The physical basis for transparency of the crystalline lens

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The transmission of light has been studied in tissue-cultured lenses of albino rabbits. The interaction of light with matter can be resolved into the two processes of absorption and scattering. Scattered light was measured from both whole lenses and soluble lens proteins at varied concentrations with a light-scattering photometer. Transmitted light was measured with a recording spectrophotometer. The reduction in intensity of the beam in the visible portion of the spectrum that was transmitted through the albino rabbit lens is shown to be, primarily, a result of light scattering. The back-scattered light from the lens is shown to increase with age. The soluble proteins which comprise most of the interior of the lens fiber act as small particles that scatter light. A paracrystalline state of these proteins with a high degree of spatial order in the intact fiber is inferred to explain the transparency of the fiber. The extinction of light from large particle scattering by the walls of the lens fiber is minimized by their regular spacing. The regularity was shown to have a characteristic diffraction pattern which forms the lenticular halo. Opacification is discussed in terms of quantitative changes in light scattering.

Measurements of light transmission through the crystalline lens were reported by Ludvigh and McCarthy in 1938 and more recently by Weale in 1954. However, there has been no analysis of the transmission in terms of the physical interactions between the light and the known structures of the lens. It is the purpose of this study to investigate the qualitative and quantitative aspects of these interactions.

The main refractive volume of the crystalline lens consists of fibers of cellular origin with a known cortical organization. The cortical fibers, derived from the lens epithelium, are surrounded by cell membranes. They have a defined protein fraction which comprises most of their cytoplasm. The morphology of the lenticular nucleus has been studied by biomicroscopy, and, to some extent, by light microscopy, but it has eluded electron microscopic study because of the technical difficulty of fixation of sites deep within the lens.

The molecular structure of the lens proteins has been intensively studied by many investigators. Mörner in 1894, first separated the proteins of the lens into three fractions, and, recently, physicochemical analyses of several of the soluble lens proteins have been reported upon. The manner in which these molecular and microscopic structures affect the traversing light wave determines the transmission characteristics of the lens. These characteristics depend upon the two processes of absorption and scattering. Absorption is the conversion of light from the...
incident beam to other forms of energy, such as heat or chemical energy. Scattering takes place when light passes over the elastically bound electrons in the atoms and molecules. The scattering interaction may be thought of as producing elastic vibrations which result in the emission of secondary light in all directions. Thus, scattering also removes energy from the traversing beam.

Exponential coefficients, $a_a$ and $a_s$, are used to describe the removal of energy from the light beam by the processes of absorption and scattering, respectively. The extinction coefficient, $a_0$, is defined as the sum of the absorption and scattering coefficients. This definition substitutes the terms extinction and absorption for the more unwieldy “absorption” and “true absorption” and is the preferred nomenclature. The intensity ($I$) of a beam of light after it traverses the lens will be less than the incident intensity $I_o$. It can be shown that $I$ depends upon the length of the optical path ($t$) according to the equation:

$$I = I_o e^{-a_0t}$$

where

$$a_0 = a_a + a_s$$

A distinction is made between light scattering by small and by large particles. Scattering by small particles occurs when the objects are smaller than the wavelength of light, such as the soluble proteins of the lens. Large particles are larger than several wavelengths in size, and are the structures that can be resolved by the light microscope.

In the present study, precise measurements were made of extinction and scattering of light by the lens. Understanding the processes in terms of known molecular and microscopic structure should lead to a qualitative explanation of transparency of the crystalline lens.

**Method**

The measurements of transmitted and scattered light were obtained from an isolated lens mounted in an optical bench. The lenses were those of New Zealand albino rabbits of various ages. Each animal was sacrificed by air embolism, the globe enucleated, and the cornea and iris removed. A scleral annulus was prepared; it contained the intact zonular lamellae that attached the lens to the ciliary body. The preparation was mounted (Fig. 1) on a stainless steel holder with stainless steel pins.

The lens holder was fastened to a machined nylon cap which held and centered the lens in a cylindrical Pyrex chamber, designed to function as a tissue culture chamber and at the same time allowed optical study of the lens. A 10 ohm nichrome coil, isolated in a glass tube at the cell bottom, served, in conjunction with a thermistor, to maintain the temperature at 35°C. The tissue-culture technique described by Schwartz and modified by Eagle's more recent amino acid mixture was used as the basis for the in vitro system.

