THEORY OF CORNEAL STRUCTURE

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Normal, healthy cornea is a specialized kind of tissue that performs several functions. For example, (see Fig. 1) it is a part of the wall of the eye, and must therefore possess the mechanical strength necessary to resist the intraocular pressure. It is the window of the eye, so that it must be transparent. Its outer surface is curved to provide most of the eye's optical focusing power, (2½ times that of the lens of the eye). Further, the cornea sustains its properties throughout life, being permeable to fluids in such a way that the waste products of metabolism continually pass outward from it while new metabolic fuel continually passes into it. In this connection, it exhibits a pronounced tendency to swell by taking in fluid and, as it swells, it becomes less transparent.

The behavior of the cornea is necessarily determined by its molecular structure. Thus, the organization of the macromolecules within the cornea, and the dependence of its physiological properties on that structure pose problems of considerable interest and importance to a basic understanding of the behavior of healthy cornea and the causes and possible cure or control of diseased cornea. The problems are sufficiently complex, however, that experimental studies have not satisfactorily solved these problems, and no physiomathematical theory that might elucidate them has heretofore been forthcoming.

The present study deals with the formulation of a structural model of the major portion of cornea, namely the stroma, which comprises about 90% of the thickness and to a large extent determines many corneal properties. The model is necessarily somewhat speculative because of the incompleteness of our knowledge, and no doubt will be improved upon

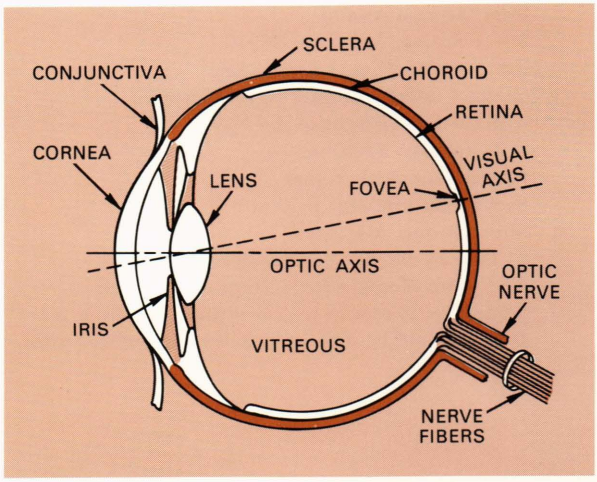

Fig. 1—Schematic diagram of the eye.

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The physical basis of corneal microstructure is investigated theoretically in an attempt to understand important physiological properties of the cornea. First, the basis for the optical transparency of the cornea is studied in terms of the molecular structure revealed by electron microscopy, which shows a quasi-ordered arrangement of collagen fibrils, with correlation extending over separation distances of the order of a few thousand angstroms. It is shown that the quasi-ordered structure is consistent with transparency, whereas a totally disordered structure is not. Second, the nature of the intermolecular forces that can be responsible for such spatially extensive order is discussed, and a theoretical molecular model is formulated. Analysis of the model leads to a theoretically derived structure approximating that shown by electron microscopy. Finally, the swelling behavior of the cornea is considered briefly in terms of the model.

as more information becomes available. Even in its present form, however, it is sufficiently representative of the stroma to permit calculation and elucidation of the structural basis of several properties. Thus, it constitutes an important first step toward the development of a more fundamental understanding of the physiological behavior of the cornea.

Structure of the Stroma as Revealed By Electron Microscopy

Comparison of electron micrographs of the transparent cornea and the surrounding opaque sclera reveals a marked difference in the size and uniformity of the collagen fibrils (Fig. 2). The corneal stroma is made up of a large number of stacked

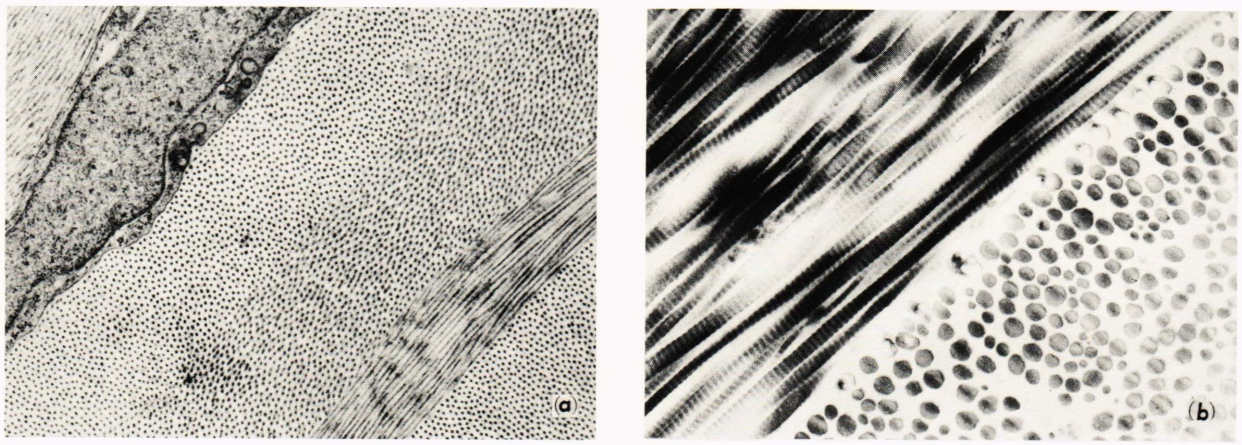

Fig. 2(a)—The collagen fibrils of the cornea of an adult rabbit. Scattered throughout the stroma and lying between the lamellae are the stromal cells, of which a portion is seen.
(b)—The collagen fibrils of the sclera taken from the same eye. The axial periodicity of ~ 700 Å in the collagen fibril is seen clearly in the sclera but not in the stroma. The section was stained with uranyl acetate and lead citrate. The magnifications of the two sections are the same; the interfibrillar center-to-center spacing in the stroma is ~ 600 Å.

