THE STRUCTURE AND TRANSPARENCY OF THE CORNEA

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The cornea, belonging to the dioptric system of the eye and being part of its structural coat, must be both transparent and tough. Like other tissues of great tensile strength, the skin and sinews, it has a fibrous structure built up of collagen. The collagen fibres of the cornea are disposed in a layer, the substantia propria or stroma, which constitutes the greater part of its thickness. Unlike these other collagenous tissues the stroma is normally optically clear, but it tends to swell when in contact with fluid and becomes cloudy on swelling. This tendency is opposed by two cellular layers which cover the corneal surfaces, for if either of these layers is destroyed or severely damaged the stroma lying under the affected area will swell. Mechanical distortion of the cornea or compression of the eye-ball can also cloud the stroma; it clears instantly when the strain is removed.

The explanation of the transparency of the stroma can have a satisfactory foundation only on a knowledge of its optical structure, that is, the geometric form, dimensions and refractive indices of its components. The first part of this paper is concerned with establishing this structure. In the second part the transmission of light across it, and the physical basis of its transparency are considered, and in the third the disturbance to the transparency caused by swelling and mechanical strain.

The treatment of the problem of transparency and its solution, as given in this paper, have been the subject of a brief preliminary report (Maurice, 1954).

STRUCTURE

Descriptions of the *chemistry* of the cornea and its appearance under the *microscope* and *electron-microscope* are available in the literature. These indicate the chemical nature, size, shape and organization of the components of the stroma. A knowledge of their *refractive index* is then all that is required to determine the optical structure. Measurements on the tissue as a whole are of limited value, and isolation of the components probably leads to changes

in their hydration and consequently in their refractive index. The value of the *birefringence* or double refraction is of greater assistance, since it is determined largely by the difference in refractive index of the components in the intact tissue. Information available in the literature concerning these optical properties is supplemented with the writer's experimental work.

The *diffusion* of substances within the stroma gives an impression of the size of its submicroscopic interstices and of the nature of the substance filling them. This is a valuable auxiliary study because the experimental techniques cause little interference with the fresh tissue. The necessary data are provided almost entirely by original experimental work.

Chemistry

The dry weight of the stroma is about one-quarter of its fresh weight. For the cornea as a whole, the more careful analyses vary only slightly according to species and observer: ox 22%, rabbit 24% (Davson, 1949, 1955a), ox 22%, rabbit 22%, cat 24% (Duane, 1949). Inorganic substances make up 0·87% (Krause, 1934) of the fresh weight of the cornea; in the extracellular fluid of the stroma they are close to being in a Donnan equilibrium with the aqueous humour (Davson, 1949). The greater part of the organic material of the stroma is collagen, which constitutes about 82% of the dry weight (Krause, 1934). Among the extractable substance a sulphated mucopolysaccharide has been identified which accounts for 4·2% of its dry weight and is combined in a complex with an equal weight of protein (Woodin, 1954). The final 10% is probably largely composed of proteins and other water-soluble components of the blood. Roughly speaking, the fresh stroma may be taken as consisting of 76% of a fluid similar to aqueous humour, 19% collagen, 2% mucoid and 3% other soluble organic substances.

Microscope

Under the ordinary microscope, sections of the cornea show that essentially the stroma is made up of lamellae with their faces parallel to its surface. In between the lamellae lie a few flattened cells, and elastic and nerve fibres can also be distinguished; these represent a very small fraction of the volume and they may be ignored for the purposes of this paper.

The lamellae are composed of a transparent substance which appears to be structureless except when treated by special methods, and it is difficult to distinguish one from the other and to determine their dimensions. Salzmann (1912) describes them as broad thin bands which cross each other at wide angles, lacing in and out at very small angles to each other, so that they vary only slightly from a course parallel to the surface. Naylor (1953) identified each lamella with certainty by means of polarization microscopy and found they had an average thickness of 5μ in the cat. Their breadth, according to

Salzmann, is most difficult to determine but is certainly far greater than 20μ. Their length is the entire width of the cornea, according to Virchow (1910).

When the lamellar substance is opened up by teasing or similar techniques it is seen to have a fibrous structure. Virchow described the fibres as running straight along the lamellae and strictly parallel to one another. Their size has never been generally agreed upon, and structural units of different diameter denoted as fibres, fibre bundles, fibrils, etc., have been described by various authors; certainly, the size of the smallest structure visible with the microscope lies close to its limit of resolution. Virchow suspected, correctly as it turned out, that the fibres were bundles of yet smaller structures and for these he reserved the name of fibrils.

Electron-microscope

The appearance of the stroma under the electron-microscope has been observed by many workers, the most complete studies being those of Schwarz (1953) and Jakus (1954; private communication, 1955). They agree that each lamella is made up of long uniform fibrils lying parallel to one another (Pl. I, fig. 1). Generally, the fibrils are embedded in an adherent ground substance which hides the details of their structure, but this can be removed by vigorous chemical or physical treatment whereupon they show the typical cross-striation of collagen. The ground substance may be identified with the corneal mucoid, since in the sclera, where very much less mucopolysaccharide is present, the untreated collagen fibrils appear free from it.

The corneal fibrils are of uniform and equal circular section and seem to be indefinitely long. According to Jakus, their true diameter is 20–23 mμ in man and 23–27·5 mμ in the rat, though slightly higher values are recorded as a result of flattening if isolated fibrils are allowed dry down on to a supporting film. For the purposes of this paper the diameter of the dry fibrils will be taken to be 25 mμ.

Refractive index

The refractive index of the stroma has been measured with the Abbé refractometer with slightly different results according to the species. Average values are 1·375 for man (Aubert & Matthieson, 1876), 1·382 for the ox and 1·373 the pig (Aurell & Holmgren, 1953). The writer has made confirmatory measurements for the last two species, using six eyes in each case. The average values for the epithelial surface of the stroma were 1·382 and 1·374 respectively, in agreement with those previously found. Measurements were also made throughout the stroma at different levels, by splitting the tissue parallel to its surface with a spatula. The values found were consistently lower than those at the surface in both species; the difference seemed to be larger, the greater the depth of the section. The average values within the tissue were 1·376 in

the ox and 1·370 in the pig. The low value in the latter case accords with a low value of the dry weight found for these specimens, 21 %.

The approximate validity of Gladstone and Dales' law of mixtures may be assumed so that the refractive index of the stroma, n_s, should be given by

$$n_s = n_c d_c + n_i d_i, \tag{1}$$

where n_c and n_i are the refractive indices of the collagen fibrils and the interstitial ground substance, and d_c and d_i their volume fractions, so that

$$d_c + d_i = 1. \tag{2}$$

The writer extracted collagen from the stroma of the ox in order to measure n_c and d_c. The tissue was cut into fragments, extracted with water or 0·01N-NaOH, and in each case allowed to dry down to a thin film which was heated to constant weight at 105° C. The specific gravity of the films was measured by weighing them in air and nitrobenzene (specific gravity 1·196) and each gave a value of 1·34. Since collagen, dried in a similar way, makes up 0·19 of the weight of the stroma, which itself has a specific gravity 1·05 (Felchlin, 1926), it follows that $d_c = 0·15$. Small pieces of the film dried in this way, and over phosphorus pentoxide, were matched in refractive index with immersion fluids, using the standard Becke line method. This led to a value for n_c of 1·55.

