the entire surface of the CNS tissue. The pia does not directly contact nerve cells or fibers, being separated from the neural elements by the very thin superficial layer of astrocytic processes (the glial limiting membrane, or *glia limitans*), which adheres firmly to the pia mater. Together, the pia mater and the layer of astrocytic end feet form a physical barrier separating CNS tissue from CSF in the subarachnoid space (Figure 9–19).

Blood vessels penetrate CNS tissue through long **perivascular spaces** covered by pia mater, although the pia disappears when the blood vessels branch to form the small capillaries. However, these capillaries remain completely covered by the perivascular layer of astrocytic processes (Figures 9–9a and 9–10c).

Blood-Brain Barrier

The **blood-brain barrier** (BBB) is a functional barrier that allows much tighter control than that in most tissues over the passage of substances moving from blood into the CNS tissue. The main structural component of the BBB is the **capillary endothelium**, in which the cells are tightly sealed together with well-developed occluding junctions, with little or no transcytosis activity, and surrounded by the basement membrane. The **limiting layer of perivascular astrocytic feet** that envelopes the basement membrane of capillaries in most CNS regions (Figure 9–10c) contributes to the BBB and further regulates passage of molecules and ions from blood to brain.

The BBB protects neurons and glia from bacterial toxins, infectious agents, and other exogenous substances, and helps maintain the stable composition and constant balance of ions in the interstitial fluid required for normal neuronal function. The BBB is not present in regions of the hypothalamus where plasma components are monitored, in the posterior pituitary which releases hormones, or in the choroid plexus where CSF is produced.

Choroid Plexus

The **choroid plexus** consists of highly vascular tissue, elaborately folded and projecting into the large ventricles of the brain (Figure 9–20a). It is found in the roofs of the third and fourth ventricles and in parts of the two lateral ventricular walls, all regions in which the ependymal lining directly contacts the pia mater.

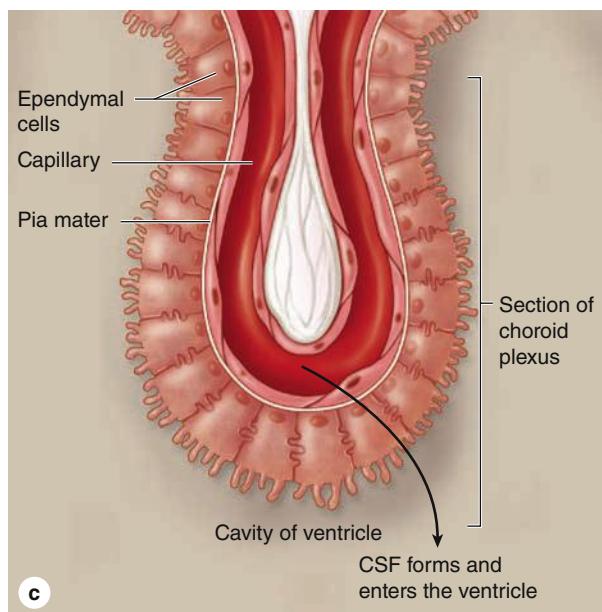
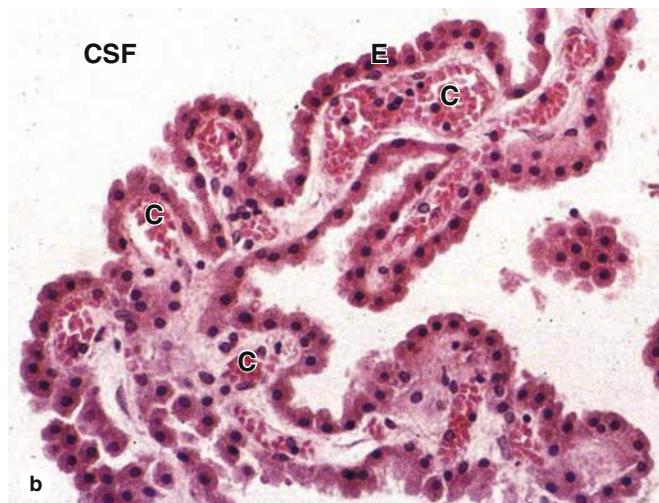
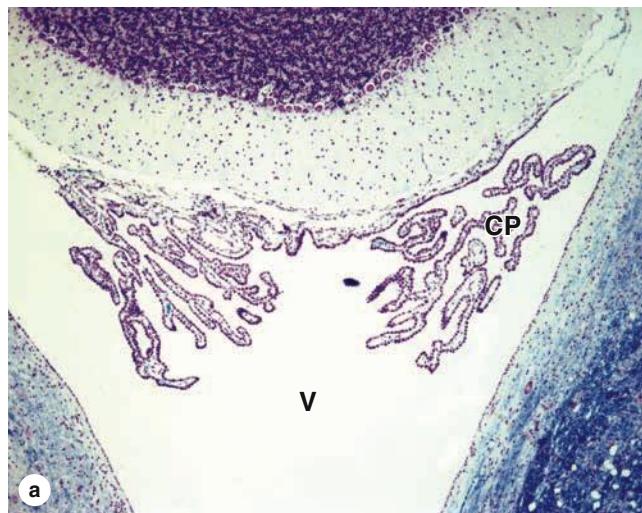
Each villus of the choroid plexus contains a thin layer of well-vascularized pia mater covered by cuboidal ependymal cells (Figure 9–20b). The function of the choroid plexus is to remove water from blood and release it as the **CSF**. CSF is clear, contains Na^+ , K^+ , and Cl^- ions but very little protein, and its only cells are normally very sparse lymphocytes. It is produced continuously and it completely fills the ventricles, the central canal of the spinal

cord, the subarachnoid and perivascular spaces. It provides the ions required for CNS neuronal activity and in the arachnoid serves to help absorb mechanical shocks. Arachnoid villi (Figure 9–19) provide the main pathway for absorption of CSF back into the venous circulation. There are very few lymphatic vessels in CNS tissue.

» MEDICAL APPLICATION

A decrease in the absorption of CSF or a blockage of outflow from the ventricles during fetal or postnatal development results in the condition known as **hydrocephalus** (Gr. *hydro*, water + *kephale*, head), which promotes a progressive enlargement of the head followed by mental impairment.

FIGURE 9–20 Choroid plexus.



The choroid plexus consists of ependyma and vascularized pia mater and projects many thin folds from certain walls of the ventricles.

(a) Section of the bilateral choroid plexus (**CP**) projecting into the fourth ventricle (**V**) near the cerebellum. (X12; Kluver-Barrera stain)

(b) At higher magnification each fold of choroid plexus is seen to be well-vascularized with large capillaries (**C**) and covered by a continuous layer of cuboidal ependymal cells (**E**). (X150)

(c) The choroid plexus is specialized for transport of water and ions across the capillary endothelium and ependymal layer and the elaboration of these as CSF.

► PERIPHERAL NERVOUS SYSTEM

The main components of the peripheral nervous system (PNS) are the **nerves**, **ganglia**, and **nerve endings**. Nerves are bundles of nerve fibers (axons) surrounded by Schwann cells and layers of connective tissue.

Nerve Fibers

Nerve fibers are analogous to tracts in the CNS, containing axons enclosed within sheaths of glial cells specialized to facilitate axonal function. In peripheral nerve fibers, axons are sheathed by **Schwann cells**, or neurolemmocytes (Figure 9–9b). The sheath may or may not form myelin around the axons, depending on their diameter.

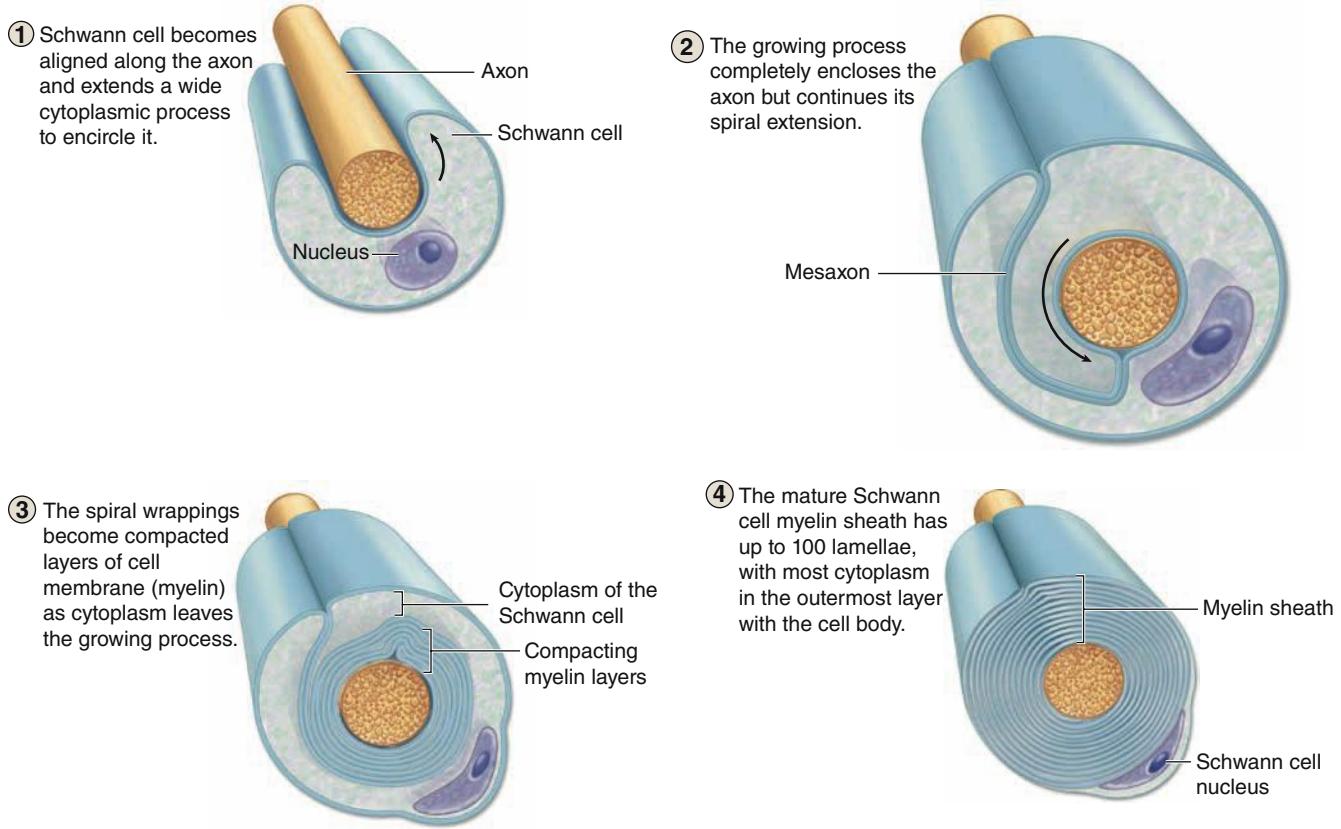
Myelinated Fibers

As axons of large diameter grow in the PNS, they are engulfed along their length by a series of differentiating neurolemmocytes and become **myelinated nerve fibers**. The plasma membrane of each covering Schwann cell fuses with itself at

an area termed the mesaxon and a wide, flattened process of the cell continues to extend itself, moving circumferentially around the axon many times (Figure 9–21). The multiple layers of Schwann cell membrane unite as a thick **myelin sheath**. Composed mainly of lipid bilayers and membrane proteins, myelin is a large lipoprotein complex that, like cell membranes, is partly removed by standard histologic procedures (Figures 9–14 and 9–17d). Unlike oligodendrocytes of the CNS, a Schwann cell forms myelin around only a portion of one axon.

With high-magnification TEM, the myelin sheath appears as a thick electron-dense axonal covering in which the concentric membrane layers may be visible (Figure 9–22). The prominent electron-dense layers visible ultrastructurally in the sheath, the **major dense lines**, represent the fused, protein-rich cytoplasmic surfaces of the Schwann cell membrane. Along the myelin sheath, these surfaces periodically separate slightly to allow transient movement of cytoplasm for membrane maintenance; at these **myelin clefts** (or Schmidt-Lanterman clefts) the major dense lines temporarily disappear (Figure 9–23).

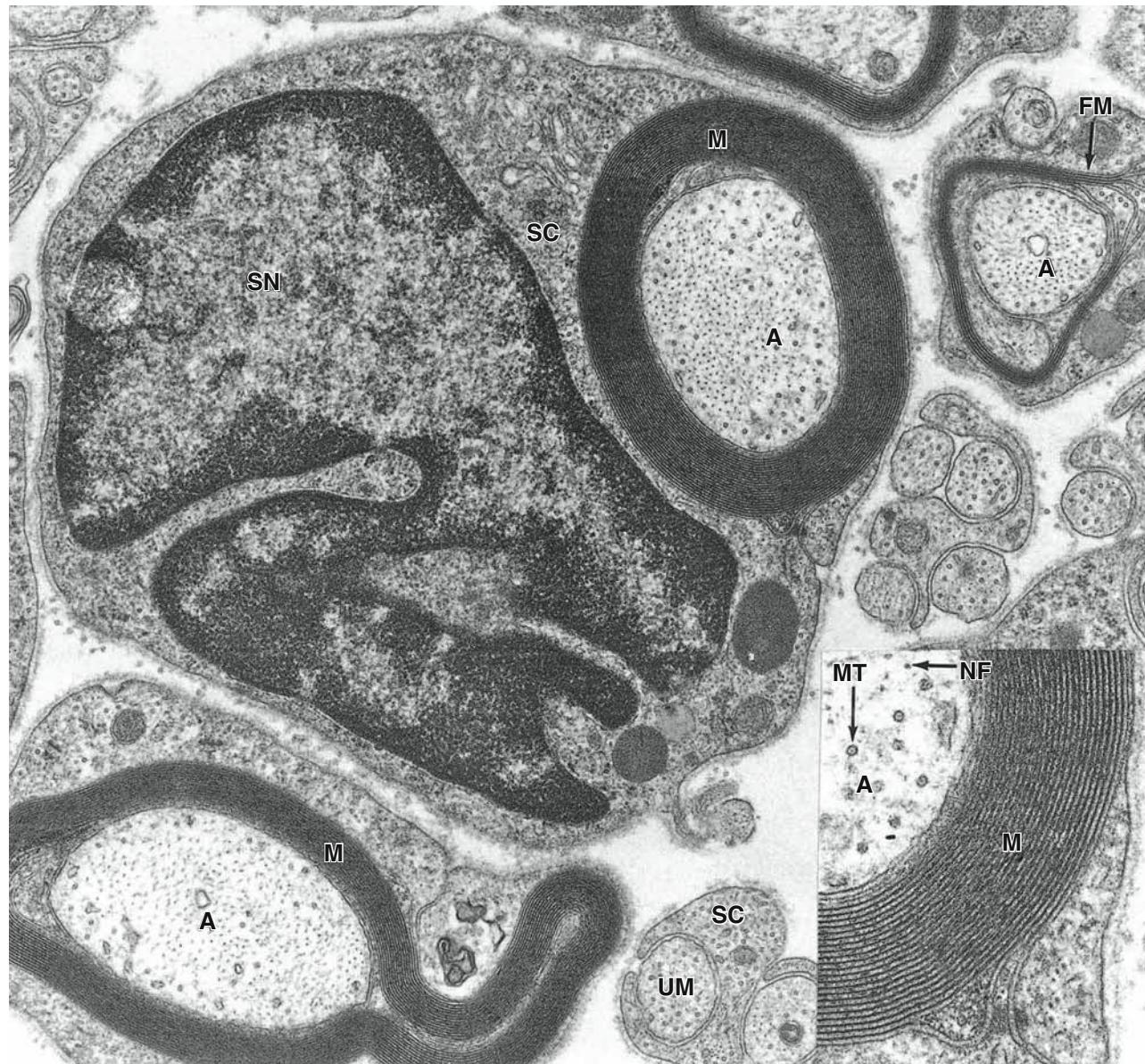
FIGURE 9–21 Myelination of large-diameter PNS axons.



A Schwann cell (neurolemmocyte) engulfs one portion along the length of a large-diameter axon. The Schwann cell membrane fuses around the axon and one thin extension of the Schwann cell elongates greatly and wraps itself repeatedly around the axon to form multiple, compacted layers. The Schwann cell membrane

wrappings constitute the myelin sheath, with the Schwann cell body always on its outer surface. The myelin layers are very rich in lipid, and provide insulation and facilitate formation of action potentials along the axolemma.

FIGURE 9–22 Ultrastructure of myelinated and unmyelinated fibers.



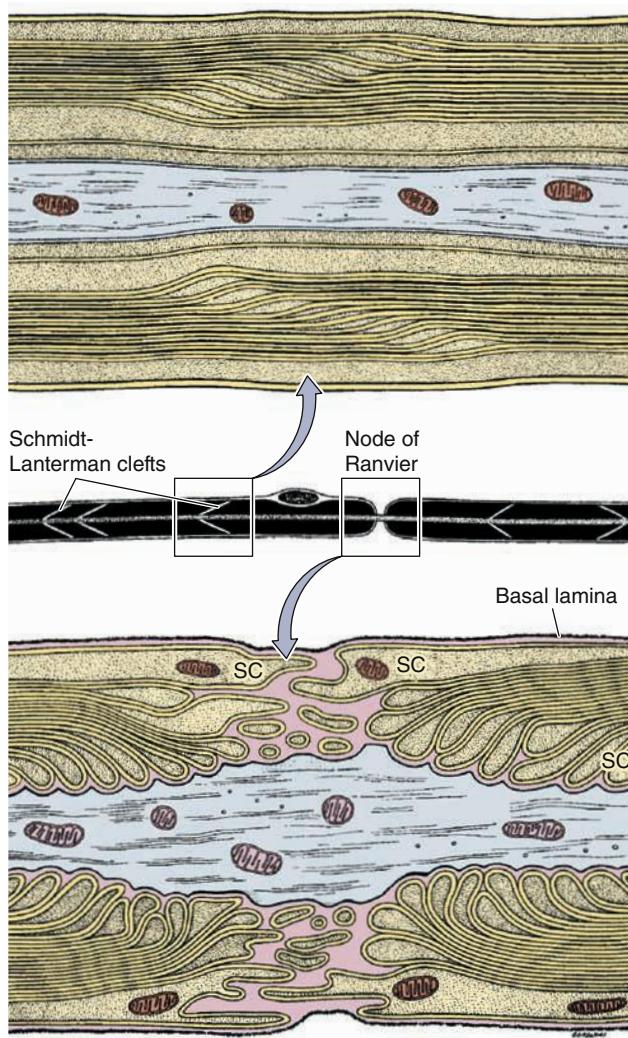
Cross section of PNS fibers in the TEM reveals differences between myelinated and unmyelinated axons. Large axons (**A**) are wrapped in a thick myelin sheath (**M**) of multiple layers of Schwann cell membrane.

The inset shows a portion of myelin at higher magnification in which the major dense lines of individual membrane layers can be distinguished, as well as the neurofilaments (**NF**) and microtubules (**MT**) in the axoplasm (**A**). At the center of the photo is a Schwann cell showing its active nucleus (**SN**) and Golgi-rich cytoplasm (**SC**). At the right is an axon around which myelin is still forming (**FM**).

Unmyelinated axons (**UM**) are much smaller in diameter, and many such fibers may be engulfed by a single Schwann cell (**SC**). The glial cell does not form myelin wrappings around such small axons but simply encloses them. Whether it forms myelin or not, each Schwann cell is surrounded, as shown, by an external lamina containing type IV collagen and laminin like the basal laminae of epithelial cells. (X28,000, inset X70,000)

(Used with permission from Dr Mary Bartlett Bunge, The Miami Project to Cure Paralysis, University of Miami Miller School of Medicine, Miami, FL.)

FIGURE 9–23 Myelin maintenance and nodes of Ranvier.



The middle diagram shows schematically a myelinated peripheral nerve fiber as seen under the light microscope. The axon is enveloped by the myelin sheath, which, in addition to membrane, contains some Schwann cell cytoplasm in spaces called **Schmidt-Lanterman or myelin clefts** between the major dense lines of membranes.

The upper diagram shows one set of such clefts ultrastructurally. The clefts contain Schwann cell cytoplasm that was not displaced to the cell body during myelin formation. This cytoplasm moves slowly along the myelin sheath, opening temporary spaces (the clefts) that allow renewal of some membrane components as needed for maintenance of the sheath.

The lower diagram depicts the ultrastructure of a single node of Ranvier or nodal gap. Interdigitating processes extending from the outer layers of the Schwann cells (SC) partly cover and contact the axolemma at the nodal gap. This contact acts as a partial barrier to the movement of materials in and out of the periaxonal space between the axolemma and the Schwann sheath. The basal or external lamina around Schwann cells is continuous over the nodal gap. The axolemma at nodal gaps has abundant voltage-gated Na^+ channels important for impulse conductance in these axons.

Faintly seen ultrastructurally in the light staining layers are the intraperiod lines that represent the apposed outer bilayers of the Schwann cell membrane.

Membranes of Schwann cells have a higher proportion of lipids than do other cell membranes, and the myelin sheath serves to insulate axons and maintain a constant ionic micro-environment most suitable for action potentials. Between adjacent Schwann cells on an axon the myelin sheath shows small **nodes of Ranvier** (or **nodal gaps**, Figures 9–9b, 9–23 and 9–24), where the axon is only partially covered by interdigitating Schwann cell processes. At these nodes the axolemma is exposed to ions in the interstitial fluid and has a much higher concentration of voltage-gated Na^+ channels, which renew the action potential and produce **saltatory conduction** (L. *saltare*, to jump) of nerve impulses, their rapid movement from node to node. The length of axon ensheathed by one Schwann cell, the **internodal segment**, varies directly with axonal diameter and ranges from 300 to 1500 μm .

