Abstract

The tissues at the ocular surface include the cornea, the conjunctiva, and the intervening zone of the limbus; the regions are shown diagrammatically and histologically in Fig. 1. The primary function of the entire region is to refract and transmit light to the lens and retina. Although the cornea and its surface tear film constitute the tissue actually performing the tasks, the limbus and conjunctiva support the cornea in these important functions. Because the cornea is such a major functional tissue of the eye and because damage to or disease of the cornea has serious visual consequences, its structure, function, and pathology have received much attention. Increased interest in the limbus and conjunctiva has yielded new information regarding the important supportive functions of the tissues surrounding the cornea. This chapter reviews the anatomy and cell biology of the three regions of the ocular surface, including some of the recently observed structural and cell biologic features. In the previous edition of this text, specific chapters dealt with the cell biology of the corneal epithelium, the corneal stroma and its connective tissue, and the corneal endothelium. In this volume, these topics are combined and the publication space is restricted. Thus, for more complete details regarding the cell and molecular biology of these tissue regions, refer to a previous edition of this text [1, 2]. More complete details regarding the gross anatomy of the region also are available [3, 4].

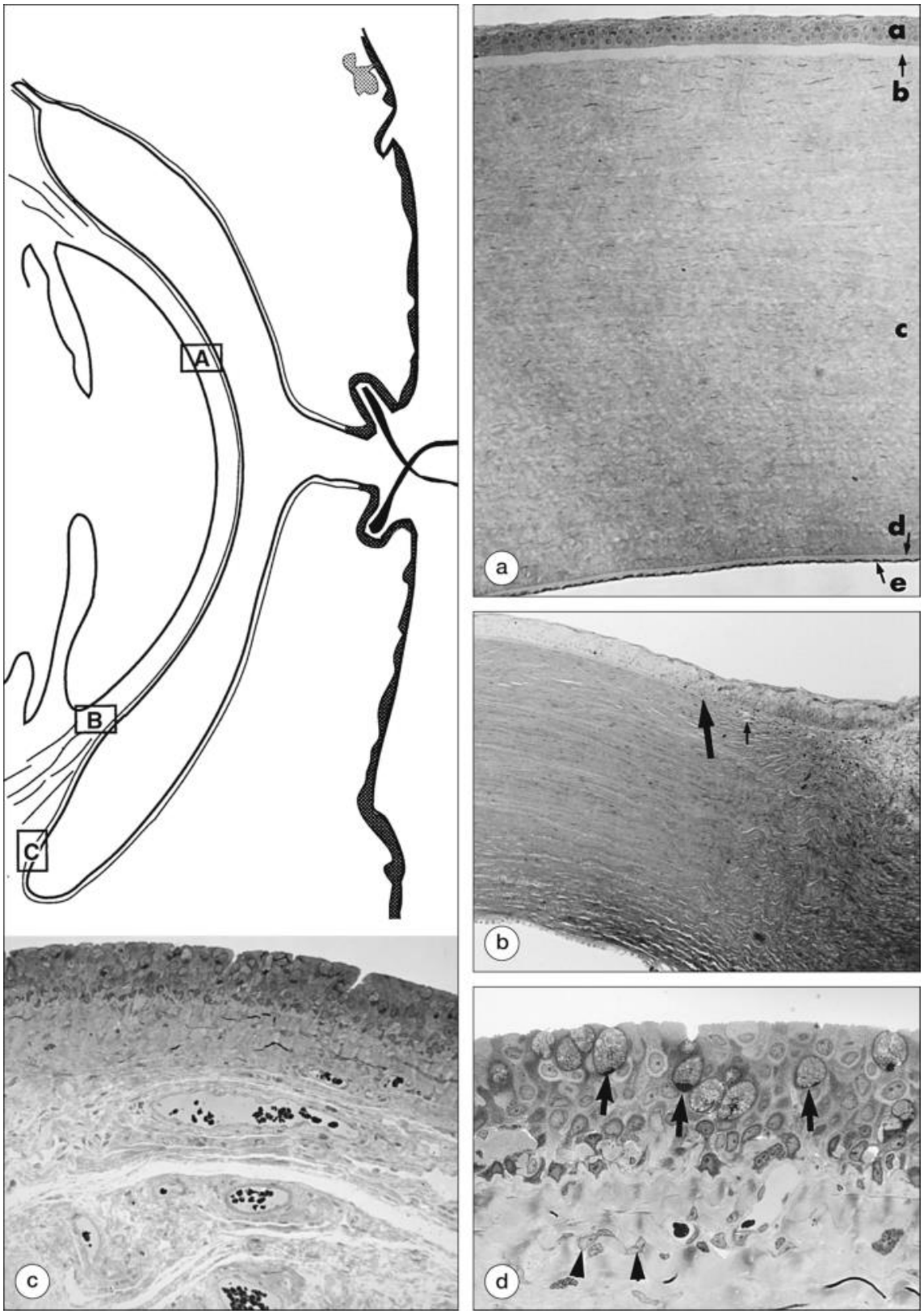

Diagram and light micrographs of ocular surface tissues. Boxes A through C correspond to the regions in the light micrographs A through C at the right; all are sections of human tissue, as is D, which shows a higher magnification of conjunctival epithelium. (**a**) Section through the central cornea: (a) epithelium, (b) Bowman's layer, (c) lamellar stroma, (d) Descemet's membrane, and (e)

endothelium. ×120. (**b**) Section through the limbus. The large arrow designates the end of Bowman's layer and the small arrow the position of the first blood vessel encountered outside the corneal stroma. ×48. (**c**) Section of bulbar conjunctiva. Note the highly vascularized connective tissue. ×120. (**d**) Section of bulbar conjunctiva demonstrating the presence of numerous goblet cells *(arrows)* within the stratified epithelium and the cellular nature of the connective tissue of the substantia propria *(arrowheads)*. ×300

Keywords

- **Cornea**
- **Ocular surface**
- **Corneal epithelium**
- **Bowman's Layer**
- **Corneal endothelium**
- **Corneal stroma**
- **Conjunctiva**
- **Limbus**
- **Glycocalyx**
- **Goblet cell**
- **Microplicae**
- **Hemidesmosome**

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Cornea

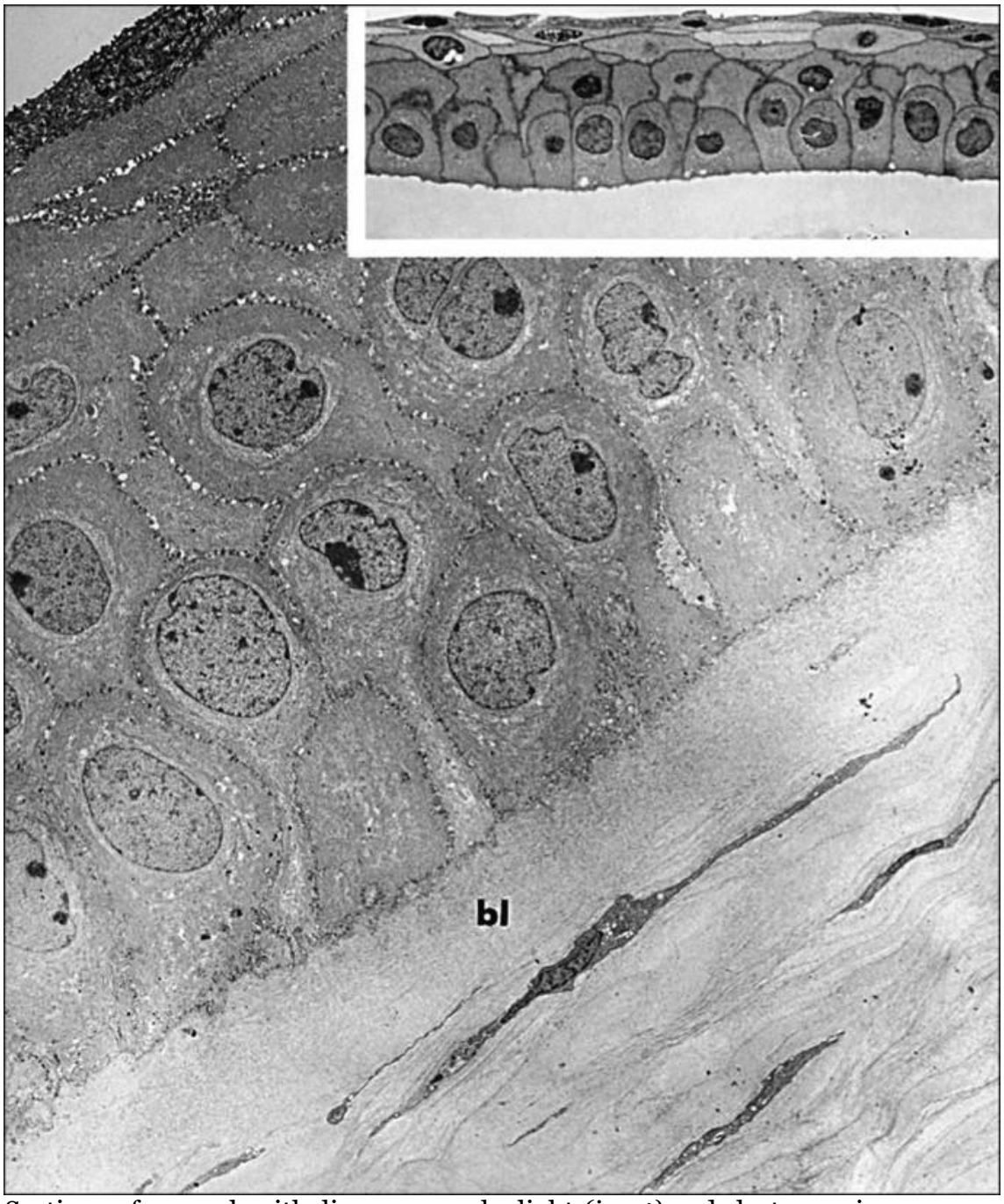
The cornea is a highly specialized tissue that refracts and transmits light to the lens and retina. In humans, it is about twice as thick at the periphery than at the center (1 mm compared with 0.5 mm) [5,6,7]. The tissue of the cornea appears simple in composition because it is composed only of an outer stratified squamous nonkeratinized epithelium, an inner dense connective tissue stroma with its resident fibroblast-like keratocytes, and a monolayered cuboidal endothelium bordering the anterior chamber (see Fig. 1). The cornea, however, actually is highly ordered and complexly arranged in comparison with other tissues of the body. Its transparency, avascularity, and highly ordered structure make it unique among all tissues of the body. Cells of all layers interact with and influence each other's functions. They do not act alone, but mediators (cytokines) expressed by one cell type influence cells of adjacent layers.

Epithelium

The surface of the cornea is covered by a stratified squamous nonkeratinizing epithelium, which in humans, rodents, and rabbits has 5–7 cell layers. The epithelium is 50–52 μm thick. The corneal epithelium has functions unique to it as well as functions that are common to all other epithelia of the body. Several of its unique functions include light refraction and transmittance and survival over an avascular bed. The unique function of light refraction is brought about by its absolutely smooth, wet apical surface and its extraordinarily regular thickness. Transparency of the epithelium to light appears to be brought about by scarcity of cellular organelles and possibly by high concentrations of enzyme crystallins [8]. The epithelium has specialized metabolic characteristics that allow it to exist over an avascular connective tissue [9]. The epithelium also has a rapid and highly developed ability to respond to wounds, and it is maintained by centripetal movement of cells derived from an adult stem-cell population located in the basal layer of the limbal epithelium (see below). In addition to its specialized functions, the corneal epithelium has the routine housekeeping functions of all epithelia that border the outside world. The layers of cells provide a barrier to fluid loss and pathogen entrance and resist abrasive pressure by tightly adhering to one another and to the underlying connective tissue stroma.

The stratified epithelium consists of three or four layers of outer flat squamous cells termed squames at its apical surface facing the tear film, one to three layers of midepithelial cells termed wing cells because of their rounded cell body and lateral wing-like cellular processes, and a layer of columnar basal cells (Fig. 2). The basal cells secrete and maintain the epithelium's basement membrane, which, compared with that of the other stratified epithelia (i.e., epidermis), is smooth and planar. Like all other stratified epithelia, the epithelium of the cornea is self-renewing, turning over in humans and rats in ~5–7 days [10]. Basal cells are the mitotically active layer; as they divide, their adhesion to the basement membrane decreases. Cells begin to detach from the basement membrane and begin a process of terminal differentiation and eventual desquamation from the apical surface. In addition to forming tight adhesions to their underlying basement membrane, basal cells also wrap their basal and basolateral cell membranes around sensory axons, referred to as intraepithelial corneal nerves (ICNs) that branch from stromal nerves whose nerve cell bodies are located in the trigeminal ganglion [11]. The density of the ICNs is 300–400 times that of the epidermis [12]. The ICNs have two components: the basal nerves that extend parallel to and above the basement membrane (basal nerves) and the intraepithelial nerve terminals that extend apically and terminate below the apical squames. Human ICN basal nerves are typically several mm long. The epithelial basal cells adhere directly to the axons via integrin–L1CAM cell–cell adhesions, and they also secrete basement membrane proteins around the nerves to insulate them. L1CAM is an IgG superfamily cell adhesion molecule that can participate in both homotypic (L1CAM–L1CAM) and heterotypic (L1CAM–integrin) cell–cell adhesions [13]. Thus, the corneal epithelial basal cells function as surrogate Schwann cells wrapping their cell membrane around axons. Since ICNs continuously grow and extend apically as intraepithelial nerve terminal, homeostasis is maintained by the nerve tips severing. The axon remnants are phagocytosed by the corneal epithelial suprabasal cells and squames where they serve as a source of raw materials and energy for the epithelial cells [11].