sheets (i.e., lamellae) of more or less uniform thickness ($\sim 10\mu$). Lying within each of the lamellae are long cylindrical fibrils whose axes are very nearly parallel to each other and to the anterior and posterior surfaces of the stroma. Between the lamellae lie the stromal cells forming a framework in which the processes of individual cells interconnect by means of special points of contact. The collagen fibrils of the sclera are significantly larger than those in the cornea and the typical band pattern which reflects the uniform sequence of the constituent amino acids is clearly seen (Fig. 2(b)). The fibrils display little uniformity in either diameter or distribution and the rare supporting cell appears scattered randomly throughout the tissue.

The ability of the cornea to support the tension caused by the intraocular pressure is generally believed to follow from the mechanical strength of the collagen fibrils. These fibrils run through the stroma much like steel reinforcing rods through concrete, and are anchored in the surrounding tissue of the eye wall. Their existence poses certain problems with respect to transparency, however, because their index of refraction differs from that of the surrounding medium (the "ground substance"), so that they must scatter light. We shall examine this question in more detail shortly.

First, however, we note that the distribution of these fibrils about each other is of special importance because it reflects the nature of the forces exerted between the collagen fibrils. A quantitative description of this distribution is provided by the so-called radial distribution function, $g(r)$, defined as the average local number density of fibril centers at a distance r from an arbitrary center to the average (bulk) number density of fibril centers. The radial distribution function obtained by analysis of the electron micrograph is shown by Fig. 3. If there were no forces of interaction between the fibrils they would be distributed randomly, and $g(r)$ would be unity for all values of r (except $r=0$); the extent to which $g(r)$ differs from unity at any distance indicates some degree of local order persisting to that distance. Thus, the radial distribution function reveals information concerning the interfibril forces and, to the extent that the electron micrographs are valid, provides the basis for one of the first tests of any theoretical model of the microstructuré of the stroma.

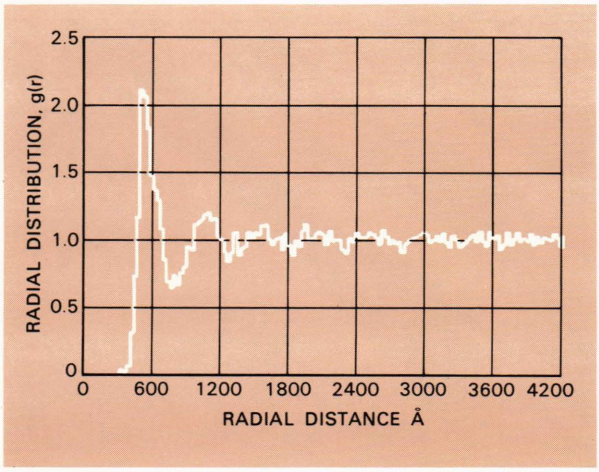

Fig. 3—Histogram of the radial distribution function of a central region of Fig. 2, as obtained by determining the ratio of local to bulk number densities of fibrils as a function of the radial distance from the middle of the reference fibril, (using 700 reference centers). (From Ref. 3)

It is important to recognize, however, that the validity of the structure as revealed by the electron microscope is questionable. This follows from the fact that, in order to obtain the electron micrographs, the stromal tissue is first infused with an electron dense substance in order to achieve contrast, then pickled, in order to preserve it, and finally saturated by a liquid plastic which then solidifies and provides dimensional stability for slicing into thin sections. Thus, it is difficult to determine how accurately the observed spatial distribution of fibrils reflects the actual distribution.

In order to investigate this question, we note that the transmission of light through the cornea will depend on the spatial distribution of the collagen fibrils. Thus, an indication that the radial distribution function obtained from the electron micrograph is at least approximately valid can be obtained if the calculated light transmission from that distribution is in close agreement with the measured transmission through freshly excised cornea.

Light Scattering in the Stroma

It is quite evident that an array of cylinders such as that shown in Fig. 2 will, in general, scatter light. In order that the cornea be essentially transparent, it is necessary that relatively little light be scattered out of the incident beam. How much light is scattered by each cylinder depends on its diameter and on how much the index of refraction of the cylinder differs from that of the ground substance; the total amount of light scattered in any direction depends on the extent to which the scattering from the individual cylinders interferes constructively or destructively in that direction. This, in turn is determined by the optical path lengths, and thus by the spatial distribution of fibrils about each other. If, for example, the spatial arrangement were crystalline, destructive interference would be essentially complete for all angles except for that of

the incident beam. In this event, the stroma would be perfectly transparent. If, on the other hand, the fibrils were distributed purely at random, the optical path lengths—the distance from source to scatterer to detector—would also be distributed purely at random (except in the direction of the incident beam), so that the individual scattered fields would add with random phase, i.e., incoherently. This possibility was investigated a number of years ago by Maurice, who showed that more than 90% of the incident light would be scattered in traversing the cornea if the fibrils were distributed purely at random. Thus we see that the transparency of the cornea must indeed depend in a rather sensitive fashion on the spatial distribution of the collagen fibrils. In fact, one explanation of transparency has assumed that the fibrils are arranged in a crystalline array, and this would imply that the randomness shown by the electron micrographs is spurious, being introduced by the fixation process.

However, since the spatial distribution shown by an electron micrograph is obviously not purely random, it is not legitimate to abandon it merely on this basis. Rather, it is necessary to calculate the scattering that would result from the observed distribution and see whether it is or is not consistent with the observed transparency of the cornea.

