REVIEW ARTICLE

THE CORNEA— STRUCTURE AND MACROMOLECULES IN HEALTH AND DISEASE

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A Review

Gordon K. Klintworth, MD, PhD

DISEASES OF THE CORNEA constitute an important cause of blindness and visual impairment and an immense economic burden on society. Although the incidence of blindness due to corneal disease is only approximately 0.7% of the total number of newly blind individuals in the United States, treatment of diseases affecting the cornea constitute approximately 25% of ophthalmic practice in this country. In the United States and Canada, corneal disease accounts for approximately 5% of blindness. In some parts of the world, such as the Middle East, the Far East, and parts of Africa, serious corneal disease is much more prevalent.[533] Like diseases in other tissues, the frequency of those of the cornea vary in different parts of the world and even within the same country. This is particularly true with regard to genetically determined conditions where the incidence depends on the gene frequency in the population. Numerous diseases of the cornea are recognized as a result of the meticulous clinical observations that have accumulated since the birth of ophthalmology. Largely as a result of keratoplasty, the morphology of many corneal diseases has been characterized by light and electron microscopy and, in some instances, also by histochemical and other methods. Some diseases are limited to the cornea; others are part of systemic disorders. An enormous and heterogenous literature pertains to them. Because of this the present review is selective. It deals mainly with some aspects of the normal and abnormal cornea that are most likely to be of interest to pathologists. A major goal of this survey is to draw attention to some intriguing facets of the cornea in health and disease and to stress the cornea's potential for providing answers to important biologic questions.

The cornea is a gold mine for the pathologist. From the standpoint of human corneal disease, several aspects are attractive to pathologists. Many human corneal diseases are still poorly understood and have not been studied to their fullest potential with the techniques of modern molecular pathology. Some have not passed beyond the phase of clinical

From the Department of Pathology, Duke University Medical Center, Durham, North Carolina.
Supported in part by Research Grant 1R01 EY00146 from the US Public Health Service.
Address reprint requests to Dr. Gordon K. Klintworth, Department of Pathology, Duke University Medical Center, Durham, NC 27710.

description. The known biochemical alterations in corneal disease remain rudimentary. Cell culture techniques have established themselves in the study of human genetic disease, but very few workers have applied this methodology to inherited corneal diseases.[214,354,361] Yet surgically excised human corneal tissue is available in many of these disorders. Corneal grafting is a frequent ophthalmologic procedure and provides material ideally suited for a variety of investigative techniques. Also, the cornea is one of the few ocular structures from which small biopsy specimens can be taken.

The cornea is appealing to the experimentalist as well. Experimental models of several disorders of the cornea exist but have not been fully explored. The cornea is particularly suitable for experimental studies of the inflammatory response and wound healing because it is avascular and relatively acellular. Quantitative counts of the invading cells can be made with relative ease. The relatively large size of the cornea in many animals permits interlamellar injections to be made readily in different locations in the cornea.

The contribution of electron microscopy to our understanding of corneal disease has recently been reviewed [359,456,534] and will not be considered in detail. Not only are specific corneal diseases appropriate for study by transmission electron microscopy, but also the inner and outer surfaces of the cornea are ideally suitable for examination by scanning electron microscopy. In contrast to many other tissues, autolytic alterations in the cornea are relatively mild and late and valuable information can be obtained from electron microscopic studies of postmortem material.[34,48,63] Electron microscopy is even a useful diagnostic tool in evaluating certain corneal diseases after the tissue has been fixed in formalin and embedded in paraffin.[145,201,312,507]

History

It was that celebrated Greek, and later Roman, physician Galen (c. 129–199 AD) who named the transparent portion in front of the eye the "cornea." The name stems from its tough, horn-like nature (Greek: *corneus*, horny). Yet this structure was recognized in antiquity, and even Hippocrates, the father of medicine, was aware of its existence. Early medical writings refer to diseases of the cornea and Galen even made reference to corneal ulcers.[233] Inflammation of the cornea has been identified since at least 1808, when the term "keratitis" was introduced by James Wardrop (1728–1869) in his "essays on the morbid anatomy of the human eye."[97]

The existence of cells within the corneal stroma only became documented in 1841.[572] In a historically important article Toynbee [572] stated

> they are better seen in sections made at right angles to the surface of the cornea than in those parallel with its surface, and they appear to be more evident after the cornea has been immersed in spirits of wine. Some of these cells are rounded, others are oval, and have fine branches radiating from them similar to the osseous and pigment corpuscles.

Despite the apparent ignorance and indifference of most pathologists to disorders of the cornea, giants of pathology such as Virchow,[591] von Recklinghausen,[593] and Cohnheim [117-120] carried out fundamental studies using this tissue. Of particular importance were investigations related to the origin of cellular components of the inflammatory reaction.

In this era of molecular pathology, many aspects of the inflammatory response are well known and appreciated by students early in their careers. Today nobody doubts that leukocytes enter tissues during inflammation from the bloodstream, yet the origin of these "pus-cells" provoked considerable attention even before Virchow succeeded in convincing the medical world that cells were the ultimate seat of disease. A few years after Toynbee [572] drew attention to the normal existence of cells in the corneal stroma, when the cellular events that accompany the inflammatory response were not known, Bowman [74] described the microscopic alterations in the injured cornea for the first time. He believed that the increased cellularity in the cornea resulted from a proliferation of normal corneal cellular elements. Especially since the motility of leukocytes and their emigration from blood vessels was still unknown, the belief that leukocytes found anywhere were descendants of tissue cells of the locality appeared logical.

A controversy followed that eventually culminated in the general agreement that leukocytes emigrated from blood vessels in the inflammatory reaction and that those associated with keratitis originate from the capillaries at the corneoscleral limbus. In studies relevant to this phenomenon more than 100 years ago von Recklinghausen [593] cast doubt on the view that the fixed stromal cells contributed entirely to the increased cellularity that occurred in the cornea after injury. He placed portions of corneal tissue from frogs, rabbits, and dogs into lymph sacs of frogs and found that they were rapidly permeated with cells. He concluded that since this took place even when corneal tissue from dead animals was used, the cells must not have arisen in the cornea but migrated into it from elsewhere.[593] Von Recklinghausen also introduced cinnabar into the lymph sacs, which was

ingested by the cells and served as a marker of the noncorneal origin of the cells.

It is to Cohnheim that we owe the first full description of the phenomenon of inflammation as studied under experimental conditions, and it was he who established beyond all possibility of doubt that the diapedesis of blood cells can occur. Experiments on the cornea played a crucial role in this regard.[117-119] Cohnheim demonstrated that leukocytes migrated into the cornea during inflammation and asserted that the cellular infiltrate was derived from cells of hematogenous origin. In order to reach this conclusion Cohnheim even replaced the blood of frogs with saline and kept them alive for as long as 3 days![118] The interpretation of many of the early experiments posed great difficulties. For instance, Böttcher [65,71] made small circumscribed lesions in the center of the cornea, hoping to avoid any role of the conjunctival vessels. He observed "pus corpuscles" in these small central opacities and, as Cohnheim [120] pointed out decades ago, it was easy to convince oneself that they were not immigrants from the periphery of the cornea. Senftleben [521] demonstrated that even in the absence of corneal stromal cells typical inflammatory reactions occurred in the cornea. He showed that if the corneal cells were killed and made to disappear by the injection of a few drops of oil of turpentine into the anterior chamber, the cornea became cloudy and swollen and densely infiltrated with pus cells. In his lectures on general pathology that were published in 1877, and which became available in English translation in 1889 and 1890,[120] Cohnheim pointed out

> As late, as the seventh decade of this century the matter was simpler; if a pus-corpuscle were met within an epithelial or other cell, it was unhesitatingly assumed to have arisen endogenously, and if two or three were found in a situation where a connective tissue cell might be expected to be present, as for example, in the position of a so-called corneal corpuscle, they were spoken of as the descendants of the latter, and were supposed to owe their origin to its division.

Cohnheim [120] drew attention to the fact that hyperemia of the conjunctival vessels surrounding the cornea invariably preceded clouding of the cornea or the appearance of "pus-corpuscles" in the tissue. He also pointed out that

> on touching the cornea with nitrate of silver, the tissue within and immediately around the corroded portion with the contained cellular elements, will be earlier, and at any rate much more powerfully influenced than will the vessels in the periphery of the cornea, to which the chemical action of the caustic extends only very gradually, and after considerable attenuation.

Cohnheim [120] also stressed the importance of the cornea to study regenerative processes. He stated,

> But there is no favorable field for the study of these regenerative processes than the cornea; because we cannot so certainly succeed elsewhere in causing considerable loss of substance, and in, at the same time, completely excluding inflammation. In fact it is precisely the anterior epithelium of the cornea that offers the most convenient opportunity for the successful study of epithelial regeneration; besides which, the regeneration of the corneal corpuscles has been traced in the first instance to Eberth,[171,172] and then with special care by Senftleben [521] in my institute at Breslau.
>
> The cornea also allows the relations of regenerative and inflammatory tissue-formation to be demonstrated very clearly. If you excise a large piece from its anterior surface, or cauterize it over a not too limited area with the red-hot button of a sound, injection of the sclerotic, and more especially of the peripheral, vessels is wont to set in very rapidly. Colourless blood-corpuscles penetrate directly, some from the periphery and some from the conjunctival sac, into the injured portion or into the tissues immediately bordering on the eschar, and here give rise to cloudiness. The regenerative proliferation of the anterior epithelium very soon commences, and after two days, at furthest, the entire surface of the wound is covered with newly formed epithelium; while, if the cautery has been employed and the eschar can be removed by constantly moistening it with liquid, the remaining defect rapidly receives an epithelial covering. As soon as this has occurred the inflammation quickly begins to subside; the pus-corpuscles that had invaded the cornea gradually disperse, as is demonstrated to the eye by the disappearance of the cloudiness; and the depression, which at first persists and may be clearly perceived on illumination from the side, also disappears during the succeeding weeks and months, owing to the regeneration, more or less energetic, of the corneal corpuscles and intercellular substance. If, on the other hand, the regeneration of the anterior epithelium is for any reason prevented, e.g. by a firmly lodged foreign body, the inflammation continues, and blood vessels shoot in from the periphery of the cornea towards the seat of injury. A so-celled *pannus vasculosus* arises, while in the inflammed area itself a connective-tissue *cicatrix*, or leucoma, is formed.

Glycosaminoglycans and Proteoglycans

Normal

The existence of mucoid material within the cornea was discovered by Mörner toward the end of the 19th century,[18,19,422] but it was not until the early 1950s that Meyer and his coworkers established that this substance consisted of chondroitin-4-sulfate (chondroitin sulfate A), keratosulfate, and a low-sulfated glycosaminoglycan (chondroitin).[149,423] Numerous investigators have extended these observations and studied corneal glycosaminoglycans in a variety of species from different standpoints.[9-15,17-19,29,37,49,50,56,61,62,88,132,139,141,142,163,255,256,264,281,282,360,461,522,540,555,605-609,612]

The glycosaminoglycans of the corneal stroma play a role in the hydration of the cornea and hence in its thickness and degree of trans-

parency.[135,407] The mutual repulsion of the glycosaminoglycan molecules with collagen fibrils contribute to the maintenance of the regular spacing of the collagen fibrils that characterize the cornea.

Glycosaminoglycans constitute 4.5% of the dry weight of the human cornea.[405] Depending on the species, keratan sulfate accounts for approximately 45 to 60% of the total sulfated glycosaminoglycans in the cornea. Chondroitin-4-sulfate has been isolated from bovine cornea, but the chondroitin sulfate moiety of man has been identified by infrared spectroscopy as chondroitin-6-sulfate.[88] Corneal tissue of several species including man produces chondroitin-6-sulfate and chondroitin-4-sulfate *in vitro*.[361] The keratan sulfate and chondroitin sulfate in the cornea both have a variable sulfate content.[608] Chondroitin has never been clearly demonstrated in any tissue other than the cornea, and its presence in the cornea is even disputed. Glycosaminoglycans are normally bound covalently to core protein as a proteoglycan macromolecule. Several investigators have isolated normal corneal proteoglycans. At least three corneal proteoglycans, comprising approximately 2% of the corneal stroma, have been identified.[20,55,512] These contain variable amounts of keratan sulfate and chondroitin-4-sulfate.

Keratan sulfate exists in cartilage and bone as well as in the cornea. The predominant structural feature of keratan sulfate in all sites is a core portion consisting of sulfated galactose and N-acetylglucosamine residues. It is now recognized that corneal keratan sulfate (keratan sulfate I) is unique to the cornea. Corneal keratan sulfate differs in several respects from cartilaginous keratan sulfate (keratan sulfate II).[56,133,282,396,522] With corneal keratan sulfate, the sulfated N-acetyllactosamine polymers are linked by alkali-stable N-glycoside bonds to asparagine residues of core protein.[56,522] The carbohydrate-peptide bond of cartilaginous keratan sulfate, on the other hand, appears to involve N-acetylagalactosamine linked to serine and threonine residues by an O-glycosidic, alkali-labile bond.[396] The average length of the polymer chains, the degree of branching, and the susceptibility to keratan-sulfate–endogalactosidase degradation are dissimilar in corneal and cartilaginous keratan sulfate.[56,133] Significant differences also exist in the predominant amino acids and in the relative amounts of glucosamine, galactosamine, and sialic acid.

As in the skin, cartilage, and other tissues, the glycosaminoglycan content of the corneal stroma varies with age.[19] During embryonic development, the quantity of glycosaminoglycans in the corneal stroma increases. This has been demonstrated by chemical analyses [10] and by histochemical techniques.[137] The relative amount of keratan sulfate increases during development.[10] The hexosamine content of the bovine

cornea decreases during early embryonic development (10 to 20 cm) but thereafter increases.[540] In the newborn human cornea the sulfate content of glycosaminoglycans is considerably less than in adults.[88]

Corneal glycosaminoglycans have been studied by incubating corneal tissue in medium containing ^{35}S-sulfate [29,132,163,166,555,606,609] or other radioactive labeled precursors of these substances.[141,166] Most investigators who have studied extracellular corneal glycosaminoglycans *in vitro* have concentrated on the glycosaminoglycans that are extractable from the corneal tissue. Some have analyzed the nutrient medium.[132,255,256,360]

Although the tissues of the body contain numerous sulfated compounds,[250,381,501] several investigators have demonstrated that ^{35}S-labeled sulfate ions are incorporated mainly as ester sulfate into sulfated glycosaminoglycans and that a negligible amount of administered ^{35}S-sulfate is utilized in the synthesis of sulfur-containing amino acids.[70,310,445] In the cornea,[35]S is incorporated predominantly into keratan sulfate and chondroitin sulfate by corneal fibroblasts.[132,163,360]

The incorporation of ^{35}S-sulfate into macromolecules is a vital energy-dependent process.[445] In the cornea, as well as elsewhere, the process has been shown to involve sodium-potassium activated ATPase.[605] The biosynthesis of sulfated glycosaminoglycans and other compounds involves the activation of sulfate by ATP in a two-step sequence requiring two separate enzymes. ATP-sulfurylase catalyzes the reaction between ATP and SO_4 to form adenosine-5′-phosphosulfate (APS). This is followed by the phosphorylation of APS by ATP to form 3′-phosphoadenosine-5′phosphosulfate (PAPS), a reaction catalyzed by the enzyme APS-phosphokinase. The activated sulfate is then transferred to the acceptor molecule by a class of enzymes termed "sulfo-transferases," which appear to be specific for each acceptor.[244,250]

Puromycin inhibits the incorporation of ^{14}C-glucosamine, ^{14}C-galactose, ^{14}C-serine, and $^{35}SO_4$ into corneal proteo-keratan sulfate (PKS) and proteo-chondroitin sulfate (PChS). The inhibition is more marked with PChS than PKS, suggesting that the synthesis of PKS protein is separate from the protein synthesis by PChS.[263] The synthesis of corneal glycosaminoglycans by corneal fibroblasts appears to be dependent on the corneal endothelium and/or epithelium.[15,360,532,605] This dependency has been shown in the cornea of the ox,[62,139] rabbit,[15,360,605] and rat.[532] The mechanism underlying this epithelial-mesenchymal interaction is not fully understood. However, trauma to the stroma caused by removal of the latter layers may account at least in part for the apparent increased synthesis that accompanies corneas that have not been stripped of their inner and outer cellular layers.[160] The synthesis of corneal glycosaminoglycans has

been shown to be influenced by light, which lowers ^{14}C incorporation into keratan sulfate. The corneal epithelium seems to play a role in this photoeffect because it does not occur when this layer of cells is removed from the corneal. The same effect has been observed when intact cornea is incubated in the presence of allopurinol, a known xanthine oxidase inhibitor. Since the epithelium contains a pteridine-like substance, which may be susceptible to xanthine oxidase digestion, the pteridine-like substance has been postulated as the target substance for the photoeffect on glycosaminoglycan synthesis.[141]

Several factors are known to affect the biosynthesis of corneal glycosaminoglycans and, especially, corneal keratan sulfate.[37,62,132,142,269,360,612] In cartilage the biosynthesis of chondroitin sulfate and keratan sulfate involves separate pathways; these mechanisms are independently influenced by adrenal corticosteroids.[150,151] This may also be true for the corneal glycosaminoglycans. Indeed, Balduini *et al.*[37] have shown that uridine diphosphate (UDP)-xylose decreases the biosynthetic rate of chondroitin and chondroitin sulfate but increases the rate of synthesis of keratan sulfate by corneal explants. The mechanism involved in this regulation is thought to be an inhibition of UDP-xylose on UDP-glucose dehydrogenase. The uptake by the cornea of sulfate is inhibited by a variety of compounds including iodacetate, salicylate, and antiinflammatory steroids (cortisone, dexamethasone, hydrocortisone).[62,597] Phenothiazines have also been shown to inhibit the synthesis of corneal glycosaminoglycans.[142] Hypoxia decreases the synthesis of keratan sulfate but not of chondroitin sulfate *in vitro*.[141]

The turnover of sulfated glycosaminoglycans by the cornea is slower than that in the sclera.[163] The avasularity of the cornea may play a role in this regard as sulfated glycosaminoglycans disappear from vascularized corneas more rapidly than from normal avascular corneas.[535]

The ability of cultured corneal fibroblasts from several species to synthesize keratan sulfate *in vitro* is lost or markedly decreased.[132,255,256,360,612] Conrad and Dorfman [132] investigated the sulfated glycosaminoglycans synthesize *in vitro* by whole corneas of 14-day-old chick embryos. They found that freshly isolated whole corneas, corneal stromas, and isolated corneal fibroblasts from chick embryos all incorporated ^{35}S-sulfate into a keratan-sulfate–like polysaccharide, but neither the corneal epithelium nor the endothelium incorporated label into it. After 48 hours *in vitro* under a variety of cultural conditions, chick corneas ceased to synthesize the keratan-sulfate–like polysaccharide. The cultured chick cells continued to synthesize chondroitin-4-sulfate and several glycosaminoglycans which are not thought to be present in normal cornea (viz, dermatan sulfate,

chondroitin-6-sulfate, and heparan sulfate). Cultured rabbit and bovine corneal fibroblasts also produce chondroitin sulfates and glycosaminoglycans not present in normal cornea.[255,256] It is clear that fetal calf serum, an essential requirement for the maintenance of cultures of corneal fibroblasts, does not prevent or appreciably influence the synthesis of the keratan-sulfate–like fraction.[360] Ascorbate, thyroxin, glutamine, freshly isolated corneal epithelium and endothelium, and sonicated preparations of freshly isolated fibroblasts do not prolong the continued synthesis of this material by embryonic chick corneas.[132] Whether the limitation of corneal keratan sulfate biosynthesis *in vitro* reflects a specific characteristic of tissue culture conditions or whether they are operable under other circumstances still remains to be determined.

