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THE TRANSPARENCY OF THE MAMMALIAN CORNEA

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SUMMARY

1. A theoretical and experimental analysis of the relationship of the corneal stromal ultrastructure with light transmission has been made in an attempt to resolve recent contradictory explanations of corneal transparency.

2. The spatial distribution of collagen fibrils in electronmicrographs of rabbit corneal stroma has been analysed in terms of a radial distribution function. The results indicate the presence of local order extending to at least 200 nm from individual fibrils.

3. The observed spatial distribution of the collagen fibrils was used as a basis to compare the theoretically derived and the experimentally determined values of light transmission. It has been found that the transparency of the normal cornea may be explained by the quasi-random structure revealed by the electronmicroscope.

4. Histograms of the collagen fibril diameter in normal rabbit corneal stroma revealed the range to be 12·5–32·5 nm and the mean value to be approximately $20 \pm 1{\cdot}5$ nm. Corneal swelling did not change the collagen fibril diameter significantly.

5. It is concluded that the size and distribution of collagen fibrils revealed in electronmicrographs are consistent with the observed transparency of normal stromas.

6. A marked heterogeneity in the spatial distribution of collagen fibrils was found in the swollen cornea. This is qualitatively consistent with the observed decrease in transparency.

INTRODUCTION

Electronmicroscopic studies have shown the mammalian corneal stroma, constituting about nine tenths of the corneal mass, to be comprised of collagen fibrils of similar diameters immersed in a ground matrix and lying parallel to the corneal surface (Pl. 1). The corneal tissue merges imperceptibly with the surrounding sclera which is also composed of a collagen-mucopolysaccharide matrix but which differs strikingly from that of the corneal stroma in both the diameter and spacing of the collagen fibrils (Pl. 1).

To account for corneal transparency it is necessary to explain the near absence of light scattering from the collagen fibrils. We will consider two possible explanations. The traditional explanation was based on the assumption that the collagen and intervening ground substance had substantially equal refractive indices (Schwarz, 1953; Davson, 1955). Although this viewpoint recently found support in calculations of the refractive indices based on electronmicroscopic and chemical analyses (Smith, 1969), the uncertainties of the experimental methods are sufficiently great that the calculation is not definitive. On the other hand, the birefrigent studies of Naylor (1953) and Maurice (1957) indicated that the corneal stroma is anisotropic and that the refractive index of the collagen fibrils is significantly greater than that of the ground substance. If this is so, each fibril scatters an appreciable amount of light. Transparency must then be a result of the spatial arrangement of the fibrils in a pattern which creates essentially complete destructive interference to light in all directions except the forward direction.

In order to determine which of the two possible explanations of transparency is correct the angular dependence of the scattered light must be considered. Experimentally the angular dependence of the freshly excised cornea shows a very pronounced sharp peak in the near forward direction (Kikkawa, 1960; Bettelheim & Vinciguerra, 1969; R. E. Walker, P. E. R. Tatham & R. McCally, personal communication). This is in contrast to the broad angular dependence which would characterize a purely random arrangement of fibrils. Thus, it is concluded that the transparency of the cornea derives from the spatial distribution of the fibrils.

The significance of the arrangement of the collagen fibrils to the transparency of the cornea was considered by Maurice (1957) who calculated that the tissue would not be transparent if the fibrils were randomly distributed, that is, if the scattered light energy was the sum of that dispersed by the individual fibrils. This led him to propose the concept that transparency of the cornea was dependent on a crystalline or lattice-like spacing of collagen fibrils.

Attempts to confirm the concept of the lattice structure proposed by Maurice have failed. Schwarz (1961) and Schwarz & Graf Keyserlingk (1969) concluded from electronmicroscopic studies that the collagen fibrils were not arranged in this configuration, and Goldmann & Benedek (1967) reported that the ultrastructure of Bowman's layer of the cornea of the shark was incompatible with the lattice concept.

The present study deals primarily with the question of whether the ultrastructure of the stroma, as revealed by electronmicroscopy, is consistent with transparency. We have formulated in quantitative terms the size and distribution of the collagen fibrils in the cornea, and we have used these data as a basis to compare the theoretically derived and experimentally observed values of light transmission. The results show that transparency can be explained on the basis of a quasi-random structure revealed by the electronmicroscope and suggest that the loss of transparency with corneal swelling is due to a decrease in the order of the collagen fibril spacing.

METHODS

Fixation and processing of tissues. The corneas used in the quantitative analyses presented in this paper were prepared as follows. The complete cornea including a 2 mm ring of sclera was excised from the anaesthetized rabbit and dropped immediately into a 1 % solution of osmic acid in isotonic veronal acetate buffer (pH 7·4) at 4° C (Zetterqvist, 1956). Fixation was continued for 60 min. Corneal thickness measurements were made periodically during fixation and no change in thickness was observed. In a group of five corneas, the mean corneal thickness was $0{\cdot}38 \pm 0{\cdot}01$ mm in the living animal and $0{\cdot}38 \pm 0{\cdot}01$ mm at the completion of fixation.

The fixed tissues were left for 15 min in a series of ethyl alcohols of increasing concentration, then for 60 min in two changes of toluene, and finally embedded in Araldite 6005 (Ciba). Corneal thickness measurements during treatment with alcohol showed that a rapid and significant decrease in thickness occurred with 50 % ethyl alcohol, whereas no change occurred in concentrations of less than 30 % ethyl alcohol. In a group of six corneas there was a mean decrease of 20 ± 3 % in corneal thickness when the concentration of ethyl alcohol was changed from 30 to 50 %.

Polymerization was undertaken at 60° C for 4 days. Sections were made with a Porter Blum microtome and diamond knives (DuPont, Wilmington, Delaware); sections were stained with lead citrate and uranyl acetate (Reynolds, 1963). The sections were examined on an RCA EMU-3f electron microscope. Comparison of corneal thickness measurements made prior to and following embedding showed that some swelling had taken place during polymerization. The degree of swelling was variable even in an individual piece of cornea. In normal corneas the final corneal thickness after embedding was between 0·07 and 0·13 mm more than in the living eye.

Experimental. Adult albino rabbits weighing 2·5–3·0 kg were used in the experimental study. Corneal thickness *in situ* was measured with a modified Maurice & Giardini (1951) instrument; the apparatus was calibrated on a series of glass shells of known curvature, refractive index and thickness. The reproducibility of readings on normal corneas was within 3 %. During fixation and embedding procedures corneal thickness was measured by direct microscopy using the vertical vernier scale

on a Zeiss microscope. Particles of lycopodium powder (diameter 1–5 μ) were dusted on the two surfaces of the cornea to define them clearly. The reproducibility of measurements on the cornea (initial thickness approximately 400 μ) was within 3 %.

