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Light scattering and reversible cataracts in the calf and human lens

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[Plates 1 and 2]

By using measurements of the intensity of light scattered from intact calf lenses, we have determined a number of reagents that induce nuclear and cortical opacification at body temperature. Diffusion of buffered saline solutions of glycerol, other glycols, urea, guanidine hydrochloride or glycine into the lens reverses the opacity of all the reagent-induced cataracts. Similar findings are obtained with lens homogenates, which have gel-like properties as determined from viscosity measurements. A 50% (by volume) glycerol or 5 M urea solution clarifies human pathologic cataractous lenses by reducing the opacification due to light scattering. These findings suggest that it may be possible, in principle, to reverse human lens cataracts chemically *in situ*. The scattering of laser light from quasi-periodic lattice of normal lens cells produces a regular diffraction pattern containing many Bragg spots whose positions are those predicted from the basis vectors of the cellular lattice. The intensity of the Bragg reflexions increases greatly when cataracts are formed in the calf and human lens, and falls greatly when the lenses are clarified. The spatial variation in the scattered light intensity of the Bragg spots and between these spots contains detailed information on the structure of the scattering elements associated with opacification.

Introduction

The turbidity of the pathologic cataractous lens generally involves both the absorption and the scattering of light. The former effect produces a change in the colour of the lens (often making it yellowish or brownish) with a consequent reduction in the intensity of the transmitted light. In the latter, the scattering of light distorts the wavefront of the light transmitted through the lens and thereby degrades the quality of the image on the retina (Miller & Benedek 1973). This degradation is the principal defect that inhibits vision through the cataractous lens. Indeed it has been shown (Reynolds *et al.* 1973) that holographic reconstruction of the distorted wavefronts can allow the formation of clear images in the focal plane of the cataractous lens.

A central objective of current research on cataracts is the physico-chemical identification of the microscopic elements within the lens responsible for the scattering of light. With the identification of these elements, it should be possible, in principle, to find reagents that inhibit or reverse the formation of the scattering elements.

The present work represents an inversion of this kind of reasoning. Here we have empirically identified reagents which will induce or reverse cataracts in a model lens system: the calf lens. Following this we have used the methods of electron microscopy and the diffraction of laser light in an effort to identify the microstructural changes produced by the opacifying or clarifying agent. We also have discovered that the reagents capable of reversing cataracts in the model system are capable of clarifying the human pathologic cataractous lens.

The calf lens exhibits the phenomenon of reversible 'cold cataract' (Zigman & Lerman 1964). As the temperature is lowered into the region of 17 °C, the turbidity of the nuclear region increases rapidly with decreasing temperature until multiple scattering occurs and the lens becomes opaque. The opacification is reversed when the temperature is raised above the cold cataract temperature T_{cat} (figure 1).

On the basis of measurements of the diffusivity of the concentration fluctuations in the intact lens, it has been proposed that the cold cataract phenomenon is the result of a first order phase separation of the concentrated protein–water mixture in the lens cytoplasm. According to this model (Tanaka & Benedek 1975) the opacification of the lens is the result of lowering the temperature below the coexistence curve of the protein–water mixture. Recently, Tanaka *et al.* (1977) have provided strong support for this view by showing a striking similarity between the coexistence curve of a concentrated lysozyme–water mixture and the coexistence curve of the young rat lens. In those experiments it was observed that the value of the critical temperature for the coexistence curve of the lysozyme–water solution was a strong function of the concentration of salt in the solution. This finding suggests that T_{cat} in the calf lens might be substantially altered, not only by changes in ionic strength, but also by changes in the concentration of a variety of reagents which can be diffused into the intact lens.

In the present paper we report how T_{cat} of the calf lens is indeed affected by a number of different reagents. These reagents can be divided into two classes:

(1) those that increase the temperature (T_{cat}) at which nuclear opacification occurs, and

(2) those that decrease T_{cat}.

In the first class are the monovalent salts LiCl, NaCl, KCl and CsCl, heavy water D_2O and methanol. By suitable choice of concentration of these 'opacifying' agents the cataract temperature of the calf lens could be raised close to or above body temperature (37 °C). In the second class are the following reagents: glycerol, ethylene glycol, 1,3-butanediol, 1,4-butanediol, urea, guanidine hydrochloride and glycine. All these 'clarifying' agents reduce the temperature at which calf lens opacification occurs.

Agents of each class were introduced into the lens by incubating the lens in phosphate buffered saline solutions containing selected concentrations of each of the reagents to be tested. The effect of each agent on T_{cat} was measured by determining the temperature at which a well-defined high degree of light scattering was produced. The macroscopic optical effect of either type of agent was characterized by photographing the image of a grid as observed through the lens. To ascertain the microscopic scattering elements within the lens responsible for opacification, we used the techniques of scanning electron microscopy and the diffraction of coherent laser light beams.

Materials and methods

Lenses

Calf lenses were obtained, fresh, from Trelagan Meat Company of Cambridge, Massachusetts, and were used immediately in our experiments. Human lenses were obtained from the Maryland Eye Bank and local hospitals.

Solutions

The standard saline solution used for incubation at physiological ionic strength was phosphate buffered saline of the following composition: 0.133 M NaCl, 0.003 M KCl, 0.016 M

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Na_2HPO_4, 0.002 M KH_2PO_4, and the pH was adjusted to 7.4 with HCl. For experiments in which the ionic strength was varied, we simply substituted the relevant salt for NaCl and adjusted the salt concentration appropriately. All chemicals were reagent grade. D_2O was obtained from Bio-Rad Laboratories, Richmond, California.

Measurement of the scattered light intensity and determination of T_{cat}

The intensity of the light scattered by an intact lens whose temperature could be varied from −10 °C to 60 °C was measured with an experimental setup essentially identical to that described in Tanaka & Benedek (1975). The light scattered at 90° passes through the collection aperture and is focused by a lens on to the surface of a photomultiplier. The scattering volume was at the very centre of the nuclear region. The output current of the photomultiplier is proportional to the scattered light intensity. The proportionality constant depends on the light collecting optics and the photomultiplier gain. This proportionality constant was maintained at a fixed value in all our experiments by standardizing the photocurrent with a 0.1 gm/l solution of 910 Å† latex spheres. To determine the value of T_{cat} for the lenses being examined, we measured the photocurrent produced by the scattered light as a function of the temperature. In our setup, when the photocurrent exceeded 10 μA, the multiple scattering within the lens was so great that the intensity of the transmitted beam became fully attenuated. Hence, the temperature at which the photocurrent reached 10 μA was taken to be the cold cataract temperature T_{cat}.

Scanning electron microscopy

An elliptical cross section (0.5 mm thick) was cut from the centre of each freshly dissected calf lens, fixed for 16–24 h at room temperature (4 °C for cold cataract) in 4% (by volume) glutaraldehyde and post-fixed for 1 h in 10 mg/ml OsO_4 (both fixatives were in 0.15 M phosphate buffer, pH 7.4). The samples were then dehydrated by successively equilibrating in a graded series of ethanol–H_2O mixtures and then dried from 100% ethanol in a Denton critical point dryer with CO_2 as the transition fluid. The dried samples were fractured along the minor axis, coated with a thin layer of evaporated gold, and then viewed on a Cambridge Mark 2*a* Stereoscan Scanning electron microscope.

