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DIMETHYL SULFOXIDE CATARACT: A MODEL FOR OPTICAL ANISOTROPY FLUCTUATIONS

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ABSTRACT

Rat lenses incubated in dimethyl sulfoxide (DMSO)–water binary mixtures of different compositions became turbid. A slight haziness developed up to 0.062 mole fraction of DMSO; at higher concentrations dense turbidity developed. Microscopic examination of the incubated rat lenses showed damage restricted to the epithelium and outer cortex at low DMSO concentrations; maximum damage occurred, in terms of fiber cell swelling, extracellular fluid (lake) formation and disintegration of epithelium, at 0.25 mole ratio of DMSO. Scanning electron microscopic observation showed that at high DMSO concentrations, especially at 0.25 mole fraction, the contents of the fiber cells were greatly damaged. Apparently a large part of the crystallins had coalesced around the cytoskeletal bodies. Polarized light-scattering intensity measurements and their analyses indicated that when dense opacities developed at high DMSO concentrations, the major contribution to the turbidity came from the optical anisotropy fluctuations. The change in the organization of the components within the fiber cells disturbs the balance between intrinsic birefringence and form birefringence necessary for transparency. Thus, the DMSO-caused opacification can be described as a good model for the involvement of optical anisotropy fluctuations in cataractogenesis.

Keywords light scatter and fluorescence; cataract; dimethyl sulfoxide; optical anisotrophy; scanning electron microscopy.

1 INTRODUCTION

Cataractogenesis is caused by light scattering due to random fluctuations in the refractive index.[1] These fluctuations can be density or optical anisotropy fluctuations. Fluctuations in the refractive index due to density may arise because of aggregation of lens proteins[2,3] or microphase separation.[4,5] In these processes the enhanced turbidity is due to the increase in the size of aggregates or microphases. Another process, syneresis, also causes an increase in the amplitude of the density fluctuations.[6] In syneresis, water is released from the bound state in the hydration layers of lens proteins and becomes bulk water. This in turn increases the refractive index difference between the lens proteins and the surrounding fluid.[7]

Fluctuations in optical anisotropy occur when the light passing through the lens detects changes in the orientation of optically anisotropic bodies. Actin, vimentin, and other cytoskeletal bodies are optically anisotropic because their refractive index along the long axes of the cytoskeleton is different from the refractive index perpendicular to the long axes.[8] In a normal transparent lens, the optical anisotropy fluctuations caused by the change in the orientation of the contents of the fiber cells is balanced by an optical anisotropy of an opposite sign, form birefringence. This arises from the morphology of the lens in which thin membranes of high refractive index are embedded in the cytoplasm of a lower refractive index. This morphology gives rise to Wiener bodies,[9] and the optical anisotropy of Wiener bodies cancels the optical anisotropy of the fiber cell contents in a normal lens.[10] When this balance is upset, turbidity is the result. Analyses of the polarized light scattering of human cataracts[11] have shown that 15 to 30% of the turbidity results from optical anisotropy fluctuations.

There are a number of possible processes that can cause changes in the balance of optical anisotropies (different birefringences) of the lens. Calcium cataract in its early stage is accompanied by the disappearance of vimentin and possibly other cytoskeletal bodies from the fiber cells.[12] The calpain-induced degradation of vimentin diminishes the intrinsic birefringence, causing an enhanced optical anisotropy fluctuation both in its size as well as in its amplitude.[13] In spite of the demonstration that optical anisotropy fluctuations contribute to human cataracts,[11] the role of such fluctuations in cataractogenesis has not gained wide acceptance. A possible explanation for this is the absence of a clear model cataract in which the main contribution comes from optical anisotropy fluctuations. Dim-

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ethyl sulfoxide (DMSO)-caused opacification may provide such a model.

