## Demonstration of a Net Movement of Water Through the Lens

If the crystalline lens is injured mechanically or if it is incubated with general metabolic poisons, the cation and water content increases[1]. Anisotropic uptake across the anterior and posterior surfaces of the rabbit lens was reported for radioactive labeled $K^+$, $Na^+$, $Cl^-$ and $\gamma$-aminobutyric acid[2,3] and also for fluorescein[4]. The distribution of $Na^+$ and $K^+$ are not uniform within the normal lens[5] nor is $^{86}Rb$[6] after incubation in vitro with it. A pump-leak theory for active transport of cations was proposed[7] which suggested a pump across the epithelial layer of the lens and leak across all surfaces. Thus the data and theory picture a lens with both unequal distribution of actively transported substances within the lens and of anisotropic exchange of these substances across its 2 surfaces.

Experiments with tritium labeled water[2], which revealed no anisotropy of uptake into the lens, seems to be inconsistent with the active transport function of the epithelial cell layer. It has been reported[1] that the rabbit lens could maintain cation balance for a few hours in the absence of exogenous glucose. Thus the biologically inert fluid, silicone oil, was chosen as a suitable medium in which to look for secretion of a fluid by the lens epithelium. The crystalline lenses used were removed either immediately post mortem or were obtained from young rabbits' eyes which had been in a refrigerator overnight. The human lenses were removed from eyes which had been refrigerated at least 24h. The lenses were removed and handled so as to avoid even slight injury to the anterior surface. Excess fluid was removed by gently rolling the lens on hardened filter paper meagerly dampened with normal saline.

The silicone oil (Dow-Corning 360 Medical Fluid) was prepared before use by washing it 3–5 times with triple distilled water. Just before use the oil was filtered through a Millipore filter (LCN, Mitex, 10 μm pore size, in a microsyringe filter holder). During incubation, the lenses were supported by a device made from Tygon tubing or the other substances named below. At the beginning and at various times during incubation the lens surfaces were examined for fluid secretion using a stereo microscope at magnifications up to ×25. Stereographic pictures were taken of some lenses. Time lapse (2 frames/sec) cinephotography of either surface of 12 lenses were obtained using a 16 mm movie camera. After incubation the orientation of the lens was determined by placing on one surface about 2 μl of nitroblue tetrazolium chloride solution which stains[8] the epithelial cells or the fibers.

The first 20 of 20 rabbit lenses from refrigerated eyes developed drops or lakes of fluid on the anterior surface only (Figure 1) during incubation at 37 °C. 2 human lenses from different donors had identical results. Any fluid left on the posterior surface disappeared during incubation and if bits of vitreous body or ciliary processes were placed on the posterior surface they were appreciably dehydrated over a period of 2 h. The gravitational field, lens support, temperature differentials, observing light, etc., could not be the cause of the results observed because fluid accumulated on the anterior surface only, whether that surface was up or down with respect to the gravitational field or if the lens was supported in the preparation by Tygon tubing, polyethylene plastic, plexiglass, or fluorosilicone oil.

No secretion of fluid was observed if the lens preparation was kept cold. In four preparations fluid which


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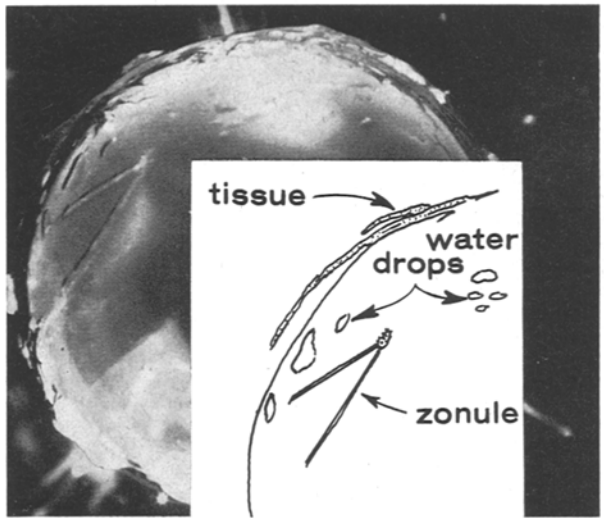


