The relationship between morphology and transparency in the nonswelling corneal stroma of the shark

Jerome N. Goldman* and George B. Benedek

The ultrastructure of the corneal stroma of the spiny dogfish, Squalus acanthias, was studied in order to determine whether there was a morphologic basis for the alleged inability of the elasmobranch cornea to swell, and for the possibly related maintenance of corneal transparency when it is immersed in distilled water. The most apparent morphologic explanation for the nonswelling properties was the presence of "sutural fibers" which seemingly bound the anterior and posterior limits of the corneal stroma. The filamentous strands connecting collagen fibers within the lamellae were more pronounced than those seen in the corneal stroma of other species. Bowman's layer was found to comprise 15 per cent of the total stromal thickness, yet the structural organization of its collagen fibers was incompatible with the "lattice theory" for corneal transparency proposed by Maurice. Densitometric studies of a slit-lamp photograph of dogfish stroma showed that Bowman's layer scatters less light than the underlying tissue. Measurements of collagen fiber size and density were made at different depths within the corneal stroma in order to provide a basis for future physiologic studies, and to determine whether there might be an explanation for transparency consistent with the collagenous ultrastructure of both Bowman's layer and the more organized corneal stroma.

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Ranvier first observed that the cornea of the skate does not swell in water. He further noted that this cornea, unlike others so tested, remains transparent upon immersion. He attributed the nonswelling property to the existence of "sutural fibers" which ran transversely from the "anterior basal membrane" (Bowman's layer) to the posterior surface of the cornea. His remarkable studies remained an obscure curiosity until Smelser nearly 80 years later, regenerated interest in the nonswelling properties of both the skate and the smooth dogfish shark. Payrau and associates confirmed the existence of the sutural fibers in several species of elasmobranchs, and elaborated on Ranvier's early descriptions with studies including electron microscopic analyses of these sutural components.

Phylogenetically, the elasmobranchs immediately precede terrestrial forms (Fig. 1). They have the most primitive verte-
Fig. 1. The family Elasmobranchii appear just before the major division of the vertebrate phylogenetic tree into aquatic and terrestrial forms. They first appeared about 350 million years ago in the Silurian Era.

Fig. 2. Since the thickness of each section (400 to 700 Å) is large compared to the diameter of the collagen fibers, the electron beam "sees" the entire cross-sectional diameter of most of the longitudinal fibers embedded in any one section. A fiber, a, which has been sectioned so as to include much less than half its diameter, will obviously be blurred on the electron micrograph, and will be selectively excluded by the observer. One would not, therefore, expect longitudinal sections to yield a measurement differing from that of cross sections.

The ultrastructure of the corneal stroma in the elasmobranch, Squalus acanthias, was studied in order to elucidate the factors involved in maintaining the transparent, nonswollen state of this corneal stroma. The construction of this stroma is so simple that several authorities attached no significance to the presence of the very apparent fibers traversing the lamellae. This primitive arrangement is associated with two properties relevant to clinical ophthalmology: an inability to swell, and the inability to lose transparency under conditions which render the corneas of more advanced species opaque. This study introduces a series of investigations aimed at determining the factors involved in maintaining the transparent, nonswollen state of the corneal stroma of the common spiny dogfish shark, Squalus acanthias, and will provide a detailed morphologic description which may be useful in future studies.

Method and materials

Preparation for microscopy. The excised cornea was fixed for 20 minutes in 3 per cent glutaraldehyde in pH 7.4 cacodylate buffer. One millimeter strips of cornea were cut and postfixed for 30 to 60 minutes in a 2 per cent osmium tetroxide solution in cacodylate buffer (pH 7.4). Dehydration in increasing concentrations of ethyl alcohol was followed by four 5 minute rinses in propylene oxide and by overnight immersion in a mixture of equal parts of propylene oxide and Epon 812. Epon 812 (Loft's 1:1 mixture) was used for embedding. Sections were cut perpendicular, oblique, and tangential to the corneal surface with glass and diamond knives on an LKB Ultramicrotome, Model 8802A, and mounted on various types of copper grids with and without parlodion films. The former were provided with a thin carbon layer. Electron photomicrographs were made with a Phillips 200 Electron Microscope operating at 60 kv. Kodak Contrast Projector Slide plates were used for most of the study. Toward the end, Kodak Electron Image plates were substituted.

