A significant number of SP-positive cells were seen in the trigeminal ganglion, and no SP-positive cells were seen in the ciliary ganglion.

**Discussion.** Previous pharmacologic studies with the rabbit have shown that SP affects the constriction of the pupillary sphincter muscle. However, the present study with squirrels failed to demonstrate a conspicuous number of SP-positive fibers to the pupillary sphincter, although a few SP-positive fibers do exist in this area. This discrepancy might be explained by species differences since another of our studies with rats has demonstrated numerous SP-positive fibers to the pupillary sphincter.

The present study has shown the existence of a small number of SP-positive fibers around the ciliary cleft. This fact suggests that SP might play an important role in the physiologic control of aqueous outflow system.

With regard to the origins of these fibers, the trigeminal ganglion cells are mentioned as a possible site because a substantial number of SP-positive cells are located in this ganglion whereas none is demonstrable in the ciliary ganglion of the squirrel. However, since trigeminal ganglion cells are primary sensory afferent neurons, other origins cannot be excluded.

The present study demonstrates the presence of SP-positive fibers in the cornea of the squirrel, which is in good agreement with the results of a previous study. Because the branches of these fibers are located in the epithelial cell layer, these fibers are presumed to have some effect on sensory transmission. There is little evidence regarding the origins of these fibers, although a recent study denied the possibility that trigeminal SP-positive cells are the origin of corneal SP.

In any case, although it is certain that SP plays an important role in the physiologic control of anterior eye segment function, the precise role of SP in this area remains obscure. To explore the function of SP in this area, further anatomic and physiologic studies are needed.

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From the Department of Ophthalmology (Y. S., Y. K., M. F., I. I.) and Department of Neuroanatomy, Institute of Higher Nervous Activity, Osaka University Medical School, Osaka, Japan. Supported by grant 577660 from the Japan Ministry of Education. Submitted for publication May 1, 1981. Reprint requests: Dr. Y. Shimizu, M.D., Department of Ophthalmology, Osaka University Medical School, 1-1-50, Fukushima, Fukushima, Osaka 553, Japan.

**Key words:** substance P, anterior eye segment, squirrel, immunohistochemistry

**References**


Ca++-induced cataract. K. R. HIGHTOWER AND V. N. REDDY.

Cataracts in cultured rabbit lenses were produced by elevation of internal calcium. Experimental procedures were successful in increasing levels of total and bound Ca++, often without significant changes in sodium, potassium, or water content. Although the excess in calcium was predominantly associated with water-soluble proteins and was freely diffusible, a significant amount was...
bound to membranes and cytosol water-insoluble proteins. Thus, in lenses with a 10-fold increase in total Ca++, the bound Ca++ increased twofold, nearly 35% of which remained fixed to water-insoluble and membrane proteins after exhaustive (72 hr) dialysis. In contrast, over 55% of the Ca++ in water-soluble protein fractions was removed by dialysis. (INVEST OPHTHALMOL VIS SCI 22:263-267, 1982.)

Excessive amounts of calcium have been shown to aggregate isolated protein of lens homogenates into high molecular weight proteins large enough to scatter light. However, calcium-induced aggregation of water-soluble proteins has not been demonstrated in situ. In fact, elevated levels of Ca++ have not been consistently observed in human cataract, although recently data were obtained showing a correlation between calcium content and severity of opacification in senescent and brunescent forms of cataract, as classified by Cooperative Cataract Research Group guidelines.

In an effort to further clarify the role of calcium in cataract development, experiments were designed to determine whether calcium elevation leads to the formation of cataract. Thus several means of raising the level of calcium in cultured rabbit lenses were employed in an attempt to produce an opacity that might be solely attributed to an accumulation of calcium. The results suggest that calcium-induced opacities develop concomitantly with an increase in total and bound calcium in the lens.

Methods. Lenses were dissected from eyes of 4-wk-old Dutch New Zealand white rabbits. Control lenses were incubated in TC199 (Gibco, Grand Island, N. Y.) supplemented with bicarbonate at 37° or 4° C for 20 hr. The Ca++ concentration measured in TC 199 was approximately 1.8 mM. Ca++-loaded lenses were obtained by incubation at 37° or 4° C with various amounts of added calcium and lanthanum chloride.