Immediately prior to use, the medium was filtered through a Millipore pressure filtration apparatus with a GS filter of 0.22µm pore diameter and a microfiber glass prefilter. The filtration had the dual function of sterilization and clarification. This reduced the turbidity of the medium, an essential process because it was part of the optical path in measurements of scattering.

The scattering measurements were made on the optical bench of an Aminco model 4-6000 light-scattering photometer (Fig. 2). This instrument had a mercury light source with suitable filters for the selection of spectral lines. An RCA 1P21 photomultiplier tube mounted on a rotatable circular plate permitted measurement of the intensity of the scattered light at angles from 15 to 145 degrees relative to the transmitted beam. A high voltage supply for the photomultiplier tube and a d.c. amplifier to measure its plate current completed the unit. The tissue culture cell was mounted in the center of the light-tight chamber surrounding the optical bench.

Measurements of scattering were also made on
Fig. 2. Design of the optical bench for the measurement of light scattered by the crystalline lens. These components are contained within a light-tight chamber. $\theta$ is the angle between the viewing photomultiplier and the transmitted beam.

Fig. 3. Extinction curve of the lens of a 2½-month-old male albino rabbit. The optical density caused by extinction of light is recorded as a function of wavelength.

soluble protein extracts with the same arrangement. The concentration of the soluble proteins was determined by the Lowry modification of the Folin-Ciocalteau method.\textsuperscript{13} A 0.5 per cent solution of polystyrene in toluene was used as a standard of scattering to calibrate the instrument.

After filtration, the medium was gassed with a mixture of 4.2 per cent CO\textsubscript{2}, 9.0 per cent O\textsubscript{2}, and 86.8 per cent N\textsubscript{2} for one hour.\textsuperscript{9} The sterilized cell was connected to the medium storage flask and to a drain reservoir. The cell was then positioned in the optical bench and filled with tissue culture medium. The lens preparation was placed in the chamber and adjusted so that the transmitted light from the mercury source fell upon the receiver port at 0 degrees.

Temperature equilibration in the tissue culture cell was reached within 90 seconds and the distribution of scattered light was determined with a receiver port which resolved 3 degrees of arc.

Data reported were the average of three runs taken between 15 and 45 minutes after equilibration. Wavelengths of 4,360 Å and 5,780 Å were used in these scattering measurements.

Extinction measurements were made by placing this lens preparation in the beam of a Cary model 14 recording spectrophotometer. The preparation was placed close to the receiver port to assure the collection of the entire transmitted beam at all wavelengths of light. The intensity of transmitted light was measured over the spectral range between 8,000 and 3,700 Å.

**Results**

**Extinction measurements.** A typical extinction curve obtained for the lens of a 10-week-old albino male rabbit (Fig. 3) shows the optical density as a continuous function of wavelength from 8,000 to 3,700 Å. A decrease in optical density was found from the infra-red at 8,000 Å to a minimum for this lens at 7,600 Å. From the latter point there is a gradual increase in the optical density with decreasing wavelength. At about 4,300 Å, the curve rises abruptly and shows that the optical density increases rapidly. The lens becomes effectively opaque for the energy available in the spectrophotometer beam at 3,700 Å and shorter wavelengths.

Absorption, as distinguished from extinction, begins at wavelengths greater than 7,000 and less than 4,200 Å. The region from 4,200 to 7,600 Å is free of significant absorption. This pattern of extinction in the central region of gradual increase in optical density is characteristic of loss of light due to scattering.

**Scattering measurements.**

**Intact lens.** Typical curves which show the angular distribution of scattered light are shown in Fig. 4. These data were scattering patterns for the lenses of a 2½-month- and a 30-month-old albino rabbit for the 2 wavelengths of 4,360 and 5,780 Å. The intensity of scattered light, $i_\theta$, is plotted as a function of the viewing angle, $\theta$, from 20 to 145 degrees relative to the transmitted beam. The intensity of the incident light was the same for all the curves.

The interrupted lines between 80 and
100 degrees estimate the scattered light at $\theta = 90$ degrees. At this angle the lens holder obstructs the view of the photomultiplier tube.