The optical transmission measurements were made with the Beckman Model DU absorption spectrophotometer. The cornea and a surrounding ring of scleral tissue were excised from the living animal after measurement of the corneal thickness. The excised tissue was immersed gently in oxygenated, buffered Ringer solution (pH 7·4) at 37° C contained in an optical cell. The cornea in the aqueous solution maintained its normal curvature and its central area was placed immediately adjacent to the slit facing the incident beam of the spectrophotometer. Light scatter from within the cell was minimized by blackening the interior except for the two areas which formed the entrance and exit slits. Control transmission readings were made on the same cell containing saline before and following corneal measurements. The transmission measurements were completed within 20–30 min of excision of the cornea. Corneal thickness increase during this period was less than 5 %.

Measurement of collagen fibril diameter and spacing. Transparencies made from electronmicroscopic studies were used in the quantitative analysis. An Oscar-N data reduction instrument (Benson Lehner Co.) with a digital readout was used to record the x, y co-ordinates and the diameters of each fibril. Histograms of collagen fibril diameters were made by conventional procedures.

The spacing of the fibrils was analysed by determining the radial distribution function as follows. Initially, a centrally located fibril was chosen as a 'reference centre'. The fibril served as the co-ordinates' origin. The co-ordinates of the other fibrils were recalculated with respect to this reference fibril. Other fibrils were then selected as reference centres. The process was repeated for several hundred reference centres. An example of the printout of the computer memory from 351 reference centres, displayed in Text-fig. 1, shows that the average distribution of fibrils around one another appears to be dependent only on the radial separation distance. The Figure reveals a considerable departure from a purely random distribution. A large space exists in the neighbourhood of the origin (void radius is $\sim$ 40 nm, whereas the fibril diameter is $\sim$ 20 nm). A diffuse ring of greater than average number density is visible at a radius of $\sim$ 55 nm, and a diffuse ring of less than average density is visible at a radius $\sim$ 90 nm. Numerical evaluation of the radial distribution function $g(r)$ was completed by incorporating a greater number of reference centres (in order to improve the statistics), counting the number of fibril co-ordinates in shells of 3 nm thickness, and dividing by the bulk number density.

For the radial distribution function to provide a valid characterization of the fibril arrangement, it is necessary that $g(r)$ be sensibly independent of those fibrils selected as reference centres. Numerical explorations of the electronmicrographs of lamellae of rabbit stromas that we have used indicate that this criterion is approximately satisfied. There are, in general, several conditions that must be met.

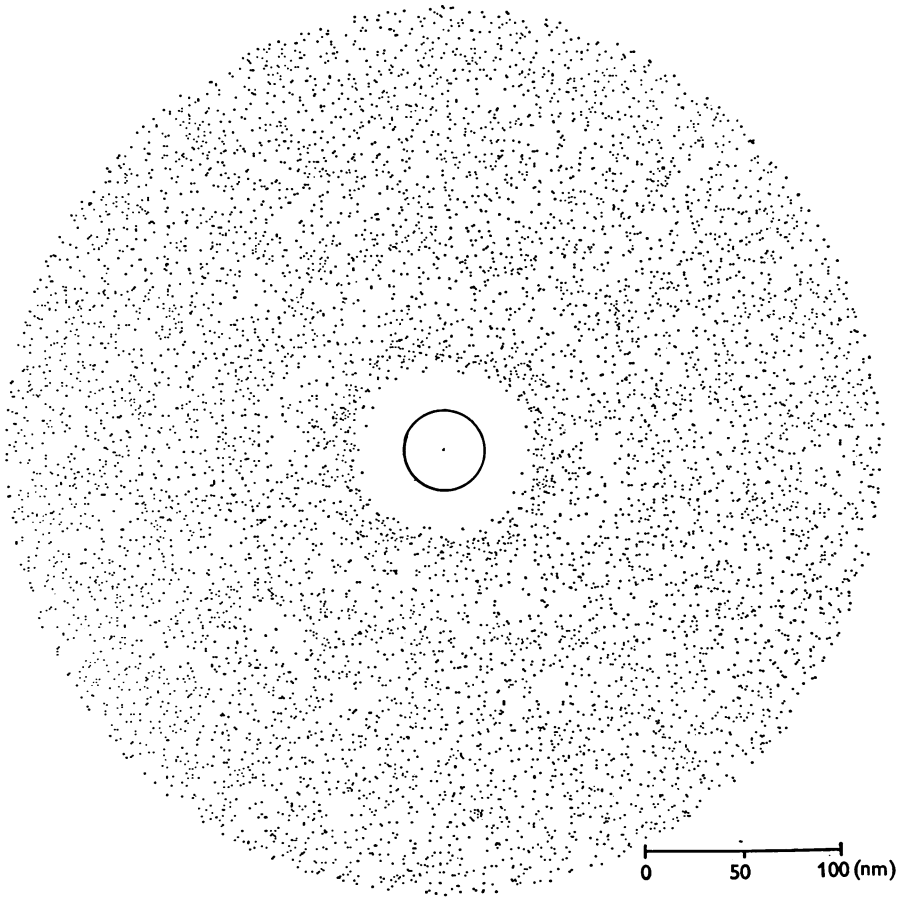
(1) Since the distribution about fibrils near the boundary of a lamella will necessarily be different from that about centrally located fibrils, it is important that the area covered by the lamella be large compared with the distance over which $g(r)$ differs sensibly from unity, so that only a negligible fraction of fibrils are in the 'boundary zone'. This condition will be violated in certain fish corneas (Goldmann & Benedek, 1967).

(2) The lamella must be spatially homogeneous. This condition is not usually met in swollen corneas (Pl. 2*b*).

(3) The distribution of fibrils about each other must be sensibly independent of angle, depending only on the radial separation. We have found this condition to be

well satisfied for lamellae of corneas of normal thickness (cf. Text-fig. 1), but not for swollen corneas, in which there tends to be a preferred direction of swelling.

Theory. The theoretical calculation of the transmissivity for an unpolarized light-wave incident on an array of cylinders embedded in a homogeneous ground substance has been presented (Hart & Farrell, 1969). The first step involves the determination



Text-fig. 1. Radial distribution of fibril centres about a reference centre at the origin, as obtained by translating the origin of the co-ordinate system to the position of 351 reference centres. There is a large 'effective excluded volume' region successively followed by regions of greater and less than average density. The 'hard core' diameter of a collagen fibril is indicated by the circle drawn about the origin.

of the electric field arising from the presence of a single cylinder illuminated by a plane wave. The second step consists of expressing the resultant electric field by summation of the fields of the individual light scatterers, taking into account the phases which determine the extent to which the fields add constructively or destructively. These phases are determined by the distribution of optical path lengths and

thus by the spatial distribution of the fibrils. Finally, the radiant flux scattered in any direction is proportional to the square of the resultant electric field, and the energy removed from the incident beam may be determined by integration over all angles of scattering. The detailed analysis shows that the transmittance, F_T, is given by

$$F_T = \exp\,[-\bar{\sigma}_s \rho_1 \Delta], \tag{1}$$

where ρ_1 is the bulk number density of collagen fibrils in the stroma, Δ is the thickness of the stroma, and $\bar{\sigma}_s$ the average scattering cross-section (per unit length) per fibril, is given by

$$\bar{\sigma}_s = \frac{\sigma_1}{2}(1-f_1) + \frac{\sigma_2}{2}(1-f_2). \tag{2}$$