In experiments in which the amount of calcium

Table I. Ca++ and Na+ levels in calcium-induced cataracts

<table>
<thead>
<tr>
<th>Incubation conditions</th>
<th>Temperature (°C)</th>
<th>Medium</th>
<th>Ca++ (mM)</th>
<th>La+3 (mM)</th>
<th>Lens Ca++ (mM)</th>
<th>Lens Na+ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37</td>
<td>TC199</td>
<td>2</td>
<td>0</td>
<td>0.2 ± 0.01 (6)</td>
<td>14 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>TC199</td>
<td>2</td>
<td>0</td>
<td>0.6 ± 0.08 (6)</td>
<td>29 ± 5</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>TC199</td>
<td>6</td>
<td>0</td>
<td>5.0 ± 0.76 (6)</td>
<td>31 ± 4</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>TC199</td>
<td>2</td>
<td>0.1</td>
<td>0.35 ± 0.04 (6)</td>
<td>14 ± 1</td>
</tr>
<tr>
<td></td>
<td>37</td>
<td>TC199</td>
<td>6</td>
<td>0.1</td>
<td>1.8 ± 0.3 (10)</td>
<td>15 ± 2</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>Ringer</td>
<td>100</td>
<td>0</td>
<td>9.1 ± (6)</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

*Rabbit lenses cultured in media for 20 hr; number of lenses in parentheses.
Fig. 1. Rabbit lenses photographed (Polaroid) under four floods at 45° C. a, Lens after 24 hr at 4° C in TC199 and 15 min at 37° C. b, Lens after 24 hr at 4° C in TC199 with 6 mM Ca ++ in media. c, Lens incubated under the same conditions as in b and 15 min after recovery at 37° C. d, Lens after 24 hr at 37° C with 6 mM Ca ++ and 0.2 mM lanthanum. e, Lens after 3 hr at 21° C in 100 mM CaCl 2 solution (containing glucose and Mg ++ ).

faint peripheral haziness in the cortex on both anterior and posterior surfaces (Fig. 1, b). Although the nuclear opacity disappeared on warming to 37° C, the cortical opacity persisted (Fig. 1, c). The Ca ++ content of these lenses was found to increase from 0.2 to 5 mM.

Another means of elevating lens Ca ++ was to inhibit the Ca ++ pump with lanthanum. As seen in Table I, a marked increase in Ca ++ occurred when the media were supplemented with 6 mM calcium in addition to 0.1 mM lanthanum. Fig. 1, d, shows the resultant opacity after a 20 hr culture at 37° C. Calcium analysis revealed that lenticular calcium was elevated to 1.8 mM (Table I). However, sodium transport and membrane permeability were not significantly affected, since the change in sodium concentration during the 20 hr culture period was minimal.

Cortical opacities were also observed when lenses were incubated at 21° C for 3 hr in an isosmotic CaCl 2 solution (300 mOsm) containing 5 mM glucose and 2 mM Mg ++ (Fig. 1, e). The Ca ++ level in these lenses attained an average value of 9 mM without an increase in sodium. In fact, there was a considerable decrease in lens Na ++.

To determine whether the accumulated Ca ++ was loosely associated with the water-soluble protein or bound to proteins or membranes, we measured the amount of Ca ++ in soluble and insoluble protein fractions. Thus lenses having a Ca ++ concentration of nearly 1.8 mM (see Table I) were homogenized in histidine buffer and centrifuged at 1000 × g and 10,000 × g to separate membrane fragments from water-insoluble and -soluble proteins of the cytosol. Fig. 2, a, illustrates the marked increase in Ca ++ observed in the 10,000 × g supernatant from approximately 1000 to 12,000 ng of total calcium. Over 95% of this Ca ++ was easily removed from the water-soluble protein fraction after 48 hr of dialysis in a histidine buffer free of EGTA. The pellets of the 1000 × g and 10,000 × g centrifuge appeared to retain more Ca ++ in the Ca ++-induced cataract than that in the control, which is more evident from the data expressed as ng Ca ++/mg dry weight (Fig. 2, b). Thus the Ca ++ associated with the pellets has increased from 37 ng/mg to nearly 70 ng/mg in the calcium-induced cataract. Dialysis of pellets from cataracts shows that approximately 25 ng/mg is not diffusible compared with only 8 ng/mg in the control.

Discussion. The major finding of this study is that elevation of lens calcium above 0.2 mM, to at
least 1.8 mM, results in a superficial opacity of anterior and posterior surfaces of a mammalian lens. In addition, an increase in lens sodium does not appear to be a contributing factor in this type of cataract, since the calcium-induced opacities shown in Fig. 1, d and e, were produced without an accompanying increase in lens sodium.