It is assumed that the other organic substances are dissolved in the interstitial fluid and give a refractive index

$$n_i = n_a + \frac{c_s}{d_i} R, \tag{3}$$

where n_a is the refractive index of aqueous humour, c_s the concentration of these substances in the stroma, and R their specific refractive increment. Inserting values 1·335 for n_a, 0·045 g/ml. for c_s, 0·85 for d_i and 0·18 for R, between the known values for proteins (0·19) and mucopolysaccharide (0·16), a figure 1·345 is obtained for n_i. Then from equation (1) a value for n_s of 1·374 is obtained, within the range observed experimentally.

Though this gives an over-all check on the composition of the stroma, it does not necessarily follow that it is divided into dry collagen and ground substance in the way suggested. The collagen will almost certainly be hydrated with water and, perhaps, salt obtained at the expense of the ground substance. If the values of d_c and n_c for the dry collagen are written $\bar{d}_c$ and $\bar{n}_c$, the values appropriate to the fresh tissue will be

$$d_c = (1+h)\bar{d}_c, \tag{4}$$

and

$$n_c = \frac{\bar{n}_c + h n_a}{1+h}, \tag{5}$$

where h is the volume of water associated with one volume of collagen. Substituting equations (2), (3), (4) and (5) in equation (1) shows that the value of n_s is independent of the hydration of the collagen.

Aurell & Holmgren (1953) attempted to measure the refractive indices of the collagen and ground substance directly. In order to obtain samples of the latter they squeezed fluid from the cornea and found that the first drops which appeared had an index of 1·342. This is a little lower than the value calculated above for the ground substance, but there is no reason to suppose that all its constituents are present in the expressed fluid; some, in particular the mucoid, may interact with collagen fibrils and remain attached to them.

To measure the refractive index of the collagen they fixed the tissue, dehydrated it in alcohol, and then estimated its transparency when it was immersed in fluids of differing indices. The intact cornea was transparent in fluids over a range of 1·542–1·564, but if the structure of the stroma was broken up mechanically it was found to be fully transparent in a fluid of index 1·547 only. This is the refractive index of dry collagen, and it is doubtful whether the value applies in fresh tissue since it is unlikely to be unaffected by fixing and dehydration in alcohol. The results are interesting, however, in that they suggest that the transparency of the stroma is based on its internal arrangement.

Birefringence

It has been known for a long time (His, 1856) that the lamellae of the stroma are doubly refracting with their optic (slow) axis lying in the direction of the fibres. The stroma as a whole has an optic axis perpendicular to the corneal surface. Stanworth & Naylor (1953) have shown that this behaviour is to be expected if the lamellae lie upon one another with a random orientation, and proved that the value of the birefringence of the stroma should be one half that of the individual lamellae. Using this relationship, they obtained a value for the lamellar birefringence of 0·0028 from measurements on the retardation of light transmitted across the isolated cornea. Naylor (1953), by observation of the retardation of individual lamellae in thin corneal sections found a value of 0·0030.

The structure of a lamella as shown by the electron-microscope is that of a perfect mixed body of the rodlet type (Frey-Wyssling, 1948). Accordingly, it should exhibit a birefringence with the slow axis lying in the direction of the fibrils, in agreement with that found experimentally. The magnitude of this textural birefringence is given by Wiener's formula which is most readily evaluated in the form

$$\omega_t = \frac{d_c d_i (n_c^2 - n_i^2)^2}{2n_s [n_c^2 + n_i^2 - d_c(n_c^2 - n_i^2)]} \, . \tag{6}$$

The four unknown quantities d_c, d_i, n_c, n_i in this equation, and the unknown h, are connected by the four independent equations (2), (3), (4) and (5), and a knowledge of the value of ω_t is sufficient to determine them absolutely. Unfortunately, the measured birefringence comprises not only this textural

component, but also one intrinsic to the collagen molecule. The standard method of distinguishing between these components is to measure the retardation of the tissue when impregnated with fluids of differing refractive index. The retardation passes through a minimum when the textural birefringence is zero. At this point the refractive index of the impregnating fluid is equal to n_c, and the remaining retardation corresponds entirely to the intrinsic birefringence.

Naylor (1953) applied this method to the cornea and found that the minimum retardation was shown in a fluid of index 1·50–1·52, and that the intrinsic birefringence was a third to a half of the total. However, it was necessary to fix the cornea and impregnate it with non-aqueous liquids in order to obtain a high enough refractive index. It is likely that under these conditions changes in the hydration and structure of the collagen may have occurred.

Because of this uncertainty, the writer has carried out some measurements of the retardation of the fresh isolated cornea of the rabbit, details of which will be published elsewhere. The experimental method was essentially that of Stanworth & Naylor (1950, 1953) but certain refinements were added; in particular, the thickness of the cornea was measured by an optical method capable of an accuracy of 0·01 mm (Maurice & Giardini, 1951 a).

The proportions of intrinsic and textural birefringence were estimated by allowing the tissue to dry, when the ground substance becomes sufficiently concentrated for its refractive index to equal that of the fibrils. The textural birefringence then vanishes and what remains is wholly intrinsic. The cornea was allowed to dry as far as it would, at first in air and then in a desiccator, and its thickness and retardation were measured at various stages. Similar measurements were taken when the cornea was allowed to swell in water. Experimental points from two corneae are plotted in Text-fig. 1. The retardation of the stroma at its normal thickness, 0·38 mm, corresponds to a total birefringence of 0·0027, in good agreement with the values of Stanworth & Naylor.

The curves drawn in the figure were constructed according to Wiener's formula to relate the thickness with the retardation. In their calculation the following assumptions were made:

(1) The hydration of the collagen fibrils is independent of the thickness of the cornea. The values of d_c and n_c appropriate to each curve were calculated by means of equations (4) and (5).

(2) The refractive index of the ground substance obeys equation (3) when dilute, and analogous equations to (4) and (5) when concentrated. The dry interstitial material is assumed to have the same density and refractive index as dry collagen; considerable variations in these figures would have little influence on the shape of the curves.

(3) The retardation which remains when the cornea will dry no further

corresponds to the intrinsic birefringence and does not alter in value as the tissue swells.