Here, σ_1 and σ_2 are the scattering cross-sections (per unit length) of a single fibril for parallel and perpendicular polarizations, respectively, and are well approximated, (for fibril radius $\ll$ wave-length), by

$$\sigma_1 = 2\pi^2 \left(\frac{n_c + n_i}{n_i^2}\right)^2 \left(\frac{\pi n_i}{\lambda_0}\right)^3 [(n_c - n_i)a^2]^2 \tag{3a}$$

and

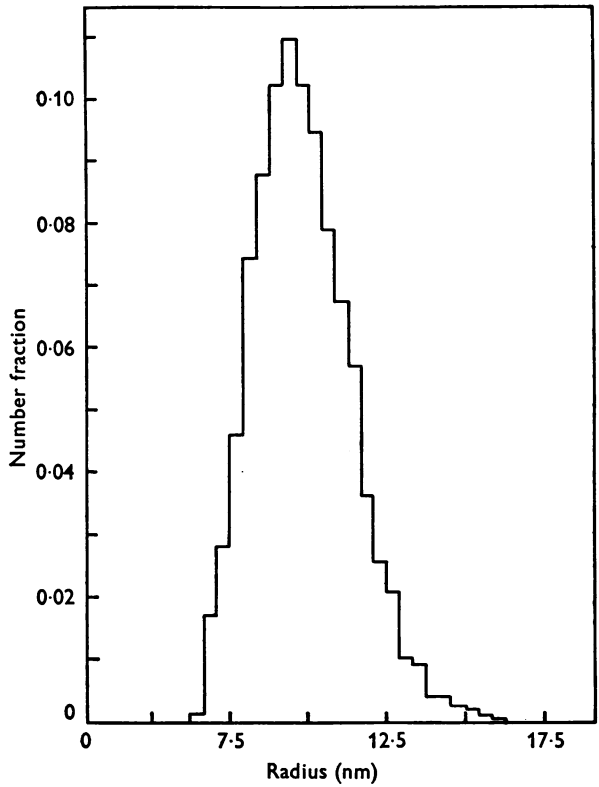
$$\sigma_2 = 4\pi^2 \left(\frac{n_c + n_i}{n_c^2 + n_i^2}\right)^2 \left(\frac{\pi n_i}{\lambda_0}\right)^3 [(n_c - n_i)a^2]^2 \tag{3b}$$

where n_c and n_i are the indices of refraction of the fibrils and ground substance, respectively, a is the fibril radius and λ_0 is the wave-length in vacuum. The numbers f_1 and f_2 involve rather complicated integrals over $g(r)$ and Bessel functions of argument $(2\pi n_i r/\lambda_0)$, and must be evaluated numerically for each case of interest. The mathematical expressions for f_1 and f_2 are given in the Appendix. It is noteworthy that f_1 and f_2 vanish if the fibril distribution is purely random, and approach unity for distributions such that destructive interference is essentially complete except in the direction of the incident beam.

RESULTS

Quantitative description of the ultrastructure of the corneal stroma. Three corneas were prepared for detailed quantitative analysis and representative cross-sections of lamellae taken from three depths of the central area of an individual cornea are shown in Pl. 3. The diameters of 37,149 fibrils were measured from six electronmicrographs of the three corneas, and a representative histogram illustrating the distribution of diameters is shown in Text-fig. 2. The range of fibril diameter was 12·5–32·5 nm but the majority of fibrils were of similar diameter. The results of analyses of the six micrographs are summarized in Table 1.

The important question arises whether the fibril diameters are consistent with the collagen content of the tissue. The electronmicrographs yield a mean collagen fibril volume fraction of 0·10 (Table 1). The collagen content of the ox corneal stroma has been reported to be approximately 13 % of the wet weight on the basis of the hydroxyproline analysis (Smits, 1957; Polatnick, La Tessa & Katzin, 1957). Assuming a similar value for



Text-fig. 2. Data analysis of 5932 fibril radii taken from an electronmicrograph of the anterior region of a normal rabbit cornea. The average fibril radius is 10·0 nm with a S.D. of 1·6 nm.

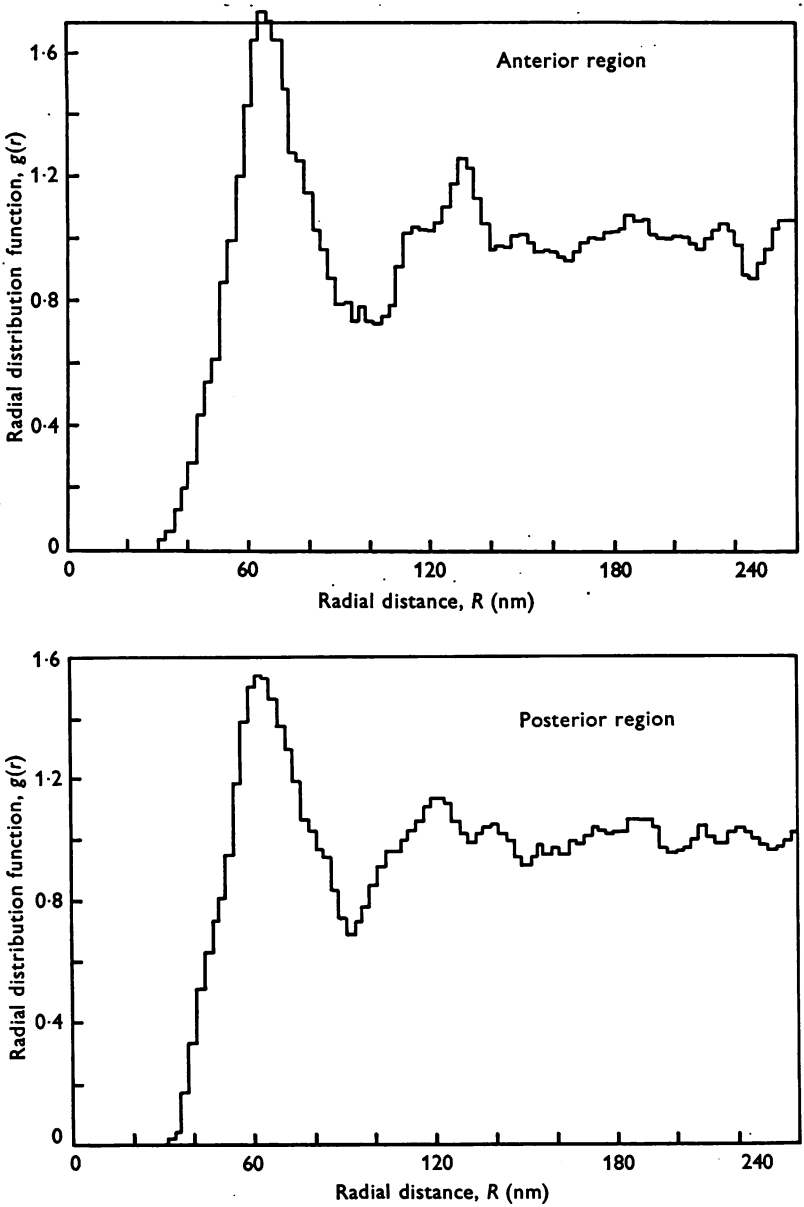
TABLE 1. Analysis of collagen fibrils in anterior, central and posterior regions of normal and swollen corneas. Fibril radii (a) are expressed in nm as arithmetic mean $\pm$ the S.D. The number of fibrils is in parentheses. Bulk density ρ_1 is expressed in $(\mu m)^{-2}$. The volume fraction $d_c = \pi a^2 \rho_1$

