Note: Large images and tables on this page may necessitate printing in landscape mode.

CONNECTIVE TISSUE: INTRODUCTION

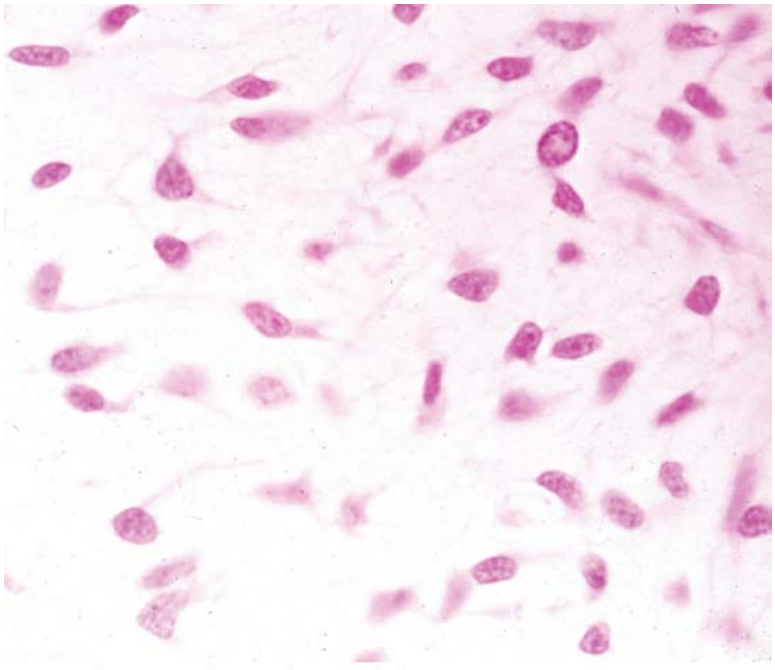
The different types of connective tissue are responsible for providing and maintaining the form of organs throughout the body. Functioning in a mechanical role, they provide a matrix that connects and binds other tissues and cells in organs and gives metabolic support to cells as the medium for diffusion of nutrients and waste products.

Structurally, connective tissue is formed by three classes of components: cells, fibers, and ground substance. 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, reticular, and elastic fibers) and **ground substance.** Ground substance is a highly hydrophilic, viscous complex of anionic macromolecules (glycosaminoglycans and proteoglycans) and multiadhesive glycoproteins (laminin, fibronectin, and others) that stabilizes the ECM by binding to receptor proteins (**integrins**) on the surface of cells and to the other matrix components. In addition to its major structural role, molecules of connective tissue serve other important biological functions, such as forming a reservoir of factors controlling cell growth and differentiation. The hydrated nature of much connective tissue provides the medium through which nutrients and metabolic wastes are exchanged between cells and their blood supply.

The wide variety of connective tissue types in the body reflects variations in the 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.

The connective tissues originate from the **mesenchyme,** an embryonic tissue formed by elongated undifferentiated cells, the **mesenchymal cells** (Figure 5–1)**.** These cells are characterized by oval nuclei with prominent nucleoli and fine chromatin. They possess many thin cytoplasmic processes and are immersed in an abundant and viscous extracellular substance containing few fibers. The mesenchyme develops mainly from the middle layer of the embryo, the **mesoderm.** Mesodermal cells migrate from their site of origin in the embryo, surrounding and penetrating developing organs. In addition to being the point of origin of all types of connective tissue cells, mesenchyme develops into other types of structures, such as blood cells, endothelial cells, and muscle cells.

Figure 5–1.



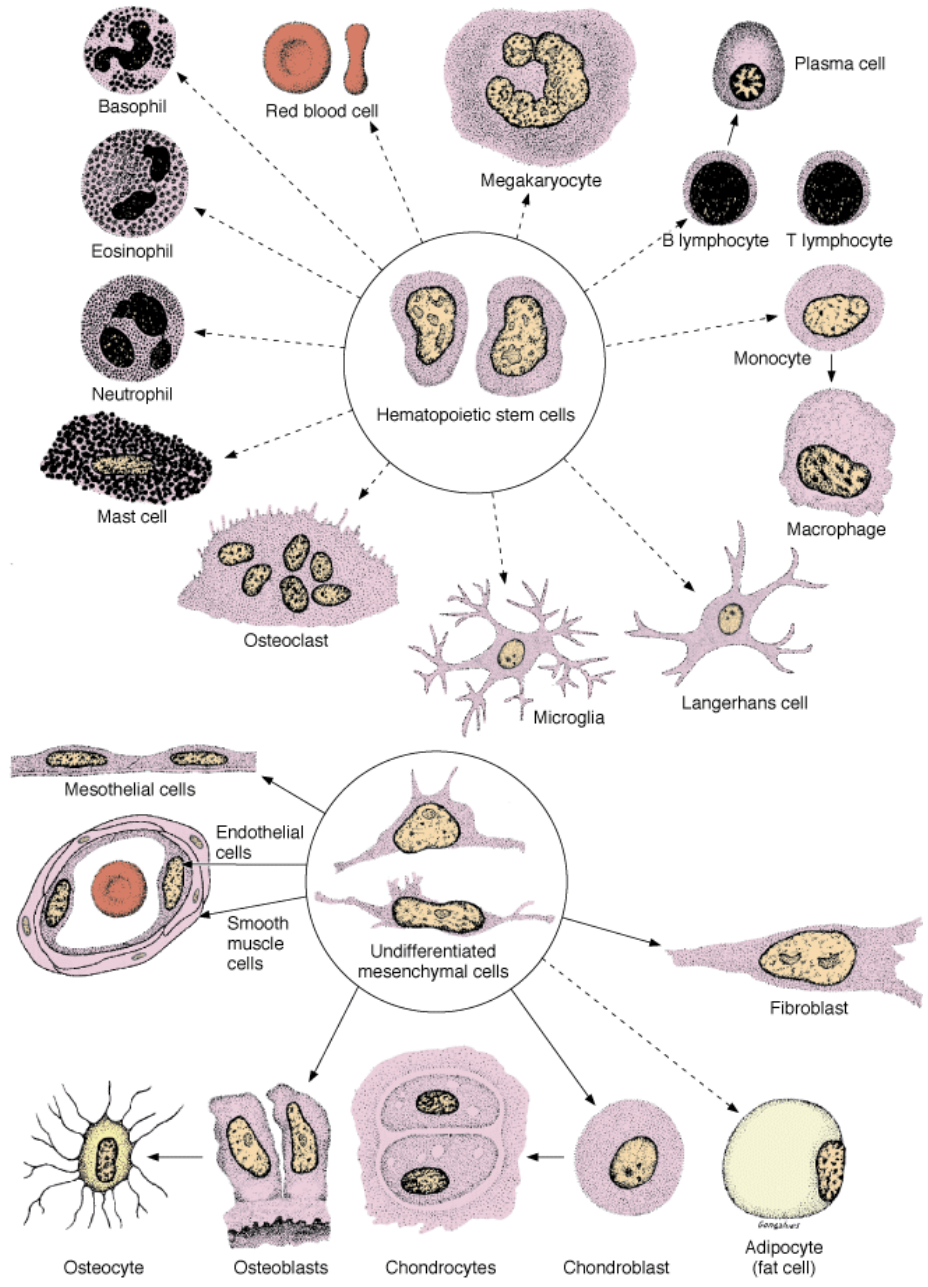
Embryonic mesenchyme.

Mesenchyme consists of a population of undifferentiated cells, generally elongated but with many shapes, having large euchromatic nuclei and prominent nucleoli which indicate high levels of synthetic activity. These cells are called **mesenchymal cells**. Mesenchymal cells are surrounded by an extracellular matrix which they produced and which consists largely of a simple ground substance rich in hyaluronan (hyaluronic acid). This section is stained with Masson trichrome which stains collagen fibers blue and the lack of collagen in mesenchyme is apparent. X200.

CELLS OF CONNECTIVE TISSUE

A variety of cells with different origins and functions are present in connective tissue (Figure 5–2 and Table 5–1). **Fibroblasts** originate locally from undifferentiated mesenchymal cells and spend all their life in connective tissue; other cells such as **mast cells**, **macrophages**, and **plasma cells** originate from hematopoietic stem cells in bone marrow, circulate in the blood, and then move into connective tissue where they remain and execute their functions. White blood cells (leukocytes) are transient cells of most connective tissues; they also originate in the bone marrow and move to the connective tissue where they reside for a few days, then usually die by apoptosis.

Figure 5–2.



Lineages of connective tissue cells.

This simplified representation of the connective tissue cell lineage includes cells from the multipotential embryonic **mesenchyme cells** and **hematopoietic stem cells** of bone marrow. Dotted arrows indicate that one or more intermediate cell types exist between the examples illustrated. The cells are not drawn in proportion to actual sizes, eg, adipocyte, megakaryocyte, and osteoclast cells are significantly larger than the other cells illustrated.

Table 5–1. Functions of connective tissue cells.

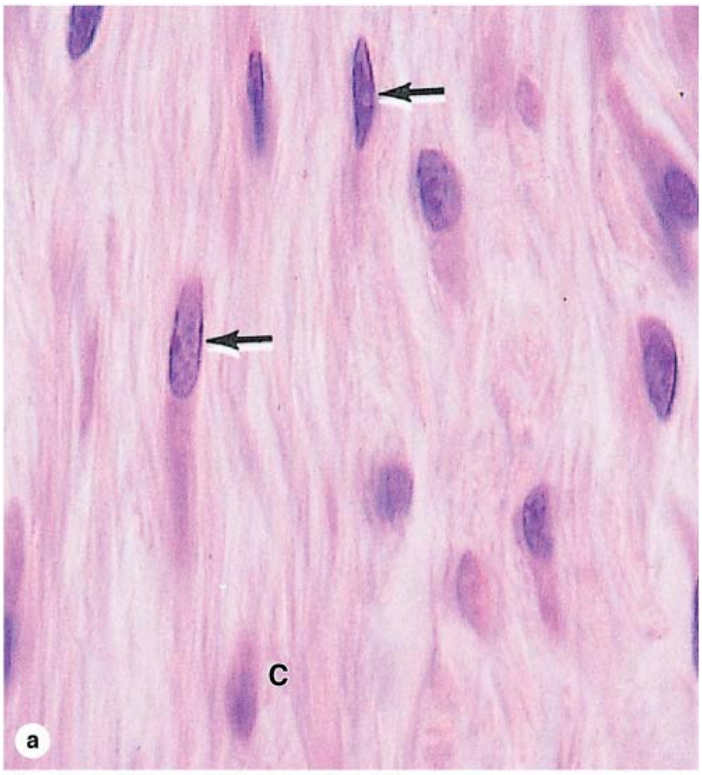
Cell Type	Representative Product or Activity	Representative Function
Fibroblast, chondroblast, osteoblast, odontoblast	Production of fibers and ground substance	Structural
Plasma cell	Production of antibodies	Immunologic (defense)
Lymphocyte (several types)	Production of immunocompetent cells	Immunologic (defense)
Eosinophilic leukocyte	Participation in allergic and vasoactive reactions, modulation of mast cell activities and the inflammatory process	Immunologic (defense)
Neutrophilic leukocyte	Phagocytosis of foreign substances, bacteria	Defense
Macrophage	Secretion of cytokines and other molecules, phagocytosis of foreign substances and bacteria, antigen processing and presentation to other cells	Defense
Mast cell and basophilic leukocyte	Liberation of pharmacologically active molecules (eg, histamine)	Defense (participate in allergic reactions)
Adipocyte	Storage of neutral fats	Energy reservoir, heat production

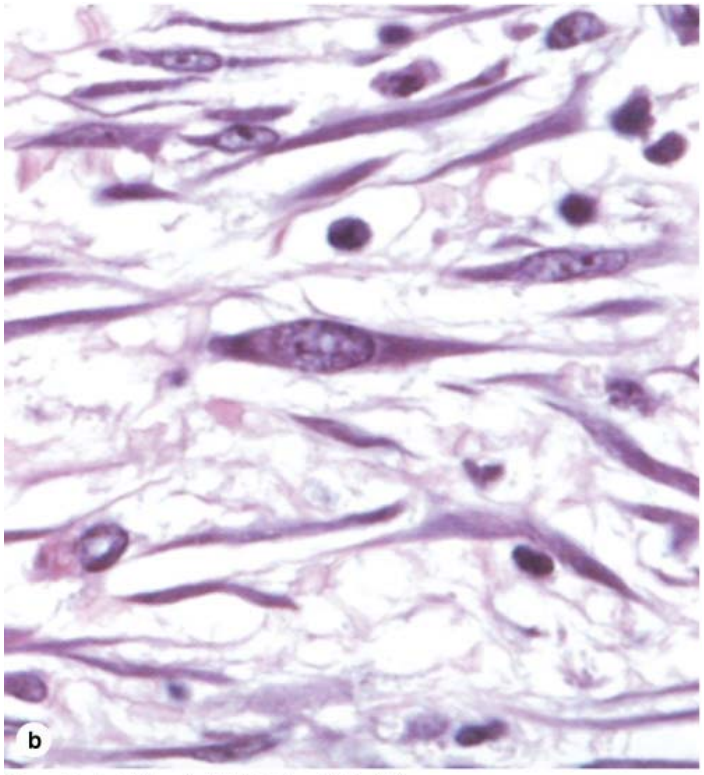
Fibroblasts

Fibroblasts synthesize collagen, elastin, glycosaminoglycans, proteoglycans and multiadhesive glycoproteins. Fibroblasts are the most common cells in

connective tissue (Figure 5–3) and are responsible for the synthesis of extracellular matrix components. Two stages of activity—active and quiescent—are often observed in these cells (Figure 5–3b). Cells with intense synthetic activity are morphologically distinct 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.

Figure 5–3.





Fibroblasts.

Connective tissue where parallel bundles of collagen are being formed. **(a):** Fibroblasts typically show large active nuclei and eosinophilic cytoplasm tapering off in both directions along the axis of the nucleus, a morphology usually called "spindle-shaped." The nuclei (arrows) are clearly seen, but the cytoplasmic processes resemble the collagen bundles (C) that fill the extracellular matrix 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 are large cells with large, euchromatic nuclei and basophilic cytoplasm, whereas inactive fibroblast or fibrocytes are smaller with less prominent, heterochromatic nuclei. The very basophilic round cells in (b) are leukocytes. Both X400. H&E.

The active fibroblast has an abundant and irregularly branched cytoplasm. Its nucleus is ovoid, large, and pale-staining, with fine chromatin and a prominent

nucleolus. The cytoplasm is rich in rough ER, and the Golgi apparatus is well developed. The quiescent fibroblast or fibrocyte is smaller than the active fibroblast and is usually spindle-shaped. It has fewer processes; a smaller, darker, elongated nucleus; and more acidophilic cytoplasm with much less RER.

Fibroblasts synthesize most components of connective tissue ECM, including proteins, such as collagen and elastin, which upon secretion form collagen, reticular, and elastic fibers, and the glycosaminoglycans, proteoglycans, and glycoproteins of the ground substance. Fibroblasts are targets of various **growth factors** that influence cell growth and differentiation. In adults, fibroblasts in connective tissue rarely undergo division; mitosis can resume when the organ requires additional fibroblasts as in wound healing.

MEDICAL APPLICATION

The regenerative capacity of the connective tissue is clearly observed when tissues are destroyed by inflammation or traumatic injury. In these cases, the spaces left after injury to tissues whose cells do not divide (eg, cardiac muscle) are filled by connective tissue, which forms a scar. The healing of surgical incisions depends on the reparative capacity of connective tissue. The main cell type involved in repair is the fibroblast.