It can be seen that the experimental points conform very well to the shape of the theoretical curves and lend support to the above assumptions. The value of the hydration which gives the best fit to the points is 0·55, which leads to a value for n_c of 1·47, d_c of 0·23, and a fibril diameter of 31 mμ.
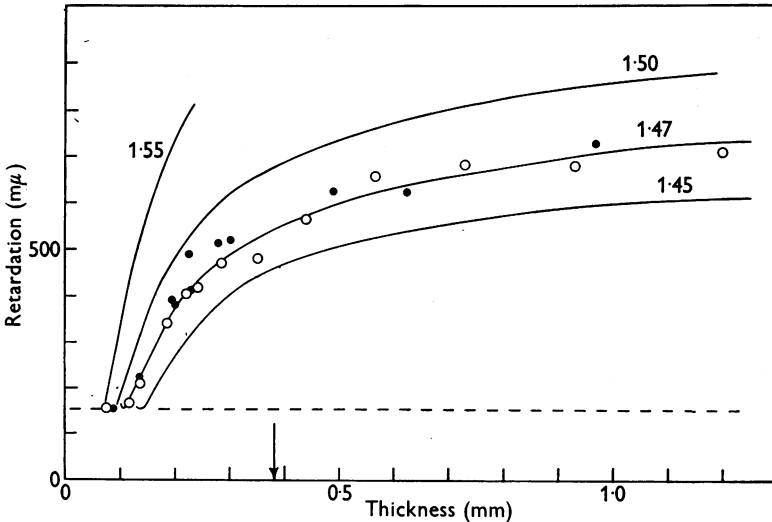

Fig. 1. Relationship of retardation and thickness of stroma; normal thickness, 0·38 mm. The points are experimental values from two corneae. The curves are derived from Wiener's formula as described in the text. Each curve is labelled with the refractive index of the fibrils and corresponds to a different state of their hydration.

The evidence of double refraction, then, accords with the picture given by the electron-microscope of the stromal lamellae being made up of straight parallel collagen fibrils. In addition, it provides information about their state of hydration. It does not necessarily confirm the state of subdivision of the fibrils shown by the electron-microscope, since the birefringence would have the same value whatever their size, so long as they approximated to cylinders of uniform refractive index.

Diffusion

The writer has carried out some studies on the diffusion of haemoglobin in the cornea with the following results. When a very small volume of concentrated mammalian haemoglobin was injected into the stroma *in vivo* or *in vitro* it was observed to diffuse both along and across the tissue. If a thick cornea such as that of the ox was used and it was sectioned at the point of injection an hour or two later, it was seen that the diffusion took place more or less at the same speed in the two directions. The rate of spreading of

haemoglobin along a thin strip of cornea from a drop injected into its mid point, was measured photo-electrically. This led to a diffusion constant of 3.6×10^{-8} cm²/sec at 0° C, about 10 times lower than its rate in free solution. If a cornea was allowed to dry a point was reached, when it was about 50 % of its original thickness, at which haemoglobin would be no longer spread, the boundaries of an injected volume remaining sharp after several days. Diffusion both across and along the tissue appeared to be prevented at the same time.

Similar experiments were carried out with the haemoglobin of the mollusc *Planorbis* which has molecules of uniform weight, 1,630,000, which is 24 times greater than that of mammalian haemoglobin (Svedberg & Pedersen, 1940). It was found that a small volume of its solution injected into a cornea would not diffuse through the stroma in either direction. If the cornea was allowed to swell in saline, however, the haemoglobin started to diffuse when it was about 150 % of its original thickness; the movement along the stroma began slightly before that across it.

This evidence shows that diffusion in the stroma is limited by a structural system which becomes more open as the tissue swells. The equal freedom of movement in each direction suggests that it is taking place in the substance of the lamellae and not in any spaces between them. Since it is probable that the swelling takes place in the interstitial substance and leads to a separation of the fibrils, it is tempting to assume that these are the structures limiting the diffusion. If the dimensions of the spaces between the fibrils are considered, this view seems tenable.

The hydrated fibrils occupy 0.23 of the stromal volume, and are 31 mμ in diameter; if they are equally spaced from one another, the distance between the axes of neighbouring fibrils will be about 62 mμ. The gaps between the fibrils will therefore be 31 mμ on the average. The molecule of a mammalian haemoglobin has been estimated to be a cylinder 5.7 mμ in diameter (Wyman, 1948); when hydrated this would be increased to about 6.4 mμ. *Planorbis* haemoglobin has a molecular weight 24 times that of mammalian haemoglobin, so that the diameter of its molecules will be about 18.5 mμ. The diameter of the largest molecules which can diffuse in the stroma in its normal state is, then, probably of the order of 12 mμ.

There are several experimental results that suggest that when dimensions are of this order structures are capable of restricting the movement of molecules much smaller than would be indicated by a strictly geometrical relationship (Pappenheimer, 1953; Maurice, 1953). The discrepancy in the stroma, the abolition of diffusion of molecules $2\frac{1}{2}$ times smaller than the tissue spaces, is comparable with that found in other cases. It is possible, however, that the movement of haemoglobin is restricted further by the ground substance.

The diffusion of small molecules across the stroma is also informative. The

only substance for which the diffusion rate has been explicitly calculated is the tracer ion [24]Na (Maurice, 1951). It was estimated that it diffused eight times more slowly than in free solution. The value taken for the thickness of the cornea in that paper has since been shown to be too small (Maurice & Giardini, 1951*b*) and a better estimate would be five to six times more slowly. It is possible also to calculate the diffusion rate of heavy water across the excised cat's stroma from the experiments of Cogan & Kinsey (1942*a*). Taking its thickness to be 0·6 mm, this leads to a value five times slower than that in free solution at the same temperature.

The obstacle the fibrils present to the diffusion of such small particles within the stroma can be derived from the formulae of Rayleigh (1892). In the direction along the fibrils the diffusion rate will be unaltered from that in the interstitial fluid; across the fibrils it will be reduced by the factor $1 + d_c$, that is 1·23. The greater part of the resistance to diffusion must therefore lie in the ground substance; since the resistance to Na and heavy water is similar, it seems to be in the nature of a viscosity.

TRANSPARENCY

A tissue which is made up of components of submicroscopic dimensions, which are transparent but of different refractive indices, may be expected to diffuse light by the process of scattering. Objective and subjective estimates of the *corneal scattering* agree that it is very small. If, on the other hand, the *scattering by the collagen fibrils* is theoretically calculated on the assumption that the scatter by each fibre is independent of its neighbours, and is summed up for the whole cornea, it leads to the conclusion that the cornea should be opaque. Attempts to reconcile current *explanations of transparency*, particularly that the stroma has a uniform refractive index, with the experimental evidence for its structure are not satisfactory. The conclusion has to be drawn that the fibrils do not scatter energy independently of one another and a '*lattice theory*' of the transparency is put forward in which the scattered light is suppressed as a result of mutual interference. Preliminary evidence of the arrangement of the fibrils in a lattice is provided by *X-ray diffraction*.

Corneal scattering

The healthy cornea appears to be perfectly transparent under ordinary illumination. However, in an intense beam of focused light, such as the slit-lamp provides, the stroma appears slightly opalescent. The writer has estimated the light scattered back from the human cornea by focusing a slit-lamp beam perpendicularly upon it, and bringing the image of the illuminated area on to a photo-cell by means of a microscope (Langham & Wybar, 1954). The intensity and distribution of the light was compared with that scattered from a flat surface covered with magnesium oxide. It was found that 0·3 % of the

incident light energy was scattered back if it was blue and 0·1% if red. Part of this light may have been returned from the superficial layers of the cornea and their interfaces, and part from the cells of the stroma. These, therefore, represent maximum limits for the lamellar substance itself. It is difficult to estimate the total light scattered by the cornea since it is probable that the greater part is directed forward and much at a small angle to the transmitted beam (p. 280). This low angle scatter would form part of the 'ciliary corona' which surrounds a point of bright light in the darkness and has not yet been ascribed to any particular structure of the eye (Simpson, 1953). Nevertheless it is unlikely that the total light scattered at all but very small angles exceeds 1% of that incident upon the cornea.