We have carried through the necessary theory, as described in detail in another publication.[1] The general nature of the theoretical analysis consists in first obtaining the solution of Maxwell's equations for the electromagnetic field arising from the presence of a single cylinder (e.g., fibril) illuminated by an incident plane wave, and then summing the individual fields arising from the many cylinders whose spatial distribution is characterized by $g(r)$.

Figure 4 is illustrative of the degree of correspondence between experimentally measured and theoretically calculated transmission vs. wavelength curves for rabbit cornea. (The theoretical results are only semiquantitative except at a wavelength of 5000 Å, because the index of refraction of the fibrils has been measured only at this wavelength, and was held constant at that value for the calculations.) Since the behavior of $g(r)$ was found to vary somewhat from cornea to cornea and from one local region to another within the same cornea, some variation also was found in theoretical curves. Data analysis indicates that a major source of the variability derives from some inhomogeneity in the electron micrographs. Part of this inhomogeneity may be real and part may be a fixation artifact, but in any case its effects on light transmission were not large, and in no case yet investigated

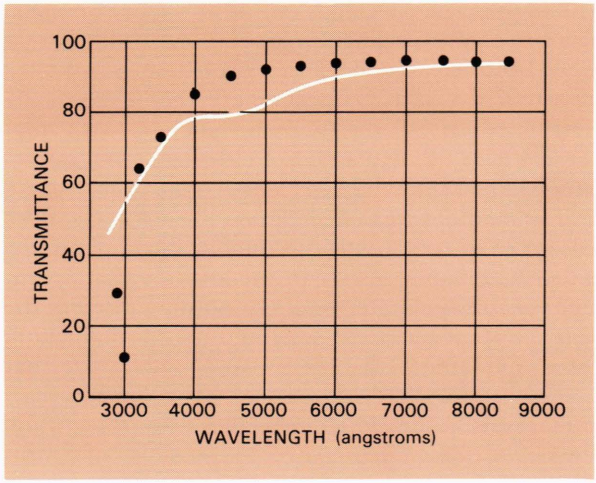

Fig. 4—Theoretically calculated light transmission through the stromal region of the cornea (smooth curve) compared with experimental data from two freshly excised rabbit corneas, (from Ref. 1).

(3 corneas, 7 regions) was the calculated transmission at $\lambda = 5000$ Å less than that shown on Fig. 4. Thus, we have developed a new theory of light scattering in the stroma, and shown that, at least with respect to the fibrils, the quasi-ordered-quasi-random structure revealed by the electron micrographs may indeed be a reasonable approximation to the actual structure.

A Model of Stroma

Accepting, therefore, the hypothesis that the fibrils of stroma are distributed more or less as shown by the radial distribution function obtained from electron micrographs, we were led to consider the question of how that distribution arises. In order to answer this question, we constructed a theoretical macromolecular model for which the radial distribution can be calculated.[2,3]

GENERAL APPROACH—The techniques of statistical mechanics provide a mathematical formalism for calculating the radial distribution function of a system of particles when the force laws characterizing the inter-particle forces are known. As will be discussed, there is a considerable body of more or less indirect experimental evidence suggesting that the collagen fibrils of stroma are held together by mucopolysaccharide polymeric chains extending between and fastened to them. The essential mechanical properties of such chains are known from polymer theory in terms of parameters whose

[1] R. W. Hart and R. A. Farrell, "Light Scattering in the Cornea," to appear in *J. Opt. Soc. Am.* (in press).

[2] M. E. Langham, R. W. Hart, and J. Cox, "The Interaction of Collagen and Mucopolysaccharides," to appear in *The Cornea*, M. E. Langham, Ed., The Johns Hopkins Press, Baltimore, 1969 (in press).

[3] R. A. Farrell and R. W. Hart, "On the Spatial Organization of Macromolecules in the Cornea," (to be published).

values can be inferred, at least approximately, from related experimental studies. Accordingly, we have been led to model the stroma in terms of a network of chains, and compare the theoretically calculated radial distribution function with that obtained experimentally from the electron micrograph.

The mathematical formulation is via the canonical ensemble of Gibbs, wherein the likelihood of finding a thermodynamic system in any arbitrary configuration is expressed in terms of the number of ways the configuration can arise, weighted by the Boltzmann factor containing the energy associated with that configuration. The theoretical (two-dimensional) radial distribution function is found by integrating the Gibbs phase function over all possible configurations for which a fibril center lies in the interval dr at a distance r with respect to a reference center $r=0$, (and dividing by the average number of fibril centers in that interval), subject to the constraint specifying the bulk number density of fibrils.

As will be described, rather good agreement has been obtained for network topologies similar to those found in other connective tissues, using parameter values that are thought to be representative of stroma. Thus far, the central result is that the observed spatial distribution of collagen fibrils can be explained, at least semiquantitatively, in terms of a theoretical model in which the fibrils are held together by polymeric chains extending between them. The detailed topology of the chain-fibril connections remains partially open, however, and probably will be determined ultimately only by new and improved techniques.

THE ORIGIN OF THE FORCES BETWEEN FIBRILS—In order to carry through this approach, it is necessary to define a theoretical model of the stroma for which the configurational energy can be formulated. Thus, the initial question concerns the nature of the interfibril forces that determine the configurational energy, and that must be responsible for a significant degree of order extending over distances of more than a thousand angstroms. These forces are not likely to be primarily the usual van der Waals forces between the fibrils, which have ranges of the order of only a few angstroms. Further, the forces between the fibrils are not likely to be primarily electrostatic forces, which have a range (i.e., a Debye shielding length) of less than about 10 Å in an electrolyte of ionic strength $\sim$ 0.15 N, such as that of normal stroma. As discussed,[2,3] the essential clue may be found in the fact that many properties of the stroma depend sensitively on the mucopolysaccharide constituent of the ground substance, which may be regarded as the "glue" that holds the stroma together. These molecules exist, typically, in the form of linear polymeric chains, and are known to bond to collagen, so that the forces associated with the stretching of polymeric chains of mucopolysaccharide or with mucopolysaccharide constituent, would provide long range interfibril forces.