When it is adequately stimulated, such as during wound healing, the fibrocyte reverts to the fibroblast state, and its synthetic activities are reactivated. In such instances the cell reassumes the form and appearance of a fibroblast. The **myofibroblast**, a cell with features of both fibroblasts and smooth muscle cells, is also observed during wound healing. These cells have most of the morphological characteristics of fibroblasts but contain increased amounts of actin microfilaments and myosin and behave much like smooth muscle cells. Their activity is responsible for wound closure after tissue injury, a process called **wound contraction**.

Adipocytes

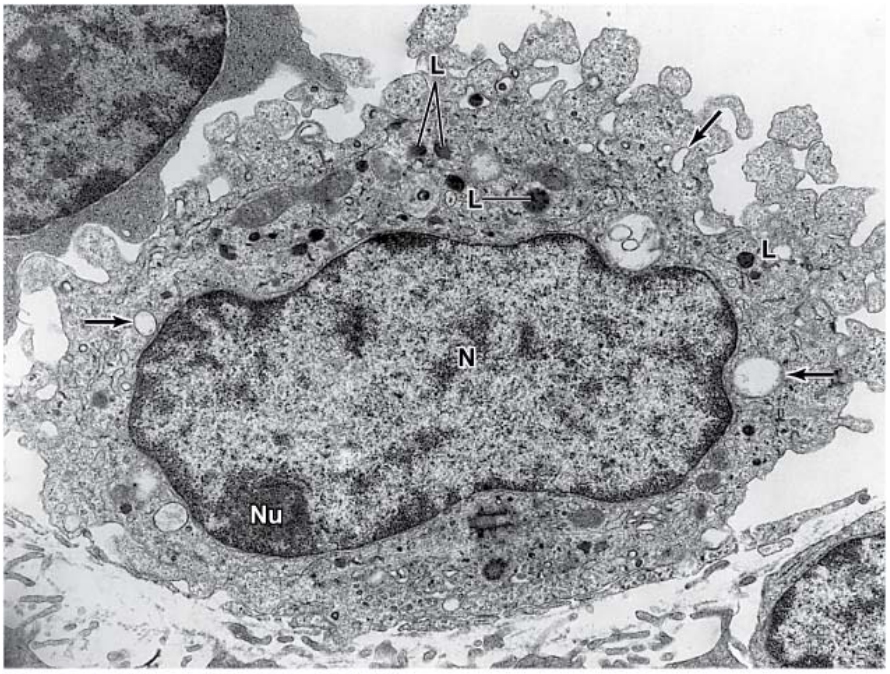
Adipocytes (L. *adeps,* fat, + Gr. *kytos,* cell) are connective tissue cells that have become specialized for storage of neutral fats or for the production of heat. Often called **fat cells,** they have considerable metabolic significance and are discussed in detail in Chapter 6.

Macrophages & the Mononuclearphagocyte System

Macrophages were discovered and initially characterized by their phagocytic ability. They have a wide spectrum of morphologic features that correspond to their state of functional activity and to the tissue they inhabit.

In the electron microscope, macrophages are characterized by an irregular surface with pleats, protrusions, and indentations, a morphologic expression of their active pinocytotic and phagocytic activities. They generally have a well-developed Golgi apparatus, many lysosomes, and rough ER (Figure 5–4).

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.

Macrophages derive from bone marrow precursor cells that divide, producing **monocytes** which circulate in the blood. These cells cross the wall of venules and capillaries to penetrate the connective tissue, where they mature and acquire the morphologic features of **macrophages.** Therefore, monocytes and macrophages are the same cell in different stages of maturation. Macrophages are also sometimes referred to as "histiocytes."

Macrophages are distributed throughout the body and are present in most organs. Along with other monocyte-derived cells, they comprise a family of cells called the **mononuclearphagocyte system** (Table 5–2). All are long-living cells and may survive for months in the tissues. In most organs these cells are highly important for the up-take, processing, and presentation of antigens for lymphocyte activation. The macrophage-like cells have been given different names in different organs, eg, Kupffer cells in the liver, microglial cells in the central nervous system, Langerhans cells in the skin, and osteoclasts in bone tissue. However, all are derived from monocytes. 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. A typical macrophage measures between 10 and 30 μm in diameter and has an oval or kidney-shaped nucleus located eccentrically.

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

Cell Type	Location	Main Function
Monocyte	Blood	Precursor of macrophages
Macrophage	Connective tissue, lymphoid organs, lungs, bone marrow	Production of cytokines, chemotactic factors, and several other molecules that participate in inflammation (defense), antigen processing and presentation
Kupffer cell	Liver	Same as macrophages
Microglia cell	Nerve tissue of the central nervous system	Same as macrophages
Langerhans cell	Skin	Antigen processing and presentation
Dendritic cell	Lymph nodes	Antigen processing and presentation
Osteoclast	Bone (fusion of several macrophages)	Digestion of bone
Multinuclear giant cell	Connective tissue (fusion of several macrophages)	Segregation and digestion of foreign bodies

MEDICAL APPLICATION

When adequately stimulated, macrophages may increase in size and are arranged in clusters forming **epithelioid** cells (named for their vague resemblance to epithelial cells), or several may fuse to form **multinuclear giant cells.** Both cell types are usually found only in pathological conditions.

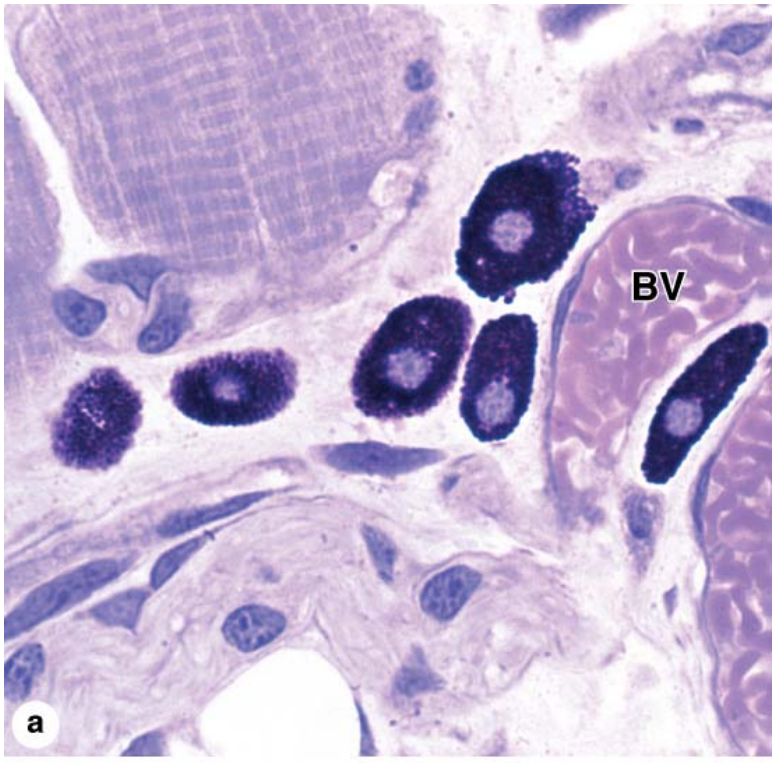
Macrophages act as defense elements. They phagocytose cell debris, abnormal extracellular matrix elements, neoplastic cells, bacteria, and inert elements that penetrate the organism. Macrophages are also antigen-presenting cells that participate in the processes of partial digestion and presentation of antigen to other cells (see Chapter 14). A typical example of an antigen-processing cell is the macrophage present in the skin epidermis, called the Langerhans cell (see Chapter 18). Although macrophages are the main antigen presenting cells, under certain circumstances many other cell types, such as fibroblasts, endothelial cells, astrocytes, and thyroid epithelial cells, are also able to perform this function. Macrophages also participate in cell-mediated resistance to infection by bacteria, viruses, protozoans, fungi, and metazoans (eg, parasitic worms); in cell-mediated resistance to tumors; and in extrahepatic bile production, iron and fat metabolism, and the destruction of aged erythrocytes.

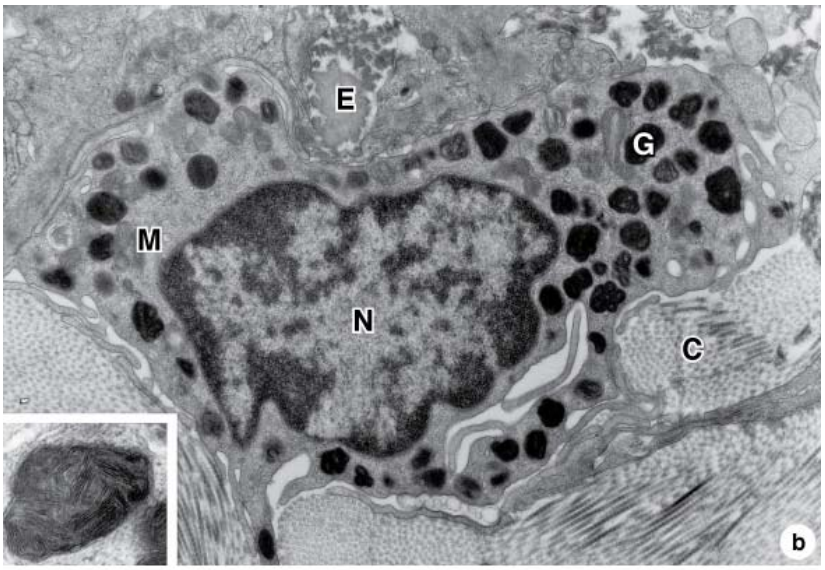
When macrophages are stimulated (by injection of foreign substances or by infection), they change their morphological characteristics and metabolism. They are then called **activated macrophages** and acquire characteristics not present in their nonactivated state. These activated macrophages, in addition to showing an increase in their capacity for phagocytosis and intracellular digestion, exhibit enhanced metabolic and lysosomal enzyme activity. Macrophages also have an important role in removing cell debris and damaged extracellular components formed during the physiological involution process. For example, during pregnancy the uterus increases in size. Immediately after parturition, the uterus suffers an involution during which some of its tissues are destroyed by the action of macrophages. Macrophages are also secretory cells that produce an impressive array of substances, including enzymes (eg, collagenase) and cytokines that participate in defensive and reparative functions, and they exhibit increased tumor cell–killing capacity.

Mast Cells

Mast cells are large, oval or round connective tissue cells, 20–30 μm in diameter, whose cytoplasm is filled with basophilic secretory granules. The rather small, spherical nucleus is centrally situated and may be obscured by the cytoplasmic granules (Figure 5–5).

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).

The secretory granules are 0.3–2.0 μm in diameter. Their interior is electron-dense and heterogeneous. Mast cells function in the localized release of many bioactive substances with roles in the inflammatory response, innate immunity, and tissue repair.

Because of their high content of acidic radicals in their sulfated glycosaminoglycans, 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 are frequently difficult to identify. Mast cell granules contain a wide variety of paracrine compounds that promote different aspects of a local inflammatory response. A partial list of important molecules released from these granules includes:

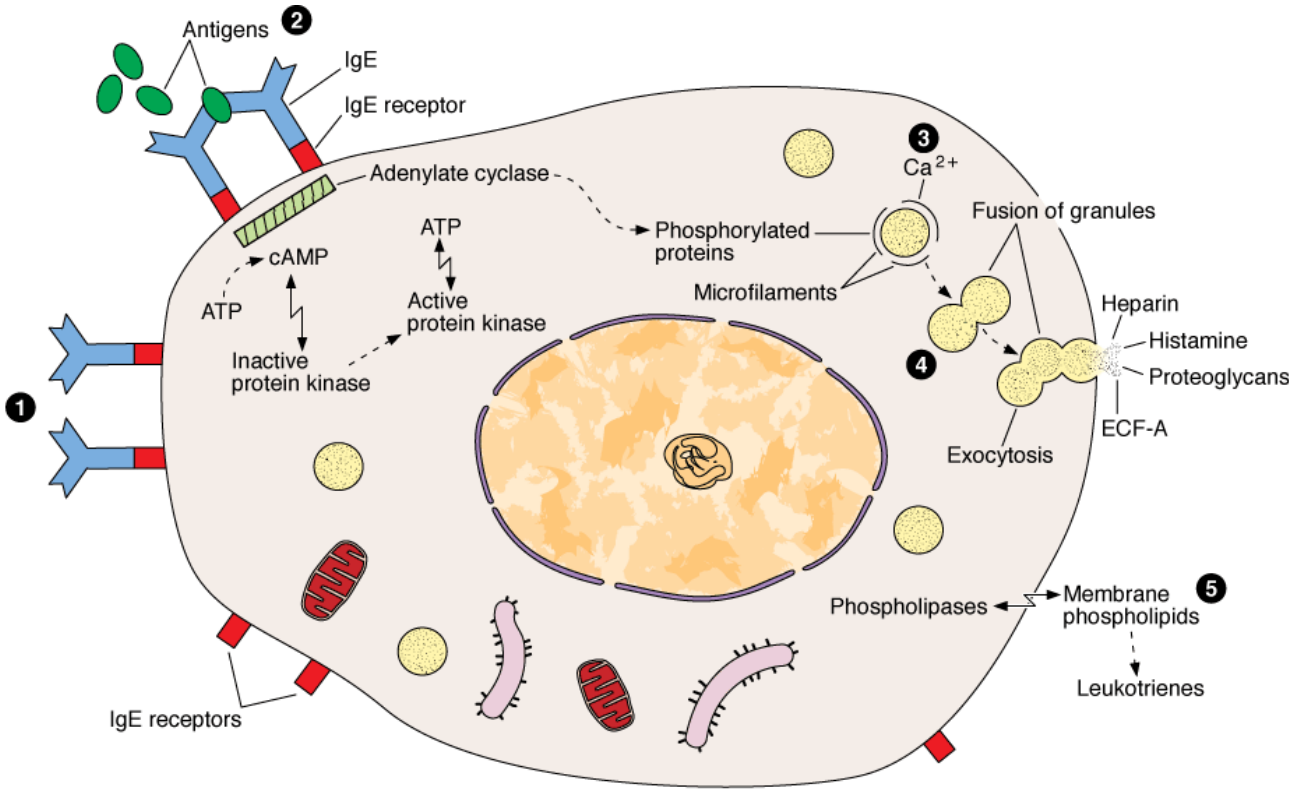
- **Heparin**, a sulfated glycosaminoglycan that acts locally as an anticoagulant
- **Histamine**, which promotes increased vascular permeability and smooth muscle contraction
- **Serine proteases**, which activate various mediators of inflammation
- **Eosinophil** and **neutrophil chemotactic factors** which attract those leukocytes
- **Leukotrienes C_4, D_4, and E_4** (or the slow-reacting substance of anaphylaxis, SRS-A) which also trigger smooth muscle contraction.

Mast cells occur in many connective tissues, but are especially numerous near small blood vessels in skin and mesenteries (**perivascularmast cells**) and in the mucosa lining digestive and respiratory tracts (**mucosal mast cells**). The average size and granular content of these two populations differ somewhat.

Mast cells originate from progenitor cells in the bone marrow. These progenitor cells circulate in the blood, cross the wall of venules and capillaries, and penetrate connective tissues, where they proliferate and differentiate. Although they are in many respects similar to basophilic leukocytes, they have a separate stem cell.

Release of the chemical mediators stored in mast cells promotes the allergic reactions known as **immediate hypersensitivity reactions,** because they occur within a few minutes after penetration by an antigen of an individual previously sensitized to the same or a very similar antigen. There are many examples of immediate hypersensitivity reaction; a dramatic one is anaphylactic shock, a potentially fatal condition. The process of anaphylaxis consists of the following sequential events: The first exposure to an antigen (allergen), such as bee venom, results in production of the IgE class of immunoglobulins (antibodies) by plasma cells. IgE is avidly bound to the surface of mast cells. A second exposure to the antigen results in binding of the antigen to IgE on the mast cells. This event triggers release of the mast cell granules, liberating histamine, leukotrienes, ECF-A, and heparin (Figure 5–6). Degranulation of mast cells also occurs as a result of the action of the complement molecules that participate in the immunological reaction cited in Chapter 14. Histamine causes contraction of smooth muscle (mainly of the bronchioles) and dilates and increases permeability (mainly in postcapillary venules). Any liberated histamine is inactivated immediately after release. Leukotrienes produce slow contractions in smooth muscle, and ECF-A attracts blood eosinophils. Heparin is a blood anticoagulant, but blood clotting remains normal in humans during anaphylactic shock. Mast cells are widespread in the human body but are particularly abundant in the dermis and in the digestive and respiratory tracts.