Scattering by the collagen fibrils

If a lamella has the structure shown by the electron-microscope of long, straight, round, collagen fibrils embedded in ground substance, the light it should scatter may be calculated. The general case of the scattering of a plane electromagnetic wave falling perpendicularly on a dielectric cylinder of infinite length has been worked out theoretically by Schaefer (1909) and submitted to an experimental check (Schaefer & Grossmann, 1910). He gave the results in terms of a series of Bessel functions, but if the circumference of the cylinder is small compared to the wave-length they may be simplified.

There are two cases to be considered, distinguished by the plane of polarization of the incident wave. If the electric vector is parallel to the fibril axis the light is scattered uniformly at all angles to the axis and its electric amplitude at a distance r is given by

$$E_{\parallel} = \tfrac{1}{2}E\left(\frac{\lambda}{4r}\right)^{\tfrac{1}{2}}\left(\frac{2\pi\rho}{\lambda}\right)^{2}(n_c^2 - n_i^2), \tag{7}$$

where E is the amplitude of the incident light in the stroma, λ its wave-length *in vacuo*, and ρ the radius of the fibril, and n_c and n_i the refractive indices of the two media, as before.

If the electric vector is perpendicular to the fibril axis the amplitude of the light scattered at an angle ϕ to the direction of the incident wave is

$$E_{\perp} = E\left(\frac{\lambda}{4r}\right)^{\tfrac{1}{2}}\left(\frac{2\pi\rho}{\lambda}\right)^{2}\left(\frac{n_c^2 - n_i^2}{n_c^2 + n_i^2}\right)\cos\phi. \tag{8}$$

The energy scattered by each length l of fibril when unpolarized light falls on it is, then

$$\sigma = \int_{-\pi}^{\pi} \frac{n_s c (E_{\parallel}^2 + E_{\perp}^2)}{4\pi}\, lr\, d\phi$$

$$= \frac{n_s c E^2}{4\pi}\frac{\pi l\lambda}{8}\left(\frac{2\pi\rho}{\lambda}\right)^{4}(n_c^2 - n_i^2)^2\left[1 + \frac{2}{(n_c^2 + n_i^2)^2}\right], \tag{9}$$

where c is the velocity of light, and n_s is the refractive index of the stroma.

This equation is applicable when l is finite but very large compared to λ. The scattered light will be confined within the two planes passing through the ends of the length l perpendicularly to the fibril.

In a layer of tissue of width across the fibrils, w, and thickness Δt there will be a number $\dfrac{d_c w \Delta t}{\pi \rho^2}$ of fibrils. If each scattered energy independently of the others, the total light scattered by a length l of the tissue layer will be this number times σ.

The amount of energy in the incident beam falling on the area lw will be $\dfrac{n_s c E^2}{4\pi} lw$ for each plane of polarization. The fraction of the incident light scattered is, therefore,

$$\Delta S = \frac{d_c \pi^4 \rho^2}{\lambda^3}\left[1 + \frac{2}{(n_c^2 + n_i^2)^2}\right](n_c^2 - n_i^2)^2 \Delta t$$

$$= k\Delta t. \tag{10}$$

For the total thickness t of the stroma the fraction of the incident light scattered will be

$$S = 1 - e^{-kt}. \tag{11}$$

It may be noted that only the terms $d_c \rho^2 (n_c^2 - n_i^2)^2$ of the scattering constant k vary markedly with the internal constitution of the stroma. Similarly, only the numerator in equation (6) varies markedly with the constitution; when d_c is small this reduces to $d_c(n_c^2 - n_i^2)^2$. The similarity arises because both the scattering and birefringence depend upon the dipole moment induced in the fibrils by the electromagnetic field.

Inserting in equations (10) and (11) the values derived previously for d_c, n_c, n_i, and ρ, with a representative value for λ, 500 mμ, and the thickness of the human stroma, 0·46 mm, the fraction S becomes 94 %. If, therefore, the optical structure of the stroma established previously and the assumptions made in deriving the formulae are correct, the cornea should scatter most of the light falling upon it and be opaque.

Explanations of transparency

The oldest and most generally accepted theory which accounts for the transparency of the stroma is that all its components have the same refractive index. Before the electron-microscope revealed the fundamental structure of the tissue, these components were supposed to be the interstitial fluid and a structural unit—the fibre or lamella. Other writers allowed that the components could have different indices, but postulated that refraction at their surfaces was reduced by their close apposition (Ranvier, 1881) or the reduction of the fluid component to a minimum (Cogan & Kinsey, 1942b). These considerations are superfluous to the essential problem of transparency as set out

in the previous section. More recently, Schwarz (1953) and Davson (1955b) have applied the uniform refractive index theory to the structure seen by the electron-microscope, and have suggested the function of the mucopolysaccharide is to raise the index of the ground substance to that of the collagen.

Equation (10) shows that the stroma could be transparent if either ρ or $(n_c - n_i)$ were very small. The former condition may be held to correspond to a uniform refractive index as much as the latter, since in any clear solution there exist differences in refractive index over regions of molecular size. The possibility of reconciling either of these conditions with the experimental evidence must be explored before the hypothesis of equal refractive index throughout the stroma can be rejected.

To reduce the scattered light to the value actually found, the diameter of the dry fibrils would need to be 2 mμ or less, n_c and n_i retaining the values corresponding to the birefringence. These could correspond to the protofibrils which are believed to be the basic elements of collagen structure (Bear, 1952). If this was true, the fibrils seen by the electron-microscope would be artifacts, and in the fresh tissue they would be divided into more than a hundred strands, separated from one another so that their distribution throughout the stroma is more or less uniform. On the contrary, the electron-microscope picture is of extremely well-defined structures, and no worker has reported any evidence of their subdivision. The diffusion of haemoglobin in the stroma also argues against this possibility and it would be necessary to suppose the existence of a system of channels running in all directions in the tissue to account for its movement.

The second possibility, that the light scattering is low because $n_c - n_i$ is smaller than previously estimated, implies that the fibrils are very hydrated. By equation (5) they would need to take up 4·5 times their own volume of water in order for the value of n_c to fall to 1·374, the refractive index of the stroma. The fibrils would then approximate to cylinders of 60 mμ diameter. This structure is compatible with the appearance under the electron-microscope since the specimens are necessarily dried in their preparation and the hydration of the collagen in the fresh tissue is irrelevant. On the other hand, the reduction in the refractive index difference would make the textural birefringence very small, and the birefringence of the lamellae would be almost entirely intrinsic to the collagen fibrils. This conflicts with the experimental evidence previously described. Another argument against this very hydrated structure is that the diameter of the fibrils, 60 mμ, is about the same as the average distance between their centres, 65 mμ. This is difficult to reconcile with the experimental finding that there is little more resistance to the diffusion of mammalian haemoglobin, diameter 6·4 mμ, than to the Na ion in the stroma.