Cornea	Region of stroma	Fibril radii	Bulk number density	Volume fraction
		Normal cornea		
1	Anterior	10·0 ± 1·6 (5932)	301	0·09456
2	Anterior	9·3 ± 1·5 (5203)	318	0·08641
3	Anterior	9·5 ± 1·6 (5085)	391	0·11086
2	Central	9·9 ± 1·7 (7627)	372	0·11454
2	Posterior	8·9 ± 1·2 (5838)	379	0·09431
2	Posterior	8·9 ± 1·5 (7464)	388	0·09655
		Swollen cornea		
4	Anterior	9·6 ± 1·6 (5085)	—	—
5	Anterior	9·8 ± 1·5 (4550)	—	—
6	Posterior	8·8 ± 1·4 (5100)	—	—

the rabbit corneal stroma, that the specific gravity of the normal stroma is 1·05 (Maurice, 1957) and that the specific gravity of dry collagen is 1·41 (Bear, 1956), the volume fraction of collagen would be 0·10. In this respect, the agreement between the two values indicates that the fibrils as seen in the electronmicrograph are unswollen. This conclusion is in keeping with the observations of Pirie (1947) that corneal collagen is minimally swollen and reactive at physiological pH.

This conclusion is, however, in disagreement with the recent conclusions of Smith & Frame (1969). These authors stated that the collagen fibrils occupied 33 % of the total area of the extracellular matrix and calculated that only 31 % of each collagen fibril in the rabbit cornea is collagen. Unfortunately, the authors do not give sufficient data to allow one to account for the discrepancy.

We turn now to the characterization of the spatial distribution of the fibrils. The transmissivity of the cornea depends on the spatial distribution of the collagen fibrils because the phase of the light scattered by each fibril is determined by the optical path lengths. The distribution of optical path lengths may be described quantitatively in terms of the radial distribution function, $g(r)$. It is the ratio of local (number) density of fibril axes at a radial distance r from the axis of a reference fibril at $r = 0$, to the bulk number density of fibrils. Thus $g(r)$ specifies the relative likelihood of finding two fibril axes separated by a distance r. Since two fibril axes cannot approach each other more closely than the fibril diameter, it will certainly vanish for $r < 2a$, where a is the fibril radius. Since the presence of a fibril at the origin is not expected to influence the likelihood of finding another fibril at great distances, $g(r)$ will approach unity for large r. If the fibrils were spatially distributed purely at random, $g(r) \to 1$, whereas if they were distributed on a perfect lattice, $g(r)$ would vanish except for the discrete lattice spacing distances, where it would be arbitrarily large. Representative results for two regions of two corneas are shown in Text-fig. 3*a*, *b* (another may be found in Hart & Farrell, 1969). Except for the large distance scale, these distribution functions are quite similar to those of other disordered structures such as liquids. The Figure suggests the presence of local order extending out to perhaps 200 nm or more, in general agreement with the structural theory presented elsewhere (Farrell & Hart, 1969; Langham, Hart & Cox, 1969). At the larger distances, the undulations of $g(r)$ become obscured by scatter of the data. Ideally, this scatter could be reduced by increasing the number of reference centres, but in practice the structural detail then tends to become obscured by scatter arising from some degree of spatial heterogeneity. Whether the latter is a fixation artifact or not is unknown.



Text-fig. 3. Radial distribution functions obtained from data analysis of electronmicrographs of rabbit stroma. Text-fig. 3*a* is obtained from an anterior region using 506 reference centres, while Text-fig. 3*b* is from a posterior region using 732 reference centres.

Estimation of refractive indices. It was noted in the introduction that whereas the amount of light scatter is influenced by even slight changes in the difference between the refractive index of the collagen fibrils and that of the ground substance, the value of this difference is not accurately known. In the present calculation, we shall evaluate the refractive indices on the assumption that the fibrils are as the electron-microscope shows them to be. Since analysis of the electronmicrographs leads to a collagen volume fraction $d_c \approx 0{\cdot}10$ corresponding to the volume fraction of 'dry' collagen, we assign the fibrils an index of refraction corresponding to that of dry collagen, namely 1·547. The index of refraction of the ground substance, n_i, is then calculated using the Gladstone–Dale law in the form

$$[(n_c - n_i)d_c] = n_s - n_i \approx 1{\cdot}374 - n_i \tag{4}$$

where $n_s \approx 1{\cdot}374$ is the refractive index of the stroma (cf. Maurice, 1957; Smith, 1969).

It might appear that assignment of a maximal value to the refractive index of the fibrils would cause a serious over-estimate of the scattering since the fibrils *in vivo* may well be somewhat hydrated and therefore have an appreciably smaller index of refraction. In fact, however, the scattering depends primarily on the difference between the refractive indices of fibrils and ground substance multiplied by the volume fraction occupied by the fibrils. Thus, the diminished refractive increment resulting from hydration tends to be compensated for by the larger volume required by hydrated fibrils. This aspect may be illustrated most simply by giving attention to the parameter groupings that characterize transmissivity for a purely random distribution. Setting $f_1 = f_2 = 0$ in eqn. (2), the transmissivity for this case may be written

$$F_T = \exp\{-A[d_c(n_c - n_i)]^2\} \tag{5a}$$

where (using $d_c = \pi a^2 \rho_1$)

$$A = \frac{\Delta n_i}{\rho_1}\left(\frac{\pi}{\lambda_0}\right)^3 (m+1)^2\left[1 + \frac{2}{(m^2+1)^2}\right], \quad \text{with} \quad m = n_c/n_i. \tag{5b}$$

This result differs slightly from Maurice's result owing to a minor error in his eqn. (10), where the bracket should read

$$\left[\frac{1}{n_i} + \frac{2n_i^3}{(n_i^2 + n_c^2)^2}\right].$$

Values must be assigned to A and to $[d_c(n_c - n_i)]$.