Dimethyl sulfoxide and its aqueous solutions have long been known to have interesting solvation properties. DMSO has been used as a vehicle to enhance drug delivery through the skin[14,15] and to maintain viability during cryopreservation.[16–19] DMSO perturbs phospholipid bilayers[20] and changes protein conformations.[21–23] Such conformational changes are in evidence when enzymes act in aqueous DMSO solutions.[24–25] For example, both the solubility of trypsin as well as its catalytic activity diminish at about a 0.3 mole fraction of DMSO, the point at which DMSO and water form a complex (1 DMSO:2 H_2O). This can be seen from physical-chemical properties such as viscosity,[26–27] dielectric constant,[28] and heat of mixing and freezing point.[22] It occurred to us that such interesting solvation properties may cause some alterations either in the membranes of the fiber cells or in the conformations of the lens proteins within the fiber cells, or both. In any case, such perturbation may upset the balance between the intrinsic birefringence and the form birefrigence and thus give rise to optical anisotropy fluctuations and as a consequence to opacification of lenses. Indeed, DMSO-caused cataract formations have been reported in the literature,[29–31] In those cases, whether DMSO was administered orally or applied to the skin or the eye, the lens nucleus seemed to separate from the cortex, but both remained clear. In the following discussion, a model DMSO-induced cataract is described and analyzed in which turbidity is caused by the lens being in direct contact with DMSO–water systems.

2 MATERIALS AND METHODS

Lenses were obtained from 4-week-old Sprague-Dawley rats. They were placed in different organ culture media immediately after dissection from freshly enucleated eyes. Modified TC-199 media[32] adjusted to a final osmolarity of 298±2 mosmol served as a control. Other media contained DMSO:TC-199 media in the following molar ratios: 1:2, 1:3, 1:9, 1:15, 1:30, and 1:60. The incubation was at 37 °C in a 5% CO_2 atmosphere overnight.

Lenses were taken from the different incubation media and placed in 5% glutaraldehyde buffered by 50 mM cacodylate at pH 7.2, containing also 4% sucrose and 2 mM $CaCl_2$. They were stored at 4 °C for 2 weeks. The lenses were fractured for scanning electron microscopy by immersing them in liquid nitrogen and fracturing them with a razor blade. The fractured specimens were then dehydrated in an ethanol series, critical point dried, and examined after sputter coating (gold-palladium) with a JEOL T-330 scanning electron microscope.

For light microscopic studies, the rat lenses incubated in different media were fixed in 2.5% glutaraldehyde solutions in 50 mM cacodylate buffer at pH 7.2, containing 4% sucrose and 2 mM $CaCl_2$. The fixation time was 4–6 h at room temperature, after which the lenses were transfered into buffered formalin (10%). The lenses were embedded in methylmethacrylate. Sections (ca. 2 μm) were stained with hematoxylin and eosin.

Lenses from the different media were also taken for light-scattering studies. Four lenses were used from each incubation medium. The lenses were sectioned at −5 °C in a refrigerated microtome, progressing from the anterior pole to the nucleus. Sections of 40 μm were placed on microscope slides. A cover slip with a 40-μm spacer was placed over each sample. The first 20-μm section of each lens as well as one 20-μm section after each sample section was discarded. Only the first three 40-μm sections of each lens were studied for their light-scattering properties, since microscopic observations have shown that DMSO damage is largely limited to the epithelium and cortex. Light-scattering intensities of the rat lens sections were obtained in a Phoenix-Brice type apparatus (Model 3000, Wood Mfg. Co, Newton, Pennsylvania) as a function of scattering angles. The 546-nm line of an Hg lamp and later the 634-nm light of an He-Ne laser served as light sources. Light-scattering intensities were obtained in two modes: I_+ when the polarizer and analyzer were set 90 deg apart and $I_\parallel$ when they were parallel to each other. Scattering intensities were read twice at each scattering angle from 10 to 35 deg in 2 deg increments. Triply distilled water served as a standard; its light-scattering intensity was observed at 90 deg to convert the observed intensities into Rayleigh ratios.