Unmyelinated Fibers

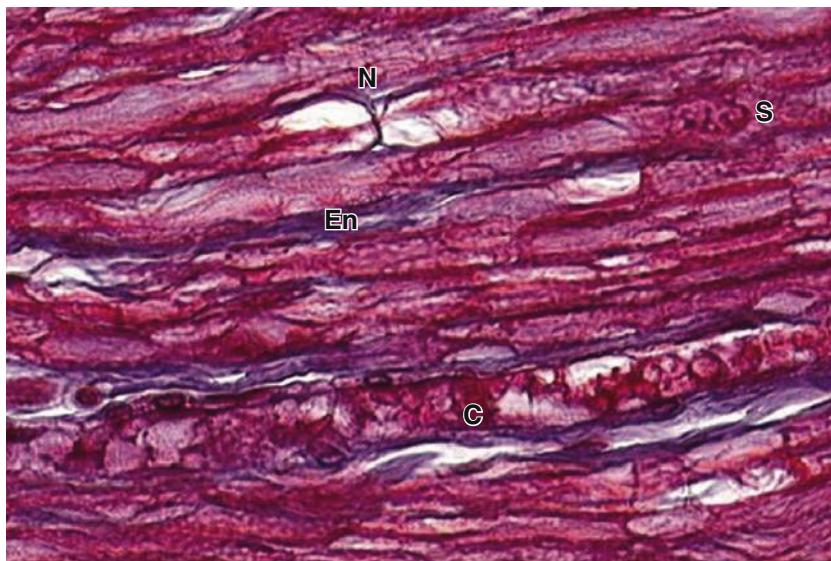
Unlike the CNS where many short axons are not myelinated at all but run free among the other neuronal and glial processes, the smallest-diameter axons of peripheral nerves are still enveloped within simple folds of Schwann cells (Figure 9–25). In these **unmyelinated fibers** the glial cell does not form the multiple wrapping of a myelin sheath (Figure 9–21). In unmyelinated fibers, each Schwann cell can enclose portions of many axons with small diameters. Without the thick myelin sheath, nodes of Ranvier are not seen along unmyelinated nerve fibers. Moreover, these small-diameter axons have evenly distributed voltage-gated ion channels; their impulse conduction is not saltatory and is much slower than that of myelinated axons.

Nerve Organization

In the PNS nerve fibers are grouped into bundles to form **nerves**. Except for very thin nerves containing only unmyelinated fibers, nerves have a whitish, glistening appearance because of their myelin and collagen content.

Axons and Schwann cells are enclosed within layers of connective tissue (Figures 9–24, 9–26, and 9–27). Immediately around the external lamina of the Schwann cells is a thin layer called the **endoneurium**, consisting of reticular fibers, scattered fibroblasts, and capillaries. Groups of axons with Schwann cells and endoneurium are bundled together as **fascicles** by a sleeve of **perineurium**, containing flat fibrocytes with their edges sealed together by tight junctions. From two to six layers of these unique connective tissue cells regulate diffusion into the fascicle and make up the **blood-nerve barrier** that helps maintain the fibers' microenvironment. Externally, peripheral nerves have a dense, irregular fibrous coat called the **epineurium**, which extends deeply to fill the space between fascicles.

Very small nerves consist of one fascicle (Figure 9–28). Small nerves can be found in sections of many organs and often show a winding disposition in connective tissue.

FIGURE 9–24 Node of Ranvier and endoneurium.

A longitudinally oriented nerve shows one node of Ranvier (N) with the axon visible. Collagen of the sparse endoneurium (En), blue in this trichrome stain, surrounds the Schwann cells and a capillary (C). At least one Schwann cell nucleus (S) is also clearly seen. (X400; Mallory trichome)

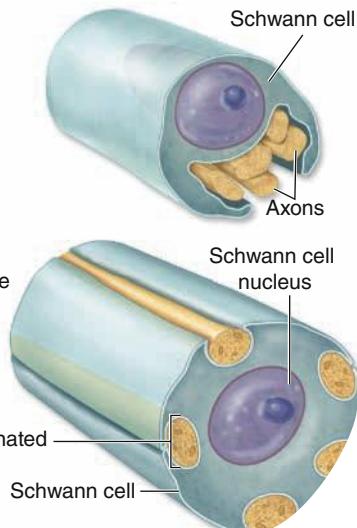
Peripheral nerves establish communication between centers in the CNS and the sense organs and effectors (muscles, glands, etc). They generally contain both afferent and efferent fibers. **Afferent** fibers carry information from internal body regions and the environment to the CNS. **Efferent**

fibers carry impulses from the CNS to effector organs commanded by these centers. Nerves possessing only sensory fibers are called **sensory nerves**; those composed only of fibers carrying impulses to the effectors are called **motor nerves**. Most nerves have both sensory and motor fibers and are called **mixed nerves**, usually also with both myelinated and unmyelinated axons.

FIGURE 9–25 Unmyelinated nerves.

Unmyelinated axons

- ① Schwann cell starts to envelop multiple axons.
- ② The unmyelinated axons are enveloped by the Schwann cell, but there are *no* myelin sheath wraps around each axon.



During development, portions of several small-diameter axons are engulfed by one Schwann cell. Subsequently the axons are separated and each typically becomes enclosed within its own fold of Schwann cell surface. No myelin is formed by wrapping. Small-diameter axons utilize action potentials whose formation and maintenance do not depend on the insulation provided by the myelin sheath required by large-diameter axons.

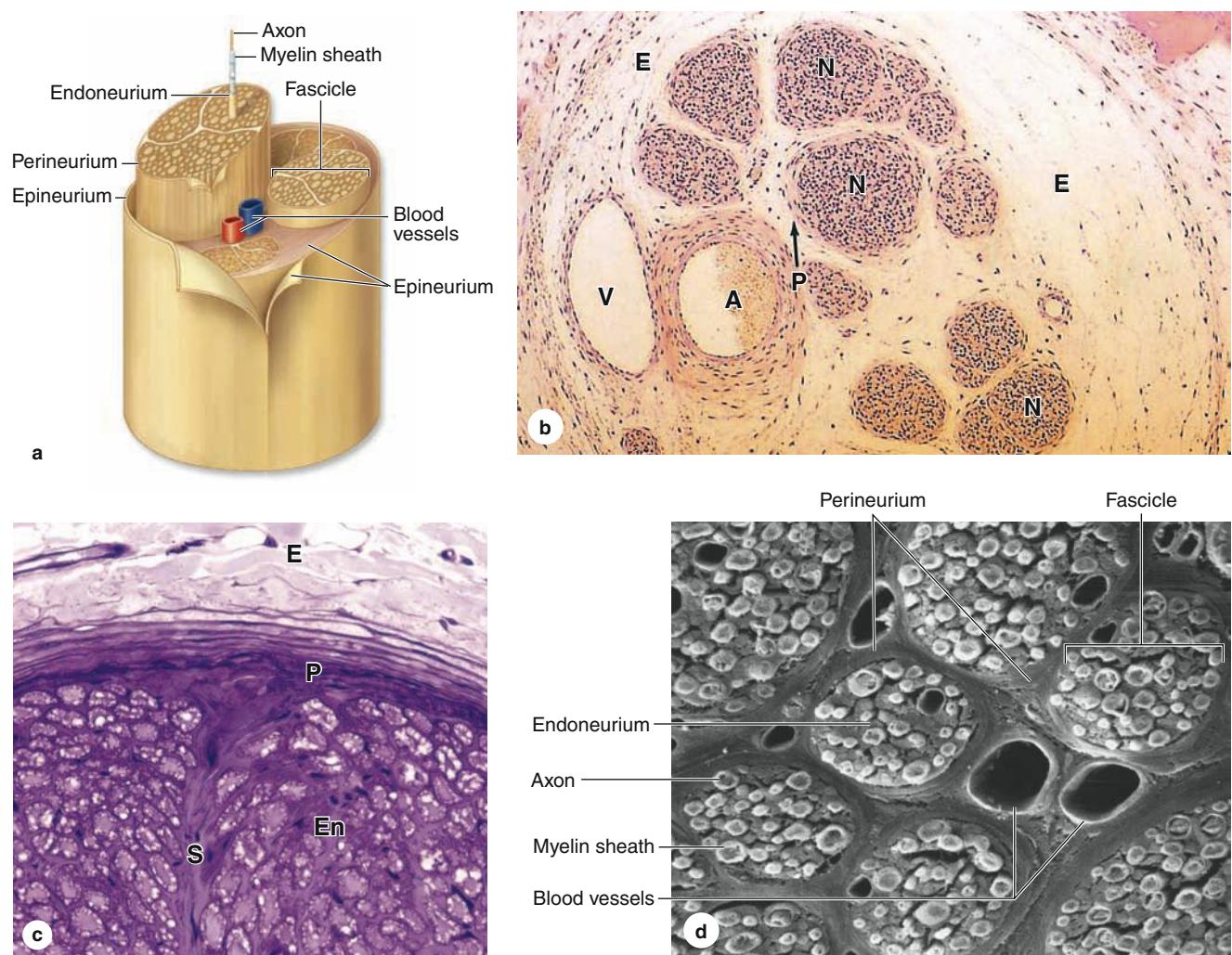
Ganglia

Ganglia are typically ovoid structures containing neuronal cell bodies and their surrounding glial satellite cells supported by delicate connective tissue and surrounded by a denser capsule. Because they serve as relay stations to transmit nerve impulses, at least one nerve enters and another exits from each ganglion. The direction of the nerve impulse determines whether the ganglion will be a **sensory** or an **autonomic** ganglion.

Sensory Ganglia

Sensory ganglia receive afferent impulses that go to the CNS. Sensory ganglia are associated with both cranial nerves (cranial ganglia) and the dorsal roots of the spinal nerves (spinal ganglia). The large neuronal cell bodies of ganglia (Figure 9–29) are associated with thin, sheet-like extensions of small glial **satellite cells** (Figures 9–9b and 9–13). Sensory ganglia are supported by a distinct connective tissue capsule and an internal framework continuous with the connective tissue layers of the nerves. The neurons of these ganglia are pseudounipolar and relay information from the ganglion's nerve endings to the gray matter of the spinal cord via synapses with local neurons.

FIGURE 9–26 Peripheral nerve connective tissue: Epi-, peri-, and endoneurium.



(a) The diagram shows the relationship among these three connective tissue layers in large peripheral nerves. The epineurium (**E**) consists of a dense superficial region and a looser deep region that contains the larger blood vessels.

(b) The micrograph shows a small vein (**V**) and artery (**A**) in the deep epineurium (**E**). Nerve fibers (**N**) are bundled in fascicles. Each fascicle is surrounded by the perineurium (**P**), consisting of a few layers of unusual squamous fibroblastic cells that are all joined at the peripheries by tight junctions. The resulting blood-nerve barrier helps regulate the microenvironment inside the fascicle. Axons and Schwann cells are in turn surrounded by a thin layer of endoneurium. (X140; H&E)

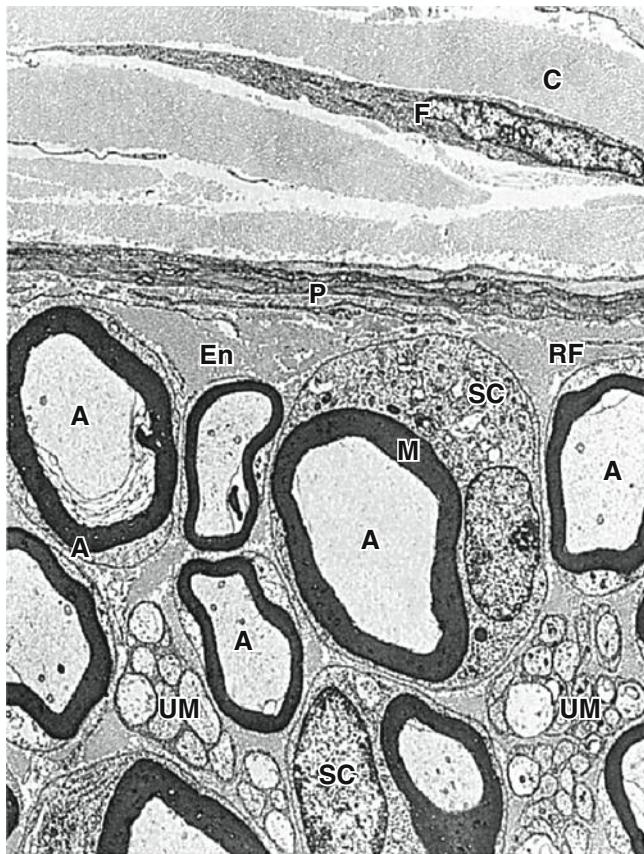
(c) As shown here and in the diagram, septa (**S**) of connective tissue often extend from the perineurium into larger fascicles. The endoneurium (**En**) and lamellar nature of the perineurium (**P**) are also shown at this magnification, along with some adjacent epineurium (**E**). (X200; PT)

(d) SEM of transverse sections of a large peripheral nerve showing several fascicles, each surrounded by perineurium and packed with endoneurium around the individual myelin sheaths. Each fascicle contains at least one capillary. Endothelial cells of these capillaries are tightly joined as part of the blood-nerve barrier and regulate the kinds of plasma substances released to the endoneurium. Larger blood vessels course through the deep epineurium that fills the space around the perineurium and fascicles. (X450)

Autonomic Ganglia

Autonomic (Gr. *autos*, self + *nomos*, law) nerves effect the activity of smooth muscle, the secretion of some glands, heart rate, and many other involuntary activities by which the body maintains a constant internal environment (**homeostasis**).

Autonomic ganglia are small bulbous dilations in autonomic nerves, usually with multipolar neurons. Some are located within certain organs, especially in the walls of the digestive tract, where they constitute the **intramural ganglia**. The capsules of these ganglia may be poorly defined among

FIGURE 9–27 Peripheral nerve ultrastructure.

This low-magnification TEM shows a fibroblast (F) surrounded by collagen (C) in the epineurium (E) and three layers of flattened cells in the perineurium (P) which form another part of the blood-nerve barrier. Inside the perineurium the endoneurium (En) is rich in reticulin fibers (RF) that surround all Schwann cells. Nuclei of two Schwann cells (SC) of myelinated axons (A) are visible as well as many unmyelinated axons (UM) within Schwann cells. (X1200)

the local connective tissue. A layer of satellite cells also envelops the neurons of autonomic ganglia (Figure 9–29), although these may also be inconspicuous in intramural ganglia.

Autonomic nerves use two-neuron circuits. The first neuron of the chain, with the **preganglionic fiber**, is located in the CNS. Its axon forms a synapse with **postganglionic fibers** of the second multipolar neuron in the chain located in a peripheral ganglion system. The chemical mediator present in the synaptic vesicles of all preganglionic axons is acetylcholine.

As indicated earlier autonomic nerves make up the **autonomic nervous system**. This has two parts: the **sympathetic** and the **parasympathetic divisions**. Neuronal cell bodies of preganglionic sympathetic nerves are located in the thoracic and lumbar segments of the spinal cord and those of the parasympathetic division are in the medulla and midbrain and in

the sacral portion of the spinal cord. Sympathetic second neurons are located in small ganglia along the vertebral column, while second neurons of the parasympathetic series are found in very small ganglia always located near or within the effector organs, for example in the walls of the stomach and intestines. Parasympathetic ganglia may lack distinct capsules altogether, perikarya and associated satellite cells simply forming a loosely organized plexus within the surrounding connective tissue.

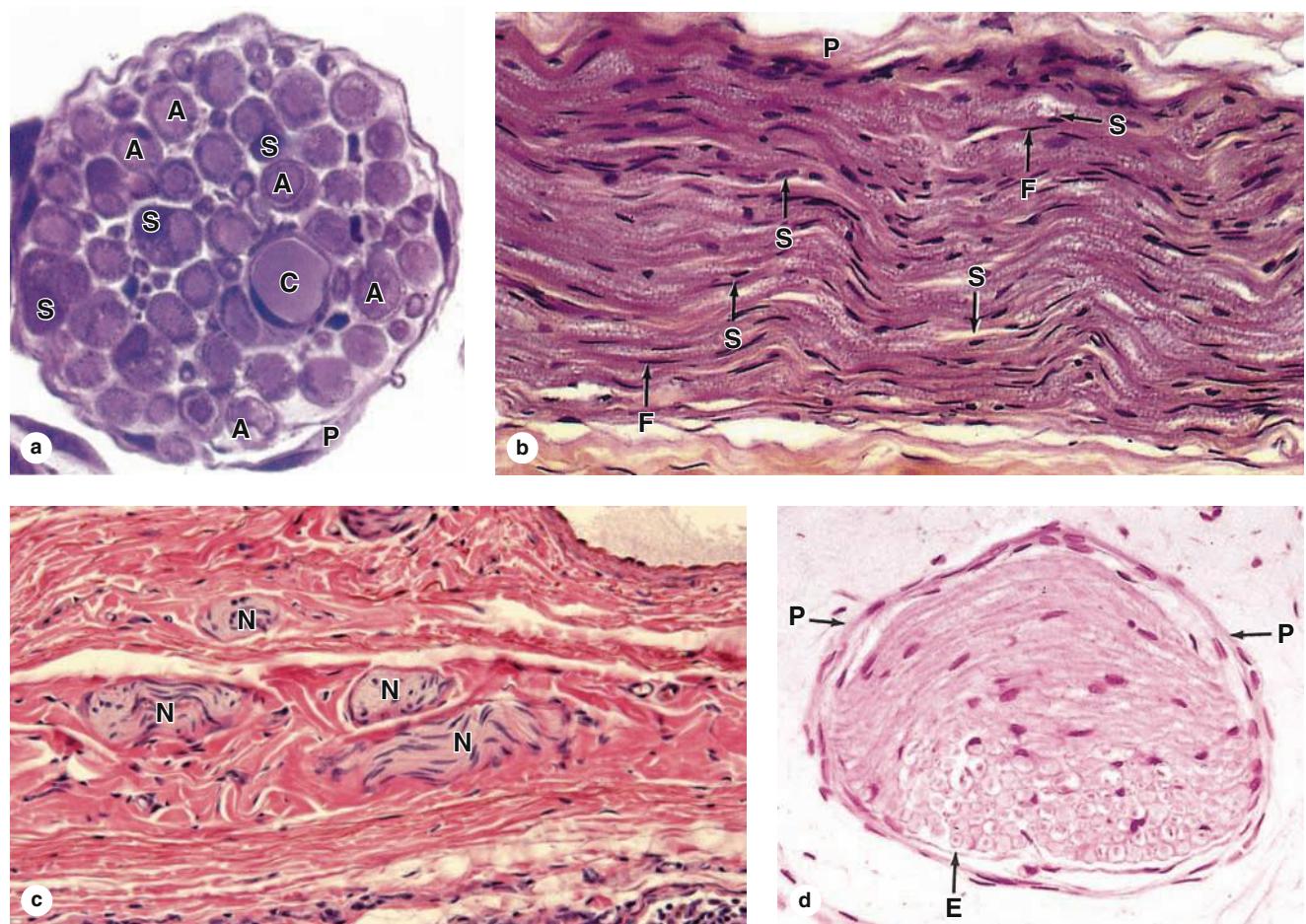
► NEURAL PLASTICITY & REGENERATION

Despite its general stability, the nervous system exhibits neuronal differentiation and formation of new synapses even in adults. Embryonic development of the nervous system produces an excess of differentiating neurons, and the cells that do not establish correct synapses with other neurons are eliminated by apoptosis. In adult mammals after an injury, the neuronal circuits may be reorganized by the growth of neuronal processes, forming new synapses to replace ones lost by injury. Thus, new communications are established with some degree of functional recovery. This **neural plasticity** and reformation of processes are controlled by several growth factors produced by both neurons and glial cells in a family of proteins called **neurotrophins**.

Neuronal stem cells are present in the adult CNS, located in part among the cells of the ependyma, which can supply new neurons, astrocytes, and oligodendrocytes. Fully differentiated, interconnected CNS neurons cannot temporarily disengage these connections and divide to replace cells lost by injury or disease; the potential of neural stem cells to allow tissue regeneration and functional recovery within the CNS components is a subject of intense investigation. Astrocytes do proliferate at injured sites and these growing cells can interfere with successful axonal regeneration in structures such as spinal cord tracts.

In the histologically much simpler peripheral nerves, injured axons have a much greater potential for regeneration and return of function. If the cell bodies are intact, damaged, or severed PNS axons can regenerate as shown in the sequence of diagrams in Figure 9–30. Distal portions of axons, isolated from their source of new proteins and organelles, degenerate; the surrounding Schwann cells dedifferentiate, shed the myelin sheaths, and proliferate within the surrounding layers of connective tissue. Cellular debris including shed myelin is removed by blood-derived macrophages, which also secrete neurotrophins to promote anabolic events of axon regeneration.

The onset of regeneration is signaled by changes in the perikaryon that characterize the process of **chromatolysis**: the cell body swells slightly, Nissl substance is initially diminished, and the nucleus migrates to a peripheral position within the perikaryon. The proximal segment of the axon close to the wound degenerates for a short distance, but begins to grow again distally as new Nissl substance appears and debris is removed. The new Schwann cells align to serve as guides for the regrowing axons and produce polypeptide factors that

FIGURE 9–28 Small nerves.

Small nerves can be seen in sections from most organs.

