through the vessel wall, permeates the intercellular clefts of the tapetum lucidum and stains Bruch's membrane as a continuous dark band. The tortuous infoldings of the basement membrane are likewise filled with the tracer, although none is present in cytoplasmic vesicles. In the pigmented epithelial layers, dense reaction product outlines the intercellular channels and proceeds to the zonula occludens. No reaction product was found beyond these junctions,indicating the HRPO most probably does not permeate into the retinal area.

Figure 2 is a higher power micrograph (x10,000) showing a channel between the lateral walls of two adjoining pigmented cells which is filled with reaction product up to the impermeable zonula occludens, again no product is observed between the villi or in the vicinity of the outer rod segments. It is concluded that in the skate, the morphologic site of the blood-retina barrier to molecules of the size of HRPO corresponds to the junctions encircling the apices of the retina pigment epithelial cells.

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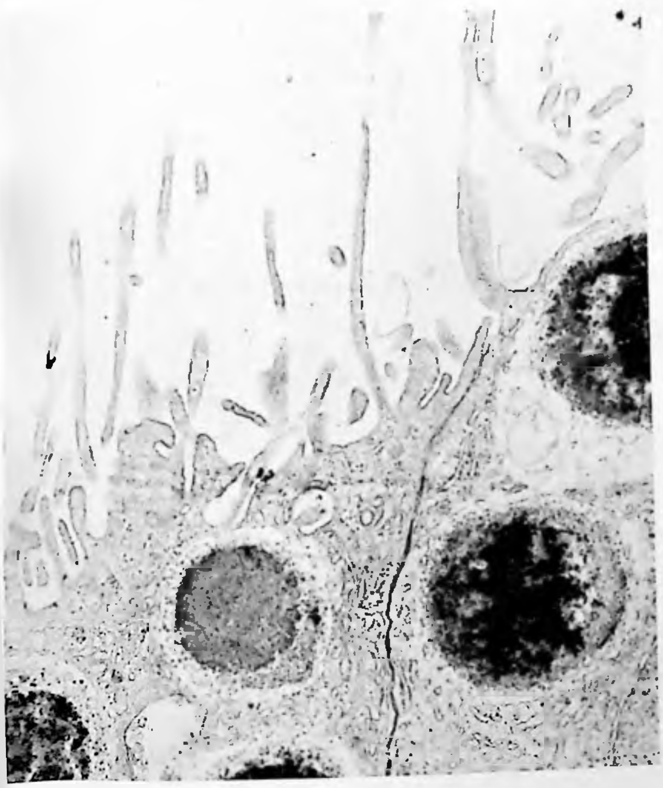

Figure 2

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## The Calcium Content of Ocular Tissues, Ocular Fluids and Plasma in some Fishes and Frogs with Reference to the High Calcium Content of the Retina Pigment Epithelium

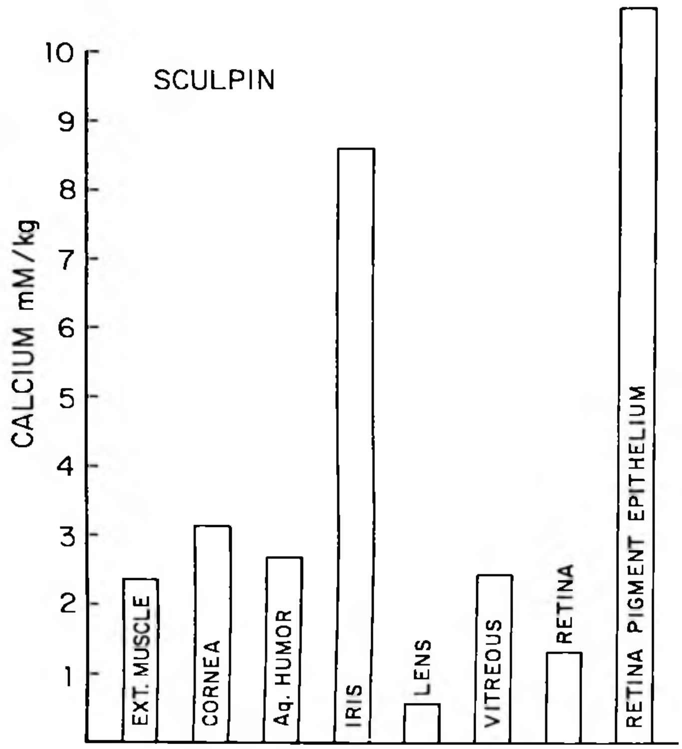
Jose A. Zadinaisky and Laura J. Zadunaisky, New York University Medical Center, New York, N.Y.