Determination of lamellar thickness, collagen fiber diameter, and collagen fiber density. A single thin section was made through the entire thickness of the central cornea and put on a copper grid on which a parlodion film had been placed, enabling one to see all the lamellae of the same section. Thus, it was possible to determine at what depth of the corneal stroma (e.g., lamella 4, 10, or 20) electron micrographs were being made. Care was taken to orient the tissue so that sectioning was perpendicular to the corneal surface. The entire thickness of the stroma, including Bowman's layer, was then photographed at different magnifications.

In order to determine collagen fiber size, 8 by 10 inch prints were made of the entire thickness of each lamella and the prints were coded. A minimum of twenty clearly demarcated collagen fibers, in either a cross or longitudinal section, were selected from each photograph. An investigator, knowing code numbers only, measured the
diameters of the selected collagen fibers with an ocular micrometer and an operating microscope. Where there were differences in the measurements of two axes of the cross-sectioned collagen, only the narrower of the two was considered the fiber diameter. Where both types of sections were present in one lamella, the dimensions were determined for both in the same lamella (Fig. 2).

Statistical analysis of the diametric measurements of cross and longitudinally sectioned fibers within the same lamella showed that there was no significant difference ($s < 0.05$) between them. Values for both were, therefore, combined. Means and standard deviations were determined for each of the 25 lamellae before the code was broken (Table I). Some lamellae (Nos. 6 and 17) did not have fibers which could be accurately measured because of the extreme obliquity of their course in the section.

For the determination of collagen fiber density (the number per given area), the same coded 8 by 10 inch prints were arbitrarily divided into a grid on 1 inch ruled squares. Wherever the photographic enlargement of any one lamella provided a minimum of ten 1 inch squares containing collagen fibers with circular cross-sectional diameters, the number of fibers in each square was counted and recorded. If a horizontally or obliquely sectioned fiber appeared within the outlines of the grid, or if a marked irregularity in the pattern of the fibers was found, the square was excluded. Means and standard deviations and the number of 1 inch squares counted on each print were determined before the code was revealed.

**In vivo determination of light scattered by the corneal stroma.** After a portion of the epithelium had been removed, slit-lamp photographs of the cornea of a living dogfish shark were taken (Fig. 3) (Zeiss photoslit-lamp with Kodak Tri-X film in a special 35 mm camera body). Positive print enlargements magnified twelve times were studied with a Photovolt densitometer employing a 1.0 mm. pupillary aperture. The density curve was drawn with an xy plotter. The translation of the photograph was transmitted to the x motion of the plotter through a micropotentiometer in which ten turns corresponded to 10 mm. on the photographic enlargement. This was expanded to 300 mm. on the plotter.

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**Table I. Lamellar thickness and collagen fiber diameter in one adult elasmobranch cornea**

<table>
<thead>
<tr>
<th>Lamella No.</th>
<th>Thickness (µm)</th>
<th>Cross-section diameter, A (mean ± S.D.) (No.)</th>
<th>Longitudinal-section diameter, A (mean ± S.D.) (No.)</th>
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<tr>
<td>Bowman's layer</td>
<td>18,000</td>
<td>298 ± 25 (20)</td>
<td>263 ± 26 (20)</td>
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<td>1</td>
<td>1,456</td>
<td>327 ± 21 (20)</td>
<td>296 ± 22 (20)</td>
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<td>2</td>
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<td>295 ± 22 (20)</td>
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<td>288 ± 25 (20)</td>
<td>289 ± 25 (20)</td>
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<td>314 ± 21 (20)</td>
<td>295 ± 22 (20)</td>
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<td>295 ± 22 (20)</td>
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<td>5,615</td>
<td>280 ± 17 (20)</td>
<td>261 ± 24 (20)</td>
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<td>25</td>
<td>332</td>
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**Fig. 3.** Slit-lamp photograph of the anterior segment of the dogfish eye; a = the slit image of the lens capsule, b = the corneal slit image where the epithelium is in place, c = the portion of epithelium-free cornea at which densitometry was performed for Fig. 26, and d = the iris.
Results

When thick sections of epon-embedded material were examined with the light microscope, the total thickness of the central cornea was found to be 0.20 to 0.25 mm. This correlated well with the dimensions calculated from electron photomicrographs, as well as with in vivo optical determinations made with the Maurice-Giardi di pachometer. Forty to 55 per cent of the total corneal thickness consisted of epithelium. Fifteen per cent of the remaining corneal thickness resembled Bowman's layer in man. The main portion of the stroma contained 24 to 25 lamellae when counted under the higher magnifications of the electron microscope. The thickness of each layer varied according to its depth in the cornea. Alternate lamellae appeared wrinkled together, like crêpe paper. These wrinkled lamellae consisted chiefly of cross sections of collagen. (Fig. 4).