(a) In cross section an isolated, resin-embedded nerve is seen to have a thin perineurium (**P**), one capillary (**C**), and many large axons (**A**) associated with Schwann cells (**S**). A few nuclei of fibroblasts can be seen in the endoneurium between the myelinated fibers. (X400; PT)

(b) In longitudinal sections the flattened nuclei of endoneurial fibroblasts (**F**) and more oval nuclei of Schwann cells (**S**) can be distinguished. Nerve fibers are held rather loosely in the endoneurium and in low-magnification longitudinal section are seen to be wavy rather than straight. This indicates a slackness of fibers within the

nerve, which allows nerves to stretch slightly during body movements with no potentially damaging tension on the fibers. (X200; H&E)

(c) In sections of mesentery and other tissues, a highly wavy or tortuous disposition of a single small nerve (**N**) will be seen as multiple oblique or transverse pieces as the nerve enters and leaves the area in the section. (X200; H&E)

(d) Often, a section of small nerve will have some fibers cut transversely and others cut obliquely within the same fascicle, again suggesting the relatively unrestrained nature of the fibers within the endoneurium (**E**) and perineurium (**P**). (X300; H&E)

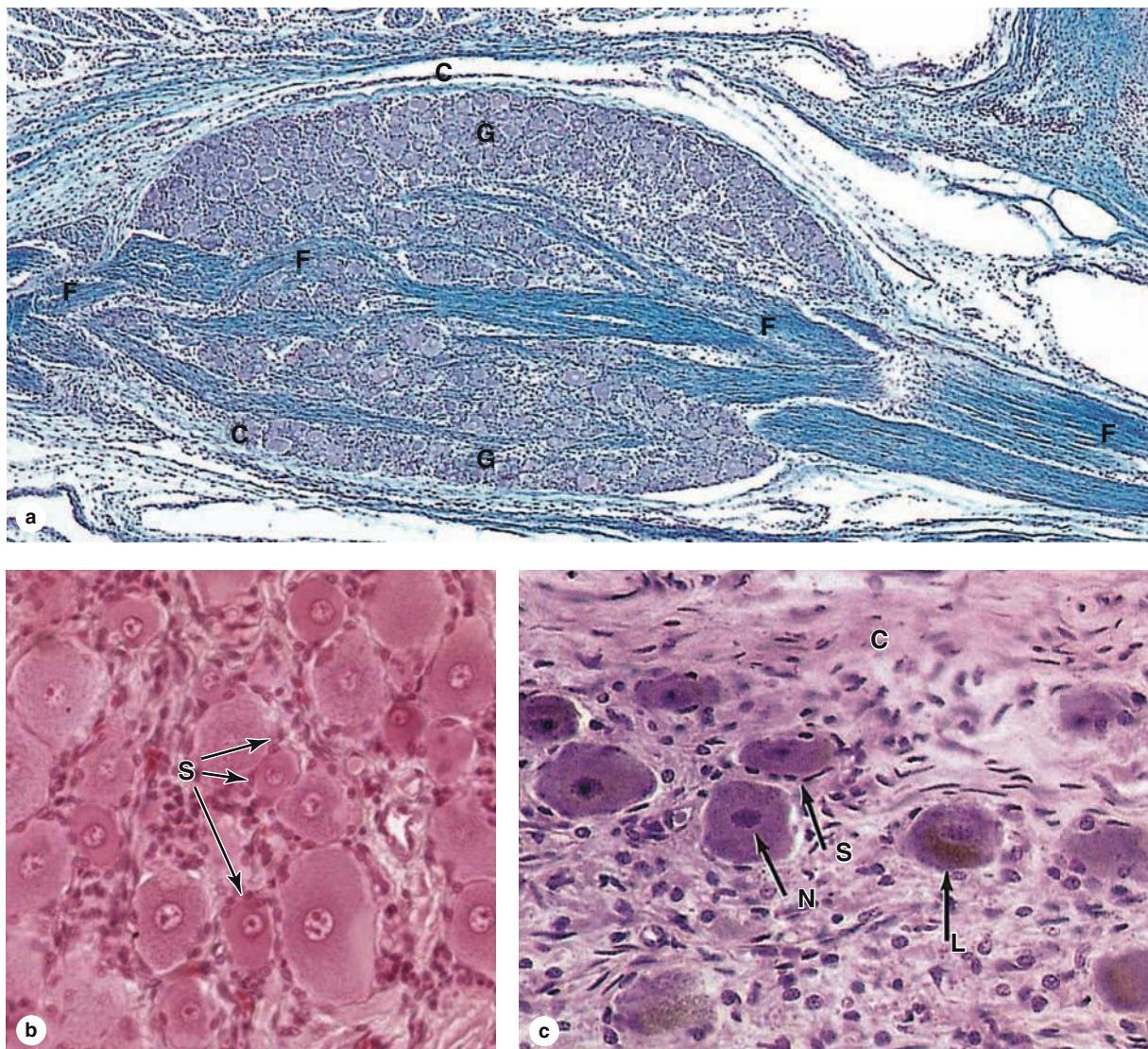
promote axonal outgrowth. Motor axons reestablish synaptic connections with muscles and function is restored.

» MEDICAL APPLICATION

Regeneration of peripheral nerves is functionally efficient only when the fibers and the columns of Schwann cells are directed properly. In a mixed nerve, if regenerating sensory

fibers grow into columns formerly occupied by motor fibers connected to motor end plates, the function of the muscle will not be reestablished. When there is an extensive gap between the distal and proximal segments of cut or injured peripheral nerves or when the distal segment disappears altogether (as in the case of amputation of a limb), the newly growing axons may form a swelling, or **neuroma**, that can be the source of spontaneous pain.

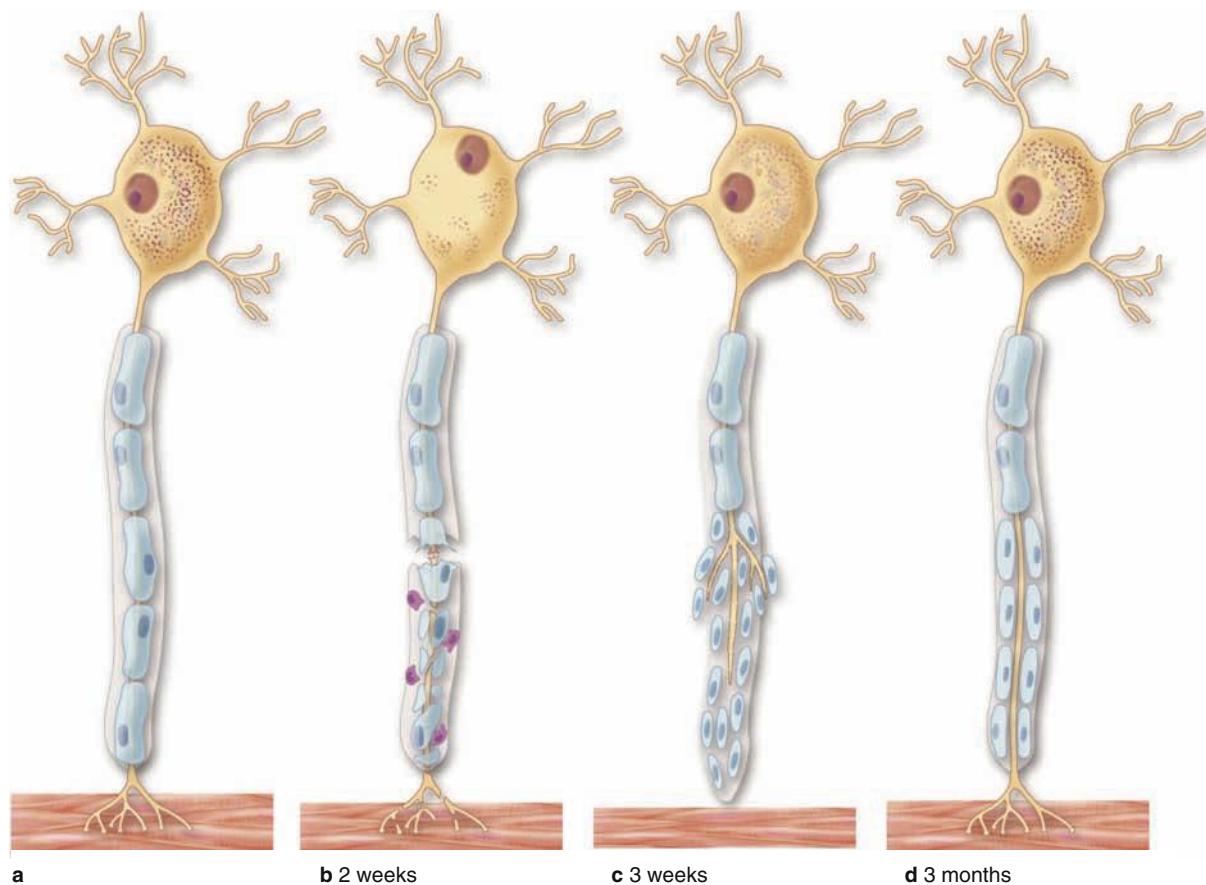
FIGURE 9-29 Ganglia.



(a) A sensory ganglion (**G**) has a distinct connective tissue capsule (**C**) and internal framework continuous with the epineurium and other components of peripheral nerves, except that no perineurium is present and that there is no blood-nerve barrier function. Fascicles of nerve fibers (**F**) enter and leave these ganglia. (X56; Kluver-Barrera stain)

(b) Higher magnification shows the small, rounded nuclei of glia cells called satellite cells (**S**) that produce thin, sheet-like cytoplasmic extensions that completely envelop each large neuronal perikaryon. (X400; H&E)

(c) Sympathetic ganglia are smaller than most sensory ganglia but similar in having large neuronal cell bodies (**N**), some containing lipofuscin (**L**). Sheets from satellite cells (**S**) enclose each neuronal cell body with morphology slightly different from that of sensory ganglia. Autonomic ganglia generally have less well-developed connective tissue capsules (**C**) than sensory ganglia. (X400; H&E)

FIGURE 9–30 Regeneration in peripheral nerves.

In an injured or cut peripheral nerve, proximal axon segments can regenerate from their cut ends after a delay. The main changes that take place in an injured nerve fiber are shown here.

(a) Normal nerve fiber, with its perikaryon, extensive RER (Nissl substance), and effector cell (muscle).

(b) When the axon is injured, the RER is greatly reduced initially and the nerve fiber distal to the injury degenerates along with its myelin sheath. Debris is phagocytosed by macrophages (shown in purple).

(c) In the following weeks after injury, muscle fiber shows denervation atrophy, but Schwann cells proliferate to form a compact cord penetrated by the regrowing axon. The axon grows at the rate of 0.5–3 mm/d.

(d) After some months, the nerve fiber regeneration is successful and functional connections with the muscle fiber are restored.

Nervous System SUMMARY OF KEY POINTS

Development of Nerve Tissue

- Nervous tissue develops in the early embryo when the dorsal ectoderm **neural plate** folds lengthwise to form the **neural tube**, the precursor of the CNS, and releases **neural crest cells**, precursors for much of the PNS.

Neurons

- There are many kinds of **neurons**, but all consist of a **cell body (perikaryon)** containing the nucleus, a long cytoplasmic extension called the **axon**, and one or more shorter processes called **dendrites**.
- Neurons use the common cell property of **excitability** to produce and move an **action potential (nerve impulse)** along the axon to excite another neuron or other effector cell.

- Such nerve communication is transmitted to another neuron or effector cell via a **synapse**, where **neurotransmitter** is released at the **presynaptic membrane** and binds receptors on the **postsynaptic** cell, initiating a new action potential there.

Glial Cells

- **Glial cells (glia)**, required to support neurons in many ways, consist of six major types:
 - **Oligodendrocytes** wrap processes around portions of axons in the CNS, forming **myelin sheaths** that insulate the axons and facilitate nerve impulses.
 - **Astrocytes**, the most numerous cell of the CNS, all produce hundreds of processes to cover and provide regulated microenvironments for neuronal perikarya, synapses, and capillaries.

- **Ependymal cells** are epithelial-like cells, lacking basement membranes, which line the fluid-filled cerebral ventricles and central canal of the spinal cord.
- **Microglia** differs from all other glial cells in originating from blood monocytes, not from neural tissue precursors; they mediate immune defense activity within the CNS.
- **Schwann cells (neurolemmocytes)** enclose all axons in nerves of the PNS, producing **myelin sheaths** around large-diameter axons, whose impulse conductivity is augmented at the **nodes of Ranvier** between successive Schwann cells.
- **Satellite cells** are located within PNS **ganglia**, aggregated sensory or autonomic neuronal cell bodies, where they enclose each perikaryon and regulate its microenvironment.

Central Nervous System

- Within the brain and spinal cord, regions rich in neuronal perikarya and astrocytes comprise the **gray matter** and regions containing tracts of myelinated axons comprise **white matter**.
- Hundreds of different neurons make up the CNS; large, unique **Purkinje neurons** characterize the cortex of the cerebellum, and layers of small **pyramidal neurons** form the cerebral cortex.
- The CNS is completely enclosed by three connective tissue layers called **meninges**: (1) the tough external **dura mater**; (2) the middle **arachnoid layer**; and (3) the delicate **pia mater** that directly contacts neural tissue.
- The **arachnoid layer** contains much CSF, which helps **cushion** the CNS within its bony enclosure.
- The **choroid plexus** consists of elaborate folds of vascularized **pia mater** covered by **ependyma** that project from walls of the cerebral ventricles; there water is removed from capillaries and transferred into the ventricles as **cerebrospinal fluid (CSF)**.
- In most CNS regions, neurons are also protected by the blood-brain barrier, consisting of the **perivascular feet of astrocytic processes** and the nonfenestrated capillary endothelial cells' **tight junctions**.

Peripheral Nervous System

- **Peripheral nerves** consist of axons from motor neurons (in the spinal cord), sensory neurons, and autonomic neurons (in ganglia); all the axons are enclosed within a series of **Schwann cells**, but only large (myelinated) axons have myelin sheaths and nodes of Ranvier.
- **Endoneurium** is a thin connective tissue layer immediately surrounding Schwann cells in peripheral nerves, containing a few non-fenestrated capillaries and much reticulin.
- Groups of axons (with Schwann cells and endoneurium) are surrounded by **perineurium**, consisting of layered, squamous fibroblastic cells joined by **tight junctions** to make a **blood-nerve barrier**.
- In large peripheral nerves, groups of axons are subdivided as **fascicles**, each of which is surrounded by perineurium.
- Surrounding the perineurium is a thick, outermost layer of dense irregular connective tissue, the **epineurium**.
- **Ganglia**, which can be either sensory or autonomic, contain neuronal cell bodies and their **satellite cells** and are surrounded by connective tissue continuous with that of nerves.

Neural Plasticity & Regeneration

- Certain regions of the CNS, such as near the ependyma, retain rare **neural stem and progenitor cells** that allow some replacement of neurons throughout life; **neural plasticity** involving formation and remodeling of synaptic connections is also prevalent throughout life.
- The complexity and distances of the neuronal and glial interconnections with the CNS make regeneration and restoration of function within this tissue after major injury very difficult.
- The more simply organized peripheral nerves have better capacity for **axonal regeneration**, a process involving reactivation of the perikaryon, Schwann cells, and macrophages.

Nervous System ASSESS YOUR KNOWLEDGE

1. Which of the following is characteristic of the chromatophilic material called Nissl substance in neural tissue?
 - a. Found throughout neurons
 - b. Site of mRNA translation for proteins of the axolemma
 - c. Most abundant in unipolar neurons
 - d. Becomes more abundant as an individual gets older
 - e. An example of intermediate filament proteins
2. Which of the following events occurs immediately after an action potential reaches a synapse at an axon terminal?
 - a. Vesicle fusion with the presynaptic terminal membrane
 - b. Calcium ion influx at the presynaptic terminal
 - c. Neurotransmitter binding to receptors on the postsynaptic membrane
 - d. Neurotransmitter release into the synaptic cleft
 - e. Binding of the neurotransmitter at the presynaptic terminal
3. A report from a hospital pathology laboratory indicates that a microscope slide with a small specimen of neural tissue contains "numerous GFAP-positive" cells. What is the most likely source of this specimen?
 - a. A region of white matter
 - b. A sensory ganglion
 - c. An autonomic ganglion
 - d. A region of gray matter
 - e. Pia mater
4. In the choroid plexus water from capillaries is transported directly into the cerebrospinal fluid by what structure(s)?
 - a. Ependyma
 - b. Astrocytes
 - c. Cells of the arachnoid mater
 - d. Lining of the central canal
 - e. Microglial cells
5. What term applies to collections of neuronal cell bodies (somata) in the central nervous system?
 - a. Ganglia
 - b. Neuroglia
 - c. Nodes
 - d. White matter
 - e. Nuclei
6. Which structure contains trabeculae around which cerebrospinal fluid (CSF) flows?
 - a. Arachnoid mater
 - b. Ependyma
 - c. Dura mater
 - d. Pia mater
 - e. Gray matter

7. Which of the following is a characteristic of the connective tissue layer that surrounds individual fascicles in large peripheral nerves?
 - a. A delicate region of connective tissue in contact with Schwann cells
 - b. Called the dura mater
 - c. Important as part of the blood-nerve barrier in the nerve
 - d. Rich in myelin
 - e. The thickest sheath of connective tissue in the nerve
8. A 35-year-old woman presents with weakness and spasticity in the lower left extremity, visual impairment and throbbing in the left eye, difficulties with balance, fatigue, and malaise. There is an increase in cerebrospinal fluid (CSF) protein, elevated gamma globulin, and moderate pleocytosis. MRI confirms areas of demyelination in the anterior corpus callosum. Imaging identifies plaques which are hyperintense on T2-weighted and fluid attenuated inversion recovery (FLAIR) images, and hypointense on T1-weighted scans. Which of the following cells are specifically targeted in her condition?
 - a. Microglia
 - b. Oligodendrocytes
 - c. Astrocytes
 - d. Schwann cells
 - e. Multipolar neurons
9. A 22-year-old man receives a severe, traumatic compression injury to his radial nerve during a motorcycle crash. He shows an advancing Tinel sign. Which one of the following characterizes regeneration of axons after this nerve injury?
 - a. It occurs in the absence of motor nerve action potentials.
 - b. It occurs at a rate of about 100 mm/d.
 - c. It occurs in the segment distal to the site of axon damage.
 - d. It occurs by a process that involves Schwann cell proliferation.
 - e. It occurs in conjunction with degeneration and phagocytosis of the endoneurium.
10. A 2-year-old boy presents with hearing impairment, poliosis (a white shock of hair), complete heterochromia and sectoral heterochromia, hypertelorism, a low hairline with eyebrows that touch in the middle, white pigmentation of the skin, and suspected neurologic deficits. He is diagnosed with Waardenburg syndrome with a mutation in the *PAX-3* gene that affects neural crest differentiation. Which of the following structures would most likely also be affected in this patient?
 - a. Purkinje cells
 - b. Pyramidal neurons
 - c. Ventral horns of the spinal cord
 - d. Astrocytes
 - e. Neurons and satellite cells of the spinal ganglion

10 Muscle Tissue

| | | | |
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Muscle tissue, the fourth basic tissue type with epithelia, connective tissues, and nervous tissue, is composed of cells that optimize the universal cell property of **contractility**. As in all cells, actin microfilaments and associated proteins generate the forces necessary for the muscle contraction, which drives movement within organ systems, of blood, and of the body as a whole. Essentially all muscle cells are of mesodermal origin and differentiate by a gradual process of cell lengthening with abundant synthesis of the myofibrillar proteins actin and myosin.

Three types of muscle tissue can be distinguished on the basis of morphologic and functional characteristics (Figure 10–1), with the structure of each adapted to its physiologic role.

- **Skeletal muscle** contains bundles of very long, multi-nucleated cells with cross-striations. Their contraction is quick, forceful, and usually under voluntary control.
- **Cardiac muscle** also has cross-striations and is composed of elongated, often branched cells bound to one another at structures called **intercalated discs** that are unique to cardiac muscle. Contraction is involuntary, vigorous, and rhythmic.
- **Smooth muscle** consists of collections of fusiform cells that lack striations and have slow, involuntary contractions.

In all types of muscle, contraction is caused by the sliding interaction of thick myosin filaments along thin actin filaments. The forces necessary for sliding are generated by other proteins affecting the weak interactions in the bridges between actin and myosin.

As with neurons, muscle specialists refer to certain muscle cell organelles with special names. The cytoplasm of muscle cells is often called **sarcoplasm** (Gr. *sarkos*, flesh + *plasma*,

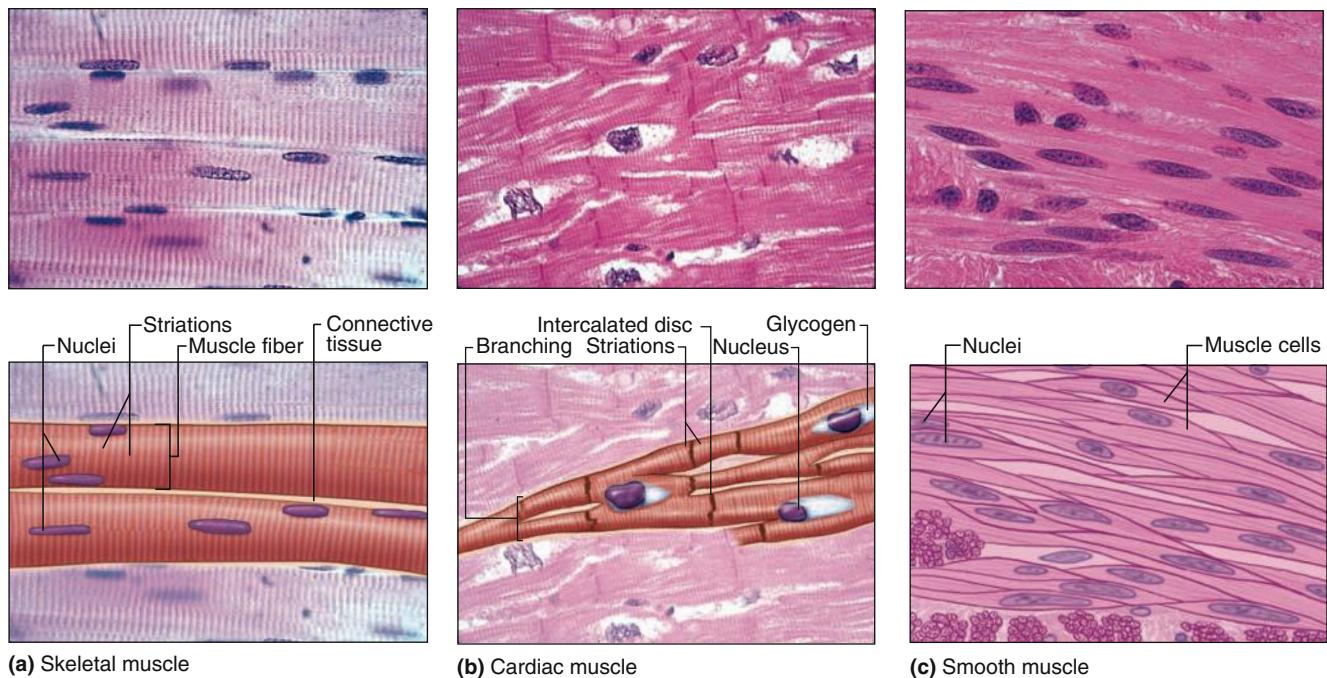
thing formed), the smooth ER is the **sarcoplasmic reticulum**, and the muscle cell membrane and its external lamina are the **sarcolemma** (*sarkos* + Gr. *lemma*, husk).