Examination of eqn. (5*b*) reveals that the value to be assigned to A can be determined with satisfactory accuracy because it is insensitive to the fibril hydration. For example, consider a light wave-length (in air) of $\lambda_0 = 500$ nm, a normal rabbit corneal thickness $\Delta \approx 0{\cdot}037$ cm, and a representative fibril density $\rho_1 \sim 350/\mu^2$ (cf. Table 1). The index of refraction of the fibrils must lie between that of water and that of dry collagen ($\sim 1{\cdot}547$), whereas the index of refraction of the ground substances must be greater than but close to that of water. Thus, $A \sim 2 \times 10^3$.

This large value of A when inserted into eqn. (4*a*) indicates the importance of evaluating $[(n_c - n_i)d_c]$ with care, although for the living, normal eye, neither the fibril volume fraction d_c nor the small difference between the refractive indices are accurately known. The product of the two unknowns is estimated using the Gladstone–Dale law of mixtures. We note that the refractive index of normal stroma is independent of fibril hydration and has been fairly accurately determined to be $n_s \sim 1{\cdot}374$ (cf. Maurice, 1957). The quantity $d_c(n_c - n_i)$ in eqn. (5*a*) can be evaluated from the

Gladstone–Dale law expressed by eqn. (4), where the index of refraction of the ground substance is estimated from

$$n_1 = n_a + \frac{C_0 R_0}{1-d_c} \approx 1{\cdot}335 + \frac{C_0(0{\cdot}18)}{1-d_c}, \tag{6}$$

where $n_a \approx 1{\cdot}335$ is the index of refraction of the aqueous humor (cf. Maurice, 1957), $R_0 \approx 0{\cdot}18$ is the specific refractive increment (cf. Maurice, 1957) and $C_0 \sim 0{\cdot}05$ (Maurice, 1957) to 0·1 (Smith, 1969) is the concentration of (non-collagenous) organic material in the stroma. Equation (6) shows that $[(n_c - n_1)d_c]$ tends to be rather insensitive to fibril hydration for reasonably small values of d_c and C_0, although not for the larger values assumed by Smith (1969). Thus, our evaluation of the transmissivity for a purely random distribution is in qualitative agreement with that of Maurice (1957).

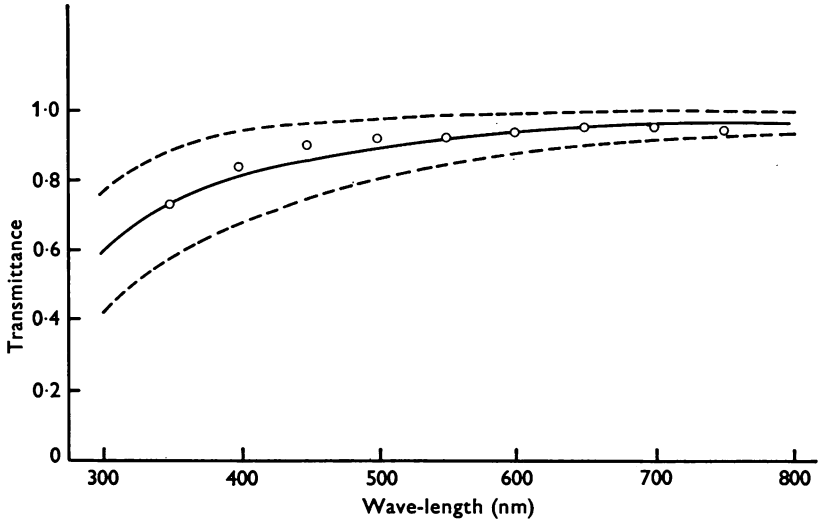
Transmissivity for fibril distributions obtained from electronmicrographs. Low transmissivity for a purely random distribution of fibrils implies that a high transparency of the stroma results because the light scattered by the individual fibrils essentially interferes destructively in all directions except that of the incident beam. A crystalline array would exhibit this property, and it might seem highly unlikely that a pseudo-random distribution of fibrils such as Pl. 1 reveals would behave like a lattice. Nevertheless, straightforward mathematical analysis leads directly to this perhaps surprising result.

The analytical and numerical details have been carried through elsewhere (Hart & Farrell, 1969). The results are summarized by Text-fig. 4 which presents the mean (and S.D. from the mean) of the theoretically calculated transmissivity *vs.* wave-length determined from six electronmicrographs of various regions of three normal rabbit corneas. Text-fig. 4 also shows the mean experimental results of transmissivity measurements on six rabbit corneas. It is evident that the agreement between theory and experiment is reasonably satisfactory, at least over the visible range. The theoretical calculations are only semi-quantitative because the variation of indices of refraction with wave-length is unknown and the refractive indices were assigned the constant values cited previously. The conclusion, therefore, is that the spatial distribution of the collagen fibrils seen on the electron-micrographs is consistent with transparency.

Decreased transparency and corneal swelling. In previous studies relevant to the problem of the cause of the increased light scatter with swelling, it has been found that corneal swelling induced by either mechanical damage to the limiting layers of the living animal or to decreased metabolism in the excised eye caused similar qualitative changes in the spatial distribution of the collagen fibrils (Langham & Cox, 1966; Langham *et al.* 1969). In all cases corneal swelling was confined within the lamellae with no demonstrable swelling of the keratocytes or the interlamellar regions.

Within the swollen lamellae the collagen fibrils were spaced more randomly (Pl. 2). Islands or domains devoid of collagen fibrils were found at all depths and regions of the swollen corneas. In a typical cross-section of an individual swollen lamella the long axis of the collagen-free spaces lay approximately parallel to the corneal surface and extended several microns. The short axis was generally no more than 100–200 nm.

Complete recovery of normal corneal thickness and spatial distribution of the collagen fibrils in the living animal could be demonstrated. In the enucleated eye corneal swelling induced by lowered temperature could be



Text-fig. 4. A curve of corneal transmittance *vs.* wave-length. The continuous curve is obtained by taking the average theoretical transmittance calculated from eight electronmicrographs. These included electronmicrographs of the anterior, central and posterior regions. The dashed curves represent the S.D. of the data about the continuous curve. The circles are the mean experimental transmittance obtained by averaging over six rabbit stromas.

reversed with a 70% recovery of corneal thickness (Langham & Taylor, 1956) but ultrastructural studies showed that collagen-free spaces remained. The role of the mucoproteins, mucopolysaccharides, and metabolism in determining this spatial distribution has been presented in previous papers (Farrell & Hart, 1969; Langham *et al.* 1969).

Detailed analysis of the collagen fibril diameters of normal and swollen corneas are tabulated in Table 1. Even in grossly swollen tissue no significant change in collagen fibril diameter was found.

If the increase in corneal thickness were associated with a uniform change in the spacing of the collagen fibrils, it may be calculated that the trans-

mission (500 nm) would decrease from 92 to 91 % for a swelling of 50 %. In spectrophotometric measurements at this wave-length, however, the mean transmission of six normal corneas of mean thickness $0{\cdot}40 \pm 0{\cdot}01$ mm was 88 ± 2 % and in the same corneas swollen to a mean value of $0{\cdot}62 \pm 0{\cdot}03$ mm the transmission was 67 ± 3 %.

