COMMUNICATIONS
THE POLARIZATION OPTICS OF THE ISOLATED CORNEA*

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It has long been known that the cornea, like most biological tissues, changes polarized light passing through it, but the possibility that these changes vary with the intra-ocular pressure (Stanworth, 1949) justifies a further examination of the subject.

Previous Studies of the Isolated Cornea

Brewster (1815) was the first to report that the cornea was anisotropic, but the first adequate study was that of Valentin (1861), who found that when the cornea was placed between a polarizer and crossed analyser a dark cross-shaped figure with peripheral concentric coloured bands was produced. A figure similar to this is also seen when convergent light is incident on a uniaxial crystal plate cut perpendicular to the optic axis, i.e., perpendicular to the direction along which no double refraction

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takes place. Valentin therefore postulated that the cornea behaves as a curved uniaxial crystal plate with the optic axis at any point always perpendicular to the plane of the cornea.

His (1856) found, on the other hand, that when sections of the cornea were examined in polarized light the corneal lamellae were doubly refracting with the optic axis along their length, and therefore in the plane of the cornea. In view of this, Valentin's conclusions seemed unlikely, and Rollett (1871) pointed out that the shape of the interference figure could be explained if the corneal lamellae, having an optic axis along their length, were arranged in a predominantly radial manner. Since, however, there was no anatomical evidence of such a radial arrangement, the explanation was not entirely satisfactory.

Fleischl (1880) also studied the interference figure, and came to the conclusion that it was seen only when the cornea was under tension, the unstretched cornea showing no double refraction. That this is not the case was shown by Schiotz (1882), using calves' and pigs' eyes. He devised a pressure chamber in which the excised cornea was supported by pins passing through adjacent sclera; pressure could then be applied to the posterior surface of the cornea by filling the chamber with saline and connecting it to a manometer. He found that when the applied pressure approximated to the normal intra-ocular pressure, a well-defined interference figure could be produced, though the clarity of the figure did increase when the pressure was raised. He also found that the interference figure was not a simple cross except when the polarizer and analyser were parallel to two mutually perpendicular directions in the cornea. In all other positions of the polarizer and analyser, the cross was replaced by hyperbolas.

These investigators made no attempt to measure the magnitude of the birefringence of the cornea. Nordenson (1921) did make such measurements, using mainly the method of estimating the birefringence by noting the colour of the tissue observed in the polarizing microscope and comparing it with the Michel-Levy colour chart. Estimates of colours are, however, notoriously subjective and the method is only an approximate one, although Nordenson did check some of his results by using a Berek compensator. A more serious objection is that his measurements were not made under physiological conditions, and, as we shall show, the position in the cornea at which the measurements are made, and the direction of the incident and emergent light, are of considerable importance.

Cogan (1941) noted that an interference figure could be seen in the living eye in polarized light, and briefly reviewed the literature on the figure in the isolated cornea. He was unable to put forward any adequate theory to explain its presence.
Present Investigations

In order to study the birefringence of the isolated cornea adequately, a method of suspension is required which maintains it in as physiological a condition as possible. The pressure chamber shown in Fig. 1 was used. It consists, in principle, of two chambers between which the isolated fresh cornea is suspended by clamping an attached rim of sclera between two roughened surfaces (R), of such radii that the sclera fits perfectly to form a watertight joint. The chambers are closed at the front and back of the apparatus by strain-free glass plates (T), and can be filled with any required fluid at any desired pressure. In order to maintain physiological conditions as far as possible, Ringer's solution is used, at a pressure in the posterior chamber of about 20 mm. Hg. The anterior chamber is filled with this solution at atmospheric pressure, and so fulfills the double function of keeping the anterior surface of the cornea moist and minimizing its refractive effect.
The optical arrangement for studying the cornea as a whole is shown in Fig. 2. The light from a slit lamp source (S) is collimated by the lens (L), passes through a polarizer (P), and then through the specimen, which is observed through the analyzer (A). The polarizer and analyzer are rotatable and their positions can be measured by means of verniers.

For measurements of the birefringence, the arrangement in Fig. 3 was used. The additional apparatus is a pinhole (D), a low-power—2 in.—objective (O), a quarter-wave plate (W), and an ocular (E) containing cross wires. Two auxiliary items not shown in the figure are a green filter (Wratten 62) and a sensitive tint plate. The former is inserted in the light path (LP) to increase the accuracy of measurement by making the light almost monochromatic. The latter is substituted, in diagonal position, for the quarter-wave plate when it is required to ascertain the orientation of the principal vibration directions.

The method of measuring birefringence was that of Goranson and Adams (1933). It consists essentially of placing the polarizer at 45° to a principal vibration direction of the specimen at the point to be measured; the quarter-wave plate is placed parallel to the polarizer and the analyzer is then rotated until the light is extinguished. The retardation in degrees is then twice the angle the analyzer makes with its original "crossed" position, measurements being made either clockwise or anti-clockwise according to the relative orientation of the principal vibration directions of the specimen and the quarter-wave plate.