Fig. 2



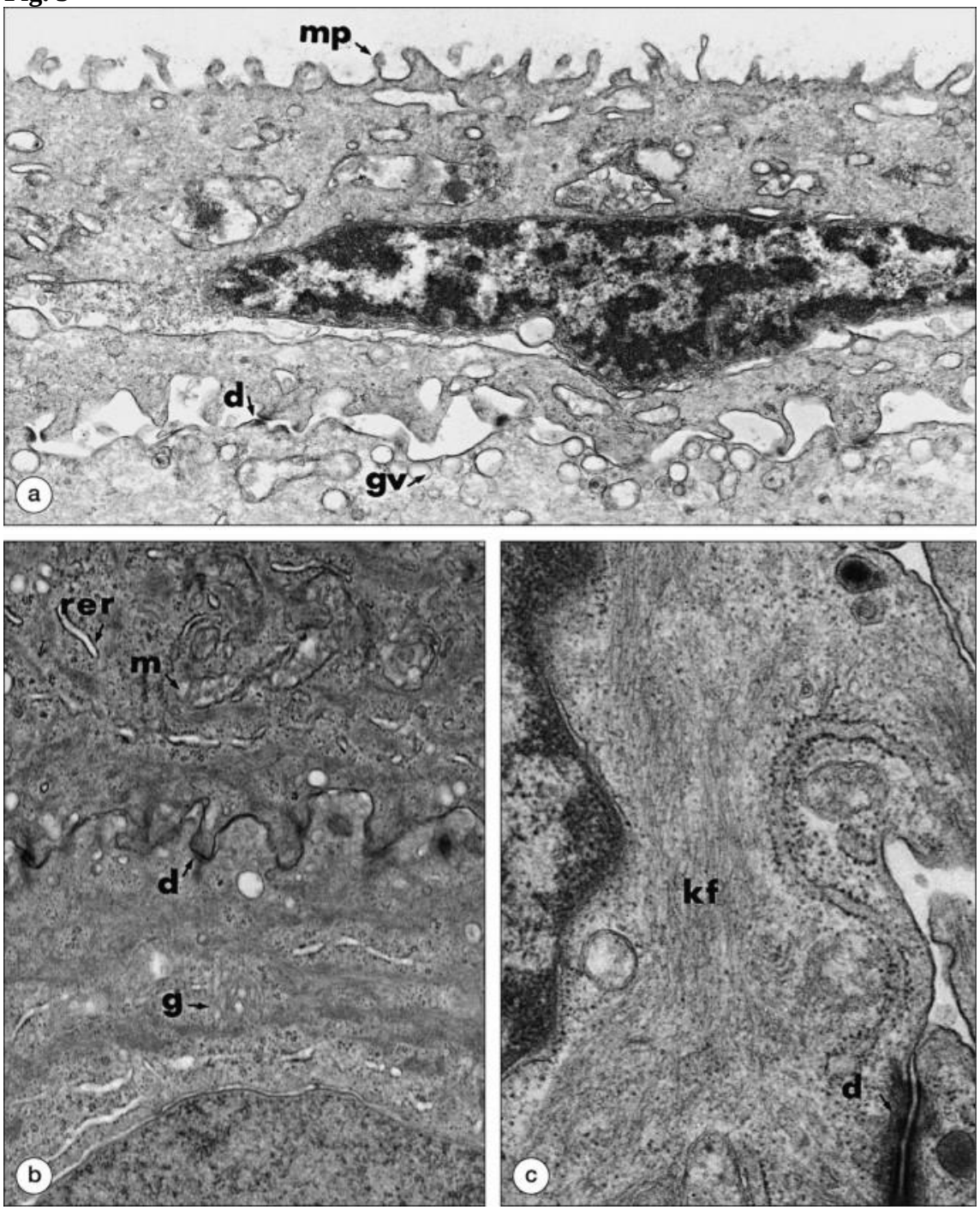
Sections of corneal epithelium as seen by light *(inset)* and electron microscopy showing superficial, wing, and basal cell layers and Bowman's layer (bl). In the electron micrograph, note the surface microplicae and interdigitating cell membranes with electron-dense desmosomes. Electron-lucent profiles of endoplasmic reticulum are widely scattered, primarily within basal and wing cells. Electron-dense hemidesmosomes are prominent along the basal cell membrane of the columnar basal cells adjacent to the basal lamina. ×300; *inset* ×2700

Full size image

All cell layers of epithelium have a sparse accumulation of cytoplasmic organelles. Endoplasmic reticulum and mitochondria are sparsely distributed around the cytoplasm,

with a Golgi apparatus present in a supranuclear position, particularly in the basal cell layer (Fig. 3). In the apical cell layers, Golgi cisternae and small membrane-bound vesicles consistent in size and structure with Golgi-associated vesicles are especially prominent (see Fig. 3).

Fig. 3



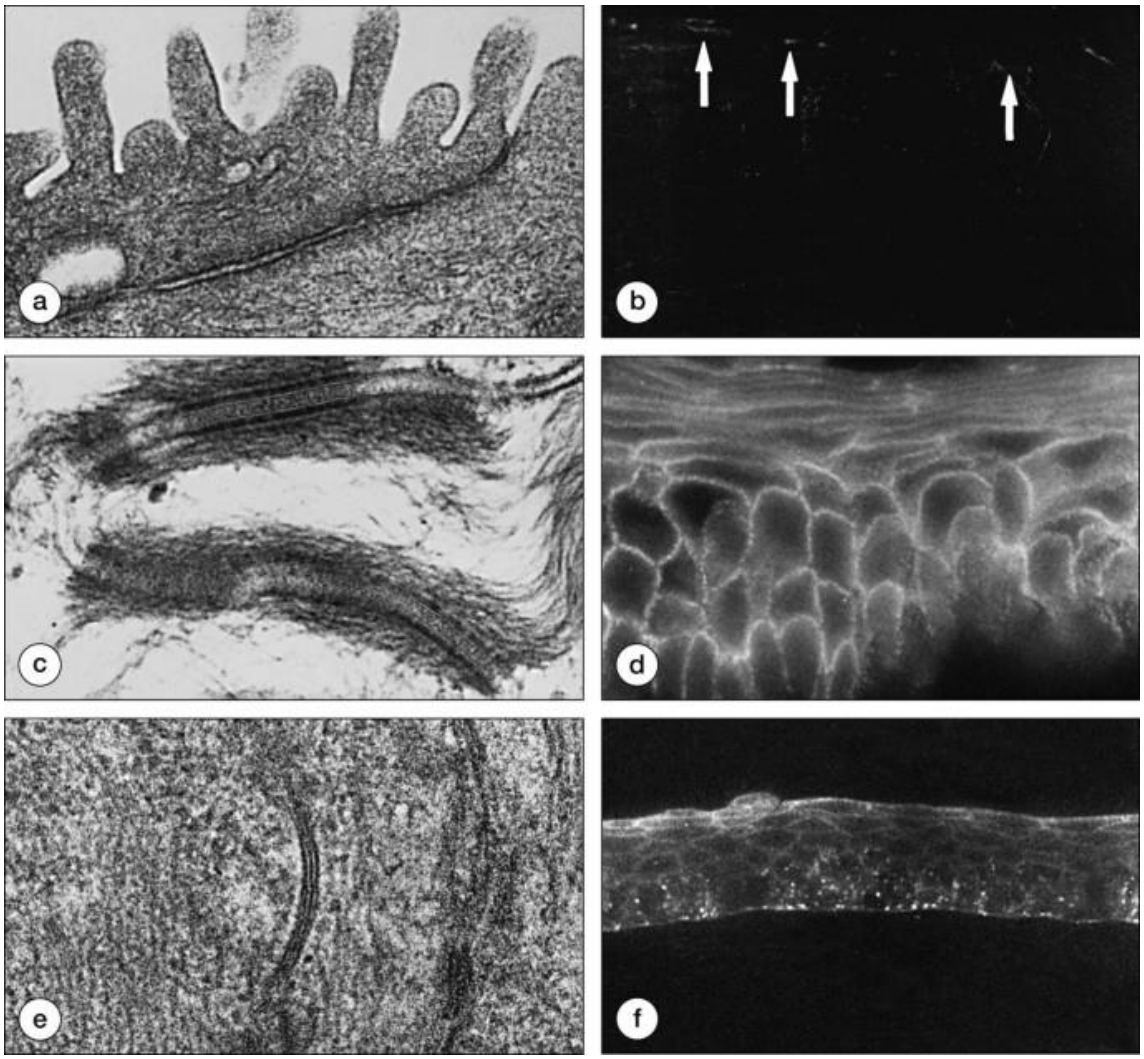
Electron micrographs demonstrating aspects of the ultrastructure of the corneal epithelium of apical cells (**a**) and wing cells (**b**) and (**c**). (**a**) Portion of an apical cell and the cell immediately beneath it. Note microplicae (mp) and Golgi vesicles (gv). ×21,000. (**b**) Elaborate interdigitation of membranes of adjacent cells, characteristic of wing and squamous cells, shows mitochondrion (m), Golgi apparatus (g), and rough endoplasmic reticulum (rer). ×21,000. (**c**)

Higher-magnification electron micrograph demonstrating that the cytoplasm of epithelial cells is rich in keratin filaments (kf). ×42,000. All these micrographs show the presence of the cell-to-cell adhesion junctions known as *desmosomes* (d), which are present along interdigitating cell membranes. Desmosomes of corneal epithelia appear similar to those of all other stratified squamous epithelium

Full size image

Of the three cytoplasmic filament types within all cells, actin filaments, keratin filaments, and microtubules, keratin intermediate filaments are the major type within the cytoplasm of cells of the corneal epithelium. On electron micrographs, the cell cytoplasm of all layers of the corneal epithelium appears full of these filaments, and keratin proteins, which polymerize to form the filaments, are among the most abundant proteins of the tissue. The keratin family of proteins that form intermediate filaments is a complex family of ~30 polypeptides, which are of two classes: type I, or acidic, and type II, or neutral and basic. Intermediate filaments within ectodermally derived epithelia are formed by the pairing of two specific keratin proteins, one from each class. In the corneal epithelium, as basal cells differentiate to apical cells, two keratin pairs are expressed sequentially. First, K5 and K14 are expressed in basal cells; subsequently, suprabasal cells express K3 and K12 [14,15,16,17,18]. K12, a 64-kDa keratin, is believed to be cornea specific [19]. The cytokeratin filaments not only increase the tensile strength of the epithelial cells but also, by keeping the nucleus and other organelles in their proper positions, affect the overall organization of the cell. They also provide a scaffold upon which associated proteins are organized and regulated to control cell metabolic and homeostatic activities [20]. Another major role of the intermediate filaments of the corneal epithelium is to provide the cytoskeletal component of the system that anchors cells tightly to one another and to their substrate through the desmosome and hemidesmosome (Figs. 4 and 5). Such tight anchorage is critical to a stratified epithelium that borders the outside world and is subject to the abrasive pressures from lid movement and eye rubbing.

Fig. 4



Cell-to-cell junctions of the corneal epithelium as demonstrated by electron microscopy (**a**, **c**, **e**) and immunolocalization of cell-to-cell junction components (**b**, **d**, **f**). (**a**) Areas of apparent membrane fusion at the tight junction are obvious. ×66,000. (**b**) Vinculin, a component of the adherens junction, can be seen in the immunofluorescence micrograph on the lateral membranes of apical cells *(arrows)*. ×600. (**c**) Desmosomes are prominent along cell membranes. ×66,000. (**d**) Localization of the desmosome component desmoplakin is demonstrated in the immunofluorescence micrograph. ×1000. (**e**) A gap junction *(arrow)* between two basal cells is shown in the electron micrograph. ×105,000. (**f**) The immunolocalization of the gap junction protein connexin 43 is shown in a section of chick corneal epithelium. Note the punctate binding, particularly along the membranes of basal cells. (Antibody against connexin 43 was provided by D. A. Goodenough, Ph.D. (**b**) From Zieske JD, Bukusoglu G, Gipson IK: Enhancement of vinculin synthesis by migrating stratified squamous epithelium. J Cell Biol 109:571, 1989. Reproduced from The Journal of Cell Biology by copyright permission of The Rockefeller University Press)

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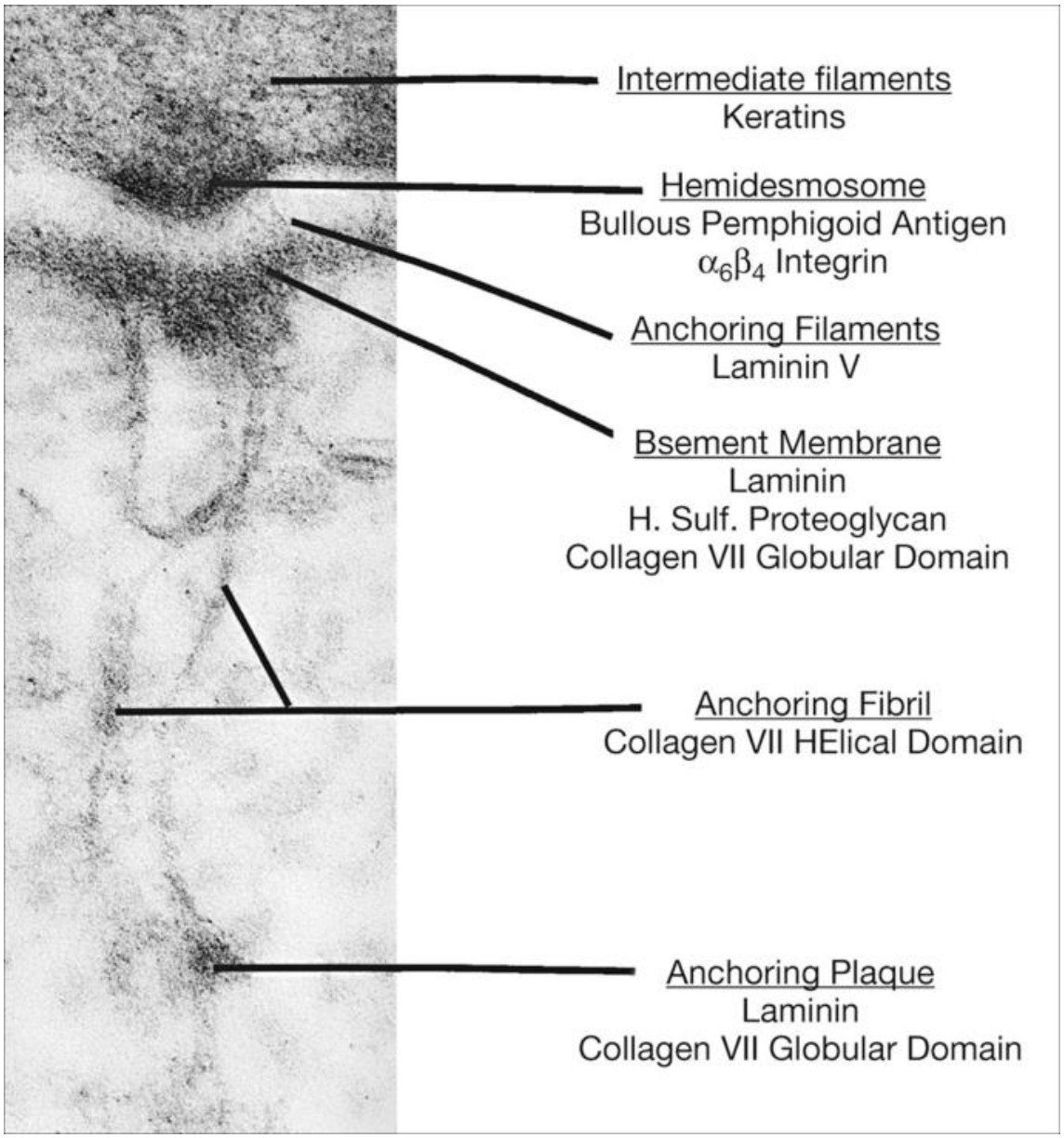
Fig. 5