THE CONFIGURATIONAL ENERGY—Recalling that we must formulate the configurational energy of the stroma, it is evident that we are faced with two main kinds of problem. The first concerns specification of the mechanical behavior of an individual chain, and the second concerns the geometrical layout of the chain-fibril connections. We shall discuss these two problems in turn.

The first problem demands an expression for the configurational free energy of a polymer chain in terms of the end-to-end length of the chain. In polymer theory, this free energy is usually represented as the sum of two components. The first is the free energy in the absence of monomer-solvent and long-range monomer-monomer interactions. It is the free energy of a "phantom" chain, and is easily calculated. The second term, the so-called free energy of mixing, corrects for the neglect of these interactions. Its relative importance depends especially on the monomer-solvent interaction, and thus on the nature of the solution in which the cornea is immersed, and is very difficult to estimate on the basis of existing information. If the excised cornea is immersed in a "good solvent," the free energy of mixing will be of major importance, tending to cause the cornea to swell to a sufficiently large volume until tension in the collagen fibrils and in the phantom chains results in a net force balance. If the cornea is immersed in a rather "poor solvent," the free energy of mixing is relatively small. In the present theory, where we are concerned with the radial distribution function of the electron micrograph, we shall assume that the final fixation bath is a sufficiently poor solvent so that free energy of mixing is negligible. This assumption is more or less arbitrary, although the fact that the baths of the fixation process are so chosen that the cornea maintains itself at essentially constant volume suggests that our neglect of the free energy of mixing may not be very serious in the present case.

The relationship between the stretching force and the length of a phantom chain is known from studies of other polymers (such as rubber), where it has been shown that in this respect a phantom chain is like an ideal spring with the stretching force being proportional to the distance from one end of the chain to the other, i.e., $F = -Kh$, where K is the "spring constant" and h is the chain length.[4] Thus,

[4]H. M. James, "Statistical Properties of Networks of Flexible Chains," *J. Chem. Phys.* **15**, 1947, 651–668.

the configurational energy to be associated with the j-th chain is

$$\varphi_j = \frac{1}{2} K_j h_j^2, \qquad (1)$$

where K_j is the spring constant and h_j is the length of the j-th chain. We shall assume for our model that all of the chains have identical spring constants, recognizing that this assumption no doubt assigns to the model somewhat less randomness than is actually present in the stroma. As a result of this, and other idealizations to be discussed subsequently, the theoretical radial distribution function will no doubt exhibit somewhat greater order in the fibril arrangement than does the experimental one.

It will be recalled that the spring constant of a phantom chain [4] is given by

$$K = \frac{3\,kT}{\langle h_0^2 \rangle},$$

where k is Boltzmann's constant, T is temperature, and $\sqrt{\langle h_0^2 \rangle}$ is the root mean square (end-to-end) length that the chain would have if its ends were free. Its order of magnitude may be estimated from the results of viscosity measurements of free chains of free mucopolysaccharides in bovine cartilage. When the stroma is denatured by extraction of its major mucopolysaccharide constituents, two major components are found. One of these (chondroitin sulfate) is found to have a molecular weight of $\sim 4 \times 10^4$ and the other (keratan sulfate) is found to have a molecular weight of $\sim 2 \times 10^4$. Measurements of the viscosity of free chondroitin sulfate chains of molecular weight of $\sim 5 \times 10^4$ (obtained from bovine cartilage) correspond to a root-mean-square end-to-end distance of ~ 250 Å, so that the value of $\sqrt{\langle h_0^2 \rangle}$ characterizing the stromal chains is presumed to be comparable to 250 Å. There is, of course, considerable uncertainty in this estimate, especially because the chains in natural stroma may well have a protein as well as a mucopolysaccharide constituent.

To complete the formulation of the configurational energy of the network, it is necessary to consider the configurational energy associated with stretching the fibrils. For this purpose, each fibril is thought of as being divided into a large number of segments whose lengths equal the axial distance between chain connection points. Each connection point may, if we like, be thought of as a "molecule," each interacting with other "molecules" to which it is paired by virtue of being connected by chains or segments. The segments are assumed to be identical. (This assumption, like the assumption of identical chains, no doubt introduces somewhat less randomness into the model than actually exists in stroma.) Since a collagen fibril is made up of a complex of polymeric chains, the pair-potential associated with the stretching of a segment is assumed to depend on the distance between its ends. It is not necessary, however, to specify the precise functional form of this dependence. Rather, the pair-potential associated with the stretching of segments is assumed to be a general function of the distance between the endpoints. This function is expanded in a Taylor's series about the most probable free length, and the relevant coefficients are evaluated in terms of K on the assumption that the tissue is in stress-strain equilibrium at constant volume under the influence of no external forces.

Topology of Chain-Fibril Connections—In order to evaluate the radial distribution function characterizing the distribution of the fibrils, it is necessary to relate the chain lengths (i.e., the h_j's) to the separation distances between fibrils. For this purpose, we must specify the topology of the chain-fibril connections.