**Results**

The general appearance of the cornea between a polarizer and a crossed analyzer is that described by previous authors, and is
shown for the cat cornea in Fig. 4. The interference figure is made up of two parts, one consisting of a series of roughly circular concentric colour. rings or bands, the other of a dark cross-shaped figure. The former are bands connecting points of equal retardation, the magnitude of which determines the colour, and by analogy with photo-electric stress patterns and crystal optics can be termed “isochromatics”. Similarly, the dark bands are analogous to “isogyres” or “isoclinics”, and connect points at which the principal vibration directions of the cornea coincide with the directions of the polarizer and analyser. The dark bands form an almost perfect cross for all positions of the polarizer and crossed analyser, though when they are examined carefully, they can usually be resolved into two rectangular hyperbolas, with only a
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slight separation between them. In the human, dog, and rabbit corneas the isogyres are almost identical with those in the cat, but in the ox and sheep corneas, a marked separation of the hyperbolas is seen. In general the cross-shaped isogyres in the human and cat cornea are equivalent to those seen when a uniaxial crystal is examined in convergent light, the hyperbolic type of isogyres corresponding to a biaxial crystal.

The colours of the isochromatics indicate that the retardation increases from a very small value in the optical zone of the cornea to a maximum (in the cat) of about 2 wave-lengths retardation at the periphery. This is also seen in Fig. 5, A which shows the retardation at various distances from the centre of the cornea, measured by the method described above. This does not, however, indicate that the essential birefringence of the corneal substance at the periphery is necessarily greater than in the centre, as the light passing through the periphery is traversing a much greater thickness of the cornea. This is due not only to the increased peripheral thickness but also to the gradually increasing angle at which the light meets the cornea.

In fact, measurements of the retardation normal to the surface of the cornea (Fig. 5, B) indicate that the retardation in the whole of the optical zone is very small, and it can be calculated from these results that the effective birefringence of the cornea, expressed as

![Graph showing effects of 3.5% formalin on original retardation.](http://bjo.bmj.com/)

**Fig. 6.** Effects of 3.5% formalin on the original retardation.
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the difference between the ordinary and extraordinary refractive indices, varies from zero in the centre to about 0.00012 at the edge of the optical zone, and, allowing for the increased thickness, to about 0.00027 at the extreme periphery. Unfortunately it was not possible to reach the periphery of the cornea using the physiological method of suspension described above: measurements at the periphery could only be done by using a cornea fixed in formalin, which retains its shape when the attached scleral rim is pinned on to a cork ring mounted on a microscope slide. It is from measurements made on such corneas that the values of the retardation in Fig. 5, B for points on the cornea more than 3 mm. from the centre are derived. That this procedure gives readings approximating to those of the normal cornea is seen from Fig. 6, which shows the effects on the original retardation (A) of 3 per cent. formalin in the apparatus for one day (B) and six days (C).

Discussion

Since His (1856) has shown from the study of sections that the most important birefringent elements of the cornea are the lamellae, the interference figure must obviously be explained in terms of these lamellae, the distribution of which must result in the observed shape of the isogyres and a total birefringence such as described above.

That the retardation produced by the cornea is so small can be explained by a consideration of the summation of the effects of numerous superimposed layers of lamellae lying in different directions. If two lamellae of equal retardation are situated at right angles, there will be no retardation for light passing perpendicular to the plane in which they lie, as the retardation produced by one will cancel that produced by the other. Consequently the small retardation for light normal to the cornea could be produced either by a random arrangement of the lamellae, or by lamellae running in alternating layers at approximately right angles, such as described by Virchow (1910). Nevertheless, provided that this is the total effect, such arrangements are not excluded as that described by Kokott (1938) wherein the superficial layers are predominantly vertical, the middle layers running mainly in directions corresponding to the insertions of the recti, and the deep layers showing predominantly circular fibres in the periphery.

We have already noted that Nordenson (1921) also found the birefringence of the cornea to be low, and he gave a mean value of 0.00059 with only a small variation from this figure. This is much higher than we have found, probably because Nordenson used an excised piece of cornea spread on a glass sphere; also it is not certain that all his measurements were made normal to the cornea.
Nordenson concluded from this low value for the birefringence that it was of little importance during normal use of the eyes. Boehm (1940), however, as a result of observations on the effect of crystal plates of various retardations on the entoptic phenomenon of Haidinger's brushes in blue light, concluded that the ocular media produced in some way the effect of approximately a one-eighth-wave plate (a retardation of about 50 μ). He thought that this effect was probably due to the cornea and that the lens was excluded as the source of the phenomenon by the fact that the latter was still present in aphakia. It appears unlikely, however, in view of our results, that the cornea could have an effect of this magnitude; nevertheless, owing to the angle between the fixation and the optic axes, the macula is not quite symmetrically placed with regard to the cornea, and some slight retardation might be produced by the obliquity of the rays reaching the fovea. The same mechanism might also produce an additional retardation due to birefringence of the lens, which together with that due to the cornea, might amount to the magnitude suggested by Boehm. In that case the persistence of this retardation after the removal of the lens may be due to post-operative astigmatism of the cornea, which may well, as we shall see, convert the cornea from a uniaxial to a biaxial type and hence produce the observed retardation.