By dissecting out one sutural fiber, Ranvier demonstrated that the sutural fibers were continuous through the thickness of the corneal stroma in the skate. With the use of polarized light, he also showed that the portions of sutural fibers seen in alternate lamellae were continuous from one lamella to the next. Our studies confirm those of Payrau and associates: The sutural fibers traverse as many as ten adjacent lamellae, and probably run the entire thickness of the cornea of the dogfish. The attachments of these fibers, which were not found by Payrau and co-workers, will be described in detail. When the cornea

Fig. 4. Low power view of five lamellae in the middle portion of the dogfish stroma. Wrinkles appear in alternate layers in which the collagen fibers have been cut in cross section. The "sutural fibers" (S) cross the lamellae in a wavy path in this photograph. (Original magnification x6,000.)

Fig. 5. Flat preparation of dogfish endothelium fixed in 5 per cent glutaraldehyde in dogfish Ringer's solution diluted to 991 mOsm. (20 minutes) and postfixed with 2 per cent osmium tetroxide (5 minutes). (Original magnification x100.)
was fixed conventionally for paraffin- or epon-embedding, no endothelial cells remained attached to Descemet's membrane. Since a rather typical endothelial mosaic was discernible by specular reflection on the slit lamp, various methods were used in an effort to demonstrate its existence. The most successful of these required fixation of the entire fish in glutaraldehyde. With this technique, we observed a cellular arrangement similar to the mosaic found in flat endothelial preparations of higher species (Fig. 5).

**Basal lamina and Bowman's layer.** The basal lamina compares structurally with that found in the human cornea, but is proportionally thicker in the dogfish. It measures 2,800 to 4,200 Angstrom units (Å). Anteriorly, it is represented by the cell membrane of the basal epithelial cell. Posterior to this is a narrow (600 Å) light zone and a narrower (200 Å) dense zone.
which blends into the thick, moderately dense region comprising the major portion of the structure. The lamina ends in a condensation of dark material which abuts the underlying Bowman’s layer. These relationships are demonstrated in Fig. 6. In this electron photomicrograph, the fine fibrillar component of the sutural complex can be seen blending into a conical evagination of the most posterior dense layer of the basal lamina. This cone represents the anterior terminus of the fine fibrillar component. No other specific morphologic structure appears at this point of attachment.

Bowman’s layer is 30 to 45 times thicker than the basal lamina, measuring between 0.01 and 0.02 mm. centrally in different individuals of the species. As in the human cornea, the collagen fiber feltwork comprising Bowman’s layer has no particular orientation (Fig. 7), except in its posterior...
portion where there is a preponderance of fibers aligned parallel to the corneal surface. Most of these fibers blend into the most anterior lamellae of the stroma. A few of them join the fine fibrillar component of the sutural fibers within the anterior two lamellae and pass with them through the thickness of at least one lamella (Fig. 8). The relationship of these thick fibers to the collagen fibers comprising the corneal lamellae is not clear. The collagen fibers of Bowman's layer are thinner than those in the adjacent anterior lamellae. Their diameter is about 270 Å.

In Bowman's layer as in other lamellae there is no significant statistical difference between the diameters measured in cross section and those measured in longitudinal section.

We have also estimated the volume fraction \( d_c \) of fibers in the Bowman's zone using the electron micrograph in Fig. 7. This was done by counting the number of fibers per unit area \( (N_A) \) whose axes are oriented within some pre-selected solid angle \( (\Delta \Omega) \). If \( p \) is the radius of a fiber, then the volume fraction of fibers \( d_c \) is given in terms of \( N_A \) and \( \Delta \Omega \) by

\[
d_c = N_A \left( \frac{\pi p^2}{4\pi/\Delta \Omega} \right).
\]

We can identify those fibers lying in a solid angle \( \Delta \Omega \) by counting for each unit area only those fibers whose photographic image ranges between a perfect circle (vertical alignment of the fiber) to an ellipse of a pre-selected length-to-width ratio. In our measurement we took this ratio to be 3. The fibers having this projected length to width ratio were inclined an angle

\[
\Delta \theta_m = \tan^{-1} \left( \frac{4p}{L_T} \right)
\]

where \( L_T \) is the thickness of the epon section photographed. We counted all fibers whose orientation was between vertical and this maximum angle. The solid angle \( \Delta \Omega \) which was included in our measurement is related to \( \Delta \theta_m \) by

\[
\Delta \Omega = 2 \pi \left( 1 - \cos \Delta \theta_m \right).
\]

In this way we found the volume fractions of fibers to be about \( (40 \pm 10) \) per cent. The lower limit of 30 per cent corresponds to an epon section of 500 Å, while the upper limit of 50 per cent corresponds to a section of thickness 700 Å. The volume fraction of fibers in the shark stroma, \( d_c = 35 \) per cent, falls within these limits.