3 RESULTS

All rat lenses incubated for 12 h in DMSO-containing media became opaque. The density of opacification increased from slight haziness to frank opacity with an increase in DMSO concentration of the media.

Figure 1 shows the average light-scattering intensities (in arbitrary units, millivolts) of lenses incubated in a DMSO:water mixture (0.062 mole fraction of DMSO). The standard deviations at each point are indicated by the vertical lines. The difference between the intensities of the parallel and perpendicular modes is typical for all lenses examined; at high DMSO concentrations, the intensities in the perpendicular mode increase significantly at wider scattering angles. This can be seen from the values of the Rayleigh ratios presented in Table 1. The Rayleigh ratios give the values of scattering coming from a unit volume. The intensities of scattered light in both modalities, I_+ and $I_\parallel$, were converted to Rayleigh ratios, R_θ,

$$R_\theta = (I_\theta / I_0)(r^2 / V),$$

where I_θ is the intensity of scattered light at an angle θ, I_0 is the intensity of the incident beam at 0

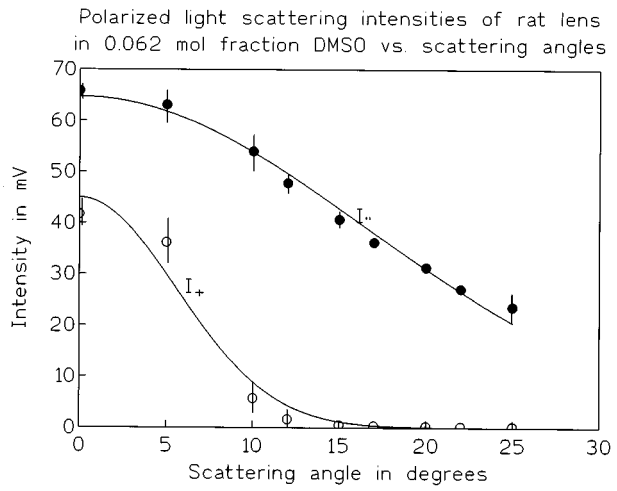

Fig. 1 Average polarized light scattering intensities (in arbitrary units, millivolts) of the first section from a rat lens incubated in 0.062 mole fraction DMSO solution as a function of scattering angle in degrees. The solid circles represent the $I_{\|}$ and the empty circles the I_{+} modes, respectively. The bars indicate the standard deviations.

angle, r is the distance between the detector and the scattering volume, V. With the aid of the instrument constant, k,

$$k=(r^2/V)/I_0$$

all scattering intensities could be converted into Rayleigh ratios. In this denotation the scattering intensities are independent of the instrument design. The instrument constant was obtained from the measured intensity of the scattered light of water at 90 deg, I_w, and the absolute value of water scattering, R_w, at 90 deg using the 546-nm light: 1×10^{-6} cm^{-1}.[33]

$$k=R_w/I_w.$$

Instrument constants at other angles were similarly obtained; corrections were also made for the difference between the refractive index of water and that of the lens sections.

The second sections of the rat lenses in different media also gave similar results. The third sections of the lenses that cut the inner cortex and outer nucleus did not give reproducible data and the experimental points were more scattered.

The polarized light-scattering intensities can be analyzed in terms of the theory of random density and orientation fluctuations,[3,34] which is an extension of the original Debye-Bueche theory.[1] If the fluctuations in density and in orientation of the optically anisotropic entities are not correlated, the following equations give the intensities of light scattering in the two modalities:

$$R_{\|}=K\left\{\boldsymbol{\eta}^2\int\gamma(r)\,\frac{\sin \mathrm{hr}}{\mathrm{hr}}\,r^2dr+\frac{4}{45}\,\boldsymbol{\delta}^2\int f(r)\left[\frac{\boldsymbol{\eta}^2}{\alpha^2}\,\gamma(r)+1\right]\frac{\sin \mathrm{hr}}{\mathrm{hr}}\,r^2dr\right\}$$

$$R=K\left\{\frac{1}{15}\,\boldsymbol{\delta}^2\int f(r)\left[\frac{\boldsymbol{\eta}^2}{\alpha^2}\,\gamma(r)+1\right]\frac{\sin \mathrm{hr}}{\mathrm{hr}}\,r^2dr\right\}.$$