It could be postulated that the fibrils were swollen to this diameter, and that the water taken up was not bound to the collagen but separated it into

protofibrils of high refractive index which recombined into single fibrils on drying. If a system of channels to permit diffusion was presumed to exist it would seem that all the experimental findings could be explained. This solution, however, meets a further objection which is put forward in the final section of this paper (p. 283).

Caspersson & Engström (1946) have advanced a different kind of explanation of the transparency, suggested by the evidence that it is dependent on an internal arrangement in the stroma (p. 267). They supposed, on the basis of a peak in the absorption spectrum of the cornea, that the diameter of the fibrils was 300 mμ. It was postulated that these fibrils were strictly arranged so that their centres were spaced at equal distances along straight lines perpendicular to the corneal surface. According to this hypothesis, a ray of light passing down the row of fibrils would be refracted to and fro and would finally emerge undeviated. Reflexions at the fibril surfaces would be avoided by an envelope of mucoid whose refractive index tapered from that of the fibril to that of the interstitial fluid.

This theory is not satisfactory on its own terms, since it makes no provision for rays falling other than perpendicularly on the corneal surface, and it necessitates a determined number of fibrils in the row, else the emergent ray will be deviated. More important is that the transmission of light across structures as small as 300 mμ in diameter cannot be treated in terms of rays, and this applies with greater force to those of 30 mμ in diameter which the electron-microscope shows to be present.

It cannot be decided readily whether a region of tapering refractive index around the fibril would affect the amount of light it scatters. The writer knows of no theoretical treatment of this problem, but since both the birefringence of the tissue and its light-scattering properties are linked to the dipole moment induced in the fibrils by the electric component of the electromagnetic field, it is unlikely that the latter could be reduced very markedly from the value calculated earlier without a corresponding change in the former.

Lattice theory

The discussion in the previous section failed to find a satisfactory mechanism whereby the light scattering of the individual fibrils could be reduced which was compatible with the experimental evidence. This makes it necessary to question the validity of an assumption made in calculating the scattering caused by the stroma—that the fibrils scattered energy independently of one another.

The electromagnetic wave radiated by a fibril is determined by the dipole moment induced in it, this is dependent only on its radius, its refractive difference from the surrounding medium, and the amplitude of the electric component of the oscillatory electromagnetic field which surrounds it. Only the last can be influenced by neighbouring fibrils, and the magnitude of this

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interaction may be assessed by considering the disturbance to the incident field produced by any one fibril. By analogy with the standard treatment of the Herzian dipole (e.g. Joos, 1934) it should generate an electromagnetic field which contains both a propagated and a non-propagated component. The electric amplitude of the propagated component is given by $E_\parallel$ and $E_\perp$ of equations (7) and (8), which fall off as the square root of the distance from the axis of the fibril and amount to 1 or 2% of E, the incident amplitude, at its surface. The non-propagated component is that of dipole induced in the fibril by the electric field of the incident wave. When this is perpendicular to the axis of the fibril this dipole produces a local field which falls off as the square of the distance from the axis, and has extreme values of $\pm \dfrac{n_c^2 - n_i^2}{n_c^2 + n_i^2} E$, or 9% of E, at the fibril surface (Rayleigh, 1892). When the incident electric field is parallel to its axis, the dipole induced in the fibril causes no local variations in the field; within a lamella the field is everywhere tangential to the fibril surfaces and, therefore, everywhere equal. In the extreme case then, where one fibril touches another, it will alter the electric field surrounding it and hence its polarization by only a few per cent. If the value for E, the electric amplitude, is taken to be that in the stroma, equations (7) and (8) will apply with sufficient closeness.

In general, it is the amplitude of the scattered wave as expressed by these equations, not its energy, that is the constant quantity associated with the radiation from a fibril. The waves emitted by the separate fibrils will mutually interfere and, according to whether this leads to their reinforcing or destroying one another, more or less energy will be scattered. The total scattered energy is equal to the sum of that which would be radiated by the fibrils if isolated from one another only if their axes are distributed in a random manner within the lamella. If there is any marked regularity in their arrangement the amount and distribution of the energy may be profoundly modified. For example, if n fibrils join together to form a bundle whose diameter is still small compared to the wave-length, the energy they scatter will be n² times that of a single fibril, not n times. This follows from equation (9), since the scattered waves reinforce one another giving rise to one wave of n times the amplitude; the same result is obtained if the bundle is considered as a single fibril of $\sqrt{n}$ times the radius.

If, on the other hand, the fibrils are spaced out in a regular order within the lamella (Text-fig. 2) the individual scattered waves will interfere destructively in all directions except that of the incident beam. The scattered light will be projected entirely in this direction and the tissue will appear transparent.

This may be shown by the methods used in the elementary treatment of diffraction, each fibril being considered as the source of a real Huygens'

wavelet. More simply, it may be demonstrated by treating each row of the arrangement as a diffraction grating. It is the characteristic of a grating that it gives rise to energy maxima at angles θ to a normally incident beam given by $\sin \theta = m\lambda/d$, where m is an integer and d is the spacing of the grating elements. In a row of fibrils d/λ is less than unity, so this equation will be satisfied only when m is zero and θ is 0° or 180°.

If the fibrils were spaced out regularly in one dimension only, the tissue would reflect a fraction of the light falling on it in any but along this direction. This would be avoided if the arrangement of the fibrils was in a two-dimensional lattice. A regular hexagonal order is illustrated in section in Text-fig. 2, but any other would function as well.
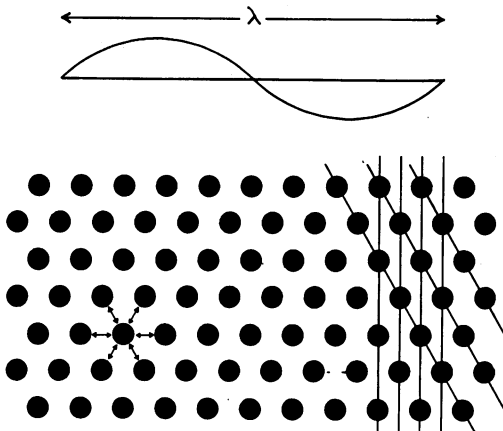

Fig. 2. Arrangement of fibrils in lattice, shown in section, proposed to explain transparency of cornea. A hexagonal lattice is shown; the lines passing through the fibrils are two sets of lattice planes. The arrows between the fibrils indicate the system of forces which is supposed to maintain the regularity of the structure. The wave-length of light is drawn above for comparison.

For a tissue to be transparent it is necessary that its fibrils are parallel, equal in diameter, and have their axes disposed in a lattice. The sclera is opaque because, although the fibrils are parallel over limited regions, a range of diameters of 28–280 mμ is revealed by the electron-microscope (Schwarz, 1953); the greater light-scattering by the larger fibrils itself adds to the whiteness of the tissue. The freedom of the fibrils of the sclera from interstitial mucoid makes it unlikely, furthermore, that there is any order in their arrangement.

X-ray diffraction

If the explanation given above is correct, the lamellae should act as diffraction gratings for electromagnetic waves whose wave-length is less than the interfibrillar spacing, and produce first and higher order spectra. It is possible to put this to the test with X-rays.