**Stroma.** The three adult dogfish corneas in which the layers of the stroma were actually counted under the electron microscope each had 25 lamellae. In order to distinguish the anterior two from the posterior two, magnification of \( \times 20,000 \) was required. The thickness of consecutive lamellae measured in several corneas revealed variation from one cornea to another, but showed similar distribution to that tabulated for one cornea (Fig. 9, Table 1). Every precaution was taken to insure that sections were made perpendicular to the corneal surface. Any deviation from the perpendicular in the sections used for measurement or counting is probably small. As previously stated, the entire stromal thickness could be seen within one parlodion thickness could be seen within one parlodion-supported grid opening, so that the numerical position of each lamella could be ascertained.

Within the lamellae, the collagen fiber size varied according to the location of the lamella. With the exception of Bowman's layer, the anterior lamellae contained larger fibers than the posterior ones. The best fitting parabola

\[
D = aL^2 + bL + c
\]

(\textit{where} \( D \) = diameter and \( L \) = lamella number), was computed and fitted between the measured points (Fig. 10). The parabola was considered as three slices (slice No. 1 = lamellae 1 to 8, slice No. 2 = lamellae 9 to 16, and slice No. 3 = lamellae 17 to 25) and analyzed. The collagen fiber diameters within each slice differed statistically from those in the other two slices.

In analyzing the density of collagen fibers, two slices were considered (slice...
Fig. 8. The thick fibers (F) of the sutural complex originate in the posterior portion of Bowman's layer (B). They join the fine fibrils (f) as they pass through the anterior layers (numbered 1, 2, and 3). In this micrograph, the process (p) of a poorly fixed keratocyte accompanies the principal bundle through the second and a portion of the third lamella. (Original magnification x29,000.)

No. 1 = lamellae 1 to 16, and slice No. 2 = lamellae 17 to 25). The two slices, which appeared to differ from one another when represented graphically (Fig. 11), were significantly different upon statistical analysis. The fibers were more concentrated in the posterior lamellae.

As a rule, the collagen fibers in each lamellar ribbon of the shark stroma are more parallel to one another than in human or rabbit corneas, but changes of direction (Fig. 12) and individual fibers traveling perpendicular to the general direction are frequently seen. These fibers may be part of the thick component of the sutural complex, which will be described in the next section. Unlike the lamellae of human and rabbit corneas, the ribbons of collagen fibers do not appear to interweave. Rarely, a group of fibers is
Fig. 9. Graphic representation of the distribution of lamella thickness in one dogfish cornea. This pattern exists for the stroma of other adult members of this species.

Fig. 10. The mean diameter of collagen fibers decreases progressively in the posterior lamellae. Bowman's fibers compare in diameter with the smaller fibers found in lamellae 18 through 25.

Fig. 11. The significant change in collagen fiber density in the posterior lamellae.
Fig. 12. Part of a keratocyte lying between the twenty-first and twenty-second lamellae. Collagen fibers appear in perpendicular (*) and horizontal (***) section in adjacent portions of the same lamella. One vertically extending process has an accumulation of free ribosomes at its base; \( N \) = nucleus, \( p \) = process, \( g \) = golgi apparatus, and \( v \) = surface vesicle. (Original magnification \( \times 29,000 \).)

observed to pass obliquely across a lamella (Fig. 13). Occasionally, even in the central cornea, rows of collagen only one, two, or three fibers thick, which represent the lateral excursions of the thick component of the sutural complex, are found. The width of the transversely running ribbons of collagen fibers making up one lamella exceeded the width of the sectioned tissue, so that their dimensions could not be de-
Fig. 13. A bundle of collagen fibers of one lamella in the anterior portion of the stroma crossing obliquely to a third lamella. The individual thick fibers (single arrows) and a thin row of thick fibers (double arrows) are also seen. (Original magnification ×24,000.)

Fig. 14. Interstices between collagen fibers within one lamella are filled with a reticulum of fine strands. The suggestion of a collagen fiber substructure appears in the micrograph. (Original magnification ×120,000.)
The thickness of the entire cornea doubled near the periphery with apparent proportionate increase in the thickness of each of the individual lamellae, but the number of lamellae remained the same.