The intensity of the scattered light is greater for the older lens at all angles. This is especially true in the region from 100 to 145 degrees in which the rapid increase in scattering intensity introduces a marked asymmetry to the curve. Because this is light scattered back toward the source, it is called “back scatter.” If ratios are made of the back-scattered light at 135 degrees to the forward-scattered light at 45 degrees, the asymmetry can be compared for different animals. In Fig. 5, this ratio is plotted as a function of age for 6 rabbits ranging in age from 2 to 30 months. The ratio can be seen to increase with age in the samples studied. No systematic attempt was made to explore the change in the scattering pattern with time in the in vitro system.

**Extracted proteins.** The scattering at 90 degrees per unit concentration soluble protein, $i_{90}/c$, was measured as a function of $c$, the weight concentration of the protein. The results are summarized in Table I. At low concentrations, the scattering per unit concentration protein is constant; it decreases with increasing concentration of soluble protein.

**Discussion**

Lenticular transparency can be satisfactorily understood only when the size, shape, and composition of all the microscopic and molecular components of the lens are known. However, a simple model of the lens can be made which incorporates many known features of the lens structure.

A phase photomicrograph of the unstained cortex of a 2-year-old rabbit’s lens shows the hexagonal organization of the cortical fibers (Fig. 6). Salzmann has described these fibers as radially oriented with a width of 8 to 12 $\mu$ and a thickness of 2 to 5 $\mu$. Electron micrographs confirm these dimensions. Unfortunately, the microscopic structure of the nucleus of

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**Table I.** $i_{90}/c$ varying with concentration soluble protein

<table>
<thead>
<tr>
<th>Protein concentration (%)</th>
<th>0.19</th>
<th>0.38</th>
<th>0.76</th>
<th>1.51</th>
<th>3.02</th>
<th>6.05</th>
<th>12.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$i_{90}/c$</td>
<td>1.6</td>
<td>1.5</td>
<td>1.5</td>
<td>1.25</td>
<td>0.95</td>
<td>0.74</td>
<td>0.46</td>
</tr>
</tbody>
</table>
the lens has not been so clearly delineated as it has been of the cortex.

The main cytoplasmic constituent of the fibers is the physicochemical fraction which contains the soluble proteins of the lens. The insoluble proteins, which constitute about 9 per cent of the total lens protein, are thought to comprise part of the fiber walls and the membranous intracellular structures.

Microscopic and submicroscopic structures cause the extinction of light which determines the transmission characteristics of the lens. This extinction derives from the many processes summarized in Fig. 7. As described in the results, the shape of the experimental extinction curve (Fig. 3) indicates the absence of absorption and the major role of scattering in the extinction of visible light by the albino rabbit’s lens. The physics of light scattering must be examined to understand the extinction characteristics of the lens. Therefore, light scattering by small particles, viz., soluble proteins, and large particles, viz., protein membranes of the lens fibers, is discussed in detail.

Small particle scattering. The equation describing the scatter of light by independent, optically isotropic particles has been derived by Gans as a modification of Rayleigh’s original scattering law for point scatterers. It is known as the Rayleigh-Gans scattering law:

\[ I(\theta, \lambda) = \frac{L}{r^2} \frac{2\pi n_0^2 \left( \frac{dn}{d\lambda} \right)^2}{\lambda^4 N} P(\theta, \lambda) \quad M \quad (1 + \cos^2 \theta) \]

This describes the intensity, \( I_0 \), of the light scattered at the angle \( \theta \) at a distance \( r \) from the scattering proteins of weight concentration \( c \) and molecular weight \( M \). The scatterers are illuminated by an incident light of intensity, \( I_0 \), and wavelength \( \lambda \). \( n_0 \) is the refractive index of the solvent and \( \frac{dn}{dc} \) is the specific refractive increment of the scattering molecules. \( N \) is Avagadro’s number. \( P \), the particle scattering factor, is a function of the particle size, \( \theta \), and \( \lambda \). For very small particles, \( P \) reduces to 1 and the Rayleigh-Gans equation reduces to the Rayleigh scattering formula. Rayleigh scattering then is proportional to the inverse fourth power of the wavelength. However, Rayleigh-Gans scattering is not proportional to the inverse fourth power of wavelength because \( P \) is a function of wavelength.