Diffraction measurements

The diffraction patterns were obtained by focusing the laser beam into an appropriate region of whole lenses or thick sections to determine the dimensions of lens cells in the clear lens. 100 μm thin sections were used to follow the changes in the diffraction pattern during opacification. Two kinds of section cuts were used: anterior–posterior section at the nucleus. This gives a one dimensional diffraction pattern. The second cut was a coronal section of the cortex or nucleus. This gives a two dimensional diffraction pattern, as described below. The angular directions of the Bragg peaks was measured geometrically after the spots were located on a screen. Their intensity was measured with a Schottky Photodiode PIN8LC (United Detector Technology).

† 1 Å = 10^{-1} nm = 10^{-10} m.

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Experimental results

Effects of various reagents on lens transparency

In figure 1 we demonstrate the behaviour of the light intensity scattered from the nucleus of a calf lens as a function of temperature, incubated in our physiological ionic strength solution and showing cold cataract formation.

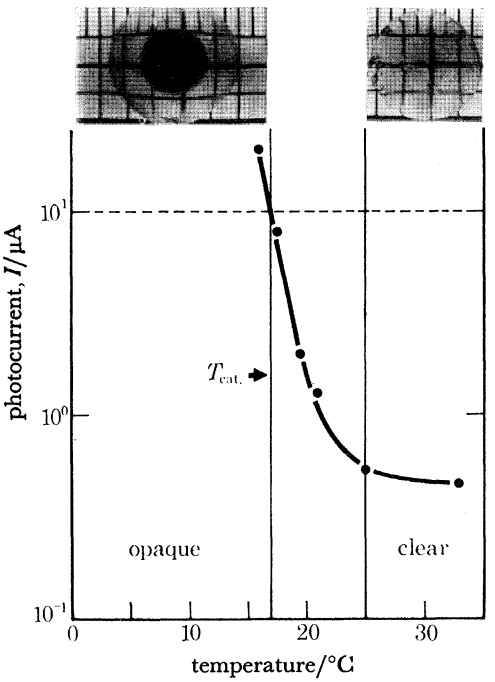

Figure 1. Intensity of light scattered from the centre of a calf lens as a function of temperature, measured for 90° scattering angle. The intersection of I against T curve with 10 μA photocurrent level defines T_{cat}.

First, we present those reagents which increase the cold cataract temperature, T_{cat}, in the nucleus of the calf lens (*a–c*) and a reagent that produces cortical cataracts (*d*).

(*a*) KCl. In view of the fact that salt concentration markedly shifts the coexistence curve of lysozyme–water solutions (Tanaka *et al.* 1977), we undertook to measure the effect of salt on T_{cat}. In figure 2 we present measurements of the intensity of light scattered at 90° near the nucleus of a calf lens as a function of the temperature for the following concentrations in the incubating system: 0.1 M KCl, 0.15 M KCl, 0.3 M KCl and 0.6 M KCl. The intersection of each curve with the 10 μA photocurrent level defines T_{cat}. In figure 3 we plot T_{cat} against the salt concentration. The dependence of T_{cat} on KCl concentration is seen to be roughly linear in concentration with a slope of $\Delta T_{cat}/\Delta[\mathrm{KCl}] = +25\ ^\circ\mathrm{C/M}$. It is to be noted that for salt concentrations in excess of 0.8–0.9 M the calf lens is opaque at body temperature. The effect of salt is reversible in the sense that, when the salt-treated lens is subsequently incubated in the physiological ionic strength solution, T_{cat} for that lens returns close to 17 °C.

(*b*) NaCl. Incubation of calf lenses in 0.1 M NaCl ($T_{cat} = 16\ ^\circ$C), 0.2 M NaCl ($T_{cat} = 21\ ^\circ$C), 0.4 M NaCl ($T_{cat} = 28\ ^\circ$C) and 0.6 M NaCl ($T_{cat} = 31\ ^\circ$C) results in a shift of T_{cat}, similar to the effect of KCl, yielding a value of $\Delta T_{cat}/\Delta[\mathrm{NaCl}] = 25\ ^\circ\mathrm{C/M}$. The same dependence of T_{cat} upon molar concentration is found for salts of monovalent cations such as LiCl and CsCl.

(*c*) D_2O. Incubation of a calf lens in a solution of physiological saline containing 40 % (22 M) by volume increases T_{cat} to 32 °C. Consequently, we obtain $\Delta T_{cat}/\Delta[D_2O] = +0.9$ °C/M.

(*d*) $CaCl_2$. In view of our previous work on the role of $CaCl_2$ in inducing opacification of solutions of lens proteins (Jedziniak *et al.* 1972), we undertook to investigate the effect of $CaCl_2$ on the intact lens. Treatment of calf lenses with a solution of $CaCl_2$ having a concentration $> 10^{-3}$ M produces a cortical cataract whose turbidity is independent of temperature from 0 °C to 37 °C.

Now we present the effect of agents that reduce the T_{cat} in the nuclear region of the calf lens.

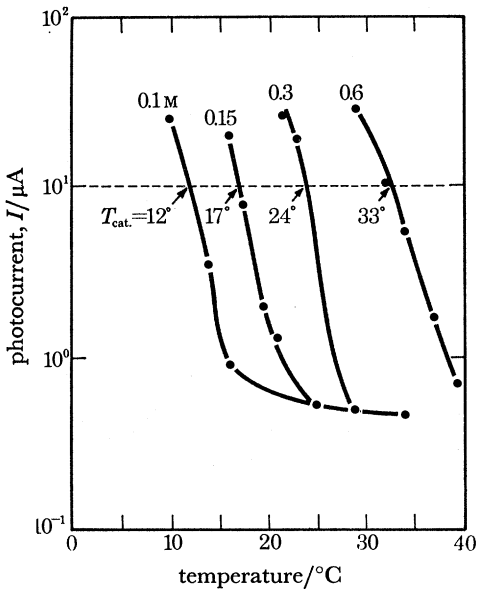

FIGURE 2. Intensity of light scattered from the centre of a calf lens, measured for 90° scattering angle as a function of temperature for lenses incubated in 0.1 M KCl, 0.15 M KCl, 0.3 M KCl and 0.6 M KCl.

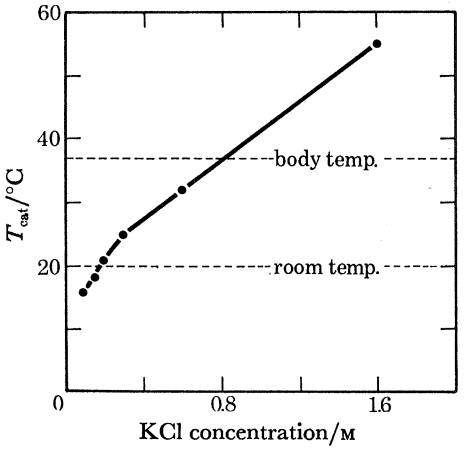

FIGURE 3. Dependence of T_{cat} on concentration of KCl in incubating solution. This plot applies also for NaCl, LiCl and CsCl.