Figure 5–6.



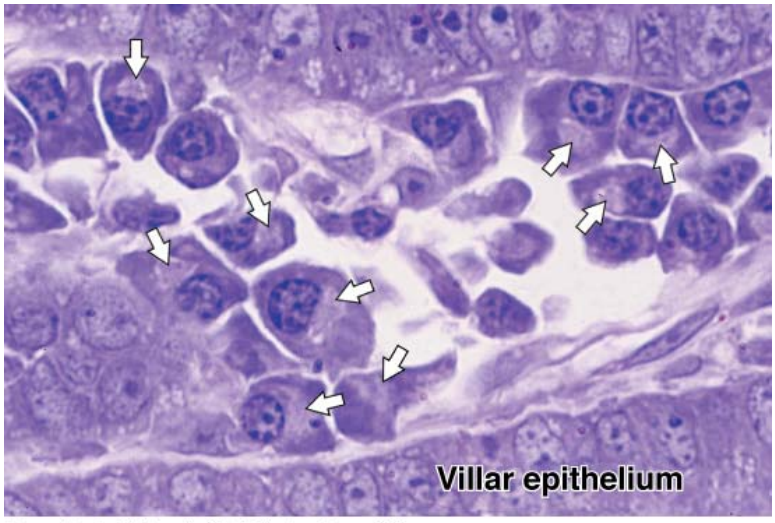
Mast cell secretion.

Mast cell secretion is triggered by re-exposure 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 and (**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 which together normally comprise part of the inflammatory process called the immediate hypersensitivity reaction. ECF-A, eosinophil chemotactic factor of anaphylaxis.

Plasma Cells

Plasma cells are large, ovoid cells that have a basophilic cytoplasm due to their richness in rough ER. The juxtanuclear Golgi apparatus and the centrioles occupy a region that appears pale in regular histologic preparations (Figure 5–7).

Figure 5–7.



Plasma cells.

Plasma cells are abundant in this portion of an inflamed intestinal villus. The plasma cells are characterized by their abundant basophilic cytoplasm involved in the synthesis of antibodies. A large pale Golgi apparatus (arrows) near each nucleus is the site of the terminal glycosylation of the antibodies (glycoproteins). Plasma cells can leave their sites of origin in lymphoid tissues, move to connective tissue, and produce the antibodies that mediate immunity. X400 PT.

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, a configuration that can give the nucleus of a plasma cell the appearance of a clock-face. There are few plasma cells in most connective tissues. Their average lifespan is short, 10–20 days.

MEDICAL APPLICATION

Plasma cells are derived from B lymphocytes and are responsible for the synthesis of antibodies. Antibodies are immunoglobulins produced in response to penetration by antigens. Each antibody is specific for the one antigen that gave rise to its production and reacts specifically with molecules possessing similar epitopes (see Chapter 14). The results of the antibody-antigen reaction are variable. The capacity of the reaction to neutralize harmful effects caused by antigens is important. An antigen that is a toxin (eg, tetanus, diphtheria) may lose its capacity to do harm when it combines with its respective antibody.

Leukocytes

Connective tissue normally contains leukocytes that migrate from the blood vessels by diapedesis. Leukocytes (Gr. *leukos,* white, + *kytos*), or white blood corpuscles, are the wandering cells of the connective tissue. They leave blood by migrating between the endothelial cells lining capillaries and postcapillary venules to enter connective tissue by a process called **diapedesis**. This process increases greatly during inflammation, which is a vascular and cellular defensive reaction against foreign substances, in most cases pathogenic bacteria or irritating chemical substances. The classic signs of inflammation were first described by Celsus in the first century as redness and swelling with heat and pain (*rubor et tumor cum calore et dolore*).

Inflammation begins with the local release of **chemical mediators of inflammation,** substances of various origin (mainly from local cells and blood plasma proteins) that induce some of the events characteristic of inflammation, eg, **increase of blood flow** and **vascular permeability, chemotaxis,** and **phagocytosis**.

MEDICAL APPLICATION

Increased vascular permeability is caused by the action of vasoactive substances; an example is histamine, which is liberated from mast cells and basophilic leukocytes. Increases in blood flow and vascular permeability are responsible for local swelling (edema), redness, and heat. Pain is due mainly to the action of chemical mediators on nerve endings. **Chemotaxis** (Gr. chemeia, *alchemy,* + taxis, orderly arrangement), the phenomenon by which specific cell types are attracted by some molecules, is responsible for the migration of large quantities of specific cell types to regions of inflammation. As a consequence of chemotaxis, leukocytes cross the walls of venules and capillaries by diapedesis, invading the inflamed areas.

Leukocytes do not return to the blood after arriving in connective tissue except for the lymphocytes. These cells circulate continuously in various compartments of the body: blood, lymph, lymphatic organs, and the interstitial fluid of connective tissue. Lymphocytes are particularly abundant in the connective tissue of the digestive tract. A detailed analysis of the structure and functions of leukocytes and lymphocytes is presented in Chapters 12 and 14.

FIBERS

The connective tissue fibers are formed by proteins that polymerize into elongated structures. The three main types of connective tissue fibers are **collagen, reticular,** and **elastic fibers.** Collagen and reticular fibers are both formed by the protein **collagen,** and elastic fibers are composed mainly of the protein **elastin.** These fibers are distributed unequally among the types of connective tissue and the predominant fiber type is usually responsible for conferring specific properties on the tissue.

Collagen

The collagens constitute a family of proteins selected during evolution for the execution of several (mainly structural) functions. During the process of evolution of multicellular organisms, a family of structural proteins was selected by both environmental influences and the functional requirements of the animal organism and developed to acquire varying degrees of rigidity, elasticity, and strength. These proteins are known collectively as **collagen,** and the chief examples among its various types are present in the skin, bone, cartilage, smooth muscle, and basal lamina.

Collagen is the most abundant protein in the human body, representing 30% of its dry weight. The collagens are produced by several cell types and are distinguishable by their molecular compositions, morphologic characteristics, distribution, functions, and pathologies. More than 20 types of collagen have been identified and designated with Roman numerals; the most important of these are listed in Table 5–3. They are classified into the following four categories according to their structure and general functions.

Table 5–3. Collagen types.

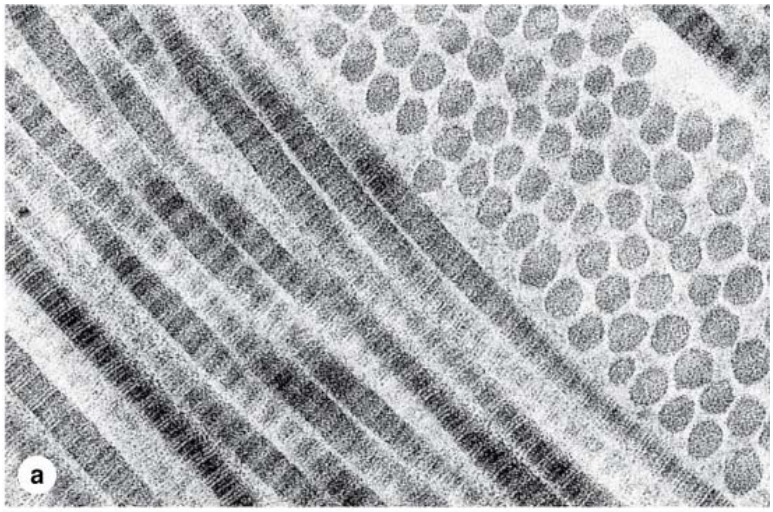
Type	Molecule Composition	Structure	Optical Microscopy	Representative Tissues	Main Function
Collagens that form fibrils					
I	$[\alpha 1\ (I)]_2[\alpha 2\ (I)]$	300-nm molecule, 67-nm banded fibrils	Thick, highly picrosirius birefringent, nonargyrophilic fibers	Skin, tendon, bone, dentin	Resistance 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 fibers	Skin, muscle, blood vessels, frequently together with type I	Structural maintenance in expansible 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	[α1 (XI)] [α2 (XI)] [α3 (XI)]	300-nm molecule	Small fibers	Cartilage	Participates in type II collagen function
Fibril-associated collagens					
IX	[α1 (IX)] [α2 (IX)] [α3 (IX)]	200-nm molecule	Not visible, detected by immunocytochemistry	Cartilage, vitreous body	Bound glycosaminoglycans; associated with type II collagen
XII	$[\alpha 1\ (XII)]_3$	Large N-terminal domain; interacts with type I collagen	Not visible, detected by immunocytochemistry	Embryonic tendon and skin	Interacts with type I collagen
XIV	$[\alpha 1\ (XIV)]_3$	Large N-terminal domain; cross-shaped molecule	Not visible; detected by immunocytochemistry	Fetal skin and tendon	
Collagen that forms anchoring fibrils					
VII	$[\alpha 1\ (VII)]_3$	450 nm, globular domain at each end	Not visible, detected by immunocytochemistry	Epithelia	Anchors skin epidermal basal lamina to underlying stroma
Collagen that forms networks					

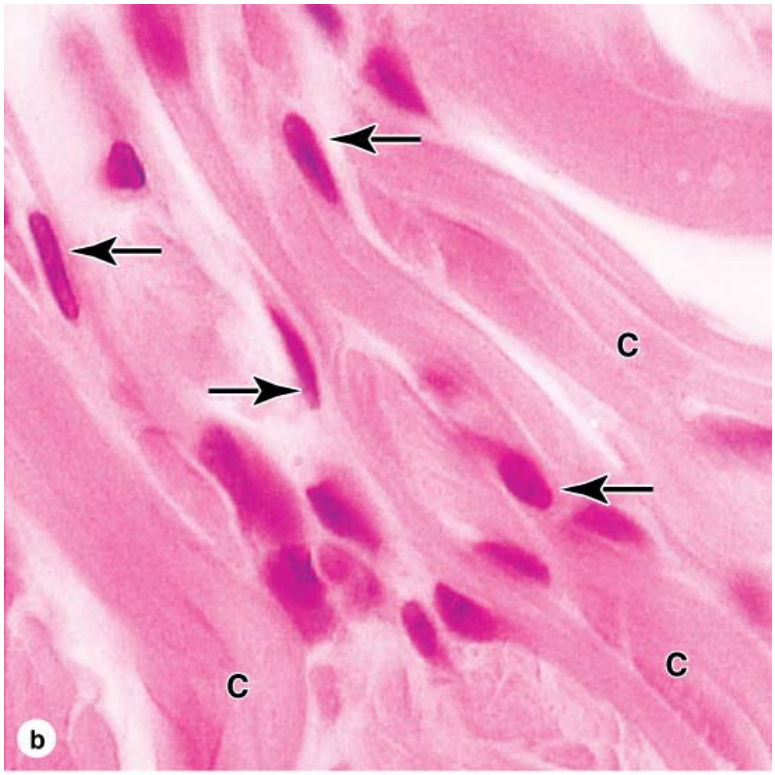
Type	Molecule Composition	Structure	Optical Microscopy	Representative Tissues	Main Function
Collagens that form fibrils					
IV	$[\alpha 1\ (VII)]_2\ [\alpha 1\ (IV)]$	Two-dimensional cross-linked network	Not visible, detected by immunocytochemistry	All basement membranes	Support of delicate structures, filtration

COLLAGENS THAT FORM LONG FIBRILS

The molecules of long fibril–forming collagens aggregate to form fibrils clearly visible in the electron or light microscope (Figure 5–8). Collagen type I is the most abundant and has a widespread distribution. It occurs in tissues as structures that are classically designated as **collagen fibers** forming structures such as tendons, organ capsules, and dermis.

Figure 5–8.





Type I collagen.

Molecules of type I collagen, the most abundant type, assemble to form much larger structures. **(a):** TEM shows fibrils cut longitudinally and transversely. In longitudinal sections the fibrils display alternating dark and light bands that are further divided by cross-striations and in cross-section the cut ends of individual collagen molecules can be seen. Ground substance completely surrounds the fibrils. X100,000. **(b):**In H&E stained tissues, type I collagen fibrils can often be seen to aggregate further into large collagen bundles (C) of very eosinophilic fibers. Subunits for these fibers were secreted by fibroblasts (arrows) associated with them. X 400.

FIBRIL-ASSOCIATED COLLAGENS

Fibril-associated collagens are short structures that bind the surfaces of collagen fibrils to one another and to other components of the ECM. Molecules in this

category are also known as FACIT collagens, an acronym for "fibril-associated collagens with interrupted triple helices."

COLLAGENS THAT FORM ANCHORING FIBRILS

Anchoring collagen is type VII collagen, present in the anchoring fibrils that bind the basal lamina to reticular fibers in the underlying connective tissue (Figure 4–2).

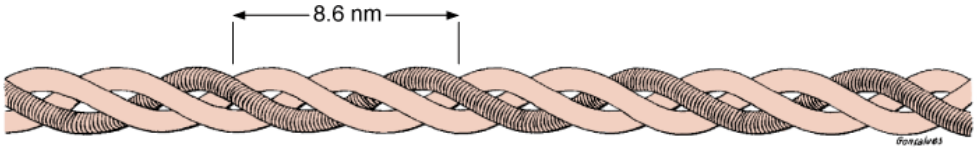
COLLAGENS THAT FORM NETWORKS

An important network-forming collagen is type IV collagen, whose molecules assemble in a meshwork that constitutes a major structural component of the basal lamina.

Collagen synthesis, an activity once thought restricted to fibroblasts, chondroblasts, osteoblasts, and odontoblasts, has now been shown to occur in many cell types. The polypeptides initially formed on ribosomes of the rough ER are called **procollagen α chains**, which intertwine in ER cisternae to make triple helices. Every third amino acids in the α chains is glycine; two other small amino acids abundant in collagen are hydroxylated post-translationally to form **hydroxyproline** and **hydroxylysine.** Many different α chains have been identified, encoded by related genes and varying in length and amino acid sequence.

The triple helix of α chains forms a rod-like procollagen molecule**,** which in type I and II collagen measures 300 nm in length and 1.5 nm in width. Procollagen molecules may be homotrimeric, with all three chains identical, or heterotrimeric, with two or all three chains having different sequences (Figure 5–9). Different combinations of the many procollagen α chains in procollagen molecules are largely responsible for the various types of collagen with different structures and functional properties. In collagen types I, II, and III, collagen molecules aggregate and become packed together to form **fibrils.** Hydrogen bonds and hydrophobic interactions are important in the aggregation and packing of these subunits. In a subsequent step, this structure is reinforced by the formation of covalent cross-links, a process catalyzed by lysyl oxidases.

Figure 5–9.