Perhaps the first question that arises concerns how many chains should be assumed to connect to the end of each fibril segment. Although we have investigated other possibilities, the assumption that six chains terminate on each fibril segment leads to the best agreement between the theoretical and the experimental radial distribution functions, as will be discussed later. It will be noted that the symmetry of this topology implies that the minimum energy configuration will be that of a centered-hexagonal lattice, such as is found in other connective tissue,

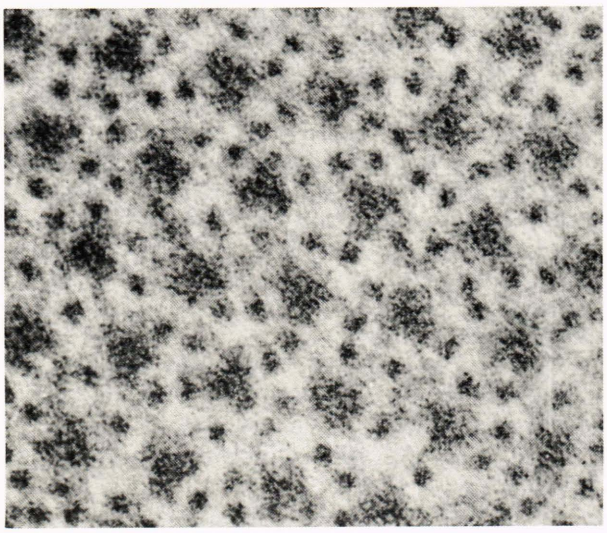

Fig. 5—The lattice-like disposition of fibrils in frog muscle, (from Ref. 5), showing the thick fibrils to be arranged in a somewhat disordered centered-hexagonal array. (Published by permission of Prof. H. E. Huxley.)

[5] H. E. Huxley, "The Mechanism of Muscle Contraction," *Scientific American* **213**, December 1965, 18–27.

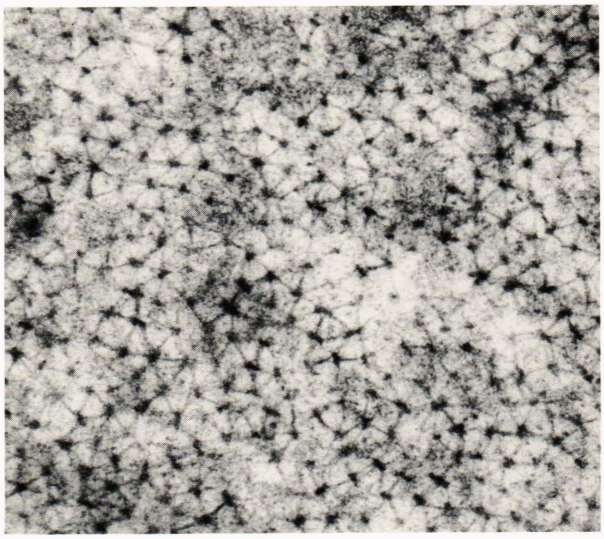

Fig. 6—The lattice-like disposition of collagen fibrils in Descemet's membrane of bovine cornea, (from Ref. 6), showing the collagen fibrils to be arranged in a somewhat disordered centered-hexagonal array. Here, macromolecular bridges between most fibrils are clearly visible, six bridges extending from each fibril to neighboring fibrils. (Published by permission of Dr. Marie A. Jakus.)

e.g., in muscle (Fig. 5) and in Descemet's membrane of the cornea (Fig. 6).

We must now consider whether the chains extend directly from one collagen fibril to another, or whether the connection is accomplished through the intermediary of a noncollagenous protein core, as has been observed in certain other connective tissue. In particular, in bovine cartilage (and also in muscle) there are believed to be long thin cylindrical protein molecules between the thick (e.g., collagen) fibrils, with their axes aligned substantially parallel to each other. One end of a bridging molecule is attached to a collagen fibril and the other end to the intermediary "protein core." In the absence of a definitive answer to this question for stroma, we consider in the theory four possibilities, shown schematically in Fig. 7.

1. Direct connections, fibril-chain-fibril, (i.e., no protein core), as suggested by the electron micrograph of Descemet's membrane, Fig. 6).

2(a). Indirect connections with two chains terminating on each core.

2(b). Indirect connections with three chains terminating on each core, (which leads to the well-known "double lattice" of muscle).

2(c). Indirect connections with six chains terminating on each core.

[6]M. Jakus, *Ocular Fine Structure,* plate 25, Little, Brown & Co., Boston, 1964.

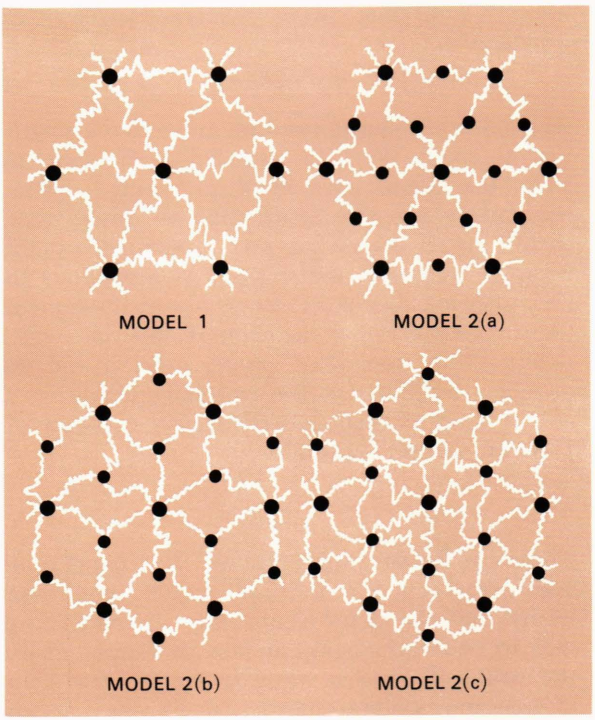

Fig. 7—Schematic representation of the four topologies of fibril-bridge connections considered in the theory. The large dots depict collagen fibrils and the small dots depict the protein cores.