» MEDICAL APPLICATION

The variation in diameter of muscle fibers depends on factors such as the specific muscle, age, gender, nutritional status, and physical training of the individual. Exercise enlarges the skeletal musculature by stimulating formation of new myofibrils and growth in the diameter of individual muscle fibers. This process, characterized by increased cell volume, is called **hypertrophy** (Gr. *hyper*, above + *trophe*, nourishment). Tissue growth by an increase in the number of cells is termed hyperplasia (*hyper* + Gr. *plasis*, molding), which takes place very readily in smooth muscle, whose cells have not lost the capacity to divide by mitosis.

» SKELETAL MUSCLE

Skeletal (or **striated**) **muscle** consists of **muscle fibers**, which are long, cylindrical multinucleated cells with diameters of 10–100 µm. During embryonic muscle development, mesenchymal myoblasts (L. *myo*, muscle) fuse, forming myotubes with many nuclei. Myotubes then further differentiate to form striated muscle fibers (Figure 10–2). Elongated nuclei are found peripherally just under the sarcolemma, a characteristic nuclear location unique to skeletal muscle fibers/cells. A small population of reserve progenitor cells called muscle **satellite cells** remains adjacent to most fibers of differentiated skeletal muscle.

FIGURE 10–1 Three types of muscle.

(a) Skeletal muscle

(b) Cardiac muscle

(c) Smooth muscle

Light micrographs of each type, accompanied by labeled drawings. **(a) Skeletal muscle** is composed of large, elongated, multinucleated fibers that show strong, quick, voluntary contractions. **(b) Cardiac muscle** is composed of irregular branched cells bound together longitudinally by intercalated discs and shows strong,

involuntary contractions. **(c) Smooth muscle** is composed of grouped, fusiform cells with weak, involuntary contractions. The density of intercellular packing seen reflects the small amount of extracellular connective tissue present. ([a, b]: X200; [c]: X300; All H&E)

Organization of a Skeletal Muscle

Thin layers of connective tissue surround and organize the contractile fibers in all three types of muscle, and these layers are seen particularly well in skeletal muscle (Figures 10–3 and 10–4). The concentric organization given by these supportive layers resembles that in large peripheral nerves:

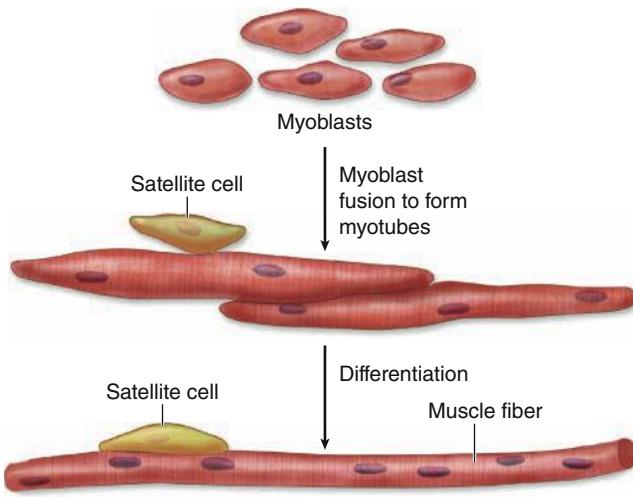
- The **epimysium**, an external sheath of dense irregular connective tissue, surrounds the entire muscle. Septa of this tissue extend inward, carrying the larger nerves, blood vessels, and lymphatics of the muscle.
- The **perimysium** is a thin connective tissue layer that immediately surrounds each bundle of muscle fibers termed a **fascicle** (Figure 10–3). Each fascicle of muscle fibers makes up a functional unit in which the fibers work together. Nerves, blood vessels, and lymphatics penetrate the perimysium to supply each fascicle.
- Within fascicles a very thin, delicate layer of reticular fibers and scattered fibroblasts, the **endomysium**, surrounds the external lamina of individual muscle fibers. In addition to nerve fibers, capillaries form a rich network in the endomysium bringing O₂ to the muscle fibers (Figure 10–5).

Collagens in these connective tissue layers of muscle serve to transmit the mechanical forces generated by the contracting muscle cells/fibers; individual muscle fibers seldom extend from one end of a muscle to the other.

All three layers, plus the dense irregular connective tissue of the deep fascia which overlies the epimysium, are continuous with the tough connective tissue of a tendon at **myotendinous junctions** which join the muscle to bone, skin, or another muscle (Figures 10–3 and 10–4c). Ultrastructural studies show that in these transitional regions, collagen fibers from the tendon insert themselves among muscle fibers and associate directly with complex infoldings of sarcolemma.

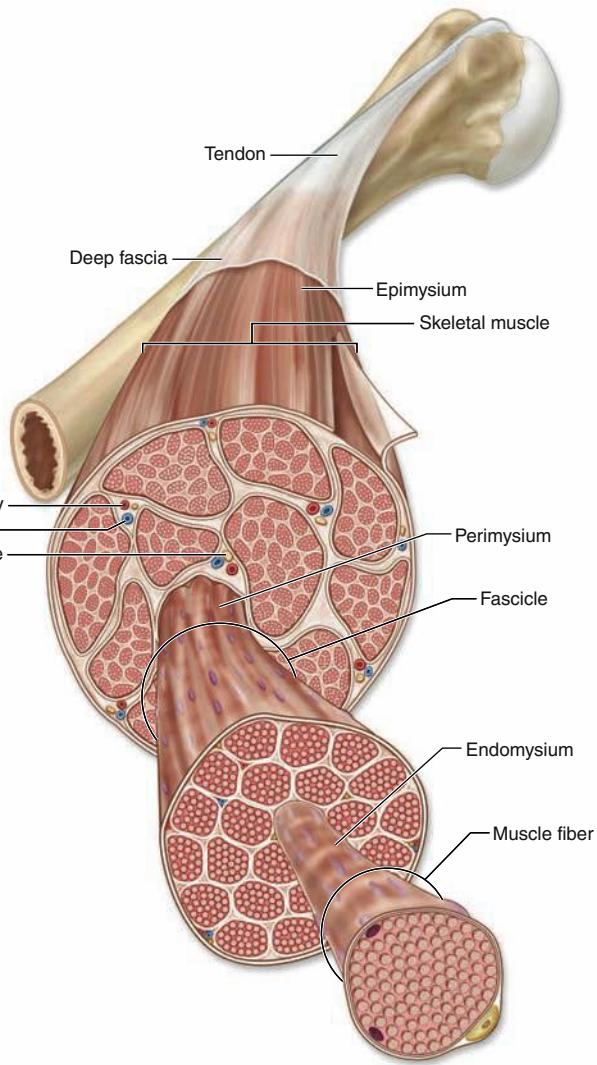
Organization Within Muscle Fibers

Longitudinally sectioned skeletal muscle fibers show striations of alternating light and dark bands (Figure 10–6a). The sarcoplasm is highly organized, containing primarily long cylindrical filament bundles called **myofibrils** that run parallel to the long axis of the fiber (Figure 10–6b). The dark bands on the myofibrils are called **A bands** (*anisotropic* or *birefringent* in polarized light microscopy); the light bands are called **I bands** (*isotropic*, do not alter polarized light). In the TEM (Figure 10–6c), each I

FIGURE 10–2 Development of skeletal muscle.

Skeletal muscle begins to differentiate when mesenchymal cells, called **myoblasts**, align and fuse together to make longer, multinucleated tubes called **myotubes**. Myotubes synthesize the proteins to make up myofilaments and gradually begin to show cross-striations by light microscopy. Myotubes continue differentiating to form functional myofibrils, and the nuclei are displaced against the sarcolemma.

Part of the myoblast population does not fuse and differentiate but remains as a group of mesenchymal cells called muscle **satellite cells** located on the external surface of muscle fibers inside the developing external lamina. Satellite cells proliferate and produce new muscle fibers following muscle injury.

FIGURE 10–3 Organization of skeletal muscle.

band is seen to be bisected by a dark transverse line, the **Z disc** (Ger. *zwischen*, between). The repetitive functional subunit of the contractile apparatus, the **sarcomere**, extends from Z disc to Z disc (Figure 10–6c) and is about 2.5- μm long in resting muscle.

Mitochondria and sarcoplasmic reticulum are found between the myofibrils, which typically have diameters of 1-2 μm . Myofibrils consist of an end-to-end repetitive arrangement of sarcomeres (Figure 10–7); the lateral registration of sarcomeres in adjacent myofibrils causes the entire muscle fiber to exhibit a characteristic pattern of transverse striations.

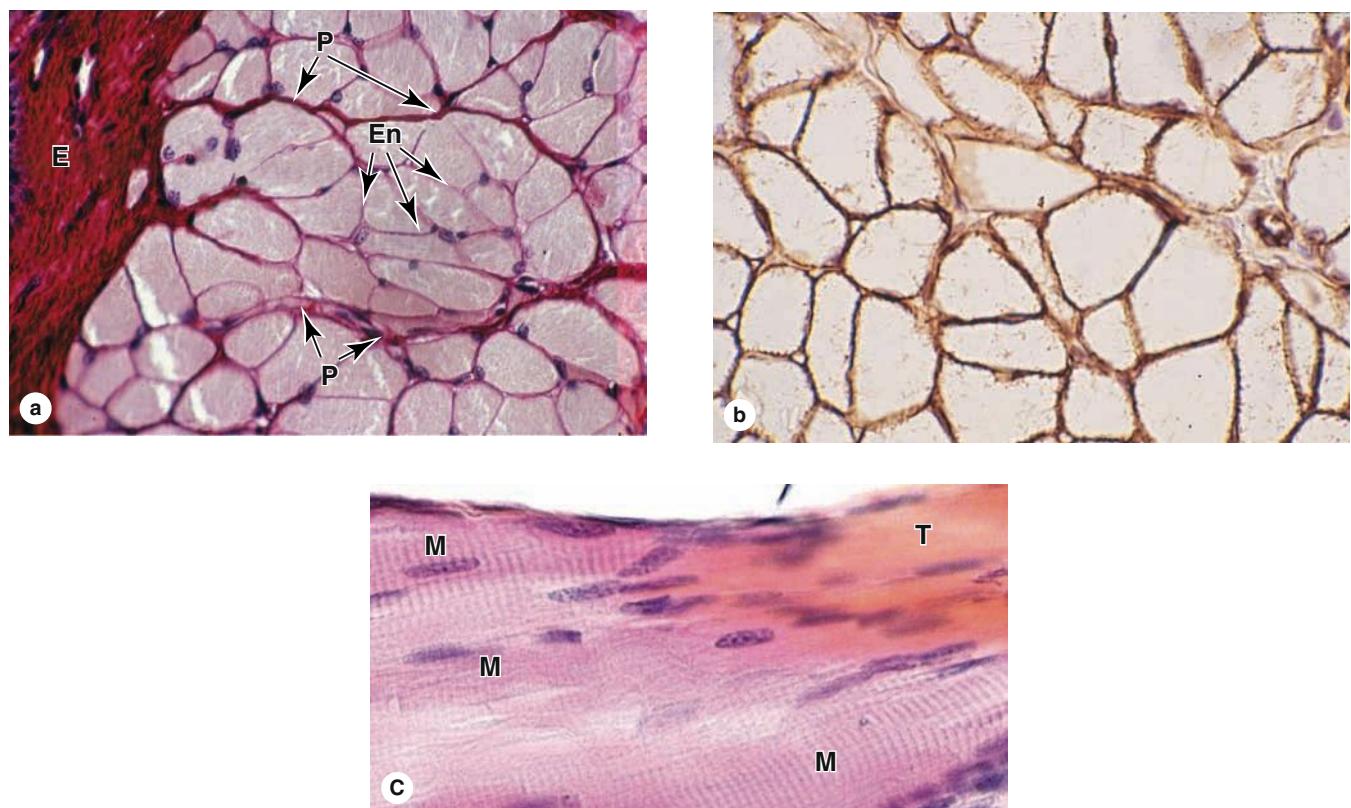
The A and I banding pattern in sarcomeres is due mainly to the regular arrangement of thick and thin **myofilaments**, composed of **myosin** and **F-actin**, respectively, organized within each myofibril in a symmetric pattern containing thousands of each filament type (Figure 10–7).

The thick myosin filaments are 1.6- μm long and 15-nm wide; they occupy the A band at the middle region of the sarcomere. **Myosin** is a large complex (~500 kDa) with two identical heavy chains and two pairs of light chains. Myosin heavy chains are thin, rodlike motor proteins (150-nm long and 2-3 nm thick) twisted together as myosin tails (Figure 10–7). Globular projections containing the four myosin light chains form a head at one end of each heavy chain. The myosin heads bind both actin, forming transient crossbridges between the

An entire skeletal muscle is enclosed within a thick layer of dense connective tissue called the **epimysium** that is continuous with fascia and the tendon binding muscle to bone. Large muscles contain several **fascicles** of muscle tissue, each wrapped in a thin but dense connective tissue layer called the **perimysium**. Within fascicles individual muscle fibers (elongated multinuclear cells) are surrounded by a delicate connective tissue layer, the **endomysium**.

thick and thin filaments, and ATP, catalyzing energy release (**actomyosin ATPase activity**). Several hundred myosin molecules are arranged within each thick filament with overlapping rodlike portions and the globular heads directed toward either end (Figure 10–7a).

The thin, helical actin filaments are each 1.0- μm long and 8-nm wide and run between the thick filaments. Each G-actin monomer contains a binding site for myosin (Figure 10–7b).

FIGURE 10–4 Skeletal muscle.

(a) A cross section of striated muscle demonstrating all three layers of connective tissue and cell nuclei. The endomysium (**En**) surrounds individual muscle, and perimysium (**P**) encloses a group of muscle fibers comprising a fascicle. A thick epimysium (**E**) surrounds the entire muscle. All three of these tissues contain collagen types I and III (reticulin). (X200; H&E)

(b) An adjacent section immunohistochemically stained for laminin, which specifically stains the external laminae

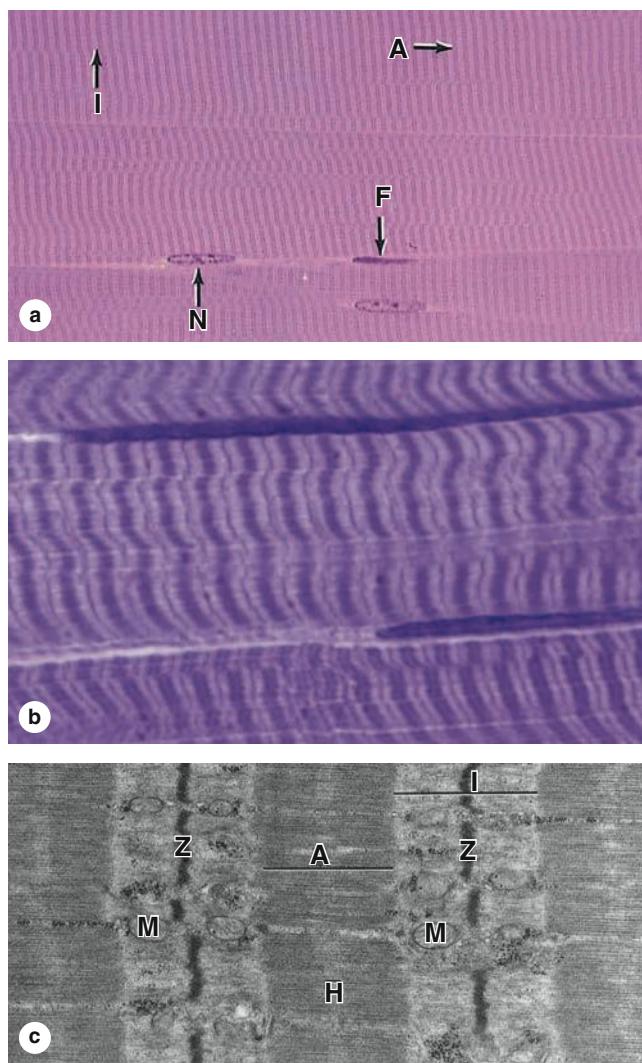
of the muscle fibers, surrounded by endomysium. (X400; Immunoperoxidase)

(c) Longitudinal section of a **myotendinous junction**. Tendons develop together with skeletal muscles and join muscles to the periosteum of bones. The dense collagen fibers of a tendon (**T**) are continuous with those in the three connective tissue layers around muscle fibers (**M**), forming a strong unit that allows muscle contraction to move other structures. (X400; H&E)

FIGURE 10–5 Capillaries of skeletal muscle.

The blood vessels were injected with a dark plastic polymer before the muscle was collected and sectioned longitudinally. A rich network of capillaries in endomysium surrounding muscle fibers is revealed by this method. (X200; Giemsa with polarized light)

FIGURE 10–6 Striated skeletal muscle in longitudinal section.



Longitudinal sections reveal the striations characteristic of skeletal muscle.

(a) Parts of three muscle fibers are separated by very thin endomyium that includes one fibroblast nucleus (F). Muscle nuclei (N) are found against the sarcolemma. Along each fiber thousands of dark-staining A bands alternate with lighter I bands. (X200; H&E)

(b) At higher magnification, each fiber can be seen to have three or four myofibrils, here with their striations slightly out of alignment with one another. Myofibrils are cylindrical bundles of thick and thin myofilaments that fill most of each muscle fiber. (X500; Giemsa)

(c) TEM showing one contractile unit (**sarcomere**) in the long series that comprises a myofibril. In its middle is an electron-dense A band bisected by a narrow, less dense region called the H zone. On each side of the A band are the lighter-stained I bands, each bisected by a dense Z disc which marks one end of the sarcomere. Mitochondria (M), glycogen granules, and small cisternae of SER occur around the Z disc. (X24,000)

(Figure 10-6c, used with permission from Mikel H. Snow, Department of Cell and Neurobiology, Keck School of Medicine at the University of Southern California, Los Angeles.)

The thin filaments have two tightly associated regulatory proteins (Figure 10-7b):

- **Tropomyosin**, a 40-nm-long coil of two polypeptide chains located in the groove between the two twisted actin strands
- **Troponin**, a complex of three subunits: TnT, which attaches to tropomyosin; TnC, which binds Ca^{2+} ; and TnI, which regulates the actin-myosin interaction

Troponin complexes attach at specific sites regularly spaced along each tropomyosin molecule.

The organization of important myofibril components is shown in Figure 10-8. I bands consist of the portions of the thin filaments that do not overlap the thick filaments in the A bands, which is why I bands stain more lightly than A bands. Actin filaments are anchored perpendicularly on the Z disc by the actin-binding protein **α -actinin** and exhibit opposite polarity on each side of this disc (Figure 10-8c). An important accessory protein in I bands is **titin** (3700 kDa), the largest protein in the body, with scaffolding and elastic properties, which supports the thick myofilaments and connects them to the Z disc (Figure 10-8c). Another large accessory protein, nebulin, binds each thin myofilament laterally, helps anchor them to α -actinin, and specifies the length of the actin polymers during myogenesis.