Corneal Glycosaminoglycans in Pathologic States

Systemic Mucopolysaccharidoses

Corneal opacification is a significant clinical feature in several systemic mucopolysaccharidoses, including mucopolysaccharidoses Type I-H (Hurler syndrome) and Type I-S (Scheie syndrome) but not mucopolysaccharidosis Type II (Hunter syndrome). Ultrastructural alterations occur in the cornea in those mucopolysaccharidoses that are associated with corneal opacification. The reason corneal involvement occurs in some but not all of the mucopolysaccharidoses remains to be explained, particularly in regard to heparan sulfate and dermatan sulfate, which are not normally found in the cornea and which accumulate throughout the body and are excreted in excess in mucopolysaccharidoses Type I-H (Hurler syndrome), Type I-S (Scheie syndrome), and even in Type II (Hunter syndrome). Numerous membrane-bound vacuoles, sometimes enclosing finely fibrillary or granular material, have been identified by electron microscopy in the stromal keratocytes in the systemic mucopolysaccharidoses Type I-H (Hurler syndrome),[508,581] Type IV (Morquio syndrome),[247] and in Type VI (Maroteaux-Lamy syndrome).[479] In addition to these clear cells (Hurler cells), peculiar cells characterized by membrane-bound bodies filled with amorphous, electron-dense material have been described in mucopolysaccharidosis Type I-H (Hurler syndrome).[397] Membrane-bound vacuoles occur in the endothelium in mucopolysaccharidosis Type I-H (Hurler disease).[508,581]

Macular Corneal Dystrophy

One of the inherited corneal diseases, i.e., macular corneal dystrophy, appears to be a localized mucopolysacchardosis. Opacities usually first

become evident in both corneas at puberty. Initially irregular ill-defined areas of diffuse clouding appear in the central cornea. With time they progressively become confluent and eventually involve the entire corneal stroma. The disease culminates in severe visual impairment usually before the fifth decade. Vision can be restored by corneal grafting; the excised corneal tissue is typified morphologically by distinctive abnormalities. Recurrences of the disease in the graft have been reported,[278,388] but convincing histologic documentation of the disease in the grafted tissue is lacking.

The basic abnormality in macular corneal dystrophy clearly resides in the nuclear DNA. In 1964 Klintworth and Vogel [362] proposed that this disease is a genetically determined metabolic storage disorder manifestly restricted to the cornea and characterized by an intracellular and extracellular accumulation of excessive quantities of glycosaminoglycans (mucopolysaccharides). This view was based on a consideration of the genetic characteristics, light and electron microscopic morphologic attributes, and the histochemical characteristics of the disease. Intracytoplasmic accumulations, that have tentatively been identified cytochemically as glycosaminoglycans, occur within the corneal fibroblasts (also known as keratocytes) and usually in the endothelium of the cornea.[64,212,213,237,246,279,315,316,351,362,388,430,520,543,565,579] The accumulations stain positively with periodic acid–Schiff, alcian blue, and metachromatic dyes and possess an affinity for colloidal iron.[208,212,237,246,259,279,315,316,362,430,520,565,579] When viewed via transmission electron microscopy, the corneal fibroblasts are consistently and conspicuously distended by intracytoplasmic vacuoles in macular corneal dystrophy. With adequate fixation, microfibrillogranular material can be discerned within the vesicles.[259] The corneal endothelium often contains similar material [246,351,543,579,581] but some endothelial cells are spared, even when the adjacent ones are severely affected. Deposits of material with similar cytochemical characteristics aggregate extracellularly between the collagen fibers and commonly within portions of Descemet's membrane, which very often has excrescences on its inner surface (cornea guttata). In macular corneal dystrophy Descement's membrane is studded by numerous vacuoles that give it a honey-combed appearance.[246,351,543,579] The electron-lucent areas probably represent sites where extracellular glycosaminoglycans dissolved out during tissue processing. The accumulations can be stained for electron microscopic examination with the periodic acid-Schiff-thiocarbohydrazide-silver proteinate [259] and periodic acid-silver methenamine techniques.[359,581] Also, the fact that corneal stromal and endothelial cells synthesize glycosaminoglycans [360,612] makes it reasonable to accept the premise that the corneal

deposits in macular corneal dystrophy are products of the cornea's own cellular elements.[351]

By drawing from the analogy between macular corneal dystrophy and the systemic mycopolysaccharidoses, one is led to suspect an enzymatic defect in the degradation of one or more corneal glycosaminoglycans. The cornea contains several of these polysaccharides which warrant consideration, namely keratan sulfate, chondroitin-4-sulfate (chondroitin sulfate A), chondroitin-6-sulfate (chondroitin sulfate B), and low-sulfated glycosaminoglycans.[360] The persistence of the staining qualities of the accumulations after testicular hyaluronidase digestion [237,362] precludes hyaluronic acid, chondroitin-4-sulfate, and chondroitin-6-sulfate as significant components, while their failure to become digested by chondroitin ABC lyase rules out dermatan sulfate also.[356] By exclusion the primary suspect is keratan sulfate, which accounts for the greatest quantity of the sulfated glycosaminoglycans in the normal cornea. This contention is supported by the affinity of the deposits for alcian blue at low pH with magnesium chloride concentrations of up to 0.8 M:an attribute of keratan sulfate.[237,356] Also, of the aforementioned histochemical quartet that demonstrates the deposits, the periodic acid-Schiff may react with keratan sulfate, while chondroitin sulfates, hyaluronic acid, and other glycosaminoglycans are not usually visualized by this reaction.[361]

A storage disease involving corneal keratan sulfate would account for the apparent limitation of the disease to the cornea. From a clinical standpoint the disease is restricted to corneal opacification. If lesions of comparable size exist in other nontransparent structures, they could easily be asymptomatic and overlooked in hematoxylin-and-eosin–stained tissue sections. A detailed examination of all tissues in patients with macular corneal dystrophy has not been performed. Particularly important would be an examination of cartilage, a tissue with many chemical similarities to the cornea. Histologic observations on several tissues (skin, breast, fallopian tube, ovary, uterus, oral mucosa, thyroid) excised from patients for miscellaneous diseases and a single autopsy, although incomplete, have not disclosed lesions in tissues other than the cornea.[246,356,362]

Graf *et al.*[259] have argued that the propensity of the deposits to stain with periodic acid-Schiff and alcian blue favors the stored material to be a glycoprotein rather than an acid mucopolysaccharide (glycosaminoglycan). But negative cytochemical reactions for proteins [237,362] attest against a significant proteinaceous component in the stored material. To some extent the question of whether the disease involves a glycoprotein or a glycosaminoglycan is academic. The polymers of keratan sulfate, like those of other glycosaminoglycans, are linked covalently to core protein.

Also, despite the traditional consideration of keratan sulfates as glycosaminoglycans, there is an increasing amount of evidence to indicate that it would be more realistic to regard them as sulfated glycoproteins. They lack uronic acid, a constituent of other glycosaminoglycans, and contain sugars characteristic of glycoproteins, including galactose, glucosamine, galactosamine, mannose, sialic acid, and fucose.[547]

The literature contains very few reports on the application of tissue culture techniques to macular corneal dystrophy.[147,213,214,354,361] Danes [147] studied cultured fibroblasts from the cornea, conjunctiva, and skin of 6 patients with macular corneal dystrophy and reported that the uronic acid content was similar to that in controls. Francois *et al.*[214] claimed that they could detect differences between normal corneal fibroblasts and those from individuals with macular corneal dystrophy by culturing cells in medium containing the vital dye acridine orange. However, their observations were not confirmed in a study involving cultured corneal fibroblasts from 3 patients with macular corneal dystrophy and from appropriate controls.[357] In many inherited diseases attention has been given to the presence of metachromatic granules in cultured cells.[354] Metachromatic granules have been observed in some cultured fibroblasts with macular corneal dystrophy at a pH that does not diminish the catabolism of sulfated glycosaminoglycans,[385] but indistinguishable granules have been observed in cultured fibroblasts from normal corneas.[361] In an unpublished study, Cotlier *et al.*[136] explored the activity of several glycosidases (lysosomal enzymes degradative for certain glycosaminoglycans and glycoproteins) in cultured limbal conjunctival fibroblasts from a patient with macular corneal dystrophy. They detected a relative deficiency of α-galactosidase, which they also found to be defective in a fragment of corneal tissue. However, this observation could not be confirmed in other specimens from patients with macular corneal dystrophy.

As macular corneal dystrophy appears to be a localized mucopolysaccharidosis,[351,362], Klintworth and Smith [361] studied cultured corneal fibroblasts from patients with this disease with techniques that have yielded valuable data about several inherited disorders of glycosaminoglycan metabolism.[38,39,215-217,435,436] Morphologic, cytochemical, and ^{35}S-sulfate incorporation studies were performed on corneal fibroblasts isolated from patients with macular corneal dystrophy and from corneas that were free from overt disease.[361] In addition they investigated the sulfated glycosaminoglycans produced by a corneal explant of a patient with macular corneal dystrophy. Many normal and abnormal cultured corneal cells contained material with the cytochemical characteristics of glycosaminoglycans. In contrast to the corneal tissue obtained at keratoplasty from patients with

macular corneal dystrophy, the deposits within cultured corneal fibroblasts were not resistant to testicular hyaluronidase or chondroitin ABC lyase digestion. Moreover, unlike the inherited systemic disorders of glycosaminoglycan metabolism, mucopolysaccharidoses, Type I-H (Hurler syndrome), and Type II (Hunter syndrome), the corneal fibroblasts from patients with macular corneal dystrophy failed to accumulate abnormal quantities of ^{35}S-sulfate–labeled glycosaminoglycans. As the synthesis of keratan sulfate by the cornea of man and other species either ceases or markedly decreases in culture [132,255,256,360,612] the data remain consistent with the contention that this disease is a disorder of keratan sulfate I catabolism. If cultured corneal fibroblasts in a patient devoid of keratan sulfate do not synthesize significant quantities of keratan sulfate, its intracytoplasmic accumulation obviously will not occur, even if keratan sulfate cannot be degraded because of an inherited deficiency of a critical enzyme.

Glycosaminoglycans in Other Disorders

Abnormalities in the corneal glycosaminoglycans have been reported in other pathologic states. Anseth and Laurent [19] found a) a decrease in both glucosaminoglycans and galactosaminoglycans in the healing area of perforating corneal wounds and b) the principal glycosaminoglycan of scar tissue to be chondroitin sulfate. In wound healing, Anseth and Fransson [17] detected a reduced keratan sulfate and increased chondroitin-4-sulfate synthesis. With injury to Descemet's membrane and the endothelium, dermatan sulfate production occurs. Abnormalities have also been described in patients with keratoconus,[476] swollen opaque corneas,[476] experimentally induced corneal edema,[12] unsuccessful corneal grafts,[13] and viral keratitis.[14] Differences in the relative amounts of different glycosaminoglycans have been reported *in vivo*. Dohlman and Praus [165] noted that the incorporation of ^{35}S-sulfate into the glycosaminoglycans of corneal wounds differ markedly from the normal pattern. Particularly between 1 and 3 weeks after corneal wounding, during the peak of fibroblastic activity, these investigators noted a greatly increased uptake of ^{35}S-sulfate into the galactosaminoglycan fraction (chondroitin sulfate) compared with that of the glucosaminoglycan fraction (mainly keratan sulfate).

Collagen and Its Disorders

Normal

The structural integrity and tensile strength of the cornea is due to collagen. It accounts for approximately 68.1% of the dry weight of the

human cornea.[405] Collagen is located mainly in the corneal stroma, where the collagenous fibrils differ from those of most other tissues in that they have an extremely regular diameter. They vary slightly in thickness from area to area, but most are of an almost uniformly small diameter (24 to 32 nm).[308,516] The collagen fibers are separated from each other by a regular spacing of approximately 55 nm. Maurice [405] has explained the transparency of the cornea by this precise orderly relationship between respective collagen fibrils. It is not known why the individual fibers do not thicken with age as do those in other tissues. Like collagen fibers elsewhere, those of the cornea normally display a macroperiodicity of 55 to 64 nm in tissue sections. The collagen in the cornea is arranged in regular lamellae. These are most evident in the posterior corneal stroma, while the anteriorly located collagen is less compactly interwoven into bundles. The collagen fibrils and adjacent lamellae are minimally interlaced in many vertebrate corneas, but in man interweaving and branching of lamellae are pronounced in the anterior third of the stroma. Some collagen lamellae in the anterior stroma crisscross and terminate in Bowman's layer.[584] The central cornea contains less collagen lamellae than the peripheral part.[268]

The structure and biosynthesis of collagen warrant a brief description. More detailed reviews are available elsewhere.[261,411,590a] Fibrous collagen is a highly symmetric, insoluble protein composed basically of identical subunits. The fundamental molecular units of collagen, known as tropocollagen, align themselves in a precise, staggered arrangement that causes the prominent cross striations of collagen that are 68 nm apart. A gap of approximately 41 nm occurs between the end of one tropocollagen molecule and the first part of the following one in the same line. Each rod-shaped tropocollagen unit is 300 nm long and 1.5 nm wide and is composed of three polypeptide chains coiled together in the form of a helix.

At least four different molecular species of collagen are recognized in vertebrate tissues (Types I through IV).[577] These molecules differ from one another in their composition and in the primary structure of their component polypeptide chains. Type I collagen contains two identical polypeptides or α-chains designated α-1 Type or α-1 (I) and an additional one of a different primary structure termed α-2. The second species, Type II collagen, consists of three identical α-chains named α-1 (II) with a primary structure dissimilar to α-1 (I) and α-2. A third variety of collagen is composed of α-chains of unique primary structure termed α-1 Type III. Type IV collagen, the fourth molecular species of collagen, contains three identical α-1 Type IV polypeptide chains which differ from other α-chains. Type I collagen (α-1 $[I]_2$ α-2) has been identified in the corneal

stroma of the chick,[577] the ox,[325] and man.[578] Basement membranes contain Type IV collagen (α_1 [IV]$_3$), and this type has been isolated from Descemet's membrane. The α-1 and α-2 chains differ in amino acid content, but each contains approximately 1000 amino acids. Noteworthy aspects of the amino acid composition of collagen are the high percentage of glycine (33% of all amino acid residues), the sizable content of proline and hydroxyproline (approximately 22%), the presence of hydroxylysine, the comparatively small amount of tyrosine, and the absence of tryptophan and cysteine.

The biosynthesis of collagen occurs in a series of sequential intracellular steps: assembly on ribosomal complexes of a polypeptide precursor (procollagen), hydroxylation of appropriate proline and lysine residues to hydroxyproline and hydroxylysine, and substitution of some hydroxylysine residues with galactose or glucosylgalactose in O-glycosidic linkage prior to the extrusion of the molecule from the cell. Collagen is initially synthesized in the cell as a precursor molecule larger than α chains. This so-called procollagen is composed of polypeptide chains termed "pro-α chains." These chains have a molecular weight approximately 15 to 20% greater than that of α-chains. Procollagen molecules contain cystine and remain in solution under conditions in which collagen molecules spontaneously precipitate to form fibers.[427] Incubation of pro-α chains with the proteolytic enzyme pepsin results in products with chromatographic properties similar to α-chains.[427] The extension of the NH_2-terminal end of the transport form is not cleaved off until after the molecule leaves the cell. Under artificial conditions the long, rigid, triple-helical molecules of tropocollagen in the presence of adenosine triphosphoric acid (ATP) at low pH align in perfect transverse register to form crystallites, designated segment long-spacing collagen, that are the same length as the collagen molecule. When stained with phosphotungstic acid or uranyl acetate, such crystallites reveal characteristic band patterns that are remarkably similar for collagens derived from particular sources.[86] The microfibrils that form spontaneously from tropocollagen possess little tensile strength. For the latter to develop covalent bonds, "cross-links" need to form between adjacent tropocollagen molecules. This is achieved by the enzymatic synthesis of aldehydes by removal of the terminal amino groups of several of the lysyl or hydroxylysyl residues in tropocollagen. This reaction is catalyzed by amine oxidase, an enzyme inhibited by nitriles. Bonds form between adjacent molecules by reactions with these aldehydes obtained from lysine and hydroxylysine.

The collagen fibers that form in the cornea, as in other tissues, develop in a matrix that includes proteoglycans, serum protein, and glycoproteins. Glycosaminoglycans, e.g., chondroitin sulfate and keratan sulfate,[604] and

proteoglycans [389] markedly influence the rate at which collagen fibers form from tropocollagen *in vitro* and in all likelihood have an important effect on fiber formation *in vivo*.[305]

Several investigators have analyzed the chemical composition of the normal corneal collagen.[40,66,69,102,156,226,260,325,336,382,384,428,438,461,492,514,546,577,578] Such studies have generally required large quantities of tissue and have not been applicable to individual corneas. Many early analyses on corneal collagen utilized insoluble tissues that were difficult to evaluate because of enormous obstacles in removing noncollagenous material such as glycoproteins and glycosaminoglycans from such tissue. Corneal collagen was thought to be highly glycosylated and discrepant data resulted from some analyses. In recent years bovine corneal collagen has been analyzed using isolated α and β chains which could be highly purified by ion exchange and molecular sieve chromatography. Katzman *et al.*[325] found glucose and galactose to be the only detectable monosaccharides. Approximately 1.7% of residues of bovine collagen have been found to be hexoses.[260,325] Robert and Dische [492] found approximately 2% carbohydrate in trichloracetic-acid–extracted, insoluble calf corneal collagen, with the carbohydrate being composed of equimolar amounts of glucose and galactose. With human corneal collagen Schofield *et al.*[514] found a higher hexose content (2.4%), including hexoses other than glucose and galactose in addition to hexosamines.