Each lamella should behave similarly to a three-dimensional lattice of the type familiar in crystallography but degenerated to two dimensions (James, 1948). The nature of the diffraction pattern will be determined by the lattice planes, the sets of parallel planes that pass through the fibril axes, two of which are indicated in section in Text-fig. 2. The spacing of the planes in a set determines the angles the diffraction maxima make to the incident beam as if it was the spacing of the elements in a linear grating. Only certain maxima are produced, however, according to the orientation of the lattice plane relative to the X-ray beam. The dimensions of any particular lattice are fixed if the dry fibrils have a diameter 25 mμ and occupy 15% of the volume; in a regular hexagonal lattice the largest lattice plane separations are 54 and 31 mμ, and in a square lattice 57 and 40 mμ.

Some X-ray diffraction pictures were taken of the fresh cat's cornea, using the diffraction apparatus of Franks (1955), which produces a very intense beam of X-rays focused at a point. Unfortunately, after the very first exposures were made the instrument became unavailable. These first pictures, nevertheless, showed diffraction rings surrounding the central spot and indicate that a randomly orientated regular spacing is present in the specimen (Pl. 1, fig. 2). The diameter of the rings corresponds to spacings of 20·5 and 13·5 mμ, suggesting that they are 2nd and 3rd order diffraction maxima of a 41 mμ spacing in the specimen; the 1st order maxima would have too small a diameter to be resolved. This spacing is quite distinct from the typical periodicity of 64 mμ shown by the collagen molecule, and suggests the existence of a square lattice with its diagonal perpendicular to the corneal surface. It is desirable that an attempt be made to obtain better defined exposures and that the values for the percentage of collagen and the diameter of the fibrils in the stroma of the cat be confirmed before any definite conclusions are drawn.

<div align="center">DISTURBANCES OF TRANSPARENCY</div>

The normal cornea is in a compressed state and there is a tendency to *swelling* of the stroma if it is interfered with. The swelling is accompanied by *clouding* on which some original data are provided. *Explanations of clouding* can be given in terms of the uniform refractive index theory or the lattice theory of transparency. It is also possible to produce *clouding by mechanical strain* which seems only to be compatible with the lattice theory.

<div align="center">*Swelling*</div>

If the excised cornea is placed in saline or water its stroma will absorb the fluid and swell perpendicularly to the corneal surface. The cornea *in situ* also swells if its limiting layers are damaged, or after death. The swelling of the excised stroma can be prevented or reversed by pressing on its surface, and the change in weight of a cornea when immersed in saline and compressed

beneath a plunger was measured by Kinsey & Cogan (1942). Their results show that after five days, when the changes were greatest, the normal thickness was maintained by a pressure of about 80 g/cm². An increase to about 650 g/cm² reduced the thickness to about two-thirds, and a decrease of 13 g/cm² allowed it to swell to $1\frac{1}{2}$ times normal.

The same workers showed that the limiting cellular layers of the cornea acted as semi-permeable membranes, and postulated that the swelling pressure of the stroma was balanced by an osmotic pressure difference between it and the aqueous humour (Cogan & Kinsey, 1942b). They suggested that this difference arose because the newly formed aqueous humour was hypertonic to the blood, but it is now generally accepted that it results from the activity of the cellular layers of the cornea which continuously pump substances out of the stroma (Maurice, 1951; Davson, 1949, 1955a, b). It does not seem to have been noted previously that a stabilizing mechanism for the corneal thickness is provided by the very rapid alteration in swelling pressure which accompanies its variations. A 10% change in the secretory activity of the cellular layers, for example, might lead to an equal variation in the osmotic difference between cornea and aqueous humour; the change in thickness of the cornea would need to be only 1 or 2% in order to adjust the swelling pressure to the new circumstances.

It may be seen from Text-fig. 1 that the hydration of the fibrils is unchanged while the cornea swells from almost dryness to three times its normal thickness. The swelling therefore takes place in the ground substance. The same conclusion was reached by Heringa, Leyns & Weidinger (1940), who found that the swelling capacity of a cornea from which the mucopolysaccharide had been extracted was very much reduced. Also in agreement are the observations of François, Rabaey & Vandermeersche (1954), who examined surface replicas of swollen and normal sections of stroma under the electron-microscope, and could distinguish no difference in the fibril diameter in the two cases.

Clouding

When the stroma swells it scatters more light. This is first observed subjectively as an increased haze round bright lights when the thickness of the cornea is increased by about 5%, as a result of wearing contact lenses, for example. When it is more swollen the increase in cloudiness of the stroma can also be seen objectively, most clearly with the aid of the slit-lamp. The relationship between thickness and clouding was observed in enucleated rabbits' eyes using the apparatus of Maurice & Giardini (1951a) to measure the thickness of the cornea, and the photometric method of Langham & Wybar (1954) to measure the scattering. The slit-lamp beam was directed normally on to the corneal surface and the light scattered at an angle of 60° to the normal was collected and used as an estimate of the clouding. The results are shown in

Text-fig. 3. The general run of the points suggests that the scattering increases almost immediately the cornea swells above its normal value.

Experiments on isolated swollen corneae showed that a beam of light falling perpendicularly on the surface is scattered predominantly in the forward direction. Even when the cornea is swollen to its maximum thickness nearly all the scattering was restricted to a cone of 20° half-angle. The scattering is markedly greater for the shorter wave-lengths as is evident from the colour of a swollen cornea, smoky blue by direct light and red by transmitted light.
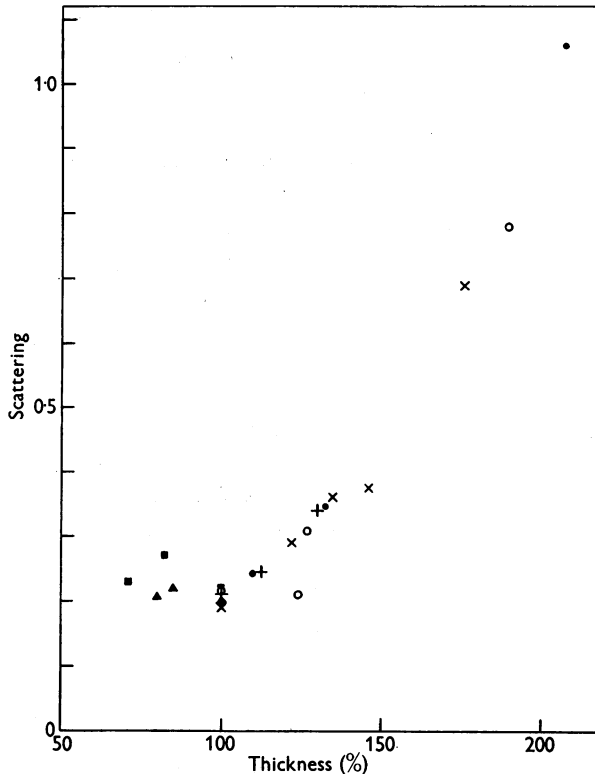
Fig. 3. Relationship between light scattered back from cornea and swelling. Green light shone normally on to the surface of rabbit's cornea and light scattered at angle 60° measured photoelectrically. Ordinate: measured light as percentage of that scattered from MgO surface. Abscissa: thickness of cornea as percentage of normal. Different symbol for each of six eyes. Corneae swollen by placing eyeball in saline, shrunk by replacing aqueous humour by air and leaving at room temperature.