Fig. 1. A frame taken from cinephotograph of fluid secretion on the anterior surface of a rabbit lens incubated (37 °C, 30 min) in silicone oil. When placed in the oil the whole surface area was smooth except for the zonule and excised ciliary tissue at the equator. The identification of some of the structures are indicated in the line drawing inset. A few min after this picture was taken most of the drops shown coalesced into a single pool of fluid.

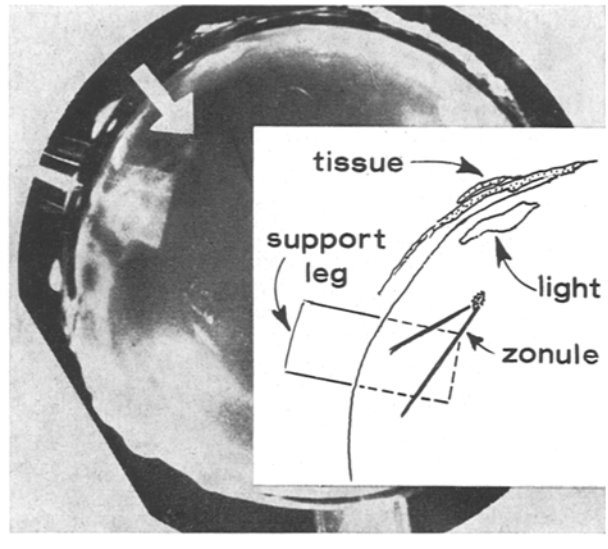


Fig. 2. The same lens shown in Figure 1 photographed after 24 h refrigeration plus 30 min of incubation at 37 °C in silicone oil. When this frame was taken the V-shaped zonule was covered by a pool of fluid. When the preparation was placed for cinephotography there was no detectable fluid on the surface. The frame was printed so that the V-shaped zonule was in the same relative position in Figure 1. The line drawing inset identifies some structures.

appeared on the anterior surface while incubated at 37 °C disappeared on storage of the preparation in the refrigerator. The fluid reappeared promptly upon reincubation at 37 °C. (Figure 2). In one preparation this reversal was observed 4 times over a period of 2 days. Loss of the ability of the lens to secrete fluid resulted from storage of the lens in the refrigerated eye for 4 days or longer (rabbit or human lenses), and for rabbit lenses, removal of the lens capsule, exposure of the lens to formaldehyde vapors for 10–15 sec just prior to placing the lens in silicone oil or injury to the lens epithelium. Deliberate excision of a small part of the lens with a very sharp blade did not always result in total failure to secrete fluid except near the injury.

Lenses excised rapidly post mortem secreted fluid more readily if a source of fluid were present on the posterior surface. The secreted fluid was always directly opposite the source, providing the source was placed nearer to the equator than to the posterior pole. The secreted fluid had the same shape as the fluid source, (Figure 3), on 5 of 5 lenses. Thus it appears that net fluid movement through the peripheral portions of the lens has a 'fiber-optics'-like property.

The experiments reported here were not designed to measure the magnitude nor the rate of fluid secretion. However, we did measure changes in drop diameter with time on cinephotographs such as Figure 1, and from those data estimated a fluid secretion rate of 30–50 µl/lens $h^{-1}$. Data reported for the rabbit lens[2] for the half time for exchange of water ($t\frac{1}{2}$ = 15 min) means that the average exchange rate is at least 550 µl/lens $h^{-1}$ or about 10–20 times more rapid than the estimated rate of secretion. These calculations suggest why anisotropy of water uptake into the lens was not detectable[2].