Collagen isolated from Descemet's membrane of sheep has been identified as Type IV. Collagen from Descemet's membrane, as in other basement membranes, is composed of three identical α-chains possessing the chromatographic properties on carboxymethyl cellulose of α-1 chains isolated from interstitial tissue. The molecular weight of basement membrane α-1 chains (108,000) is higher than that of Type I collagen by an amount attributed to the excess of hexose. Such collagen contains unusually high amounts of hydroxylysine and hydroxyproline (including a significant percentage of 3-hydroxyproline), a small amount of alanine, and eight residues of half-cystine. The total carbohydrate content is approximately 12%, consisting of equimolar amounts of glucose and galactose. Approximately 95% of the hexose is in the form of glucosyl-galactosyl-hydroxylysine; the remainder exists as galactosyl-hydroxylysine.[336] The sum of lysine and hydroxylysine is higher than in Type I collagen. As in other collagen, glycine accounts for one third of all amino acid residues. Alanine is low and corresponds to only approximately 25% of the amount found in Type I collagen. The extractibility, glycosylation, and aldehyde content of the collagen in Descemet's membrane differ from those of stromal collagen.[428]

Several recent communications have reported the successful harvesting of procollagen and collagen from corneal fibroblasts [99,225,552] and corneal endothelial cells [336a,447a,483] in culture. Human,[552] bovine,[99] and rabbit [225] stromal fibroblasts produce Type I collagen in culture; cultured rabbit corneal endothelial cells synthesize Type IV collagen.[483]

Abnormal

Corneal collagen has been largely neglected in pathologic states except from the standpoint of its appearance in transmission electron micrographs.[359] In contrast to the normal corneal stroma, which contains orderly arranged collagen fibrils of uniform thickness, pathologic corneas manifest a variety of morphologic abnormalities of collagen. The structure of individual collagen fibers, and the arrangement of them to each other, can differ from the normal. In many keratopathies, including keratoconus,[472] the collagen fibrils are of conventional diameter, while in others they are thicker [323,337] or thinner [63,578] than usual. Another situation in which abnormal corneal collagen has been detected is in scar tissue. In corneal scars the collagen has marked variations in diameter, with most fibers being abnormally thick.[307] Cintron [102] found a lower content of glycosylgalactosyl hydroxylysine than normal in rabbit corneal scar collagen. Although the ratio of lysine to total hydroxylysine in normal and scar collagen was approximately the same, the relative proportion of glycosylated hydroxylysine to nonglycosylated hydroxylysine was different. In the developmental anomaly known as sclerocornea, the corneal tissue takes on the attributes of the sclera and the individual collagen fibers vary in diameter, with some being thicker than usual, even reaching dimensions of those ordinarily located in the sclera (up to 150 nm).[323] The fibrous protein elastin does not exist in the normal cornea, but electron-dense masses thought to be elastic fibers have been described in the anterior stroma in sclerocornea.[323] In congenital central corneal leukoma (Peters anomaly),[337,433,473,553] collagen fibers that are thicker than normal and of variable diameter are randomly arranged among the thinner fibrils. When the cornea becomes edematous, its stroma thickens markedly and the corneal lamellae disunite from each other. The individual collagen fibers disperse and appear in disarray, separated by electron-lucent regions considered to represent pockets of fluid. In most forms of edema, the individual collagen fibrils retain their usual thickness. However, an increase in collagen fibril diameter accompanies the stromal edema of congenital hereditary endothelial dystrophy,[337] but this is probably part of the developmental anomaly rather than a reflection of the fluid accumulation.

The crystalline lens influences the development of the cornea. If it is

removed from chick embryos after 4 days of incubation, Descemet's membrane and the corneal stroma and endothelium fail to form. A retrocorneal sheet of connective tissue with collagen fibrils with cross-sectional diameters that are 1.8 to 3.0 times that of the normal corneal stroma develop.[615] Collagen may form fibrous or nonfibrous threadlike units with a cross-striational spacing of 256 nm (fibrous long-spacing or segment long-spacing collagen). This occurs due to a failure of the tropocollagen units to line up in the same direction and overlap each other by one quarter of their lengths. The latter presumably occurs when the extracellular matrix is abnormal and contains excessive negatively charged polymers. Fibrous long-spacing collagen with a periodicity of 100 to 110 nm can form within the corneal stroma in several diseases.[79,247,259,299,473,475,509,581] It is not always possible to discern the banded pattern of the collagen fibers in diseased corneas by electron microscopy, but this may reflect technical problems related to fixation and tissue processing rather than to something inherent in the collagen fibers. The collagen lamellae can be markedly reduced in number, as in keratoconus.[472] Sometimes the collagen bundles are not arranged in normal parallel lamellae but in a wavy Z-shaped pattern.[253] The possibility of the latter being an artifact of tissue processing still needs to be excluded.

From the standpoint of the cornea, osteogenesis imperfecta congenita is the only well-defined example of a genetically determined post-translational modification of collagen. This rare inherited disorder with an autosomal recessive mode of inheritance is characterized by bones that are prone to fracture and other effects of a generalized disorder of collagen. The sclera is sufficiently thin to permit the underlying choroid to appear light blue (blue sclera). Several abnormalities of the cornea have been described in this disease. These include megalocornea, keratoconus, and a reduced thickness of the cornea.[411] Several lines of evidence have indicated a molecular abnormality of collagen in this disease. At least two posttranslational modifications of the collagen molecule have been identified in one case in purified native collagen from several sources including the cornea.[578] These are an excessive hydroxylation of lysine and subsequent glycosylation of the hydroxylysine. Electron microscopic observations of corneal tissue from individuals with osteogenesis imperfecta have varied.[63,176,489,578] The lamellar organization of the fibrils is typical of the normal. In some cases abnormalities in corneal fibril diameter have been noted.[63,489] The majority (95%) of collagen fibrils in the cornea of the patient studied by Trelstad *et al.*[578] were within the normal range (24.2 to 32.3 nm). Five percent of fibrils were narrower than usual (12 to 16 nm). Trelstad *et al.*[578] observed that most collagen fibrils in the cornea manifest

67-nm cross-striations. The precise nature of the thinner fibrils remained in question as convincing examples cut along the fibril were not identified. Blümcke *et al.*[63] found the diameter of the corneal collagen fibers in osteogenesis imperfecta to be reduced approximately 30%.

Corneal collagen becomes destroyed by enzymatic hydrolysis in a variety of situations, including marginal degeneration, Mooren's ulcer, and alkali burns. Tissue collagenases have been implicated in human corneal ulceration [53,530] and in experimentally produced alkali burns in rabbits.[52,291] Such enzymes cleave native collagen at approximately one fourth of the length of tropocollagen from the carboxyl end of the macromolecules. Zinc and calcium ions are required for the enzymatic activity, and practical use of this fact has been made by the application of metal-binding agents to prevent and treat corneal ulcers.[51] Human granulocytes have been shown to possess specific collagenase;[377] these cells are found in inflamed corneas and are clearly an important source of the enzyme. Alkali burns of the cornea are an important clinical problem that can result in deep corneal ulceration and perforation. Both of the latter have been shown to be caused by collagenolytic enzymes produced in the cornea.[52,53,81,82,530] Collagenase inhibitors prevent the usually inevitable development of alkali-induced corneal ulceration in the rabbit. When enzymes harvested from ulcerated alkali-burned corneas are injected into alkali-burned but intact rabbit corneas, they cause a full-thickness ulcer within 12 hours. However, if the same amount of collagenase is injected into the normal rabbit cornea, there is little or no effect on the integrity of the cornea. The difference between the effect of collagenase on the alkali-burned cornea and the normal rabbit cornea may be due to differences in the content of proteoglycans in the corneas in the two situations. Corneal proteoglycans apparently protect collagen from breakdown by collagenase, and alkali-burned corneas have considerably fewer of these macromolecules.

Corneal Amyloidoses

Since even before Virchow's introduction of the term "amyloid," this hyaline, glassy eosinophilic extracellular substance has attracted considerable attention. Amyloid deposits in the cornea in a variety of nonspecific long-standing ocular and corneal lesions.[129,236,304,412,413,484,548] In such instances it occurs most conspicuously immediately beneath the corneal epithelium, but the amyloid may also be within the substantia propria corneae.

The lattice corneal dystrophies are inherited varieties of corneal amyloidosis. At least two types of lattice corneal dystrophy exist. To conform

with the nomenclature of other inherited metabolic diseases, these are designated lattice corneal dystrophy Type I (Biber-Haab-Dimmer) and Type II (Meretoja). Lattice corneal dystrophy Type I has an autosomal dominant mode of inheritance and is manifest clinically by characteristic opacities that affect particularly the central part of the cornea. The cornea typically contains a network of delicate double-contoured interdigitating filamentous structures that form a reticular pattern within the corneal stroma. Other types of corneal deposits may develop in some individuals with linear opacities or in blood relatives.[349] This lattice pattern, from which the disease derives its name, may be unilateral, with the other cornea remaining clear or containing discrete opacities.[373a] The lattice pattern of the corneal deposits resembles corneal nerves on cursory examination. This, together with a frequent diminution of corneal sensation, led some earlier investigators to postulate a primary lesion of nerves. This view was strengthened by an argyrophilia of lesions in histologic silver impregnation preparations. Electron microscopy by many observers has, however, failed to reveal nerves in the affected regions. Recurrent epithelial erosions may complicate the superficial corneal deposits. Lattice corneal dystrophy Type I usually begins clinically in both eyes at the end of the first decade of life but sometimes not until middle life. The disease is slowly progressive, and marked visual impairment eventually ensues before the fifth or sixth decade. A considerable individual variation in the clinical course exists even within affected families. Most patients require keratoplasty after the age of 40; some need a corneal graft by the age of 20.[373a]

More than 30 years ago, Franceschetti and Babel[205] stated that the stromal lesions in lattice corneal dystrophy were partially colored by gentian violet and became yellow-brown with Lugol's iodine "as if they were amyloid." In 1961, two publications in the foreign literature stated that the deposits in lattice corneal dystrophy were amyloid.[519,544] Owing largely to the flimsy nature of these investigations, the conclusions of these papers were not accepted by ophthalmologists or pathologists. Being unacquainted with these reports that were buried in the foreign literature, I independently came to the conclusion that lattice corneal dystrophy is a localized hereditofamilial form of amyloidosis.[348] This interpretation was subsequently confirmed by many individuals,[72,79,154,210,213,236,242,373a, 393,467,538,599-601] and it is now accepted that this corneal disease is an inherited form of amyloidosis. Amyloid has not been observed in surgically excised tissue from several sites (skin, lymph nodes, cartilage, nerve, muscle, and the wall of Baker's cyst) in subjects with this disease.[348,418] A detailed tissue examination has, however, not been performed on a pa-

tient who died with lattice corneal dystrophy Type I. Amyloid deposits can occur in the grafted donor tissue in patients with lattice corneal dystrophy Type I.[278,356,373a,388] There is usually no associated systemic disease, but in one family three sisters with lattice corneal dystrophy also had amyotrophic lateral sclerosis and cutis hyperelastica.[346]

Since the recognition of lattice corneal dystrophy as a form of amyloidosis, another variety of lattice corneal dystrophy with systemic amyloidosis has been discovered in Finland*[417-420] and Holland.[600] In 1969, Meretoja[416] drew attention to this condition now designated lattice corneal dystrophy Type II. Like lattice corneal dystrophy Type I, it also has an autosomal dominant mode of inheritance, but the two entities vary in several respects. The corneal manifestations in lattice corneal dystrophy Type II are less severe; visual acuity is good until late in life, with keratoplasty generally not indicated. Histologic confirmation of amyloid in the cornea in this condition has been established in eyes examined at necropsy.[418,420] A masklike facial expression with blepharochalasis, lobby ears, and protuding lips is characteristic. Cranial and peripheral nerve palsies develop. The facial nerve is usually affected at approximately the fifth decade. The skin is dry and itchy with lichen amyloidosis and cutis laxa. In heterozygous individuals the onset of the disease is later than in lattice corneal dystrophy Type I, but the disease may begin earlier than usual in persons homozygous for the mutant gene. Widespread amyloidosis has been detected at postmortem examination of patients with lattice corneal dystrophy Type II. Histopathologic and clinical findings in lattice corneal dystrophy Types I and II have been reviewed by Meretoja.[418]

The most consistent morphologic alterations in the lattice corneal dystrophies are the extracellular deposits of amyloid in the corneal stroma. The light and electron microscopic morphologic characteristics and the tinctorial attributes of the corneal deposits are identical to those of other forms of amyloid. The lesions exhibit an affinity for Congo red (with birefringence and dichroism), metachromasia, argyrophilia, and autofluorescence and have a fibrillary ultrastructure comparable to that observed in spontaneous and experimentally produced amyloidosis.[348] The largest deposits of amyloid are predominantly beneath the corneal epithelium, while the smallest accumulations are scattered throughout the substantia propria. Bowen *et al.*[72] studied frozen corneal tissue in this disease with fluorescein-labeled antisera to human amyloidotic spleen and liver. Using

* I visited Dr. Meretoja in Finland in April 1975 and studied some of these patients and tissue sections obtained from them. I confirmed that the condition is indeed different from lattice corneal dystrophy Type I.

this technique they professed specific fluorescence of the amyloid deposits.

Aside from lattice corneal dystrophy, another familial variety of corneal amyloidosis exists. It is characterized by multiple subepithelial deposits of amyloid.[400,432] This condition has been recognized in Japan [400,432] and the United States.[344] In Japan the disease has been referred to as "gelatinous drop-like dystrophy of the cornea." Kirk *et al.*[344] unfortunately referred to the condition as "primary familial corneal amyloidosis," a term that had been suggested for lattice corneal dystrophy several years earlier.[349] The literature contains an interesting, but single, example of bilateral corneal amyloidosis coupled with hypergammaglobulinemia.[364]

In the various forms of amyloidosis, amyloid has similar tinctorial properties when examined by light microscopy. The resemblance is retained at the ultrastructural level; by electron microscopy amyloid is seen to be composed of long, nonbranching fibrils, 80 to 100 Å in width. The diversity of situations under which amyloid occurs spontaneously and experimentally suggests that amyloid is heterogenous. Variations in its affinity for some stains point to differences in chemical composition. Analyses of amyloid isolated from cases of human and experimentally produced amyloidosis have established its proteinaceous nature and disclosed differences in different types of amyloidosis. However, the composition of the proteins in amyloid has only been elucidated in those forms of amyloidosis in which adequate quantities of the material are available for isolation and analysis. Amino acid sequence analysis has revealed a difference between the components of primary and secondary amyloid fibrils. The fibrils of human primary amyloid are homologous to variable segments of κ light chains of immunoglobulin.[528] The fibrils in secondary amyloidosis, on the other hand, do not have the identity of this amyloid protein of immunologic origin but are composed of a unique protein with an amino acid sequence different from other proteins. This protein, designated protein AA, crossreacts immunologically with a serum protein SAA. Normal serum contains minute quantities of SAA, but sera from patients with secondary amyloidosis and patients with inflammatory diseases have elevated levels.[529]

The amyloid in various corneal diseases has yet to be characterized. The sequence of events involved in its formation also remains to be determined. The deposition of amyloid in the absence of systemic disease suggests a local synthesis. Particularly in the inherited varieties of amyloidosis the amyloid is undoubtedly an end product of a complex interrelated chain of biochemical reactions. The presence of amyloid in an avascular tissue composed mainly of fibroblasts and collagen rekindles the well-

known association of amyloid with collagen or reticulin. Structurally intact collagen fibers are contiguous with extracellular deposits of corneal amyloid and occasionally intermingled with them. Because of this the possibility of the amyloid resulting from collagen disintegration or of the precursors of collagen being components of the amyloid has been raised on ultrastructural grounds. Despite its fibrillary nature, however, studies of amyloid elsewhere have not only failed to demonstrate collagen as a nidus of amyloid formation but also have shown that amyloid fibrils are resistant to collagenase digestion and differ ultrastructurally as well as chemically from collagen. Based on electron microscopic observations, some investigators have concluded that the amyloid is a product of corneal fibroblasts.[467] This possibility is supported by the intimate association of some corneal fibroblasts with the amyloid and the prominent rough-endoplasmic reticulum within these cells.[286,348,467] Several investigators have demonstrated the cellular origin of amyloid from a multitude of tissues, including fibroblasts.[44,523] The question of whether the amyloid reaches the cornea from elsewhere, as by way of the aqueous humor or limbal vessels, remains open. Possibly relevant to the pathogenesis of corneal amyloidosis is the recent observation of Kaunisto[327] of Finland that permanent corneal changes in lattice corneal dystrophy are preceded by acute episodes reminiscent of iritis or scleritis. During these incidents Kaunisto observed crystals in the cornea, bulbar conjunctiva, and aqueous humor. The crystals stained positively with the performic acid alcian blue method and are hence thought to contain cystine or cysteine.