(*e*) *Glycerol*. In figure 4 we show the temperature dependence of the intensity of light scattered from a calf lens incubated in 10 % glycerol solutions by volume, in comparison with a lens in physiological ionic strength solution. This figure shows that a 10 % glycerol solution lowers T_{cat} to *ca.* 4 °C as compared with 17 °C for untreated lenses. If a calf lens is incubated in 50 % glycerol solution, there is no measurable increase in the scattered intensity (the lens remains transparent) as the temperature is lowered from 37 °C to −10 °C. Using the result from the 10 % (1.37 M) glycerol solution we find $\Delta T_{cat}/\Delta[C_3H_8O_3] \approx -10$ °C/M.

The effect of glycerol in these experiments was found to be reversible in the following sense. if a calf lens is incubated in 50 % glycerol and physiological ionic strength salt solution for 36 h, and if the glycerol is then dialysed out for 16 h at 4 °C, then the T_{cat} in the 0.15 M ionic strength solution (20 °C) returns nearly to the value of T_{cat} appropriate to the normal lens, which is 17 °C.

The data in figure 5 show clearly that glycerol maintains its ability to reduce the cold cataract temperature even for the lenses whose T_{cat} is raised near body temperature by the effect of salt (0.6 M KCl). Incubation in 10 % glycerol solution acts to reduce T_{cat} by about 20 °C. In the case of the lens incubated in physiological ionic strength solution, addition of 10 % glycerol

decreases T_{cat} by about 13 °C (figure 4). We do not regard this quantitative difference in the glycerol effect as being significant in view of the experimental uncertainty in determining T_{cat}. Multiple scattering effects can easily produce the observed flattening of the I against the T curves near the 10 μA scattered photocurrent level, as shown in figure 5. This flattening in turn reduces the reliability in the determination of T_{cat} in these lenses. On the other hand, the 20% glycerol data clearly suggest that the glycerol effect on T_{cat} may be stronger than linear for glycerol concentrations higher than 10%. In fact, for 20% and 50% glycerol the lens remained completely transparent over the entire temperature range studied. The glycerol effect is reversible in the sense that on returning any of the glycerol and salt treated lenses to a 0.6 M salt solution without glycerol, the value of T_{cat} returned to 33 °C.

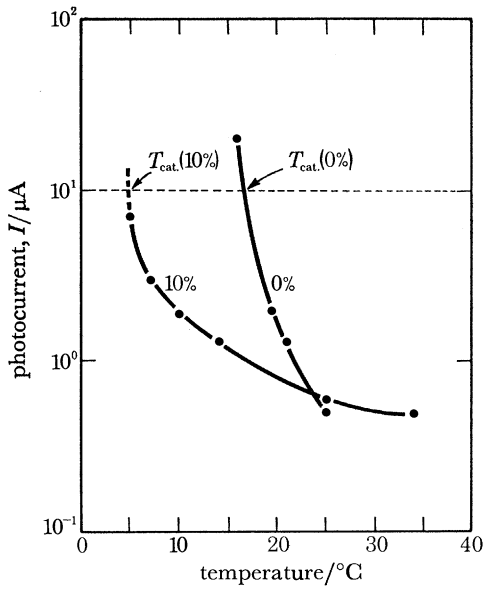

FIGURE 4. Effect of glycerol on the temperature dependence of the intensity light scattered from a calf lens. The cold cataract temperature decreases from 17 °C to *ca.* 4° on incubation in a 10% glycerol solution.

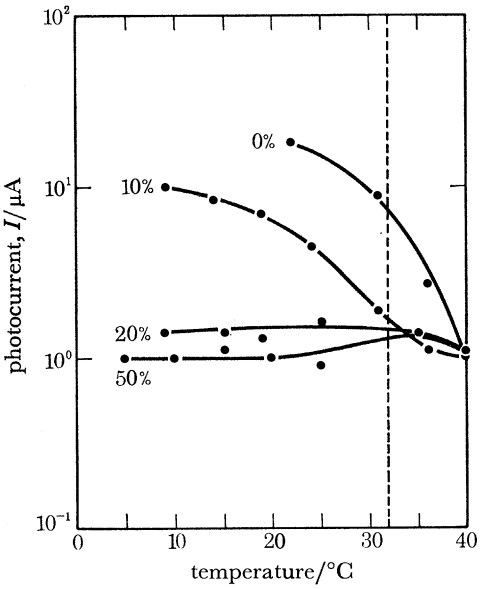

FIGURE 5. Temperature dependence of the scattered intensity from calf lenses incubating in 0.6 M KCl solution, which also contains glycerol in 0%, 10%, 20% or 50% concentration by volume.

(*f*) *Ethylene glycol*, 1,3-*butanediol*, 1,4-*butanediol*. All these glycols are similar to glycerol in that they lower the value of T_{cat}. Specifically, we have found that the change of T_{cat} per mole is given by −4.5 °C/M, −5.8 °C/M and −6.5 °C/M respectively for each of the glycols named above. In the absence of the glycols the value of T_{cat} was 17 °C.

(*g*) *Urea*. Lenses incubated in physiologic ionic strength salt solution to which was added 0.5 M or 1.0 M urea were opacified by lowering the temperature below T_{cat}. The actual value of T_{cat} appropriate to each urea solution was obtained by raising the temperature until clarification occurred. In figure 6 we plot T_{cat} obtained in this way against the urea concentration. The dependence of T_{cat} on urea concentration is linear with $\Delta T_{cat}/\Delta[CO(NH_2)_2] = -22$ °C/M.

The usefulness of urea in decreasing the cold cataractous temperature was suggested by the findings of Zigman *et al.* (1965) who studied the cold precipitable fraction in the dogfish lens. In this lens the urea concentration is 0.25–0.30 M, which is high compared to the human lens

where the urea concentration is about 0.02 M (Kuck 1975). When the urea was dialysed out of dogfish lens homogenates this material becomes opaque at 10 °C, which is the average ambient environmental seawater temperature. On the other hand, increase of the urea concentration to normal levels in the homogenate prevented the opacification at 10 °C. It is also of interest to note (Cooper *et al.* 1966) that the addition of urea into a high galactose diet in test rats delays the formation of galactose cataracts. Furthermore, on discontinuing the galactose diet, the rats which were fed both urea and galactose showed clarification of lens opacity in 10 days instead of 2 or 3 months as in the case of the rats on a urea-free galactose diet.

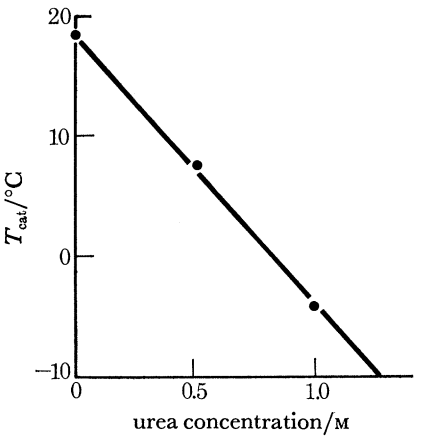

FIGURE 6. Dependence of T_{cat} on urea concentration for a normal calf lens incubated in physiological ionic strength salt solution plus urea. The slope of the lens is $\Delta T_{cat}/\Delta[CO(NH_2)_2] = -22$ °C/M.