Electron micrograph demonstrating adhesion complex of the corneal epithelium. The linked structures of the complex and their known molecular components are identified. ×165,000

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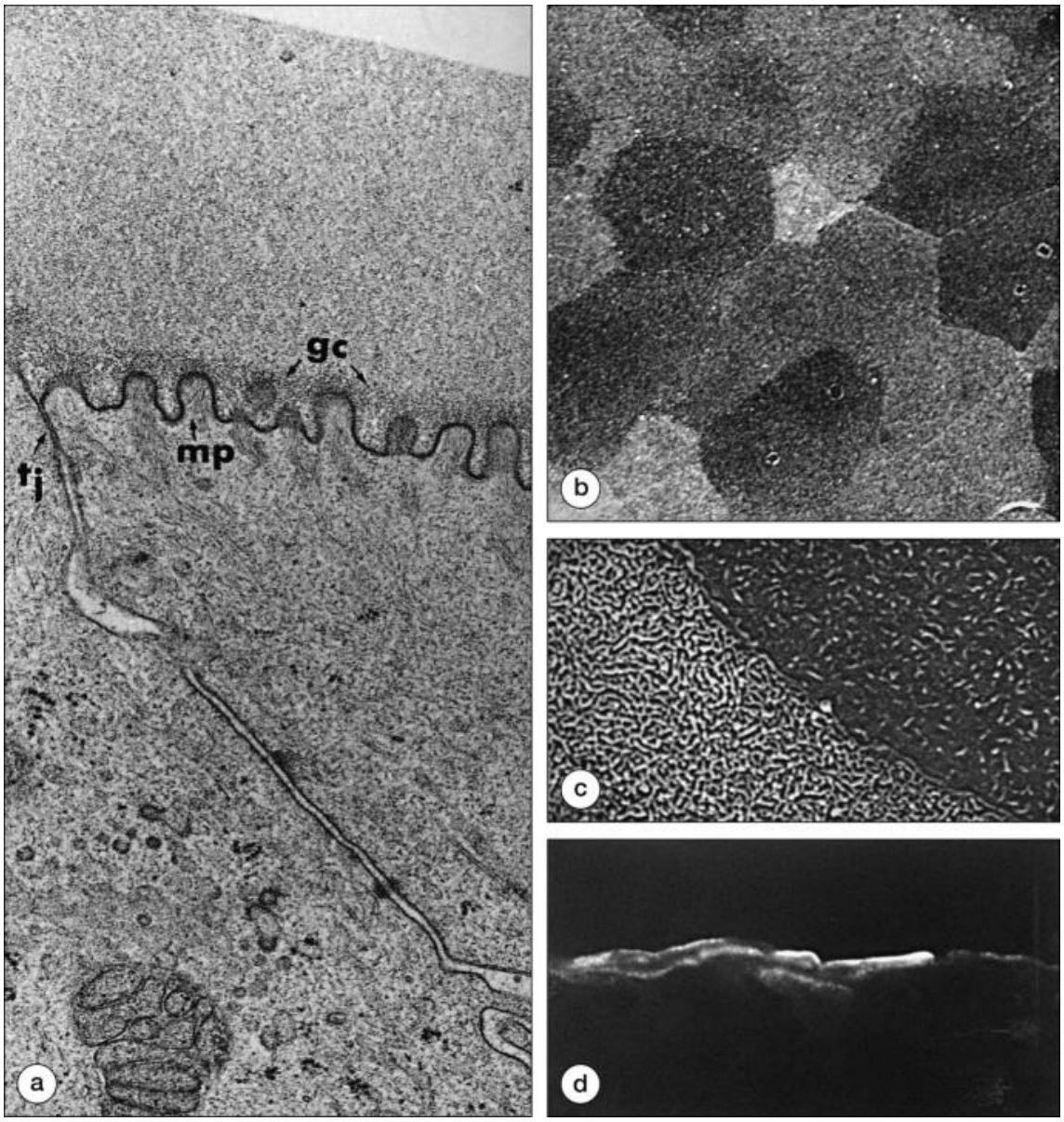
As with all cells, actin filaments are present throughout the cytoplasm of cells of the corneal epithelium. They are particularly prevalent as a network along the apical cell membranes of the epithelial squames, where they extend into microplicae (Fig. 6), and at the junction of the lateral membranes, where they associate with adherens and tight junctions [21]. The actin filament system is particularly important in providing the cytoskeletal connection of cell adhesion molecules, such as the integrins and cadherins, and the cytoskeletal component of adherens and tight junctions in epithelia.

Fig. 6



Micrographs showing specialization of the apical membrane of apical cells of the ocular surface. (**a**) Electron micrograph of mucin layer preserved on apical membranes of guinea pig conjunctiva. Note microplicae (mp) in cross-section and electron density of the glycocalyx (gc) region at the tips of the microplicae. Note tight junction (tj) between adjoining cells. ×56,000. (**b**) and (**c**) Low-magnification (**b**, ×750) and high-magnification (**c**, ×6200) scanning electron micrographs of apical cells of a rabbit cornea. In (**b**), cells vary in the amount to which they scatter electrons, leading to a mosaic with cobblestone appearance. In (**c**), this degree of scatter correlates to the density of microplicae on the surfaces of the cells. (**d**) Immunofluorescence micrograph demonstrating specific molecules along the apical membrane. Cells in this section of human cornea have been labeled with antibody to the membrane-spanning mucin termed MUC1. A similar pattern of labeling is seen with antibodies to MUC16. ×300

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Composed of both α- and β-subunits of the proteins known as tubulins, microtubules are the third major cytoskeletal element within all cells [22]. Although they are not obvious in the corneal epithelial cell cytoplasm on electron micrographs, they can be observed within the spindles of mitotic basal cells, where they provide the cytoskeletal framework for chromosome segregation. They do not appear to play a significant role in cell migration after corneal epithelial wound healing since studies using drugs that depolarize the microtubules do not delay wound closure [23].

The corneal epithelium, like all other epithelia, has intercellular junctions that function not only in cell adhesion but also in cell communication and barrier formation. Four junction types are present (see Fig. 4). Desmosomes, which are present along the lateral membranes of all corneal epithelial cells, function in cell-to-cell adherence; adherens junctions, which are present along the lateral membrane of the apical cells of the epithelium, function to maintain cell-to-cell adherence in the region of the tight junctions; the tight junctions are present along with adherens junctions in apical cell lateral membranes, where they function to provide a paracellular permeability barrier; and gap junctions, which function in cell-to-cell communication, allow intercellular passage of small molecules up to 2000 Da. The latter are present along lateral membranes of all cells of the epithelium. Basal cells have gap junctions with a different molecular composition (connexin 43) than suprabasal cells (connexin 50). For a more complete description of the molecular composition of the four junction types, see [1] and [24]. Additional integral membrane proteins located outside the specialized junctions mentioned above are present which also function in cell-to-cell adhesion including cadherins (specifically, E-cadherin), several integrin heterodimers [25], and L1CAM. While E-cadherin functions exclusively in cell–cell adhesion, L1CAM and integrins can mediate epithelial cell–cell adhesion as well as epithelial–axonal cell adhesion.

The apical and basal surfaces of the corneal epithelium have specializations indicative of their function in the epithelium. The apical surface facing the tear film is specialized to optimize the spreading of the tear film [26] providing the extraordinarily smooth refractive surface of the cornea. To facilitate this function, the apical cell membrane has short ridgelike folds, termed microplicae, that form regular undulations of the membrane when viewed in cross-section (see Fig. 6). In addition, microvilli (finger-like projections of the membrane) up to ~1 m in length are present. These two membrane specializations presumably supply an increased surface area for adherence of the mucous layer of the tear film. Scanning electron microscopic studies of the corneal surface demonstrate that apical cells scatter electrons to varying degrees (see Fig. 6). Cells that scatter electrons to a lesser degree are termed dark cells. Light cells, which scatter more electrons, have a higher density of surface microplicae and microvilli [27]. It has been hypothesized that the dark cells with fewer surface membrane specializations represent the “oldest” cells of the ocular surface and therefore are about to desquamate [28]. The undulating, specialized apical membrane bears a prominent glycocalyx that is intimately associated with the tips of the microplicae and with the mucous layer of the tear film (see Fig. 6). The corneal cells express three membrane-spanning mucins, designated MUC1, MUC4, and MUC16, which are present in the apical cell membrane. The latter is a major component of the glycocalyx and is particularly prevalent on the tips of microplicae [29].

The basal surface of the epithelium is specialized to provide tight anchorage of the epithelium to the stroma [30, 31]. A series of linked structures, termed the anchoring complex, extends from the cytoplasm of the basal cell, through the basal cell membrane, then through the basal lamina and into the anterior of Bowman's layer at the anterior region of the stroma. The structures of the anchoring complex visible by electron microscopy include keratin filaments that insert into the hemidesmosome plaque; the hemidesmosome, which is the specialized anchoring junction on the basal membrane; anchoring filaments, which extend from the hemidesmosome to the basement membrane; and anchoring fibrils, which extend from the basement membrane into Bowman's layer. These anchoring fibrils form an intertwining network and terminate distal to the basement membrane in anchoring plaques. The linked structures and their molecular components are shown diagrammatically and by electron microscopy in Fig. 5. While hemidesmosomes provide the structural integrity needed to maintain epithelial adhesion to the basement membrane during homeostasis, when the cornea is injured, hemidesmosomes disassemble to allow sheet movement and cell migration and reassemble after migration is complete.

Stroma

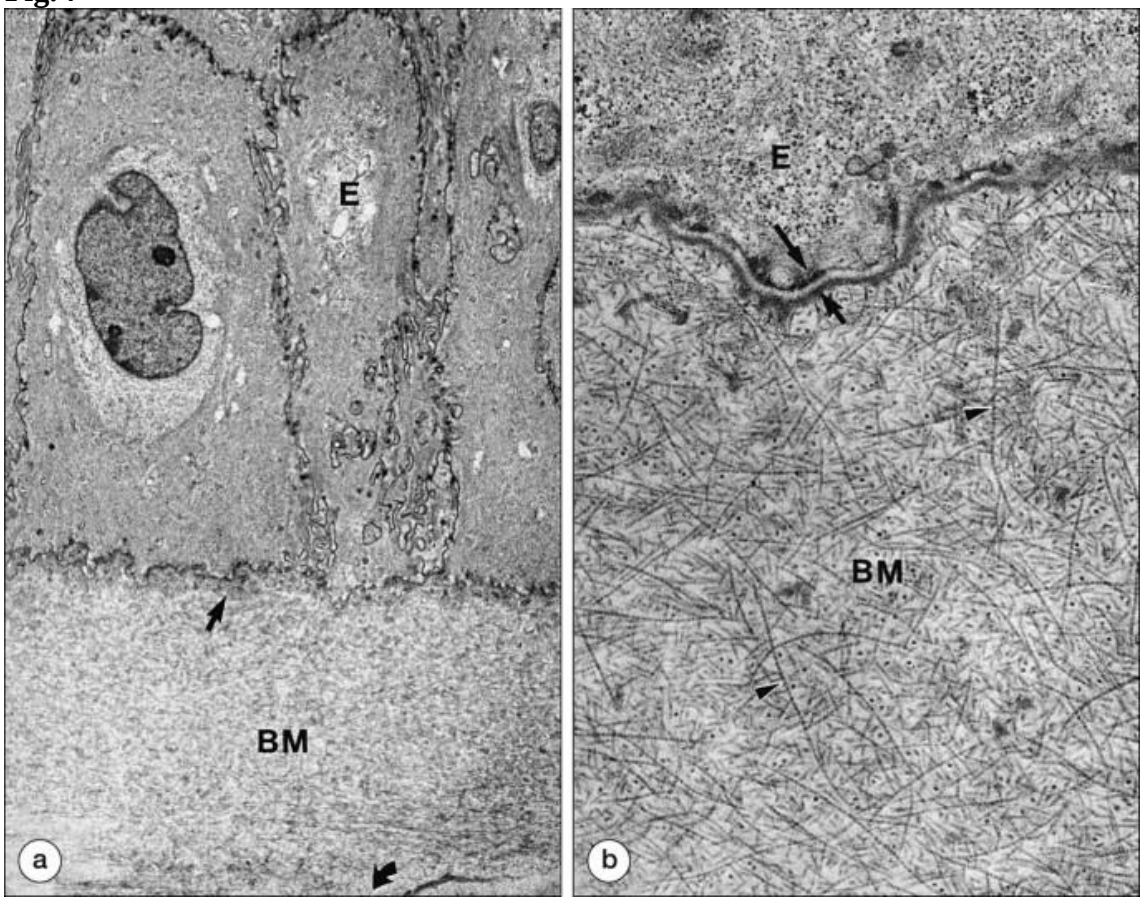
The corneal stroma (see Fig. 1a) is the dense regular connective tissue located between the epithelial basal lamina and the thick extracellular matrix secreted by the endothelial monolayer called Descemet's membrane. The stroma comprises ~90% of the corneal thickness and includes both Bowman's membrane and the lamellar stroma. The major functions of the stroma are to maintain the proper curvature of the cornea as the primary lens of the eye, to provide mechanical resistance to intraocular pressure, and to transmit light into the eye without significant absorbance. Corneal transparency is dependent on the maintenance of a low level of stromal hydration and on the orderly arrangement of collagen fibers within the stroma.

Bowman's Membrane

Bowman's membrane (Fig. 7) is an 8- to 10-μm acellular zone of randomly arranged collagen fibrils that forms an interface between the basal lamina of the epithelium and the subjacent lamellar stroma. Constituents of this layer, as well as the epithelial basement membrane, are synthesized and secreted by both epithelial cells and stromal keratocytes [32, 33]. Bowman's membrane contains several collagen types, including types I, V, and VII [34, 35] and proteoglycans, such as chondroitin sulfate proteoglycan [36]. Both Bowman's membrane and the lamellar stroma contain fibrils composed of collagen types I and V; however, the fibrils in Bowman's membrane are smaller in diameter (~20 nm) than those in the stroma (25–30 nm) [37]. Fibril diameter appears to be regulated by the relative ratio of type V to type I collagen, the greater the amount of type V, the smaller the fibril diameter [38, 39]. Studies using competitive polymerase chain reaction to quantify messenger RNA (mRNA) from avian corneal epithelial cells and stromal cells indicate that the amount of mRNA for type V collagen relative to that for type I collagen is higher in epithelial cells than in stromal fibroblasts [34]. This finding suggests that epithelial cells synthesize and secrete type I and V collagen fibers in Bowman's membrane and that the higher ratio of type V to type I collagen produced by these cells accounts for the smaller fibril diameter in Bowman's layer. Type VII collagen-containing anchoring fibrils connect epithelial hemidesmosomes to anchoring plaques located 1–2 μm into the anterior portion of Bowman's membrane. These

anchoring fibrils intertwine with type I collagen fibrils, forming a network that stabilizes the association between the surface epithelium and the underlying lamellar stroma [30, 40]. Bowman's layer is prominent in primates, including humans, but is thin or nonexistent in other mammals. The specific function of this layer is not clearly understood, but its feltwork of collagen fibrils may stabilize the transition between the epithelial and stromal layers, ensure adhesion of the overlying epithelial cells to the stromal matrix, and contribute to the smooth curvature of the corneal surface.