Corneal Avascularity and Vascularization

The process of new vessel formation and the factors that influence the growth of vascular endothelium are of fundamental biologic importance. The existence of man and of other metazoa depends on them. Neovascularization also occurs during embryonic growth and in pathologic states such as inflammation and neoplasia. The failure of some transplanted tissues to elicit an invasive vascular response may play a cardinal role in the inability of fragments of tissue such as liver and kidney to survive when implanted into ectopic sites.[562,563]

The avascularity of the normal cornea and its vascularization in certain pathologic states have attracted attention for longer than a century.[293,572] In most species, blood vessels are absent from the cornea, except at its extreme periphery. The armadillo, gecko, and manatee are alleged to have vascularized corneas.[405] At birth an active invasion of the cornea by blood vessels, followed by their regression as few days later, is said to occur in the Rocky Mountain bighorn sheep *(Ovis canadensi Shaw)*.[405]

Capillaries invade the cornea in many diseases that cause blindness and poor visual acuity. Corneal vascularization is common in infections such as trachoma (the world's leading cause of blindness) and keratitis due to herpes simplex or injuries due to trauma or alkali or other chemicals and sometimes complicates corneal grafts. In diseases which affect the superficial cornea the vessels come across the limbus from the conjuctiva, while those disorders which affect the stroma are characterized by deep vessels that arise from anastomoses of the anterior and long posterior ciliary arteries. Corneal graft rejection due to immunologic reactions is much more prone to occur in vascularized corneas than in those that are nonvascularized.[122] The vascularized cornea is also prone to lipid deposition in patients with hyperlipemia.[115]

Several investigators have studied the ultrastructure of the blood vessels that invade the cornea.[297,398,399,513] Electron microscopic alterations in corneal vascularization have been reviewed recently and will not be considered here.[537]

Models

Corneal vascularization has been induced in a wide variety of experimental situations (Table 1). These have included injuries to the cornea from chemicals, microorganisms, physical methods, intracorneal inoculations, nutritional deficiencies, toxic states, and immunologic reactions. Folkman and his co-workers concerned with angiogenesis induced by neoplasms have used the rabbit cornea to bioassay potential factors that stimulate or inhibit vascular proliferation.[28,76,248] New capillaries grow into the avascular cornea toward neoplasms, such as Brown-Pearce and V2 rabbit carcinomas (homologous to the rabbit) and mouse tumors, that have been implanted in corneal pockets created between the collagen lamellae.[28,76,248]

The vascularization provoked in these models varies in degree and in reproducibility. For example, blood vessels do not constantly invade the cornea following ascorbic acid deficiency,[557] complete starvation,[54] vitamin A deficiency,[227] or intracorneal installation of saline.[356,614]

A number of important observations have been made in experimentally produced corneal vascularization. Particularly important were two findings of Campbell and Michaelson.[94] They found that following a small focal corneal injury the blood vessels tended to grow toward the lesion within an area resembling an isoceles triangle. They also noted that the induction of corneal vascularization depended on the proximity of the lesion to the corneoscleral limbus. Campbell and Michaelson observed that if the lesion was relatively close to the limbus, vascularization would

Table 1—Models for Corneal Vascularization

Model	References
Chemical	
Acetic acid	294
Alloxan	2,25,27,122,227,262,277, 372,373,441,535
Sodium hydroxide	81,107,184,227,294,376
Silver nitrate	126,227,228,513
Ethanol	174
Chloroquine	209
Mustard gas	395
Iodine	539
Hydrochloric acid	107,124,125,294
Lactic acid	294
Hyaluronidase	422
Colchicine	277,409
Biogenic amines	
Acetylcholine	614
Histamine	614
Serotonin	614
Bradykinin	614
Water	107
Nitrogen	25
Physiologic saline	25
Extracts of normal and vascularizing rabbit corneas	25
Sulfated derivative of hyaluronic acid	560
Serum	25,75
Pyruvic acid	294
Microbiologic	
Herpes simplex	107
Tubercle bacilli	383,387
Staphylococcus	265
Head-killed tubercle bacilli	383,488
Bacterial nucleoprotein	320
Physical injuries	
Hyperthermal burn	94,107,127
Corneal abrasion	290
Hypothermal	403
Radiation	
Radiofrequency burns	356,440
Carbon dioxide laser irradiation	192
Creation of cleavage plane in cornea with probe	25
Removal of corneal endothelium and Descemet's membrane with blunt curette	25
Deficiency states	
Vitamins	
Vitamin A	73,602
Riboflavin	54,73,227,598
Ascorbic acid	557
Amino acids	
Lysine	91,283,558,570
Methionine	47,558
Tryptophan	4,5,91,570
Histidine	404
Heavy metals	
Zinc	200

Table 1—Continued

Model	References
Protein	267
Total starvation	558
Toxic states	
Thallium	91
Tyrosine	90,249,292
Immunologic reactions	
Intracorneal antigen in sensitized animal	227,245,319,320,596
Immunologically provoked corneal graft rejection	297
Intravitreal injection of antigen	290,297
Intracorneal instillation of cells or components of them	
Polymorphonuclear leukocytes	229
Macrophages	103,463
Neoplasms	28,76,248
Nonhistone proteins from malignant cell nuclei	588
Miscellaneous	
Intracorneal injection of lacrimal fluid	559
Implants in hamster cheek pouch	353
Long-term use of contact lenses	157,158
Encirclement of the eyeball by a rubber tube	2,196,380
Intracorneal placement of suture materials	426
Needle tracts penetrating into anterior chamber	25
Full-thickness keratoplasties (with donor material from normal or vascularized corneas)	25
Corneal homografts transplanted into vascularized skin	59

follow, while a more central lesion of comparable size often did not provoke vascularization. This finding has been confirmed many times in different situations. In hamster cheek pouches, newly formed capillaries commonly extend toward and into the corneal explants, consistent with the hypothesis that specific substances possess the ability to stimulate vascular growth.[353] Gimbrone *et al.*[248] also stressed that when tumors are implanted in the corneal stroma, the time taken to vascularize is directly proportional to the distance between the tumor and the limbus. Implants in the central cornea (4 to 5 mm from the limbus) sometimes remain unvascularized for up to 2 months. Cogan [107] pointed out that corneal edema accompanies and precedes clinically and experimentally produced corneal vascularization. This observation has also been confirmed subsequently by many investigators. Several years ago I inserted a variety of normal, injured, and nonviable corneas into hamster cheek pouch chambers and unexpectedly found that the vascularization of the transplanted tissues was virtually independent of the nature of the explants.[353] The crucial factor appeared to be the host's reaction to the transplanted corneal tissue. Unless it provoked an inflammatory cell infiltrate, corneal vascularization did not occur. Since then a review of experimental models of corneal vascularization has revealed that the inflammatory reaction

occurs in most of them prior to neovascularization.[227,355] Several factors seem to influence the degree of new vessel formation.

Pathogenesis

Many factors influence capillary growth and numerous theories have been proposed to account for the formation of new capillaries in different situations.[92,511] It is a generally accepted biologic fact that directional growth occurs from a region where a chemical substance is in low concentration toward a zone containing this substance in high concentration. The phenomenon has been substantiated in many different situations. It is highly likely that one or more diffusible mediators capable of initiating directional capillary growth are involved in many normal and diseased tissues. In 1893, Loeb [386] first suggested that some tropic chemical substance might be responsible for vascularization in general. Several investigators have alluded to the presence of such factors in the potentially vascularized (but not normal) cornea,[25,94,197,227-229,248,294,353,355,408,614] and available evidence favors their existence. The belief that corneal vascularization is dependent on chemical mediators that the normal cornea lacks was originally proposed by Campbell and Michaelson [94] because of observations previously described. Like other theories, this one has provoked criticism. For example, many years ago, Cogan [107] observed that a small injection of sodium hydroxide produced a swollen zone around the site of injection and that the blood vessels grew into the edematous region rather than into the injured area. This finding, which is not consistent,[408] cannot be used to argue against an angiogenic factor. As pointed out by Ashton,[23] vessels do not grow into necrotic tissue. Also, the angiogenic factor may not arise at the site of injury. Some investigators have wondered why vessels cease migrating beyond a certain point. Others have questioned this theory since the newly formed blood vessels do not develop beyond the corneoscleral limbus.[25] But an angiogenic factor diffusing toward the peripheral cornea could drain away without reaching a sufficient concentration to provoke an effect on neighboring conjunctival and scleral blood vessels. Some individuals have stressed that the factor should have been isolated by now. But, those who raise this objection clearly do not appreciate the formidable technical difficulties.

Source of Angiogenic Factor

In theory the angiogenic factor(s) responsible for corneal vascularization could arise from one or more sources, viz, the denatured tissue, the injurious agent, corneal cells, cells of noncorneal origin, serum, tears, or the aqueous humor.

From a biologic standpoint, a circulatory system evolved of necessity as a functional adaptation in metazoa that were composed of many cells.

Likewise, embryonic tissues and solid neoplasms vascularize only after reaching a critical size. All the aforementioned have in common an increased cellularity followed by vascularization. One would expect vast quantities of cells in close proximity to each other to compete for oxygen and existing nutrients and for certain metabolites to accumulate and alter the milieu of cells and possibly cause the cells to liberate some factor capable of inducing directional vascular growth. Since the normal corneal stroma is relatively acellular, could it be that an increased cellularity contributes to the invasion of the cornea by blood vessels? During the past few years much evidence has accumulated to indicate that leukocytes may play a crucial role in this regard.[227-229,353,355] In studies on corneas implanted into hamster cheek pouch chambers, I was especially inspired by the observation that corneal vascularization was invariably accompanied by a cellular infiltration of the corneal stroma. When the vascular invasion involved only part of the cornea, the blood vessels were at the site of the cellular infiltrate. If cells did not penetrate the corneal tissue under investigation, it consistently remained avascular. This view was reinforced in experiments performed with Fromer.[227-229] The events that preceded and accompanied corneal vascularization in several apparently diverse established experimental models of corneal vascularization in rats and rabbits were essentially similar. After exposure of corneas to silver nitrate, alkali, alloxan, colchicine, hyperthermal or radiofrequency burns, or intracorneal antigens in sensitized and nonsensitized animals, leukocytes enter the corneas before capillaries. Leukocytes also precede blood vessels when rats are maintained on Vitamin A or riboflavin-deficient or high-tyrosine diets.[127,227,249,292,355,602] These different models vary in several respects, including the degree of associated corneal edema and the latent period required for vascular invasion. Yet a pronounced leukocytic infiltrate of the cornea, as a rule, precedes and accompanies the corneal vascularization. The leukocytic infiltration manifests a variable time course after corneal injury. In almost all models of corneal vascularization that have been thoroughly studied, leukocytes have invaded the cornea before blood vessels. The location of the leukocytic invasion corresponds to the same site as the blood vessel invasion. The suppression of the leukocytic invasion following corneal injury suppresses corneal vascularization.[228,524] The localization, depths of stromal involvement, and direction of the vascular invasion from the limbus correspond extremely well with the pattern of the leukocytic infiltration which these injuries induce. Discrete single corneal injuries such as silver nitrate or electrocauterization stimulate a localized leukocytic and vascular infiltration into the damaged corneal stroma from the nearest region of the corneoscleral limbus. Mod-

els that result in a diffuse, circumferential leukocytic infiltration into the cornea such as topical alloxan or colchicine administration activate a vascular invasion into the periphery of the entire cornea. When leukocytes infiltrate the entire thickness of the corneal stroma, as when antigen is instilled into the corneas of sensitized animals, the vascular ingrowth extends throughout the depth of the corneal stroma. The amount of vascularization is also directly proportional to the leukocytic infiltrate. The injection of antigen into the corneas of sensitized animals, but not nonsensitized animals, provokes a marked leukocytic and vascular infiltration into the cornea to a degree which surpasses that of other models. The onset of corneal vascularization is also temporally related to the time of the initial leukocytic infiltration. For example, injection of antigen into sensitized animals produces an early leukocytic infiltration and corneal vascularization, while a longer latent period antecedes both the leukocytic and vascular invasion in rats on a riboflavin-deficient diet. In this regard it is of interest that leukocyte extracts promote cell multiplication of fibroblasts. As pointed out many years ago by Carrel,[95,96] leukocytes and extracts of leukocytes contain substances capable of stimulating cell proliferation.

An attempt has been made to pinpoint specific cell types responsible for angiogenic activity. Polymorphonuclear leukocytes, lymphocytes, or components of leukocytes have been injected intracorneally into Fisher albino rats whose circulating leukocytes had been depleted by total body x-irradiation. Evidence exists to suggest that polymorphonuclear leukocytes play an important role in corneal vascularization.[229] When polymorphonuclear leukocytes isolated from glycogen-induced peritoneal exudates and a heat-labile fraction isolated from them were injected into corneas of rats with radiation-induced leukopenia, blood vessels invaded the corneas.[229] These studies suggest the polymorphonuclear leukocyte may provoke corneal vascularization directly or indirectly. It remains to be determined whether the cornea is a special site with susceptibility to factors from the polymorphonuclear leukocyte that possess angiogenic activity or whether the phenomenon is more generalized. Fromer and Klintworth [229] found that lymphocytes isolated from thymus, spleen, and lymph nodes of normal rats did not have this vascular effect. However, evidence to implicate other leukocytes in angiogenesis also exists. Intracorneal injections of activated macrophages, or conditioned medium derived from them in culture, produce corneal vascularization in a high percentage of animals (60 to 80%).[463] Endothelial proliferation occurs at the height of the delayed sensitivity reaction in the skin of guinea pigs.[462] Sidsky and Auerbach [525] have documented that the intradermal transfer of

immunocompetent lymphocytes induces endothelial cell proliferation, which they have termed "lymphocyte-induced angiogenesis (LIA)." Corneal implants of homologous, but not autologous, adult rabbit lymph nodes also induce corneal angiogenesis.[198] Vascular proliferation is also a prominent feature of the recently recognized lymphoma-like syndrome called angioimmunoblastic (immunoblastic) lymphadenopathy.[391,485,515] These lesions are characterized by the entire range of immunologically reactive cells (including lymphocytes, plasma cells, and immunoblasts [large transformed lymphocytes]) within lymph nodes as well as a marked vascular proliferation. Capillary proliferation is also a prominent feature of some cases of cutaneous lymphoplasia (lymphocytoma).[392] An unusual hemangiomatosis occurs in skin with plasmacytosis.[442] Furthermore, splenic and bone marrow explants that are rich in leukocytes also vascularize.[561]

In noncorneal tissue a variety of cell types are suspected of producing vasoformative factors. Aside from leukocytes these include neoplastic cells,[26,76,248] the epidermis of skin,[511] and mast cells.[511] Mast cells are not likely to be a source of an angiogenic factor for corneal vascularization, as the normal cornea lacks them, and they only enter it after the blood vessels have entered.[539] However, in urticaria pigmentosa and related conditions, an increased vasculature frequently surrounds mast cell aggregates.[511] Blood vessels are alleged to invade the cornea in the absence of leukocytes after neoplastic cells are instilled into the cornea.[248] Although adequate sequential morphologic studies have not yet been documented under these conditions, the induced angiogenesis has little bearing on naturally occurring corneal vascularization. Neoplasms of the cornea are extremely rare, and neoplastic cells themselves probably possess the capability of stimulating vascular proliferation.

The question of whether leukocytes are needed to initiate corneal vascularization remains to be clarified. Experiments by Fromer and Klintworth [228] disclosed that corneal vascularization did not follow corneal cauterization with silver nitrate in the absence of leukocytes. In normal Fischer albino rats, cauterization of the cornea with silver nitrate consistently produces a vascular ingrowth within 3 days. When the same procedure was performed on rats whose circulating leukocytes had been eliminated by an adequate dosage of total body x-irradiation, neither leukocytes nor blood vessels invaded the corneas. When the leukopenic effect of irradiation reached its maximum, i.e., when circulating leukocytes were virtually absent, the corneas were cauterized with silver nitrate. Under such conditions neither leukocytes nor blood vessels invaded the corneas. On the other hand, if the corneal cauterization was per-

formed before the circulating leukocytes were totally eliminated by x-irradiation, both the leukocytic and vascular invasions occurred. Corneas cauterized after only the heads of rats received a similar dosage of x-irradiation ruled out the possibility of irradiation-induced limbal vascular endothelial damage as the explanation for the vascular suppression observed by x-ray treatment. Other rats were given subconjunctival methylprednisolone acetate immediately after or 24 hours after corneal injury with silver nitrate. This corticosteroid inhibited the infiltration of leukocytes and the subsequent vascular invasion of the corneal stroma if administered immediately after cauterization. On the other hand, it did not prevent the invasion of the cornea by blood vessels if it was instilled 1 day after cauterization, at which time leukocytes had already infiltrated the cornea. However, under such circumstances the corneal leukocytic and vascular ingrowth was less severe than in the nonglucocorticoid-treated corneas. In a study on rats made leukopenic with antileukocytic serum and total body x-irradiation (using a lower dosage than Fromer and Klintworth [228]), Sholley *et al.*[524] found that leukocytes were not essential to initiate corneal vascularization, although the degree of vascularization was less than normal. Eliason [178] has also observed that blood vessels can invade the cornea of leukopenic rabbits in the absence of leukocytes. The latter studies suggest that leukocytes may not initiate the vascular proliferation. But a problem in interpreting the observations stems from the fact that once the cornea becomes vascularized, the blood vessels apparently remain throughout life. They are frequently devoid of blood, but their presence can be detected by slit-lamp examination and is a testimony to the previous seat of corneal inflammation. Such vessels may loose their circulation but rapidly regain it as part of the hyperemic response to a subsequent corneal injury. Because of this, studies concerned with the early events in corneal vascularization need to exclude the presence of existing corneal capillaries in experimental animals.

The question of whether angiogenic factors are components of the inflammatory exudate and enter the cornea with serum has not been fully investigated but needs to be, particularly since corneal edema due to an increased vascular permeability is a fundamental and apparently consistent event that precedes the vascular invasion of the cornea. The possibility of an angiogenic mediator arising from injured corneal tissue has been raised but lacks adequate support. In experiments in which open plastic tubes were implanted radially into rabbit corneas, Maurice *et al.*[408] observed that by repeatedly wounding the tissue close to the central end of the open, but not closed, tubes, vessels grew into the open plastic tubes. This was interpreted as evidence in favor of a vasostimulatory

factor being liberated at the wound and passing down the lumen of the tube to the responding vessel walls. Maurice *et al.*[408] suggested that a vasostimulatory factor is produced by the cells and diffuses in direct proportion to the molecular weight of the particles according to the laws of diffusion. If an angiogenic factor arises in damaged tissue, one would anticipate that a particular injury would consistently provoke corneal vascularization. But the nature of the corneal injury does not influence the presence or absence of vascularization of corneal tissue that is implanted into hamster cheek pouches.[353] At least with regard to silver-nitrate–induced corneal vascularization, other factors seem to be involved. Cauterization with this compound consistently provokes corneal vascularization in normal rats but not in the radiation-induced leukopenic ones or corticosteroid-treated animals.[228]

It seems unlikely that a mediator of corneal vascularization would arise from the tears or aqueous humor since it would probably be considerably diluted. Such a source should not promote focal areas of corneal vascularization. Nevertheless, Szeghy[559] produced corneal vascularization by the intracorneal injection of lacrimal fluid from rabbits and humans and maintained that prior boiling eliminated the effect. Some noxious agents may possess angiogenic activity, but most injurious agents are unlikely to stimulate vascular endothelial cell proliferation themselves. As pointed out earlier, a wide variety of injuries culminate in corneal vascularization. If corneal cells themselves produce the factor, normal cells certainly do not liberate significant quantitatives of biologically active material.

Nature of Chemical Mediator

The initial event that triggers pericorneal blood vessels to invade the cornea occurs early in the inflammatory response, but virtually nothing is known about the nature of the postulated corneal angiogenic factor(s). The complexity of the associated events, which include all aspects of the inflammatory response, makes it extremely difficult to dissect the various components in the chain of events that culminate in corneal vascularization. Many speculations have been made but there is little or no evidence to support them.

Factors that stimulate vascular endothelial cell proliferation and migration into the cornea still have to be isolated and identified. Even the postulated tumor angiogenic factor of Folkman, which has been much more extensively investigated than angiogenic substances of different origin, has not yet been identified with certainty. A difficulty that faces investigators of tissue vascularization is the need for a sensitive bioassay system for angiogenic factors. Currently available methods include the use of hamster cheek pouch chambers,[353] chorioallantoic membranes of

chick embryos,[30] cell culture of aortic endothelial cells,[177] and rabbit corneal implants.[199] All these methods, and other methods, have their shortcomings.