In the above equations $\boldsymbol{\eta}^2$ and $\boldsymbol{\delta}^2$ are the average deviations in density and orientation fluctuations, respectively, that is, an amplitude factor of the fluctuations. $\gamma(r)$ and $f(r)$ signify the correlation functions of density and orientation fluctuations, respectively. They have a mathematical form indicating that when the separation of the scattering units is zero, r=0, the correlation function has a value of 1 and when the separation is infinite, the value of the correlation functions is zero. The scattering function $h=(4\pi/\lambda)\sin(\Theta/2)$ is dependent on the wavelength of light λ in the medium and on the scattering angle. One can assess the relative role of density and orientation fluctuations in opacification by taking the ratios of the scattering intensities of the two modalities. If there is no contribution from orientation fluctuations, the ratio is zero. On the other hand, if there is no contribution from density fluctuations, the ratio is 0.75.

Figure 2 shows the dependence of this ratio of two modalities on the DMSO concentration in the

Table 1 Rayleigh ratios of polarized light scattering intensities of first sections of rat lenses incubated in DMSO:water mixtures as a function of scattering angles and concentration of DMSO.

	$R_{\|}\times10^3$ cm^{-1}				$R_{+}\times10^4$ cm^{-1}			
Mole fraction	at angles				degrees			
of DMSO	10	15	20	25	10	15	20	25
0.00	2.07	1.17	0.53	0.29	1.78	0.15	0.06	0.04
0.016	2.18	1.67	1.45	1.11	2.44	0.21	0.13	0.08
0.032	2.20	1.48	1.07	0.77	2.53	0.38	0.26	0.20
0.062	2.16	1.63	1.25	0.94	2.33	0.22	0.12	0.08
0.100	2.18	1.85	1.60	1.31	5.45	3.70	1.83	1.50
0.25	2.32	2.32	2.25	2.15	17.4	17.1	16.9	15.1
0.33	2.5	2.42	2.37	2.29	13.5	11.4	10.3	8.76

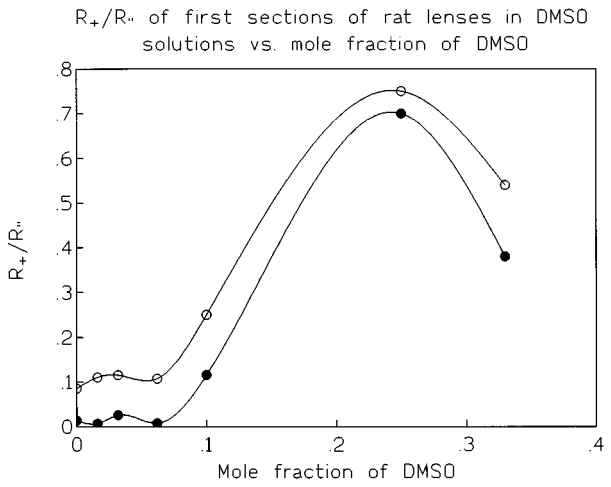

Fig. 2 The intensity ratios of the two modalities, $R_+/R_\parallel$, of the first sections (anterior) of rat lenses incubated in different media as a function of DMSO concentration in the media. The empty circles represent data obtained at 10 deg and the filled circles at 25 deg scattering angles.

media at two scattering angles. The maxima observed in Fig. 2 at 0.25 mole fraction of DMSO are near this theoretical maximal value, indicating that the turbidity is the result of fluctuations in optical anisotropy. On the other hand, the slight haziness caused by incubation in media with low DMSO concentrations is largely due to density fluctuations.