(*h*) *Water concentration in the lens.* We also have investigated the effect of water concentration on changing T_{cat} in the nucleus of the calf lens. The water concentration of the nucleus was reduced by air drying, the water loss was measured by weighing. The dried nucleus required a long time to reach the new equilibrium state on lowering the temperature below T_{cat}. Consequently, we measured T_{cat} for the nucleus by determining the temperature at which clarification occurs on raising the temperature. In this way we found that a 10% reduction in the water content of the calf nucleus produces a 20 °C decrease of the cold cataract temperature. If water is added to the dried nucleus by incubating in water at 40 °C, and observing the increase in nucleus lens weight, it is found that T_{cat} is a reversible function of the water content.

(*i*) *Human pathologic cataracts.* In view of the clarifying effect of glycerol and urea on the calf lens, we undertook to determine the effect of these agents on pathologic human cataractous lenses. Fifteen cataractous lenses were obtained postoperatively. Of these 14 were crudely characterized as 'cortical' and one as nuclear cataract. These lenses were incubated in solutions containing 50% glycerol at 4 °C overnight. In each case the glycerol very markedly improved the transparency of the lens at all temperatures between 4 °C and 37 °C. We present in figure 7 a photographic representation of the clarifying effect of glycerol. Figure 7 shows the cataractous lens resting on a rectangular test pattern (*a*) before and (*b*) after treatment with 50% glycerol. The remarkable improvement in visibility of the test pattern is quite evident.

The clarifying effect of glycerol is reversible in the sense that removal of the glycerol resulted in the return of the lens to the opaque state which existed prior to the glycerol treatment. Also, we have found that lenses in 50% glycerol bathing solution can be stored without deterioration for periods at least as long as 2 weeks. This behaviour is to be contrasted with the fact that lenses

stored in physiologic saline at 4 °C with antibiotic added show marked cortical deterioration after a few days. The glycerol does not affect the spatial distribution of the colour (usually brunescent) associated with the absorption of light by the human cataractous lens. Its effect is confined to the reduction of the 'cloudiness' associated with the scattering of light within the lens. Treatment with 50% glycerol also increased the transparency of the cortical cataracts, produced by $CaCl_2$, in calf lenses. Urea also acts to clarify the $CaCl_2$ induced cortical cataacts on the calf lens.

Scanning electron microscopy

In order to gain insight into the microstructural changes associated with the scattering of light, we undertook to obtain scanning electron microscope photographs of treated and untreated lenses. The cells of the calf lens are very long, closely packed fibres, which are hexagonal in cross section with typical basis vectors of the lattice being 4 μm and 7 μm. Adjacent cells are interlocked by two types of structures: the first are protrusions along the edges of the cells, and the second are interdigitations on the cell membrane surfaces (Dickson & Crock 1973).

In figure 8 we show scanning electron microscope photographs of calf lens cells. In figure 8*a* we show the cellular lattice of a normal calf lens, in 8*b* the lattice of a cold cataract calf lens and in 8*c* the structure of a lens originally opacified with 0.6 M KCl and then clarified by treatment with 50% glycerol. There seem to be no gross differences in the structure of the lens fibre cells in an opaque region as compared to those in a transparent region of the lens. The differences in cell size and shape seen in these micrographs depends on the exact location of the cells in the lens (Harding 1976). The differences in structure associated with opacity are probably subtle, involving alterations in the cell membrane, small changes in intercellular spacing, possible changes of structure within the cytoplasm or membrane associated protein aggregation.

Optical diffraction studies

Previous work (Vinciguerra & Bettelheim 1971; Philipson 1973) have shown the possibility of obtaining diffraction spots in the clear calf and human lens respectively. They could determine the dimensions of the cells in the intact lens from the position of the Bragg spots. We were interested in following the intensity of those spots during the formation of the cold cataract and in the cataractous human lens in order to gain insight into the microstructural changes associated with opacification.

(*a*) *Determination of cell dimensions in the clear calf lens.* Optical micrographs and scanning electron microscope photographs (figure 8*c*) have shown that the lens cells are packed together in a rather regular rhomboidal lattice. Because the mean index of refraction in the lens cytoplasm and the cell membrane are different from one another, the index of refraction is modulated by the same periodicity as the cellular lattice. The precise form of the diffraction pattern from such a lattice depends on the orientation of the incident beam relative to the basis vectors of the lattice.

In figure 9 we show the basis vector of the rhomboidal lattice of lens cells and denote the basis vector as $\boldsymbol{a}$ and $\boldsymbol{b}$ and the angle of acuity as γ. In directions perpendicular to the plane of $\boldsymbol{a}$ and $\boldsymbol{b}$ the cells are macroscopic in length. The most useful diffraction patterns can be obtained by orienting the incident beam either parallel to $\boldsymbol{a} \times \boldsymbol{b}$ or perpendicular to $\boldsymbol{a}$ or $\boldsymbol{b}$ in the $\boldsymbol{a}$–$\boldsymbol{b}$ plane. The former geometry can be achieved by allowing the light to pass perpendicularly through coronal sections (Warwick & Williams 1973). The latter geometry can be