Teleologically one would expect an organism to respond to oxygen deprivation by stimulating angiogenic factors to provide better oxygenation of the tissue by vascularizating it. Ashton has pointed out that retinal neovascularization occurs in conditions associated with relative hypoxia. Ashton [22,24] suggested that the proliferation of newly formed blood vessels occurs, at least in the retina, in response to a critical concentration of one or more chemical mediators that are produced in tissue during anaerobic metabolism[22,24,25] The possibility of tissue hypoxia playing a role in neovascularization of the cornea has also aroused consideration. One might expect a product of anaerobic metabolism to stimulate corneal vascularization, especially since the corneas of rats deprived of riboflavin, a coenzyme of several respiratory enzymes, become vascularized. The finding of increased concentrations of lactic acid adjacent to the corneoscleral limbus before corneal vascularization [380] has been taken as evidence of decreased aerobic glycolysis. Since the intracorneal injection of lactic acid or pyruvic acid can produce corneal vascularization, Imre [294] has implicated them. But these acids do not consistently cause corneal vascularization when injected intracorneally. Oxygen has not influenced experimentally produced corneal vascularization.[26,321,376,424] In a study of rabbit corneas with silicone-filled anterior chambers, Maurice *et al.*[408] maintained that hypoxia (or suboxidation) did not constitute a stimulus to corneal vascularization.

Several vasoactive amines increase vascular permeability. The possibility of histamine [443,511,614] or other biogenic amines such as acetylcholine, serotonin, and bradykinin [614] promoting vascularization has been raised. To support this view Zauberman *et al.*[614] inserted into rabbit corneas plastic tubes that released active substances from their tips. Acetylcholine, histamine, 5-hydroxytryptamine (serotonin), and bradykinin each caused the growth of vessels from the corneal limbus into the lumen of the tube in one third of the experiments. Control tubes filled with saline did not elicit this effect. These investigators considered, however, that the amines may have caused nonspecific tissue damage that might be the source of the angiogenic factor.

Inhibitors of Vascularization

Does the cornea normally contain a substance which inhibits vascularization? Is this substance absent or present in insufficient quantities in certain disease states which permit blood vessels to invade the cornea? Chemical similarities between the cornea and other avascular tissues

prompted investigation of this possibility. Meyer and Chaffee,[422] impressed by the abundance of mucopolysaccharides (more precisely termed "glycosaminoglycans") in the cornea and certain other avascular tissues such as cartilage and Wharton's jelly, thought that these compounds might inhibit the growth of blood vessels into the cornea. They also found that the intracorneal injection of hyaluronidase caused corneal vascularization, whereas the instillation of inactivated enzyme usually provoked only a transient reaction.[422] Bachsich and his colleagues [35,36] later explained this concept. If the hypothesis is correct, one might expect some difference between the glycosaminoglycan content of the normal cornea and of the vascularized cornea. Some early studies claiming the normality of glycosaminoglycans in vascularized corneas leave much to be desired.[598] But reputable investigators have pointed out that the corneal glycosaminoglycans are not diminished prior to vascularization, or in the area in front of the invading blood vessels, by histochemical methods [23] or from the standpoint of existing ^{35}S-sulfate labeled glycosaminoglycans.[532,535] For these reasons the theory of a natural inhibitor of corneal vascularization fell into disrepute. A critical review of the data, which led to the downfall of the theory, reveals many loopholes. An objection was the failure of hyaluronidase to effect corneal vascularization,[425] but the main glycosaminoglycan component of the cornea, keratan sulfate, is not degraded by this enzyme. During the past few years it has become appreciated that glycosaminoglycans normally exist in a form covalently bound to proteins as proteoglycans. The claim that mucopolysaccharides do not inhibit corneal vascularization has also not been adequately tested experimentally. The techniques used by Ashton [23] and Smelser and Ozanics [532,535] are not as sophisticated or as precise as those that can now be applied to the problem. The basic concept of a vasoinhibitory substance in the normal cornea still warrants serious consideration, expecially in the light of the recent demonstration of a vasoinhibitory effect of cartilaginous proteoglycans in several situations in which this material has been evaluated.[76,177,371] Brem and Folkman [76] observed that a tiny piece of neonatal cartilage (but not boiled cartilage) placed in the corneal stroma between the limbus and the tumor explant prevented some tumors (28%) from vascularizing. Langer *et al.*[371] have isolated and partially purified a fraction from cartilage that inhibits neoplasm-induced corneal vascularization. Also noteworthy is the fact that macular corneal dystrophy and the systemic mucopolysaccharidoses (characterized by an excessive corneal accumulation of material with the cytochemical attributes of glycosaminoglycans) are associated with avascular corneas.

Because mast cells are present at the corneoscleral limbus, the possibility of them inhibiting corneal vascularization was raised several years

ago by Smith.[539] He proposed that their massive destruction in corneal injury caused blood vessels to invade the cornea. This view lacks adequate support. Although it still remains an open question whether the normal cornea does or does not possess vasoinhibitory factors, certain compounds, e.g., corticosteroids [27,439] and the alkylating agent thiotepa triethylene thiophosphoramide, diminish corneal vascularization.[373,375] Ashton *et al.*[27] found that cortisone markedly reduced alloxan-induced corneal vascularization but did not completely inhibit it.

Almost 30 years ago Cogan [107] proposed that the avascularity of the normal cornea resulted not from a chemical inhibitor but from a mechanical barrier which the compact lamellae of corneal collagen offer to vascular invasion. He suggested that capillaries entered the cornea if the lamellae of collagen become separated by edema. This hypothesis was based on the observations that some avascular tissues, such as fingernails and cartilage, offer a mechanical barrier to blood vessels and that corneal edema consistently accompanies and frequently precedes corneal vascularization. Despite the attractiveness of this theory, it has become increasingly apparent that it has shortcomings. Corneal vascularization does not always follow corneal edema, and the latter is hence not a sufficient stimulus to provoke corneal vascularization.[197,290,353,372,402,408] Additional evidence against edema being a stimulus for corneal vascularization is Baum and Martola's [43] finding by pachometry that corneas with vascularization were thinner than nonvascularized corneas with bullous keratopathy. Moreover, the degree of vascularization is not directly related to the amount of corneal edema. Also corneal tissue does not necessarily vascularize when transplanted into hamster cheek pouch chambers,[353] even when corneal lamellae are separated in a swollen stroma. In addition, during the early development of the cornea of the chick embryo, the corneal stroma becomes edematous,[138] yet blood vessels do not invade this swollen tissue.

Finally, Ashton and Cook [25] pointed out that it is unlikely that the vascular endothelial cells will proliferate in response to a reduction in tissue compactness. Nevertheless, stromal edema antecedes and accompanies corneal vascularization. The loosening of the stromal framework that results from the edema undoubtedly provides capillaries with space into which they may extend. There is ample reason to suspect that vessels will grow preferentially in planes of diminished resistance.

Corneal Transplantation

Subsequent to the first corneal graft in man in 1838,[345] many refinements have taken place in the transplantation of corneal tissue from one person to another. Corneal homografts (allografts) have become an estab-

lished method of treating many corneal diseases and are frequently successful despite immunologic differences between the donor and the recipient. Why the transplantation of corneal tissue between antigenically dissimilar individuals should ever be successful has provoked considerable discussion.[59] Several factors probably play a role. Transplantation and tissue-specific antigens within homografts are capable of providing an immunologic stimulus. As the cornea contains relatively few cells, particularly if the epithelium is denuded, corneal homografts have relatively few antigenic determinants. The possibility of stromal glycosaminoglycans interfering with the access of transplantation antigens to immunologically competent cells has also been raised. Moreover, the lack of lymphatics and blood vessels in a normal cornea interposes a barrier between the antigens of the graft and the immunologically competent cells of the recipient. Assuming that the avascular recipient bed permits corneal homograft success, one would expect rejection in the presence of corneal vascularization. The accumulated clinical experience of ophthalmologists indicates that this is the case. Vascularization also plays a prominent role in homograft rejection of other tissues. Furthermore, vascularized corneas also contain lymphatics which drain into the regional lymph nodes. The presence of these lymphatics may partly account for the increased corneal graft rejection rate in vascularized corneas.[122] Antigens can reach immunologically competent cells by this or other routes. Corneal homografts can become opaque due to an immune rejection. This usually occurs at least 2 weeks after the operation. In nonvascularized corneas estimates of the rejection rate range from 12 to 35%.[590] Under experimental conditions it is possible to provoke corneal graft rejection. For example, corneal grafts in rabbits will become cloudy when skin from the donor is grafted to the recipient 2 weeks later.[401] This effect decreases with time. Sensitized lymphocytes have been shown to play a role in corneal graft rejection, and opacification of the graft usually begins in the endothelial layer at the periphery of the graft.[449,456] Aggregates of lymphocytes accumulate at the graft junction and the destroyed endothelial cells ultimately become replaced by fibroblast-like cells.[295,296,340,453,456,457] The possible contribution of humoral responses in graft rejection remains unclear. In addition to immunologic mechanisms, corneal grafts may be unsuccessful for many other reasons.[464]

The success rate of corneal grafts in nonselected donor–recipient pairs is high. This, together with the relative scarcity of adequate donor material for corneal grafts, has made it not practical for most ophthalmologists to try to match donors and recipients antigenically as is needed for transplants of organs such as the kidney.

The ultimate fate of the cells and other constituents in the graft is naturally of interest from several standpoints. Does the grafted tissue persist or eventually disappear after serving as a scaffold to be taken over by tissues of the host? Several investigators have addressed this question.[21,41,272,450,458,536] The problem has been approached by utilizing isotopically labeled donor material. Early studies using ^{32}P-labeled donor cells suggested a rapid replacement of the grafted cells.[159] However, in experiments using ^{3}H-thymidine, an isotope that is incorporated into DNA (a more stable cellular component), it became apparent that donor corneal stromal and endothelial cells survive for prolonged periods in the graft in the absence of rejection. With time, corneal homografts can become populated by cells of the host.[41] The destiny of donor cells (keratocytes and corneal endothelium) has been followed in grafts in which the transplanted cells were identified by sex chromatin (Barr body)[42,181] or by labeling the DNA with ^{3}H-thymidine,[272,458,459] Using the Barr body as a marker, the stromal cells have been shown to persist for at least 3 months in the cat.[42] In the rabbit, using the sex chromatin as a marker, donor epithelial cells have been shown to be replaced by the host after the seventh day.[21] Espirutu *et al.*[181] also used the Barr body as a marker and observed that the corneal endothelial cells after penetrating keratoplasty persist in rabbits for 4 months but become almost completely replaced by the seventh month. However, ^{3}H-thymidine labeled corneal stromal and endothelial cells can be detected in the rabbit in undiminished numbers for as long as 12 to 13 months (duration of the experiment).[272,458] ^{3}H-thymidine can be retained in the corneal endothelium in the rabbit for as long as 4 years.[450] Little is known about the destiny of human corneal grafts, but corneal fibroblasts in the human graft may survive as long as 6 years.[41] The turnover of ^{35}S-labeled glycosaminoglycans in corneal transplants has been studied by several investigators.[159,182,374] In rabbits these macromolecules have been shown not to persist as long as normal by scintillation counting [159] and autoradiography.[182] This diminution of labeled glycosaminoglycans occurs particularly during the first 2 postoperative weeks.[159,374] By 7 weeks Espiritu *et al.*[182] could demonstrate practically no ^{35}S in the autoradiographs of labeled corneal grafts. In the rabbit, ^{14}C-glycine labeled donor collagen persists in clear, but not opaque, corneal grafts for at least 17 months.[536] Smelser *et al.*[536] found that hazy grafts, in contrast, underwent some removal and/or synthesis of new collagen.

The recurrence of disease within corneal grafts is of particular interest, especially in inherited corneal dystrophies, where it has bearing on the pathogenesis of the disease. In several corneal dystrophies the condition

has recurred in the graft.[84,278,388,507,554,585] Such recurrences can in theory be due to the invasion of the graft by host cells that are responsible for the particular disease. But the possibility of substances diffusing into the cornea from elsewhere and depositing in this tissue still needs to be excluded in some of these dystrophies.

Corneal Hypersensitivity

Circulating antibodies play a role in several corneal diseases. After a soluble antigen is introduced into the cornea, it diffuses toward the corneoscleral limbus. Despite the absence of lymphatic channels in the normal cornea, substances injected into the cornea can diffuse to the corneoscleral limbus and drain to regional lymph nodes by way of lymphatic vessels which are normally present in the conjunctiva. This can be demonstrated by the unilateral intracorneal injection of foreign protein. After this procedure, antibody-producing cells can be found in the homolateral draining lymph nodes.[541] Certain antigens elicit antibody production after being injected intracorneally in amounts that are usually inadequate to stimulate antibody production when injected systemically.[541] In the presence of antibodies, a ring of opacification develops in the cornea between the site of inoculation and the limbus (immune ring, Wessely ring). If the animal is sensitized, this occurs within hours; if the animal is not sensitized, the immune ring develops many days later, after the development of sensitization to the intracorneally injected antigen. The zone of precipitation is analogous to that which occurs after antigens and antibodies diffuse toward each other in gels (Ouchterlony's diffusion system). The immune ring is composed of precipitated antigen-antibody-complement complexes and a pronounced infiltration of polymorphonuclear leukocytes that results from this reaction. When the antigens are instilled eccentrically into the cornea, the ring extends into the corneoscleral limbus where focal hemorrhage and hyperemia are conspicuous due to an Arthus phenomenon in the pericorneal blood vessels.[227] In 1962 using light microscopy, Germuth *et al.*[245] concluded that collagen damage accompanied the immune ring. This observation, although not supported in one electron microscopic study,[431] has been confirmed in a more recent investigation using newer techniques.[429] The immune ring only occurs in the presence of detectable antibody in the serum. After the intracorneal injection of antigens into rabbits, antigen-antibody-producing cells are found in the homolateral preauricular lymph nodes, cornea, and uveal tract. Antibody production may be elicited by amounts of antigen which are inadequate to stimulate antibody production after systemic administration. Although Wessely described this immune ring in

1911, rings had been documented around corneal lesions prior to that time. For example, Boettcher [65] described a ring of concentrated cells around focal corneal lesions in 1875. Such a ring, however, may not be due to immunologic mechanisms.

Abnormalities of Corneal Curvature

Different aberrations of corneal curvature exist. These include astigmatism, cornea plana, and keratoconus (conical cornea). Tissue studies have been most frequently performed in keratoconus. This clinically important disease is characterized by an ectasia of the central portion of the cornea and an abnormal thinning of the corneal stroma. It is usually bilateral and has an onset in youth or adolescence. The cause and pathogenesis remain obscure, but it is often familial. There are documented cases of keratoconus in association with a variety of disorders of connective tissue, including Ehlers-Danlos syndrome (especially Types II [mitis type] and VI [ocular]), Marfan syndrome, and osteogenesis imperfecta congenita (van DeHoeve syndrome).[232,317,567,568,502,510] Association with atopic dermatitis,[46,67,134,206,234,366,429,526,545] vernal conjunctivitis,[57,204,207,234,251,257] Down syndrome,[143,155,207,285,482,517,527] and other conditions has also been reported. The light microscopic appearance of corneal tissue in this disease has been well known for a long time. The most striking abnormality in keratoconus is the diminution of collagen fibers in the thinned portion of the corneal stroma. Corneal edema frequently develops as a late complication; when this occurs the stroma becomes thickened. Dehiscences in Bowman's zone are common. The morphology of the cornea in keratoconus has been evaluated extensively by electron microscopy.[253,300,307,308,332,414,446,468,469,472,564] Ultrastructural abnormalities have been described in all corneal layers. The corneal epithelium is frequently of irregular thickness and rests on an abnormal thickened basement membrane. In a recent electron microscopic study of 14 corneal buttons of clinically typical cases of keratoconus, Iwamoto and DeVoe [300] documented the presence of peculiar enveloped particles surrounded by electron-dense finely filamentous zones within a thickened epithelial basement membrane and occasionally deeper in the Bowman's zone or in the connective tissue that replaced portions of that layer. The particles were identified in all of these corneas and were numerous in 8 cases. Similar particles were occasionally found within and between corneal epithelial cells. The particles have yet to be identified and have not been observed by others who have examined corneal tissue by electron microscopy. But Iwamoto and DeVoe [300] confined their observations to the central cone area, unlike other observers.

Multiple breaks in Bowman's zone are a prominent morphologic feature of this disease, and these disrupted portions of Bowman's layer are often replaced by stromal connective tissue and fibroblasts.[300,308,414] It is generally agreed that the individual stromal collagen fibers are of normal thickness (25 to 30 nm) but that there are fewer collagen lamellae than normal. In contrast to the normal cornea, which contains approximately 40 lamellae of collagen in this central part and approximately 60 in its periphery, the number of lamellae becomes markedly reduced to approximately 26 centrally and 51 peripherally in keratoconus.[472] The periodicity of the individual collagen fibers appears to be normal. The distance between collagen fibers may be increased because of an accumulation of microfibrillary granular material that is sometimes conspicuous.[470,472] Descemet's membrane and the corneal endothelium may be abnormal in advanced stages of the disease. Some observers have been impressed by the greater than normal morphologic variation of the corneal stromal cells.[472] Fine granular material is present among collagen fibers, as well as around abnormal corneal fibrocytes. Some investigators regard this observation as cardinal to the disorder. Relatively crude chemical analyses by modern standards have been performed on corneas with keratoconus.[88,476,500,603] As one would anticipate there is less collagen than usual. In their analysis of corneal buttons with keratoconus, Robert *et al.*[500] found several abnormalities. These included a) an increase in the glycoprotein/collagen ratios, b) a decrease in uronic acid, c) increases in hexoses and extractable hexosamines with 0.9% NaCl, d) a decrease in the hydroxylation of lysine, e) a decrease in the glycosylation of hydroxylysine, and f) a decrease in the glycosylation of collagen. The detailed chemical structure of corneal collagen in keratoconus has not yet been documented. The glycosaminoglycans have been essentially normal in some studies;[88] other investigators have reported abnormalities such as a relative increase in the glucosaminoglycan (keratan sulfate) fraction, a significant decrease in the galactosaminoglycan (chondroitin sulfate) fraction,[476] and a decrease in total hexosamine sulfate.[88,603]

The possibility of a macromolecular structural abnormality deserves serious consideration. For the normal cornea to maintain its characteristic curvature against the intraocular pressure, the tissue needs an adequate tensile strength. This is probably provided as in other collagenous tissues by the formation of covalent bonds or cross links between adjacent tropocollagen molecules and possibly between adjacent collagen microfibrils. Yet very few investigators have attempted to correlate the clinical, morphologic, and macromolecular anomalies. The only extensive studies in this regard were performed by Pouliquen *et al.*[470,472] and Robert *et al.*[500] These scientists at the Centre de Recherches d'Ophthalmologie in Paris

conducted electron microscopic as well as biochemical analytic studies on normal corneas and on those with keratoconus. They also studied the biosynthesis of the macromolecules of normal and pathologic stroma. They postulate that keratocytes which synthesize excessive quantities of one type of macromolecule produce less of others and that disorders in the relative synthetic rates of the different macromolecules can result from disturbances in the metabolic controls. When this occurs the chemical composition and physiologic properties of the cornea are thought to become abnormal. This view is supported by studies on opacified grafts in which animals were preimmunized against corneal constructural glycoproteins in the donor cornea. Such opacified grafts possess a synthetic activity similar to that of sclera or tendon.[493,494,496,497] Robert *et al.*[500] have proposed that a similar metabolic shift may account for the corneal findings in keratoconus, i.e., a diminution in corneal collagen fibers and an increase in noncollagenous interfibrillary substances. Robert *et al.*[500] correlated the findings with the increase in microfibrillary material that has been detected in electron microscopic investigations of the disease.[470] The French scientists interpreted the findings as indicating a defect in the quantitative control of structural glycoproteins and collagens, in which there was a relative decrease in the synthesis of corneal collagen and relative increase in the synthesis of structural glycoproteins.