Data for electrolyte concerntration in ocular tissues is rather scarce in general and in particular in fishes and frogs. Values for the composition of the aqueous humor and vitreous in some species is available. However, the calcium concentrations have not been well studied. On the other hand, there are some recent reports on the high calcium content of the retina pigment epithelium (Hess, H:ARVO, 1975, p.33) and it was thought useful to determine calcium concentration in eye tissues and fluids and compare them to plasma in elasmobranchs, teleosts and amphibians. Eyes were enucleated from specimens of sculpins *Myoxocephalus octodecmspinosum* and *Myoxocephalus scorpius*) after severing the spinal cord. These teleosts were collected by sea bottom dragging or were caught in lobster traps and were kept in running sea water in plastic tanks at the station. Eyes from the spiny dogfish, *Squalus acanthias*, were obtained immediately after they were beheaded. Frogs (*Rana catesbeina*) were collected in local ponds and kept in an aquarium with fresh water. The aqueous humor was obtained by suction with needle and syringe after paracenthesis. Portions of the different tissues of the eye were placed in vials, weighed and dissolved in 0.5 ml of concentrated nitric acid. After 8-24 hours, the samples were made up to a total volume of 2 to 2.5 ml with 1%lanthaum oxide in an acid medium and the calcium content determined with a Perkin-Elmer atomic absorption spectrophotometer. Appropriate standards, nitric acid blanks and glass distilled water were used.

The results presented in Table 1 and Fig. 1 show that in the teleosts, elasmobranchs and amphibians studied, the retina pigment epithelium as well as the iris, have a very high calcium content, when compared to other tissues and fluids of the eye and plasma. The values for Ca content of the pigment epithelium in dogfish and frog are approximately ten times greater than in plasma, while in the sculpin they are some 4 times greater than plasma. It is important to notice also that the iris of fishes and frogs had a higher calcium concentration than the plasma and other tissues and fluids of the eye.

It is probable that the presence of pigment is the reason for the high calcium concentration. In fact, recently, it has been shown that light sensitive neurons of Aplysis californica, have a high calcium content and that calcium shifts are apparently responsible for the excitation of these neurons. Similarly, there is evidence that the response to light of the pigment epithelium has a continuous action spectrum, most probably due to the capture of light by melanin than any other pigment. Finally, pigment aggregation in the frog requires the presence of calcium. (Snyder and Zadunaisky, 1975 p. 58). If calcium is indeed associated with the melanin granules of the pigment epithelium and iris, the role of the action in visual excitation will be extended to tissues other than the rhodopsin containing photoreceptors. It appears then that a high calcium region in the pigment epithelium separates the lower calcium containing plasma and retina. The function for this calcium resevoir could be to act as a sink for the calcium movements needed for retinal excitation or as mentioned above could be related to the electrical responses of the pigmented tissues to light.

In the aqueous and vitreous humors, the calcium




concentrations were similar to plasma, although, in the dogfish, they were relatively higher than in plasma. The cornea has more calcium than other tissues of the eye, both in the frog and in the dogfish, perhaps denoting the high level of collagen in this tissue. The sclera also had higher calcium content than the plasma of aqueous humor in both the sculpin and the frog. The retina showed the lowest concentration of all ocular tissues.

Two other cations, Na and K, analyzed in some of the samples by flame photometry did not show the same pattern of distribution in the eye tissues as described for calcium.

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CALCIUM CONTENT in mM/kg. OF OCULAR TISSUES AND FLUIDS AS WELL AS OF PLASMA OF SOME FISHES AND THE FROG.

| | RPE mM/kg | Retina mM/kg | Cornea mM/kg | Iris mM/kg | EOM mM/kg | Sclera mM/kg | O.N. mM/kg | Aqueous mM/l | Vitreous mM/l | Plasma mM/l |
|---|---|---|---|---|---|---|---|---|---|---|
| SCULPIN | | | | | | | | | | |
| (7) | 8.90 | 1.05 | 2.58 | 6.69 | 1.84 | 4.04 | 1.72 | 2.33 | 1.82 | 2.61 |
| | ±0.71 | ±0.16 | ±0.26 | ±0.99 | ±0.25 | ±0.99 | ±0.56 | ±0.25 | ±0.18 | ±0.09 |
| DOGFISH | | | | | | | | | | |
| (4) | 19.4 | 1.90 | 4.96 | -- | 1.98 | -- | 2.34 | 2.30 | 2.65 | 1.82 |
| | ± 4.9 | ±0.19 | ±0.70 | | ±0.14 | | ±0.08 | ±0.14 | ±0.09 | ±0.06 |
| FROG | | | | | | | | | | |
| (2) | 6.4 | 0.50 | 1.83 | 7.5 | 1.18 | 3.8 | 1.87 | 0.51 | 0.49 | 0.62 |
| | ±0.12 | ±0.09 | ±0.24 | ±2.8 | ±0.33 | ±0.50 | ±0.25 | ±0.01 | ±0.01 | ± 0.08 |