The total light scattered from independent scatterers is the sum of the separate intensities of light scattered from each molecule. If the soluble proteins of the lens are assumed to act as independent scatterers, their expected transmission at in vivo concentrations may be calculated.
At 4,360 Å the particle scattering factor will be nearly equal to one because the largest protein has a maximum dimension about 700 Å. The refractive index \( n_0 \) for the solvent is estimated to be 1.34. The specific refractive increment for most proteins in an aqueous solution is 0.18. Substituting these values in the Rayleigh-Gans equation, we find:

\[
\frac{I_{0\text{scattering}}}{I_0} = 8.7 \times 10^{-2},
\]

This quantity is directly related to the scattering coefficient \( \alpha \) which may be evaluated as equal to 1.45.

If the rabbit’s lens is taken to be about 0.8 cm. thick, then

\[
I = I_0 e^{-\alpha t} = I_0 e^{-1.45} = 0.31 I_0
\]

If the protein molecules scatter light in an independent fashion, we can expect that the total amount scattered will be 69 per cent of the incident light, and, hence, only 31 per cent will be transmitted. The assumption of independent scattering has been tested and the data are shown in Table I. The decrease in scattering per unit concentration as the protein concentration rises above 1 per cent means that the Rayleigh-Gans equation does not apply for higher protein concentrations. If the scattering coefficient is recalculated for a 12 per cent solution, then

\[
\alpha = 0.138
\]

and for an 0.8 cm. lens

\[
I = I_0 e^{-0.138} = 0.90 I_0
\]

This calculation represents the extinction due to scattering by the soluble proteins at high concentrations. Only 10 per cent of the light is seen to be scattered as compared with 70 per cent if independent scattering is assumed. Interference of this scattered light occurs with the increased local order that exists at high concentrations of proteins. Under these conditions, the scattering and extinction of light decreases and transmission increases. Completely regular crystalline matter will change only the velocity of traversing light without removing energy by scattering. It can be concluded that the high degree of light transmission of the intact lens fibers results from the spatial order of lens proteins in their normal state.

The spatial order of protein molecules can be described by \( \rho(r) \), the probability that two protein molecules are a distance \( r \) apart. The reduction in scattering due to local order has been derived by Zernike and Prins in a general form.

\[
4\pi N \int \frac{\sin ksrdr}{ksr} [1 - \frac{V}{4\pi} \int \rho(r) r^2 dr]
\]

This formula expresses the reduction of scattering of \( N \) particles in a volume \( V \), where \( k = 2\pi/\lambda \), and \( s = 2\sin \theta/2 \). The distribution function \( \rho(r) \) is normalized to unity when all \( r \)'s are equally probable. This is the dilute solution in which this factor reduces to one, and no external interference of scattered light occurs.

Solving this expression for the lens proteins is a very complex problem. It has been solved for a hard sphere model, but this solution is not valid at the high tissue concentration of protein. Quantitative application of the Zernike-Prins factor to the lens proteins in the intact state is not now feasible because the exact dimensions and the spatial order of the proteins in the intact fiber are unknown. Qualitatively, the high concentration of the soluble proteins in the lens fiber must be accompanied by a degree of local order approaching a paracrystalline state. This results in the interference of scattered light and the transparency of the fibers.

**Large particle scattering.** Although the nature of the physical interaction is the same, large particle scattering calls for a mathematical treatment different from that for small particle scattering. Rays incident on an isotropic particle give rise to the phenomena of diffraction and reflection. The reflection is accompanied by refraction at the large particle surface. Diffraction and reflection can be considered special cases of scattering. The phase contrast photomicrograph of an unstained section...
emphasizes those structures which cause large particle scattering (Figs. 6 and 8). The numerous lens fiber membranes diffract and reflect light which is being transmitted by the lens. The diffraction and reflection remove light from the traversing wave and comprise part of the extinction phenomenon.

**Diffraction.** Diffraction, with its characteristic angular distribution of light, is produced at the portion of the wave front which is eliminated when light traverses a large particle. Diffracted light patterns, which are generally more intense than reflected light, are confined to a narrow cone in the forward direction around $\theta = 0$ degrees. The diffracted light contaminates measurements of the intensity of light transmitted by the lens.