The isogyres have given rise to some speculation and have usually been considered to give an indication of the fibre directions in the cornea. Their appearance with various positions of the polarizer and crossed analyser, however, leads essentially to a determination of the principal vibration directions (Figs 7 and 8). Fig. 7, which corresponds to the cross-shaped isogyres,
showed that at any point the principal vibration directions are radial and perpendicular to the radius, and this, as we have seen, has led to the supposition that the lamellae must run predominantly radially (Rollett, 1871). For the hyperbolic figure, on the other hand, the principal vibration directions are as shown in Fig. 8, the distance between points H and I being a measure of the separation of the hyperbolas. A figure somewhat similar to this, evolved by Schiotz (1882), suffers from the defect that at some places three lines pass through the same point: he too identified these as the possible directions of the birefringent elements, which he thought to be probably the lamellae.

It is not necessary, however, to interpret these diagrams as indicating the directions of the lamellae. If a ray of light passes at an angle through a combination of two lamellae at right angles, such as described above, the retardation produced will not in general be zero, and the principal vibration directions of the cornea for such a ray of light will be in the plane of incidence of the ray and perpendicular to this plane. Owing to the curvature of the cornea, rays of parallel light meet the cornea in such a way that the plane of incidence is always radial to the cornea, and hence, if the cornea is examined in plane polarized light, the principal vibration directions must always be radial and perpendicular to the radius. The cross-shaped isogyres are thus explained without any preferential arrangement of lamellar directions.

If this be the case, it is easy to understand why a rise in pressure in the posterior chamber of the suspension apparatus fails, as Schiotz observed, to alter the shape of the isogyres, for however much it may alter the birefringence of the individual lamellae (Stanworth, 1949), the combination will still produce zero retardation for light passing normal to the cornea, and hence will give rise to a cross-shaped figure in the same manner as that already described. A change in the birefringence of the individual lamellae could only be detected by using rays of light passing through the cornea at a large angle, so that they were affected more by some lamellae than by others, thus making an approach to isolating lamellae running in one direction only.

In the case of the hyperbolic isogyres additional factors must be involved. It may be of some significance that the more asymmetrical the cornea, the larger the separation between the hyperbolas. Nevertheless, this does not fully explain the shape of the isogyres, though it would result in elliptical rather than circular isochromatics, as is indeed seen in these corneas. Some degree of lamellar orientation such as postulated by Schiotz may thus occur, though it should be remembered that the actual lamellae need not run along the principal vibration directions, but need only be
arranged in such a manner that these directions are the resultant of their individual effects. It is equally possible, moreover, that a change from a uniaxial to a biaxial type of interference figure is due not so much to the way in which the lamellae are arranged as to the effects of stress on the birefringence. As has previously been shown (Stanworth, 1949), tension on a strip of cornea results in a marked increase in the birefringence of the lamellae, and if this occurs more in some lamellae than in others, it may then alter the shape of the interference figure to that of a biaxial type. In this case the lines shown in Fig. 8 may give the directions of the principal stresses at any point, and would be described in photoelastic analysis as "isostatics". If the distance between the points H and I is small these lines approximate to those in Fig. 7, indicating an almost radial and circular distribution of the stresses. That the stresses on the cornea may be responsible for the shape of the isogyres is indicated by the fact that the figure tends to become biaxial if the method of suspension is not physiological, if the cornea is dried, if it is fixed in formalin, or if it is markedly asymmetrical.

It is also possible that the lamellar arrangement and the influence of stresses may both be involved in producing the figure, for during the development of the cornea the lamellae may grow into their final positions along the lines of stress produced in the formative cornea by the rising intra-ocular pressure. Such an influence of stresses on growth has been postulated in the case of nerve fibres by Weiss (1934).

Summary

1. An apparatus is described for investigating the isolated cornea under near-physiological conditions in polarized light.

2. The effective birefringence for light passing normally through the cornea is extremely small, but increases rapidly with increasing obliquity of the light.

3. The "interference figure" seen between a polarizer and a crossed analyser is discussed in terms of photoelastic stress analysis and crystal optics, and is shown to be due primarily to the effect of the oblique incidence of the ray of light falling on the anisotropic corneal lamellae. Stresses in the cornea and the arrangement of the corneal lamellae may be secondary factors.

4. It is shown that though the interference figure is unchanged by a rise in pressure behind the cornea, this does not exclude a change in the birefringence of the individual corneal elements. This can only be studied by light passing obliquely through the
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cornea so as to approach an isolation of the birefringence of lamellae running in one direction only.

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