Fig. 7



(**a**) Bowman's membrane (BM) forms an acellular interface between the basal cells of the epithelium (E) with its basement membrane *(straight arrow)* and the lamellar stroma *(curved arrow)*. Note the relative thickness and feltwork-like appearance of Bowman's membrane. ×5800. (**b**) The random arrangement of collagen fibrils *(arrowheads)* is shown. Also note the close association of the hemidesmosomal structures *(large arrow)* on the basal aspect of the epithelial cells, the highly organized extracellular matrix *(small arrow)* of these cells, and Bowman's membrane. ×31,000

Full size image

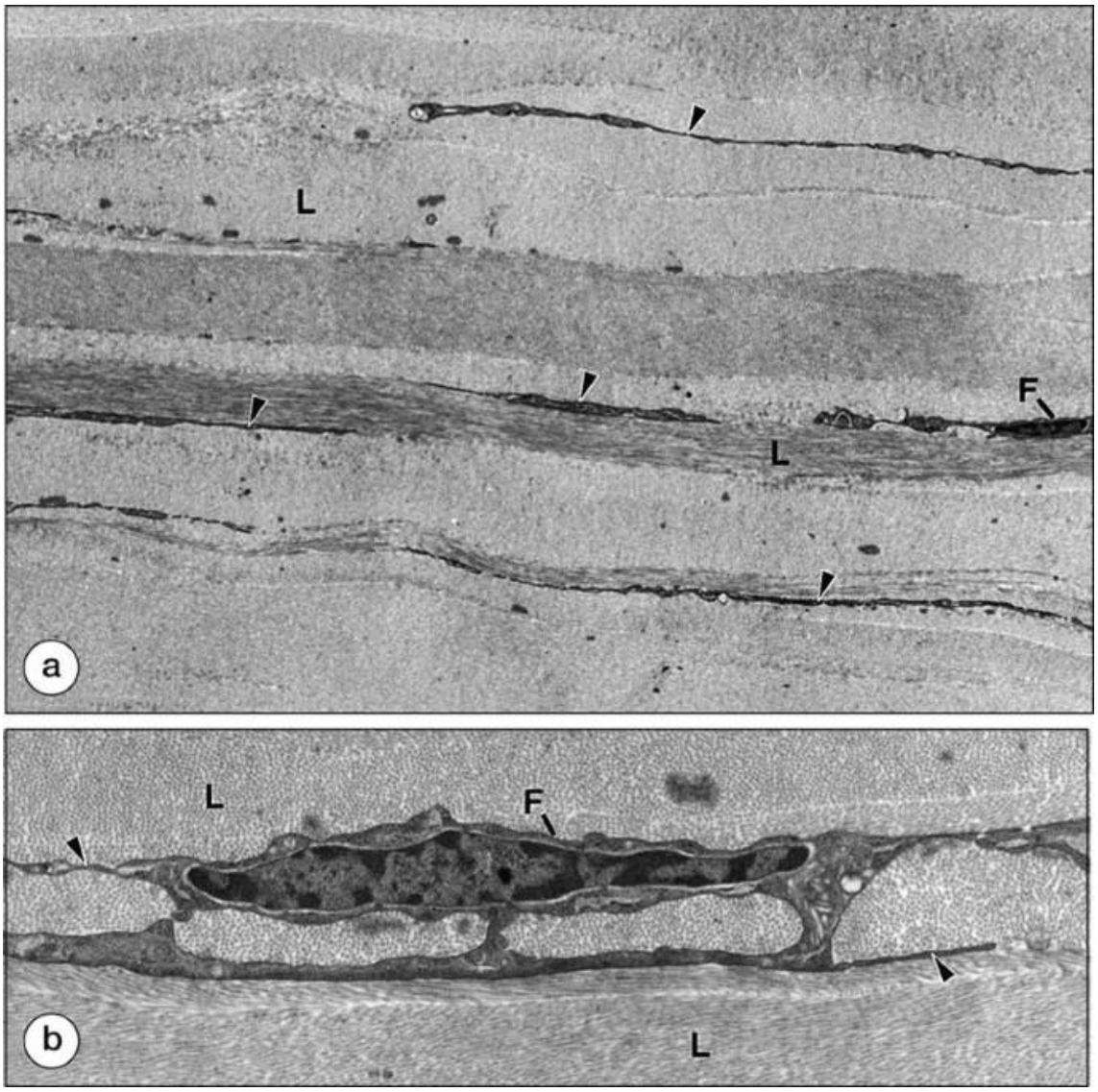
Lamellar Stroma

The lamellar stroma is the thick collagenous layer posterior to Bowman's membrane. Collagen types I and V are the predominant fibrillar collagens in the lamellar stroma, although small amounts of other fibrillar collagens, including type III, also may be present [41]. The stroma contains collagen type XII, which cross-links fibrillar

collagens, and type VI, which forms microfibril networks [42]. Keratan sulfate proteoglycans are the predominant proteoglycans within the corneal stroma. Lumican and keratocan are the core proteins of keratan sulfate proteoglycans, with lumican regulating keratocan expression [43].

As shown in Fig. 8a, the constituents of the lamellar stroma are organized precisely. The basic structural unit of the fibrillar collagens is tropocollagen, an asymmetric molecule ~300 nm long and 1.5 nm in diameter. Fibrillar collagens are composed of three polypeptide chains coiled in a triple helix. These molecules polymerize to form elongated collagen fibrils with diameters of 25–30 nm. The uniformity of collagen fibril diameter appears to result from specific interactions between type V collagen, located toward the center of the fibril, and type I collagen, on the fibril exterior. As mentioned previously, the relative ratio of type V to type I collagen appears to regulate fibril diameter. The interfibrillar distance also is highly uniform and may be maintained by apposing interactions at the fibril surface. In the chick cornea, type XII collagen binds to type I on the fibril exterior and may form lateral “bridges” between fibrils, thus limiting interfibrillar distance [44]. Proteoglycans bind to the exterior surfaces of collagen fibrils. The polyanionic nature of the glycosaminoglycan side chains attracts cations and water molecules and may exert a swelling pressure on the collagen fibrils, which is balanced by the interactions between collagen types I and XII. Microfibrils composed of type VI collagen also associate with type I collagen [45, 46] but the specific function of these fibrils is not known. Collagen fibrils are packed in parallel bundles extending from limbus to limbus, and the bundles are arranged in layers, or lamellae. The stroma of the human eye contains 200–250 lamellae. Lamellae in the middle and posterior regions of the stroma are arranged at approximate right angles, whereas those in the anterior stroma are arranged at less than right angles. The small diameter of the collagen fibrils and their close, regular packing create a lattice or three-dimensional diffraction grating [47]. The “lattice theory” of Maurice [48] suggests that the ability of the cornea to scatter 98% of incoming light results from equal spacing of the collagen fibers. Scattered light waves interact in an ordered fashion, eliminating destructive interference. The lamellar organization of the stroma also produces a uniform tensile strength across the cornea, withstanding intraocular pressure and maintaining appropriate corneal curvature.

Fig. 8



Sections of corneal stroma showing collagen bundles arranged in lamellae (L), which are oriented at different angles. (**a**) Micrograph illustrating the stacked lamellae and long, attenuated processes *(arrowheads)* of the stromal fibroblasts (F) located between the lamellae. ×4800. (**b**) Collagen bundles in the upper lamella (L) are sectioned crosswise, whereas those in the lower lamella are sectioned at an angle. Junctions between the cytoplasmic processes of neighboring fibroblasts form a network of communicating cells. ×13,000

Full size image

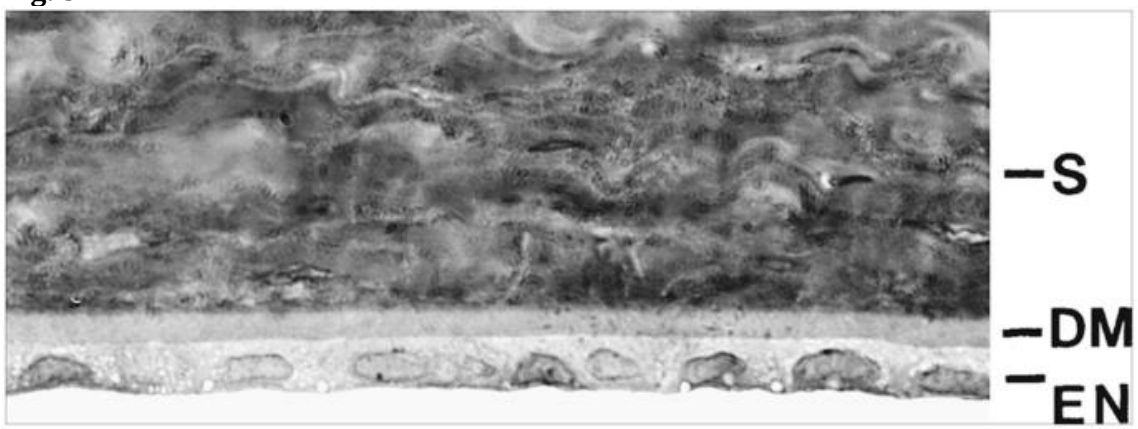
The matrix components of the lamellar stroma are secreted and maintained by stromal cells often referred to as keratocytes. As shown in Fig. 8b, these long, attenuated cells are arranged parallel to the corneal surface and are located between the collagen lamellae. The keratocyte cell body contains an elaborate rough endoplasmic reticulum and Golgi apparatus, reflecting its active synthetic function. Keratocytes extend slender cytoplasmic processes and can form gap junctions with neighboring cells, resulting in a network of communicating cells [49]. An ultrastructural study [50] of human cornea demonstrated the presence in central stroma of unmyelinated nerve fibers that run parallel to the collagen bundles. While unmyelinated, the stromal nerves are not without

the support of Schwann cells. Non-myelinating Schwann cell nuclei are observed wrapping around the stromal nerves. These stromal nerve fibers pass through Bowman's membrane and the basal lamina of the epithelium, and, as they penetrate the epithelial cell layer, they branch to become the basal nerves and intraepithelial nerve terminals of the ICNs. Stromal nerve fibers innervate stromal keratocytes. Thus, the stroma contains as resident cells quiescent keratocytes and non-myelinating Schwann cells. Both keratocytes and non-myelinating Schwann cells are derived during development from neural crest cells. Because both cell types share numerous neural crest markers, determining the relative contribution of Schwann cells and keratocytes in the stroma has proven difficult. Recently, bone marrow-derived cells were also demonstrated in the corneal stroma [51]. These cells are of both monocytic and myeloid lineages, demonstrating surface markers of the dendritic cell (antigen-presenting cells) and monocytes/macrophage type. It is not clear whether the bone marrow-derived cells function in immunologic defense or play a role in the induction of tolerance and the immune-privileged state of the cornea [51].

Endothelium

The endothelium (Fig. 9) is the single layer of cells located at the posterior of the cornea that forms a barrier between the corneal stroma and anterior chamber. The endothelial cell monolayer acts as a "leaky" barrier, permitting the passage of nutrients from the aqueous humor into the avascular corneal stroma [52, 53]. The endothelium is responsible for maintaining the relatively low level of stromal hydration required for corneal transparency. The tendency of the corneal stroma to swell is balanced by the removal of excess stromal fluid via the activity of "ionic pumps" located at the endothelial plasma membrane. The relatively high extracellular ion concentration produced by these pumps draws water from the stroma, thus maintaining the highly organized collagen lamellar structure required for corneal transparency. The endothelium also secretes components of the thick basal lamina, termed Descemet's membrane, which lies between the endothelium and posterior stroma. Developmentally the corneal endothelium is of neural crest origin [53].

Fig. 9



Low-magnification electron micrograph illustrating the posterior portion of the cornea. The stroma (S) is closely associated on its posterior-most aspect with Descemet's membrane (DM), the thick extracellular matrix secreted by the endothelial cells (EN). The endothelium is the monolayer of cells located at the

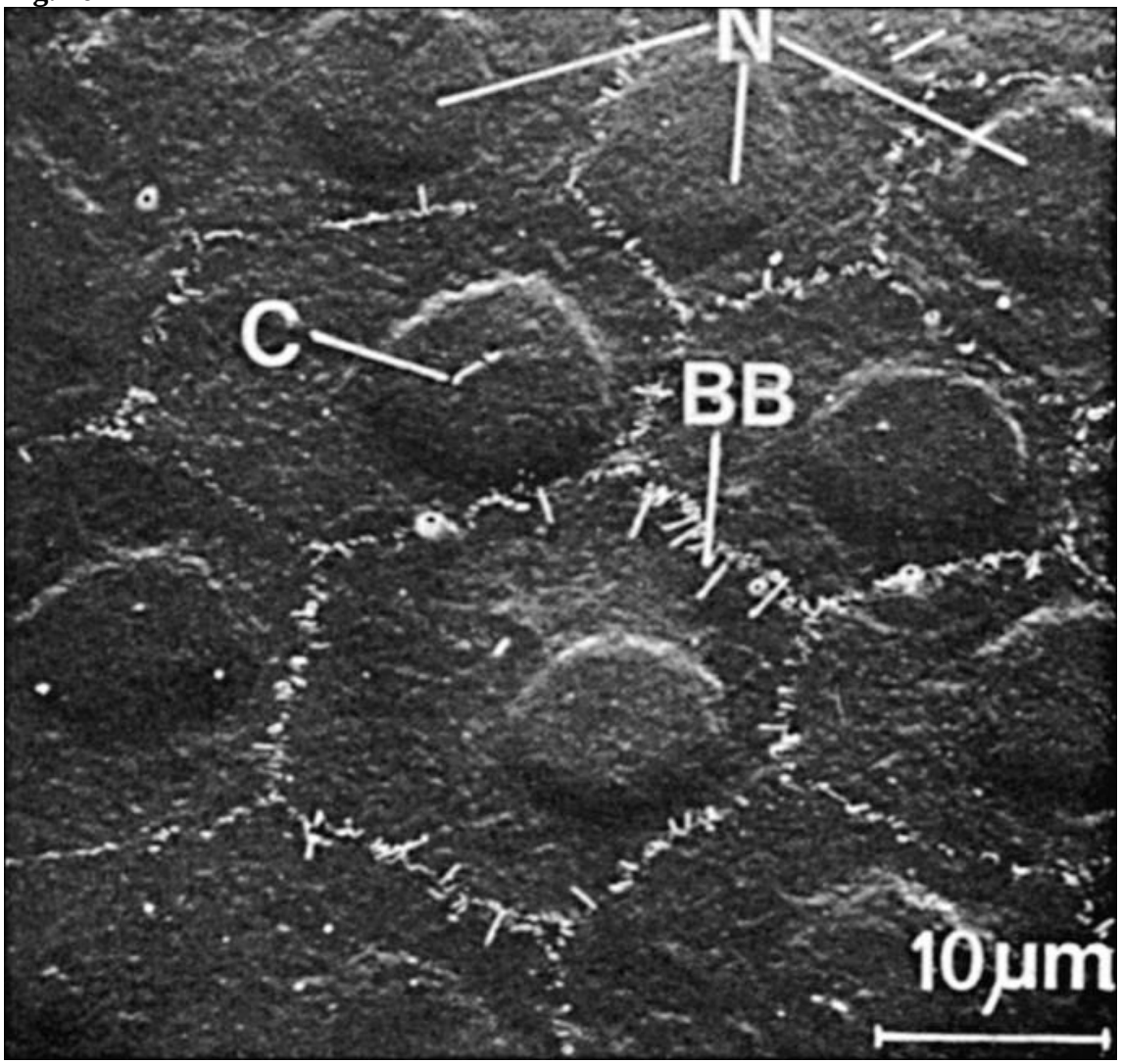
posterior of the cornea; it acts as a barrier between the aqueous humor and overlying corneal tissues. ×750