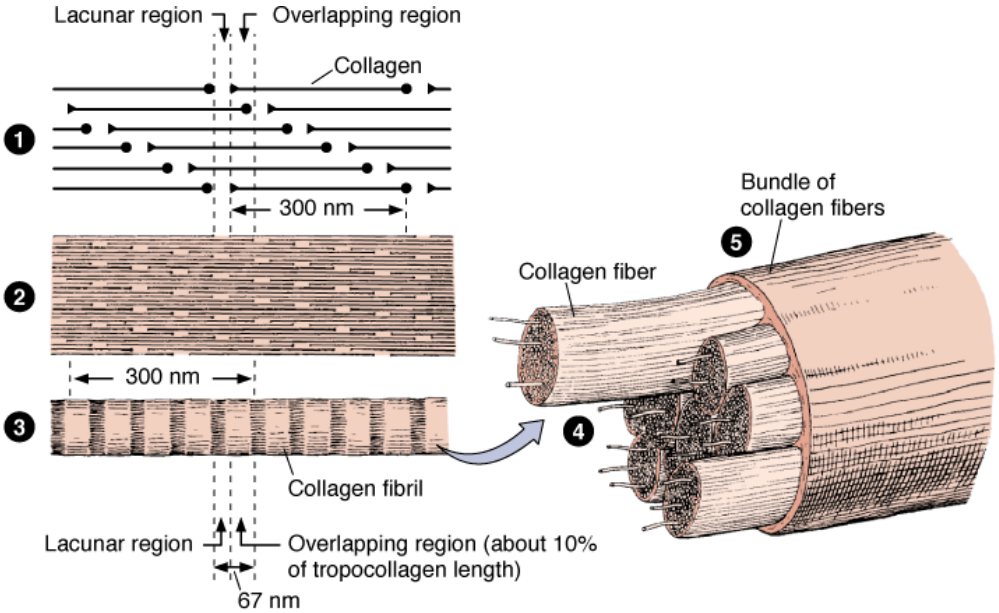


Procollagen.

In the most abundant form of collagen, type I, each procollagen molecule is composed of two α1 and one α2 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. Each complete turn of the helix spans a distance of 8.6 nm. The length of each tropocollagen molecule is 300 nm, and its width is 1.5 nm.

Collagen fibrils are thin, elongated structures with diameters ranging from 20 to 90 nm and can be several micrometers in length; they have transverse striations with a characteristic periodicity of 64-68 nm (Figure 5–10). The striations are caused by the regular, overlapping arrangement of the collagen molecule subunits (Figure 5–10). The dark (electron-dense) bands retain more of the lead-based stain used in TEM studies because their more numerous free chemical groups react more intensely with the lead solution than do the light bands. In some collagen types these fibrils associate further with FACIT collagens to form fibers. In collagen type I, the fibers can form large bundles (Figure 5–10). Collagen type II (present in cartilage) occurs as fibrils but does not form fibers or bundles. Collagen type IV, present in all basement membranes, assembles as a lattice-like network in the basal lamina.

Figure 5–10.



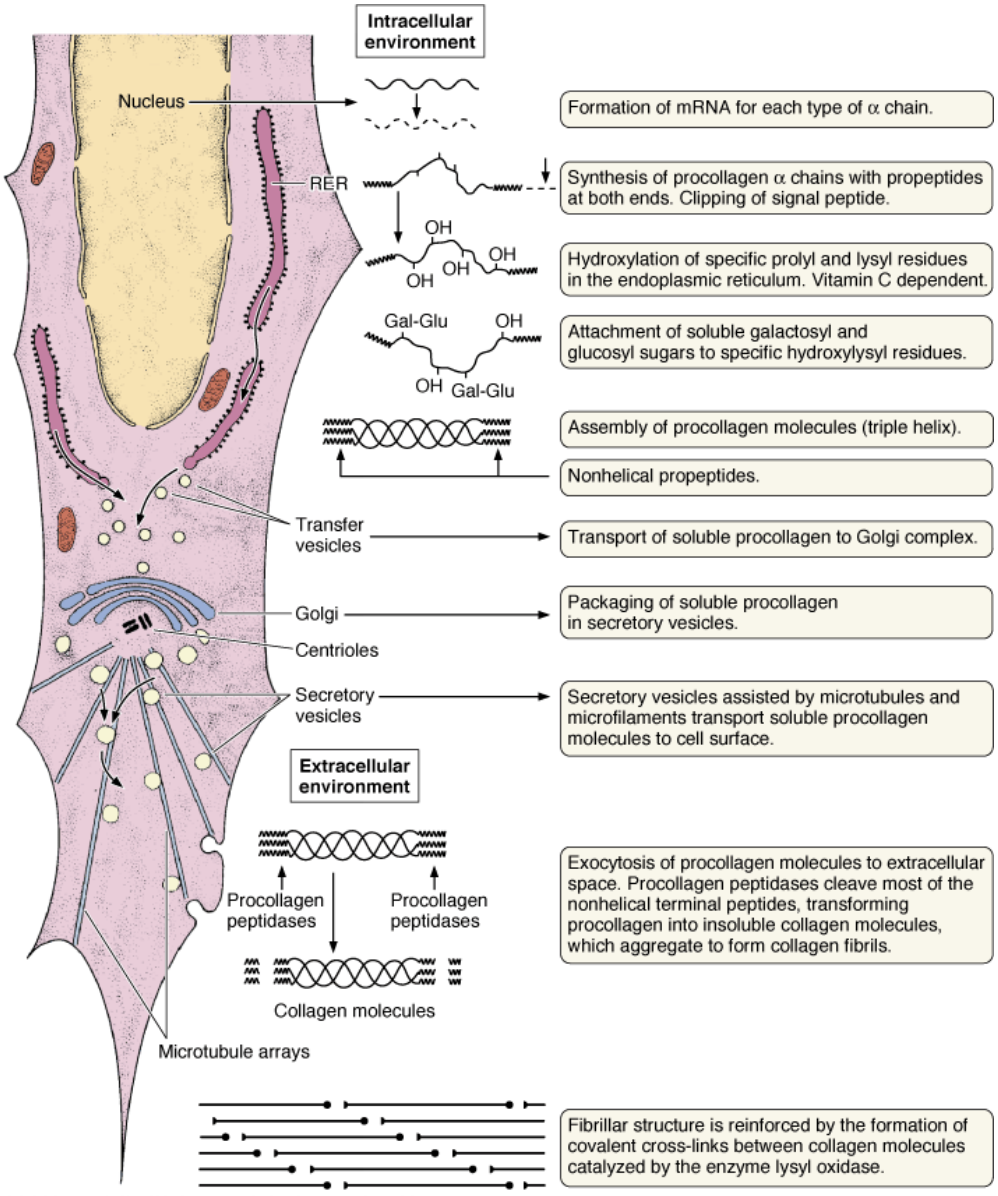
Assembly of collagen molecules into collagen fibers.

This diagram shows an aggregate of collagen molecules, fibrils, fibers, and bundles. There is a stepwise overlapping arrangement of rodlike collagen molecules, each measuring 300 nm (**1**). This arrangement results in the production of alternating spaces and overlapping regions (**2**), which cause the cross-striations characteristic of collagen fibrils and confer a 67-nm periodicity of dark and light bands when the fibril is observed in the electron microscope (**3**). Fibrils aggregate and are covalently cross-linked to form fibers (**4**), which in collagen type I aggregate further to form bundles (**5**) routinely called collagen fibers when seen by light microscopy.

Because collagen type I is so abundant, its synthesis has been studied most thoroughly. Synthesis of this important protein involves several steps, which are summarized in Figure 5–11:

1. Polypeptides called procollagen α chains are produced on polyribosomes bound to membranes of RER and translocated into the cisternae and the signal peptide is clipped off.
2. Hydroxylation of proline and lysine begins after the peptide chain has reached a certain minimum length and is still bound to the ribosomes. The enzymes involved are **prolyl hydroxylases** and **lysyl hydroxylase** and the reactions require O_2, Fe^{2+}, and ascorbic acid (vitamin C) as co-factors.
3. Glycosylation of some hydroxylysine residues occurs, with the various collagen types having different amounts of galactose linked to hydroxylysines.
4. Both the amino- and carboxyl-terminal ends of each α chain make up nonhelical portions of the polypeptides, sometimes called the extension propeptides, which may help ensure that the appropriate α chains (α1, α2) assemble in the correct position as a triple helix. In addition, the nonhelical propeptides make the resulting **procollagen molecule** soluble and prevent its premature intracellular assembly and precipitation as collagen fibrils. Procollagen is transported through the Golgi network and undergoes exocytosis to the extracellular environment.
5. Outside the cell, specific proteases called **procollagen peptidases** remove the extending propeptides, converting the procollagen molecules to collagen molecules. These are now capable of self-assembly into polymeric collagen fibrils, usually in specialized niches near the cell surface.
6. In some collagen types, fibrils aggregate to form fibers. Certain proteoglycans and types of collagen (types V and XI) participate in the aggregation of collagen molecules to form fibrils and in the formation of fibers from fibrils. FACIT collagens help stabilize the molecules in collagen fibrils and fibers and bind these structures to other components of the ECM.
7. Fibrillar structure is reinforced further by the formation of covalent cross-links between assembled collagen molecules, a process catalyzed by the extracellular enzyme **lysyl oxidase**.

Figure 5–11.



Collagen synthesis.

Hydroxylation and glycosylation of procollagen α chains and their assembly into triple helices occurs 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 post-translational events involving several other enzymes, many diseases involving defective collagen synthesis have been described.

The other fibrillar collagens are formed in processes similar to that described for collagen type I. In summary, collagen synthesis involves a cascade of unique

post-translational modifications of the original procollagen polypeptides. All these modifications are critical to the structure and function of normal mature collagen. 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.

Although fresh collagen fibers are colorless strands, when present in large numbers (eg, in tendons) they appear white. The highly regular orientation of subunits in collagen fibers makes them birefringent under the polarizing microscope (Figure 1–7). In the light microscope, collagen fibers are acidophilic; they stain pink with eosin, blue with Mallory trichrome stain, green with Masson trichrome stain, and red with Sirius red. Because of the long and tortuous course of collagen bundles, their length and diameter are better studied in spread preparations than in histologic sections (Figure 1–7). 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. Mesentery consists of a central portion of connective tissue lined on both surfaces by a simple squamous epithelium, the mesothelium. The collagen fibers in a spread preparation appear as elongated and tortuous cylindrical structures of indefinite length, with a diameter that varies from 1 to 20 μm.

MEDICAL APPLICATION

Collagen synthesis depends on the expression of several genes and several posttranslational events. It should not be surprising, therefore, that a large number of pathological conditions are directly attributable to insufficient or abnormal collagen synthesis.

Certain mutations in the α1 (I) or α2 (I) genes lead to **osteogenesis imperfecta**. Many cases of osteogenesis imperfecta are due to deletions of all or part of the α1 (I) gene. However, a single amino acid change is sufficient to cause certain forms of this disease, particularly mutations involving glycine. Glycine must be at every third position for the collagen triple helix to form.

In addition to these disorders, several diseases result from an over-accumulation of collagen. In **progressive systemic sclerosis,** almost all organs may present an excessive accumulation of collagen (**fibrosis**). This occurs mainly in the skin, digestive tract, muscles, and kidneys, causing hardening and functional impairment of the implicated organs.

Keloid is a local swelling caused by abnormal amounts of collagen that form in scars of the skin. Keloids, which occur most often in individuals of black African descent, can be a troublesome clinical problem to manage; not only can they be disfiguring, but excision is almost always followed by recurrence.

Vitamin C (ascorbic acid) deficiency leads to scurvy, a disease characterized by the degeneration of connective tissue. Without this vitamin, fibroblasts synthesize defective collagen, and the defective fibers are not replaced. This process leads to a general degeneration of connective tissue that becomes more pronounced in areas in which collagen renewal takes place at a faster rate. The periodontal ligament that holds teeth in their sockets has a relatively high collagen turnover; consequently, this ligament is markedly affected by scurvy, which leads to a loss of teeth. Ascorbic acid is a cofactor for proline hydroxylase, which is essential for the normal synthesis of collagen. Table 5–4 lists a few examples of the many disorders caused by failure of collagen biosynthesis.

Table 5–4. Examples of clinical disorders resulting from defects in collagen synthesis.

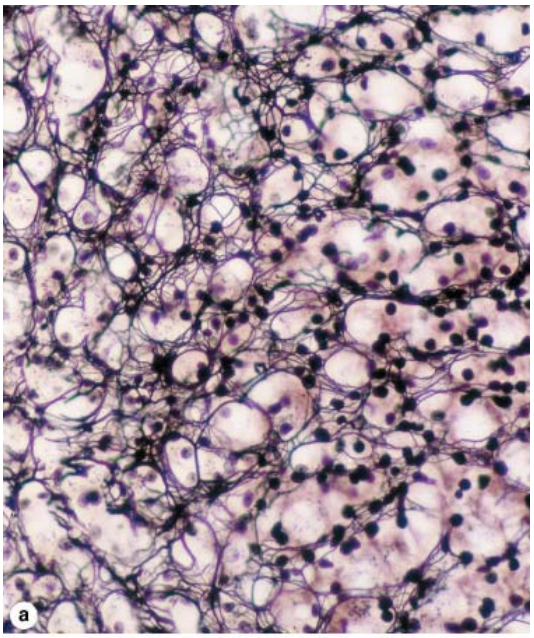
Disorder	Defect	Symptoms
Ehlers-Danlos type IV	Faulty transcription or translation of collagen type III	Aortic and/or intestinal rupture
Ehlers-Danlos type VI	Faulty lysine hydroxylation	Augmented skin elasticity, rupture of eyeball
Ehlers-Danlos type VII	Decrease in procollagen peptidase activity	Increased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

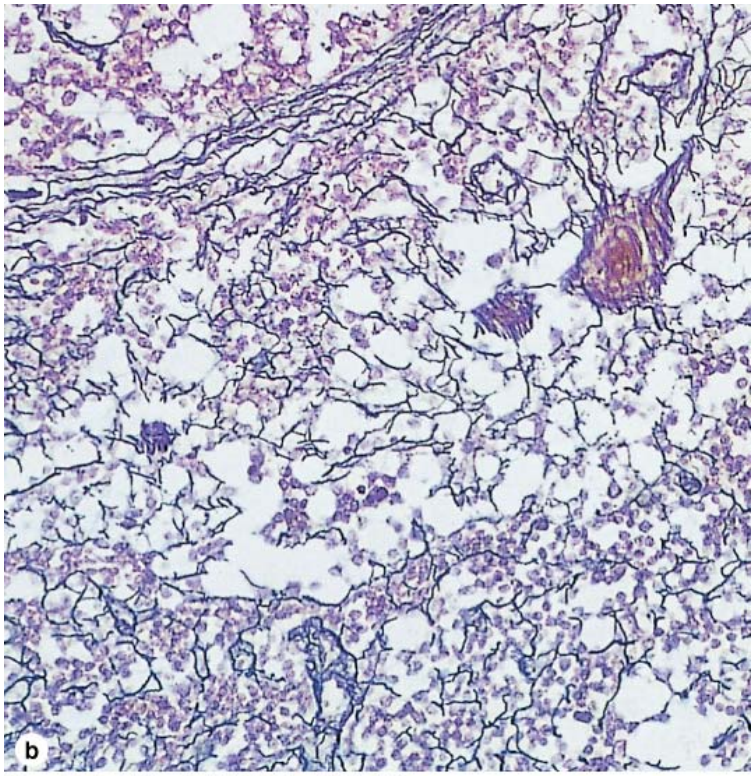
Collagen turnover and renewal in normal connective tissue is generally a very slow process. In some organs, such as tendons and ligaments, the collagen is very stable, whereas in others, as in the periodontal ligament surrounding teeth, the collagen turnover rate is very high. To be renewed, the collagen must first be degraded. Degradation is initiated by specific enzymes called **collagenases**, which are members of an enzyme class called matrix metalloproteinases or MMPs. Collagenases clip collagen molecules in such a way that they are then susceptible to further degradation by nonspecific proteases.

Reticular Fibers

Once thought to be uniquely distinct from collagen, **reticular fibers** are now known to consist mainly of collagen type III, which forms extensive networks of extremely thin (diameters 0.5–2 μm) and heavily glycosylated fibers in certain organs. They are not visible in hematoxylin-and-eosin (H&E) preparations but can be easily stained black by impregnation with silver salts (Figure 5–12). Because of their affinity for silver salts, these fibers are called **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 associated with these fibers. Reticular fibers contain 6–12% hexoses as opposed to 1% in most collagen fibers.