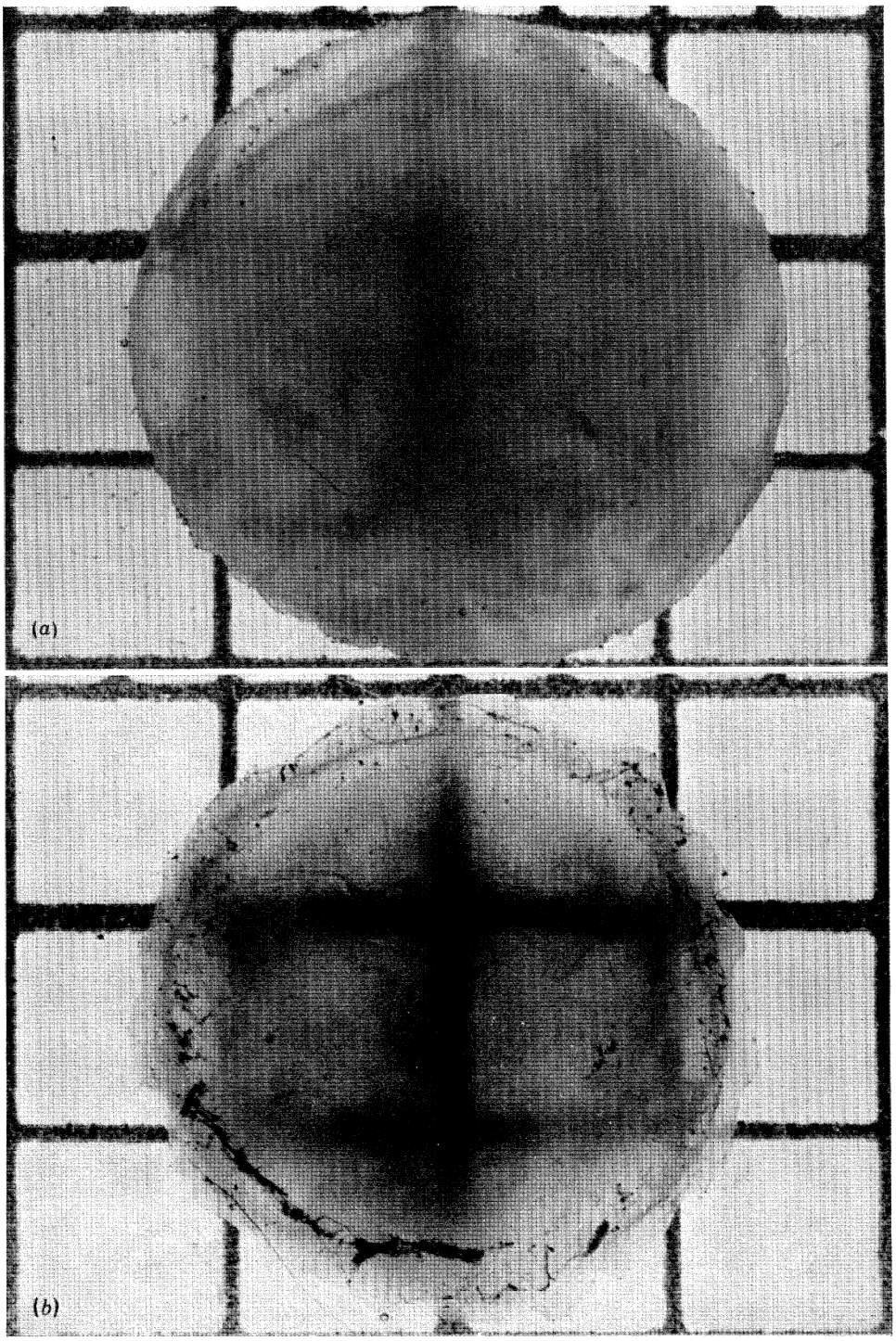

FIGURE 7. Effect of glycerol on the transparency of the human pathologic cataractous lens (*a*) before treatment, (*b*) after treatment in 50 % glycerol in physiological ionic strength salt solution at pH 7.4.

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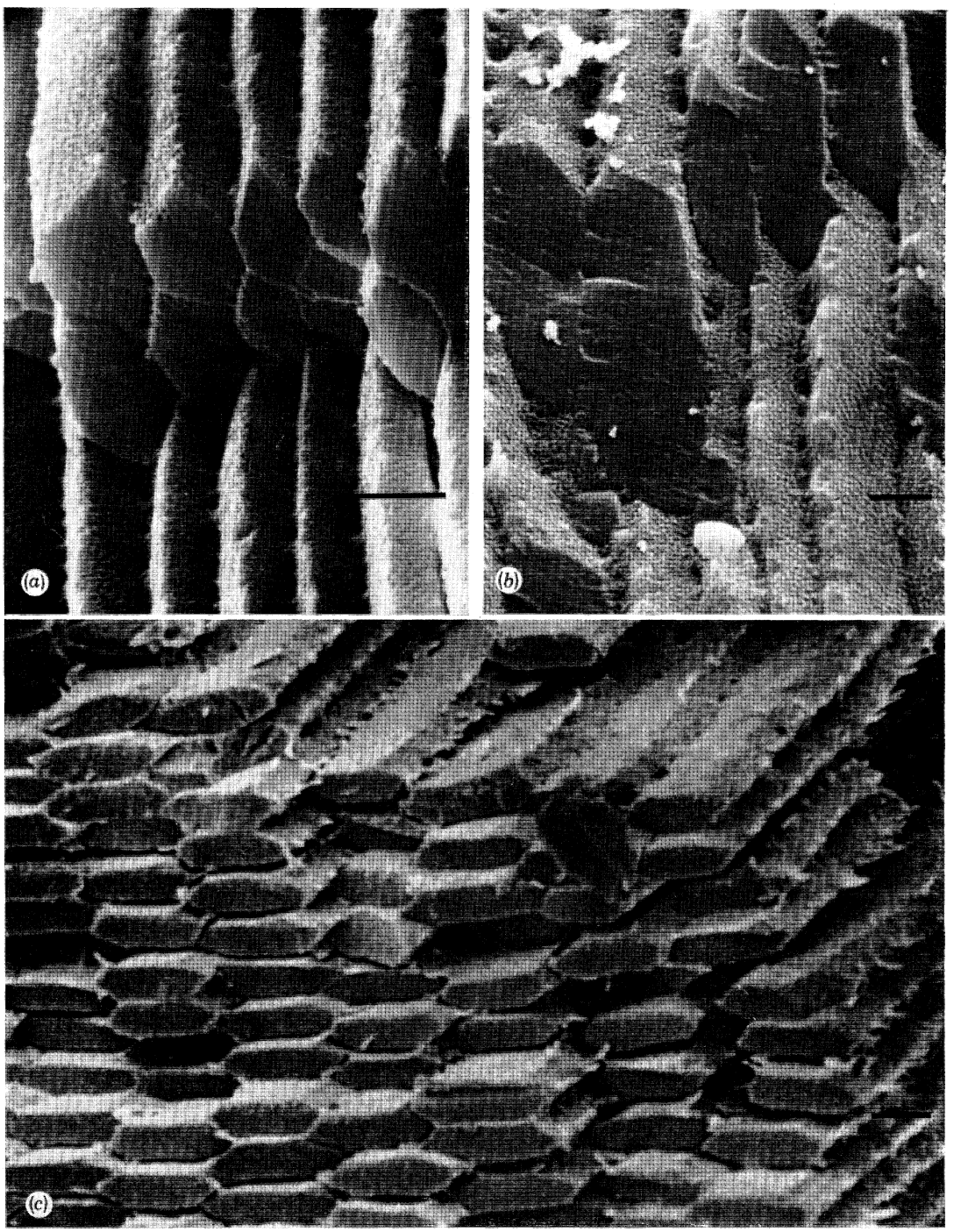

FIGURE 8. Scanning electron micrographs of normal (*a*), cataractous (*b*), and glycerol reversed cataractous (*c*) calf lenses. The general morphology of the lens fibre cells in the transparent lenses (*a* and *c*) is very similar to the morphology of the lens fibre cells in the cataractous lens (*b*). Slight changes in the surface and cytoplasmic structure may be related to lens opacity, but major structural alterations are not present.

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achieved by passing the light perpendicular to the basis vector a and to the anterior–posterior midline.

In figure 10 we show the diffraction pattern produced by 2 mm section of lens tissue after one day of incubation in physiological ionic strength solution when the light is oriented in accordance with the first configuration above. This figure shows 8 first order spots and 16 second order spots having respectively intensities 0.1 and 0.01 times that of the transmitted beam.

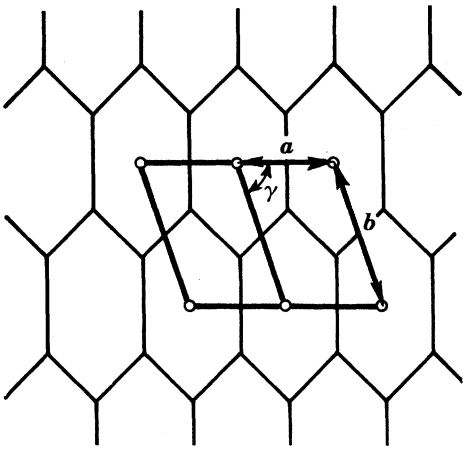

FIGURE 9. Schematic diagram of the rhomboidal cellular lattice of the lens cell according to the scanning electron microscope graph of figure 8. $\boldsymbol{a}$ and $\boldsymbol{b}$ are the translational vectors and γ is the angle of acuity.