The characteristic diffraction pattern of the lens has been known for many years. The lenticular halo, first attributed to the lens in the mid-nineteenth century, was ascribed by Druault \(^{21}\) in 1898 to diffraction by the regularly spaced lens fibers. Recently, the quantitative aspects of lenticular diffraction have been reviewed by Simpson \(^{22}\) who observed a pronounced lenticular halo and studied the angular spacing of the halo rings. Assuming a radial diffraction grating model of the lens, he calculated a grating spacing of about 9.4 μ. This compares with a fiber width of 8 to 10 μ in the human lens \(^{14}\) and 9.2 μ in the rabbit lens.\(^ {15}\)

**Reflection.** Light striking a large particle surface will be reflected and refracted according to the laws of geometrical optics. These laws can be shown to be derived from scattering of light.\(^ {23}\) The membranes of the fibers of the lens cortex can be considered to be large particles which form a stratified dielectric medium, whereas the ultimate structure of the nucleus is unknown.

The amount and distribution of reflected light energy depends upon the form, composition, and surface quality of the stratified membranes. If the surface is smooth with respect to the wavelength of light, there will be specular reflection. Irregular surfaces will diffuse reflect light in many directions. The sutures are visible because the irregular interdigitating membranes\(^ {24}\) reflect light diffusely, whereas the surrounding, ordered fiber walls reflect light specularly. Adjoining membranes of two fibers are about 200 A thick measured in electron micrographs. They are smooth and show no irregularities with dimensions comparable to those of visible light's wavelength. For this model, the membrane pair formed by adjacent fibers will be considered to form a uniform, 200 A thick membrane. Light will be reflected from both surfaces of these membranes, and this also will contribute to the removal of light from the transmitted beam, and, hence, the extinction of light by the lens.

The net reflected wave (r, Fig. 8) is the resultant of the waves reflected at the membrane interfaces. The reflected wave at the denser medium undergoes a 180 degree phase shift, and there is a path difference equal to n2d where d is the thickness of the fiber wall. This path difference is about 640 A or 1/9 of a wavelength. The reflected waves at both surfaces will be out of phase and will interfere...
to reduce the intensity of light extinction at the individual fiber interface.

The observed regular spacing of the many fiber layers results in the interference of specularly reflected light from successive membrane pairs. This interference reduces extinction of light by these structures.

Light reflected from the diagonal walls of the lens fibers ($r_2$ in Fig. 8) is about 60 degrees from the transmitted beam. This effect may account for the small rise in scattering intensity observed between 55 and 60 degrees in the scattering-intensity diagram (Fig. 4).

**Opacity.** It is of interest to conjecture what in the structure of the lens can vary to produce extinction changes that will decrease the light energy the lens can transmit.

The human lens, unlike the albino rabbit's lens, absorbs light in the blue and has a distinct yellow color. This absorption has been shown to increase with age. Therefore, the aged lens will transmit less light in the blue region of the spectrum. The nature of this absorbing pigment and its spectral limits have not been determined.

Another factor is the increased back scattering, such as that observed in the rabbit's lens with increasing age. The intensity of the slit-lamp beam must be increased with the age of the subject. The increased scattering means that less light will be available for retinal imagery and more light will be scattered around the image.

Several factors mentioned in the discussion of small particle scattering will increase the light scatter if changed even slightly. One sensitive variable is $\rho(r)$, the probability function measuring the spatial relationship of the scattering centers. Should there be aggregation of the soluble proteins or any change tending to decrease their order, there will be a marked increase in scattering intensity. Any process tending to increase the weight concentration or molecular weight of these proteins will increase the scatter of light.

Changes in the fiber walls can increase large particle scattering. If the thickness of the fiber wall increases, there will be less destructive interference from both sides of the membrane pair and the net reflected wave will be of greater intensity. If the fiber thickness should vary or the cell wall become irregular, the rays reflected from successive membrane pairs will change their phase relationships. This may lead to increased large particle scatter and decreased transmission of light.

Similar considerations have been made by Maurice in his discussion of the transparency of the cornea. He attributed corneal transparency to the extreme regularity of the corneal stroma, and concluded that any process tending to interfere with the regularity would decrease the external interference of the individual scattering stromal elements. Increased scatter and visible clouding of the cornea would follow.

This discussion on the dependence of the optical properties of the lens upon its microscopic and molecular structure has of necessity, in part, been speculative. Additional details of ultrastructure will add to the understanding of the mechanisms responsible for transparency and opacification.

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