Full size image

Morphologic and Ultrastructural Characteristics

The average density of corneal endothelial cells at birth is ~4000 cells/mm^2 [54]. Each cell is 4–6 μm thick and ~20 μm wide and has a surface area of ~250 μm^2. Scanning electron microscopy of the monolayer surface (Fig. 10) reveals that cells assume a hexagonal shape and possess numerous lateral, interdigitating cellular processes [54,55,56]. These processes increase the area of contact between neighboring cells and resemble interlocking fingers. Numerous small microvilli are present on the posterior (apical) cell surface, which faces the aqueous humor. This surface appears to be covered by a glycocalyx layer ~0.5 μm thick [57]. MUC1 is at least one component of this layer and is believed to have a protective function [58]. A single, centrally located cilium, ~2–7 μm long, has been observed on the apical surface of endothelial cells at the corneal periphery. This cilium exhibits the ultrastructural characteristics of other primary cilia [59] but its function in corneal endothelium is unclear.

Fig. 10

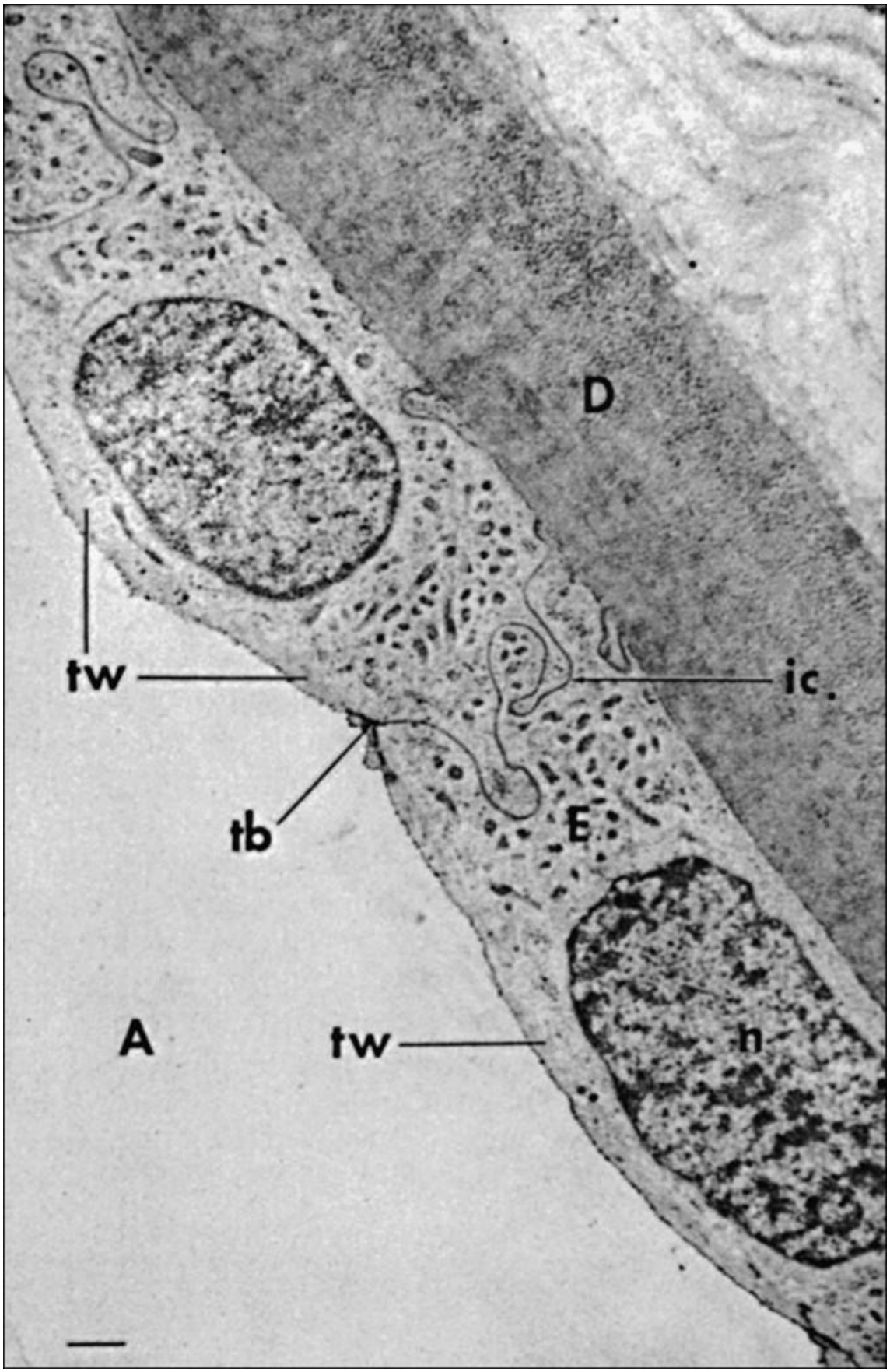


Scanning electron micrograph of the surface of the corneal endothelium illustrating the hexagonal shape of the cells as well as the other surface features, including nuclei (N) that bulge from the cell surface, a single cilium (C), and long lateral projections (BB) that bridge from one cell onto the body of adjacent cells. (From Svedbergh B, Bill A: Scanning electron microscopic studies of the corneal endothelium in man and monkeys. Acta Ophthalmol Scand 50:321, 1972)

Full size image

The ultrastructural features of the endothelium reflect its functions [60]. Numerous mitochondria within the cytoplasm indicate that these cells are metabolically active (Fig. 11). The cytoplasm also contains extensive rough and smooth endoplasmic reticulum, numerous ribosomes, and a prominent Golgi apparatus reflective of a high level of protein synthesis (Fig. 12). A circumferential band of actin-containing microfilaments is located beneath the apical plasma membrane at the cell periphery. These microfilaments help maintain cell shape and mediate cell movement [61,62,63]. An intermediate filament network comprised primarily of vimentin forms a basket-like structure that surrounds the nucleus and anchors at the apical junctions [62,63,64,65]. This network appears to be responsible for nuclear centration and, in part, for maintenance of cell–cell junction stability.

Fig. 11



Low-magnification transmission electron micrograph illustrating the general orientation and ultrastructural features of the corneal endothelium and Descemet's membrane. A band of actin-containing filaments, termed

the *terminal web* (tw), is present in the anterior aspect of the cells and excludes other cell organelles. Junctional complexes located on the apical aspect of the lateral plasma membranes are visible at this low magnification as a terminal bar (tb). The intercellular border (ic) formed between adjacent cells is long and sinuous. A, anterior chamber; E, endothelium; n, nucleus; D, Descemet's membrane. Bar = 1 µm. (From Iwamoto T, Smelser GK: Electron microscopy of the human corneal endothelium with reference to transport mechanisms. Invest Ophthalmol Vis Sci 4:270, 1965)

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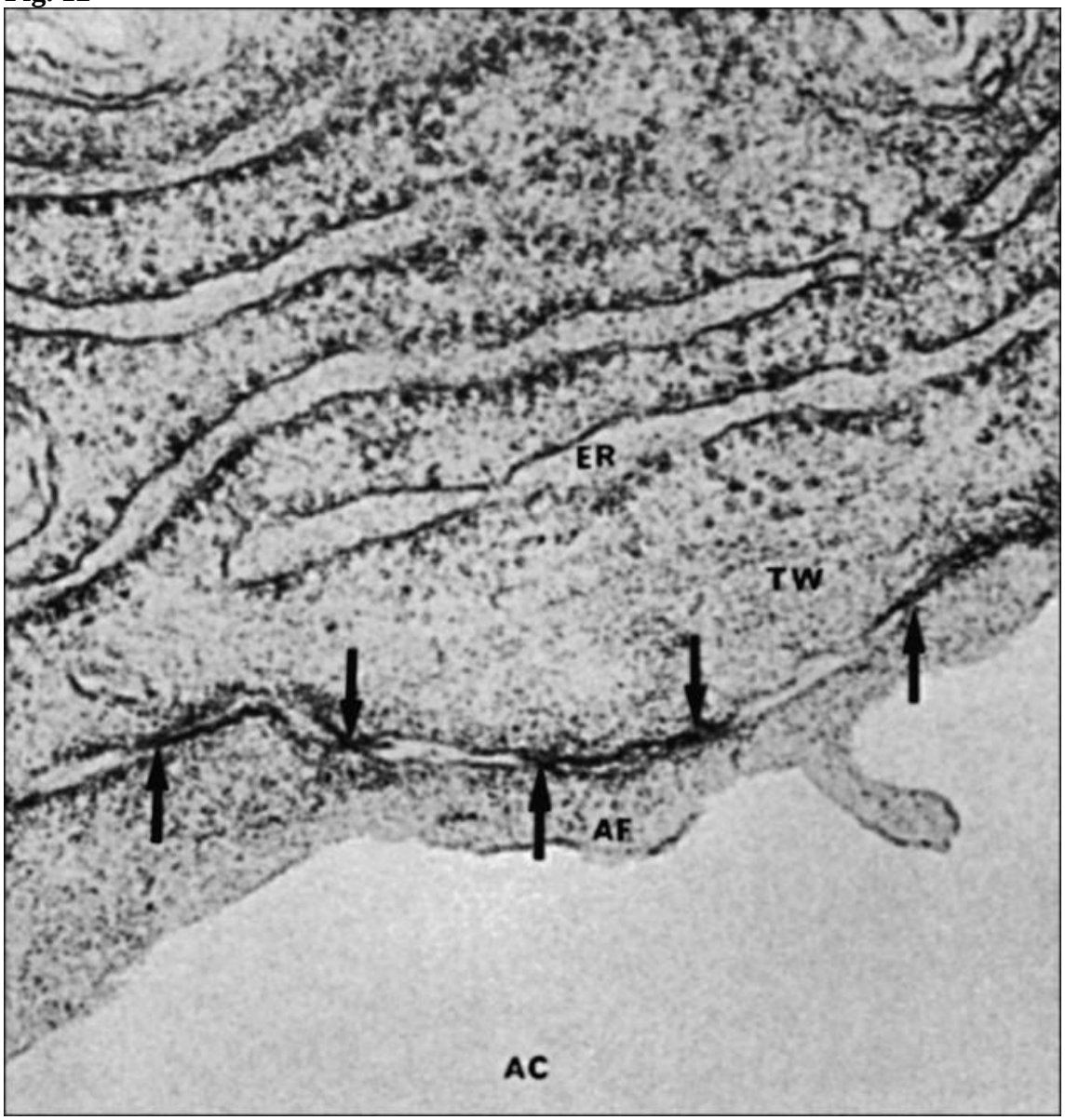
Fig. 12



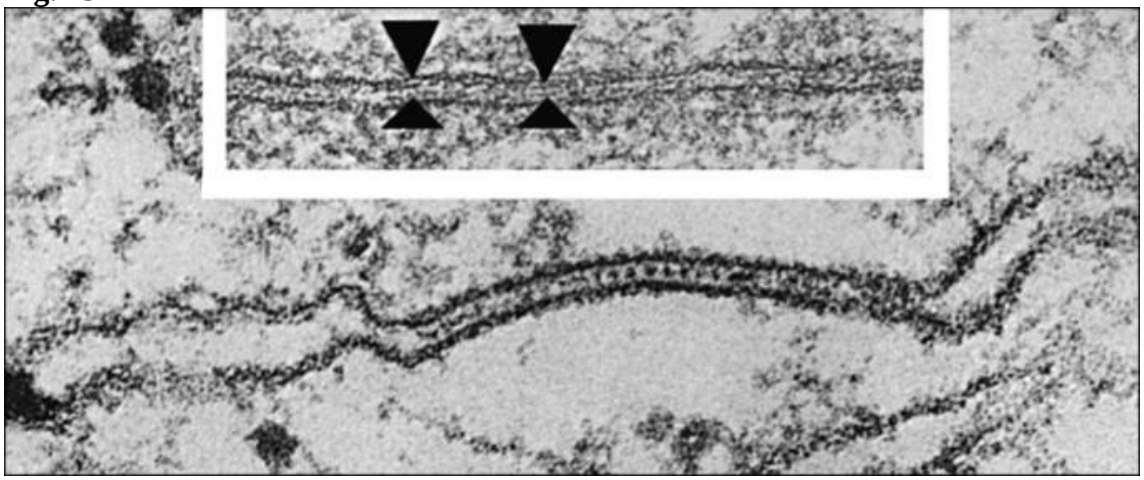
Anterior portion of an endothelial cell illustrating the extensive endoplasmic reticulum (ER) with its associated ribosomes as well as focal tight junctional complexes *(arrows)* and apical folds (AF) where adjacent cells interdigitate. AC, anterior chamber; TW, terminal web. ×80,000. (From Hirsch M, Renard G,

Faure JP, et al: Formation of intercellular spaces and junctions in regenerating rabbit corneal endothelium. Exp Eye Res 23:385, 1976)

Full size image

Relatively little is known about the molecular basis of adhesion of the endothelium to Descemet's membrane. Focal areas of increased electron density are present on the cytoplasmic aspect of the basal plasma membrane [63] and may represent a form of adhesion plaque anchoring the endothelium to Descemet's membrane. Cytoplasmic processes extend from the basal aspect of the cells and penetrate Descemet's membrane contributing to increased adhesiveness of the monolayer [66]. Corneal endothelial cells express numerous integrins including α4, α6, αv, and α1 integrins [67, 68] as well as the ECM proteins that make up Descemet's membrane. Focal tight junctions (Fig. 12) on the apical aspect of the lateral membranes are small areas in which the outer leaflets of the plasma membranes of adjacent cells appear to fuse, obliterating the extracellular space [66, 69,70,71]. These junctional complexes do not form "belts" or rings extending around the cell, as found in other simple epithelia [72, 73]. Rather, they occur as small zones of membrane fusion found around the cell circumference. There have been few studies to specifically identify the protein constituents of tight junctions in corneal endothelium; however, it is known that ZO-1 (zonula occludens-1) and occludin are components of these structures [63, 74]. In fact, focal tight junctions can be visualized by the discontinuous immunostaining of ZO-1. Electrical resistance across the endothelial monolayer is low (73 ± 6 W/cm^2) [71] compared to that across the corneal epithelium (1.6–9.1 kW/cm^2), reflecting the different organization of tight junctions in these two tissues [75, 76]. Adherens junctions are also located at the lateral plasma membrane. These appear to be composed of N-cadherin [77], α- and β-catenin, and plakoglobin [63]. Gap junctions (Fig. 13) are located at all levels of the lateral plasma membrane below the tight junctions [66, 69]. These junctions stain positively for connexin 43 [78, 79], possess a characteristic pentalaminar structure, and are the site of electrical and metabolic coupling, which facilitates cell-to-cell communication [79, 80].