Several histological changes were observed in the lenses incubated in DMSO media (Figure 3). Control rat lenses incubated in TC-199 maintained a normal epithelium and cortex in both the anterior and equatorial regions (Fig. 3A and B). Lenses in the 0.032 (Fig. 3C and D) and 0.062 (Fig. 3E and F) mole fraction DMSO solutions showed damage mostly to the epithelial layer. The epithelial cells were swollen, had rounded nuclei, and were displaced from the capsule to large, newly formed intercellular vacuoles (lakes) and other intercellular spaces where they appeared singly or in clumps. The normal, single-cell arrangement of epithelium was disrupted throughout. The extent of damage to the epithelium was greater in the 0.062 than in the 0.032 mole fraction DMSO medium. In the latter, fiber cells swollen but otherwise intact seem to be pushing the damaged swollen epithelial cells into disorganized entities. The rat lenses incubated in the high DMSO-containing media (0.25 and 0.33 mole fractions) show extensive damage not only in the epithelia and outer cortex but all the way up to the nucleus. Here the epithelial cells, although swollen and perhaps containing vacuoles, still maintain their unilayer organization. The cortical fiber cells in both the anterior and equatorial regions show not only extensive swelling but also emptying of the cytoplasm (Fig. 3G and H). The damage in the cortical fiber cells goes deeper in the 0.33-mole fraction DMSO media than in the 0.25-mole fraction media.

Figure 4 represents a collection of scanning electron micrographs of fiber cells of rat lenses incubated in different DMSO media. The control lenses in TC-199 media exhibit normal fiber cells of approximately 3 μm diameter in the anterior (Figure 4A) as well in the equatorial region (Figure 4B). In the 0.032 mole fraction DMSO medium, the fiber cells show some swelling in the outer cortex to about 4 to 5 μm diameter and minor disorganization but otherwise retain integrity of the fiber cells (Fig. 4C and D). Similar observations can be made of the lenses incubated in the 0.062 mole fraction DMSO medium. Here, the fiber cells are somewhat swollen and one can perceive some separation between them although they still seem to maintain their integrity. The fiber cells under the vacuoles (lakes) in the anterior cortex showed some disorganization (Figure 4E). The equatorial cortical fiber cells in this medium also exhibited incipient intracellular vacuole formation (Figure 4F). On the other hand, at high concentrations of DMSO, especially at 0.25 mole fraction, fiber cell disintegration is evident. Not only do we see enhanced invaginations of the fiber cell membranes, but the fiber cells themselves are full of vacuoles. It seems that the cytoplasm has coalesced around the cytoskeletal bodies, leaving behind a ghostly scaffolding (Fig. 4G and H).

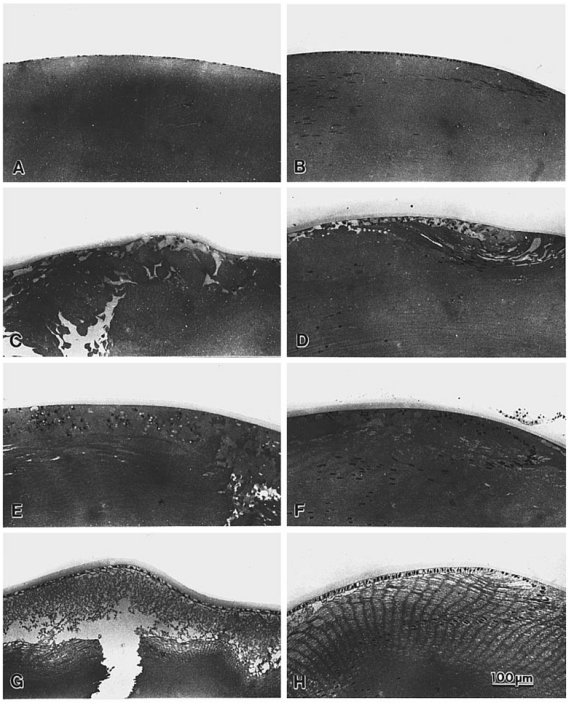

Fig. 3 Micrographs from the anterior (A,C,E,G) and equatorial (B,D,F,H) regions of rat lenses incubated in the following media: (A and B) Control (TC-199); (C and D) 0.032 mole fraction DMSO; (E and F) 0.062 mole fraction DMSO; (G and H) 0.25 mole fraction of DMSO. ×124; bar=100 μm for all micrographs.