Fine strands connect adjacent collagen fibers within any one lamella. These are best seen in oblique or cross sections (Fig. 14). The strands resemble those seen between the collagen fibers in human and rabbit corneas. A great many more of them are found in the shark than in the corneas of the other two species. Within the central shark cornea, the concentration of filamentous strands between collagen fibers is greater in the posterior lamellae. These collagen fibers occasionally display an electron dense peripheral ring suggestive of a substructure (Fig. 14).

**The sutural fiber complex.** The sutural fiber as seen by Ranvier with the light microscope was found to contain the two types of fibers described by Payrau, which will be referred to as the fine and thick components of the sutural complex. On horizontal section, the buttonhole-shaped sutural complexes are seen to be irregularly spaced, their long axes parallel to one another within one lamella. There are about fifteen sutural complexes per square millimeter (Fig. 15). The montage of such sections (Fig. 16) demonstrates the variety of relationships within the sutural complex. Thick fibers are seen only on the two pointed ends of the buttonhole, and never within or around the buttonhole on the flattened sides except at the intersection of lamellae, where the sutural complex assumes a triangular shape. The cylindrical core of the fine fibrils is referred to by Payrau and associates as the principal bundle. Its narrowest diameter varies from 3,500 Å to 4,500 Å. The longitudinal diameter may be four times as great. One bundle contains 60 to 100 fibrils, each of which measures about 75 Å in diameter. A bundle attaches anteriorly to the anterior dense layer of the basal lamina and extends posteriorly into Bowman's layer, where it is joined by the thick fibers. Both components terminate in an interweaving network beneath the most posterior lamella and Descemet's membrane (Fig. 17). Rarely, smaller bundles of fine fibrils are seen in cross section in the middle of a posterior lamella, isolated from the thick component of the sutural complex. These fine fibrils do not show the collagen peri-
Fig. 16. The varied relationships of the fine (f) and thick (F) fibrillar components of the sutural complex. In a, b, and c interfibrillar bridges can be distinguished among the thick fibers in the apical triangle of the complex. A cross section of a vertical process (p) of a keratocyte appears in c. The manner in which the thick fibrillar components leave the complex near the interlamellar interface can be appreciated in d. The rounding of the principal bundle at this level suggests that its flattened appearance elsewhere is the result of lateral compression. (Original magnification a and b, ×64,000; c, ×34,000; and d, ×46,000.)
Fig. 17. The fibers of the sutural complex plunge past the process of a keratocyte to their termination in the network overlying Descemet's membrane (D). Groups of fine fibrils (f) can be seen amidst the thick fibers in this network. The endothelial monolayer was not present in this preparation. (Original magnification x31,000.)

Odicity seen in the fibers of the thick component.

The thick fibers take several pathways, some of which are described by Payrau. As previously stated, the thick component originates anteriorly in the posterior portion of Bowman's layer. Some thick fibers leave the sutural complex at or near every interlamellar interface. A few leave within the center of the lamella. Whether or not any of them traverse the entire thickness of the cornea cannot be ascertained from observations made here. No individual thick fiber has been traced for more than two lamellae. When a thick fiber has left the sutural complex, it blends in with the mass of parallel collagen fibers of the lamella proper. The anteroposterior path of some thick fibers can be quite straight for the thickness of one or two lamellae. In
the same cornea, other thick fibers show as many as four twists within one lamella (Fig. 18). This observation, when correlated with the direction of the thick fibers in the horizontal sections of the sutural complexes of Fig. 16, suggests that a large number, if not the bulk of the thick fibers, twist about as they descend within the triangle on one side of the core of a sutural complex. There is no evidence that a thick fiber on one side of the principal bundle passes to the other side, except at the interlamellar interfaces.

Paraffin-embedded dogfish corneas fixed in CPC-formalin were histochemically examined in order to determine the composi-
Fig. 19. Scleral collagen demonstrates typical periodicity, loose organization, twisting, and variable cross-sectional diameter of fibers. (Original magnification ×60,000.)

However, the sutural complex could be seen in sharp contrast. The dark, linear staining extended through Bowman’s membrane to the base of the epithelium in some sections. This extension suggests that the thin component was being stained. The...
Fig. 20. Varying diameters of scleral collagen fibers seen in cross section. Fibrillar accumulations (arrows) adjacent to the scleral fibroblasts (Fib.) may be collections of mucopolysaccharide, protocollagen, or elastin. (Original magnification ×25,000.)