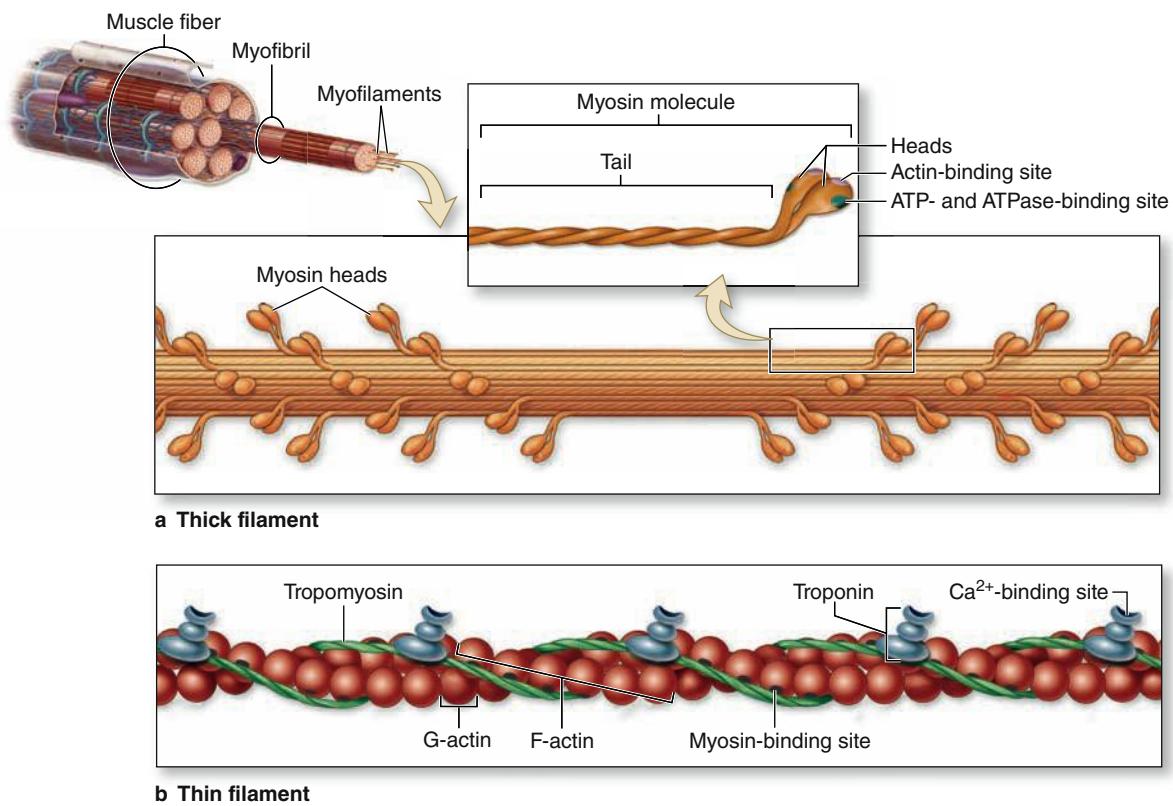
The A bands contain both the thick filaments and the overlapping portions of thin filaments. Close observation of the A band shows the presence of a lighter zone in its center, the H zone, corresponding to a region with only the rod-like portions of the myosin molecule and no thin filaments (Figure 10-8c). Bisecting the H zone is the M line (Ger. *Mitte*, middle; Figure 10-8d), containing a myosin-binding protein **myomesin** that holds the thick filaments in place, and **creatine kinase**. This enzyme catalyzes transfer of phosphate groups from phosphocreatine, a storage form of high-energy phosphate groups, to ADP, helping to supply ATP for muscle contraction.

Despite the many proteins present in sarcomeres, myosin and actin together represent over half of the total protein in striated muscle. The overlapping arrangement of thin and thick filaments within sarcomeres produces in TEM cross sections hexagonal patterns of structures that were important in determining the functions of the filaments and other proteins in the myofibril (Figures 10-8b and 10-8e).

Sarcoplasmic Reticulum & Transverse Tubule System

In skeletal muscle fibers the membranous smooth ER, called here **sarcoplasmic reticulum**, contains pumps and other proteins for Ca^{2+} sequestration and surrounds the myofibrils (Figure 10-9). Calcium release from cisternae of the sarcoplasmic reticulum through voltage-gated Ca^{2+} channels is triggered by membrane depolarization produced by a motor nerve.

To trigger Ca^{2+} release from sarcoplasmic reticulum throughout the muscle fiber simultaneously and produce

FIGURE 10–7 Molecules composing thin and thick filaments.

Myofilaments, which include both thick and thin filaments, consist of contractile protein arrays bundled within myofibrils. **(a)** A thick

myofilament contains 200-500 molecules of **myosin**. **(b)** A thin filament contains **F-actin**, **tropomyosin**, and **troponin**.

uniform contraction of all myofibrils, the sarcolemma has tubular infoldings called **transverse** or **T-tubules** (Figures 10–9 and 10–10). These long fingerlike invaginations of the cell membrane penetrate deeply into the sarcoplasm and encircle each myofibril near the aligned A- and I-band boundaries of sarcomeres.

Adjacent to each T-tubule are expanded **terminal cisternae** of sarcoplasmic reticulum. In longitudinal TEM sections, this complex of a T-tubule with two terminal cisternae is called a **triad** (Figures 10–9 and 10–10). The triad complex allows depolarization of the sarcolemma in a T-tubule to affect the sarcoplasmic reticulum and trigger release of Ca^{2+} ions into cytoplasm around the thick and thin filaments, which initiates contraction of sarcomeres.

Mechanism of Contraction

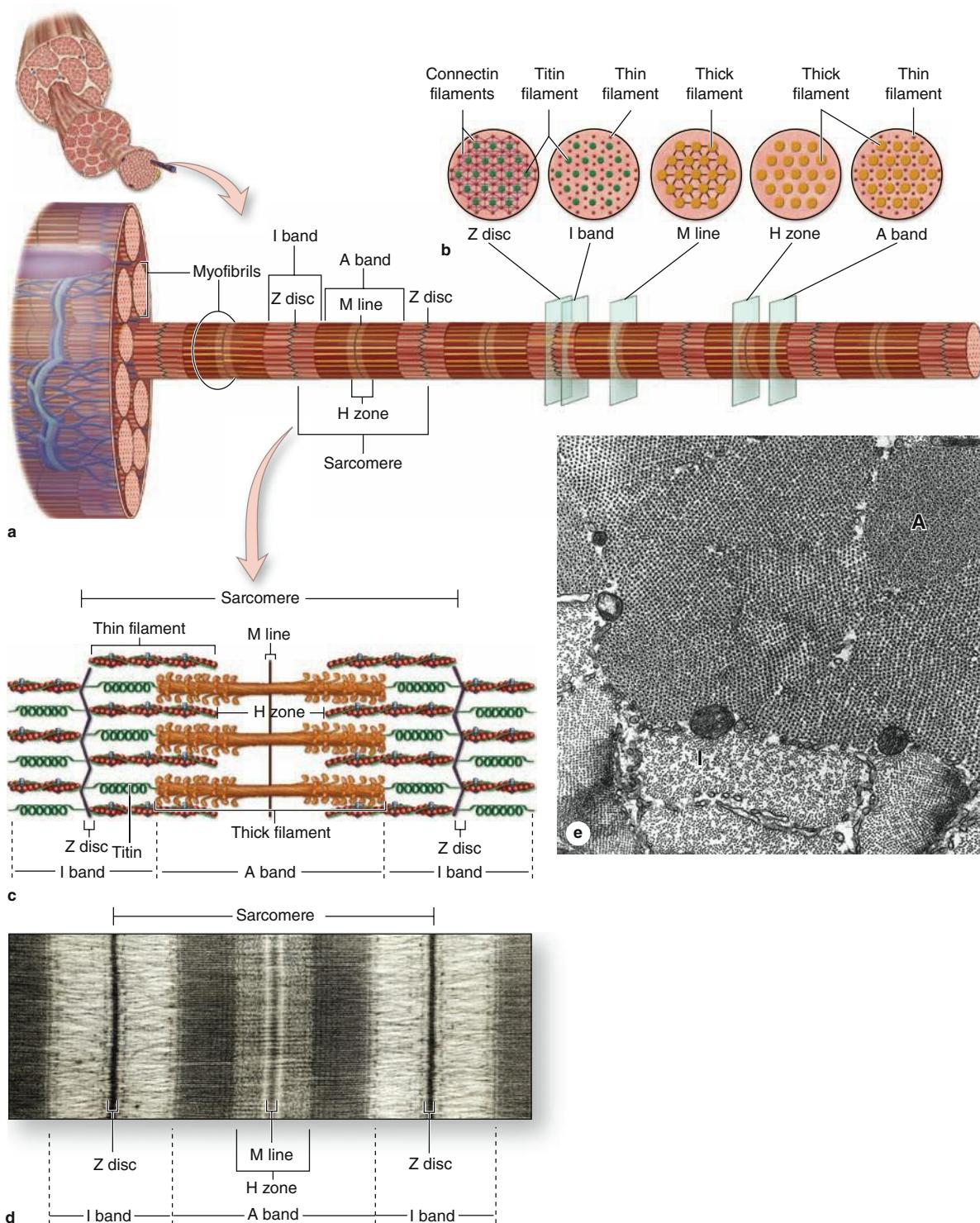
Figure 10–11 summarizes the key molecular events of muscle contraction. During this process neither the thick nor thin filaments change their length. Contraction occurs as the

overlapping thin and thick filaments of each sarcomere slide past one another.

Contraction is induced when an action potential arrives at a synapse, the **neuromuscular junction (NMJ)**, and is transmitted along the T-tubules to terminal cisternae of the sarcoplasmic reticulum to trigger Ca^{2+} release. In a resting muscle, the myosin heads cannot bind actin because the binding sites are blocked by the troponin-tropomyosin complex on the F-actin filaments. Calcium ions released upon neural stimulation bind troponin, changing its shape and moving tropomyosin on the F-actin to expose the myosin-binding active sites and allow crossbridges to form. Binding actin produces a conformational change or pivot in the myosins, which pulls the thin filaments farther into the A band, toward the Z disc (Figure 10–11).

Energy for the myosin head pivot which pulls actin is provided by hydrolysis of ATP bound to the myosin heads, after which myosin binds another ATP and detaches from actin. In the continued presence of Ca^{2+} and ATP, these attach-pivot-detach events occur in a repeating cycle, each lasting about

FIGURE 10–8 Structure of a myofibril: A series of sarcomeres.



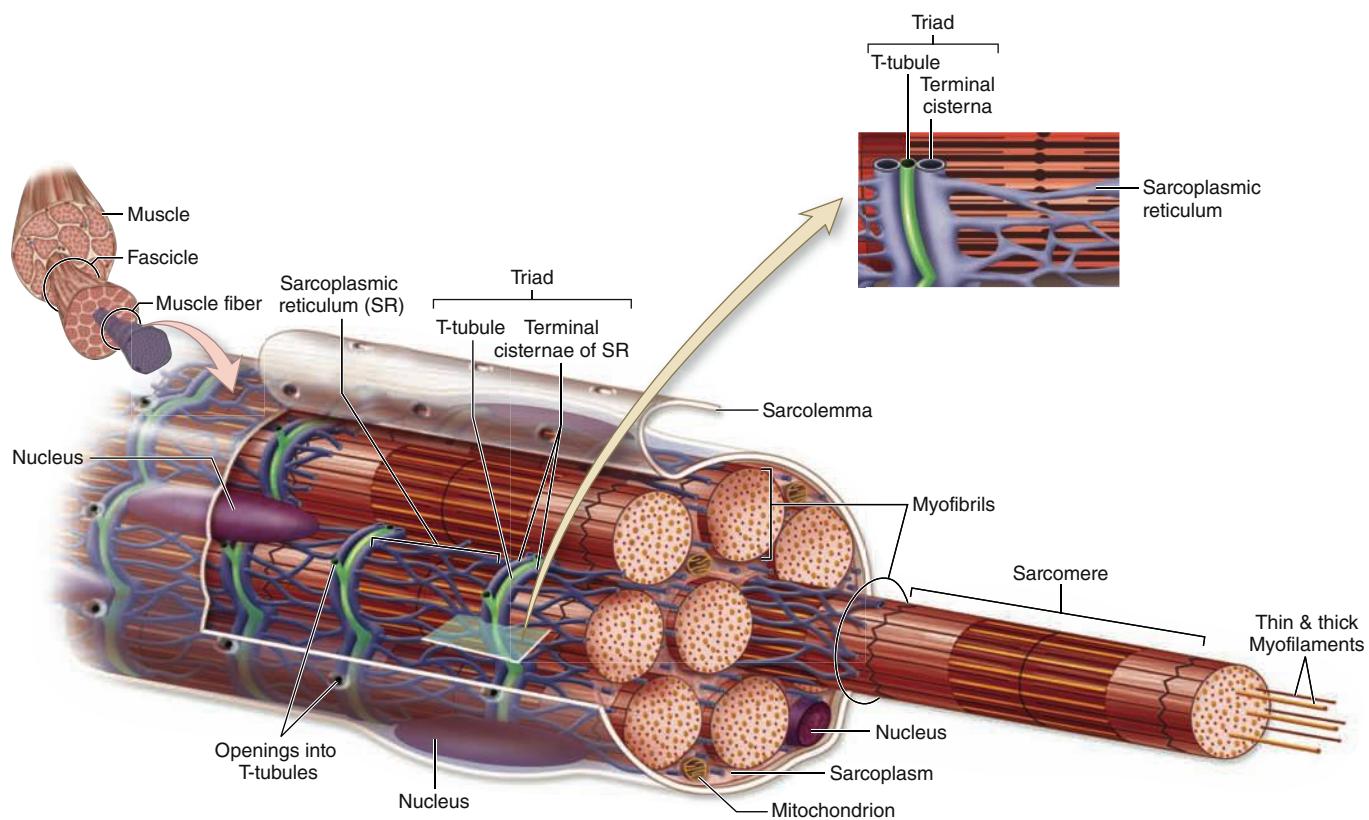
(a) The diagram shows that each muscle fiber contains several parallel bundles called **myofibrils**.

(b) Each myofibril consists of a long series of sarcomeres, separated by Z discs and containing thick and thin filaments that overlap in certain regions.

(c) Thin filaments are actin filaments with one end bound to **α -actinin** in the Z disc. Thick filaments are bundles of myosin, which span the entire A band and are bound to proteins of the M line and to the Z disc across the I bands by a very large protein called **titin**, which has springlike domains.

(d) The molecular organization of the sarcomeres produces staining differences that cause the dark- and light-staining bands seen by light microscopy and TEM. (X28,000)

(e) With the TEM an oblique section of myofibrils includes both **A** and **I** bands and shows hexagonal patterns that indicate the relationships between thin and thick myofilaments and other proteins, as shown in part **b** of this figure. Thin and thick filaments are arranged so that each myosin bundle contacts six actin filaments. Large mitochondria in cross section and SER cisternae are seen between the myofibrils. (X45,000)

FIGURE 10–9 Organization of a skeletal muscle fiber

Skeletal muscle fibers are composed mainly of myofibrils. Each myofibril extends the length of the fiber and is surrounded by parts of the sarcoplasmic reticulum. The sarcolemma has deep invaginations called T-tubules, each of which becomes associated with two

terminal cisternae of the sarcoplasmic reticulum. A T-tubule and its two associated terminal cisterna comprise a “triad” of small spaces along the surface of the myofibrils.

50 milliseconds, which rapidly shorten the sarcomere and contract the muscle (Figures 10–11 and 10–12). A single muscle contraction results from hundreds of these cycles.

When the neural impulse stops and levels of free Ca^{2+} ions diminish, tropomyosin again covers the myosin-binding sites on actin and the filaments passively slide back and sarcomeres return to their relaxed length (Figure 10–11). In the absence of ATP, the actin-myosin crossbridges become stable, which accounts for the rigidity of skeletal muscles (**rigor mortis**) that occurs as mitochondrial activity stops after death.

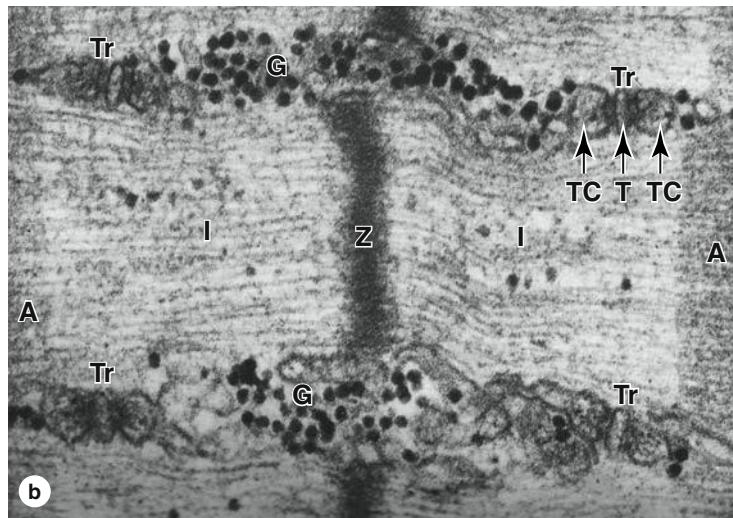
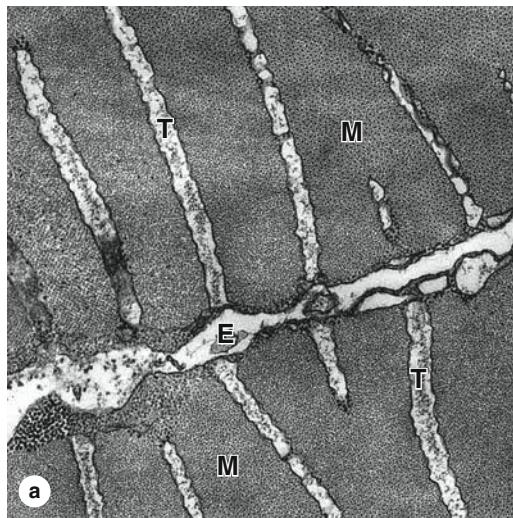
Innervation

Myelinated motor nerves branch out within the perimysium, where each nerve gives rise to several unmyelinated terminal twigs that pass through endomysium and form synapses with individual muscle fibers. Schwann cells enclose the small axon

branches and cover their points of contact with the muscle cells (Figure 10–13); the external lamina of the Schwann cell fuses with that of the sarcolemma. Each axonal branch forms a dilated termination situated within a trough on the muscle cell surface, which are part of the synapses termed the neuromuscular junctions, or **motor end plates (MEP)** (Figure 10–13). As in all synapses the axon terminal contains mitochondria and numerous synaptic vesicles; here the vesicles contain the neurotransmitter **acetylcholine**. Between the axon and the muscle is the **synaptic cleft**. Adjacent to the synaptic cleft, the sarcolemma is thrown into numerous deep **junctional folds**, which provide for greater postsynaptic surface area and more transmembrane acetylcholine receptors.

When a **nerve action potential** reaches the MEP, acetylcholine is liberated from the axon terminal, diffuses across the cleft, and binds to its receptors in the folded sarcolemma. The **acetylcholine receptor** contains a nonselective cation channel that opens upon neurotransmitter binding, allowing influx

FIGURE 10–10 Transverse tubule system and triads.



Transverse tubules are invaginations of the sarcolemma that penetrate deeply into the muscle fiber around all myofibrils.

(a) TEM cross section of fish muscle shows portions of two fibers and the endomysium (E) between them. Several transverse or T-tubules (T) are shown, perpendicular to the fiber surface, penetrating between myofibrils (M). (X50,000)

(b) Higher-magnification TEM of skeletal muscle in longitudinal section shows four membranous triads (Tr) cut transversely near the A-band–I-band junctions. Each triad consists of a central

transverse tubule (T) and two adjacent terminal cisterns (TC) extending from the sarcoplasmic reticulum. Centrally located is the Z disc. Besides elements of the triad, sarcoplasm surrounding the myofibril also contains dense glycogen granules (G).

Components of the triad are responsible for the cyclic release of Ca^{2+} from the cisternae and its sequestration again that occurs during muscle contraction and relaxation. The association between SR cisternae and T-tubules is shown diagrammatically in Figure 10–11. (X90,000)

of cations, depolarizing the sarcolemma, and producing the **muscle action potential**. Acetylcholine quickly dissociates from its receptors, and free neurotransmitter is removed from the synaptic cleft by the extracellular enzyme **acetylcholinesterase**, preventing prolonged contact of the transmitter with its receptors.

As discussed with Figure 10–11, the muscle action potential moves along the sarcolemma and along T-tubules that penetrate deeply into sarcoplasm. At triads the depolarization signal triggers the release of Ca^{2+} from terminal cisterns of the sarcoplasmic reticulum, initiating the contraction cycle.

An axon from a single motor neuron can form MEPs with one or many muscle fibers. Innervation of single muscle fibers by single motor neurons provides precise control of muscle activity and occurs, for example, in the extraocular muscles for eye movements. Larger muscles with coarser movements have motor axons that typically branch profusely and innervate 100 or more muscle fibers. In this case the single axon and all the muscle fibers in contact with its branches make up a **motor unit**. Individual striated muscle fibers do not show graded contraction—they contract either all the way or not at all. To vary the force of contraction, the fibers within a muscle fascicle do not all contract at the same time. With large

muscles composed of many motor units, the firing of a single motor axon will generate tension proportional to the number of muscle fibers it innervates. Thus, the number of motor units and their variable size control the intensity and precision of a muscle contraction.

Key features of skeletal muscle cells, connective tissue, contraction, and innervation are summarized in Table 10–1.

» MEDICAL APPLICATION

Myasthenia gravis is an autoimmune disorder that involves circulating antibodies against proteins of acetylcholine receptors. Antibody binding to the antigenic sites interferes with acetylcholine activation of their receptors, leading to intermittent periods of skeletal muscle weakness. As the body attempts to correct the condition, junctional folds of sarcolemma with affected receptors are internalized, digested by lysosomes, and replaced by newly formed receptors. These receptors, however, are again made unresponsive to acetylcholine by similar antibodies, and the disease follows a progressive course. The extraocular muscles of the eyes are commonly the first affected.