Not only do opacities arise in calcium-loaded lenses, but like human cataract they also contain more protein-bound calcium than does a clear lens with low levels of calcium. Although the increase in bound calcium in these lenses is considerably smaller than that observed in human cataracts the extent and severity of the opacity are also considerably less. It is conceivable that much longer incubation periods might result in more bound Ca++ and perhaps more extensive opacification.

It is noteworthy that in a short incubation period (20 hr), a significant amount of the Ca++ has become associated with fiber cell membranes, representing nearly a twofold increase. To the extent that such binding occurs in situ, calcium probably has a deleterious effect on membrane integrity and membrane-bound enzymes such as (Na-K)-ATPase. The results of Clark et al., who used 3 hr postmortem calf lenses, suggest that membrane structure is altered in the presence of high calcium concentrations, which causes lens opacification. It should be noted, however, that these lenses were incubated in the absence of glucose and K++, which might also contribute to the observed disruption of fiber cell membranes. Duncan and Bushell also reported that lens opacities were produced by freezing and thawing bovine lenses prior to culture in a calcium medium. It is difficult, however, to distinguish between the effects of high calcium and the nonspecific effects of the physiologic condition employed to raise internal calcium levels. This is most evident in Duncan's observation that significant changes in Na+, Cl−, and K+ levels in calcium-loaded lenses were also apparent.

In a study by Fagerholm, the structure of individual lens fibers incubated in media containing high calcium was observed to breakdown within 2 hr. However, fiber degeneration occurred even at low Ca++ concentrations, although at a slower rate than that in high Ca++ concentrations. If such a
breakdown was occurring in the intact calcium-induced cataract one would certainly expect accompanying changes in Na⁺ or K⁺.

Since little if any change in hydration or cation levels was observed, it is postulated that in the earliest stage of opacification, scattering centers might result from calcium-protein interactions both in the membranes and the cytosol. This hypothesis is presently being pursued in a study involving histologic examination to determine whether localized disorder of fiber cells might contribute to light scattering in this cataract.

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From the Institute of Biological Sciences, Oakland University, Rochester, Mich. The study was supported by Research Grants EY-00481, EY-00483, and EY-00484 from the National Eye Institute of the National Institutes of Health and is part of the Cooperative Cataract Research Group activity. Submitted for publication Aug. 10, 1981. Reprint requests: Kenneth R. Hightower, Institute of Biological Sciences, Oakland University, Rochester, Mich. 48063.

Key words: cataract, calcium, lens, rabbit, proteins

REFERENCES


Decrease in canine corneal endothelial cell density and increase in corneal thickness as functions of age. ROBERT M. GWIN, IRVING LERNER, J. KAY WARREN, AND GLENWOOD GUM.

Fifty-nine normal dogs, ranging in age from 6 weeks to 132 months were examined with contact specular microcopy to determine the relationship of age to corneal endothelial cell density, morphology, and corneal thickness. Canine corneal endothelial cells appear quite similar to those of other species studied, including man. The hexagonally shaped canine endothelial cells tend to enlarge with age, with the population in young animals ranging around 2500 cells/mm² and the number of cells in older dogs being frequently below 2100 cells/mm². A significant increase in corneal thickness was observed with age. Healthy canine corneal endothelial cells appear to maintain a functional monolayer by enlargement and migration and represent a reasonable model for future endothelial cell study. (Invest Ophthalmol Vis Sci 22: 267-271, 1982.)

With the popularity of advanced surgical techniques such as phacoemulsification, intraocular lens implantation, and corneal transplantation and the development of the clinical specular microscope, trauma to corneal endothelial cells by these procedures has been well documented.

Several studies show that normal human endothelial cell populations decrease with age, resulting in a significantly larger size of individual cells. These data indicate that human endothelial cells maintain a functional monolayer by the process of enlargement and migration, rather than by active mitosis.

Endothelial cell regenerative activity appears to vary in animal species. In the rabbit, mitotic activity of endothelial cells is extensive after endothelial cell loss. In contrast to man, the number of rabbit corneal endothelial cells increases with age. It has been shown, however, that the cat and primate have limited capacities to actively divide and respond to injury in a manner more similar to man. The regenerative capacity of canine corneal endothelial cells is unknown.

The purpose of this paper is to describe the normal aging changes of canine corneal endothelial cells with regards to cell density, morphology, and corneal thickness.

Materials and methods. Dogs used in this study were either normal beagles and schnauzers obtained from dog colonies at the University of Florida or were dogs of various breeds and ages.