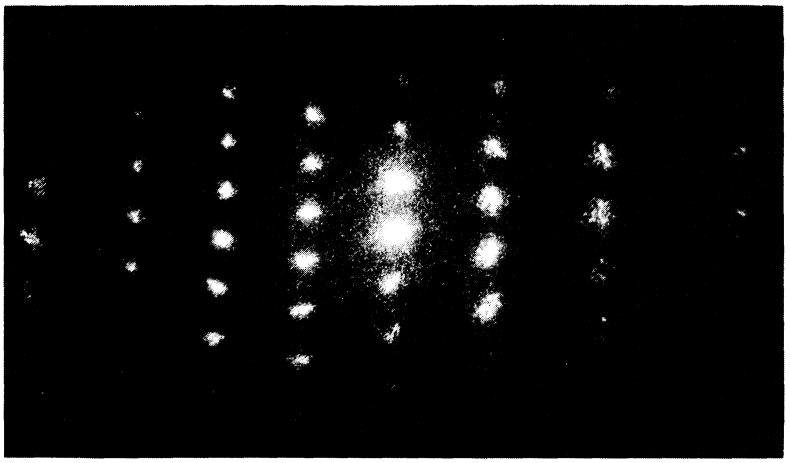

FIGURE 10. Diffraction pattern produced by a 2 mm thick peripheral section of a calf lens in the transparent state after 1 day of incubation in a physiological ionic strength salt solution. The orientation of the fibres is shown in figure 9 and the laser beam traverses the section parallel to the $\boldsymbol{a} \times \boldsymbol{b}$ direction.

We also obtained spots associated with the third and fourth order. The total intensity associated with all Bragg spots is 50 % of the incident beam intensity. It should be recognized that, despite the fact that 50 % of the incident light is scattered into Bragg spots, the lens appears quite transparent when viewed in ambient white light. The spatial and temporal coherence of the incident laser beam permits a great enhancement of the diffraction spots over that which is produced by the spatially and temporally incoherent ambient illumination. From the angular position of the spots we deduce the following values for $\boldsymbol{a}$, $\boldsymbol{b}$ and γ for a cortical region of the lens: $a = 4\ \mu m$, $b = 7.3\ \mu m$ and $\gamma = 72.5°$. These results are in satisfactory agreement with the values previously obtained using light and electron microscopy (Trokel 1962; Kuwabara

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1975) and laser diffraction. The results presented above can also be found in intact whole calf lenses properly illuminated near the lens periphery. The diffraction spots described above cannot be obtained when lenses are stored for a couple of hours at very low temperatures (−30 °C) and then thawed.

(*b*) *Effect of cold cataract on diffraction patterns.* If the temperature of the thick sections of whole lenses is reduced below the T_{cat}, the incident beam becomes very heavily attenuated in passing through the lens. Multiple scattering occurs and the diffraction pattern completely loses contrast as light is scattered between the Bragg spots. To overcome this difficulty, we reduced the size of the section studied to 100 μm. Under this condition multiple scattering and beam attenuation was not significant. On passing below T_{cat} we observed that the positions of the Bragg spots do not change, but that their intensity increases roughly by a factor of 100 on opacification. The effect is reversible in the sense that cycling above and below T_{cat} always reproduces the intensity and the position of each spot. In these thin sections we are normally able to detect photographically first and second order Bragg spots.

When the calf lens sample is warmed from below to above T_{cat} rapidly, we observe that the section immediately clarifies and the scattered intensity between the lowest order Bragg spots also decreases simultaneously. The intensity of light in the Bragg spots only falls after a delay of 10–20 s. This suggests that those structural elements which produce the Bragg spots are related but are not the same as those elements that reduce the image forming properties of the lens.

Finally, diffraction patterns from thin sections of human pathologic cataractous lenses also show a higher diffracted light intensity on the Bragg spots than similar sections of clear human lenses.

Viscosity measurements

The mechanical resistance against homogenization by hand of the lens nucleus with a tissue grinder is markedly temperature dependent. As the temperature is lowered towards T_{cat}, the resistance against homogenization becomes increasingly more difficult. At temperatures below T_{cat} homogenization of the nucleus becomes practically impossible.

To investigate this effect further we undertook to measure the viscosity of homogenates of the lens diluted 1 : 1 with 0.15 M saline buffer as a function of the temperature by using a falling ball viscosimeter in capillary tubes. As the temperature is lowered below T_{cat} the viscosity rises markedly. Apparently the state below T_{cat} is metastable in the sense that it takes 1 h before the full viscosity is established. For temperatures between T_{cat} and $T_{cat}-5$ °C the viscosity rises quite sharply. Below $T_{cat}-5$ °C the ball sticks in the viscosimeter. This marked increase in viscosity is like that reported for the lysozyme–water system (Tanaka *et al.* 1977) near the temperature of the phase transition. It is also consistent with the formation of a highly viscous mechanical structure such as that in non-covalently linked viscoelastic gels. The phenomenon described above is reversible in the sense that the viscosity falls again as the temperature is increased above T_{cat}.

In this connection we should also note that we have measured the mean intensity and amplitude of the intensity fluctuations in the light scattered by an opaque calf lens section and opaque whole lens into essentially a single coherence area in the scattered field. This measurement indicated that static scattering elements are responsible for over 99% of the scattered light intensity by the opaque lens. This static character is consistent with the holographic reconstruction experiments of Reynolds *et al.* (1973).

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Discussion and conclusions

The effects of various agents on the cold cataract temperature T_{cat} are summarized in table 1. The agents that increase T_{cat} can be used to produce opacification of the calf lens at body temperature. On the other hand, the agents that decrease T_{cat} can be used to clarify at body temperature those lenses made opaque by the former agents. The effect of all the agents listed in table 1 correspond to changes in the location of the coexistence curve in the phase diagram of the protein–water system in the nuclear region of the calf lens. Other lines of evidence (Zigman & Lerman 1965) suggest that the γ-crystallin are primarily involved in this phase separation phenomenon.

Table 1. Compilation of chemical reagents which shift the cold cataract temperature of the nucleus in intact lenses

agent	change in T_{cat} (°C mol^{-1})
KCl, NaCl, LiCl, CsCl	*ca.* +25.0
D_2O	*ca.* +0.9
glycerol	*ca.* −10.0
ethylene glycol	*ca.* −6.0
1,3-butanediol	*ca.* −5.8
1,4-butanediol	*ca.* −6.5
urea	*ca.* −22.0
guanidine hydrochloride	*ca.* −22.0
glycine	> −15.0

Our measurements of the strong temperature dependence of the viscosity of lens homogenates near T_{cat} suggest, in addition, that the opacification is associated with the formation of a highly viscous phase. Thus, the phase transition involved may be between a solution and a gel phase. Since our measurements show that 99% of the light scattered from the opaque material is static in character, this suggests that the new phase contains substantial static spatial inhomogeneities in the refractive index.