The heterogeneity of the collagen fibril spacing in the swollen cornea precluded the application of the radial distribution analysis used in the normal tissue. Alternative theoretical approaches to this problem are now being considered and preliminary results indicate that a solution may be obtained giving a satisfactory correlation between the theoretically derived and experimentally determined values of light transmission in the swollen cornea.

DISCUSSION

It is evident that the transparency of the normal mammalian cornea to the visual wave-lengths may be explained from the ultrastructure shown in the electronmicrograph. Further, the change in the quasi-ordered–quasi-random arrangement of the collagen fibrils with swelling provides the basis for the explanation for the associated decrease in transparency.

These studies resolve the criticisms of the concept put forward by Maurice (1957) that the corneal transparency resulted from an arrangement of equal-sized collagen fibrils spaced in the form of a crystalline lattice. The lack of close order in the electronmicrographs of the corneal stroma led Maurice to conclude that the disorder was likely to be due to artifact in preparation of the electronmicrograph rather than its absence in the living state. On the other hand, Schwarz & Graf Keyserlingk (1966, 1969), Goldmann & Benedek (1967) and Smith (1969) considered electronmicrographs to be representative of the tissue to the extent that the explanation of corneal transparency could not derive from a crystalline lattice of collagen fibrils. The present results give strong support to the conclusion that the order of the collagen spacing is not crystalline but show that sufficient regularity is present in electronmicrographs of normal tissue to account for the observed transparency of the living tissue. Thus, it becomes unnecessary to postulate the existence of a crystalline arrangement that is somehow randomized during the process of preparing the tissue for electronmicroscopy.

The conclusion of Maurice that a random array of collagen fibrils would cause a marked decrease in the transmission of light through the cornea is supported by the present observations. This conclusion had been questioned by others primarily on the grounds that the fibrils might be more hydrated than Maurice assumed them to be, thereby having a smaller index of refraction and scattering less light (Smith, 1969). However, light scatter

tends to be independent of the degree of swelling of the collagen fibrils because, although the index of refraction of a fibril does indeed decrease with swelling, the effect is offset by the corresponding increase in fibril diameter.

Provided the collagen fibril diameters remain a small percentage of the wave-length of visible light, it is evident both theoretically and experimentally that the fibril diameters may vary considerably without modifying normal transparency. In individual corneas the range of fibril diameters was found to vary threefold, and in comparative corneal studies (Pl. 4) the mean collagen fibril diameter has been found to range from approximately 10 nm in the cornea of the glass cat fish to a mean of approximately 32·5 nm in man. Thus, whereas the collagen fibril diameters in the corneal stroma are much less than in the sclera (Pl. 1), indicating a different morphogenetic mechanism in the two tissues, it is apparent that the cellular and metabolic factors regulating the size of the collagen fibrils in the cornea allow a significant variation in the number of protofibrils comprising individual collagen fibrils both within a given species and in different animal species.

APPENDIX

The purpose of this appendix is to give the mathematical expressions relating the degree of destructive interference between the scattered waves to the radial distribution function. The influence of the radial distribution function is embodied in the functions f_1 and f_2 which are the ratios of the interference-to-incoherent contributions to the scattering efficiency per fibril for parallel and perpendicular polarization, respectively. These functions are discussed in Hart & Farrell (1969) and may be approximated by

$$f_1 = 2\pi\rho_1 \int_0^R r[1-g(r)]\{J_0^2(k_0 r) + 2\left(\frac{k_0 a}{2}\right)^2 J_1^2(k_0 r)\}\,\mathrm{d}r \qquad \text{(A 1)}$$

and

$$f_2 = 2\pi\rho_1 \int_0^R r[1-g(r)]\{J_0^2(k_0 r) + J_2^2(k_0 r) + \left(\frac{k_0 a}{2}\right)^2 [(2+m^2)J_1^2(k_0 r) + J_3^2(k_0 r)]\}\,\mathrm{d}r, \qquad \text{(A 2)}$$

where J_i is the Bessel function of the first kind of order i, and R is sufficiently large that there is essentially no correlation in the positions of particles for separation distances greater than R. Since the radial distribution function is obtained from data analysis of the fibril positions shown by the electronmicrographs (as described in the text), the integrals in eqns. (A 1) and (A 2) are evaluated numerically.

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EXPLANATION OF PLATES

PLATE 1

Electronmicrographs of the normal corneal stroma (inset) and sclera of an adult rabbit. The sections were taken from the same eye and have the same magnification (× 15,000). Sections were stained with uranyl acetate and lead citrate. Note the stromal and scleral cells.

PLATE 2

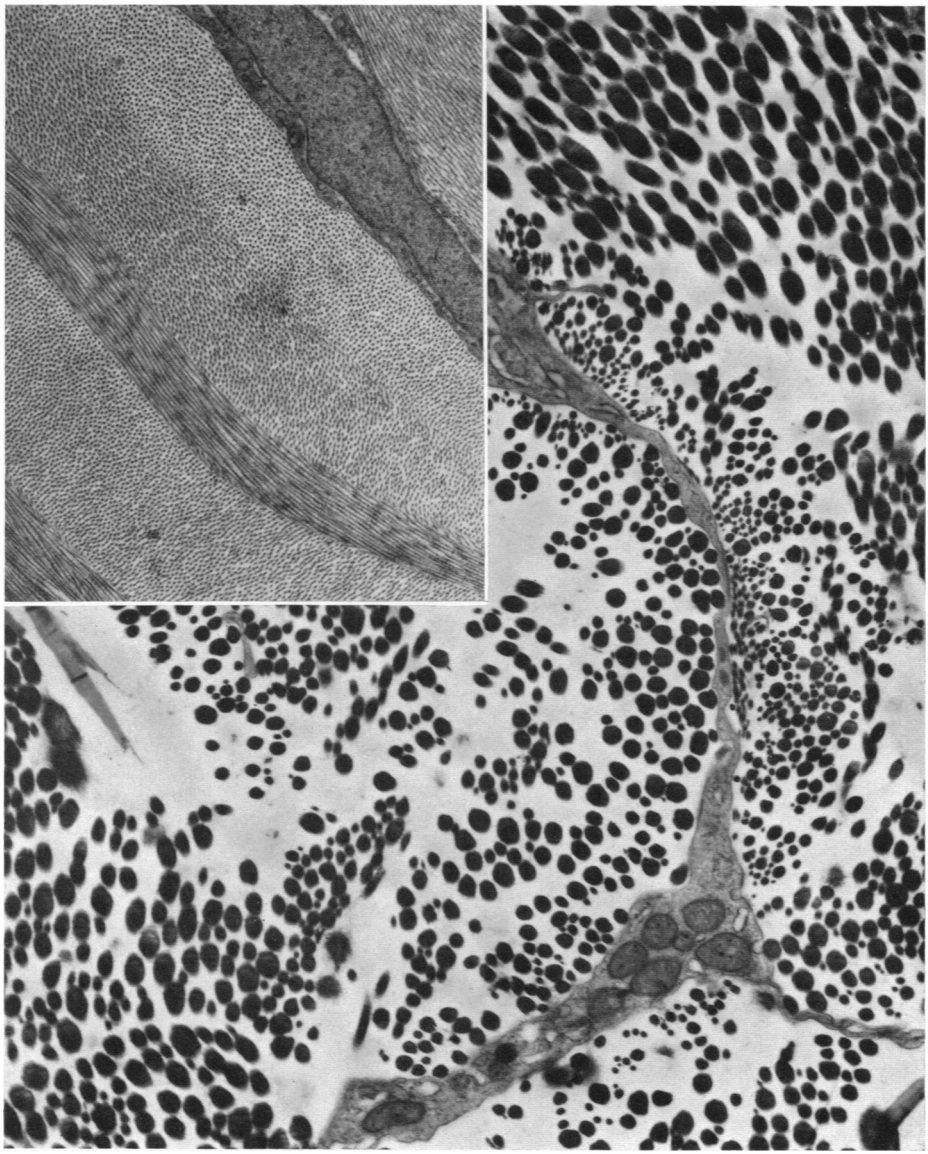
Comparison of the ultrastructure of a normal (*a*) and swollen (*b*) cornea of a pair of eyes taken from an individual rabbit. Swelling was induced by removal of the corneal epithelium 24 hr previously. Sections are of the same magnification (× 30,000).