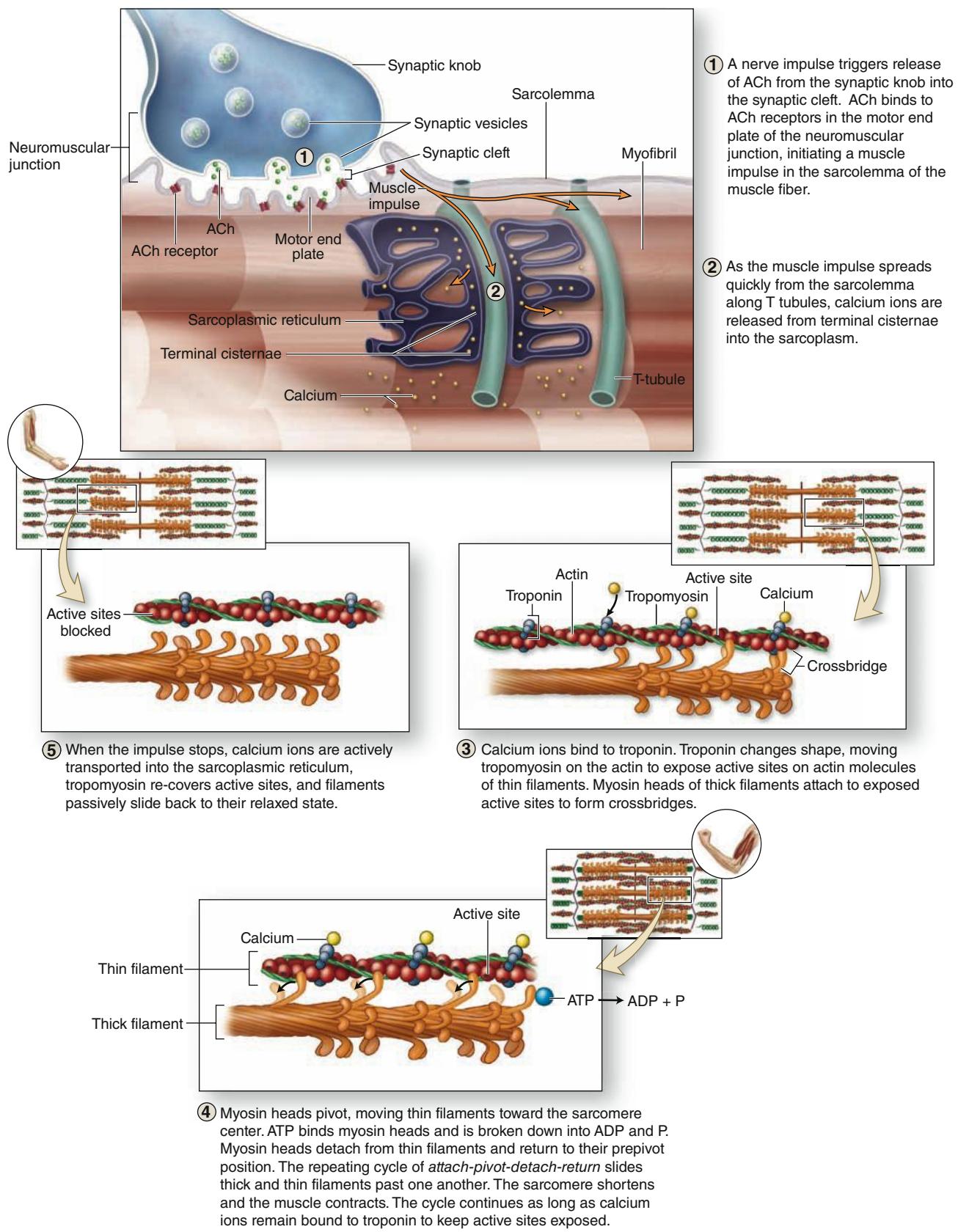
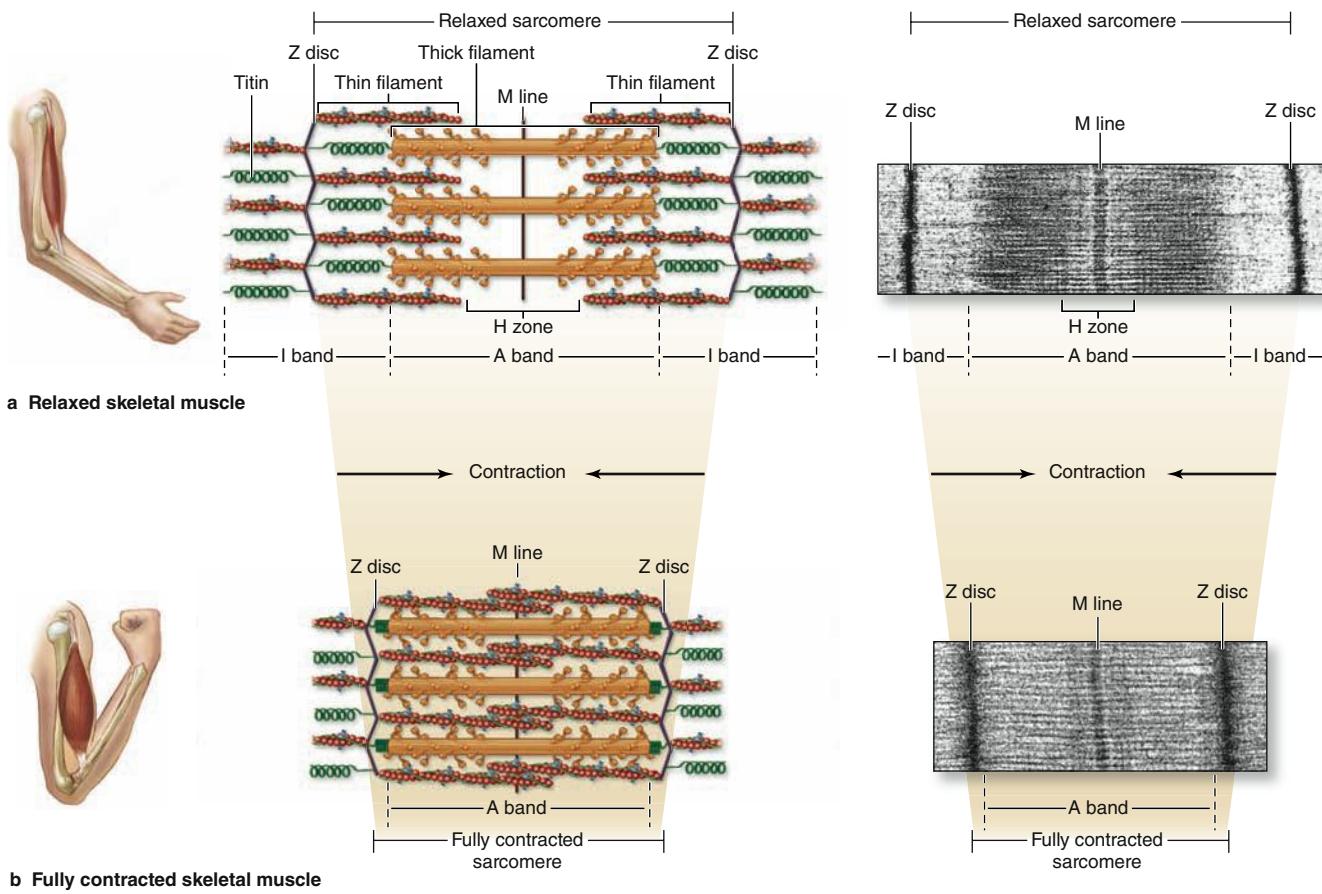
FIGURE 10–11 Events of muscle contraction.

FIGURE 10–12 Sliding filaments and sarcomere shortening in contraction.

Diagrams and TEM micrographs show sarcomere shortening during skeletal muscle contraction. (a) In the relaxed state the sarcomere, I band, and H zone are at their expanded length. The springlike action of titin molecules, which span the I band, helps pull thin and thick filaments past one another in relaxed muscle. (b) During

muscle contraction, the Z discs at the sarcomere boundaries are drawn closer together as they move toward the ends of thick filaments in the A band. Titin molecules are compressed during contraction.

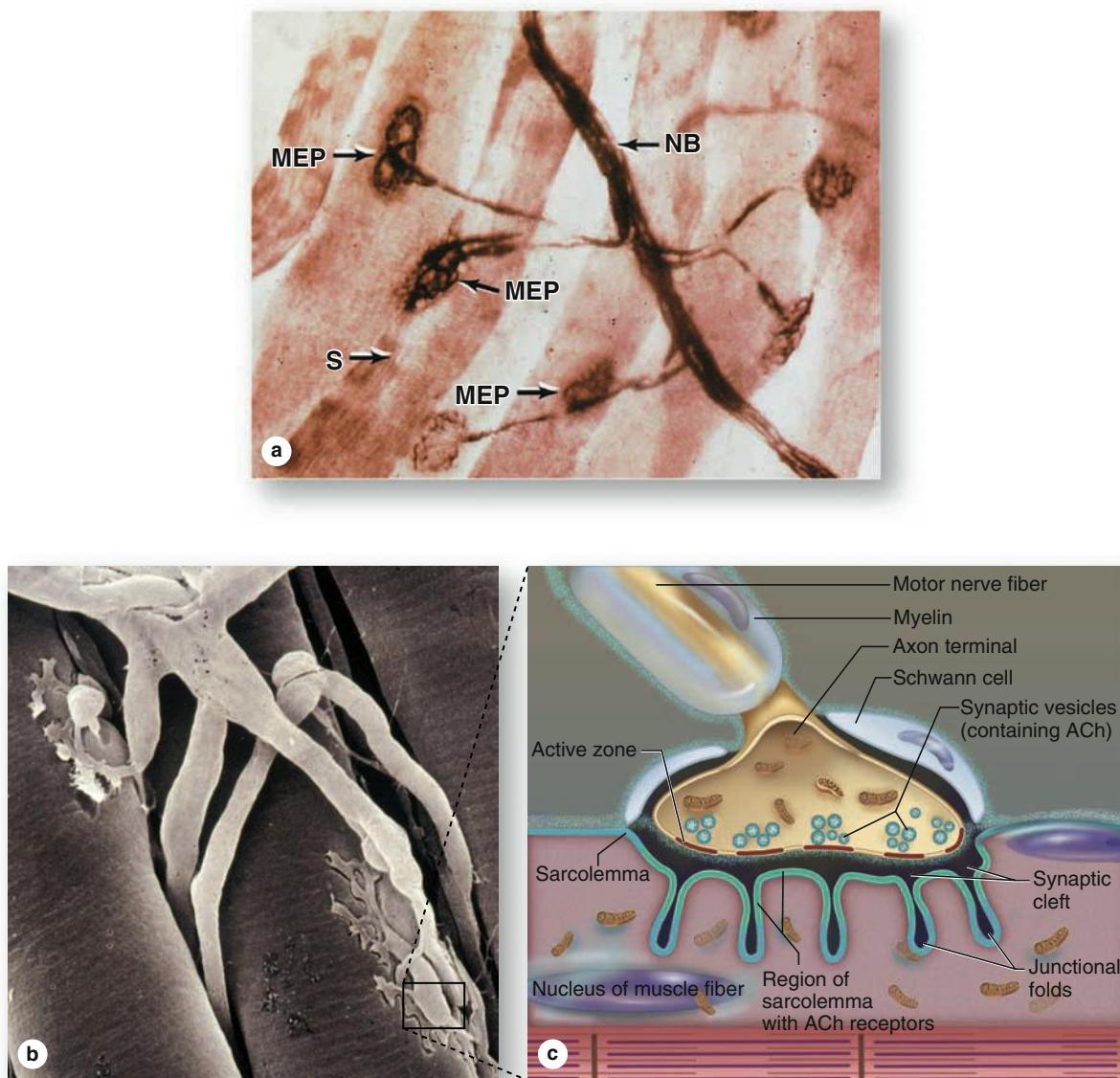
Muscle Spindles & Tendon Organs

Striated muscles and myotendinous junctions contain sensory receptors acting as proprioceptors (*L. proprius*, one's own + *capio*, to take), providing the central nervous system (CNS) with data from the musculoskeletal system. Among the muscle fascicles are stretch detectors known as **muscle spindles**, approximately 2-mm long and 0.1-mm wide (Figure 10–14a). A muscle spindle is encapsulated by modified perimysium, with concentric layers of flattened cells, containing interstitial fluid and a few thin muscle fibers filled with nuclei and called **intrafusal fibers** (Figure 10–14). Several sensory nerve axons penetrate each muscle spindle and wrap around individual intrafusal fibers. Changes in length (distension) of the surrounding (extrafusal) muscle fibers caused by body movements are detected by the muscle spindles and the

sensory nerves relay this information to the spinal cord. Different types of sensory and intrafusal fibers mediate reflexes of varying complexity to help maintain posture and to regulate the activity of opposing muscle groups involved in motor activities such as walking.

A similar role is played by **Golgi tendon organs**, much smaller encapsulated structures that enclose sensory axons penetrating among the collagen bundles at the myotendinous junction (Figure 10–14a). Tendon organs detect changes in tension within tendons produced by muscle contraction and act to inhibit motor nerve activity if tension becomes excessive. Because both of these proprioceptors detect increases in tension, they help regulate the amount of effort required to perform movements that call for variable amounts of muscular force.

FIGURE 10–13 The neuromuscular junction (NMJ).



Before it terminates in a skeletal muscle, each motor axon bundled in the nerve forms many branches, each of which forms a synapse with a muscle fiber.

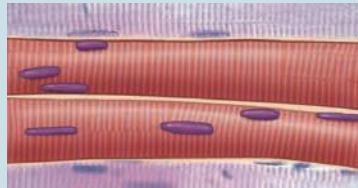
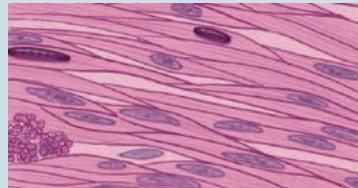
(a) Silver staining can reveal the nerve bundle (**NB**), the terminal axonal twigs, and the motor end plates (**MEP**, also called neuromuscular junctions or NMJ) on striated muscle fibers (**S**). (X1200)

(b) An SEM shows the branching ends of a motor axon, each covered by an extension of the last Schwann cell and expanded

terminally as an MEP embedded in a groove in the external lamina of the muscle fiber.

(c) Diagram of enclosed portion of the SEM indicating key features of a typical MEP: synaptic vesicles of acetylcholine (**ACh**), a synaptic cleft, and a postsynaptic membrane. This membrane, the sarcolemma, is highly folded to increase the number of ACh receptors at the MEP. Receptor binding initiates muscle fiber depolarization, which is carried to the deeper myofibrils by the T-tubules.

TABLE 10-1 Important comparisons of the three types of muscle.

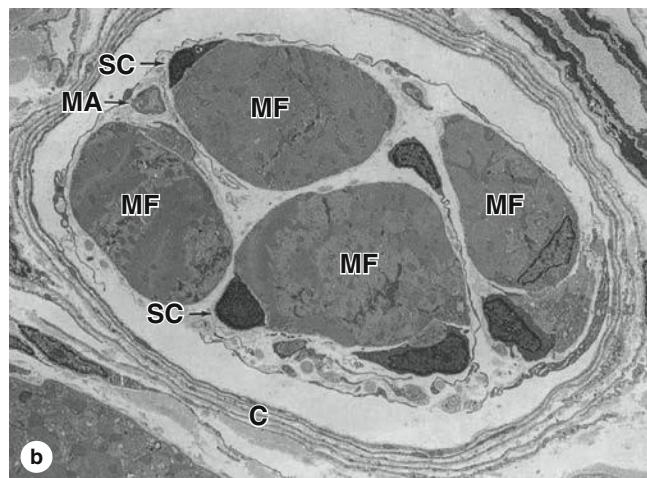
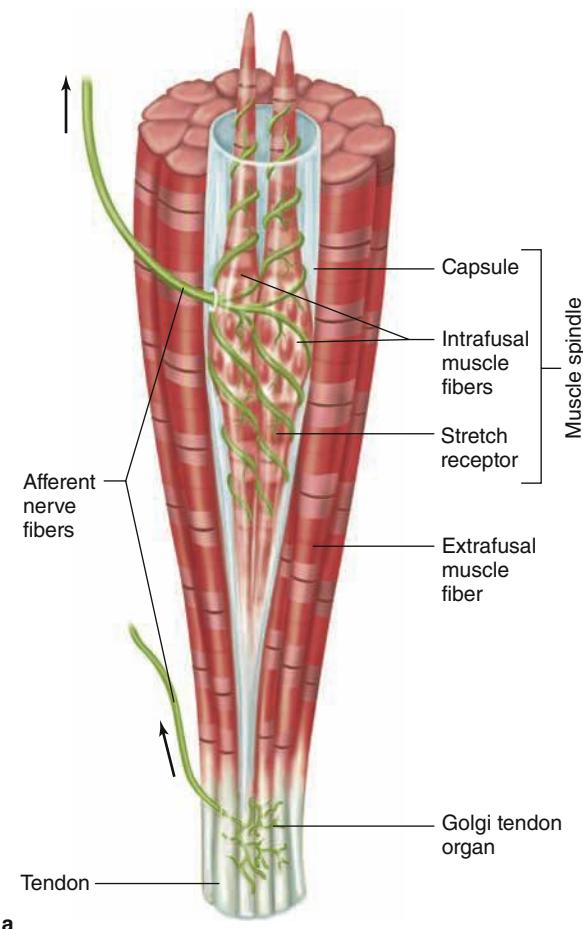
| | Skeletal Muscle | Cardiac Muscle | Smooth Muscle |
|--|--|---|--|
| |  |  |  |
| Fibers | Single multinucleated cells | Aligned cells in branching arrangement | Single small, closely packed fusiform cells |
| Cell/fiber shape and size | Cylindrical, 10-100 µm diameter, many cm long | Cylindrical, 10-20 µm diameter, 50-100 µm long | Fusiform, diameter 0.2-10 µm, length 50-200 µm |
| Striations | Present | Present | Absent |
| Location of nuclei | Peripheral, adjacent to sarcolemma | Central | Central, at widest part of cell |
| T tubules | Center of triads at A-I junctions | In dyads at Z discs | Absent; caveolae may be functionally similar |
| Sarcoplasmic reticulum (SR) | Well-developed, with two terminal cisterns per sarcomere in triads with T tubule | Less well-developed, one small terminal cistern per sarcomere in dyad with T tubule | Irregular smooth ER without distinctive organization |
| Special structural features | Very well-organized sarcomeres, SR, and transverse tubule system | Intercalated discs joining cell, with many adherent and gap junctions | Gap junctions, caveolae, dense bodies |
| Control of contraction | Troponin C binds Ca^{2+} , moving tropomyosin and exposing actin for myosin binding | Similar to that of skeletal muscle | Actin-myosin binding occurs with myosin phosphorylation by MLCK triggered when calmodulin binds Ca^{2+} |
| Connective tissue organization | Endomysium, perimysium, and epimysium | Endomysium; subendocardial and subepicardial CT layers | Endomysium and less-organized CT sheaths |
| Major locations | Skeletal muscles, tongue, diaphragm, eyes, and upper esophagus | Heart | Blood vessels, digestive and respiratory tracts, uterus, bladder, and other organs |
| Key function | Voluntary movements | Automatic (involuntary) pumping of blood | Involuntary movements |
| Efferent innervation | Motor | Autonomic | Autonomic |
| Contractions | All-or-none, triggered at motor end plates | All-or-none, intrinsic (beginning at nodes of conducting fibers) | Partial, slow, often spontaneous, wavelike and rhythmic |
| Cell response to increased load | Hypertrophy (increase in fiber size) | Hypertrophy | Hypertrophy and hyperplasia (increase in cell/fiber number) |
| Capacity for regeneration | Limited, involving satellite cells mainly | Very poor | Good, involving mitotic activity of muscle cells |

» MEDICAL APPLICATION

Dystrophin is a large actin-binding protein located just inside the sarcolemma of skeletal muscle fibers which is involved in the functional organization of myofibrils. Research on **Duchenne muscular dystrophy** revealed that mutations of the dystrophin gene can lead to defective linkages between the cytoskeleton and the extracellular matrix (ECM). Muscle contractions can disrupt these weak linkages, causing the atrophy of muscle fibers typical of this disease.

Skeletal Muscle Fiber Types

Skeletal muscles such as those that move the eyes and eyelids need to contract rapidly, while others such as those for bodily posture must maintain tension for longer periods while resisting fatigue. These metabolic differences are possible because of varied expression in muscle fibers of contractile or regulatory protein isoforms and other factors affecting oxygen delivery and use. Different types of fibers can be identified on the basis of (1) their maximal rate of contraction (fast or slow fibers) and (2) their major pathway for ATP synthesis (oxidative

FIGURE 10–14 Sensory receptors associated with skeletal muscle.

(a) The diagram shows both a **muscle spindle** and a **tendon organ**. Muscle spindles have **afferent sensory** and efferent motor nerve fibers associated with the **intrafusal fibers**, which are modified muscle fibers. The size of the spindle is exaggerated relative to the extrafusal fibers to show better the nuclei packed in the intrafusal fibers. Both types of sensory receptors provide the CNS with information concerning degrees of stretch and tension within the musculoskeletal system.

(b) A TEM cross section near the end of a muscle spindle shows the capsule (**C**), lightly myelinated axons (**MA**) of a sensory nerve, and the intrafusal muscle fibers (**MF**). These thin fibers differ from the ordinary skeletal muscle fibers in having very few myofibrils. Their many nuclei can either be closely aligned (nuclear chain fibers) or piled in a central dilation (nuclear bag fibers). Muscle satellite cells (**SC**) are also present within the external lamina of the intrafusal fibers. (X3600)

phosphorylation or glycolysis). Fast versus slow rates of fiber contraction are due largely to myosin isoforms with different maximal rates of ATP hydrolysis.

Histochemical staining is used to identify fibers with differing amounts of “fast” and “slow” ATPases (Figure 10–15). Other histological features reflecting metabolic differences among muscle fibers include the density of surrounding capillaries, the number of mitochondria, and levels of glycogen and **myoglobin**, a globular sarcoplasmic protein similar to hemoglobin which contains iron atoms and allows for O₂ storage.