Because of the mathematical difficulties associated with a general treatment of Models 2(a) to 2(c), we have so far considered only Models 2(a) and 2(b) in the limit of an axially weak protein core, and Model 2(c) in the limit that the force law of the protein core is identical to that of the collagen fibril.

THE "REFERENCE" LATTICE—One further feature of the model is now to be introduced in order to make it possible to carry through integrations of the Gibbs phase function over the configurations of the network. Since the configuration energy is quadratic in the position coordinates, the integrals can be carried out by standard techniques (used in the statistical mechanics of ferromagnetism), if we can assign definite numerical labels to the various sites that are interconnected by the chains and segments. For this purpose, we assume that any possible configuration is achievable by deformation of an array in which the chains connect only nearest neighbors. Thus, for numbering purposes only, we may order the connection sites according to a perfect "reference lattice." The stroma may well not be assembled in quite such an ideal fashion, of course, and we expect that this assumption, like the others preceding it, will introduce somewhat more order into the arrangement of fibrils in the

model than will actually be found to occur in the stroma. Nevertheless, an assumption of this kind appears to be necessary for purposes of mathematical tractability, and it leads to the simple numbering system shown in Fig. 8, which illustrates a plane of the reference lattice, cut transverse to the fibril axis direction and passing through segment ends to display connection sites.

RECAPITULATION—We have now completed the specification of a model of the stroma. From the purely physical standpoint, it may be visualized as a network of more or less elastic fibrils held together by a matrix of polymeric chains (with or without a protein core), interconnected according to one of four topological schemes. From the standpoint of mathematical analysis, the network can be represented in terms of a regular lattice with quadratic form interactions between nearest neighbor sites.

THE THEORETICAL EXPRESSION FOR THE RADIAL DISTRIBUTION FUNCTION—We shall pass over, here, the tedious but straightforward mathematical manipulations that stand between the formulation of the configurational energy and the final expression for the radial distribution function, $g(r)$.[3] The form of the final result is, in general, rather complicated and therefore tends to be unilluminating. For this reason, we shall limit our discussion to an approximation to $g(r)$ that is accurate for $r \gtrsim 150$ Å. We find

$$g(r) = \frac{1}{2\pi\sigma_c r} \sum_{\tilde{\ell}} \sum_{\tilde{m}} \frac{1}{\sqrt{\pi}\,\Delta_{\tilde{\ell},\tilde{m}}} \exp\left[-\left(\frac{r-\tilde{r}_{\tilde{\ell},\tilde{m}}}{\Delta_{\tilde{\ell},\tilde{m}}}\right)^2\right],$$

excluding $\tilde{m} = \tilde{\ell} = 0$

$$r \gtrsim \frac{\Delta^2_{\tilde{\ell},\tilde{m}}}{2\,\tilde{r}_{\tilde{\ell},\tilde{m}}}, \quad \tilde{\ell},\tilde{m} = 0, \pm 1, \pm 2, \ldots . \qquad (2)$$

where σ_c = number of collagen fibrils per unit area ($\approx 3.51 \times 10^{-6}$ (Å)$^{-2}$ for Fig. 2); $r_{\tilde{\ell},\tilde{m}}$ = the radial distance from the reference lattice site of a reference fibril, say the (ℓ',m')-th, to the reference lattice site of the (ℓ,m)-th fibril, with $\tilde{\ell}=\ell-\ell'$, $\tilde{m}=m-m'$ (see the numbering scheme of Fig. 8). For the centered hexagonal case, $r_{\tilde{\ell},\tilde{m}} = b_c\sqrt{\tilde{\ell}^2+\tilde{m}^2+\tilde{\ell}\tilde{m}}$, where $b_c = \left(\frac{2}{\sigma_c\sqrt{3}}\right)^{1/2}$ = mean distance between centers ≈ 574 Å for Fig. 2. $\Delta_{\tilde{\ell},\tilde{m}}$ is a rather complicated function of the spring constant K, the fibril and chain number densities, their manner of interconnection, and $\tilde{\ell}$ and $\tilde{m}$. It is a measure of the mechanical looseness of the network, as will be discussed.

THEORY VS. EXPERIMENT—RADIAL DISTRIBUTION FUNCTION—For the purposes of making a comparison between theory and experiment, it is