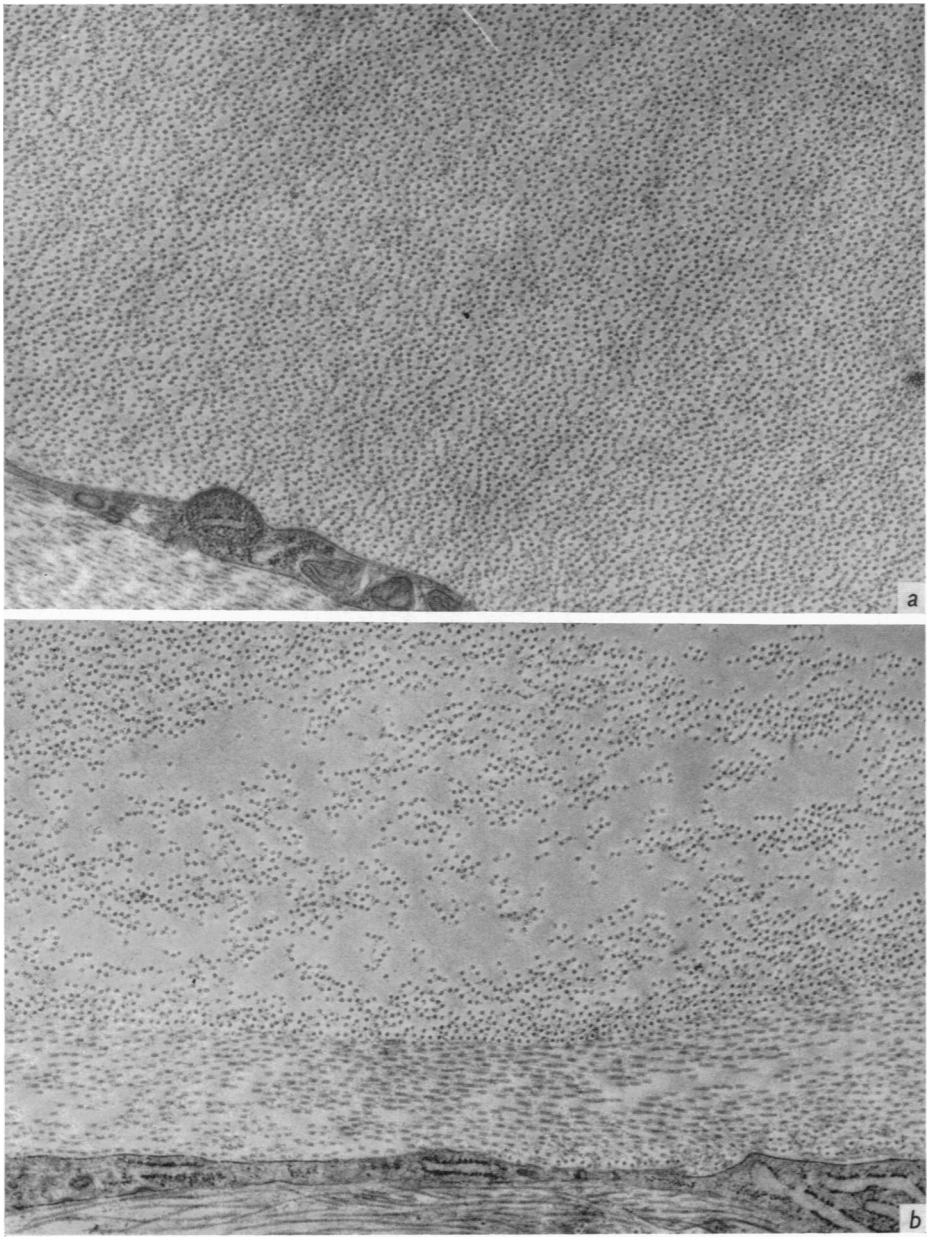
PLATE 3

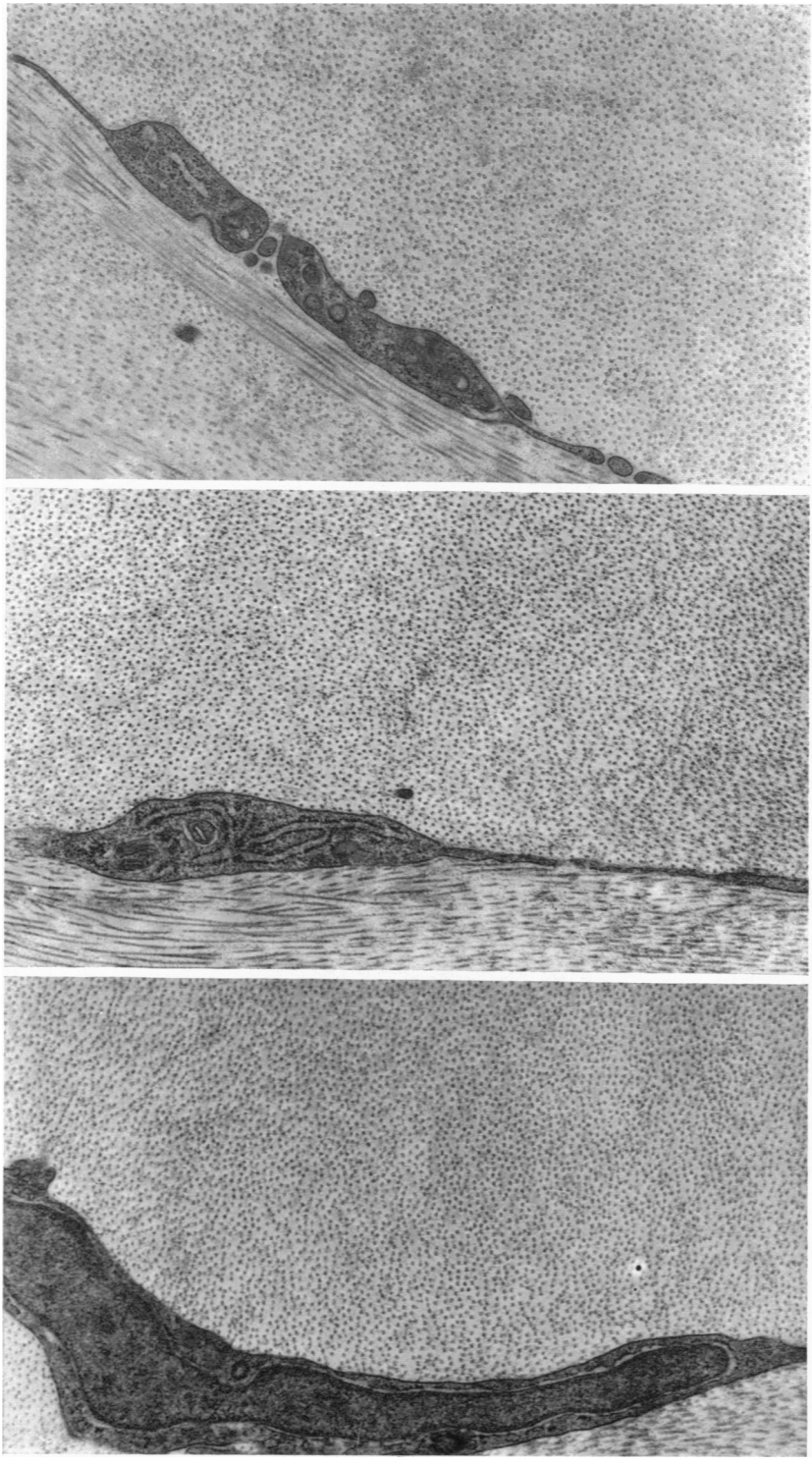
Electronmicrographs of collagen fibrils within lamellae located in the anterior, middle and posterior depth of the central area of a normal rabbit cornea. The fibrils are of essentially uniform diameter, ~ 20 nm, and are arranged in a quasi-random fashion, with an average