Our data also show that the light scattered into the Bragg spots increases by about a factor of 100 as the lens is made opaque either by lowering the temperature or increasing T_{cat}. In addition, the opaque lens produces considerable scattering of light into directions between the Bragg spots. On suddenly raising the temperature above T_{cat} the scattering between Bragg spots falls and the lens clarifies before the intensity in the Bragg spots decreases to the value appropriate to the normal lens section at equilibrium. These findings imply that the scattering elements within the lens have two components, one having the periodicity of the cellular lattice and the other one, associated with opacity, without the periodicity of the lattice. At present we have not deduced the form factor or the pair correlation function for either of these scattering fluctuations. Nevertheless, it seems clear that the analysis of the intensity variations in and between the Bragg spots can produce valuable information on the microscopic structure of the elements associated with lens opacification.

In contrast, divalent ions such as $CaCl_2$ at concentrations as low as 10^{-3} M induce cortical cataracts that are opaque over the entire temperature range from 4 to 37 °C. Furthermore, cataracts induced by these ions are not reversible upon incubation in physiologic ionic strength salt solution. These findings are consistent with previous experiments (Jedziniak *et al.* 1972) on

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protein fractions of the calf lens, and suggest that the cortical opacification may be associated with the aggregation of the α-crystallin in the cortex. Thus the effect of the divalent ions appears to be quite different phenomenologically from that of the monovalent ions. Nevertheless, we have observed that some of the agents that reduce T_{cat} in the lens nucleus region (glycols and urea) also are capable of clarifying the cortical cataract produced by the divalent ions.

Finally we have discovered that the glycols and urea, which decrease T_{cat} in the nuclear region of the calf lens, are also capable of producing a marked increase in the transparency of human pathologic cataracts obtained postoperatively and designated crudely as cortical or nuclear cataracts. We believe that this finding clearly demonstrates the possibility of chemical clarification of the human cataract *in situ*. At present we cannot propose a physico-chemical mechanism for the clarifying effect of these agents. In view of the fact that the clarification occurs over a wide range of glycol or urea concentrations, it is clear that these agents have a clarifying effect by means of a mechanism other than simple index matching.

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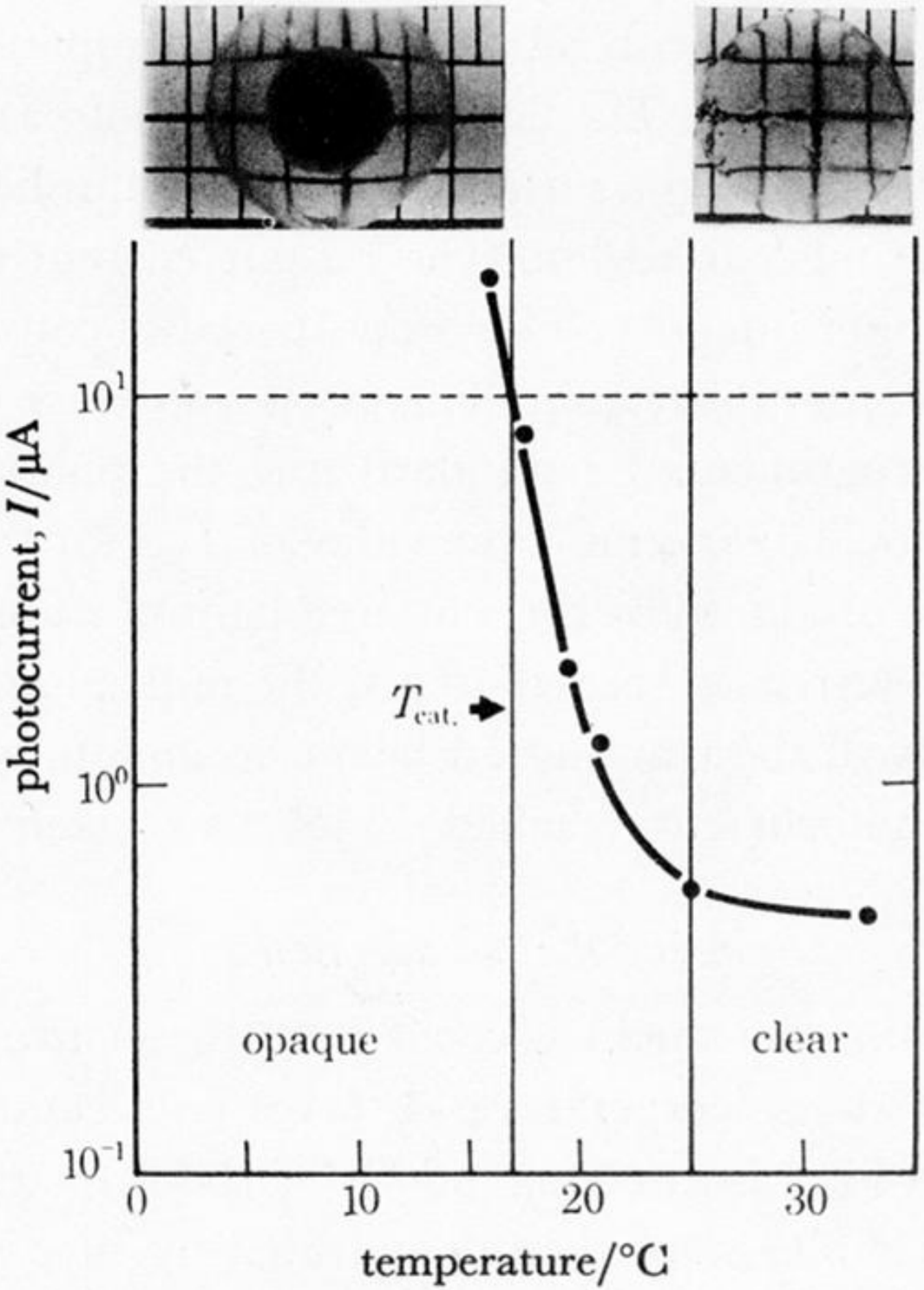
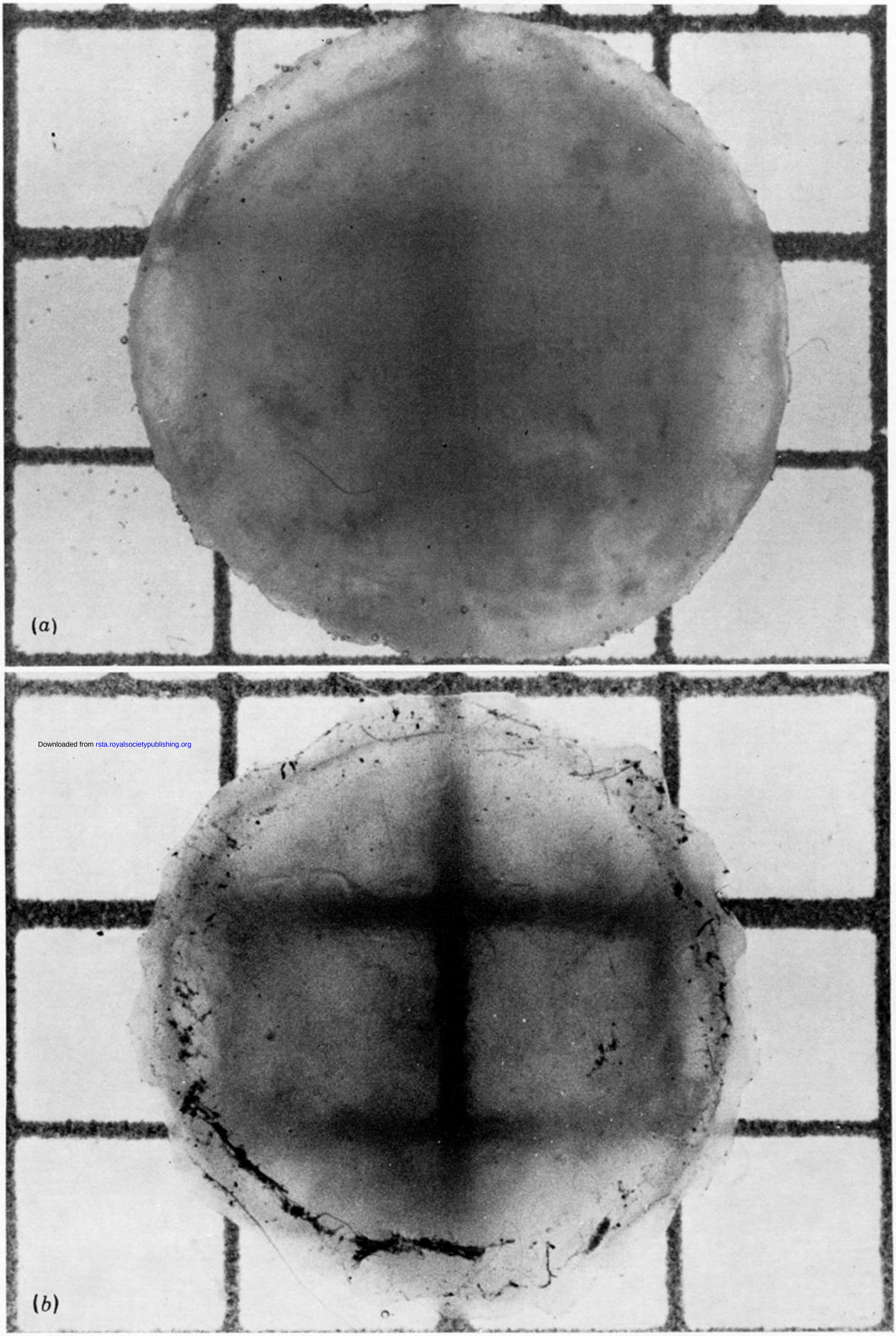