Figure 5–12.





Reticular fibers.

In these silver-stained sections of both adrenal cortex **(a)** and lymph node **(b),** the prominent feature is a network of reticular fibers which provides a framework for cell attachment. Reticular fibers contain type III collagen that is heavily glycosylated, which produces the argyrophilia. Cell nuclei are also dark but cytoplasm is unstained. X100.

Reticular fibers constitute a network around the parenchymal cells of various organs (eg, liver, endocrine glands) and are particularly abundant in the framework of hematopoietic organs (eg, spleen, lymph nodes, red bone marrow). In the latter sites the network is produced by fibroblast-like cells called **reticular cells**. The loose disposition of reticular fibers creates a flexible network in these organs and others that are subject to changes in form or volume, such as the arteries, uterus, and intestinal muscle layers.

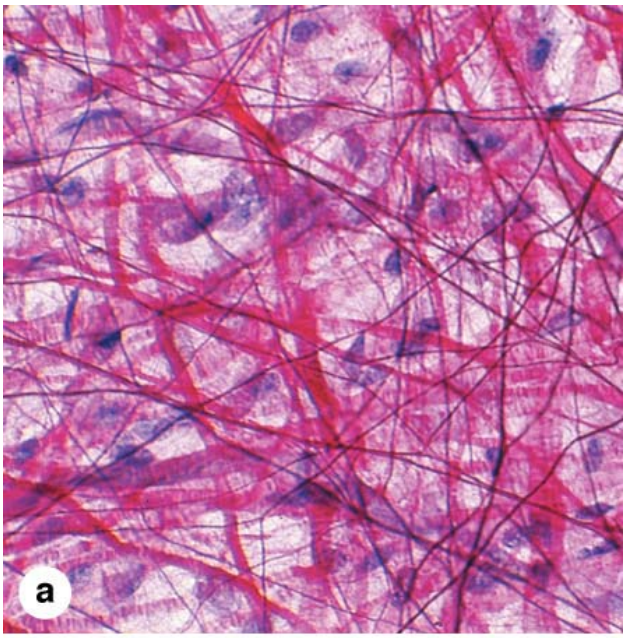
MEDICAL APPLICATION

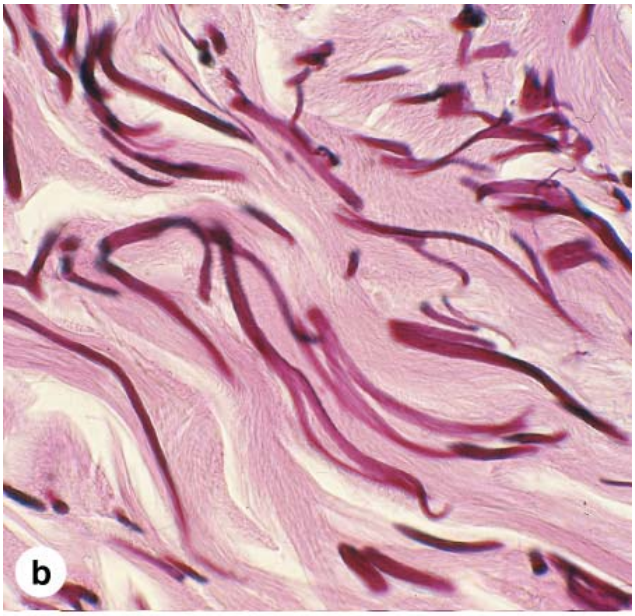
Ehlers–Danlos type IV disease, a deficiency of collagen type III, is characterized by ruptures in arteries and the intestine (Table 5–4), both structures rich in reticular fibers.

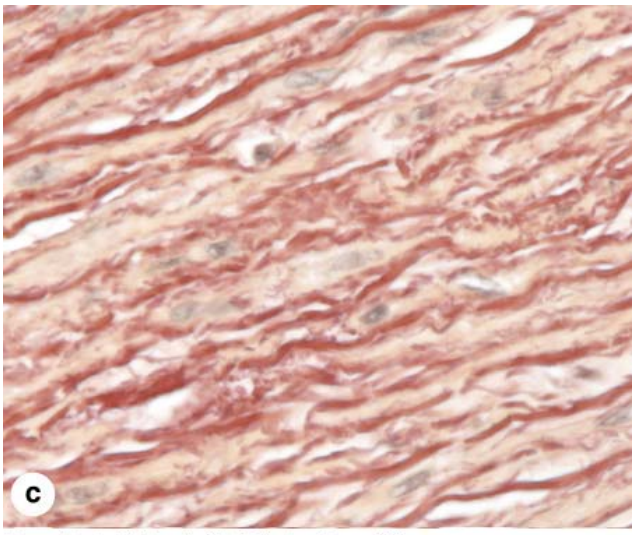
Elastic Fibers

Elastic fibers are also thinner than the average collagen fiber and form sparse networks interspersed with collagen bundles in many organs subject to much bending or stretching, such as the wall of large arteries. The name indicates the major functional property such fibers impart to these resilient organs (Figure 5–13).

Figure 5–13.





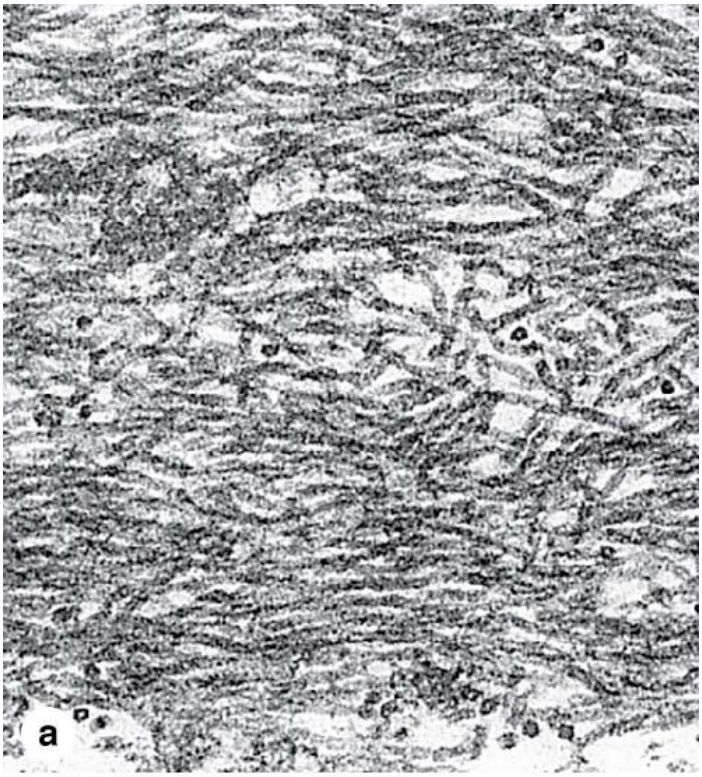


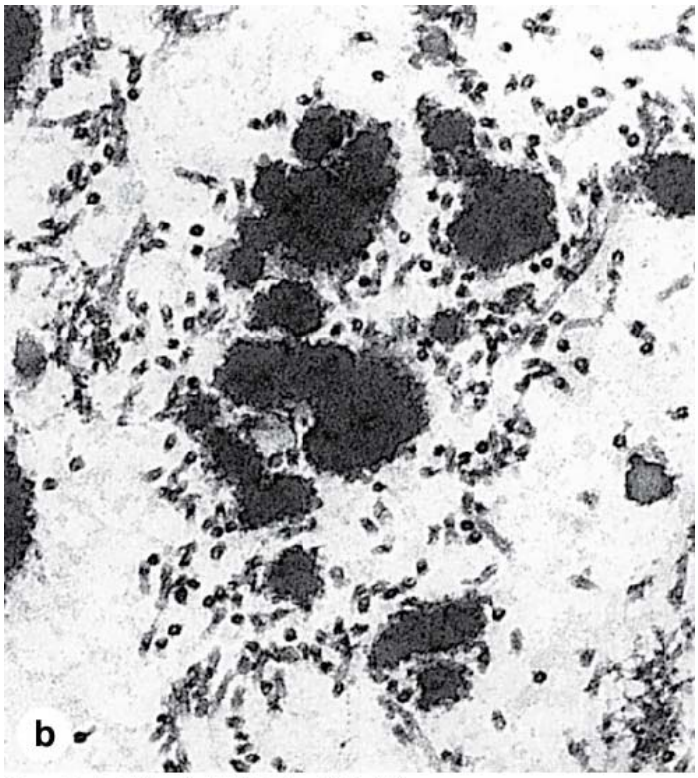
Elastic fibers.

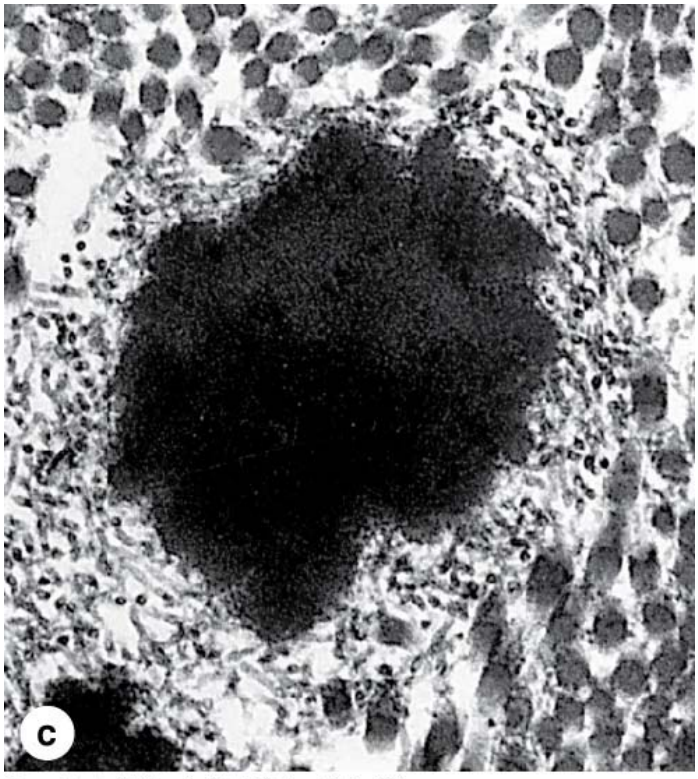
Elastic fibers or lamellae (sheets) add the resiliency to connective tissue. They are difficult to discern in H&E stained material and are usually demonstrated in preparations made using compounds such as aldehyde fuscin which stains elastin a dark magenta. **(a):** The length and density of fine elastic fibers is best seen in spread preparation of connective tissue in a thin mesentery. X200. Orcein-H&E. **(b):** At higher magnification, sectioned elastic fibers can be seen among the eosinophilic collagen bundles in dermis. X400. Aldehyde fuscin & eosin. **(c):** Elastic fibers and lamellae are abundant between layers of smooth muscle in the wall of elastic arteries such as the aorta. X200. Van Gieson-H&E.

Elastic fibers develop through successive stages. In the first stage, a core of 10-nm microfibrils forms from several different glycoproteins, notably the large glycoprotein called **fibrillin** (350kDa). Fibrillin binds elastin and forms the scaffolding necessary for the deposition of elastin. Defective fibrillin results in the formation of fragmented elastic fibrils. In the second stage of development, the protein **elastin** is deposited between the microfibrils, forming larger fibers. During the third stage, elastin gradually accumulates until it comprises most of the fiber bundles, which are further surrounded by a thin sheath of microfibrils. These are the mature **elastic fibers,** the most numerous component of the elastic fiber system. Stages of elastic fiber formation are shown in Figure 5–14. In the wall of large blood vessels, especially arteries, elastin also occurs as fenestrated sheets called **elastic lamellae**.

Figure 5–14







Formation of elastic fibers.

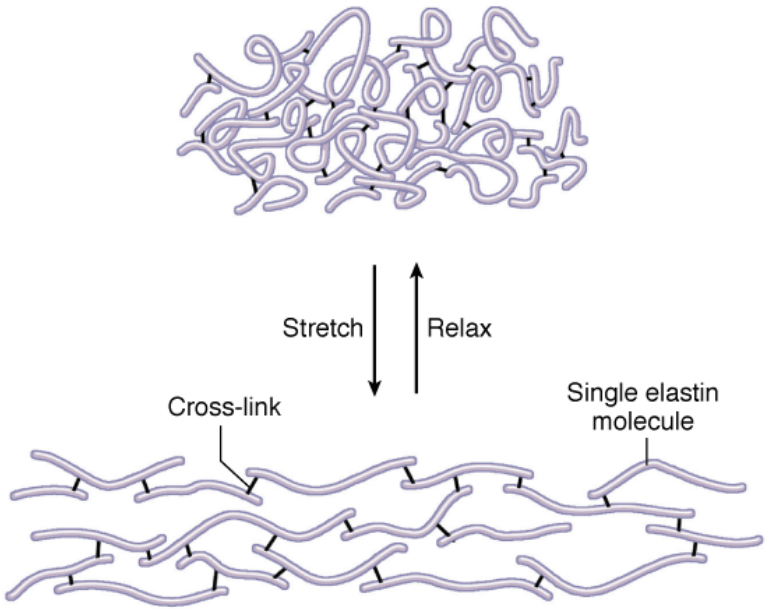
Stages in the formation of elastic fibers can be seen by TEM. **(a):** Initially a developing fiber consists of many small microfibrils composed of the glycoprotein **fibrillin** secreted by fibroblasts, smooth muscle cells or other cells. **(b):** With further development, to the microfibrils are added amorphous deposits of **elastin**. Elastin is secreted by the cells and like procollagen molecules quickly polymerizes. **(c):** Elastin accumulates and ultimately occupies the center of an elastic fiber, which retains fibrillin microfibrils at the surface. Collagen fibrils, seen in cross section, are also present. X50,000.

Microfibrils of fibrillin alone are used in some organs, such as to hold in place the lens of the eye. Such microfibrils are not elastic but are highly resistant to pulling forces, whereas the mature elastic fibers stretch easily in response to tension. By using different proportions of fibrillin and elastin, a family of fibers can be formed whose variable functional characteristics are adapted to local tissue requirements.

Like collagen elastin matures in the ECM. Elastin molecules are globular (molecular mass 70 kDa) and are secreted by fibro-blasts in connective tissue and by smooth muscle cells in the walls of blood vessels. Elastin molecules are rich in glycine and proline, with many regions having random-coil conformations (like that of natural rubber). Elastin molecules polymerize to form fibers or sheet-like structures, both of which can be stretched by external forces. Elastin contains

two unusual amino acids, **desmosine** and **isodesmosine,** which are produced when covalent cross-links are formed among four lysine residues in different elastin molecules (Figure 5–15). These effectively cross-link the subunits of elastin and help account for the rubberlike qualities of this protein. Elastin is resistant to digestion by most proteases, but is easily hydrolyzed by pancreatic **elastase**.

Figure 5–15.



Molecular basis of elasticity.

Subunits of the glycoprotein elastin are joined by covalent bonds formed among lysine residues of different subunits, catalyzed by lysyl oxidase. This produces an extensive and durable cross-linked network of elastin. (Such bonds give rise to the unusual amino acids desmosine and isodesmosine.) Each elastin molecule in the network has multiple random-coil domains which expand and contract; this allows the entire network to stretch and recoil like a rubber band.

