Small-angle light-scattering patterns of corneas of different species. FREDERICK A. BETTELHEIM AND ROBERT MAGRILL.

Light-scattering patterns of corneas of different species have been obtained. The different small-angle light-scattering (SALS) patterns were classified into four groups on the basis of the angular dependence of the intensity of light scattered in the \( 1^+ \) and \( I_u \) modes. At present, only two types of patterns can be explained on the basis of theoretical models. The need to develop a general model that can account for all four types of SALS patterns is discussed.

Normal corneas scatter light appreciably only at small angles (\( 0^\circ \) to \( 4^\circ \)). Scattering occurs both in the \( I \) and \( I_u \) modes, that is, when polarizer and analyzer are aligned perpendicular or parallel.\(^1\) This means that the scattering is due both to density and optical anisotropy fluctuations. Thus, in principle, scattering patterns can be used to analyze the ultrastructure of cornea. Electron microscopic investigations\(^5\) seem to imply similar alignment of collagen fibrils in the lamellae and similar packing of the lamellae in the cornea of different species. On the other hand, corneas of three different species\(^5\) had distinct and different SALS patterns that would imply distinct and different ultrastructures. Bovine cornea showed a light-scattering pattern that can be accounted for by a model of nonrandom assembly of anisotropic rods.\(^5\) Rabbit cornea\(^6\) implied a sheaflike morphology. Rat cornea\(^7\) yielded a SALS pattern that could not be accounted for by either of these models. Clearly there is a need to develop a model that can account for all types of corneal light-scattering patterns. The first step in this direction requires the investigation of the number of and nature of different light-scattering patterns yielded by corneas of different species.

Bovine (\( B. taurus \)) and lamb (\( O. aries \)) eyes were obtained from slaughterhouses less than 12 hours post-mortem. Sea bass (\( M. salmoides \)), sea trout (\( S. trutta \)), and bluefish (\( P. saltatrix \)) were obtained from fishing vessels less than 10 hours post-mortem while the fish were kept in seawater. Carp (\( C. carpio \)), chicken (\( G. gallus \)), turkey (\( M. gallo-pavo \)), duck (\( A. platyrinchus domesticus \)), pigeon (\( C. leucocephala \)), rat (\( R. rattus \)), and frog (\( R. temporaria \)) were killed just before enucleation. Human corneas (\( H. sapiens \)) were obtained less than 24 hours post-mortem.\(^6\)

Small-angle light-scattering (SALS) patterns were obtained as described earlier.\(^6\)

The SALS patterns of corneas of different species can be classified on the basis of the variation of the intensities in the \( I \) and \( I_u \) modes with scattering angle \( \theta \) and azimuthal angle \( \Omega \). Group I (Fig. 1, a and b) is characterized by a four-lobe scattering pattern in the \( I \) mode in which one set of the lobes is more prominent than the other set normal to it. The corresponding \( I_u \) patterns do not have this four-lobe pattern but have a geometric anisotropy along the direction of one set of the lobes in the \( I \) pattern. Both \( I \) and \( I_u \) patterns have only one intensity maximum. The patterns in Fig. 1, a and b, come from a bovine cornea, and the distribution of such patterns was studied extensively.\(^1\) Human corneas provide in essence the same SALS patterns as bovine corneas.\(^6\)

SALS patterns of Group II (Fig. 1, c to h) are characterized by a cloverleaf arrangement in the \( I \) mode. This cloverleaf pattern has five intensity maxima as compared to the one maximum of the first group. On the basis of more detailed structure, Group II can be divided into three subgroups. Group IIa (Fig. 1, c and d) has an \( I \) pattern in which the cloverleafs are in a \( 0^\circ \) to \( 90^\circ \) arrangement. The corresponding \( I_u \) pattern is nondescript. Chicken, turkey, duck, bluefish, sea bass and rat corneas yield such patterns.

Group IIb differs from IIa only in the \( I \) mode. The four lobes of the cloverleaf are not in a \( 0^\circ \) to \( 90^\circ \) arrangement but at \( 0^\circ \) to less than \( 90^\circ \) arrangement (Fig. 1, e and f). Otherwise the \( I_u \) of this group is just as nondescript as it was in Group IIa. Frog and carp corneas provide SALS patterns belonging to this group.