Morphologic observations have failed to provide an adequate explanation for the chain of events that culminate in keratoconus. To some extent this is a result of the facts that corneal grafting is performed late in the disease and that most patients undergo conservative treatment, especially with contact lenses, prior to keratoplasty. Abnormalities have varied considerably from observer to observer, making the interpretation of the primary abnormality difficult, if not impossible. For instance, the cardinal abnormality has been attributed to a degeneration of the basal epithelial cells,[564] to an alteration in the superficial stroma,[503] and to a metabolic disorder of the corneal fibroblasts.[472]

Chronic Actinic Keratopathy (Chronic Climatic Keratopathy, Climatic Droplet Keratopathy, Noncalcific Band Keratopathy)*

In an entity notorious for its profuseness of names, white, gray, or yellow particles accumulate in the interpalpebral portion of the corneal of

* The multitude of other terms which have been used to identify this condition include Bietti's nodular dystrophy, degeneratio hyaloidea granuliformis corneae, degeneratio primaria oleoguttata centrale et superficiale, degeneratio sphaerularis elaiodes, elastotic band keratopathy, elastotic keratopathy elastoid degeneration, hyaline degeneration of the cornea, hyaline nodular dystrophy, keratinoid corneal degeneration or keratopathy, Labrador keratopathy, Nama keratopathy, nodular band-shaped degeneration in tropical countries with arid soil, nodular hyaline band-shaped degeneration, proteinaceous corneal degeneration, spheroid degeneration, blindness of Dahalach, tropical corneal dystrophy, type II white limbus gridle of Vogt.[3,58,98,145,185,186,218-221,235,239,270,314,367,390,477,503,506,556]

both eyes.[1,3,58,85,98,146,185,186,218-221,223,235,240,270,314,352,477,503,504,506,611] The opacities first become clinically evident in the peripheral cornea in the horizontal meridian, where they appear often like droplets of oil on both sides of the cornea. The deposits increase in number and size with advancing age and may eventually extend toward the center of the cornea, forming a nontransparent horizontal band. Pingueculae and other conjunctival lesions are commonly associated [1,85,219,314,352,503,611] but have not always been clinically evident.[220,503,506] The condition frequently occurs in the absence of other ocular disease and usually without clinical features of inflammation.

Clinical studies on the disease in various parts of the world have revealed that the condition occurs predominantly in men who have spent their working lives outdoors.[221,611] In areas where the disease is common, affected individuals have often been fishermen, divers, or trappers. In Labrador the only women who are affected are those who spend a great deal of time outdoors in all weather.[314] Even in Britain, where the disease has a low prevalence, a majority of the patients with the keratopathy have spent much of their lives outdoors.[239]

The most striking histopathologic finding in the diseased cornea is the presence of abundant extracellular concretions. The tintorial and cytochemical characteristics of the concretions have provided insight into their identity.[85,98,238,240,352] Cytochemical data lead to the conclusion that the concretions are predominantly proteinaceous. Phenyl, indole, guanidyl, and sulfhydryl reactive groups seem to be present. This indicates that the protein is probably composed in part of sulfur-containing amino acids, tyrosine, tryptophan, and arginine. Proteins with the latter components are not normally detectable in the cornea by histochemical procedures. Despite the resemblance of the large yellow globules in this disease to oil droplets, lipid is not a significant component.[85,352] Calcification is usually not present. Some concretions stain positively with methods that demonstrate elastic tissue (such as Verhoeff-Von Gieson stain),[85,98,352,506] but this is not a consistent attribute.[239,352] In all eyes that I have studied microscopically in which globes were sectioned in the horizontal plane, the concretions have been associated with pingueculae (conjunctival elastosis) that have contained identical concretions.[352] Identical concretions are commonly identified in other pingueculae,[352] in skin with actinic elastosis (solar elastosis),[352] and at the limbus of eyes exhibiting presumed sun-induced lesions, such as actinic keratosis, carcinoma in situ, or invasive squamous cell carcinoma and variable degrees of elastotic degeneration.[85] In one study the deposits were insensitive to elastase,[85] but this observation has no bearing on the identity of the material since the cornea had

been embedded in paraffin, making it unsuitable for enzymatic digestion. When viewed in the electron microscope, the proteinaceous deposits appear as extracellular, round to oval electron densities of varying sizes.[1,85,240,314,352] Ultrastructural evidence to suggest a synthesis of this protein by corneal cells is lacking. As with other human corneal diseases, it is not possible to obtain sufficient tissue for chemical analyses to precisely identify the concretions in this disease.

The fundamental process that takes place in this keratopathy has been glibly referred to by the cliché "degenerative."[1,85,238,506] This time-worn designation for certain pathologic processes still demands explanation in terms of the fundamental pathobiology. Some authors have maintained that the material is of collagenous origin,[145] but this view is incompatible with its molecular structure. Denaturation of collagen should not provide histochemically detectable reactive amino acid groups such as trytophan, sulphur-containing amino acids, or tyrosine. Since collagen either lacks these amino acids or contains them in minimal amounts, they clearly must have a different derivation. Are the concretions products of injured corneal and/or conjunctival cells or do they enter the damaged tissue from the peripheral circulation or elsewhere? Garner and his colleagues,[238,240] impressed by histochemical similarities to keratin, consider the epithelium as a likely source of the material, a view with which others disagree.[198,352] The histochemical evidence of a protein with a high content of amino acids not normally detectable in the cornea, together with the lack of morphologic evidence of concretion synthesis by either corneal epithelial or stromal cells, suggests that the concretions are not synthesized in the cornea or formed there by the degradation of formed components. It seems more likely that the abnormal corneal deposits are derived from tissue other than the cornea. A likely site of origin can be inferred from observations on eyes with the mildest form of the keratopathy. In such cases, which presumably reflect an early stage in the genesis of the condition, the deposits are restricted to the superficial peripheral cornea in the interpalpebral fissure. This observation and the coexistence of these corneal lesions with conjunctival elastosis containing identical concretions strongly suggest that the abnormal material arises in the pericorneal conjunctival tissue. In further support of this possibility is the fact that, occasionally, identical concretions occur in pingueculae but not in the cornea.[352] This suggests that the abnormal protein progressively diffuses into the superficial cornea and deposits in it over a prolonged period of time, with the larger globules forming by the coalescence of the smaller ones. This concept is consistent with clinical observations on individuals with variable degrees of the keratopathy. Frequently, the earli-

est clinically detectable deposits involve only the medial and lateral portions of the cornea in the interpalpebral strip, with subsequent progression occurring by centripetal extension across the cornea. The observation that some corneal concretions stain positively with dyes that demonstrate elastic tissue and the coexistence of the corneal concretions with conjunctival elastosis pose the question of whether the concretions are derived from constituents of the elastotic material. The nature of the latter substance remains unsettled, but it is thought to contain derivatives of both collagen and elastic tissue. Because both elastin and collagen lack sulfur-containing amino acids, thought to be present in the concretions on histochemical evidence, other substances should be involved. In this regard it is of interest that procollagen molecules do contain cystine, as does the glycoprotein associated with elastin in elastic fibers. Tryptophan and cysteine are present in most animal proteins.

The keratopathy is geographically widespread and probably occurs throughout the world. Its incidence and severity vary considerably in different regions. The condition has been identified in southwestern and eastern United States (Arkansas,[219] California,[1,221] and North Carolina [352]), Australia,[221] Southwest Africa (Namibia),[223] South Africa,[183,221,356] central Africa,[222] north Africa (Tunisia [222] and Libya [235]), the Middle East (Saudi Arabia,[58] Jordan,[240] Dahlak and other islands in the Red Sea,[222,503,504]), east Africa (including Somalia [185,186] and Eritrea [58]), countries bordering the Persian Gulf,[221,222,240] India (Gujarat, Tamil Nadu, and Punjab states),[221,240,356] Sicily,[222] Italy,[3,390,477] England,[239] Labrador,[220-222,314,611] Newfoundland,[220,222,314,611] Finland,[203] and the Soviet Union.[203] The superficial location of the cornea and conjunctiva predisposes these tissues to adverse effects of the climate; in some areas where the disease is severe, eyes are exposed to climate extremes, evaporation, and the traumatic effects of wind-blown sand or ice. In the Dahlak islands, there is no natural or artificial shade. There is general agreement that the keratopathy is caused by exposure to some climatic factor that is related to the proportion of time spent outdoors.[219,221,314,352] Several possible etiologic factors warrant consideration. These include evaporation in areas of low humidity, microtrauma from minute particulate matter such as wind-blown snow or ice particles (in snow-bound countries like Labrador and northern Newfoundland), or dust and sand (as in the deserts of north Africa and the Middle East), as well as solar irradiation. The climate varies in areas where the disease occurs, and environmental factors that may injure the cornea in some localities do not exist in all geographic areas in which the keratopathy is found. It follows that they are not essential to its development. For example, the condition can occur in an atmosphere devoid of excessive

particulate matter. Tear evaporation is likely in some arid areas, as well as in Labrador in the winter when air contains negligible water vapor.[221] Yet, the disease occurs in temperate areas where evaporation from the cornea does not seem to be important.[221] Not only is a low humidity absent in some regions, but also similar stromal deposits do not occur in the dry eye syndrome or in exposure keratopathy in some regions where excessive evaporation is common. However, in certain localities evaporation could predispose to the precipitation of the proteinaceous deposits in the superficial cornea as it does in calcific band keratopathy.[167] For various reasons ultraviolet light from solar irradiation is the primary suspect as the cardinal causal element.[219,220,352,504] Although the geographic areas that are particularly prone to the disease have climatic differences, abundant sunshine is prevalent in them and their inhabitants have in common the potentiality for excessive exposure to radiant energy from the sun. Ultraviolet light possessing a wavelength of less than 295 mμ is almost entirely absorbed by the cornea. The prevalence of the disease has been determined in relatively few countries.[220,223,239,270,503,610,611] Unfortunately, direct measurements of ocular exposure to solar energy in various parts of the world is lacking partly due to the extreme technical difficulties.[221,222] As a consequence, correlations with the severity of the keratopathy are not known. However, both the prevalence and the gravity of the keratopathy appear to have a direct relationship to the levels of exposure to sunlight. The incidence and severity of the condition are less in the northern parts of the arctic region than in the southern portions.[203] In the extreme northern latitudes, where other adverse climatic factors still exist, the sun does not rise high above the horizon and the effects of ultraviolet light are not as pronounced as in more southern areas. In a selected population with ocular disease in Britain, where the population is to some extent protected from ultraviolet irradiation by the almost constant blanket of clouds, Garner *et al.*[239] found the disease to be less common than in countries exposed to higher levels of sunlight. An acute keratoconjunctivitis follows excessive exposure to ultraviolet light. Because a history of this entity is usually lacking in affected individuals, Freedman [220,221] has pointed out that if ultraviolet light does produce the condition, it does so at prolonged energy levels too low to cause acute keratitis. Ultraviolet light is reflected by snow, desert, and water, and in some regions where the condition is severe these are prominent features of the external environment. A causal relationship to the absorption of radiant energy from the sun would also account for the predisposition for the exposed portion of the eye in the interpalpebral fissure and their usual bilaterality. The male preponderance [219,221,239,314,610,611] can be accounted

for, at least in part, by outdoor occupations that expose individuals to excessive ultraviolet light. Moreover, since the condition is not related to aging alone, its increased incidence with advancing years may reflect an increase in time of solar exposure.

In addition to the aforementioned, there are morphologic observations that strongly suggest that the condition is a sequel to the cumulative effect of chronic solar irradiation. As mentioned earlier, several groups of investigators have independently pointed out an association with conjunctival elastosis (pingueculae) in the same eye. The corneal concretions are similar to those that are commonly found in pingueculae and in the morphologically identical cutaneous disease actinic elastosis (solar elastosis).[352] In the absence of x-irradiation there is abundant evidence that this disturbance of connective tissue is due to prolonged exposure to sunlight.[352] It is restricted to areas of the body that are traditionally exposed to solar irradiation, the severity of the change is remarkably pronounced in parts that receive the most intense and prolonged exposure to sunlight, and it is less evident in heavily pigmented skin. These changes have been produced experimentally with ultraviolet light. In Caucasians, other probable sun-induced lesions such as cutaneous basal cell carcinomas of the face have been associated with the keratopathy.[352]

Corneal concretions indistinguishable from those in this climatically induced disease occasionally occur unilaterally as a secondary change in eyes with absolute glaucoma, phthisis bulbi, a variety of corneal diseases, including posttraumatic scars and lattice corneal dystrophy.[85,238,352,506] In areas with little actinic radiation, such concomitant corneal lesions are almost the rule.[239] The existence of such cases clearly has bearing on the pathogenesis of the climatic disorder. Seeing that actinic elastosis seems to be a unique reaction to irradiation, one might wonder whether the apparently identical corneal concretions in all situations have a common denominator. In my experience such secondary cases have occurred in exposed eyes or in situations in which visual acuity is markedly impaired for some other reason. This suggests that they also could be due to tissue denatured by solar irradiation and desiccation. The blind eye is not protected from solar irradiation like a normal one which responds to strong light by excessive blinking and blepharospasm. A subject with a normal eye is likely to close it considerably in the presence of excessive glare. If a blind eye lacks these protective mechanisms, it would be more prone to the effects of irradiation. Also relevant is the observation of similar deposits in the peripheral cornea of patients who died of alkaptonuria (ochronosis).[85] In this disease, black-brown pigment derived from polymerized homogentisic acid accumulates in various tissues, including

the interpalpebral portion of the sclera. Since pigment aids in the absorption of ultraviolet light, this observation may be more than coincidental. It is of interest that the secondary type of reaction does occur in areas such as Britain which are frequently shielded from the sun by clouds for much of the year. Some argue that this excludes solar irradiation as the cardinal etiologic agent, but even in such countries, sunshine does exist although it is scarcer than elsewhere. On the other hand, it is axiomatic that all tissues, including the cornea, possess limited responses to noxious stimuli; multiple pathogenetic mechanisms could exist. Although the reaction occurs in eyes with different underlying disorders, claims that the response occurs to a wide variety of insults [85] cannot be substantiated. The associated lesions may be nonspecific, while the characteristic changes are secondary to a common factor. Until the condition can be produced experimentally, the question of whether it is attributable to actinic damage will remain debatable and so will the relationship between the climatic keratopathy and the secondary proteinaceous "droplet" keratopathy.

Since the climatic-induced corneal disease has no universally acceptable name, a few comments in this regard seem in order. The diverse nomenclature has reflected geographic location, clinical appearance, histochemical findings, and casual factors. Many designations are clearly inappropriate and can no longer be retained for reasons that have been considered elsewhere.[86,221,229] The lack of agreement about what to designate this disease has stemmed largely from the varied backgrounds and viewpoints of the different observers, our ignorance of the precise nature of the corneal deposits, and the lack of an experimental model of the disease. For several reasons, I considered "chronic actinic keratopathy" an appropriate name for this condition when I introduced the term several years ago. *Chronic* to connote the chronicity of the disease; *actinic* in view of the widely accepted use of this adjective in analogous cutaneous lesions, the association with actinic elastosis, and the almost certain causation by solar irradiation; and *keratopathy* since it is a pathologic state of the cornea.[352] The designation "climatic droplet keratopathy," introduced by Freedman,[221,222] has some advocates [1,240] but is unacceptable to others. To many pathologists, myself included, the denomination of "climatic droplet" lacks appeal. Climatic embraces too broad a spectrum of the external milieu, yet the common climatic factor in areas where the condition prevails is excessive sun exposure. To include "droplet" in the name of the disease because of the clinical resemblance of the corneal deposits to droplets of oil seems like a reversion to the nomenclature of antiquity. Although the word "droplet" has clinical appeal, it ignores established

facts about the disease, particularly with regard to the histochemical attributes of the deposits, and indicates nothing from the standpoint of the disease's pathobiology. In addition, the adjective "droplet" is confusing because the term "drop-like" has been used by Japanese workers in the designation of a form of corneal amyliodosis ("gelatinous drop-like dystrophy of the cornea").[400,432] Testimony to this confusion already exists in the literature.[456] Other objections to this nomenclature have also been raised.[85,219] It is hoped that a more widely acceptable term will emerge in the not too distant future. Inasmuch as no other protracted corneal disease is attributed to the physical environment, chronic climatic keratopathy might have a wider drawing power that the myriad of unacceptable terms.

Normal and Abnormal Aspects of Specific Parts of the Cornea

A fascinating aspect of different corneal diseases is their affinity for variable parts in this tissue. The locality of the lesions is undoubtedly related to their causation and pathogenesis. Some disorders affect the peripheral cornea but spare the central part, while others involve mainly the central cornea. Even the different layers of the cornea are variably affected. Corneal lesions may be localized or randomly distributed throughout the cornea.

In view of the aforementioned and for the sake of simplicity, each layer of the cornea will be considered separately.