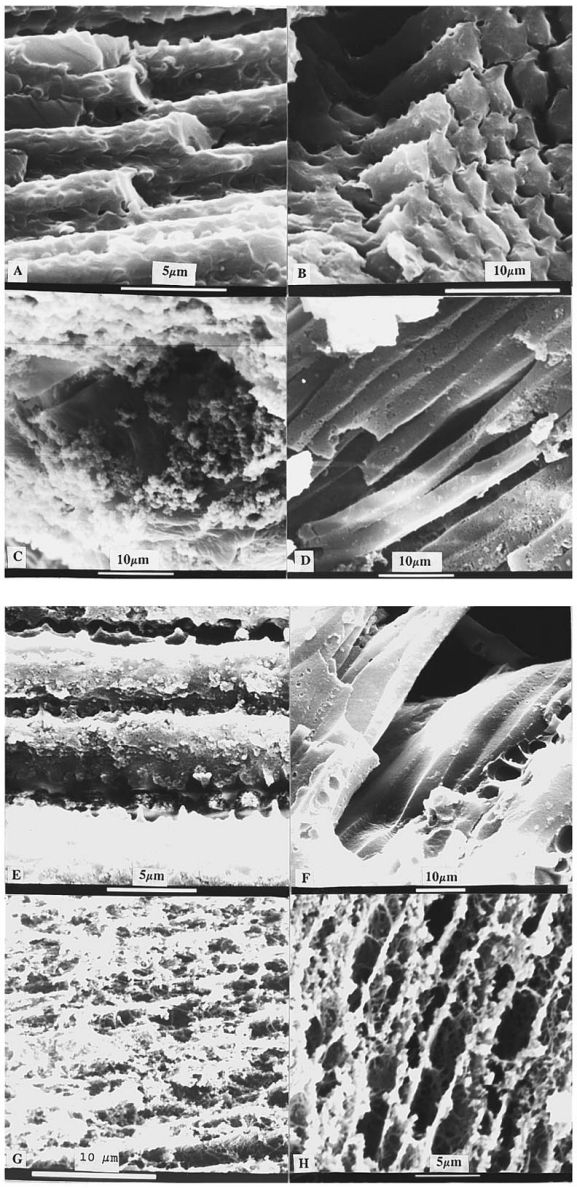

Fig. 4 Scanning electron micrographs of fiber cells from anterior (A,C,E,G) and equatorial (B,D,F,H) regions of rat lenses incubated in the following media: (A and B), control (TC-199); (C and D) 0.032 mole fraction of DMSO; (E and F) 0.062 mole fraction of DMSO; (G and H) 0.25 mole fraction of DMSO. (E) fiber cells are under a lake formed below the anterior epithelium. Bars on each micrograph indicate the distance in micrometers.

4 DISCUSSION

The selection of DMSO as an agent to cause opacification in the lens was based on its varying physical-chemical properties and solvating abilities.[22,25] DMSO is soluble in both water and organic solvents. Its solubility in water is due to the extensive hydrogen bonding ability around the ═S═O end of the molecule. On the other hand, the methyl moieties provide the nonpolar nature of the molecule which through van der Waals-type interactions has affinity for other nonpolar groups. In aqueous solutions, physical properties indicate that DMSO:2H_2O forms a compound that has the least ability to form hydrogen bonds with other compounds among all the possible mixtures of this binary system.