If the inulin space of the rabbit lens is about the same as was reported for the rat lens (10 % of lens water) then a 250 mg rabbit lens would have an inulin space of about 17 µl. If the inulin space equals the extracellular space of the lens it means that the secretion rate is sufficient for the extracellular space to be secreted each 20–30 min. Such a turnover of extracellular space would be a reasonable mechanism to aid in removal of metabolic waste products from cortical fibers and would certainly contribute to the anisotropic exchange of cations. The finding of a 'fiber optics'-like net movement of fluid through the freshly excised lens is consistent with a unidirectional turnover of extracellular fluid and is also consistent with an earlier finding of the author[10] of movement in vivo of nitroblue tetrazolium chloride along lens fibers radiating from the posterior suture line. This finding is also consistent with the reported[4], pattern of movement of fluorescein in the decapsulated calf lens. All these findings appear to be related to the fibrous structure of the lens cortex.

The observations that a lens rapidly excised post mortem secretes fluid less rapidly than if a fluid source is present on the posterior surface makes it appear that the secretion rate may be controlled by the availability of fluid to the posterior end of lens cortical fibers. If so does the composition of that fluid matter? In partial answer to this question it was observed that bits of vitreous-body ($Na^+$ rich fluid) or bits of ciliary processes ($K^+$ rich fluid) were both dehydrated at about the same rate. A practical aspect of these findings may be the observation made by eye surgeons who have used silicone oil for vitreous transplants in retinal detachment surgery[11]. They noted that a failure to leave vitreous body between the lens and the silicone fluid implant resulted in a cataractous lens.

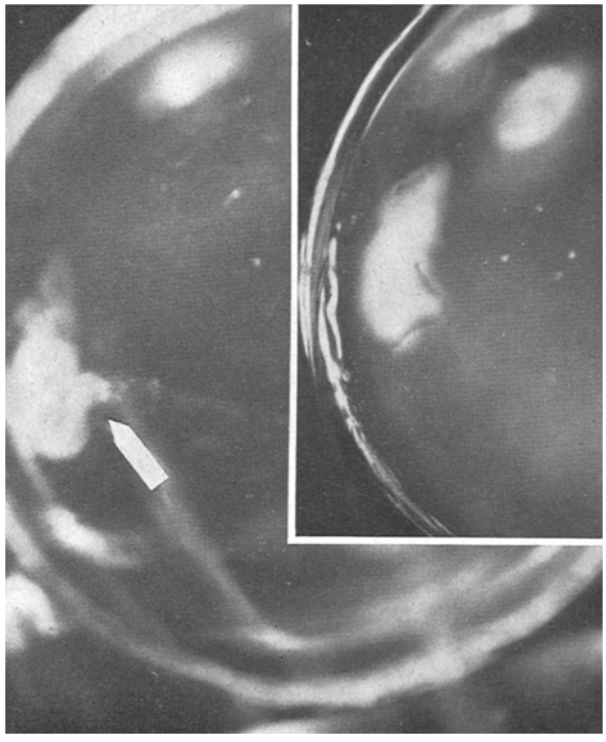

Fig. 3. A rabbit lens was excised rapidly post mortem, freed of excess surface fluid, and had a piece of ciliary process placed on its posterior surface. The ciliary process indicated by the arrow was photographed through the lens immediately after placement in silicone oil. The inset, a photograph of the anterior surface, was taken about 30 min later. It revealed a pool of fluid which began to form prior to 15 min of incubation (room temperature). This fluid was directly opposite the ciliary process on the other surface and had a very similar shape. The volume of the tissue decreased approximately by half during 30 min incubation.

*Résumé.* Des cristallins de lapins couvés dans des diméthylsilicones sécrètent un fluide à leur surface antérieure. Ce fluide peut prendre le même aspect et la même réticulation qu'un morceau du tissu de la surface postérieure, si le cristallin est excisé promptement après la mort.

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