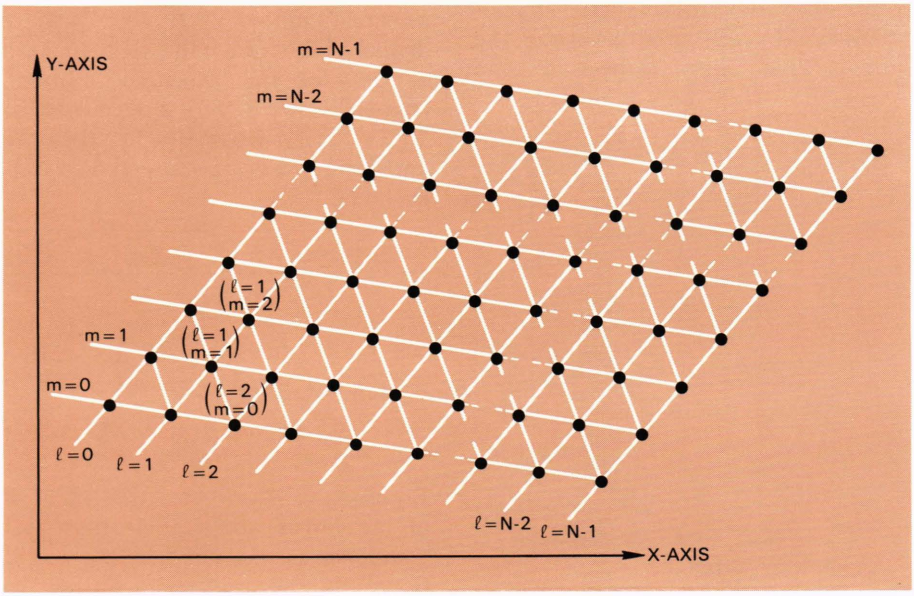

Fig. 8—Schematic representation of the labeling system for a centered-hexagonal reference lattice. The figure displays one of the transverse planes. The index ℓ labels the position in the row and the index m labels the row. A third index, N, labels the transverse plane, (from Ref. 2).

necessary to assign value to $\sqrt{\langle h_0^2 \rangle}$, and to the number densities of chains and fibrils. Only the number density of fibrils is accurately known. From various experiments, the number density of the chains (which follows from the mass fraction of mucopolysaccharide in the stroma, the fraction of it that is used up in the form of chains and the molecular weight of the chains) is estimated as $\sim 10^{-7}$ / (Å)^3. Figure 9 illustrates the comparison between theory and experiment for Model 2(a), with $\sqrt{\langle h_0^2 \rangle} \approx 370$ Å, and shows modest general agreement. As the nature of several of our approximations have led us to expect, the peaks of the theoretically derived radial distribution function of the model decrease less rapidly with distance than do those of the experimentally derived radial distribution function. Of course, some disordering is no doubt introduced by the electron micrograph fixation technique, so that it is conceivable that the theoretical model is less at fault than the experiment.

The theoretical results depend especially on the value assigned to $\sqrt{\langle h_0^2 \rangle}$, as will be discussed shortly. We note in passing, however, that curves very similar to that of Fig. 9 are obtained if we use $\sqrt{\langle h_0^2 \rangle} \approx 710$ Å, 430 Å, and 360 Å in Models 1, 2(b), and 2(c), respectively. Thus the models appear to be in rough accord with currently available data, considering the uncertainty in the values of the quantities that determine the parameters of the model, and the likelihood that the stromal structure is somewhat disordered during the fixation process.

QUALITATIVE NATURE OF THE RADIAL DISTRIBUTION OF THE MODEL—The essential nature of the theoretical result is that, with respect to the reference center as origin, other fibril centers tend to be Gaussianly dispersed about certain most probable positions that define a lattice. The lattice of most probable relative positions depends on the number of chains connecting each fibril segment with nearby segments. For example, for three, four, and six chains connecting each fibril segment with nearby segments, the lattices are simple hexagonal, simple cubic and centered-hexagonal, respectively. Since the axes of the fibrils are most likely to be found rather near the lattice sites, the type of lattice determines to a large extent where the peaks and valleys of $g(r)$ occur. Comparison of the theoretically calculated $g(r)$ with the experimentally derived $g(r)$ has shown that the centered-hexagonal structure leads to rather good accord, whereas the simple hexagonal and the simple cubic do not. Accordingly, we were led to model the stroma by assigning six chain terminations at each end of a fibril segment.

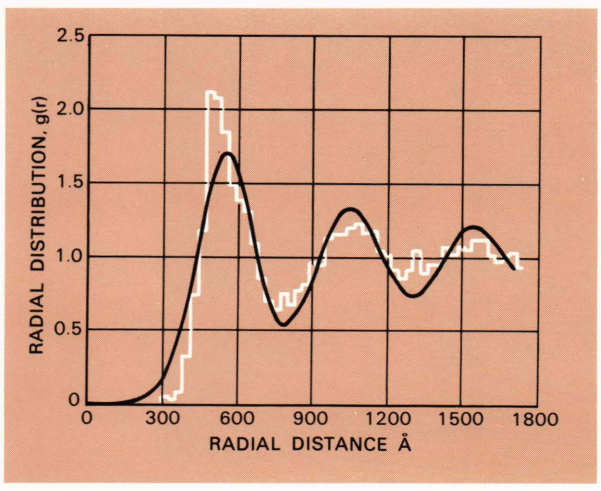

Fig. 9—Comparison of the theoretically calculated radial distribution function with that obtained by analysis of the electron micrograph of Fig. 2.

As Eq. (2) shows, the dispersion of the most probable relative positions and the height of the first maximum of $g(r)$ depend primarily on the looseness of the network (through the parameter $\Delta_{\tilde{l},\tilde{m}}$). For the present semiquantitative discussion, $\Delta_{\tilde{l},\tilde{m}}$ is approximated (to $\sim 10\%$ for $\tilde{l}$, $\tilde{m}$ not much greater than unity)by

$$\Delta_{\tilde{l},\tilde{m}} \approx \Delta_{0,1} \approx \sqrt{\langle h_0^2 \rangle} \left\{ \frac{1}{3^{3/2}\,\gamma \sqrt{1 + 0.22 \left(\frac{b_c}{\tilde{l}}\right)^2 \eta}} \right\}^{1/2}. \quad (3)$$

The parameters γ and η depend only on the assumed topology; $\tilde{l}$, the most probable axial distance between chains, depends on both the topology and on the assumed number densities of chains [$\sim 10^{-7}$ / (Å)^3] and collagen fibrils [$\sim 3.51 \times 10^{-6}$ / (Å)^2]. Estimates for these three quantities, and for $\Delta_{0,1}$ as approximated by Eq. (3), are given in the table.