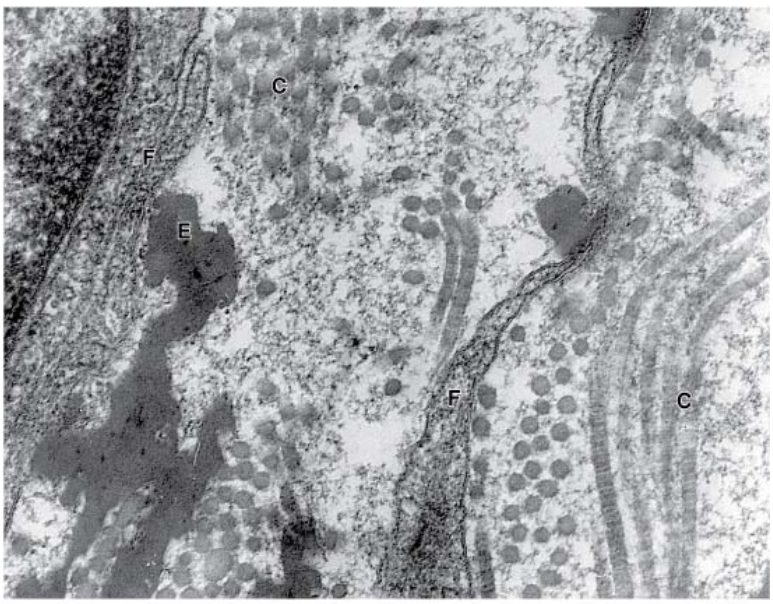
MEDICAL APPLICATION

Fibrillin is a family of proteins related to the scaffolding necessary for the deposition of elastin. Mutations in the fibrillin gene result in **Marfan syndrome**, a disease characterized by a lack of resistance in the tissues rich in elastic fibers. Because the large arteries are rich in components of the elastic system and because the blood pressure is high in the aorta, patients with this disease often experience aortic swellings called aneurysms, a life-threatening condition.

GROUND SUBSTANCE

The ground substance of the ECM is a highly hydrated, transparent, complex mixture of macromolecules, principally in three classes: **glycosaminoglycans** (or GAGs), **proteoglycans**, and **multiadhesive glycoproteins**. The complex molecular mixture of the ground substance is transparent and rich in bound water. It fills the space between cells and fibers of connective tissue and, because it is viscous, acts as both a lubricant and a barrier to the penetration of invaders. When adequately fixed for histologic analysis, its components aggregate and precipitate in the tissues as granular material that is observed in TEM preparations as electron-dense filaments or granules (Figure 5–16).

Figure 5–16.



Ultrastructure of the extracellular matrix (ECM).

TEM of the connective tissue extracellular matrix reveals ground substance as either empty or containing fine granular material that fills spaces between the collagen (C) and elastic (E) fibers and surrounds fibroblast cells and processes (F). The granularity of ground substance is an artifact of the glutaraldehyde–tannic acid fixation procedure. X100,000.

GAGs (originally called **mucopolysaccharides**) are linear polysaccharides formed by repeating disaccharide units usually composed of a uronic acid and a hexosamine. The hexosamine can be **glucosamine** or **galactosamine,** and the uronic acid can be **glucuronic** or **iduronic acid.** The largest, most unique, and most ubiquitous GAG is **hyaluronic acid** (or **hyaluronan**). With a molecular weight from 100s to 1000s kDa, hyaluronic acid is a long polymer of the disaccharide glucosamine – glucuronate. It is synthesized directly into the ECM by an enzyme complex, **hyaluronate synthase**, located in the cell membrane of many cells. Hyaluronic acid forms a dense, viscous network of polymers which binds a considerable amount of water, giving it an important role in allowing diffusion of molecules in connective tissue and in lubricating various organs and joints.

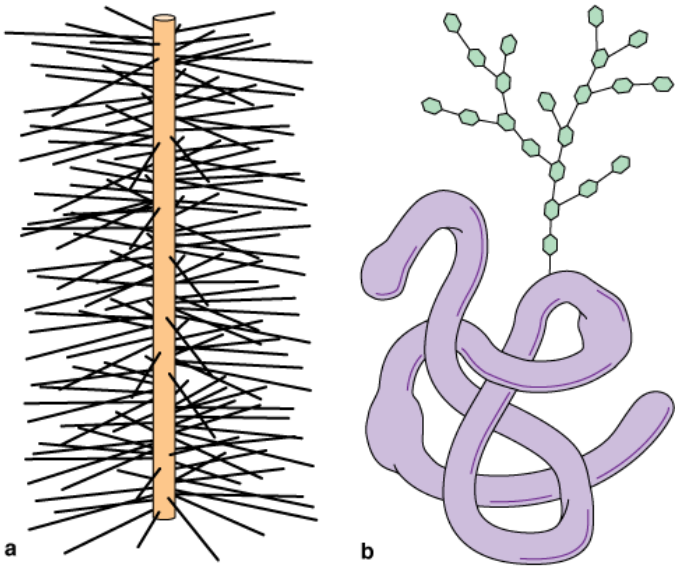
All other GAGs are much smaller (10–40 kDa), are covalently attached to proteins (as parts of proteoglycans), are synthesized in Golgi complexes, and are rich in sulfate. The four main GAGs found in proteoglycans are **dermatan sulfate, chondroitin sulfates, keratan sulfate,** and **heparan sulfate** all of which have different disaccharide units and tissue distributions (Table 5–5). Like hyaluronic acid these GAGs are intensely hydrophilic, are highly viscous, and are polyanions, binding a great number of cations (usually sodium) by electrostatic (ionic) bonds.

Table 5–5. Composition and distribution of glycosaminoglycans in connective tissue and their interactions with collagen fibers.

	Repeating Disaccharides			
Glycosaminoglycan	**Hexuronic Acid**	**Hexosamine**	**Distribution**	**Electrostatic Interaction with Collagen**
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 (cornea)	D-galactose	D-galactosamine	Cornea	None
Keratan sulfate (skeleton)	D-galactose	D-glucosamine	Cartilage, nucleus pulposus, annulus fibrosus	None

Proteoglycans are composed of a core protein to which are covalently attached various numbers and combinations of the sulfated GAGs just mentioned. Like glycoproteins they are synthesized on RER, mature in the Golgi and secreted from cells by exocytosis. The main structural differences between proteoglycans and glycoproteins are shown in Figure 5–17. In cartilage, the core proteins of secreted proteoglycans are bound via small link proteins to a hyaluronic acid chain, forming much larger structures—proteoglycan aggregates. The acidic groups of proteoglycans cause these molecules to bind to the basic amino acid residues of collagen. Proteoglycans are distinguished for their diversity and include cell-surface and ECM families. A given matrix may contain several different types of core proteins, each with different numbers of GAGs of different lengths and composition. One of the most important ECM proteoglycans is **aggrecan,** the dominant proteoglycan in cartilage. In aggrecan the core protein bears several chondroitin sulfate and keratan sulfate chains and is in turn bound via a link protein to hyaluronic acid. Cell-surface proteoglycans such as **syndecan** are present on many types of cells, particularly epithelial cells. The core protein of cell-surface proteoglycans spans the plasma membrane, with a short cytoplasmic extension. A small number of heparan sulfate chains are attached to the extracellular extension of the core protein.

Figure 5–17.



Proteoglycans and glycoproteins.

The diagram indicates the major structural features of proteoglycans and glycoproteins. **(a):** Proteoglycans contain a core of protein (vertical rod in drawing) to which molecules of sulfated glycosaminoglycans (GAGs) are covalently bound. A GAG is an unbranched polysaccharide made up of repeating disaccharides; one component is an amino sugar, and the other is uronic acid. Proteoglycans contain a greater amount of carbohydrate than do glycoproteins. In general the three-dimensional structure of proteoglycans can be pictured as a test tube brush, with the wire stem representing the core protein and the bristles representing the sulfated GAGs. **(b):** Glycoproteins are globular protein molecules to which branched chains of monosaccharides are covalently attached. Their polypeptide content is greater than their polysaccharide content. (Reproduced, with permission, from Junqueira LCU, Carneiro J: *Biologia Celular e Molecular,* 8th ed. Editora Guanabara Koogan. Rio de Janeiro, 2000.)

Besides acting as structural components of the ECM and anchoring cells to the matrix, both extracellular and surface proteoglycans also bind and sequester certain signaling proteins eg, fibroblast growth factor (FGF). Degradation of proteoglycans releases these stored growth factors which then stimulate new cell growth and ECM synthesis.

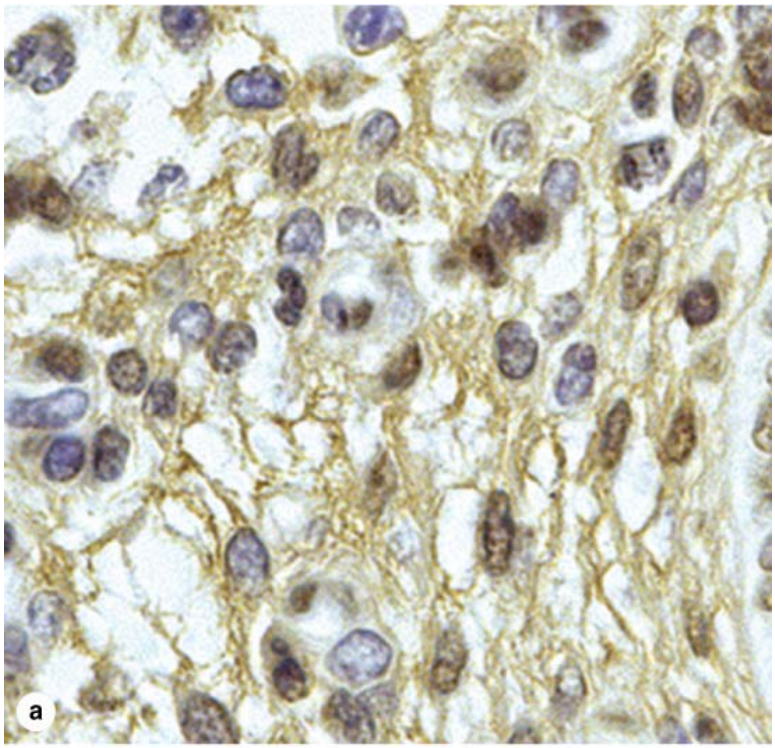
MEDICAL APPLICATION

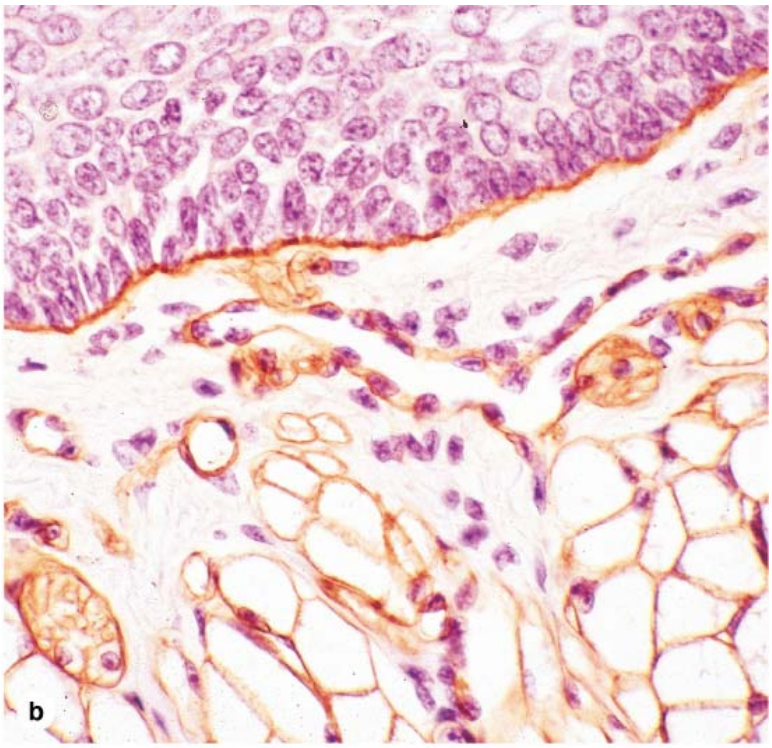
The degradation of proteoglycans is carried out by several cell types and depends on the presence of several lysosomal enzymes. Several disorders have been described in which a deficiency in lysosomal enzymes causes glycosaminoglycan degradation to be blocked, with the consequent accumulation of these compounds in tissues. The lack of specific hydrolases in the lysosomes has been found to be the cause of several disorders in humans, including Hurler, Hunter, sanfilippo, and Morquio syndromes.

Because of their high viscosity, intercellular substances act as a barrier to the penetration of bacteria and other microorganisms. Bacteria that produce **hyaluronidase,** an enzyme that hydrolyzes hyaluronic acid and other glycosaminoglycans, have greater invasive power because they reduce the viscosity of the connective tissue ground substance.

Multiadhesive glycoproteins have carbohydrates attached, but in contrast to proteoglycans the protein moiety usually predominates. The carbohydrate moiety of glycoproteins is frequently a branched structure. Several such glycoproteins have important roles in the adhesion of cells to their substrate. **Fibronectin** (L. *fibra,* fiber, + *nexus,* interconnection) is an important example synthesized by fibroblasts and some epithelial cells. This dimeric molecule, with a molecular mass of 222–240 kDa, has binding sites for collagens, certain GAGs, and integrins of cell membranes, ie, it is multiadhesive. Interactions at these sites help to mediate normal cell adhesion and migration and cause fibronection to be distributed as a network in the intercellular spaces of many tissues (Figure 5–18a). Another multiadhesive glycoprotein, **laminin** is a larger, trimeric, cross-shaped glycoprotein that participates in the adhesion of epithelial cells to the basal lamina, with binding sites for type IV collagen, GAGs, and integrins. All basal laminae are rich in laminin (Figure 5–18b).

Figure 5–18.



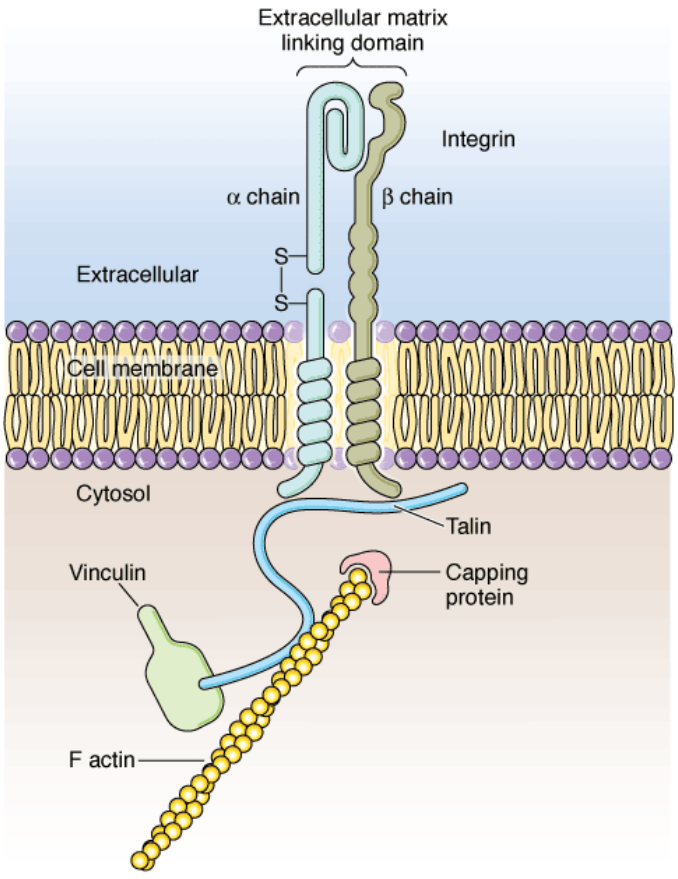


Fibronectin and laminin localization.