Acid mucopolysaccharide content of the sutural complex did not differ histochemically from the adjacent stroma when exposed to toluidine blue at pH 2.5, 4.0, and 7.0, hyaluronidase digestion, and selective blocking of alcian blue by KCl.13, 16

Scleral collagen, in contrast to corneal fibers, usually demonstrate the typical periodicity and greater variation in cross-sectional diameter. They are also more loosely organized and twist about one another (Figs. 19 and 20).
The keratocytes. The cellular elements of the dogfish stroma are quite similar to the keratocytes found in normal human and rabbit corneas. Usually the cell is situated between lamellae. There appears to be a continuous line of cells in which keratocytes maintain actual or virtual contact with the processes of adjacent keratocytes in the same plane (Fig. 21). The surrounding collagen lies close to the cell membrane. The cell organelles are similar to those of other fibroblasts.

The relationship of the keratocytes to the sutural complex suggests that the cells accommodate themselves to the fixed position of the components of the complex. In horizontal sections, portions of the keratocyte cell membrane are seen surrounding all or a portion of the sutural complex. The principal bundle of fine fibrils is often completely surrounded by keratocyte cytoplasm without any trace of the thick fibers. In the vicinity of the sutural complex, there is a dark condensation subjacent to the cell membrane suggestive of compression of the cell at that site (Figs. 22 and 23).

In perpendicular section of keratocytes, the anteroposterior dimension is greater than that of those in human or rabbit corneas, so that the cell sometimes has a plump appearance. This plump appearance is especially pronounced in the keratocyte of the embryonic shark. The sutural complex intersects the keratocyte. The fine fibrils of the principal bundle form a barrier to which the external membranes of the cell seem to accommodate themselves. These sutural complex-keratocyte relationships suggest that the cell membranes may be in motion. One unique property of dogfish keratocytes is the manner in which the processes extend in an anteroposterior direction. These vertical processes are often in close association with components of the sutural complex (Figs. 8, 16, c, and 25). Sometimes an accumulation of free ribosomes is seen at the base of the vertical cell process within the cytoplasm of the cell (Figs. 24 and 25).
Fig. 22. Horizontal section of a keratocyte. The cell membrane (c) is apparent only when folded over within the cell. The thin component of the principal bundles (f) appear unaltered. The adjacent thick fibrillar component (F) is often attenuated when seen within the confines of the keratocyte. The structure marked with an asterisk may be a mitochondrion. Islands of keratocyte cytoplasm (K) are seen in the midst of the collagen; N = nucleus. (Original magnification x23,000.)
Descemet's membrane and the endothelium. No space is discernible between the network underlying the most posterior lamella and Descemet's membrane in sections cut perpendicular to the corneal surface. In extremely oblique sections, a fiber-free region is distinguishable. The loose adherence of the very thin Descemet's membrane (3,000 Å to 44,000 Å) to the stroma might have caused this separation to occur during tissue processing. In the dogfish, Descemet's membrane consists of a homog-

Fig. 23. Two principal bundles being engulfed by the margins of a keratocyte. There is a region of increased electron density along the cell membrane adjacent to this region (single arrows). N = nucleus, ER = granular endoplasmic reticulum, m = mitochondrion. (Original magnification ×43,000.)
enous layer of fine fibrils more loosely interwoven than those seen in mammals. Posterior to Descemet's membrane, a mono-layer of cells was seen in only one embedding. A morphologic description of these cells will be waived until better fixation is achieved. A definite endothelium can be identified on slit-lamp examination and on flat endothelial preparation.

Discussion

This study was inspired by the observation that the stroma of the elasmobranch retains its transparency even under many of the conditions in which other corneas are known to swell and become opaque. This is not to say that the transparency of the dogfish stroma cannot be impaired. A needle passed obliquely through it leaves

Fig. 24. Portion of a keratocyte with vertical processes extending anteriorly and posteriorly. At the base of each vertical process, an accumulation of free ribosomes is seen. Fine fibrillar material (arrow), perhaps representing mucopolysaccharide or protocollagen, can be seen outside the cell near the base of one of the processes. N = nucleus, g = golgi apparatus, c = centriole, V = surface vesicle. (Original magnification x23,000.)
Fig. 25. Higher magnification of the region surrounding the inferior process in Fig. 24. The cytoplasmic membrane (cm) of the process can be distinguished in the midst of the fibrillar components of the sutural complex; g = golgi apparatus, r = ribosomes. (Original magnification x45,000.)
an opaque line in its path. Retention of transparency appears to be related to the natural nonswelling properties. Measurements and descriptions of the cornea of a readily obtainable and easily managed member of this unique family supplement the earlier reports of Payrau, previously cited, and are intended to provide a foundation for future experimental work on corneal transparency, swelling mechanisms, and the flow of water and electrolytes in corneal stroma.