This is shown also by measurements of the corneal absorption, which rises from the red to the blue end of the spectrum. The exact determination of the scattered light by means of absorption measurements would require particular care, however, because much of it makes small angles to the incident beam and would be included in the transmitted light by most instruments.

Explanations of clouding

On the hypothesis that the transparency of the stroma results from an equal refractive index of all its components, the explanation of the turbidity which accompanies swelling is that the absorbed fluid forms a phase with a different index. The fluid has been supposed to be taken up either between the fibres or, more commonly, the lamellae. The greater scattering of blue light by the swollen stroma indicates, on the other hand, that the scattering elements are of submicroscopic dimensions; moreover, if the fluid entered the spaces between the flat surfaces of the lamellae the cornea should assume an iridescent appearance.
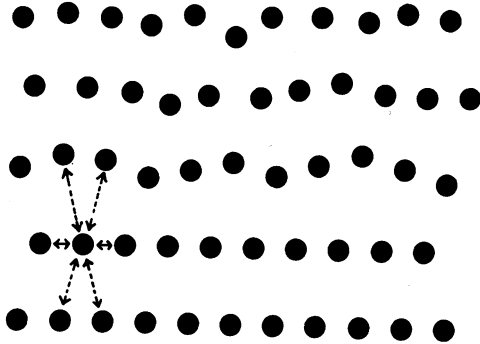

Fig. 4. Illustration of disorder produced in fibril lattice by swelling. The weakening of the forces of repulsion which keep the row in line is shown schematically.

If the fluid were supposed to enter between the fibrils this explanation would seem to be tenable. In the scheme considered earlier in which the fibrils were hydrated to such an extent that their refractive index is that of the stroma, 1·374, it was shown they would approximate to cylinders of diameter 60 mμ. If these were separated by aqueous humour of index 1·335, equation (9) shows that the cornea should be able to scatter up to a maximum of 97% of the incident light.

On the lattice hypothesis the clouding results from a derangement of the fibrils consequent on a weakening of the forces holding them in position. The simplest system of forces which will maintain the lattice is one of equal repulsion between each fibril and its neighbours, as illustrated in Text-fig. 2. When the rows are separated in swelling, as shown schematically in Text-fig. 4, the orienting forces on a fibril from those in adjacent rows will be weakened and it will be pushed out of line by the fibrils on either side of it.

The most stable arrangement of the fibrils is always when they are in a state of minimum potential energy, and this will be when the distances between all pairs of neighbours are as near equal as possible, if the forces between them are of the same nature. A hexagonal lattice of the type shown in Text-fig. 2

would have maximum stability. If it expands in a direction perpendicular to the rows, the distances between neighbours will cease to be equal and a disordered arrangement will become more stable. A square lattice with its diagonal perpendicular to the corneal surface, on the other hand, will change to a stable hexagonal lattice on swelling by 70 %. The almost immediate clouding as the cornea swells, therefore, argues in favour of the hexagonal lattice.

If the displacement of the fibrils when the structure becomes disordered were independent of one another, the light they scatter should have a broad maximum in the opposite direction to the incident beam. Since the maximum is narrow and in the direction of the incident beam it seems that these displacements must be associated (James, 1948). This is not unexpected, since an out-of-line movement of a fibril in one row should tend to move its neighbours in adjacent rows in the same direction.

It is possible that the forces between the fibrils manifest themselves externally as the swelling pressure of the cornea. A system of forces such as illustrated in Text-fig. 2 would give a resultant pressure both upon the faces and edges of a lamella; expansion in the latter direction would be prevented by its interweaving with neighbouring lamellae. This hypothesis has the attraction of connecting the two properties which distinguish the cornea from similar tissues, its transparency and its ability to swell. The swelling force is developed within the ground substance, and this must interact with the fibrils if they are to be pushed away from one another. There is physical and chemical evidence that such an interaction takes place between the fibrils and at least part of the ground substance (Woodin, 1954). It must be borne in mind, however, that the equality of the swelling pressure and the interfibrillar forces is presumptive, and it is possible that a large part of the swelling force of the ground substance is by-passed around the fibrils.

If it is allowed that the equality is true, it is possible to determine the nature of the force between two fibrils from the relation between swelling pressure and corneal thickness measured by Cogan & Kinsey. With a hexagonal lattice this leads to the result that the force falls off roughly as the thirteenth power of the distance between the fibril axes. Such a rapid change of force with separation would lead to both the formation of a very stable lattice and to its rapid derangement when it expands.

Clouding by mechanical strain

It is well known clinically that the cornea may become cloudy in conditions where the intra-ocular pressure is raised above the normal, and that its clarity is recovered when the excess pressure is relieved. Similarly, on squeezing an enucleated eyeball, the cornea will cloud for as long as the pressure is maintained and clear instantly it is released. The clarity is also disturbed in areas of local stress, for example, around the point of puncture by a needle.

Clouding by mechanical strain is much more easily elicited if the cornea is swollen. This is illustrated in Text-fig. 5 which records the results of experiments in which the light scattered back from the cornea of the rabbit's eye was measured in the manner already described, as the intra-ocular pressure was raised through a needle inserted into the vitreous humour. Some eyes were taken immediately after the animal was killed and in others the cornea was allowed to swell in saline before the experiment was started.
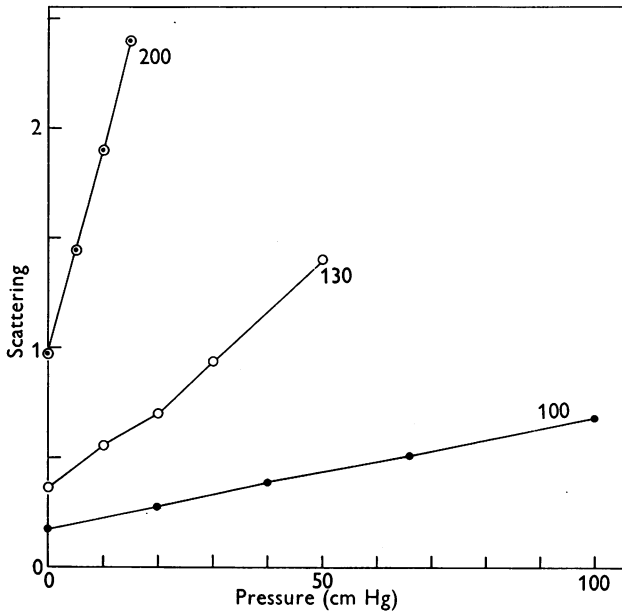
Fig. 5. Relationship between intra-ocular pressure and light scattering of cornea. Scattering measurement as in Text-fig. 4. Pressure raised through needle in vitreous body. Ordinate: percentage of light scattered from MgO surface; abscissa: intra-ocular pressure. Upper curves obtained from swollen corneae, the number drawn against them gives their thickness as a percentage of normal.