Corneal Epithelium

Externally the cornea is covered by a nonkeratinizing stratified squamous epithelium that is arranged in approximately five layers, with the outermost cells being the thinnest. Although contiguous with the conjunctival epithelium, several differences between these epithelia exist. The epithelial cells of the cornea are more densely packed and contain more desmosomes and hemidesmosomes than the conjunctival epithelium. There is also normally very little extracellular space between individual epithelial cells or the cornea. Mucin-secreting goblet cells that characterize the conjunctival epithelium are not present in the normal cornea. The surface of the epithelium is ideally suited for scanning electron microscopic examination. Numerous plicae are evident with this technique. Their pattern varies with different species.[448] The basal cells possess densities (hemidesmosomes) at irregularly spaced intervals along the posterior cell membrane, which rests on a delicate basal lamina. From the standpoint of carbohydrate metabolism, the corneal epithelium has a high level of hexose monophosphate shunt activity and a relatively sluggish tricarboxylic acid cycle.[342] The corneal epithelium, in contrast to the

conjunctival epithelium, has large glycogen stores.[566] These glycogen granules are conspicuous in electron micrographs of the basal epithelial cells. Analyses of corneal epithelium from several species have disclosed proteins of tissue origin,[211,266,288,328] and through the use of immunofluorescence microscopy, the existence of several serum proteins (IgA, IgD, IgE, IgG, and albumin) has been found to be associated with corneal epithelial cells.[7] One of the proteins that the corneal epithelium is capable of synthesizing, in at least the chick, is the ubiquitous collagen.[252,415,573,574,575] In other species, such as the rabbit, the evidence is less convincing.[254] There is evidence that the corneal epithelium interacts metabolically with the stroma. Yet in experiments in which the epithelium was removed and replaced by a glued-on contact lens, the stroma has been found to survive without the proximity of the epithelium.[160]

Normally the surface of the cornea is lubricated by a tear film composed of three layers: mucoid, watery, and oily. These components are derived from conjunctival goblet cells, major and accessory lacrimal glands, and the meibomian gland secretions. In a wide variety of localized ocular conditions and systemic disorders, the eye becomes dry. Inadequate moistening of the corneal surface of the eye causes a distinct clinical dry eye syndrome (keratoconjunctivitis sicca), in which the epithelium becomes keratinized. The causes of the dry eye syndrome include Stevens-Johnson syndrome, Sjögren syndrome, toxic epidermal necrolysis, benign mucous membrane pemphigoid, avitaminosis A, trachoma, chemical burns, rheumatoid arthritis, systemic lupus erythematosus, scleroderma, polyarteritis nodosa, familial dysautonomia (Riley-Day syndrome), irradiation of the eyelids, and poor approximation of the eyelids to the globe (entropion or ectropion).

On rare occasions the corneal epithelium becomes abnormally thickened and manifests acanthosis and individual cell keratinization. This is a prominent feature of an unusual inherited disorder known as hereditary benign intraepithelial dyskeratosis.[486] The epithelium of the cornea commonly becomes damaged. This may occur from relatively minor trauma such as inturned eyelashes (trichiasis) or from the use of contact lenses. Fragments of the corneal epithelium often exfoliate under a wide variety of circumstances including abrasions, chemical damage, and underlying corneal disease. Long-standing epithelial defects predispose to corneal infection. A pronounced extracellular epithelial edema develops in individuals with corneal endothelial dysfunction secondary to numerous causes (described below). When this occurs, the spaces between the adjacent cells widen and may contain flocculent finely granular material and products of cell degeneration.[475,583] When this spongiosis is excessive,

the epithelial cells can separate at their intercellular junctions. In a variety of conditions such acantholysis culminates in cysts within the corneal epithelium. These cysts, and others that appear following intracellular edema, have been thoroughly studied by Tripathi and Bron.[583] Intraepithelial cysts often contain cellular debris and moderately electron-dense material.[368]

A wide variety of materials accumulate in corneal epithelial cells in pathologic states. Intracytoplasmic glycogen granules are particularly abundant in some conditions, particularly in those with rapidly regenerating epithelium;[89,368] the cytoplasm contains abnormal inclusions in the keratopathy due to amyodarone;[34,571] chlorpromazine causes electron-dense melanin-like granules within the cytoplasm.[469] The stored material that corresponds to the cytochemically identifiable storage products is frequently not discernible because of its loss during the fixation and processing of the tissue. Membrane-bound cytoplasmic vacuoles are sometimes prominent in several disorders.[89,179,434,508,583] Nakanishi and Brown [434] observed vacuoles and electron-dense bodies in the cytoplasm of the corneal epithelial cells in Meesmann dystrophy. In mucopolysaccharidosis Type I-H (Hurler syndrome), membrane-bound vacuoles within the epithelium appear relatively electron-lucent but contain variable amounts of granulofibrillary or flocculent material and occasional myelin figures.[508,581] In mucopolysaccharidosis Type VI (Maroteaux-Lamy syndrome), the basal epithelial cells contain clusters of supranuclear vacuoles with fibrillogranular material.[479,509] Numerous intracellular vesicles containing granular material have also been observed in the corneal epithelium and in subepithelial "histiocytes" in G_{M1}-gangliosidosis Type I (generalized gangliosidosis).[179] In Fabry disease (glycolipid lipidosis), the corneal epithelium (especially in the basal layers between the nuclei and basement membrane) contains membranous lamellae, sometimes with a concentric arrangement.[202,595] Kenyon and Sensenbrenner [339] documented the accumulation of delicate granular noncrystalline material, resembling the form that stored cystine assumes within leukocytes of cystinotic patients, within large cytoplasmic vacuoles of corneal epithelial cells in childhood cystinosis.[339] Polygonal intranuclear crystals with a regular lattice configuration of 20-nm periodicity can occur in the corneal epithelium in gout on rare occasions.[531] In patients with hyperparathyroidism, calcium has been identified within the cytoplasm and nuclei of the corneal epithelium, where they appear by electron microscopy as multiple needle-like crystals.[48,311]

Iron in sufficient quantities to be clinically detectable accumulates in the corneal epithelium in several situations. Especially in persons more

than 50 years of age, iron within the corneal epithelium produces a line that is usually horizontal (Hudson-Stähli line). The iron can be readily demonstrated by histochemical methods [243] and has been identified within the corneal epithelium by electron microscopy as ferritin-like particles.[550] Aside from the Hudson-Stähli line, iron also deposits in the corneal epithelium in a ring around the conical portion of the cornea in approximately 50% of individuals with keratoconus (Fleischer's ring). In such Fleischer's rings, ferritin particles have been recognized in electron micrographs in widened intercellular spaces and/or in the cytoplasm of epithelial cells, especially basal cells.[301,322] An iron-containing pigment line also occurs in the corneal epithelium ahead of a pterygium (Stocker's line) or in front of a filtering bleb created in the treatment of glaucoma (Ferry's line).[191]

Electron microscopy permits the identification of infectious agents that cannot be seen by light microscopy. In this regard viral particles have been observed within human corneal epithelial cells in herpetic keratitis.[33,128,201] After treating these patients with iododesoxyuridine (IDU), Babel and Leuenberger [33] also noted peculiar pseudocrystallin structures in the cytoplasm of epithelial cells.

In conditions that are characterized by defective epithelial adherence, there is an apparent loss of hemidesmosomes in the basal epithelial cells.[582] In several situations two populations of epithelial cells ("pale cells" and "dark cells") have been identified. Their significance remains controversial; some electron-lucent cells may reflect intracellular edema.[583] Tight-fitting contact lenses deprive the corneal epithelium of oxygen and lead to pale cells with glycogen deprivation. Such cells are interpreted as having intracellular edema by some investigators.[160] Many dark cells are artifacts of fixation. Neoplasms of the corneal epithelium are rare and usually associated with involvement of adjacent conjunctiva. In epithelial neoplasms the cells not only may be abnormal in shape and size but also may possess ultrastructural deviations from the norm.[309,586]

Under pathologic circumstances foreign cells invade the corneal epithelium. Especially in the dark races and in pigmented animals, melanocytes infiltrate between the individual epithelial cells in some disorders of the cornea.[277,309,518] This is in sharp contrast to the normal situation in which the corneal epithelium is nonpigmented even in the dark races. Melanin pigmentation of the corneal epithelium (epithelial melanosis) can be readily reproduced experimentally in pigmented guinea pigs.[277,409] It also occurs spontaneously in pigmented animals with keratitis.[45] The corneal melanosis results largely from the migration of melanocytes into the corneal epithelium from the normally pigmented continuous conjunctiva

and, to a lesser extent, from the presence of melanin granules within corneal epithelial cells. Pigmentation of the corneal epithelium is preceded and accompanied by corneal vascularization.[277,409] Leukocytes antecede the vascular and melanocytic invasion of the cornea. After the migration of melanocytes into the corneal epithelium, melanin granules become transferred to the cytoplasm of epithelial cells. In an experimental study employing electron microscopy, McCracken and Klintworth [409] obtained evidence suggesting that melanin granule transfer from melanocytes to epithelial cell may follow the fusion of the membranes of the melanocytes and epithelial cells. The presence of melanocytes in the cornea may account for those rare malignant melanomas that appear to arise from this tissue.[148,370,549]

Another cell type that can become conspicuous in the corneal epithelium under abnormal circumstances is the Langerhans' cell.[80,309] This cell with its dendritic-like processes contains characteristic rod- and racquet-shaped cytoplasmic organelles (Birbeck granule or Langerhans' cell granule). It has been observed among neoplastic cells in a pigmented squamous cell carcinoma of the cornea.[309]

There is evidence that damaged epithelial cells can release enzymes, including collagenase, which are harmful to the corneal stroma.[82,83,160,530]

Basement Membrane

The corneal epithelium, like most other epithelial cells, rests on a delicate basement membrane. The latter is dramatically demonstrated in light microscopic preparations with the periodic acid–Schiff stain. It is readily identified in electron micrographs by its finely filamentous or granular appearance. The epithelial basement membrane may be irregular, thinned, or absent over long linear expanses, particularly in conditions characterized by poor adhesion of the epithelium to the underlying Bowman's zone.[388,340] In a number of unrelated pathologic states it may be thicker than normal.[192,300,302] Black granules of silver deposit in the basement membrane of the corneal epithelium after the prolonged topical application of silver-containing eyedrops. In Fabry disease, portions of the basement membrane are duplicated or form thick multilaminar masses.[202,581,595] Abnormally thickened basement membrane may contain amorphous material with short fibrous structures,[89] rounded densities,[192] and even peculiar enveloped round to oval particles.[300] In many disorders of the corneal epithelium, aberrant basement membrane forms between individual epithelial cells.[78,505] These appear clinically like fingerprints or maps, hence the clinical connotation of "fingerprint dystrophy." Intraepithelial cyst formation often coexists with the ectopic basement mem-

brane. In long-standing corneal edema, the epithelium separates from Bowman's zone forming subepithelial bullae (bullous keratopathy).

Subepithelial Pannus

In several conditions, including chronic corneal edema, subepithelial connective tissue extends between Bowman's zone and the epithelial basement membrane. Fibroblasts, leukocytes, and other cells also appear within the pannus. The individual collagen fibers in this reactive tissue vary in thickness, unlike those in the normal corneal stroma.

Bowman's Zone

Beneath the epithelial basement membrane lies an acellular 10- to 16-μ-thick modified lamella of the stroma, consisting of randomly oriented, tightly packed collagen fibrils (16 to 26 nm in diameter). This superficial acellular layer of the corneal stroma is relatively homogeneous by light microscopy and is named eponymously after Bowman. It was for a long time designated a membrane, but after electron microscopy disclosed that it is not a true membrane, the term "Bowman's zone or layer" has gained popularity. It does not occur in all species. Abnormalities of this layer are evident in numerous corneal diseases. It is absent centrally in some developmental anomalies of the cornea such as sclerocornea and Peters anomaly.[337]

Calcific deposits in the superficial cornea have a predisposition for Bowman's zone in the horizontal meridian of the interpalpebral region. The individual precipitates produce a clinically detectable band across the cornea, hence the designation "band keratopathy." In this condition the calcified precipitates are extracellular, in contrast to the findings in most reports on hyperparathyroidism. They occur in Bowman's layer and often in the adjacent superficial stroma. In addition to intracellular calcification, extracellular aggregations of calcium have also been reported in a case of idiopathic hypercalcemia of infancy.[311] By transmission electron microscopy, these appear as spherules composed mainly of electron-dense concentric rings of fine particles.[48,145,474,481] Calcium has been identified in the deposits by histochemical methods, including energy-dispersive x-ray analysis.[145] The densities have been interpreted by x-ray diffraction and electron microprobe analysis as hydroxyapatite.[48] The deposits in experimental corneal calcification possess the same ultrastructural characteristics to those of human band keratopathy.[193,589] Clinically, a mosaic of gray opacities separated by clear areas may result from calcific deposits in Bowman's zone. This appearance, known as anterior crocodile shagreen of Vogt, can be associated with a variety of underlying conditions and may

even be inherited as a mendelian dominant disorder. In a clinicopathologic study, Tripathi and Bron [584] could account for the mosaic appearance by an undulated Bowman's layer (a phenomenon prone to occur when intraocular tension is diminished), which was irregularly encrusted with calcareous deposits.

In superficial corneal injuries and ulcerations Bowman's zone may become destroyed. Once this occurs, it does not regenerate but remains absent as testimony to previous damage. Multiple breaks in this layer are rare except in keratoconus in which such fractures of Bowman's layer usually become filled by stromal connective tissue and fibroblasts. In keratoconus they are characteristic and almost diagnostic. A complete or partial disappearance of Bowman's zone and the replacement of it by newly formed collagen and other material, such as numerous microfilaments (5 to 10 nm in diameter), are consistent findings in the inherited corneal disease Reis-Bückler dystrophy. Similar alterations also happen in other conditions, including long-standing corneal edema. Cells with large vacuolar inclusions have been observed within Bowman's zone in mucopolysaccharidosis Type IV (Maroteaux-Lamy syndrome).[479] Bowman's layer is involved in some disorders of the cornea that predominantly affect the stroma. For example, pockets of extracellular electron-lucent material occur in both regions in lipid keratopathy [194] and in Schnyder dystrophy.[32,175,241] The proteinaceous deposits of granular corneal dystrophy can also be located in Bowman's zone and the corneal stroma. In arcus senilis the periphery of Bowman's layer frequently contains sudanophilic lipid.

Stroma

When corneal tissue is processed for microscopic examination, the stromal lamellae commonly become separated artifactually with the creation of a canal system in the corneal stroma. Although these spaces were regarded as possible lymphatics by early histologists, it has now been well established that the healthy cornea does not contain lymphatic vessels.

The corneal stromal cells are variably known as corneal corpuscles, keratocytes (not to be confused with keratinocytes), corneal fibrocytes, and corneal fibroblasts. Some restrict the term "corneal fibroblast" to the cell when it is metabolically active, as in wound healing. Wound healing and this transformation are delayed when the epithelium is absent.[160]

Extracellular Macromolecules

The collagen, proteoglycans, and structural glycoproteins together account for most of the dry weight of the corneal stroma. These macromole-

cules are synthesized by fibroblasts.[101,121,130,131,494,496] The glycosaminoglycans, proteoglycans, and collagen have already been discussed. Little is known about the structural glycoproteins. They have been isolated from calf cornea.[492,495] A urea-extractable glycoprotein has a characteristic amino acid and carbohydrate composition.[491] Fluorescein-labeled antibodies to this glycoprotein have localized it to the corneal stroma.[499] It is highly antigenic and may play a role in corneal graft rejection. Robert *et al.*[499] have shown that if rabbits are presensitized with this urea-soluble glycoprotein fraction of calf cornea, they reject lamellar calf corneal grafts in an accelerated fashion. In the absence of sensitization the grafts are tolerated with good transparency for up to 2 months.

The corneal stroma contains several soluble proteins derived from serum. Kawerau and Ott,[328] employing paper electrophoresis, demonstrated at least four serum proteins (albumin, siderophilin, γ-globulin, and α_1-globulin) in the corneal stroma of the ox, the pig, and man. Using column chromatography and disk gel electrophoresis, Holt and Kinoshita [288] documented the presence of serum albumin, serum transferrin, a serum lipoprotein, and 7S γ-globulin in the stroma of fresh bovine cornea. A fifth serum protein, which could not be identified, was also noted by them. The four identifiable serum proteins were calculated to comprise 56% of the soluble portein of the stroma, leaving 44% of the soluble protein intrinsic to the serum and the unidentified serum protein. Further research has revealed the existence of immunoglobulins in the corneal stroma. In a study by Allansmith *et al.*,[6] IgG and albumin were detected by direct immunoflourescence microscopy in the rabbit cornea. IgG was found evenly distributed across the entire corneal stroma. Larger amounts of albumin were present in the peripheral portions of the cornea than in the central portions of the cornea. In an extension of this work IgA, IgD, IgE, and IgG were found to possess a similar distribution pattern in the corneal stroma.[7] Occasionally, trace amounts of IgM could also be found. In several diseases the corneal stroma contains variable amounts of abnormal extracellular material that intermingles with and separates the collagen lamellae. Such substances include water, amyloid (discussed above), proteins, lipids, and glycosaminoglycans. Many compounds, including lipids and certain glycosaminoglycans, dissolve during routine tissue processing and require special handling in order to be visualized. When viewed in the electron microscope, electron-lucent spaces occur at the sites of soluble materials. These may be of characteristic shape. Thus, the sites of cholesterol crystals appear as rhomboidal areas often with a discernible notch in one corner.[241] Entities such as macular corneal dystrophy,[246,259,362,430,543,579,581] mucopolysaccharidosis

Type I-H,[508,581] G_{M1}-gangliosidosis Type I,[179] lecithin-cholesterol acyl transferase (LCAT) deficiency disease,[581] and lipid keratopathy [194] are typified by relatively soluble accumulations; clear vacuoles are common.

In some corneal diseases (including lattice corneal dystrophy and choronic actinic keratopathy reviewed in an earlier section) proteins accumulate in the corneal stroma. Characteristic electron-dense rod-shaped or trapezoid crystalline structures of a proteinaceous nature occur extracellularly in granular corneal dystrophy.[84,304,554] In these conditions, the origin and precise structure of the abnormal proteins are unknown.

In an electron microscopic study of a cornea from a patient with lecithin-cholesterol acyl transferase (LCAT) deficiency disease, numerous minute deposits consisting of elongated and irregular clusters of vesicles and small spaces were scattered throughout the depth of the stroma.[581] Extracellular rectangular spaces and small pockets of relatively electronlucent material are evident in the stroma in hereditary crystalline corneal dystrophy (Schnyder dystrophy) [32,175,241] and lipid keratopathy.[194]

Electron microscopy of the normal corneal stroma has disclosed variable amounts of electron-dense, finely granular material among the collagen fibrils and, particularly, adjacent to the corneal fibroblasts (keratocytes). This substance is thought to be composed of proteoglycans and/or structural glycoproteins. In some pathologic states the quantity of this material is increased.[31,253,414,470,498]

Invasion By Foreign Cells

In pathologic states the corneal stroma becomes invaded by cells that do not normally exist within it. Polymorphonuclear leukocytes, lymphocytes, and monocytes infiltrate the cornea as part of the inflammatory reaction. Vascularization of the corneal stroma is discussed in an earlier part of this review. In vascularized corneas, anastomosing lymphatics can also encroach beyond the corneoscleral limbus and invade the cornea.[122-126,542] Such lymphatic vessels are extensions of the lymphatic system that normally exists in the conjunctiva. By electron microscopy these channels are lined by a flattened, thin endothelium with much phagocytic activity. Pericytes and a true basement membrane are absent. Microfilamentous deposits occur along their external wall. The lumina contain lymphocytes and macrophages.[189]

Stromal Edema

The chemical constituents of the cornea, the intraocular pressure, the state of the pericorneal vascular permeability, as well as the corneal epithelium and endothelium all play a role in maintaining the fluid

balance in the cornea.[16,106,108,109,152,153,161,170,274,276,343,405,406,465,616] The excised cornea of many species, including man, is markedly hydrophilic and swells *in vitro*. This corneal swelling largely results from the marked osmotic force of the stromal glycosaminoglycans of which keratan sulfate is thought to be the most important. This belief stems from the following observations: a) the cornea of the dogfish does not swell in distilled water or salt solutions and b) the glycosaminoglycan composition of the dogfish cornea differs strikingly from that of other species. It contains relatively little keratan sulfate (25%) and much dermatan sulfate (60%).[140]

Since the cornea is bathed by aqueous humor on its posterior surface and by tears in front, it needs to remain in a critical state of relative dehydration in the intact organism. This ability of the normal cornea in the living subject to maintain a deturgescent state despite a marked hydrophilia of the corneal stroma is essential for normal vision. The edematous cornea thickens and loses its transparency. The epithelium and corneal endothelium play critical roles in maintaining normal stromal hydration by virtue of their respective barrier and pump functions.[160,173,613] In the past the corneal endothelium was thought to do this by an active transport of water, sodium ions, and possibly other electrolytes from the stroma to the aqueous humor. The only ions that have been demonstrated to be pumped into the aqueous humor are bicarbonate ions.[284] Maurice and Giardini [406] found in experimental studies on the rabbit that removal of the epithelium caused the corneas to swell to an average of 200% of their initial thickness in 24 hours, whereas they inbibed fluid to 500% after removal of the endothelium.