FIGURE 1. Intensity of light scattered from the centre of a calf lens as a function of temperature, measured for 90° scattering angle. The intersection of I against T curve with 10 μA photocurrent level defines $T_{cat.}$.



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IGURE 7. Effect of glycerol on the transparency of the human pathologic cataractous lens (*a*) before treatment, (*b*) after treatment in 50 % glycerol in physiological ionic strength salt solution at pH 7.4.

FIGURE 8. Scanning electron micrographs of normal (*a*), cataractous (*b*), and glycerol reversed cataractous (*c*) calf lenses. The general morphology of the lens fibre cells in the transparent lenses (*a* and *c*) is very similar to the morphology of the lens fibre cells in the cataractous lens (*b*). Slight changes in the surface and cytoplasmic structure may be related to lens opacity, but major structural alterations are not present.

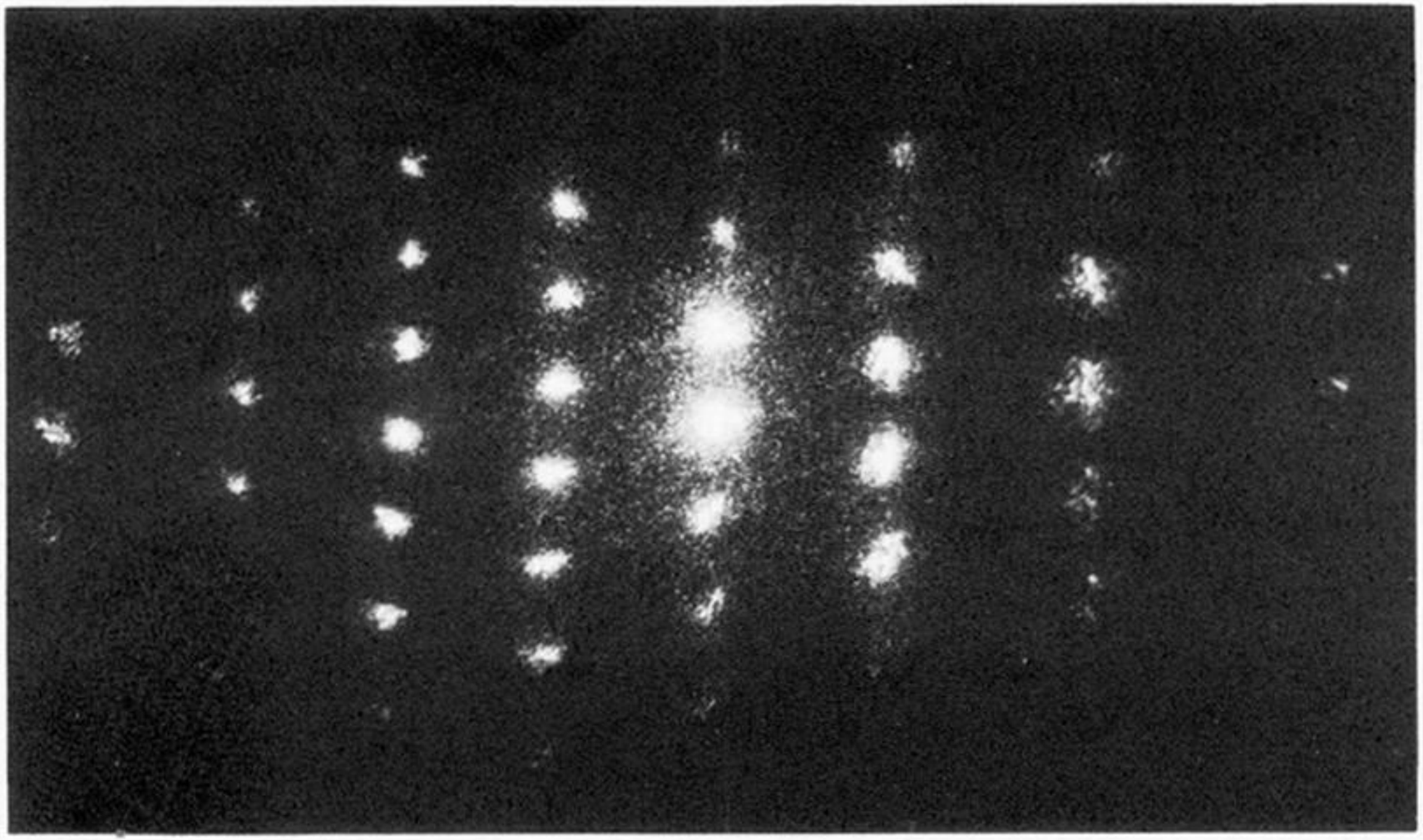

FIGURE 10. Diffraction pattern produced by a 2 mm thick peripheral section of a calf lens in the transparent state after 1 day of incubation in a physiological ionic strength salt solution. The orientation of the fibres is shown in figure 9 and the laser beam traverses the section parallel to the $a \times b$ direction.

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