On the hypothesis of equal refractive index throughout the stroma, the clouding is explained as the squeezing out of fluid from the structural element into the interstitial spaces. It is difficult to understand why this should be more marked in a swollen cornea where the spaces are supposed to have an excess of fluid in them already.

The readier clouding of the swollen cornea would be expected on the lattice theory because any tendency to derange the fibrils would be accentuated if the ordering forces were weakened. There are two ways in general by which the derangement could be brought about. In the first, the fibrils are displaced in the lattice as a result of their individual tensions. In the second, the combined force of many fibrils causes distortions in neighbouring parts of the

lattice as a result of shear or compression. The writer has so far been unable to devise a procedure for determining which of these two general mechanisms is the operative one.

<div align="center">APPENDIX</div>

<div align="center">*Acoustical analogue to corneal structure*</div>

A model has been constructed to illustrate the lattice theory of transparency. The light wave is replaced by an ultrasonic sound wave of about 30,000 c/s ($\lambda = 12$ mm). A narrow beam is conveniently generated by means of an electrostatic loudspeaker (LSH 75). It is directed at an identical loudspeaker placed about a metre away which acts as a microphone, and whose output is displayed on a cathode ray oscilloscope.

The fibrils are simulated by 1600 straight steel rods 1·5 mm in diameter and 11 cm in length. These stand on end upon a square horizontal glass plate 10×10 cm. They are supported at the top and bottom by being passed through holes in two square pieces of perforated zinc sheet which are held above the glass by corner pillars. The holes form a hexagonal pattern of $2\frac{1}{2}$ mm side so that the rods are held parallel to one another and in a regular order. The lower perforated sheet slides on the corner pillars, and may be raised into proximity with the upper one. If the model is then gently shaken the rods are sufficiently loose in their holes for their lower ends to slide on the glass bottom and become disordered.

When the model with the rods in good order is placed in front of the loud-speaker so that the beam of sound passes through its lowest part, the amplitude of the wave received by the microphone is reduced to about 70 %. When, however, the rods are disordered, the amplitude falls to less than 5 %.

<div align="center">SUMMARY</div>

1. The structure of the corneal stroma as revealed by its chemistry and its appearance under the microscope and electron-microscope is described. It is made up of collagen fibrils of uniform diameter which lie parallel to one another to form lamellae and are surrounded by a ground substance containing mucoid. Measurements of the refractive index and birefringence of the tissue indicate that the collagen is hydrated with 55 % of its volume of water, and that the fibrils have a refractive index of 1·47 and the ground substance of 1·35. The hydration of the collagen does not change when the tissue is allowed to dry partially or to swell in water. A preliminary investigation of the diffusion of haemoglobins in the stroma gives results in accordance with this structure.

2. Calculation of the light that would be scattered by the fibrils suggests that the cornea should be opaque. Previous explanations of its transparency, particularly that its components are of uniform refractive index, are examined

and shown to be unsatisfactory. The theory is advanced that the fibrils are arranged in a regular lattice so that the scattered light is destroyed by mutual interference. Evidence for this lattice structure is provided by X-ray diffraction.

3. The loss of transparency that occurs when the stroma swells in water or is mechanically stressed, is measured. It is briefly considered in the light of the hypothesis that the forces that cause the swelling are the same as those that maintain the lattice structure.

4. An acoustical analogue is described which shows how a system of parallel scattering cylinders is transparent to a sound wave when they are arranged in a regular pattern but is opaque when they are disarranged.
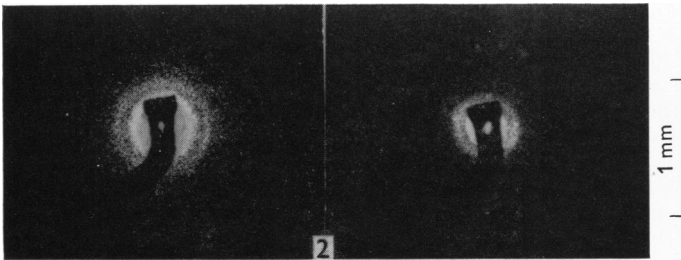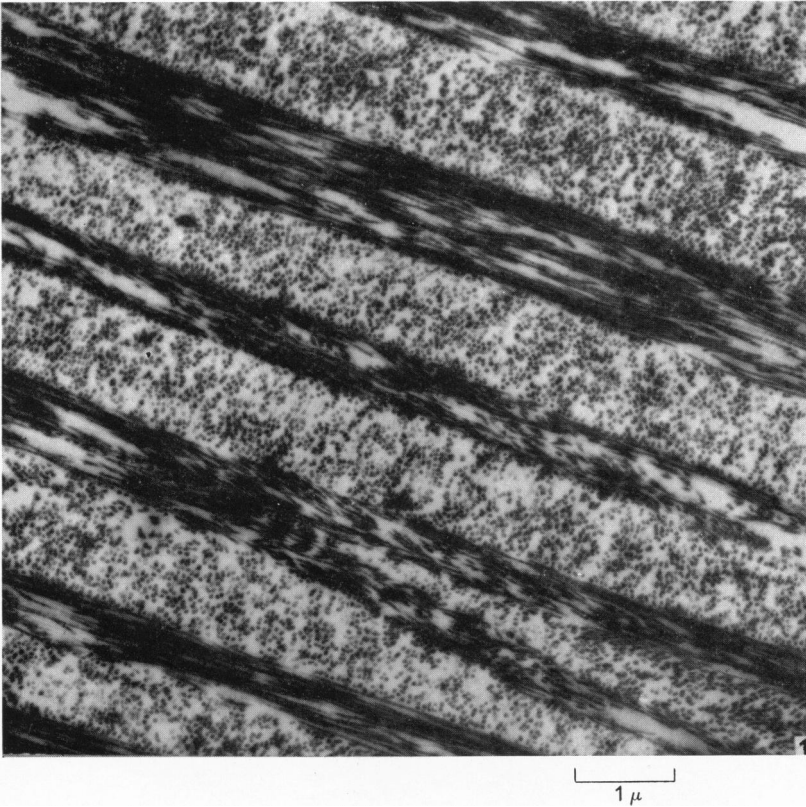
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EXPLANATION OF PLATE

PLATE 1

Fig. 1. Electron-microscope picture of cross-section of stroma (Jakus, private communication, 1955). Turtle cornea fixed and embedded in plastic, × 14,500. Owing to the fortunate orientation of the section the parallel arrangement of the fibrils in the lamellae, running alternately into and across the page, is clearly seen. The arrangement in mammalian corneae is essentially similar but because of unlucky orientation, distortion of the layers and, perhaps, a basically less regular pattern, a picture as clearly defined as this one has not yet been obtained.

Fig. 2. X-ray diffraction patterns of fresh cat's cornea (Franks). A pencil of X-rays, wave-length 0.154 mμ, is shone normally to surface of cornea which is 29 mm from film. Magnification of negative, × 15. The two figures correspond to two different exposure times. Diameters of diffraction maxima, 7 and 11 mm. correspond to randomly oriented spacings of 13·5 and 20·5 mμ in cornea.