Immunohistochemistry of sections with connective tissue shows that **fibronectin (a)** is ubiquitous throughout the ECM, while **laminin (b)** is restricted to the basal lamina of the epithelium (top of the picture) and of cross-sectioned muscle fibers, nerves, and small blood vessels (lower half of picture). Both glycoproteins (and many other similar glycoproteins) are multiadhesive, with binding sites for collagens and other ECM components and for integrins of cell surfaces. They play important roles in cell migration, in embryonic tissue formation, and in maintaining tissue structure. (a): X400, (b): X200, both hematoxylin counterstain. (Reproduced, with permission, from Junqueira LCU, Carneiro J: Biologia Celular e Molecular, 8th ed. Editora Guanabara Koogan. Rio de Janeiro, 2000.)

Cells interact with extracellular matrix components by using cell-surface molecules (**matrix receptors**) that bind to collagen, fibronectin, and laminin. These receptors are the **integrins,** a family of transmembrane linker proteins (Figures 5–19). Integrins bind their ligands in the ECM with relatively low affinity, allowing cells to explore their environment without losing attachment to it or becoming glued to it. Integrins also interact with the cytoskeleton, usually the actin microfilaments, an interaction mediated by several intracellular proteins, such as **talin** and **vinculin.** The interactions that integrins mediate between the ECM and the cytoskeleton exert effects in both directions and play an important role in orienting both the cells and the ECM in tissues (Figure 5–19).

Figure 5–19.



Integrin cell-surface matrix receptor.

By binding to a matrix protein and to the actin cytoskeleton (via talin) inside the cell, integrins serves as transmembrane links by which cells adhere to components of the ECM. The molecule is a heterodimer, with α and β chains. The head portion may protrude some 20 nm from the surface of the cell membrane into the ECM where it interacts with fibronectin, laminin, or collagens.

MEDICAL APPLICATION

The participation of fibronectin and laminin in both embryonic development and the increased ability of cancer cells to invade other tissues has been well-studied. The importance of fibronectin is shown by the fact that mice whose fibronectin gene has been inactivated die during early embryogenesis.

In connective tissue, in addition to the hydrated ground substance, there is a small quantity of free fluid—called **interstitial** or **tissue fluid**—that is similar to blood plasma in its content of ions and diffusible substances. Tissue fluid contains a small percentage of plasma proteins of low molecular weight that pass through the capillary walls as a result of the hydrostatic pressure of the blood. Although only a small proportion of connective tissue proteins are plasma proteins, it is estimated that because of its volume and wide distribution, as much as one third of the plasma proteins of the body are stored in the intercellular connective tissue matrix.

MEDICAL APPLICATION

Edema is promoted by the accumulation of water in the extracellular spaces. Water in the extracellular compartment of connective tissue comes from the blood, passing through the capillary walls into the extracellular compartment of the tissue. The capillary wall is only slightly permeable to macromolecules but permits the passage of water and small molecules, including low-molecular-weight proteins. In several pathologic conditions, the quantity of tissue fluid may increase considerably, causing edema.

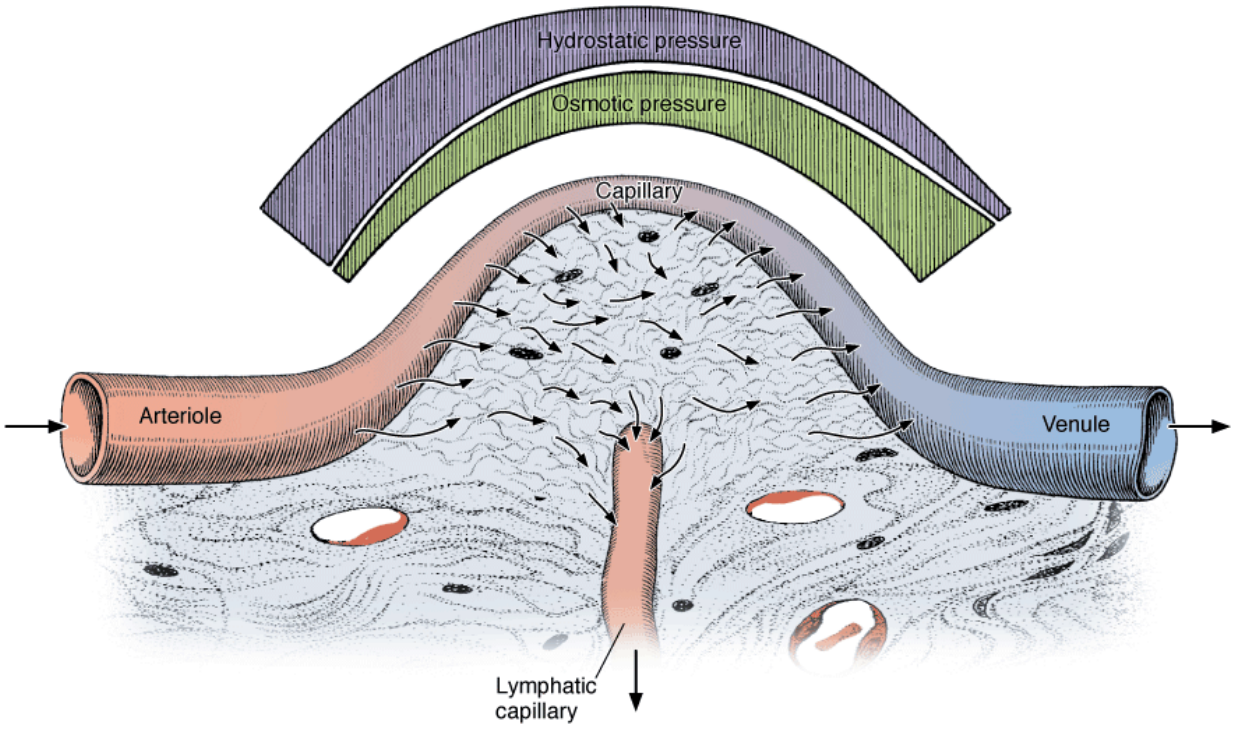
Edema may result from venous or lymphatic obstruction or from a decrease in venous blood flow (eg, congestive heart failure). It may also be caused by the obstruction of lymphatic vessels due to parasitic plugs or tumor cells and chronic starvation; protein deficiency results in a lack of plasma proteins and a decrease in colloid osmotic pressure. Water therefore accumulates in the connective tissue and is not drawn back into the capillaries.

Another possible cause of edema is increased permeability of the blood capillary endothelium resulting from chemical or mechanical injury or the release of certain substances produced in the body (eg, histamine).

Blood vessels bring to connective tissue the various nutrients required by its cells and carry away metabolic waste products to the detoxifying and excretory organs, the liver and kidneys.

Two forces act on the water contained in the capillaries: the **hydrostatic pressure** of the blood caused by the pumping action of the heart, which forces water out across the capillary wall; and the colloid **osmotic pressure** of the blood plasma, which draws water back into the capillaries (Figure 5–20). Osmotic pressure is due mainly to plasma proteins. (Because the ions and low-molecular-weight compounds that pass easily through the capillary walls have approximately the same concentration 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 colloid osmotic pressure exerted by the blood proteins—which are unable to pass through the capillary walls—is not counterbalanced by outside pressure and tends to bring water back into the blood vessel (Figure 5–20).

Figure 5–20.



Movement of fluid in connective tissue.

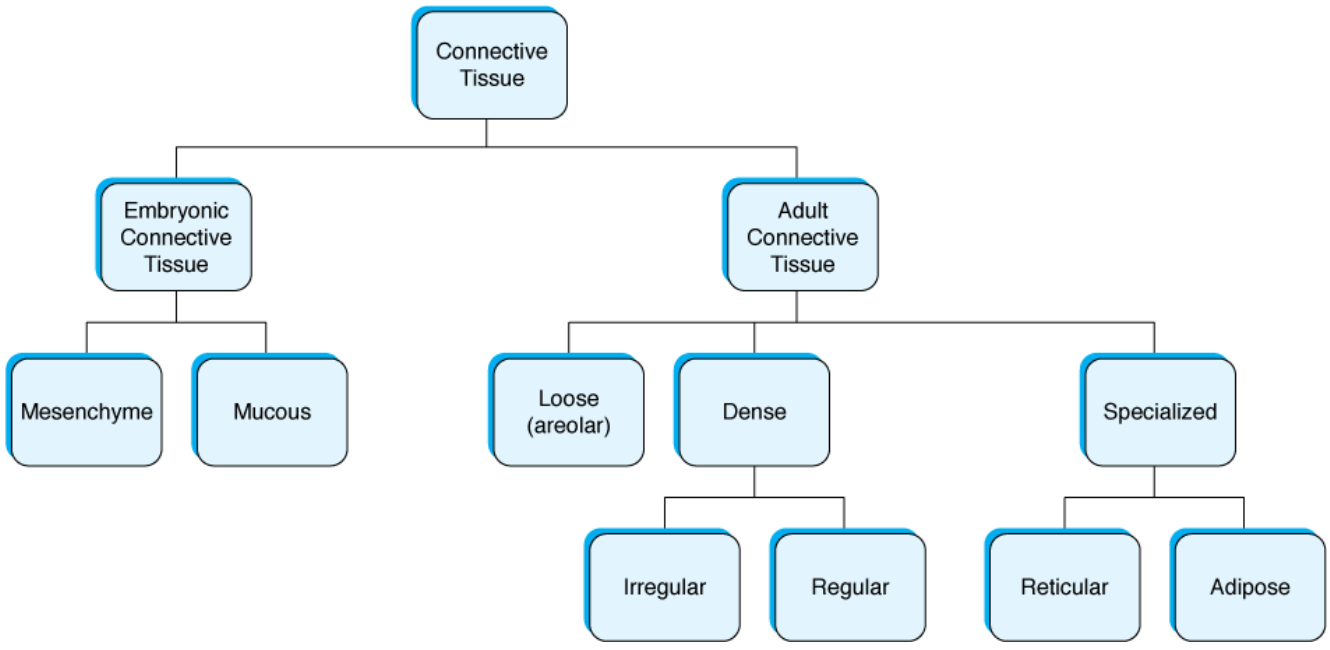
Normally, water passes through capillary walls to the ECM of the surrounding connective tissues primarily at the arterial end of a capillary, because the hydrostatic pressure there is greater than the colloid osmotic pressure. As indicated at the top of the figure, however, hydrostatic pressure decreases along the length of the capillary toward the venous end. As the hydrostatic pressure falls, osmotic pressure of the fluid in the capillary rises because the protein concentration is increasing due to the passage of water from the capillaries. As a result of the increased protein concentration and decreased hydrostatic pressure, osmotic pressure becomes greater than hydrostatic pressure at the venous end and water is drawn back into the capillary. In this way, metabolites circulate in the connective tissue, feeding its cells. 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, blind-ended vessels that arise in connective tissue and enter the one-way lymphatic system which eventually delivers the fluid (now called lymph) back to veins.

The quantity of water drawn back is less than that which passes out through the capillaries. Rather than accumulating in connective tissue, this excess fluid is continuously drained by lymphatic capillaries and eventually returned to the blood. The smallest lymphatic vessels, the lymphatic capillaries originate in connective tissue as delicate, blind-ending, endothelium-lined tubes that join increasingly larger lymphatic vessels that drain into veins at the base of the neck (see Chapter 11).

TYPES OF CONNECTIVE TISSUE

Connective tissues composed of the cells, fibers, and ground substance components already described nevertheless are quite variable in histological structure. This has led to the use of descriptive names or classifications for various connective tissue types, denoting either the major component or a structural characteristic of the tissue. Table 5-6 shows one classification commonly used for the main types of connective tissue.

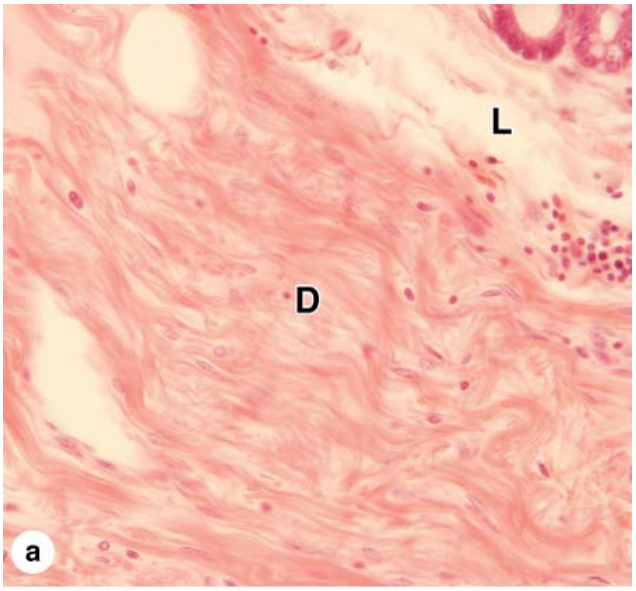
Table 5–6. Types of connective tissue.

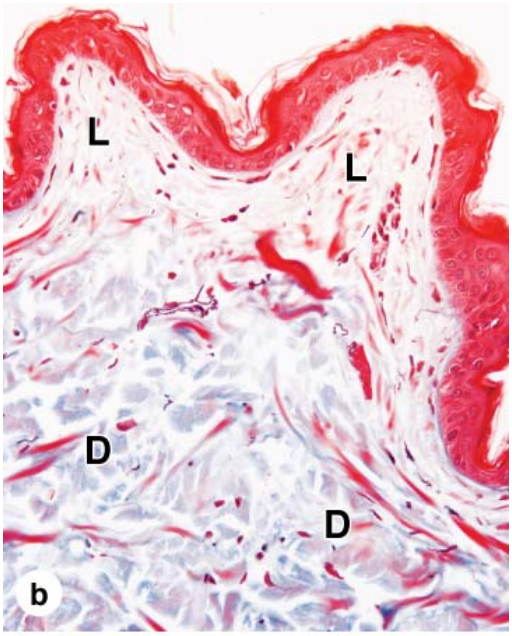


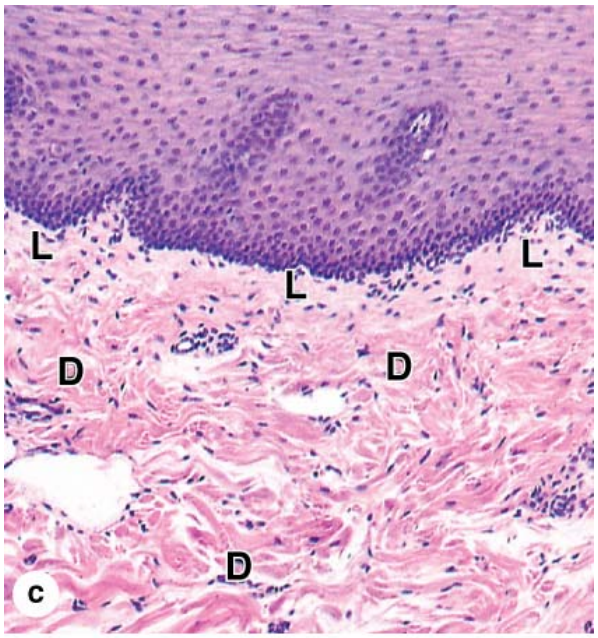
Connective Tissue Proper

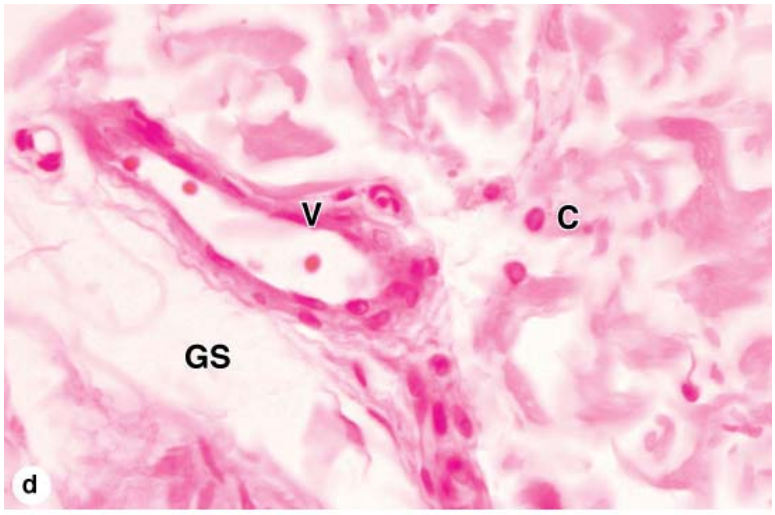
There are two general classes of connective tissue proper: loose and dense (Figure 5–21).