Fig. 13



Electron micrograph of gap junctional complexes illustrating the characteristic regular spacing of the connexin cross-bridges that draw adjacent plasma membranes into close apposition. *Inset,* Arrowheads indicate areas in which the gap between cell membranes is clearly visible. ×180,000. (From Leuenberger

PM: Lanthanum hydroxide tracer studies on rat corneal endothelium. Exp Eye Res 15:85, 1973)

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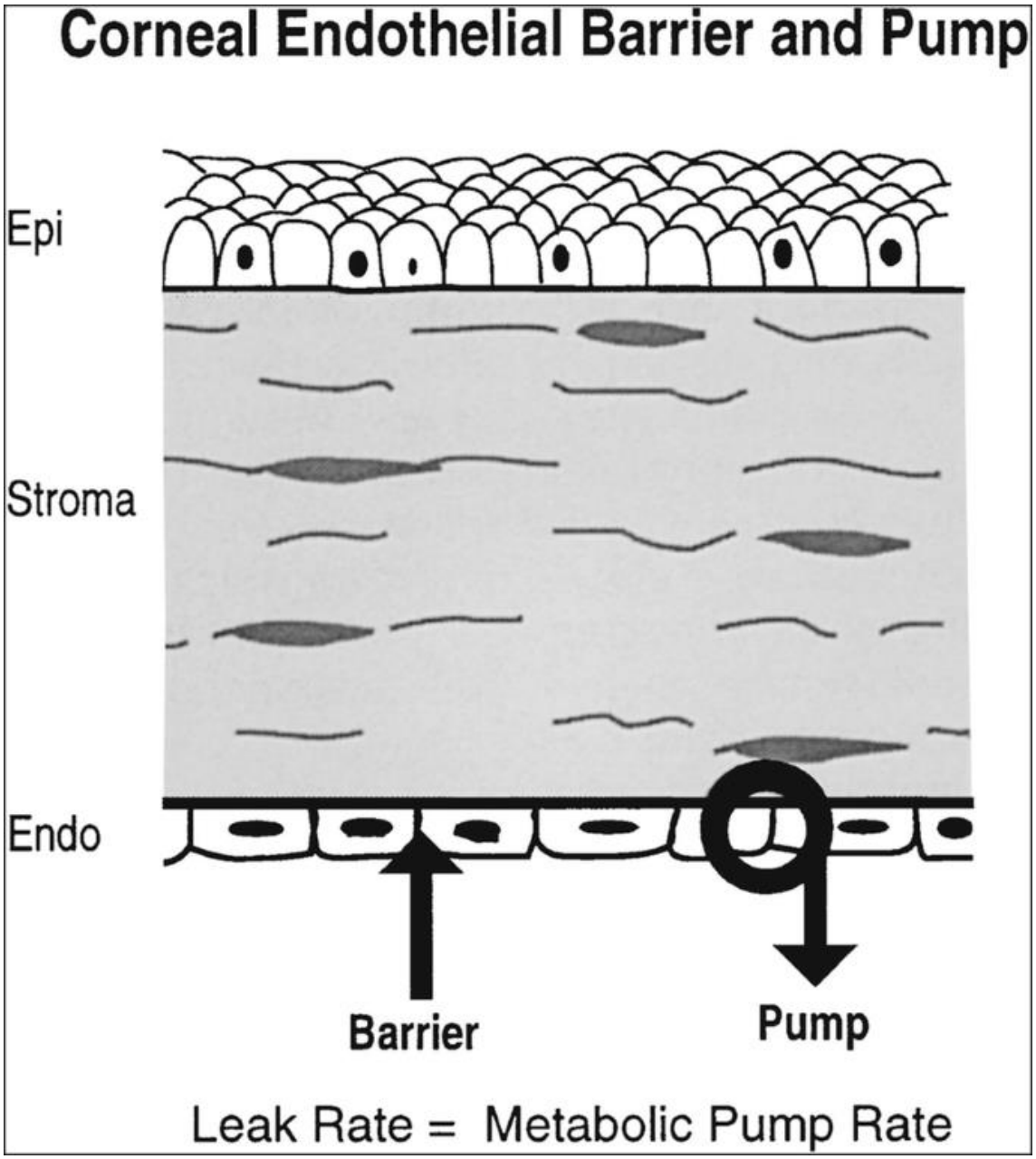
Barrier and Pump Functions

As an avascular tissue, the cornea receives oxygen mainly from the tear film [81], but its nutritional requirements are met via the aqueous humor. As such, the glucose, amino acids, vitamins, etc., needed by the epithelial cells and stromal keratocytes must traverse the corneal endothelial monolayer. This nutrient transport occurs primarily via a paracellular route, i.e., solutes move between the cells rather than by being actively transported through them. This form of transport requires that the endothelial monolayer be "leaky" to substances within the aqueous humor, but not permit bulk fluid flow into the corneal stroma. The barrier to bulk flow of fluid from the aqueous humor to the stroma is formed primarily by the focal tight junctions of the endothelium. Experiments with molecular tracers indicate that small molecules do not penetrate the tight junctions, but enter the intercellular spaces by leaking around them [66, 69, 71, 73]. Gap junctions and the sinuous, elaborate interdigitation of the lateral plasma membranes together may form a secondary barrier to fluid flow [82]. Narrowed intercellular spaces produced by the formation of gap junctions, plus the requirement that fluid must move between the inter-digitating lateral membranes, help prevent bulk fluid movement across the endothelial monolayer.

Transparency is essential for the function of the cornea as the primary lens of the eye. Transparency results from the uniformity of the tissue elements comprising the cornea and from the regularity of their spatial organization. Precise arrangement of the collagen bundles within the corneal stroma is especially important for corneal clarity [48]. This precise arrangement depends to a great extent on the maintenance of a relatively low level of stromal hydration. Proteoglycans associated with the collagen fibrils within the stroma bind water, producing a natural pressure gradient across the endothelial monolayer. As a result, loss of integrity of the endothelial cell layer can hydrate the stroma. The disorganization of collagen fibrils, which results from stromal swelling, causes light absorbance, corneal clouding, and reduced vision.

The requirement for the endothelium to permit passage of nutrients into the corneal stroma and, at the same time, maintain a barrier to the free flow of water into the stroma presents an interesting cell biological paradox. The "pump-leak" hypothesis has attempted to resolve this paradox. It states that the rate of leakage of water and solutes into the corneal stroma is balanced by the rate of pumping of excess water from the stroma back to the aqueous [83]. As long as the equilibrium suggested by this hypothesis is maintained, the corneal stroma remains relatively dehydrated and corneal clarity is maintained. Figure 14 illustrates this equilibrium.

Fig. 14



The “pump-leak” hypothesis. When the rate of fluid leakage into the stroma is balanced by the rate of fluid pumped out of the stroma, normal corneal architecture and thickness are maintained. (Adapted from Waring GO III, Bourne WM, Edelhauser HF, Kenyon KR: The corneal endothelium: Normal and pathologic structure and function. Ophthalmology 89:531, 1982)

Full size image

The endothelium maintains a low level of stromal hydration by the activity of ionic “pumps,” which mediate the transfer of Na+, K+, Mg+, Cl_2^-, and HCO_3^- (bicarbonate). Fluid flow from the stroma to aqueous humor appears to be secondary to electrolyte movement [84]; however, the specific mechanism by which movement of electrolytes is coupled to the movement of water has not been completely understood [84,85,86]. Metabolic energy is needed to maintain normal corneal thickness, indicating that at least part of the mechanism regulating stromal hydration involves an active process [87, 88].

The fluid "pump" was thought, until recently, to be dependent on the presence of $Cl_2^{-\cdot}$ and HCO_3^- [86,87,88,89]. However, newer research has demonstrated that corneal endothelial function does not have an absolute requirement for bicarbonate; rather it requires a perfusing solution with high buffering power. This facilitates lactic acid efflux, which is directly linked to water efflux. Thus, lactate flux is a component of the corneal endothelial pump [90]. In addition, the water channel protein, aquaporin-1 (AQP1), has been localized to the plasma membrane of corneal endothelium [91]; and its deletion in mice reduces corneal water permeability and delays restoration of transparency after swelling [92].

Monolayer Repair

Corneal endothelial cells are capable of normal division during fetal development; however, the total corneal endothelial cell reserve is limited, because cell division in adult cells either does not occur at all or occurs at a rate too slow to efficiently replace dead or injured cells [54, 93,94,95]. At birth, endothelial cell density is 3500–4000 cells/mm^2, whereas in adults it is reduced to 1400–2500 cells/mm^2 [54]. Beginning at about the second year of life, decreased cell density is directly related to endothelial cell loss and the inability of the endothelium to reproduce in numbers sufficient to keep pace with this loss [93, 94]. The overall rate of cell loss accelerates if the endothelium is injured as the result of trauma, disease, or dystrophy [95,96,97]. When the endothelium is injured or when cells are lost due to normal attrition, repair of the defect in the monolayer occurs mainly through enlargement and spreading of neighboring cells, causing cells to be larger in these areas [96, 98,99,100,101,102]. Polymegathism, i.e., heterogeneity in cell size [98, 103,104,105], pleomorphism [55, 106, 107], and number of multinucleated cells [95, 106,107,108], increases in the endothelium with age and as the result of damage caused by trauma, corneal infection, or disease [109,110,111]. When cell numbers are reduced, the ability to maintain or restore normal barrier and pump function can be compromised [112,113,114]. Decompensation, i.e., loss of monolayer integrity and function, can occur when cell density falls below 300–400 cells/mm^2 or when the mean cell size reaches ~3000–3500 μm^2 [52, 95]. Because of the stressed state of the endothelial monolayer under these conditions, the leak rate of fluid into the stroma becomes greater than the pump rate of fluid flow out of the stroma, producing stromal edema and corneal clouding.

Proliferative Capacity

Investigators have re-examined the relative proliferative capacity of corneal endothelial cells [115,116,117]. There is evidence to suggest that these cells can divide in vivo, but at a very slow rate [108, 118, 119]; however, the well-established observation that endothelial cell density decreases with age strongly suggests that, if endothelial cells do divide in vivo, the rate of cell division does not keep pace with the rate of cell loss. The ability to grow human corneal endothelial cells in tissue culture without requiring viral oncogene protein expression [74, 120, 121] clearly indicates that these cells retain proliferative capacity that can be harnessed under appropriate conditions. Comparative studies of cell-cycle protein expression in corneal cells suggest that endothelial cells in vivo are arrested in G1 phase of the cell cycle [122, 123]. Thus, the limited proliferation observed in this tissue in vivo appears to be due, at least in part, to microenvironmental conditions that actively maintain the endothelium in a non-proliferative state.

A number of mechanisms may contribute to inhibition of corneal endothelial cell proliferation in vivo. One inhibitory mechanism appears to be suppression of S-phase entry by transforming growth factor-β2 (TGFβ2). A role for this cytokine in negatively regulating proliferation of corneal endothelium is supported by the fact that TGFβ2 is present in relatively high concentration in aqueous humor [124, 125] and that corneal endothelial cells express the receptor types required to transmit TGFβ2-induced signals [126]. Contact inhibition is another mechanism that suppresses proliferation in corneal endothelial cells. This has been shown using an ex vivo wound model in which treatment of the endothelium with the calcium/magnesium chelator ethylenediaminetetraacetic acid (EDTA) releases cell–cell junctions and promotes cell division [127]. There are also intrinsic, age-related factors that affect the ability of human corneal endothelial cells to proliferate. A common finding has been that cells cultured from young donors grow more robustly and can be passaged more times than cells from older donors [128,129,130]. Morphometric studies [131, 132] have documented that there are differences in endothelial cell density in peripheral versus central cornea, indicating a nonuniform distribution of endothelial cells across the cornea. Cells in the far peripheral region of the endothelium close to where Descemet's membrane ends (Schwalbe's line) exhibit a higher cell density than cells in the paracentral or central regions [132]. Although cell densities from all regions decreased with age, the rate of decrease in density was slowest in the peripheral region.

Recently, the intriguing question has been raised whether there may be a difference in relative proliferative capacity between endothelial cells located in central cornea and those located in the periphery and whether there are stem cells within this population, or whether there are slow cycling cells in the periphery that can repopulate the central areas. Several lines of evidence support either hypothesis (for review see [133]), but at this writing the issue has not been resolved. Tissue culture studies [134] and ex vivo cornea studies [135] have demonstrated that human corneal endothelial cells cultured from both the central and peripheral regions exhibit proliferative capacity, regardless of donor age. In the ex vivo cornea studies, the relative percent of cells exhibiting replication competence was significantly higher in the peripheral cornea than in the central cornea, regardless of donor age. In corneas from older donors, central endothelial cells exhibited the lowest percent of replication competent cells, and low peripheral endothelial cell count is a predictor of disease severity [136]. Together, these studies suggest that, with donor age, central endothelial cells become senescent and die and that there may be a slow centripetal movement of cells from the periphery to replace them. It is not yet clear whether peripheral cells are recruited from the periphery as the result of slow cell movement and rearrangement or whether peripheral cells may divide to help replace cells lost from the central endothelium. Additional studies [137] have demonstrated telomerase activity in the far peripheral region of human corneal endothelium, suggesting that this region contains progenitor (stem-like) cells. It remains to be clearly demonstrated that stem-like cells could act as a source of cell renewal for corneal endothelium.