The association of corneal edema with corneal endothelial lesions has been observed in numerous spontaneous, iatrogenic, and experimentally produced conditions in man and other animals.[105,106,224,231,289,298,306,318,347,378,410,437,444,480] These include increased intraocular pressure; donor tissue for corneal grafts, which either lacks or has an abnormal corneal endothelium; Fuchs endothelioepithelial corneal dystrophy; and other conditions discussed later. A causal relationship between an abnormal corneal endothelium and the accompanying corneal edema is hence suspected, and there is much evidence to support the view. At one time it was suggested that the edematous fluid might be derived from aqueous humor percolating through Descemet's membrane and a damaged corneal endothelium.[169,230,378] In addition, it is thought that corneal endothelial lesions interfere with a physiologic dehydrating mechanism of the cornea.[274,275] However, in conditions with corneal endothelial cell damage the resultant stromal edema may not be entirely due to corneal endothelial cell dysfunction. The discovery by Dohlman *et al.*[164] that some types of corneal

edema can be treated satisfactorily by suturing a transparent silicone membrane directly to the posterior surface of the cornea and that the overlying stroma remains clear indicates that this is not the only mechanism of edema.

That the limbal vascular plexus contributes significantly to the normal corneal nutritional fluid is widely accepted,[135,569] yet little attention has been devoted to an increased limbal vascular permeability in the genesis of corneal edema. Langham [372] demonstrated an increased permeability of the capillary limbal vessels to the water-soluble vital dye pontamine sky blue in alloxan-induced corneal edema (a type of edema also associated with corneal endothelial lesions). The injection of the surfactant polyoxyethelene sorbitan mono-oleate (Tween 80, Atlas Chemical Industries, Inc. Wilmington, Del) into the anterior chamber of rabbit eyes produces pronounced corneal edema, which is accompanied by marked corneal endothelial cytolysis.[480] In an extensive investigation of this model Quiroga and Klintworth [480] found an increased limbal vascular permeability to intravascular markers, i.e., Evans blue, human γ-globulin, and thorium dioxide, which diffused into the cornea from the limbal vessels.

The corneal fibroblasts (keratocytes) are involved in many corneal diseases as well as in corneal wound healing. Macular corneal dystrophy,[246,259,351,362,579,581] some of the systemic mucopolysaccharidoses (Type I [Hurler syndrome],[397,508,561] Type IV [Morquio syndrome],[247] and Type VI [Maroteaux-Lamy syndrome] [479]), cystinosis,[339] chlorpromazine keratopathy,[469] hyperparathyroidism,[48,311] Niemann-Pick disease (Type A),[490] blood staining of the cornea,[258,466] lipid keratopathy,[194] G_{M1}-gangliosidosis Type I,[179] G_{M2}-gangliosidosis Type II (Sandhoff disease),[580] Fleck corneal dystrophy,[478] and other disorders of the cornea are typified by the intracytoplasmic accumulation of substances within the cytoplasm of the stromal cells. In many instances the storage is the result of an inherited deficiency of an enzyme necessary for the intracellular catabolism of specific substrates. This mechanism is not the only one operable in the cornea. In some instances the cytoplasmic accumulations represent incompletely degraded particulate matter that was phagocytosed or produced by an aberrant synthesis.

Numerous membrane-bound vacuoles, sometimes enclosing finely fibrillary or granular material, have been identified in stromal fibroblasts in G_{M1}-gangliosidosis (Type I),[179] G_{M2}-gangliosidosis (Type II),[580] and Fleck corneal dystrophy.[478] Similar cytoplasmic accumulations are evident by electron microscopy in macular corneal dystrophy and several systemic mucopolysaccharidoses (mucopolysaccharidosis Type I-H [Hurler syndrome], Type IV [Morquio syndrome], and Type VI [Maroteaux-Lamy

syndrome]), which are discussed in an earlier part of this review. All corneal fibroblasts are not always affected in an inherited storage disease, e.g., in Fleck corneal dystrophy many appear normal.[478] Round to oval laminated membranous cytoplasmic bodies have been observed in the stromal cells in Niemann-Pick disease (Type A),[490] although these cells contain needle-like calcific crystals in hyperparathyroidism [48,311] and fusiform electron-lucent profiles in childhood cystinosis.[339] In a postmortem electron microscopic study of pre-Descemet membrane dystrophy, Curran *et al.*[144] found that keratocytes in the deep peripheral cornea had many vacuoles with granular or homogeneous osmiophilic material.

In contrast to fibrocytes in other sites of the body which have not been noted for their phagocytic attributes, the corneal fibrocytes (keratocytes) manifest a pronounced capability to ingest a wide variety of foreign particulate matter.[350] This has been demonstrated by the inoculation of different types of particulate matter into the substantia propria of the cornea of the living rabbit, as well as *in vitro* tissue explants, and in confluent corneal fibroblast cultures.

The corneal cells possess the propensity to synthesize and store lipid.[8,100,111-114,116,280,358] The cornea can synthesize sterols and fatty acids from acetate and glucose.[8] Cogan and Kuwabara [111-114,116] investigated lipid storage in the cornea by incubating explants of corneal tissue from several species in media containing homologous and heterologous sera. Under the conditions of their experimental model they found that both serum and additional fatty acids were required for intracellular lipid storage to occur. These studies were later extended by Klintworth and Hijmans [358] who observed that if corneal cells were grown in medium employing homologous or heterologous serum, the cells accumulated intracytoplasmic triglycerides in direct proportion to the amount of serum used. Fatty acids need not be added to the serum for lipid storage to occur in corneal cell cultures.[358] Cogan and Kuwabara [113] questioned whether fatty acids alone were sufficient to account for increased lipid accumulation or whether some additional cofactors from the serum were also required. They suggested that oleic acid, protein, calcium, and magnesium might be involved in the sequence of events leading to the biosynthesis of the lipid.

That the cellular elements of the cornea can manifest abnormalities when exposed to normal constituents of serum raises the question whether an analogous situation will arise in the intact organism if the relevant constituents of serum reach the cornea in sufficient quantities. This may have bearing on the rare lipid corneal disorders which do not appear to be associated with hyperlipemia, hypercholesterolemia, or an underlying

corneal disease. Lipid deposition in the cornea may follow an underlying corneal lesion or occur in the absence of a known predisposing local condition. Sometimes it is associated with a disorder in the serum lipids. However, the association between lipid-containing corneal lesions and an elevated serum lipid is not constant. Either may occur in the absence of the other.

Aside from the aforementioned, other abnormalities have been observed in electron microscopic studies of human corneal stroma. A marked morphologic variation among individual corneal fibrocytes has been noted in keratoconus by Pouliquen *et al.*[472] Complete or incomplete viral particles have been identified within infected stromal cells and free in the stroma in herpes keratitis.[128]

Descemet's Membrane

Normal

The corneal endothelium is firmly attached to its basement membrane (Descemet's membrane), which thickens with age. Electron microscopy has disclosed two structurally different regions within it. The anterior, and oldest, portion of Descemet's membrane has fine collagen fibrils arranged in a vertically banded pattern (with 100- to 110-nm spacing). Rounded electron densities exist at the sites where the fibrils cross each other. When sectioned horizontally, hexagonal arrangement is displayed. The posterior portion of Descemet's membrane is finely granular and lacks this remarkable collagenous scaffolding. A major component of Descemet's membrane is collagen [162,336,428] and analyses of it are discussed in an earlier part of this review.

Abnormal

A variety of anomalies of Descemet's membrane are recognized.[79,299,313,323,337,433,447,471,473,553,594] In corneal ulcers it is highly resistant and may remain in the form of a bulging balloon-like structure, called a descemetocele, after all the other layers of the cornea are destroyed. Being a product of the corneal endothelium, it is constantly formed even over defects that occur when Descemet's membrane ruptures.

This portion of the cornea is thickened in several conditions. In specimens with congenital hereditary endothelial dystrophy, Descemet's membrane is uniformly broadened. Its anteroposterior diameter ranges from less than 3 μ to more than 40 μ. In this disease the anterior banded zone of Descemet's membrane is of normal thickness, but the nonbanded portion is replaced by a mixture of long-spacing and regular collagen.[447] In posterior polymorphous dystrophy (degeneration) of the cornea, the anterior banded portion of Descemet's membrane is normal. It rests, however, on an abnormally thick collagenous layer composed of a mixture of fibrils,

fibers, and finely granular electron-dense basal lamina substance.[68,273]

In certain disease states, notably in Fuchs endothelial dystrophy, Descemet's membrane is unevenly broadened with mushroom-shaped excrescences on the central portion of its posterior surface. The typical ultrastructure of these warts (cornea guttata) has been well described.[287,299,308,414,454] They contain regularly arranged long-spacing and regular collagen. Similar excrescences commonly occur on the peripheral portion of Descemet's membrane with aging (Hassall-Henle bodies).[190,299,308] In both Hassall-Henle bodies and cornea guttata, the thickenings of Descemet's membrane possess a banded pattern with profiles similar to that of the anterior portion of Descemet's membrane, except that the banding is in apparent disarray.

Fissures within the excrescences often contain cellular debris.[287,299] Similarities between Hassall-Henle bodies and cornea guttata exist, but ultrastructural differences between them have been pointed out.[299] Iwamoto and DeVoe [299] found fissures to be less numerous in cornea guttata than in Hassall-Henle bodies. They also observed the banded pattern of the matrix to be less obvious and only near the fissures in Hassall-Henle bodies. The anterior segment of Descemet's membrane is normal in Fuchs dystrophy. Collagen fibrils of 55-nm and 110-nm banded configurations occur in the posterior portion of Descemet's membrane in congenital hereditary endothelial dystrophy [337] and in Fuchs endothelial dystrophy.[299]

Abnormal excrescences also occur on Descemet's membrane in macular corneal dystrophy (described above). Descemet's membrane can be totally absent as a congenital anomaly.[313] In other abnormally formed corneas, such as sclerocornea, central corneal leukoma (Peters anomaly), and congenital hereditary endothelial dystrophy, Descemet's membrane is rudimentary.[323,337,433,447,471,473,553] It is excessively thin, underdeveloped, or even absent centrally in these conditions. The defect in the posterior cornea can form a crater which is sometimes filled by fibrous tissue.[553]

Deposition of Substances

In some pathologic states substances deposit within Descemet's membrane. One of the most well known is copper, which accounts for the pigmented ring (Kayser-Fleischer ring) at the periphery of the cornea in Wilson disease (hepatolenticular degeneration). Although the copper can be identified by light microscopy, electron microscopy resolves the localization better. Electron-dense granules of varying sizes accumulate within Descemet's membrane in this disease,[324,581,587] corresponding to the copper deposits. The densities tend to be aligned linearly in two or more zones parallel to the corneal endothelium. The small ones are charac-

teristically closest to the endothelium [581] but are often intermingled with the larger ones. Electron probe analysis has confirmed the presence of copper in the dense granules within Descemet's membrane.[324,581] A pigmented corneal ring is characteristic of Wilson disease but is not pathognomonic of it. Similar pigmented corneal rings can be seen clinically by slit-lamp examination in individuals with non-Wilsonian liver disease.[195] Copper has not yet been demonstrated histologically in such corneas, but elevated serum copper levels do occur secondly to the liver disease in such cases. An experimental model of the Kayser-Fleischer ring has yet to be established. In an electron microscopic study of the cornea with argyrosis secondary to the prolonged topical applications of a silver-protein solution, Hanna *et al.*[271] found spherical silver deposits limited to Descemet's membrane. Neutral fat frequently deposits in the peripheral cornea with aging (arcus senilis) and during adulthood in hyperlipoproteinemia Types II (familial hyperbetalipoproteinemia) and III (familial hyperbetalipoproteinemia and prebetalipoproteinemia) and less often in the other types of hyperlipoproteinemia. In arcus senilis the lipid is not restricted to specific structures or even to the cornea but does involve peripheral portions of Descemet's membrane. A double layer of electron-lucent vacuoles within Descemet's membrane has been observed by electron microscopy in arcus senilis.[194]

Endothelium

The posterior surface of the cornea is lined by a single layer of flattened endothelial cells that normally contain mitochondria with a condensed configuration. The corneal endothelium can be observed with remarkable clarity in the living eye with a recently refined instrument called the specular microscope. An extensive literature pertains to experimental studies on the transport of colloidal particles across the corneal endothelium.[329-335,480] These cells often possess small microvilli which are normally not prominent. Owing largely to the difficulty of obtaining adequate samples of corneal endothelium for analytic procedures, very few analyses of its protein content have been performed. Kawerau and Ott [328] found no serum proteins in a few scrapings of ox corneal endothelium.

Much has been written about the corneal endothelium,[551] and a number of disorders of this important cell layer are recognized. A common and clinically important disease of the corneal endothelium is Fuchs endothelioepithelial corneal dystrophy. It occurs mainly in middle-aged to elderly women. Due to the failure of the endothelium to remove water from the corneal stroma, there is epithelial edema especially in the central cornea. Descemet's membrane is thickened with excrescences (cornea guttata). The corneal endothelial cells decrease in number with age and

after becoming damaged from corneal graft rejection, acute glaucoma, drugs, trauma, surgical procedures, immune reaction to endothelial cells in grafts, intraocular lens implants, and other conditions. The corneal endothelium may also be damaged by therapeutic procedures such as the techniques used to remove cataracts, including phacoemulsification.[60,460] It can also follow cataract extractions in which the vitreous becomes displaced anteriorly and comes in contact with the cornea. The mechanism by which vitreocorneal contact causes endothelial cell decompensation remains to be established.[67] If a sufficient quantity of endothelial cells is lost, corneal edema ensues. The corneal endothelium in some species, such as the guinea pig [188] and the rabbit, can multiply and hence regenerate after cells are lost. This is not the case in man. When human endothelial cells desquamate or die, those that survive enlarge, and healing seems to take place by a flattening of existing endothelial cells that slide over the defective area.[326] An unusual crystalloid cytoplasmic inclusion, morphologically identical to an organelle that is characteristic of horizontal cells in the retina (Kolmer's crystalloid), has been identified within human corneal endothelial cells in a variety of apparently unrelated conditions.[312] The structure, which has not been observed in normal corneal cells, is composed of a stack of membranous tubules arranged in close proximity to each other. The unusual inclusion contains ribosomes and bears some resemblance to lamellar bodies seen in a variety of conditions in nonocular tissues.[312,363]

The cytoplasm of the corneal endothelium stores undigested metabolites or phagocytosed substances in some diseases, including macular corneal dystrophy and mucopolysaccharidosis Type I-H (Hurler syndrome) (discussed above). In Niemann-Pick disease rounded or oval laminated membranous bodies have been observed in endothelial cells.[490] Calcified crystals can accumulate within the endothelial cells in hyperparathyroidism.[48,311]

In addition to the aforementioned, the corneal endothelial cells can be absent or sparse over parts of the inner surface of the cornea. This is a consistent and prominent feature of congenital hereditary endothelial dystrophy. In other conditions, notably Fuchs endothelial dystrophy, the number of endothelial cells is also diminished. They are absent in the middle of the cornea in congenital central corneal leukoma (Peters anomaly). Another anomaly that has been noted by electron microscopy in the corneal endothelium is widening of the intercellular spaces.[287,299,471,580] Microvilli and cilia are sometimes abnormally prominent on the surface of the endothelial cells.

Electron microscopy has supported the impression that cells lining the inner surface of the cornea can undergo transformation into fibroblasts

and squamous epithelium. That the presence of cells with untrastructural characteristics of the latter two cell types occur in pathologic states is well documented. In the rare inherited disease posterior polymorphous dystrophy (degeneration), the cornea is lined by several layers of flattened cells with the fine structure of squamous epithelium.[68,273] Adjacent cells are joined by desmosomes, and the individual cellular elements contain numerous cytoplasmic fibrils indistinguishable from tonofibrils. The cornea may also be lined by a few layers of stratifed squamous epithelium as a congenital anomaly.[313] In Fuchs endothelial dystrophy and other entities, cells thought to be derived from the endothelium can possess the morphologic attributes of fibroblasts.[299] Scanning electron microscopy provides a three-dimensional view of such abnormal cells. In several conditions, including failed corneal grafts,[455] a multilayered fibrous membrane containing fibroblast-like cells develops on the endothelial surface and may replace it. Besides such metaplastic alterations, the inner lining of the cornea can contain cells of a different origin. Leukocytes adhere to the corneal endothelium (keratic precipitates) in several inflammatory disorders of the anterior segment of the eye. In corneal graft rejection these cells occur in close association with endothelial cell destruction, which they probably cause.

In diseases of the corneal endothelium, most notably in Fuchs dystrophy, the individual endothelial cells vary in size and shape. They are often absent, markedly attenuated, and partly covered by or distorted over the cornea guttata. Large intracytoplasmic vacuoles, an increased number of cytoplasmic filaments, degenerative changes, and phagocytosed melanin granules within the endothelial cells often occur in Fuchs endothelial dystrophy [287,299] and other conditions. Melanin granules derived from the iris and ciliary body are commonly adjacent to and within the cytoplasm of the endothelial cells. Although occasionally observed in the young, this endothelial melanosis is more frequent with advancing age. Pathologic states that cause cellular disintegration in the uveal tract are particularly likely to cause it. Occasionally the pigmentation is pronounced and clinically detectable. The convection currents in the anterior chamber tend to direct the free-flowing melanin granules toward the endothelial cells. When this occurs, the pigment becomes aggregated into a fusiform pattern that is almost always in the vertical meridian (Krukenberg's spindle).

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