Globular proteins change their conformations in aqueous DMSO solutions.[21–25] Hydrophobic pockets of the protein molecules are gradually opened up; and even helix–coil transitions are affected. This is why the DMSO solutions used in cryopreservations (10:90 v/v DMSO:water corresponding approximately to 0.03 mole fraction of DMSO) also have cytotoxic effects at room temperatures.[16,17,23] The 0.33-mole fraction of DMSO solution has the greatest ability to open up the hydrophobic domains, thus causing the most denaturation in proteins.[23–25]

The present study on rat lenses incubated in DMSO:water mixtures was meant to provide a model cataract based on the physical properties of the incubation media. The real cataracts in animals when DMSO was applied either orally or topically over a long period[29–31] have little in common with this model cataract. For one thing, in those animal studies both cortex and nucleus remained transparent; only the separation between the two caused cataract formation. On the other hand, it seems that in animal studies, the cataract was caused mostly by some metabolic product of DMSO[31] because oral administration was more effective than topical administration.

In the present study, the lens was in direct contact with the media. Because of the hydrophobic nature of the solvent, DMSO and its solutions perturb phospholipid bilayers,[20] not only allowing passage through such bilayers but also causing misalignments. These solvent properties can explain the opacification of rat lenses observed in this study. On the basis of the light-scattering results, one can state unequivocally that at low DMSO concentrations the turbidity is caused largely by density fluctuations. This is evident from the low values of the R_+ component of the scattered polarized light as well as the low value of the $R_+/R_\parallel$ ratio. Both microscopic and scanning electron microscopic observations support this claim. At low DMSO concentrations, the primary effect is a swollen epithelium. It is getting disorganized. This creates lake formations under the capsule while the swollen epithelial cells are pushed into the cortical areas. Such lake formations, having a lower refractive index than the swollen epithelial cells and the partially swollen outer cortical fiber cells, give rise mainly to density fluctuations.

At higher DMSO concentrations (0.1 mole fractions and above) the R_+ component of the scattered polarized light exhibits increasingly higher values, reaching a maximum at 0.25 mole fraction of DMSO. Similarly, a maximum is reached in the $R_+/R_\parallel$ ratio at this concentration. This maximum is close to the theoretical maximum 0.75, indicating

that the turbidity in the lenses incubated in these media is largely, if not solely, due to fluctuations in optical anisotropy.[13,34] The light microscopic and scanning electron microscopic pictures explain why this is happening. In the high DMSO incubation media, not only do the fiber cells swell, their membrane invaginations increase and their interfiber cell distances increase, but there is a major collapse of cytoplasmic content within the fiber cells. Such a collapse is the result of the unfolding, partial denaturation of the crystallins. The remaining scaffolding inside of the vacuolated fiber cells now causes an enhanced optical anisotropy, changing the intrinsic birefringence of the fiber cell contents. Such a change upsets the balance between the form birefringence and the intrinsic birefringence, which under normal conditions provides transparency.[8,10] The reason that the vacuolated fiber cells do not generate a large contribution from density fluctuations may be due to the refractive index of the high concentration DMSO media. Even though large density aggregates (scattering units) may be present, the refractive index difference between these units and the penetrating media may be very small, thus providing little amplitude factor to cause turbidity.

In conclusion, DMSO produces turbidity in cultured rat lenses in different ways, depending upon the concentration in the medium. At low DMSO mole fractions, the opacification is caused by lake formation, and thus by density fluctuations. At high DMSO mole fractions, the opacification is mainly due to optical anisotropy fluctuations. This is because the principal effect of the solvent penetration is to change the conformation of globular proteins,[21–25] presumably also that of the crystallins and other proteins, causing a coalescence of the fiber cell contents around the cytoskeletal scaffolding. This in turn upsets the balance between intrinsic and form birefringence, causing turbidity. Thus, the DMSO cataract in high DMSO concentrations is a good model to study opacification due to optical anisotropy (orientation) fluctuations. While the model provides an insight into what processes can lead to optical anisotropy fluctuation, it is not suggested that a similar process is definitely involved in human cataractogenesis.

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