Treatment of Endothelial Dysfunction

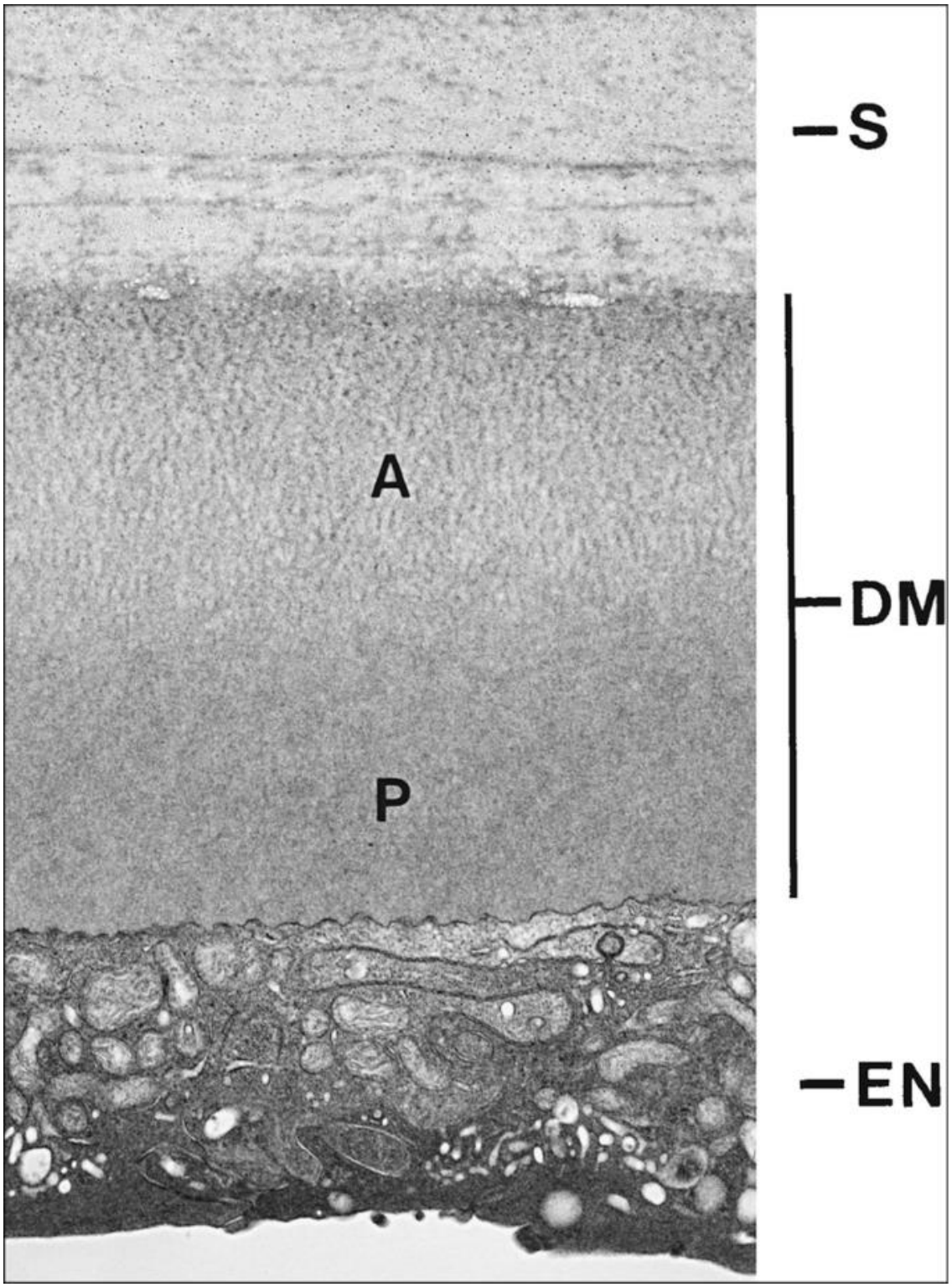
Therapy for corneal endothelial dysfunction, particularly that which occurs in Fuchs' dystrophy, has evolved in recent years. Initially total corneal transplant was employed. New methods of partial corneal transplant of posterior stroma, plus Descemet's and endothelium (DSAEK) and transplant of just Descemet's with endothelium (DMEK),

are being employed (for review see [138]). Even more recently, Descemet's stripping without transplant is being employed in subsets of patients [139] along with application of Rho-associated kinase (Rock) inhibitor [140] which has been demonstrated to maintain corneal endothelial cells in a proliferative state [141, 142].

Descemet's Membrane

Descemet's membrane is the thick extracellular matrix synthesized and secreted by the corneal endothelium (Fig. 15). In adults this matrix consists of two layers. An anterior, "banded" layer is formed during fetal development and consists of highly organized collagen lamellae and proteoglycans. A posterior "amorphous" layer is synthesized after birth and is less organized than the fetal layer. Adult Descemet's membrane contains fibronectin, laminin, type IV and type VIII collagen, as well as heparan sulfate and dermatan sulfate proteoglycan. How these constituents are assembled to form the highly ordered lattice of the fetal membrane and the more randomly organized adult membrane remains unresolved [143]. Corneal endothelial cells synthesize and secrete basement membrane material throughout life. In young adults the posterior layer measures ~2 μm, but increases to ~10 μm in older individuals. The positive correlation between age and Descemet's membrane thickness [144, 145] suggests that there is little, if any, destruction of previously formed basement membrane material. Quantifying the thickness of Descemet's membrane over time using the slit lamp can provide a type of historic record of corneal endothelial function and has been used to study the development of endothelial diseases or dystrophies. By comparing the morphology and thickness of Descemet's membrane in normal and diseased corneas, it is possible to determine the relative point in time in which the ability of corneal endothelial cells to synthesize and secrete normal Descemet's membrane is compromised. Individual endothelial cells can produce excess extracellular matrix material, resulting in the formation of focal or nodular thickenings in Descemet's membrane. These thickenings, called Hassall–Henle bodies or "warts," are frequently found in cells at the corneal periphery [54]. Similar structures are termed "guttatae" when they are located centrally within the cornea [146]. The number of these focal thickenings increases with age, in certain endothelial dystrophies, such as Fuchs' dystrophy [147,148,149], and as the result of inflammation [150].

Fig. 15



Micrograph illustrating Descemet's membrane (DM) located between the posterior aspect of the corneal stroma (S) and the underlying endothelium (EN). Two regions of Descemet's membrane are apparent in adult corneas. The anterior "banded" region (A) is secreted by the endothelial cells during fetal development and is more highly organized than the posterior "amorphous" region (P), which is secreted after birth. The posterior region increases in thickness with age as a result of continued synthesis of its constituents by the endothelium throughout life. ×9600

Full size image

Limbus

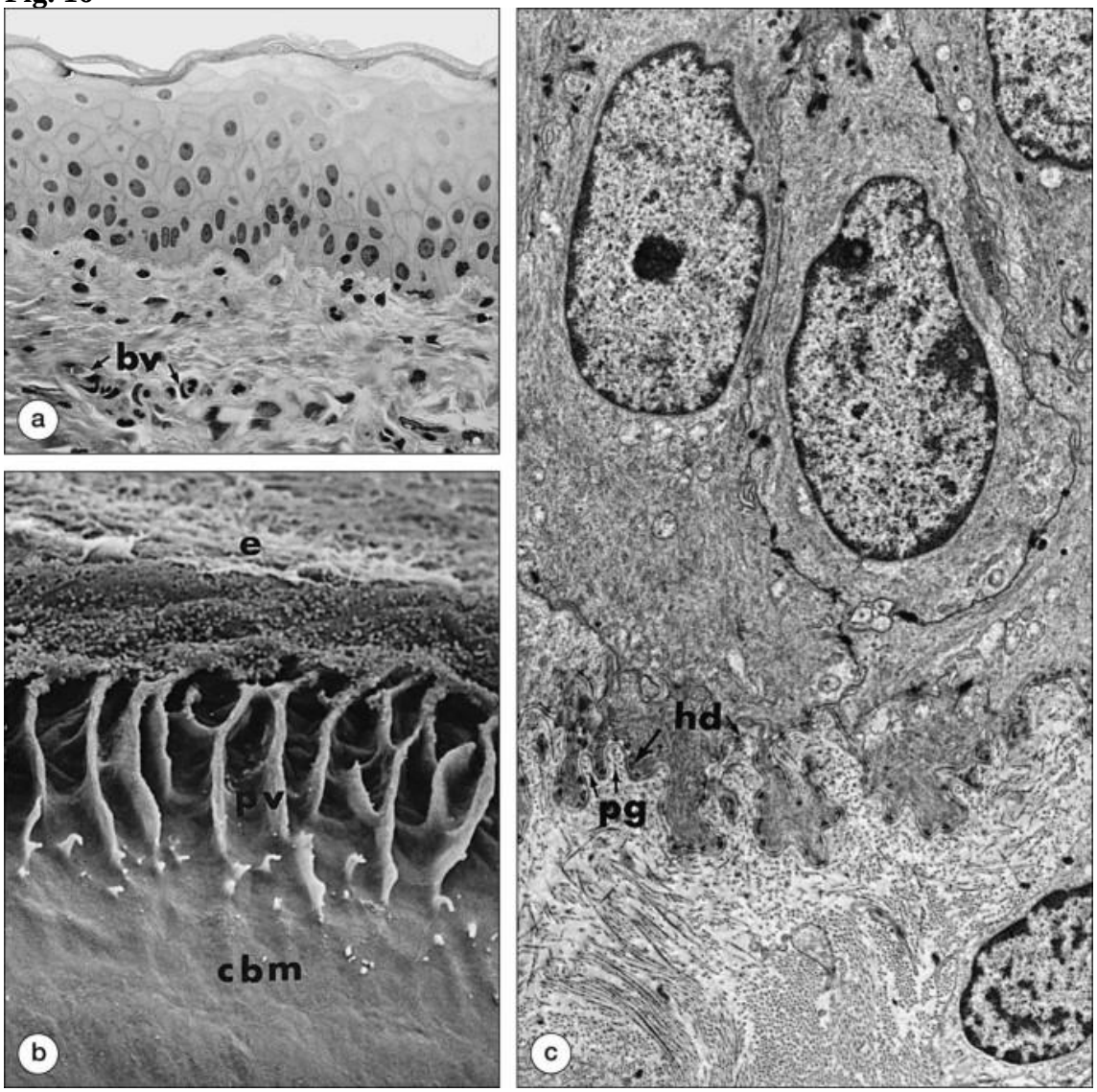
Anatomic definitions of the limbus include the anatomists' limbus, the pathologists' limbus, the histologists' limbus, and the surgeons' limbus [3, 151]. These definitions and the various angles of lines drawn on sections or diagrams of cross-sections of the region indicate that there are no definite reliable boundaries to the zone. The broadest definition of the limbus is the zone between a line drawn between the termini of Bowman's layer and Descemet's membrane, which forms the anterior border, and a line that passes parallel but 1 μm posterior to the anterior line, passing through the posterior end of Schlemm's canal. In this definition, both Schlemm's canal and the trabecular meshwork are within the limbus. This section reviews aspects of the limbus relevant to ocular surface function, specifically the superficial region, including the epithelium and loose connective tissue overlying the interface of the connective tissue at the corneoscleral junction (see Fig. 1b). The limbal region has been termed the transition zone between the cornea, conjunctiva, and sclera, and although that may be an apt description, the specialized characteristics of the limbal epithelium and its immediate subjacent connective tissue indicate that the region has specialized functions supporting the cornea and that it may be a barrier to conjunctival overgrowth of the cornea.

Epithelium

The epithelium of the limbus has many features common to corneal epithelium. It is a stratified squamous nonkeratinizing epithelium but has more cell layers than corneal epithelium making it thicker [3, 152]. Cell junctions in the limbus are similar to those in the cornea, and the apical and basal specializations present in the limbus are the same as those in the cornea. The basal cells of the limbal epithelium appear unique and are believed to be the stem and progenitor cells for maintenance of the corneal epithelium (for review, see [1, 153]). The cells appear smaller and less columnar and have more cytoplasmic organelles (Fig. 16). The basal cells sit on a basement membrane that is not flat and planar like that of the cornea; peglike interdigitations of the epithelium and the stroma are present (see Fig. 16) [3]. The limbal basement membrane is distinct in composition from the epithelial basement membrane. Compared to both the corneal and conjunctival BMs, the limbal BM has increased immunoreactivity for laminin α1, α2, β1 chains, agrin, and a specific but patchy immunoreactivity for laminin γ3 chain, BM40/SPARC, and tenascin-C [154, 155]. Probably the best indication to date that the adult stem cells are a subpopulation of limbal basal cells is their long ^{3}H-thymidine label retention time, indicating slower passage through the cell cycle than basal cells of the cornea and conjunctiva [14]. Other recent data using GFP mice show a slow centripetal movement of successive progeny of the limbal basal cells toward the center of the cornea [156]. There are differences in keratin expression in basal cells compared with suprabasal cells of the limbus and cells of the corneal epithelium [19]; and they also show enhanced presence of certain metabolic enzymes and proteins, including α-enolase, cytochrome oxidase, Na+, K+-ATPase, carbonic anhydrase, metallothionein, and glucose transporter [157,158,159,160]. Recent data suggest that the stem-cell population expresses the membrane-transporter proteins designated ABCG2 [161] or ABCB5 [153]. Another characteristic of the region is that ocular surface tumors which are rare in the central cornea occur primarily in the limbal area. Taken together, these

data and those from experiments demonstrating centripetal migration of cells from the limbal region into the cornea over time indicate that the limbal basal cells include the stem cells of the corneal epithelium. The limbal stem cells divide slowly during homeostasis; their progeny, corneal epithelial progenitor cells, migrate away from the limbus towards the corneal center. Further evidence that these basal cells are important to maintenance of the corneal epithelium comes from clinical data that demonstrate the effectiveness of limbal transplantation in the treatment of persistent, nonhealing corneal problems [162, 163]. In addition, these basal cells are protected by pigmentation and are present within deep crypts in the limbal connective tissue, termed the palisades of Vogt (see Fig. 16b).