The knowledge the measurements and relationship of the collagenous components of the cornea of Squalus acanthias permits us to evaluate the theoretical basis for corneal transparency. In a pioneering treatise, Maurice showed that if individual collagen fibers scattered light independently of one another then the corneal stroma would be opaque. The fact that the cornea is transparent indicates that phase relations must exist between the electric fields emanating from each fiber which a ray of light strikes. These phase relations result in a mutual destructive interference of the wavelets scattering from each fiber, and hence in a diminution of the intensity of the scattered light. Maurice further suggested that the physical basis for the phase relationship between the scattered wavelets was a regular or lattice arrangement of the collagen fibers.

The thickness of Bowman's layer in the shark is between 0.01 and 0.02 mm., depending on the individual specimen. This is about 15 per cent of the stromal thickness in the central cornea. In Bowman's, the axes of the fibers are randomly oriented (Fig. 7). Although their diameters are very nearly equal, they are neither parallel nor disposed in a lattice. Nevertheless, these fibers cannot be regarded as independent point scatterers.

If they were independent, then Maurice's formula (Equation 10) for the fraction $\Delta S$ of light scattered would apply:

$$\Delta S = \frac{d_c \sigma^{2} \rho^2}{\lambda^3} \left[ 1 + \frac{2}{(n_{c}^2 + n_{i}^2)^2} \right] (n_{c}^2 - n_{i}^2)^2 \Delta t.$$

We can predict the results of applying this formula in the case of Bowman's zone in the dogfish. The parameters needed are $d_c$, the volume fraction of fibers; $\rho$, the fiber radius; $\lambda$, the wavelength of light; $\Delta t$, the thickness of the zone; and $n_{c}$ and $n_{i}$, the indices of refraction of the collagen fibers and the ground substance. The values of $d_c$, $\rho$, and $\Delta t$, respectively, are: 40 ± 10 per cent, 139 ± 12 Å, and 0.015 ± 0.005 mm. We also take $\lambda = 5000$ Å. We have not measured $n_{c}$ or $n_{i}$ for the shark's Bowman's zone. However, if we take the values $n_{c} = 1.55$ and $n_{i} = 1.345$, the values given in Maurice's paper, we find that the fraction $\Delta S$ of light scattered from an incident beam is 35 per cent, with a possible range of values produced by the uncertainty in $d_c$ and $\Delta t$ of 18 per cent at the minimum and 59 per cent at the maximum. Even in the case of the minimum value of about 18 per cent, this degree of scattering is inconsistent with transparency.

In fact, when we examine slit-lamp photographs of the Squalus stroma, we find that the zone corresponding to Bowman's layer is not only transparent but that it actually scatters less light than the rest of the corneal stroma (Figs. 3 and 26). The calculation we have made suffers from the use of the values $n_{c}$ and $n_{i}$ which were obtained for the corneas of other species. The value of $n_{c}$, the collagen fiber index, is probably the same for the shark and the species used by Maurice. Of course, $n_{i}$ could be different and a measurement of it should be made for the shark. Nevertheless, we have assumed that $n_{i}$ is the same for both species. If this is the case, then the observed transparency of the shark's Bowman's zone forces us to conclude that a lattice arrangement of collagen fibrils is not a necessary condition for corneal transparency.

This conclusion is entirely consistent with the principles of optics according to which light is scattered only when the illuminated medium contains spatial fluctuations in the index of refraction over...
dimensions comparable with the wavelength of light. The fluctuations in the index of refraction as one passes from the collagen fiber to the ground substance matrix occur over dimensions of about 500 Angstrom units. This distance is small compared to the wavelength of light.

Under the conditions present in both the normal Bowman's layer and the normal stroma of the dogfish shark, the transmission and scattering of light must be calculated by summing the dipole fields radiating from each illuminated point. Only those fluctuations in the index of refraction having a spatial extent comparable with or larger than one half the dimension of the light wavelength (~2500 Å) are important in considering the relationship of structure to transparency. In fact, the theory of the scattering of light by a medium having fluctuations in its index of refraction shows that fluctuations having a wavelength of λ/2 scatter light of wavelength λ toward the backward direction, while scattering in a direction θ away from the forward direction is produced by fluctuations of wavelength 
\[
\frac{\lambda}{2 \sin \frac{\theta}{2}},
\]

i.e., by fluctuations having a wavelength longer than λ/2. One can expect to find inhomogeneities in the refractive index equal to or greater than ~2500 Å in corneas with reduced transparency. Scleral opacity results not from the absence of a lattice arrangement of collagen fibers, but rather from such large variations in collagen fiber diameter (250 Å to 4,800 Å in our specimens) that neighboring regions have different refractive indices. (Figs. 19 and 20.) The separation of adjacent bundles of scleral fibers from one another by noncollagenous spaces which are large compared to the dimensions of a wavelength of light also contributes to scleral opacity. The operative mechanisms to be considered, in this case, are refraction and reflection.