Each of these features exists as a continuum in skeletal muscle fibers, but fiber diversity is divided into three major types:

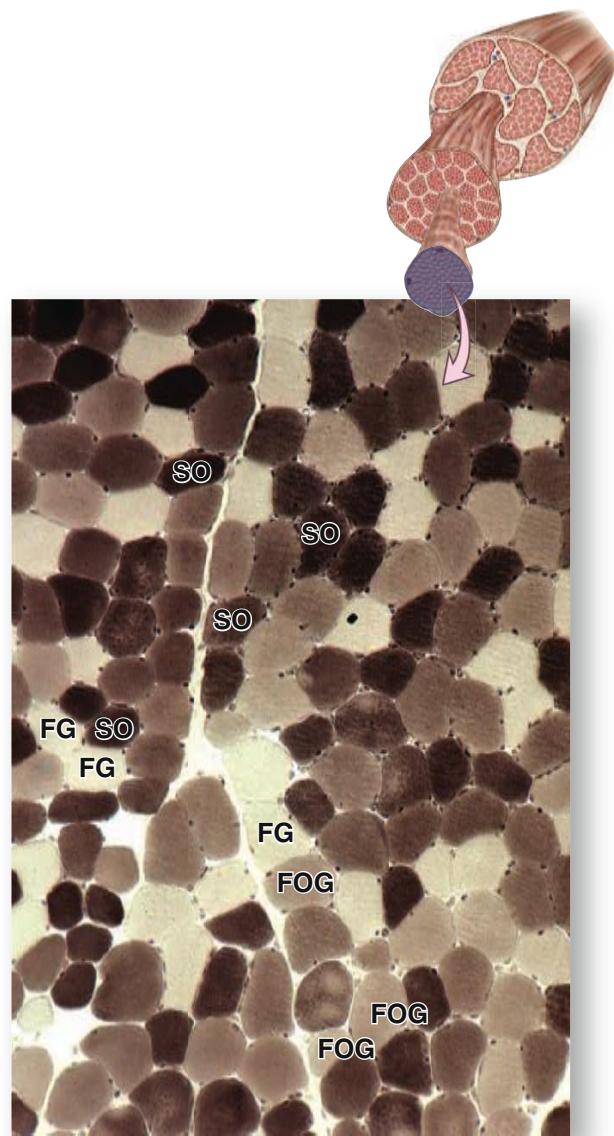
- **Slow oxidative** muscle fibers are adapted for slow contractions over long periods without fatigue, having many mitochondria, many surrounding capillaries, and much myoglobin, all features that make fresh tissue rich in these fibers dark or red in color.

- **Fast glycolytic** fibers are specialized for rapid, short-term contraction, having few mitochondria or capillaries and depending largely on anaerobic metabolism of glucose derived from stored glycogen, features that make such fibers appear white. Rapid contractions lead to rapid fatigue as lactic acid produced by glycolysis accumulates.

- **Fast oxidative-glycolytic** fibers have physiological and histological features intermediate between those of the other two types.

Table 10–2 summarizes these and other characteristics of the three skeletal muscle fiber types. The metabolic type of each fiber is determined by the rate of impulse conduction along its motor nerve supply, so that all fibers of a motor unit are similar. Most skeletal muscles receive motor input from multiple nerves and contain a mixture of fiber types (Figure 10–15). Determining the fiber types in needle biopsies of skeletal muscle helps in the diagnosis of specific

FIGURE 10–15 Skeletal muscle fiber types.



Cross section of a skeletal muscle stained histochemically for myosin ATPase at acidic pH, which reveals activity of the “slow” ATPase and shows the distribution of the three main fiber types. Slow oxidative (**SO**) or type I fibers have high levels of acidic ATPase activity and stain the darkest. Fast glycolytic (**FG**) or type IIb fibers stain the lightest. Fast oxidative-glycolytic (**FOG**) or type IIa fibers are intermediate between the other two types (X40). ATPase histochemistry of unfixed, cryostat section, pH 4.2.

myopathies (*myo* + Gr. *pathos*, suffering), motor neuron diseases, and other causes of muscle atrophy. Different fiber types also exist in cardiac muscle at various locations within the heart and in smooth muscle of different organs.

► CARDIAC MUSCLE

During embryonic development mesenchymal cells around the primitive heart tube align into chainlike arrays. Rather than fusing into multinucleated cells/fibers as in developing skeletal muscle fibers, **cardiac muscle** cells form complex junctions between interdigitating processes (Figure 10–16). Cells within one fiber often branch and join with cells in adjacent fibers. Consequently, the heart consists of tightly knit bundles of cells, interwoven in spiraling layers that provide for a characteristic wave of contraction that resembles wringing out of the heart ventricles.

Mature cardiac muscle cells are 15–30 µm in diameter and 85–120 µm long, with a striated banding pattern comparable to that of skeletal muscle. Unlike skeletal muscle, however, each cardiac muscle cell usually has only one nucleus and is centrally located. Surrounding the muscle cells is a delicate sheath of endomysium with a rich capillary network. A thicker perimysium separates bundles and layers of muscle fibers and in specific areas (described in Chapter 11) forms larger masses of fibrous connective tissue comprising the “cardiac skeleton.”

A unique characteristic of cardiac muscle is the presence of transverse lines that cross the fibers at irregular intervals where the myocardial cells join. These **intercalated discs** represent the interfaces between adjacent cells and consist of many junctional complexes (Figures 10–16). Transverse regions of these irregular, steplike discs are composed of many **desmosomes** and **fascia adherens** junctions, which together provide strong intercellular adhesion during the cells’ constant contractile activity. The less abundant, longitudinally oriented regions of each intercalated disc run parallel to the myofibrils and are filled with **gap junctions** that provide ionic continuity between the cells. These regions serve as “electrical synapses,” promoting rapid impulse conduction through many cardiac muscle cells simultaneously and contraction of many adjacent cells as a unit.

The structure and function of the contractile apparatus in cardiac muscle cells are essentially the same as in skeletal muscle (Figure 10–17). Mitochondria occupy up to 40% of the cell volume, higher than in slow oxidative skeletal muscle fibers. Fatty acids, the major fuel of the heart, are stored as triglycerides in small lipid droplets. Glycogen granules as well as perinuclear lipofuscin pigment granules may also be present.

Muscle of the heart ventricles is much thicker than that of the atria, reflecting its role in pumping blood through the cardiovascular system. T-tubules in ventricular muscle fibers are well-developed, with large lumens and penetrate the sarcoplasm in the vicinity of the myofibrils’ Z discs. In atrial muscle T-tubules are much smaller or entirely absent. Sarcoplasmic reticulum is less well-organized in cardiac compared to skeletal muscle fibers. The junctions between its terminal cisterns and T-tubules typically involve only one structure of each type, forming profiles called **dyads** rather than triads in TEM

TABLE 10–2 Major characteristics of skeletal muscle fiber types.

| | Slow, Oxidative Fibers (Type I) | Fast, Oxidative-Glycolytic Fibers (Type IIa) | Fast, Glycolytic Fibers (Type IIb) |
|-----------------------------------|---------------------------------|--|------------------------------------|
| Mitochondria | Numerous | Numerous | Sparse |
| Capillaries | Numerous | Numerous | Sparse |
| Fiber diameter | Small | Intermediate | Large |
| Size of motor unit | Small | Intermediate | Large |
| Myoglobin content | High (red fibers) | High (red fibers) | Low (white fibers) |
| Glycogen content | Low | Intermediate | High |
| Major source of ATP | Oxidative phosphorylation | Oxidative phosphorylation | Anaerobic glycolysis |
| Glycolytic enzyme activity | Low | Intermediate | High |
| Rate of fatigue | Slow | Intermediate | Fast |
| Myosin-ATPase activity | Low | High | High |
| Speed of contraction | Slow | Fast | Fast |
| Typical major locations | Postural muscles of back | Major muscles of legs | Extraocular muscles |

sections. Components of this cardiac muscle transverse tubule system have the same basic functions as their counterparts in skeletal muscle fibers.

Cardiac muscle fiber contraction is intrinsic and spontaneous, as evidenced by the continued contraction of the cells in tissue culture. Impulses for the rhythmic contraction (or heartbeat) are initiated, regulated, and coordinated locally by nodes of unique myocardial fibers specialized for impulse generation and conduction, which are discussed in Chapter 11. As with skeletal muscle fibers, contraction of individual myocardial fibers is all-or-none. The rate of contraction is modified by autonomic innervation at the nodes of conducting cells, with the sympathetic nerve supply accelerating and the parasympathetic supply decreasing the frequency of the impulses.

Secretory granules about 0.2–0.3 μm in diameter are found near atrial muscle nuclei and are associated with small Golgi complexes (Figure 10–17b). These granules release the peptide hormone atrial natriuretic factor (ANF) that acts on target cells in the kidney to affect Na⁺ excretion and water balance. The contractile cells of the heart's atria thus also serve an endocrine function.

Key features of cardiac muscle cells, with comparisons to those of skeletal muscle, are summarized in Table 10–1.

fish and amphibians, as well as newborn mice, do form new muscle when the heart is partially removed, despite the lack of satellite cells. Research on the possibility of mammalian **heart muscle regeneration** builds on work with the animal models, focusing primarily on the potential of mesenchymal stem cells to form new, site-specific muscle.

➤ SMOOTH MUSCLE

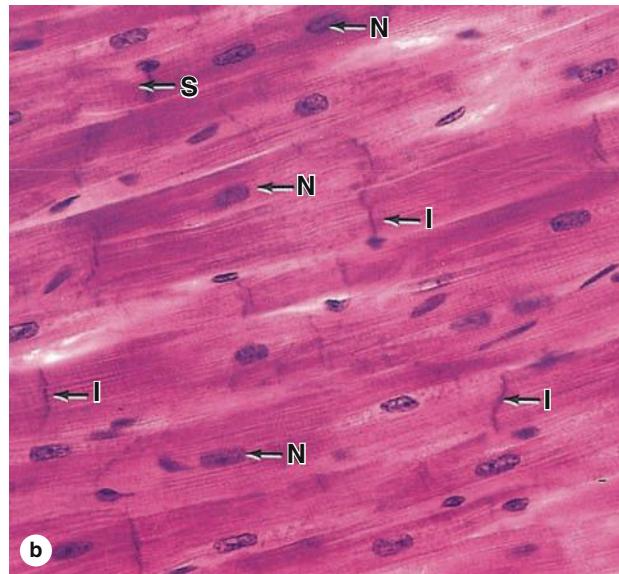
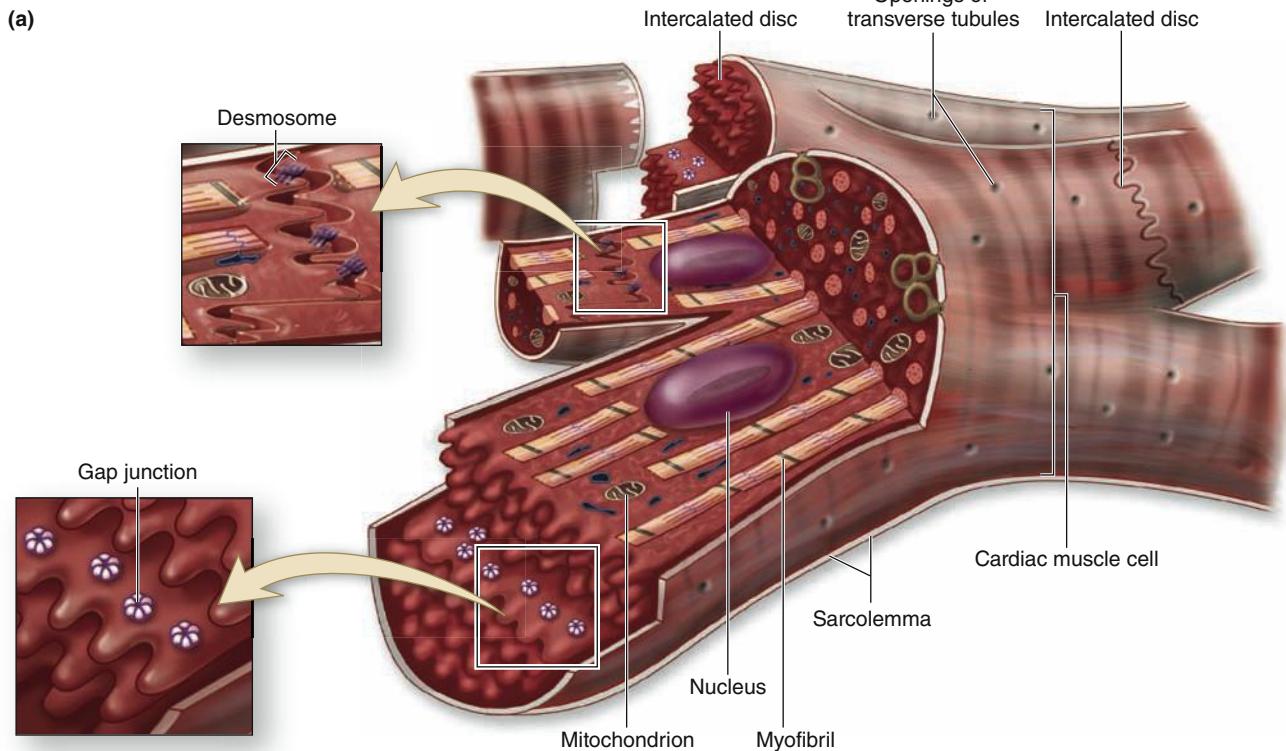
Smooth muscle is specialized for slow, steady contraction under the influence of autonomic nerves and various hormones. This type of muscle is a major component of blood vessels and of the digestive, respiratory, urinary, and reproductive tracts and their associated organs. Fibers of smooth muscle (also called **visceral muscle**) are elongated, tapering, and unstriated cells, each of which is enclosed by an external lamina and a network of type I and type III collagen fibers comprising the endomysium (Figure 10–18).

Smooth muscle cells range in length from 20 μm in small blood vessels to 500 μm in the pregnant uterus. At each cell's central, broadest part, where its diameter is 5–10 μm, is a single elongated nucleus. The cells stain uniformly along their lengths, and close packing is achieved with the narrow ends of each cell adjacent to the broad parts of neighboring cells. With this arrangement cross sections of smooth muscle show a range of cell diameters, with only the largest profiles containing a nucleus (Figures 10–18 and 10–19a). All cells are linked by numerous gap junctions. The borders of the cell become scalloped when smooth muscle contracts and the nucleus becomes distorted (Figure 10–20). Concentrated near the nucleus are mitochondria,

➤ MEDICAL APPLICATION

The most common injury sustained by cardiac muscle is that due to **ischemia**, or tissue damage due to lack of oxygen when coronary arteries are occluded by heart disease. Lacking muscle satellite cells, adult mammalian cardiac muscle has little potential to regenerate after injury. However, certain

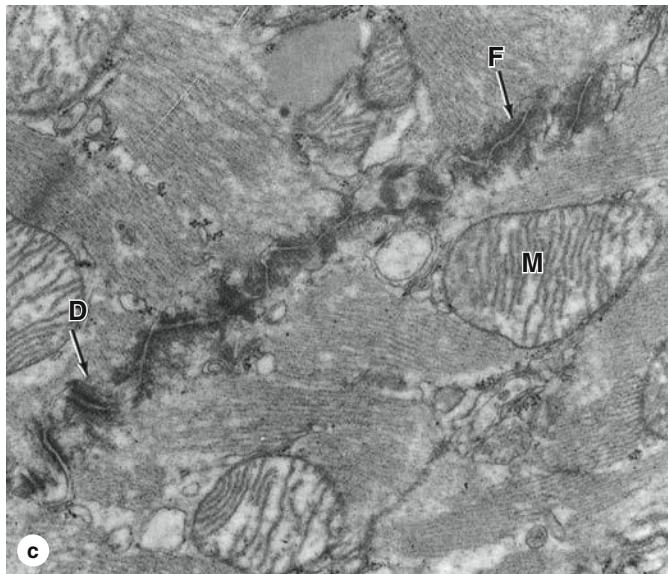
FIGURE 10–16 Cardiac muscle.



(a) The diagram of cardiac muscle cells indicates their characteristic features. The fibers consist of separate cells in a series joined at interdigitating regions called the **intercalated discs**, which cross an entire fiber between two cells. The transverse regions of the steplike intercalated disc have abundant **desmosomes** and other adherent junctions for firm adhesion, while longitudinal regions of the discs are filled with **gap junctions**.

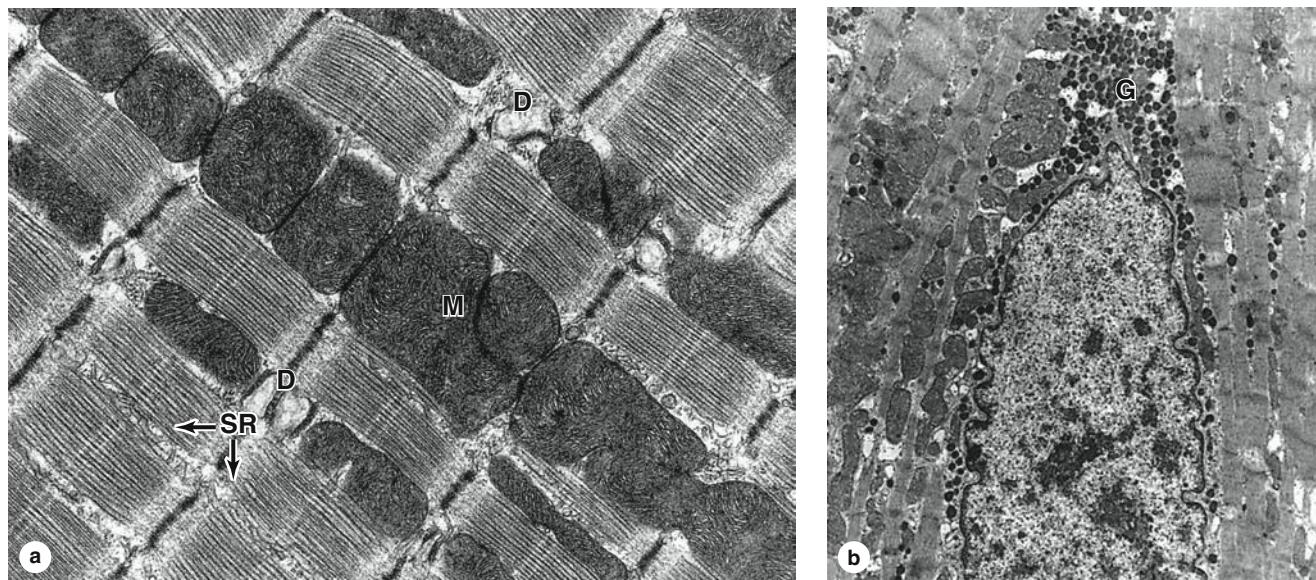
Cardiac muscle cells have central nuclei and myofibrils that are usually sparser and less well-organized than those of skeletal muscle. Also, the cells are often branched, allowing the muscle fibers to interweave in a more complicated arrangement within fascicles that produces an efficient contraction mechanism for emptying the heart.

(b) Light microscopy of cardiac muscle in longitudinal section show nuclei (N) in the center of the muscle fibers and widely



spaced intercalated discs (I) that cross the fibers. These irregular intercalated discs should not be confused with the repetitive, much more closely spaced striations (S), which are similar to those of skeletal muscle but less well-organized. Nuclei of fibroblasts in endomysium are also present. (X200; H&E)

(c) TEM showing an electron-dense intercalated disc with a step-like structure along the short interdigitating processes of adjacent cardiac muscle cells. As shown here transverse disc regions have many desmosomes (D) and adherent junctions called **fascia adherentes** (F) which join the cells firmly. Other regions of the disc have abundant gap junctions which join the cells physiologically. The sarcoplasm has numerous mitochondria (M) and myofibrillar structures similar to those of skeletal muscle but slightly less organized. (X31,000)

FIGURE 10–17 Cardiac muscle ultrastructure.

(a) TEM of cardiac muscle shows abundant mitochondria (**M**) and rather sparse sarcoplasmic reticulum (**SR**) in the areas between myofibrils. T-tubules are less well-organized and are usually associated with one expanded terminal cistern of SR, forming dyads (**D**) rather than the triads of skeletal muscle. Functionally, these structures are similar in these two muscle types. (X30,000)

(b) Muscle cells from the heart atrium show the presence of membrane-bound granules (**G**), mainly aggregated at the nuclear poles. These granules are most abundant in muscle cells of the right atrium (~600 per cell), but smaller quantities are also found

in the left atrium and the ventricles. The atrial granules contain the precursor of a polypeptide hormone, **atrial natriuretic factor (ANF)**. ANF targets cells of the kidneys to bring about sodium and water loss (natriuresis and diuresis). This hormone thus opposes the actions of aldosterone and antidiuretic hormone, whose effects on kidneys result in sodium and water conservation. (X10,000)

(Figure 10–17b, used with permission from Dr J. C. Nogueira, Department of Morphology, Federal University of Minas Gerais, Belo Horizonte, Brazil.)

polyribosomes, RER, and vesicles of a Golgi apparatus. The short plasmalemma invaginations resembling **caveolae** are often numerous at the surface of smooth muscle cells.

The fibers have rudimentary sarcoplasmic reticulum, but lack T-tubules; their function is unnecessary in these smaller, tapering cells with many gap junctions. Caveolae of smooth muscle cells contain the major ion channels that control Ca^{2+} release from sarcoplasmic cisternae at myofibrils which initiates contraction. The characteristic contractile activity of smooth muscle is generated by myofibrillar arrays of actin and myosin organized somewhat differently from those of striated muscle. In smooth muscle cells bundles of thin and thick myofilaments crisscross the sarcoplasm obliquely. The myosin filaments have a less regular arrangement among the thin filaments and fewer crossbridges than in striated muscle. Moreover smooth muscle actin filaments are not associated with troponin and tropomyosin, using instead **calmodulin** and Ca^{2+} -sensitive **myosin light-chain kinase (MLCK)** to produce contraction. The contraction mechanism, however, is basically similar to that in striated muscle.