centre to centre spacing of ~ 50 nm. Sections were stained with lead citrate and uranyl acetate (magnification × 33,600).

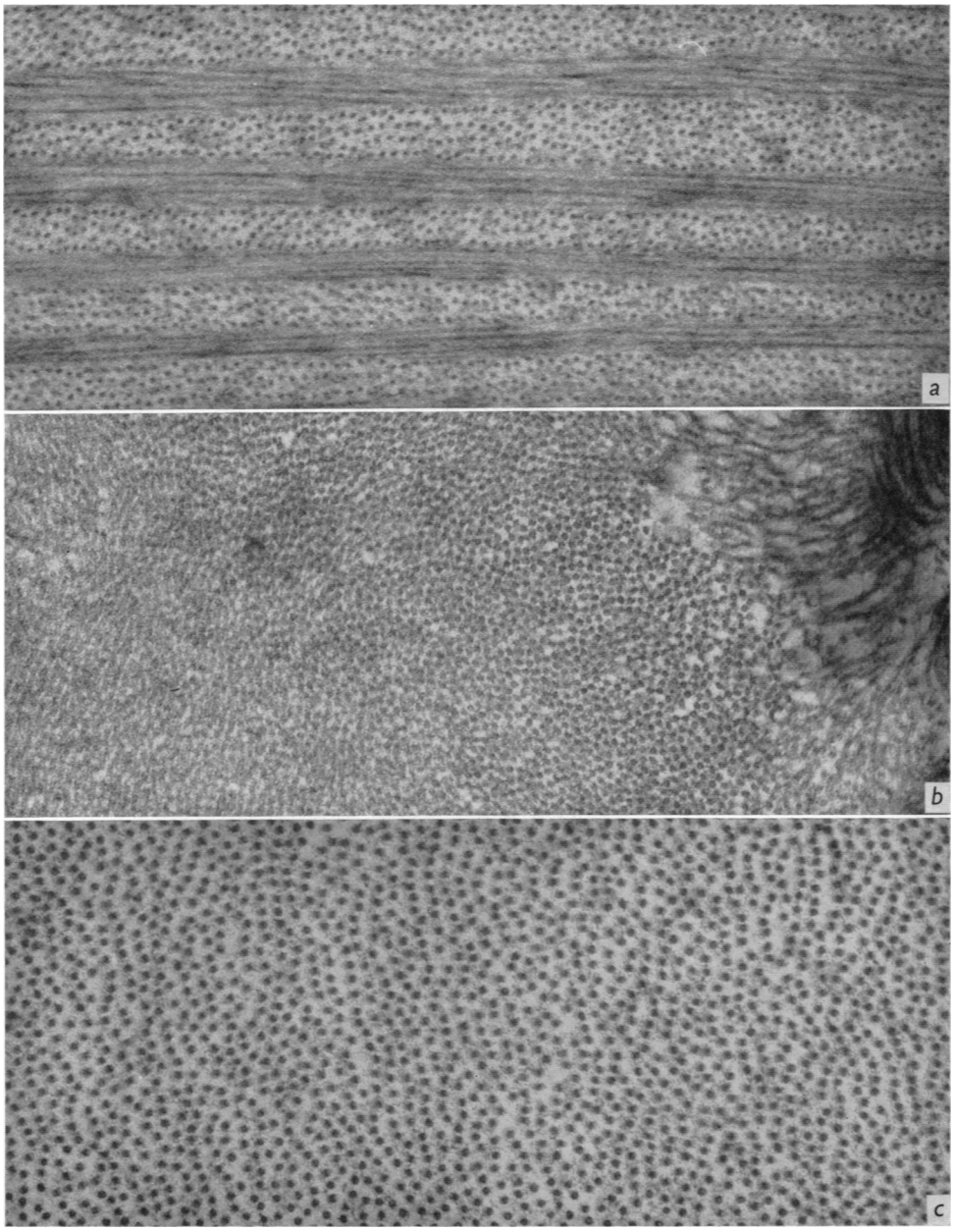
PLATE 4

Comparison of the ultrastructures of the corneal stroma of (*a*) the glassfish (*Chanda lala*), (*b*) the dogfish (*Mastelus canis*) and (*c*) the rabbit (magnification × 60,000).




a
b




a
b
c