Group IIc has \( I \) patterns in which the four lobes of the cloverleaf are at \( 0^\circ \) to less than \( 90^\circ \) arrangement, just as in Group IIb, but the \( I_u \) pattern is anisotropic (Fig. 1, g and h). The \( I_u \) pattern has a direction along one set of the cloverleaf lobes of the \( I \) pattern. In this sense there is a similarity between Group I and Group IIc, but the basic difference, that is, five maxima vs. one maximum in the \( I \) patterns, separates them. Sea trout and rabbit corneas yielded such patterns.

The less than \( 0^\circ \) to \( 90^\circ \) cloverleaf arrangement requires a special comment. It has been shown\(^7\) that deformation of the rod or spherulite structure due to elongation can cause such an effect. It is also known\(^8\) that cornea under different tensions gives rise to different SALS patterns. In our cases the excised corneas were under no tension, pos-
Fig. 1—Cont'd. G and H, Sea trout. I and J, Lamb. K and L, Pigeon.
sibly in a more relaxed state than in vivo when under ocular pressure. Thus the less than 0° to 90° cloverleaf arrangement is not an artifact due to induced orientation.

The lamb cornea produced SALS patterns that could be classified into a new group, Group III. The I mode gives a 0° to 90° cloverleaf arrangement, whereas the $I_1$ mode has a cloverleaf that is at 45° (Fig. 1, i and j). It may be possible that this group in essence is the same as Ila except that all the information provided in this $I_1$ pattern is washed out in the Group Ila patterns.

Group IV yields the most complex patterns (Fig. 1, k and l). Pigeon corneas produce SALS patterns in the I mode that are a combination of two cloverleaf arrangements. There are one in the common 0° to 90° arrangement and a stronger second set of cloverleaf at 45°. Each cloverleaf provides five intensity maxima sharing the central one at 0°. Thus the I pattern has a total of nine intensity maxima. The corresponding $I_1$ pattern contains the 45° cloverleaf, which was strong in the I mode, but the rest of the pattern is washed-out and nondescript.

In all the corneal light scattering, the $I_1$ mode is always stronger than the I mode.

The basic interest in corneal light scattering has taken two different paths. Two questions were asked. (1) Can the structures seen in electron micrographs account for the transparency of normal cornea, and as a consequence, what structural changes can cause opacity under pathological conditions? (2) What kind of information can be obtained regarding the ultrastructure of normal and pathological cornea from the angular dependence of the scattered light intensity?

In the first approach corneal structures observed in electron micrographs were utilized. The deliberation concerned what theory as applied to such structures can account for the transparency. In the second approach, the information provided by the light-scattering patterns was used to get details of the ultrastructures. In order to obtain structural parameters, model systems had to be used to which the experimental patterns can be compared. Group IIC type of scattering patterns were explained by Chang, Keedy, and Chien as due to sheaflike structures. The usefulness of our model is limited to Group I, but at least this is the group which explains the ultrastructure of human cornea. In order to build a more general theoretical model, one must know the variety of light-scattering patterns that must be accounted for. The present investigation therefore provides a useful background on which theoretical models must be built. It specifies the requirement that a successful model must accommodate the scattering patterns of all four groups.

The experimental SALS patterns obtained from corneas of different species further demonstrate that corneal ultrastructures that look similar on electron micrographs must differ in fiber or aggregate parameters in order to yield such varied light-scattering patterns.

Table I. Average $\theta$ angle at which the intensity maxima of the cloverleaf patterns occur in the I mode of light scattering of corneas of different species

<table>
<thead>
<tr>
<th>Species</th>
<th>$\theta$ (degrees)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea bass</td>
<td>2.0</td>
</tr>
<tr>
<td>Sea trout</td>
<td>2.7</td>
</tr>
<tr>
<td>Blue fish</td>
<td>3.2</td>
</tr>
<tr>
<td>Carp</td>
<td>3.3</td>
</tr>
<tr>
<td>Lamb</td>
<td>2.0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>2.4</td>
</tr>
<tr>
<td>Human</td>
<td>2.0</td>
</tr>
<tr>
<td>Cattle</td>
<td>1.8</td>
</tr>
<tr>
<td>Rat</td>
<td>2.4</td>
</tr>
<tr>
<td>Turkey</td>
<td>2.5</td>
</tr>
<tr>
<td>Duck</td>
<td>2.5</td>
</tr>
<tr>
<td>Frog</td>
<td>3.2</td>
</tr>
<tr>
<td>Pigeon</td>
<td>3.5</td>
</tr>
<tr>
<td>Chicken</td>
<td>4.5</td>
</tr>
</tbody>
</table>

*Since in human and cattle SALS patterns no cloverleaf arrangements appear, we took the corresponding centers of the four-lobe arrangement.