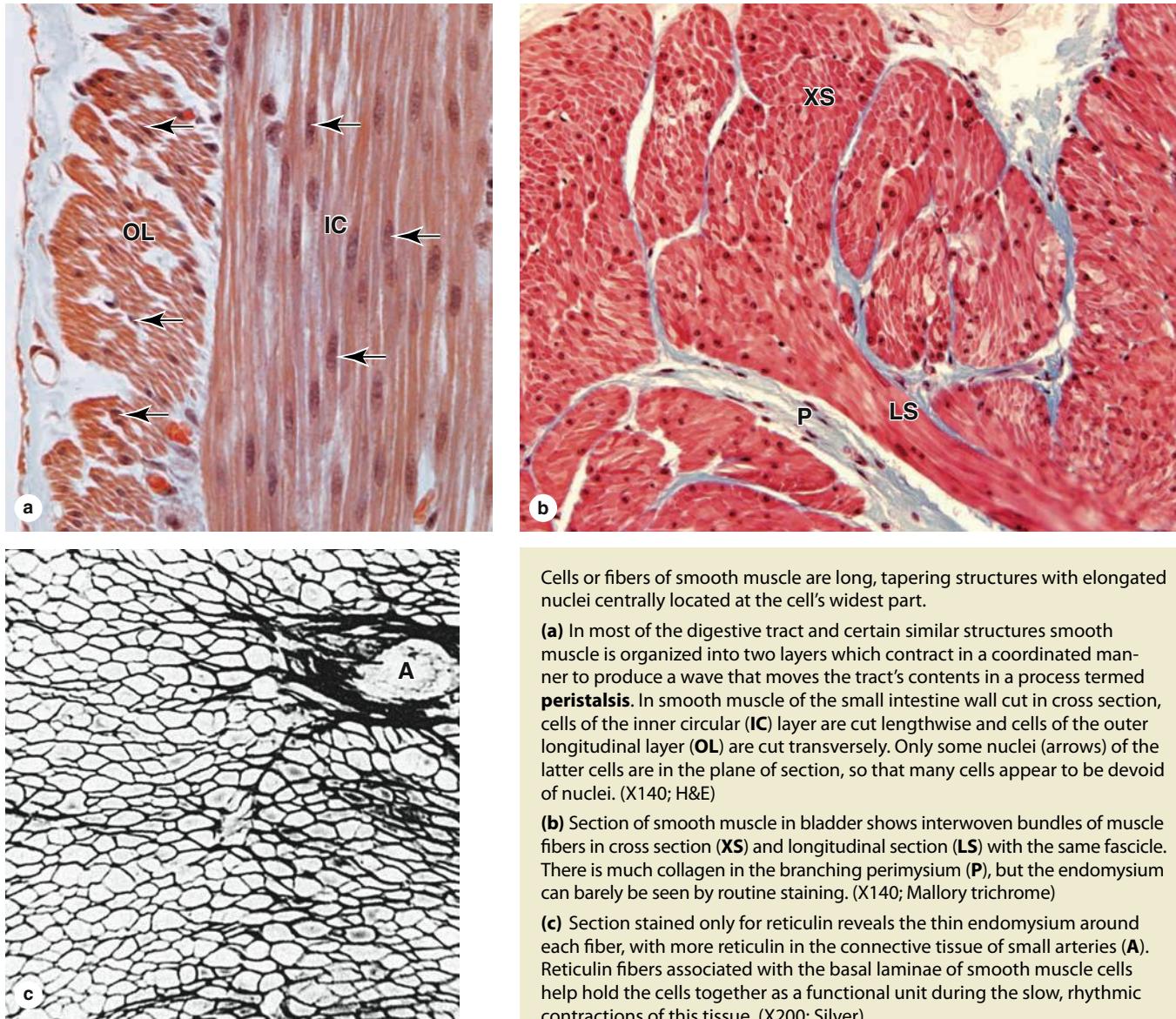
As shown in Figure 10–20 the actin myofilaments insert into anchoring cytoplasmic and plasmalemma-associated

dense bodies which contain α -actinin and are functionally similar to the Z discs of striated and cardiac muscle. Smooth muscle cells also have an elaborate array of 10-nm intermediate filaments, composed of desmin, which also attach to the dense bodies. The submembranous dense bodies include cadherins of desmosomes linking adjacent smooth muscle cells. Dense bodies in smooth muscle cells thus serve as points for transmitting the contractile force not only within the cells, but also between adjacent cells (Figure 10–20). The endomysium and other connective tissue layers help combine the force generated by the smooth muscle fibers into a concerted action, for example peristalsis in the intestine.

Smooth muscle is not under voluntary motor control and its fibers typically lack well-defined neuromuscular junctions. Contraction is most commonly stimulated by autonomic nerves, but in the gastrointestinal tract smooth muscle is also controlled by various paracrine secretions and in the uterus by oxytocin from the pituitary gland.

Axons of autonomic nerves passing through smooth muscle have periodic swellings or varicosities that lie in close contact with muscle fibers. Synaptic vesicles in the varicosities release a neurotransmitter, usually acetylcholine or

FIGURE 10–18 Smooth muscle.



norepinephrine, which diffuses and binds receptors in the sarcolemmae of numerous muscle cells. There is little or no specialized structure to such junctions. As in cardiac muscle, stimulation is propagated to more distant fibers via gap junctions which allow all the smooth muscle cells to contract synchronously or in a coordinated manner.

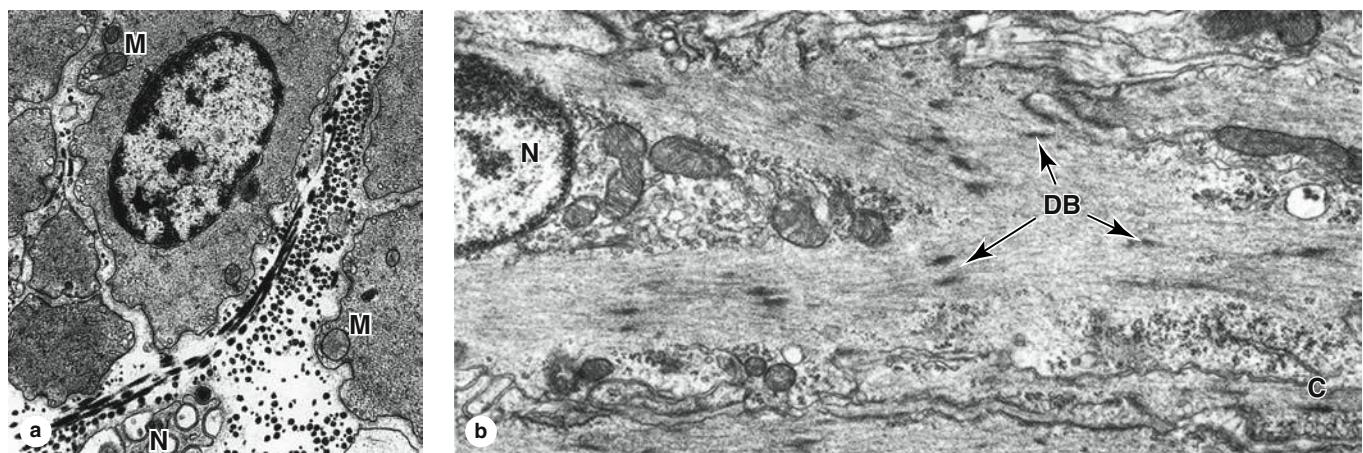
In addition to contractile activity, smooth muscle cells also supplement fibroblast activity, synthesizing collagen, elastin, and proteoglycans, with a major influence on the extracellular matrix (ECM) in tissues where these contractile cells are abundant. Active synthesis of ECM by the small cells/fibers of smooth muscle may reflect less specialization for strong contractions than in skeletal and cardiac muscle and is similar to this synthetic function in other contractile cells, such as myofibroblasts and pericytes.

Key histologic and functional features of smooth muscle, with comparisons to those of skeletal and cardiac muscle, are summarized in Table 10–1.

» MEDICAL APPLICATION

Benign tumors called **leiomyomas** commonly develop from smooth muscle fibers but are seldom problematic. They most frequently occur in the wall of the uterus, where they are more commonly called **fibroids** and where they can become sufficiently large to produce painful pressure and unexpected bleeding.

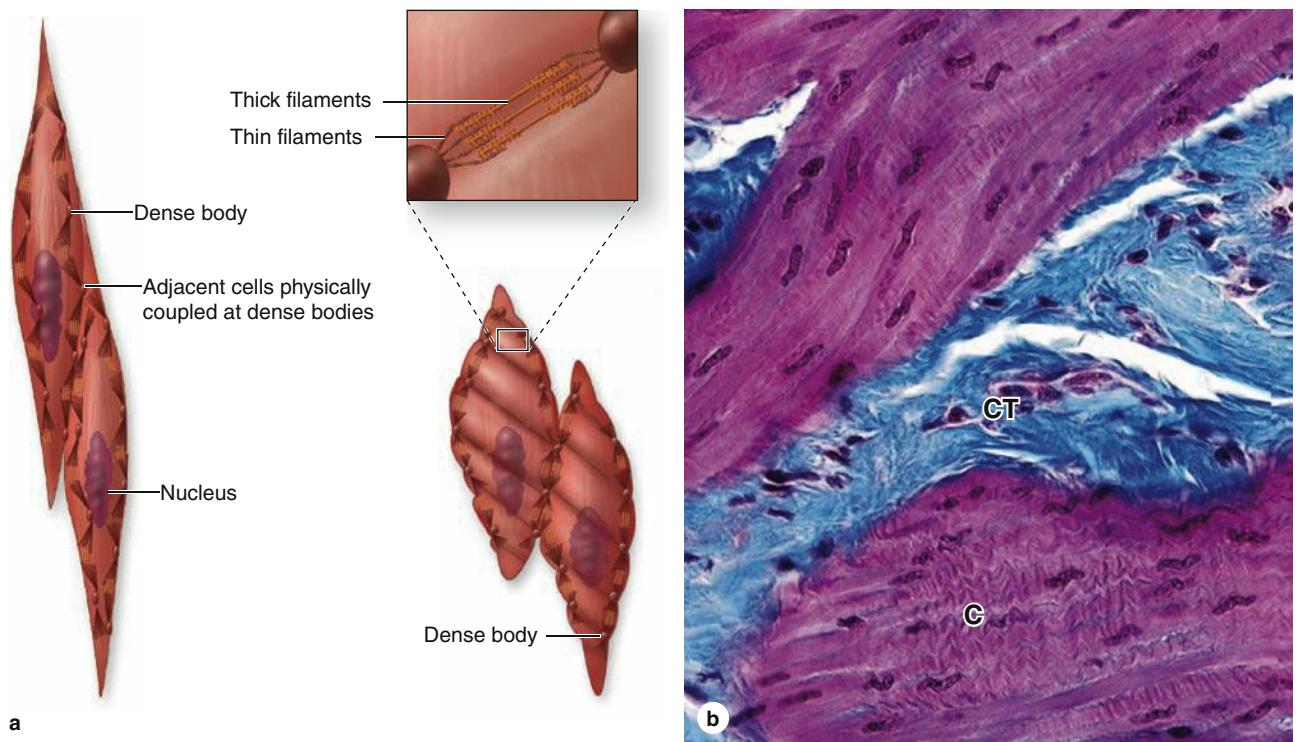
FIGURE 10–19 Smooth muscle ultrastructure.



(a) TEM of a transverse section of smooth muscle showing several cells sectioned at various points along their lengths, yielding profiles of various diameters with only the largest containing a nucleus. Thick and thin filaments are not organized into myofibril bundles, and there are few mitochondria (**M**). There is evidence of a sparse external lamina around each cell, and reticular fibers are abundant in the ECM. A small unmyelinated nerve (**N**) is also seen between the cells. (X6650)

(b) Longitudinal section showing several dense bodies (**DB**) in the cytoplasm and at the cell membrane. Thin filaments and intermediate filaments both attach to the dense bodies. In the cytoplasm near the nucleus (**N**) are mitochondria, glycogen granules, and Golgi complexes. In the lower right corner of the photo the cell membrane shows invaginations called caveolae (**C**) that may regulate release of Ca^{2+} from sarcoplasmic reticulum. (X9000)

FIGURE 10–20 Smooth muscle contraction.



Most molecules that allow contraction are similar in the three types of muscle, but the filaments of smooth muscle are arranged differently and appear less organized.

(a) The diagram shows that thin filaments attach to **dense bodies** located at the cell membrane and deep in the cytoplasm. Dense bodies contain α -actinin for thin filament attachment. Dense bodies at the membrane are also attachment sites for intermediate filaments and for adhesive junctions between cells. This arrangement of both

the cytoskeleton and contractile apparatus allows the multicellular tissue to contract as a unit, providing better efficiency and force.

(b) Micrograph showing a contracted (**C**) region of smooth muscle, with contraction decreasing the cell length and deforming the nuclei. The long nuclei of individual fibers assume a cork-screw shape when the fibers contract, reflecting the reduced cell length at contraction. Connective tissue (**CT**) of the perimysium outside the muscle fascicle is stained blue. (X240; Mallory trichrome)

► REGENERATION OF MUSCLE TISSUE

The three types of adult muscle have different potentials for regeneration after injury which are also summarized in Table 10–1.

In skeletal muscle, although the multinucleated cells cannot undergo mitosis, the tissue can still display limited regeneration. The source of regenerating cells is the sparse population of mesenchymal **satellite cells** lying inside the external lamina of each muscle fiber. Satellite cells are inactive, reserve myoblasts that persist after muscle differentiation. After injury the normally quiescent satellite cells become activated, proliferating and fusing to form new skeletal muscle fibers. Similar activity of satellite cells has been implicated in muscle growth after extensive exercise, a process in which they

fuse with existing fibers to increase muscle mass beyond that which occurs by cell hypertrophy. Following major traumatic injuries, scarring and excessive connective tissue growth interferes with skeletal muscle regeneration.

Cardiac muscle lacks satellite cells and shows very little regenerative capacity beyond early childhood. Defects or damage (eg, infarcts) to heart muscle are generally replaced by proliferating fibroblasts and growth of connective tissue, forming only myocardial scars.

Smooth muscle, composed of simpler, smaller, mononucleated cells, is capable of a more active regenerative response. After injury, viable smooth muscle cells undergo mitosis and replace the damaged tissue. As discussed in Chapter 11, contractile pericytes from the walls of small blood vessels participate in the repair of vascular smooth muscle.

Muscle Tissue SUMMARY OF KEY POINTS

- There are three major types of muscle: (1) **skeletal** or striated muscle, (2) **cardiac** muscle, and (3) **smooth** or visceral muscle.
- **Skeletal muscle cells** are very long, **multinucleated fibers**, cylindrically shaped and with **diameters up to 100 µm**.
- The **sarcolemma** of each fiber is surrounded by an external lamina and thin connective tissue, **endomysium**, containing capillaries.

Organization of Skeletal Muscle Fibers

- Groups of fibers called **fascicles** are surrounded by **perimysium**; all fascicles are enclosed within a dense connective tissue **epimysium**.
- Internally each muscle fiber is filled with myofibrils, composed of thousands of **thick myosin filaments** and **thin actin filaments**, highly organized into contractile units called **sarcomeres**.
- Within sarcomeres thick and thin filaments **interdigitate**; globular myosin heads project from the thick filaments toward the F-actin filaments, which are associated with **tropomyosin** and **troponin**.
- Sarcomeres are separated by **Z discs** that bisect the **light-staining I bands** that contain mainly the thin filaments attached to **α-actinin** in the Z disc.
- Between the two I bands of a sarcomere is the **dark-staining A band** with the thick myosin filaments; alternating light and dark bands appear as microscopic **striations** along the fibers.

Sarcoplasmic Reticulum & Transverse Tubule System

- In the sarcoplasm between parallel myofibrils are **mitochondria** and cisternae of smooth ER, called the **sarcoplasmic reticulum (SR)** specialized for **Ca²⁺ sequestration and release**.
- At each sarcomere, two **terminal cisterns** of SR contact a deep invagination of the sarcolemma called a **transverse or T-tubule**, forming a **triad** that triggers Ca²⁺ release when the sarcolemma is depolarized.

Mechanism of Contraction

- **Ca²⁺ binding to troponin** causes **tropomyosin to change shape** and allow the **myosin heads to bind the actin subunits**, forming **crossbridges** between thick and thin filaments.
- The myosin heads then pivot with **ATP hydrolysis**, which pulls the thin filaments along the thick filaments.
- With Ca²⁺ and ATP present, a **contraction cycle** ensues in which **myosin heads repeatedly attach, pivot, detach, and return**, causing the filaments to slide past one another, shortening the sarcomere.

- When the membrane **depolarization ends**, **Ca²⁺ is again sequestered**, ending contraction and allowing the sarcomeres to lengthen again as the **muscle relaxes**.
- Synapses of motor axons with skeletal muscle are called **motor end plates (MEPs)**, **neuromuscular junctions (NMJs)**, or **myoneural junctions**; the neurotransmitter is **acetylcholine**.
- A motor axon may form many terminal branches, each ending on an MEP of a muscle fiber; all fibers innervated by branches of that axon comprise a **motor unit**.

Muscle Spindles & Tendon Organs

- These are both **sensory proprioceptors** in which sensory **axons wrap around intrafusal fibers** in small specialized fascicles or around **myotendinous collagen bundles**, respectively.

Muscle Fiber Types

- Skeletal muscles contain fibers that can be physiologically classified as the three main types: (1) **slow, oxidative** (type I); (2) **fast, intermediate oxidative-glycolytic** (type IIa); and (3) **fast, glycolytic** (type IIb).

Cardiac Muscle

- **Cardiac muscle fibers** are also **striated**, but they consist of **individual cylindrical cells**, each containing **one (or two) central nuclei** and linked by adherent and gap junctions at prominent **intercalated discs**.
- **Sarcomeres** of cardiac muscle are organized and function similarly to those of skeletal muscle.
- Contraction of cardiac muscle is **intrinsic** at nodes of **impulse-generating pacemaker muscle fibers**; autonomic nerves regulate the rate of contraction.

Smooth Muscle

- **Smooth muscle fibers** are individual **small, fusiform (tapering) cells**, linked by numerous gap junctions.
- **Thin and thick filaments** in smooth muscle fibers do not form sarcomeres, and **no striations** are present.
- Thin actin filaments attach to **α-actinin** located in **dense bodies** that are located throughout the sarcoplasm and near the sarcolemma; contraction causes cells to shorten individually.
- Sarcoplasmic reticulum is less well-organized in smooth muscle fibers, and there is **no transverse tubule system**.

- Troponin is lacking in smooth muscle; proteins controlling the sliding filaments here include **myosin light-chain kinase (MLCK)** and the Ca^{2+} -binding protein **calmodulin**.

Regeneration of Muscle Tissue

- Repair and regeneration can occur in **skeletal muscle** because of a population of **reserve muscle satellite cells** that can proliferate, fuse, and form new muscle fibers.

- Cardiac muscle **lacks satellite cells** and has little capacity for regeneration.
- Regeneration is rapid in smooth muscle because the cells/fibers are small and relatively less differentiated, which allow **renewed mitotic activity** after injury.

Muscle Tissue ASSESS YOUR KNOWLEDGE

- The basal lamina of a muscle fiber is part of which structure?
 - Perimysium
 - Epimysium
 - Fascia
 - Endomysium
 - Sarcoplasmic reticulum
- With the transmission electron microscope skeletal muscle fibers can be seen to contain structures called triads. What do the two lateral components of a triad represent?
 - Attachment sites for thick myofilaments
 - Sites for calcium sequestration and release
 - Sites for impulse conduction into the fiber
 - Sites for ATP production
 - Sites for synthesis of proteins to be secreted outside the cell
- Which characteristic is unique to cardiac muscle?
 - Contain centrally located nuclei
 - Striated
 - Often branched
 - M multinucleated
 - Lack T-tubules
- In smooth muscle calcium released by the smooth ER initiates contraction by binding to what protein?
 - Actin
 - Calmodulin
 - Desmin
 - Myosin light chain kinase
 - Tropomyosin
- Which feature typifies T-tubules?
 - Evaginations of the sarcoplasmic reticulum
 - Sequester calcium during muscle relaxation, releasing it during contraction
 - Carry depolarization to the muscle fiber interior
 - Overlie the A-I junction in cardiac muscle cells
 - Rich supply of acetylcholine receptors
- Which characteristic is unique to smooth muscle?
 - T-tubules lie across Z lines
 - Each thick filament is surrounded by six thin filaments
 - Thin filaments attach to dense bodies
 - Cells are multinucleated
 - Cells have centrally located nuclei
- In one type of muscle, numerous gap junctions, desmosomes, and adherens junctions are specifically localized in which structures?
 - Myofilaments
 - Dense bodies
 - Sarcomeres
 - Neuromuscular spindles
 - Intercalated discs
- A 66-year-old man who lives alone has a severe myocardial infarction and dies during the night. The medical examiner's office is called the following morning and describes the man's body as being in *rigor mortis*. This state of *rigor mortis* is due to which one of the following?
 - Inhibition of Ca^{2+} leakage from the extracellular fluid and sarcoplasmic reticulum
 - Enhanced retrieval of Ca^{2+} by the sarcoplasmic reticulum
 - Failure to disengage tropomyosin and troponin from the myosin active sites
 - Absence of ATP preventing detachment of the myosin heads from actin
 - Increased lactic acid production
- A 5-year-old boy sustains a small tear in his gastrocnemius muscle when he is involved in a bicycle accident. Regeneration of the muscle will occur through which of the following mechanisms?
 - Dedifferentiation of muscle cells into myoblasts
 - Differentiation of muscle satellite cells
 - Fusion of damaged myofibers to form new myotubes
 - Hyperplasia of existing muscle fibers
 - Differentiation of fibroblasts to form myoblasts
- A healthy 32-year-old man lifts weights regularly as part of his workout. In one of his biceps muscle fibers at rest, the length of the I band is $1.0 \mu\text{m}$ and the A band is $1.5 \mu\text{m}$. Contraction of that muscle fiber results in a 10% shortening of the length of the sarcomere. What is the length of the A band after the shortening produced by muscle contraction?
 - $1.50 \mu\text{m}$
 - $1.35 \mu\text{m}$
 - $1.00 \mu\text{m}$
 - $1.90 \mu\text{m}$
 - $0.45 \mu\text{m}$