Although various chemical and electrical factors might contribute to nonswelling properties, the morphologic characteristics described in this paper appear to account...
for the restriction of swelling in the dogfish cornea. There is great species variation in the degree of interweaving of the lamellae and in the regularity of the arrangement of collagen fibers within a single lamella viewed with the electron microscope. The adult dogfish demonstrates the highest degree of regularity I have observed. The fine strands between the individual collagen fibers in each lamella are considerably more electron dense than those found in rabbit and human corneas prepared and examined under similar conditions. They are also more numerous in the elasmobranch. If these interfibrillar strands are actual structures, the increased electron density may very well represent greater strength and a greater tendency for adjacent collagen fibers to maintain their close relationship.

The sutural complex could account for nonswelling completely. The thin component is never kinked. It does, indeed, appear to be stretched between the basal lamina of the epithelium and Descemet's membrane. If the "sutural fibers" cannot stretch, the maximal thickness of the corneal stroma might be limited by the length of the thin bundles.

The nature of the thin fibrils is not definitely known. They do not have the periodicity typical of corneal collagen, but this may be related to their small diameter. It is more difficult to demonstrate periodicity in the corneal collagen fibers than in those of sclera. The positive reaction of the sutural complex to Wilder's stain, coupled with its greater PAS reactivity, is consistent with the histochemistry of reticulin. The fine fibrillar component is of the same order (75 Å) as has been described for this chemical analogue of mature collagen.

The fibers of the thick component appear to bridge only a few lamellae at most. Although some of them descend in a straight anteroposterior path for one or two lamellae alongside the thin fibrils, many of them follow the twisting path previously described. The twisting, along with the probability that they join together only a few adjacent lamellae, makes it unlikely that they fix the anterior and posterior limits of the stroma. They may serve to restrict the separation of normal collagen fibers within the lamellae, as well as the separation of lamellae from one another. I have observed both phenomena in human stromal swelling.

The simple arrangement of the elasmobranch stroma into 25 layers lends itself to the layer-by-layer determination of lamellar thickness, collagen fiber size, and collagen fiber density. The progressive decrease in fiber size as one proceeds posteriorly is not linear. Statistically, the fibers in each third of the stroma are closer in diameter to one another than to any others, with the exception of the fiber size of the collagen in Bowman's layer.

The observation that the collagen fibers in Bowman's layer are smaller than those of the stroma is consistent with the findings of others. Jakus found that the collagen fibers in Bowman's layer were consistently smaller than those found in the underlying stroma within the same species, but that these diameters exhibited species differences.

The different values ascribed to the size of corneal collagen fibers by various authors may be the result of variation in the preparation of the tissue for examination. No statistical difference was found in measurements made of the diameters of 20 longitudinal and 20 perpendicular fibers in the same lamella. This is in disagreement with several previous reports in which the longitudinal width is said to exceed the cross-sectional diameter. Jakus' observation of an increase in the thickness of the collagen fibers in the more posterior portions of the human corneal stroma describes a condition which is just the reverse of the progressive decrease in fiber diameter determined in this study. This species difference cannot be accounted for.

The frequent occurrence of the isolated principal bundle of the sutural complex surrounded by keratocyte cytoplasm, and
the rare occurrence of the isolated thick fibers in such surroundings, suggest that the thick fibers are either displaced or digested by the migrating keratocytes of the normal cornea. Since there is little evidence for the former situation in the electron micrographs, the latter possibility must be seriously considered. The vertical extension of keratocyte processes, only when they are associated with the sutural complex, may be related to the disappearance or renewal of the thick fibers. Whether the ribosomal accumulations at the base of these processes are indicative of activity is unknown.

The fact that the elasmobranch stroma will not swell even in the absence of the epithelium and the endothelium makes an endothelial pump unnecessary for the regulation of stromal hydration. The difficulties encountered in maintaining endothelial integrity in this study may reflect the experience of those in the past who have questioned or denied the existence of this monolayer. However, the presence of an endothelial mosaic observed on slit-lamp microscopy and on endothelial flat preparation has encouraged us to make further attempts at delineating endothelial morphology.

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