Model	*1*	*2(a)*	*2(b)*	*2(c)*
γ	1	½	⅔	1
η	1	1	1	⅓
$\tilde{l}$	105 Å	210 Å	210 Å	315 Å
$\Delta_{0,1}$	65 Å	119 Å	103 Å	102 Å

where $\sqrt{\langle h_0^2 \rangle} \approx 245$ Å, $b_c \approx 574$ Å

Equation (3) shows that the extent of the dispersion of the fibrils about their most probable relative positions, as measured by $\Delta_{0,1}$, is directly proportional to the root-mean-square length that the chains would have if they were unattached, and thus is inversely proportional to the square root of the spring constant of the individual chains. The table shows that it also depends on the scheme of chain-

fibril connections. Model 1 is the stiffest model essentially because its chains must reach all the way between fibrils, (see Fig. 7).

The first maximum of $g(r)$ is of rather special interest because it can be very easily approximated using Eqs. (2), (3),* and because disorder ensuing from the fixation process is expected to be least noticeable for small r. Equation (2) yields

$$g(r)\,|_{\text{first maximum}} \approx \frac{6}{2\pi^{3/2}\sigma_c\, b_c\, \Delta_{0,1}}, \quad (4)$$

and substituting the values listed in the table yields

$$g(r)\,|_{\text{first maximum}} \approx \begin{cases} 4.1 & \text{Model 1} \\ 2.2 & \text{Model 2(a)} \\ 2.6 & \text{Model 2(b)} \\ 2.6 & \text{Model 2(c)} \end{cases}$$

Thus, the last three models continue to agree with our estimate of $\sqrt{\langle h_0^2 \rangle}$ rather better than does Model 1, again because its chains, being required to reach all the way from one fibril to another, are stretched relatively tightly so that there is relatively little dispersion from the minimum energy configuration. Model 1 could, of course, be in good accord if its chains were composed of mucopolysaccharide and mucoprotein chains hooked in series. We are, therefore, unable with confidence to discriminate between the various models on the basis of presently available information.

Structure and Swelling Pressure

Since the previously described study led to a way of connecting the mucopolysaccharides and the collagen fibrils in such a way as to yield a reasonably satisfactory microstructure, we were led to consider the swelling properties of such a network.

If a piece of cornea is removed from the eye, denuded of its limiting layers, and placed in saline, it will swell in thickness by taking in saline. The extent of the swelling may be controlled by an externally applied force, and the pressure that is just sufficient to maintain the stroma at some fixed thickness is known as the swelling pressure associated with that thickness. The experimentally determined relationship between swelling pressure and stromal thickness for rabbit cornea in physiological saline is shown by the data points of Fig. 10. The molecular and structural basis for this behavior has remained obscure, however, in the absence of a detailed model of the stroma.

It turns out that an approximate theoretical relationship between swelling pressure and the thickness of stroma in a salt solution can be derived quite readily for our models of chain fibril topology. The basis of the swelling theory derives from the fact that swelling pressure is related thermodynamically

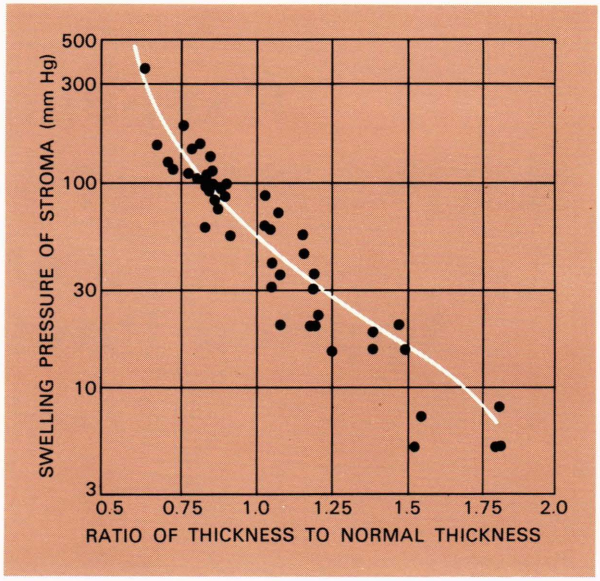

Fig. 10—Comparison of theoretically calculated dependence of swelling pressure on thickness vs. experimental data, for rabbit stroma. (Data points from Ref. 7.)

to the free energy. The free energy, in turn, can be approximated in terms of the properties of the molecular chains and the topology of their connections to the collagen fibrils, by using well-known techniques of polymer theory. The result of the calculation for Model 2(a) is shown by the smooth curve of Fig. 10, where one parameter, whose value is as yet unknown, has been arbitrarily assigned a plausible value that yields good agreement at one point, namely at normal hydration. It seems noteworthy that the agreement is satisfactory over about one and one-half decades of swelling pressure variation, so that the theory may indeed be near the truth in its essentials.

Concluding Remarks

In summary, therefore, the theoretical calculations relating to the transparency, fibril distribution, and swelling pressure support the basic validity of a model of the stroma in which the collagen fibrils are held together by polymeric chains. The detailed topology of the connections remains open, and probably will be settled ultimately only by new and improved electron microscope techniques. Nevertheless, we believe that the basic model is sufficiently representative of the stroma that it will be valuable for the illumination of the molecular and structural basis of many other physiological properties of stroma.

*Only the six nearest neighbor sites contribute appreciably to the sum of Eq. (2), and the value of r at which the peak occurs is $r \approx \bar{r}_{0,1} = b_c$.

[7] B. O. Hedbys and C. H. Dohlman, "A New Method for the Determination of the Swelling Pressure of the Corneal Stroma in vitro," *Exp. Eye Res.* **1**, 1963, 122–129.