Fig. 16



Micrographs of the limbal epithelium and subjacent connective tissue. The basal cells of the region are smaller and less columnar than those of the cornea. (**a**) Light micrograph showing the absence of Bowman's layer and the cellular stroma and blood vessels (bv). ×300. (**b**) Scanning electron micrograph of palisades of Vogt (pv); limbal epithelium was removed with ethylenediaminetetraacetic acid to demonstrate the folds or ridges in the connective tissue. Remnant epithelium (e) and denuded corneal basement

membrane (cbm) are labeled. ×2000. (**c**) Electron micrograph of basal cells of the limbal region. Note their small size and the hemidesmosomes (hd) present along the undulating basement membrane. Small, retelike pegs (pg) of stromal matrix extend into the epithelium. ×6000

Full size image

Connective Tissue

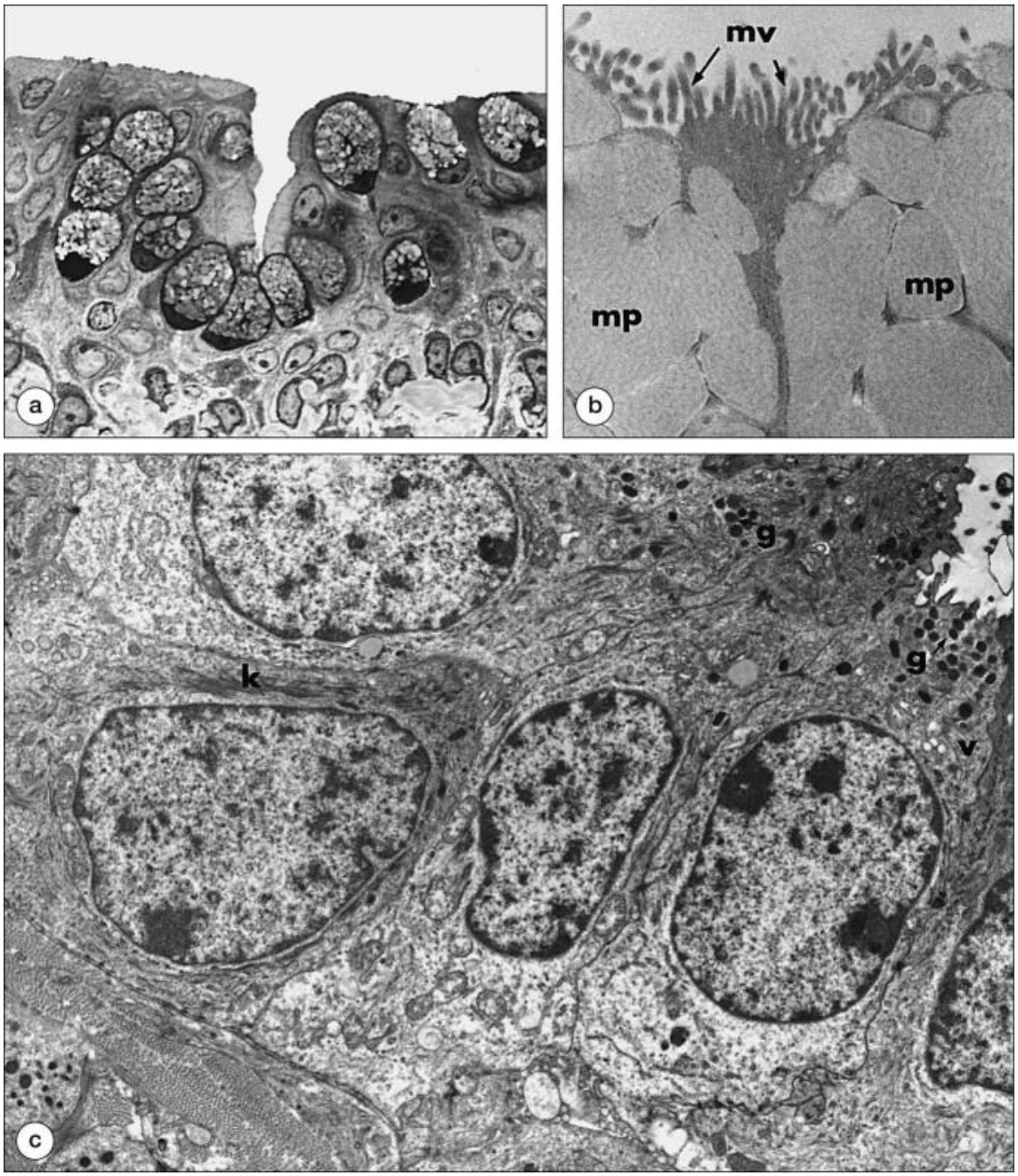
The connective tissue underlying the limbal epithelium is loose and less organized than the corneal stroma; Bowman's layer is not present. Although the molecular composition of the two limbal and stromal matrices appears to correspond, an exception is absence of the keratan sulfate proteoglycan (lumican) [164]. A major difference between the limbal stroma and that of the cornea is the presence of blood and lymphatic vessels that loop into the area of the limbal stroma. These vessels include capillaries, small arterioles and venules, and large lymphatics. Bundles of unmyelinated nerves also are present. Cellular elements within the limbal stroma are more diverse than in the corneal stroma. In addition to fibroblasts, melanocytes, mast cells, lymphocytes, and plasma cells occur routinely. The palisades of Vogt, large folds of matrix, are a unique characteristic of this area (see Fig. 16b). The outward folds of connective tissue are large enough to accommodate small blood vessels, lymphatics, and nerves, and crypts of limbal epithelium reach down into the palisades of Vogt. The deep housing of the limbal epithelium in the folds may not only protect the stem-cell population but also increase the surface area for accommodating a large cell population and increase exposure to nutrients and effector molecules derived from the vasculature. In addition to the large macroscopic folds of the palisades of Vogt, tiny rete (peglike folds or outpockets of stroma) begin in the peripheral stroma and extend through the limbal region into the conjunctiva (see Fig. 16c). These rete ridges may increase the surface area of the basal cell membrane of basal cells and may provide for additional anchoring strength in a region where hemidesmosomes are not as extensive [165].

Conjunctiva

General Characteristics and Description of Regions

The conjunctiva is the mucous membrane that covers the inner surfaces of the upper and lower lids and extends to the limbus on the surface of the globe. The two major functions of this tissue, besides connecting the lids to the globe, are provision of mucus for the tear film and protection of the ocular surface from pathogens through immune tissue. The ducts of the lacrimal, accessory lacrimal, and meibomian glands enter the conjunctival epithelium and deliver their respective products to the tear film. Three regions within the conjunctiva are recognized: the palpebral or tarsal region, which lines the inner surface of the lids; the fornical region, which lines the upper and lower surfaces of the recess or cul-de-sac known as the fornix; and the bulbar region, which lines the surface of the sclera between the fornix and the limbus. The conjunctiva has two structural components throughout all regions: the surface epithelium and the substantia propria (Fig. 17; see Fig. 1c).

Fig. 17



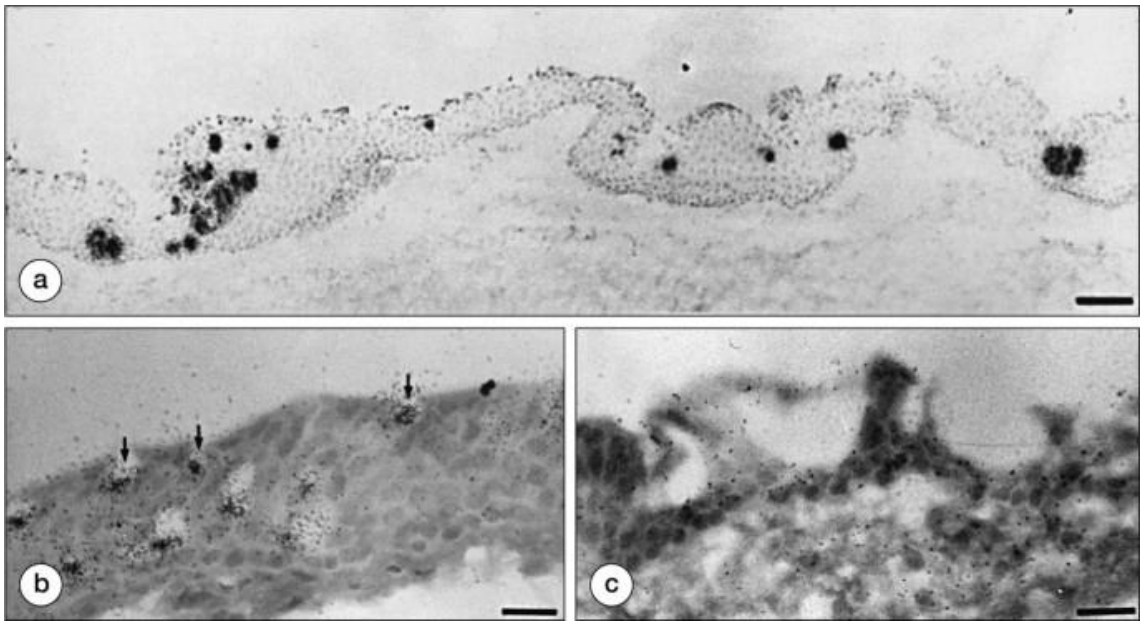
Micrographs demonstrating regions of bulbar conjunctiva. In the bulbar region, particularly in the nasal zone, goblet cells are dense. They can occur in crypts or groups, which have the appearance of acini, as demonstrated in the light micrograph (**a**). ×750. (**b**) Electron micrograph of the apical region of two adjacent goblet cells. Note the microvilli (mv) on the surfaces of cells and the fibrillar pattern in the mucin packets (mp). ×21,000. (**c**) Low-magnification electron micrograph of nongoblet cells in the conjunctiva. Note the vesicles (v) and granules (g) in the apical region of cells and clumping of keratin filaments into bundles (k). ×6000

Full size image

Conjunctival epithelium is unique among stratified nonkeratinizing epithelia in that it has goblet cells intercalated within it (see Figs. 1 and 17). The goblet cells are the major producers of mucins for the tear film. Reports of the number of cell layers in the stratified epithelium vary, especially regarding fornical and bulbar areas. These variations may result from different degrees of stretch on the tissue at time of fixation for histologic study. Cell layers of the palpebral conjunctival epithelium do not vary as much, perhaps because the substantia propria is not as loose and contractile at fixation. Reports have varied from 2 or 3 cell layers to 10–12, the latter number of layers being present at the lid margin near the junction with the epidermis covering the external lid. Langerhans' cells are present within the conjunctival epithelium [51].

Compared with cells of the corneal epithelium, the stratified cells of the conjunctiva have more cytoplasmic organelles. Keratin filaments in these cells are not as dispersed as those in corneal cell cytoplasm and often appear in bundles (see Fig. 17). Keratin proteins expressed by stratified conjunctival epithelial cells also are different, with the keratin pairs K4 and K13, and K3 and K19. K7 was previously reported to be expressed by goblet cells [166], but more recent data suggests that the keratin is not specific to the goblet cells [167]. Cell-to-cell junctions and cell-to-substrate junctions appear similar in corneal, limbal, and conjunctival epithelia, except that gap junction proteins in the conjunctiva have not been characterized [168,169,170]. The apical cells of the stratified epithelium have numerous small vesicles within their cytoplasm (see Fig. 17). It has been proposed that these vesicles (which bind Alcian blue and periodic acid–Schiff stains, indicating a highly glycosylated content) deliver mucins onto the ocular surface and thus represent a second source of mucus for the tear film. Reports indicate that the stratified epithelium is expressing membrane-spanning mucins MUC1, MUC4, and MUC16 [29, 171, 172]. The goblet cells that are intercalated within the stratified epithelium of the human conjunctiva occur as individual cells; in rodents, they occur as clusters [173]. In humans, there is a regional variation in goblet cell distribution pattern and density [174], the highest density being in the palpebral region near the tear drainage punctum and in the midfornix. In some regions, especially the temporal bulbar conjunctiva, goblet cell density is so great that the cells appear to be clustered and arranged in acini [174]. Goblet cells of the conjunctiva are plump and lack the goblet "stem" – a thin cytoplasmic extension to the basement membrane that is obvious in intestinal goblet cells. Mucin packets that fill the cytoplasm of goblet cells appear electron lucent; however, a fine filamentous network can be discerned within the packets (see Fig. 17b). Studies have demonstrated that a major mucin gene expressed by the conjunctival goblet cell is the large gel-forming mucin MUC5AC [172, 173] (Fig. 18). Tight junctions appear to be present between goblet cells and adjacent stratified cells (see Fig. 17). Knock-out of the transcription factor SPDEF causes loss of goblet cells in the rodent; these mice develop mild ocular surface inflammation and accumulate debris in their fornices, indicating the function of the cells [167, 175, 176].

Fig. 18



Distribution of messenger RNA for the mucin MUC5AC using a ^{35}S-labeled probe. (**a**) Low-magnification micrograph demonstrating dense signal distributed in patches *(arrows)* within the conjunctival epithelium. (**b**) Higher magnification of the epithelium demonstrating label specifically over goblet cells. (**c**) The control sense probe showed no binding. Bar = 100 μm

Full size image

With the accumulation of data indicating that the basal cells of the limbal epithelium are the stem cells for the corneal epithelium, interest has been generated in the location of the stem-cell population in the conjunctiva. If stem cells are present within the conjunctiva, do the stratified epithelial cells and the goblet cells derive from the same stem-cell population? Data suggest that slow-cycling stem-like cells are present in the fornical region of the rabbit conjunctiva [177]. Data from observation of conjunctival epithelial cells of GFP mice and of BRDU-labeled cells suggest that epithelial stem cells in bulbar conjunctiva are evenly distributed [178]. In addition, clonal cultures of conjunctival epithelium injected subdermally into nude mice produce cysts that contain both goblet cells and stratified cells, indicating that stem cells of the conjunctival epithelium are pluripotent and can give rise to both cell types [179]. It is not known what causes divergence of the differentiation pathway to give rise to the two cell types.

The connective tissue of the substantia propria of the conjunctiva, while similar to that of the superficial limbus, is especially rich in immune cells. The loose and highly vascularized connective tissue at this site can develop fibrosis in response to glaucoma surgery [180] and in ocular cicatricial pemphigoid [181]. Lymphocytes, mast cells, plasma cells, and neutrophils are common cell types in its matrix [152]. In fact, the substantia propria has been described as having two layers: an inner fibrous layer and an outer lymphoid layer. Although the lymphoid layer has dense accumulations of lymphocytes, these do not form lymph nodules. The accumulation of the lymphoid tissue, in addition to the phagocytic abilities of the conjunctival epithelium, demonstrates the function of the tissue in dealing with infectious agents.

Summary

The tissue organization of the cornea with its stratified epithelium, the dense connective tissue of the avascular stroma, and the simple squamous epithelium that comprises the corneal endothelium have made the cornea one of the most studied tissues by electron microscopy and other advanced imaging techniques. Combined with cell culture and animal studies, this knowledge has led to advances in our understanding of the basic cell biology underlying how basement membranes and collagen matrices assemble, how the tear film is stabilized on the ocular surface, how hemidesmosomes form, and where the stem cells for the corneal epithelium are located. Understanding the anatomy and cell biology of the cornea and conjunctiva provides insight into the function of the tissues to provide the clear, stable refractive surface needed to allow light to focus and passage to the retina and offers insight into diseases of the tissue.