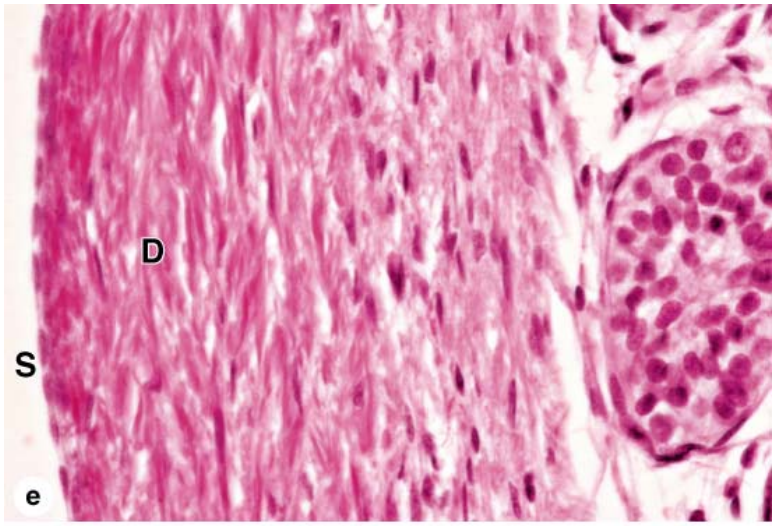
Figure 5–21.











Loose connective tissue and dense irregular connective tissue.

Shown here are three examples of connective tissue which display the types designated loose (or areolar) and dense irregular. Loose connective tissue (L) contains faint ground substance with fine fibers of collagen and is adjacent to epithelium in the examples shown here. Dense irregular connective tissue (D) underlies the thinner layer of loose connective tissue and is invariably much richer in larger bundles of collagen. **(a):** Micrograph of a mammary gland, showing a duct at the top of the figure. In the dense irregular connective tissue can be seen scattered leukocytes, and the irregular spaces of two lymphatic vessels (left). X100. H&E. **(b):** Trichrome staining of the skin demonstrates the blue staining of collagen with this method. X100. Mallory Trichrome. **(c):** Loose and dense irregular connective

tissue within the esophagus is seen below the stratified squamous epithelium. X100. H&E. **(d):** At higher magnification ground substance (GS) is more clearly seen around small blood vessels (V) and collagen bundles (C). X200. H&E. **(e):** The dense irregular connective tissue (D) capsule that surrounds the testis is shown here. Similar capsules are found around many organs and large glands. That of the testis is covered by serous mesothelial cells (S), which produce a hyaluronate-rich lubricant around the organ. X200. H&E.

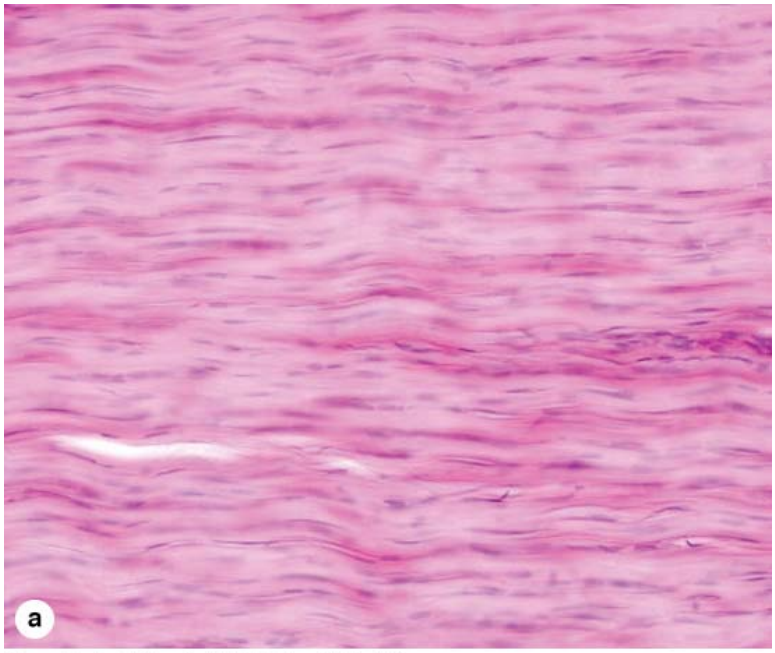
Loose connective tissue is a very common type of connective tissue that supports many structures which are normally under some pressure and low friction. It usually supports epithelial tissue, forms a layer around small blood and lymphatic vessels, and fills the spaces between muscle and nerve fibers. Loose connective tissue is also found in the papillary layer of the dermis, in the hypodermis, in the linings of the peritoneal and pleural cavities, in glands, and in the mucous membranes (wet membranes that line the hollow organs) supporting the epithelial cells.

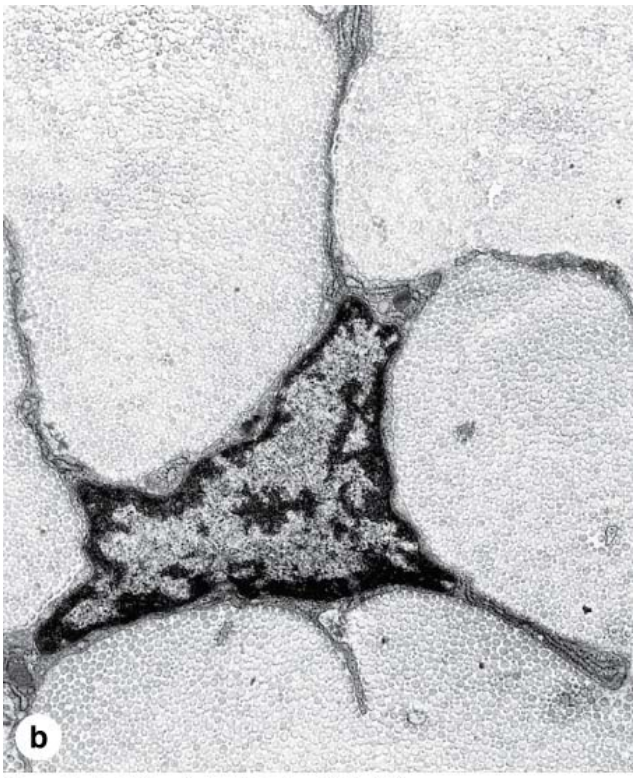
Loose connective tissue, sometimes called **areolar tissue**, has all the main components of connective tissue (cells, fibers, and ground substance) in roughly equal parts. The most numerous cells are fibroblasts and macrophages, but other types of connective tissue cells are also present. Collagen, elastic, and reticular fibers also appear in this tissue. With a moderate amount of ground substance, loose connective tissue has a delicate consistency; it is flexible, well vascularized, and not very resistant to stress.

Dense connective tissue is adapted to offer resistance and protection. It has the same components found in loose connective tissue, but there are fewer cells and a clear predominance of collagen fibers over ground substance. Dense connective tissue is less flexible and far more resistant to stress than is loose connective tissue. It is known as **dense irregular** connective tissue when the collagen fibers are arranged in bundles without a definite orientation. The collagen fibers form a 3-dimensional network in dense irregular tissue, providing resistance to stress from all directions. Dense irregular connective tissue is often found closely associated with loose connective tissue. The two types frequently grade into one another and distinctions between them are often arbitrary (Figure 5–21).

The collagen bundles of **dense regular** connective tissue are arranged according to a definite pattern, with collagen fibers aligned with the linear orientation of fibroblasts in response to prolonged stresses exerted in the same direction (Figure 5–22). This arrangement offers great resistance to traction forces.

Figure 5–22.





Dense regular connective tissue.

(a): Micrograph shows a longitudinal section of dense regular connective tissue of 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.

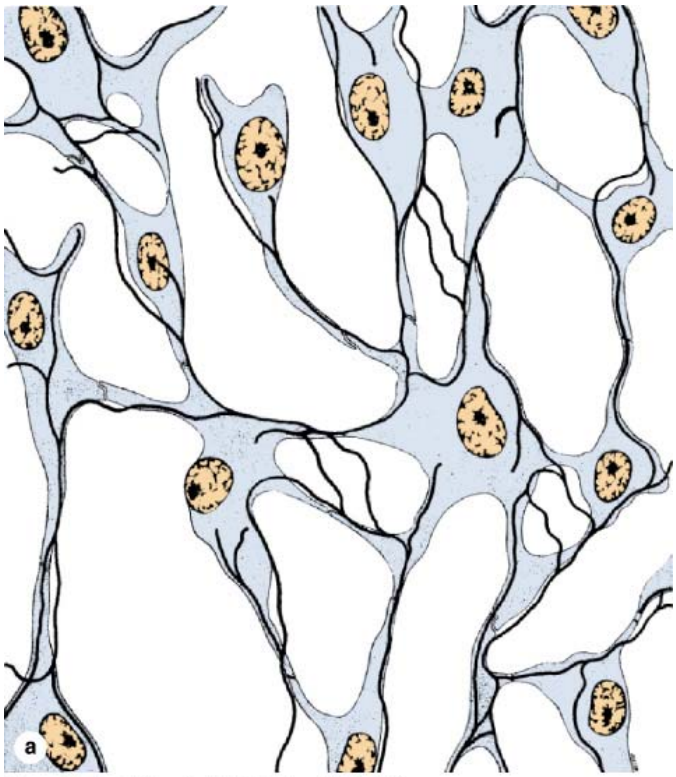
Tendons and ligaments are the most common examples of dense regular connective tissue. These elongated cylindrical structures hold together components of the musculoskeletal system; by virtue of their richness in collagen fibers, they are white and inextensible. They have parallel, closely packed bundles of collagen separated by a very small quantity of ground substance (Figure 5–22a). Their fibrocytes contain elongated nuclei parallel to the fibers and sparse cytoplasmic folds that envelop portions of the collagen bundles (Figure 5–22b). The cytoplasm of these fibrocytes is rarely revealed in H&E stains, not only because it is sparse but also because it stains the same color as the fibers. Some ligaments, such as the yellow ligaments of the vertebral column, also contain abundant parallel elastic fiber bundles.

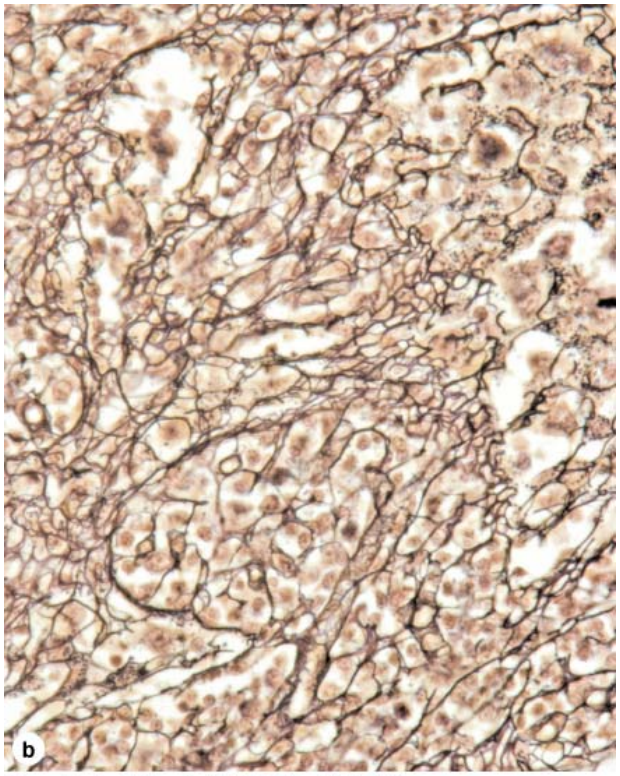
The collagen bundles of tendons vary in size and are enveloped by small amounts of loose connective tissue containing small blood vessels and nerves. Overall, however, tendons are poorly vascularized and repair of damaged tendons is very slow. Externally, the tendon is surrounded by a sheath of dense irregular connective tissue. In some tendons, this sheath is made up of two layers, both lined by flattened **synovial cells** of mesenchymal origin. One layer is attached to the tendon, and the other lines neighboring structures. The space between these linings contains a viscous fluid (similar to the fluid of synovial joints) composed of water, proteins, hyaluronate and other GAGs. This synovial secretion acts as a lubricant permitting easy sliding movements of the tendon within its sheath.

Reticular Tissue

Individual reticular fibers form delicate three-dimensional networks that support cells in **reticular tissue**. This specialized connective tissue consists of reticular fibers of type III collagen produced by specialized fibroblasts called reticular cells (Figure 5–23). The heavily glycosylated reticular fibers provide the architectural framework that creates special microenvironments for hematopoietic organs and lymphoid organs (bone marrow, lymph nodes, and spleen). The reticular cells are dispersed along this framework and partially cover the reticular fibers and ground substance with cytoplasmic processes. The resulting cell-lined system creates a spongelike structure (Figure 5–12) within which cells and fluids are freely mobile.

Figure 5–23.





Reticular tissue.

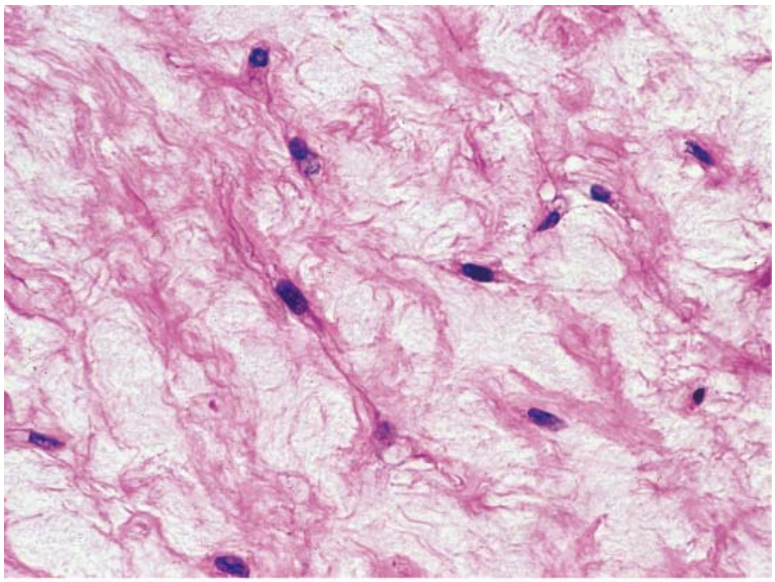
(a): The diagram shows only the fibers and attached reticular cells (free, transient cells are not represented). Reticular fibers of type III collagen are produced and enveloped by the reticular cells, forming an elaborate network through which interstitial fluid or lymph and wandering cells from blood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

In addition to the reticular cells, cells of the mononuclear phagocyte system are strategically dispersed along the trabeculae. These cells monitor the slow flow of materials through the sinuslike spaces and remove invaders by phagocytosis.

Mucous Tissue

Mucous tissue is found mainly in the umbilical cord and fetal tissues. Mucous tissue has an abundance of ground substance composed chiefly of hyaluronic acid, making it a jellylike tissue containing very few collagen fibers with scattered fibroblasts (Figure 5–24). Mucous tissue is the principal component of the umbilical cord, where it is referred to as **Wharton's jelly.** A similar form of connective tissue is also found in the pulp cavity of young teeth.

Figure 5–24.



Mucous tissue.

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