The dimension of the scattering units has been calculated for the two models mentioned above: 12 $\mu$ for human, 20 to 26 $\mu$ for bovine, and 19 to 23 $\mu$ for rabbit cornea. It is generally true, however, that the larger the scattering angle, the smaller the scattering unit. In Table I, we provided a rough comparison of the average value of the scattering maxima in patterns of Groups II to IV. A corresponding average value is also provided for human and bovine cornea. It is interesting to note that within this limited sampling there is a trend. The scattering unit in corneas increases in size when we proceed from birds to mammals to fishes.

From the Chemistry Department, Adelphi University, Garden City, N. Y. Supported in part by Public Health Service Research Grant EY00501-07 from the National Eye Institute. Submitted for publication Aug. 9, 1976. Reprint requests: Dr. Frederick A. Bettelheim, Department of Chemistry, Adelphi University, Garden City, Long Island, N. Y. 11530.

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REFERENCES


The influence of age on the sensitivity of the cornea. MICHEL MILLODOT.

Corneal touch threshold (CTT) was measured in 205 healthy people of different ages. It was found that CTT increased gradually throughout life, although more significantly after the fifth decade of life. The results are found to be in good accord with those of Boberg-Ans, thus refuting an earlier report asserting that corneal sensitivity (threshold ~ J) increased up to the fifth decade and declined thereafter.

The sensitivity of the cornea to mechanical stimuli has aroused a great deal of interest because of the widespread use of contact lenses. In this connection and from a purely gerontological point of view it is likewise important to know how this response varies with age.

Jalavisto and associates observed a marked decrease in sensitivity with age, using a puff of air as a stimulus. However, this is an equivocal type of stimulus which, moreover, stimulates a wide, undefined area of the cornea and even eyelids. Boberg-Ans used a test consisting of a nylon thread of variable length confirmed qualitatively Jalavisto’s results. Boberg-Ans did not specify in his report which area of the cornea he tested. This is important because there appears to be some difference in aging of the center as compared to aging of the edge of the cornea, at least beyond 65 years.

On the contrary, Zobel, using von Frey’s hairs, found that corneal sensitivity increases slowly up to the age of 45 to 50 and decreases sharply thereafter.

Thus it was believed that a systematic and statistical study of the variation of corneal sensitivity in a healthy population was warranted.

Methods. The cornea was stimulated by the Cochet-Bonnet aesthesiometer. The instrument consists of a nylon monofilament of 0.12 mm. diameter which can produce a pressure ranging from 11 to 200 mg./0.0113 mm.2 The aesthesiometer was mounted in a holder so that it could be moved in x, y, and z axes by means of three knobs. A corneal point near the limbus in the six o’clock position was stimulated, and the slightest bend of the nylon wire was defined as corneal contact. Stimulation of the peripheral point was chosen because this result is not affected by apprehension factors.

Measurements of touch thresholds were made subjectively. The experiment began by stimulating the cornea with the lowest pressure and continued in an ascending fashion. At each predetermined length of the nylon monofilament (with increment of 0.5 cm.) four to six contacts were made, with an occasional blank to test the subject’s reliability. From these measurements the corneal touch threshold (CTT) was defined as the length of the monofilament at which the subject responded for 50 percent of the number of stimulations. This length was converted into pressure by the use of a previously calibrated curve for the instrument.

Subjects (205) of all ages were tested. Subjects were free of any symptoms or signs of ocular conditions, and older persons with arcus senilis were not included.

Results and discussion. The mean CTT and standard deviation for the various people of all ages are shown in Table I. CTT remains practically the same between ages 7 and 40. In the fifth decade of life, however, CTT becomes significantly higher than in the fourth decade (p < 0.05), although the difference is admittedly small. But CTT continues to increase with age. By the eighth decade of life CTT has increased to almost twice what it was in children 7 to 10 years old. The latter seem to have a somewhat smaller CTT, but unfortunately it is difficult to test such young people and this result cannot as yet be conclusive, since the sample is small (n = 10). It is interesting, though, that the findings are in good agreement with those of Boberg-Ans (n = 7) as shown in Fig. 1, al-