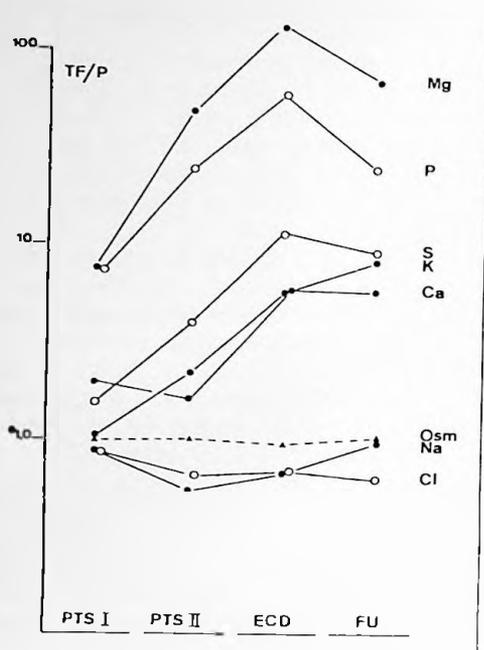


FIGURE 1



7.43,  $n = 22$  and  $24.04 \pm 2.74$ ,  $n = 22$ . This indicates that the principal site of magnesium and phosphate secretion could be localized to this segment.

Calcium TF/P was higher than unity in both PTS I and PTS II. Sulfate exhibited a pattern similar to that of magnesium and phosphate, although the absolute TF/P values are much lower.

The significant differences observed in transtubular concentration ratios of measured electrolytes arising in the PTS II show that this segment is very important in the skate nephron in excretion of divalent ions.

72

43 • 1975

### The Blood-Retina Barrier to Horseradish Peroxidase in the Eye of the skate, *Raja Erinacea*

Jose A. Zadunaisky and Leon T. Garretson, New York University Medical Center

The pigment epithelium of the retina and its basement membrane constitute an anatomical barrier to the free diffusion of solutes from the blood circulating in the choriocapillaris to the neutral retina. Previous studies on the blood-brain and blood-aqueous barriers have utilized horseradish peroxidase (MW 40,000;  $a_e$  2.5 nm) as a tracer that can be localized in the electron microscope. We have employed this method in seeking to identify the barrier to diffusion of small molecules from the choriocapillaris to the outer retinal layers at the level of the retinal pigment epithelium (RPE) in the skate, *Raja erinacea*.

Specimens of *Raja erinacea* were captured off Mount Desert Island, Maine, and housed in live cars for no more than two days prior to use. They were anesthetized with Nembutal, sea water was perfused through the opercula

and the animals placed on their backs to expose heart. Heparin was injected in the vena cava and followed by horseradish peroxidase (Sigma Chemical, St. Louis, type II) as 0.25 mg HRPO/g body weight from a prepared solution of 100 mg HRPO/ml of shark's Ringers. After appropriate time interval — 60 minutes in this study — fixation was begun by perfusing 200 ml of a diluted, then 50 ml of a concentrated solution composed of paraformaldehyde and glutaraldehyde in a sodium cacodylate buffer. The diluted fixative was one quarter (1/4) strength of the 4% paraformaldehyde and 5% glutaraldehyde concentrated fixative. The eyes were enucleated and hemisected at the ora serrata, exposing the posterior eyecup which was stored overnight in the diluted fixative. The tissues were transferred to buffer and diced for mounting on a Farquhar tissue chopper. Slices approximately 100  $\mu$  were made without freezing and were incubated with 3.3' diaminobenzidine for visualization of the exogenous peroxidase according to the method of Graham and Karnovsky (J. Histochem. Cytochem. 14: 303, 1966). Following a rinse in buffer, the slices were post-fixed in 2% osmium tetroxide, dehydrated in a graded series of ethanol solutions and infiltrated with Spurr's media. All ultramicrotome sections were stained with lead citrate prior to examination in a Siemens Elmiskop.

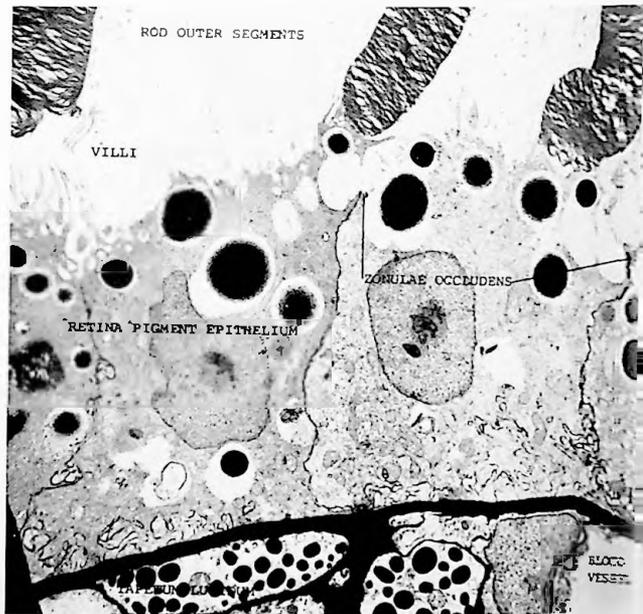


Figure 1

Figure 1 is a low power electron micrograph ( $\times 3000$ ) showing the outer retina, the pigment epithelium (RPE), tapetum lucidum and choroid vessels. The outer rod segments interdigitate with the villous processes of the retinal pigment epithelium in which the pigment granules are dispersed primarily toward the apical border. The pigment granules have been mechanically disrupted or lost during sectioning, producing in some cases, a white, halo-like artifact. The RPE rests upon its basement membrane, below which is seen the densely granulated tapetum lucidum and a section through a capillary.

The electron-opaque reaction product, having escaped

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.

This work was supported by grant #01340 of the National Eye Institute, National Institutes of Health.

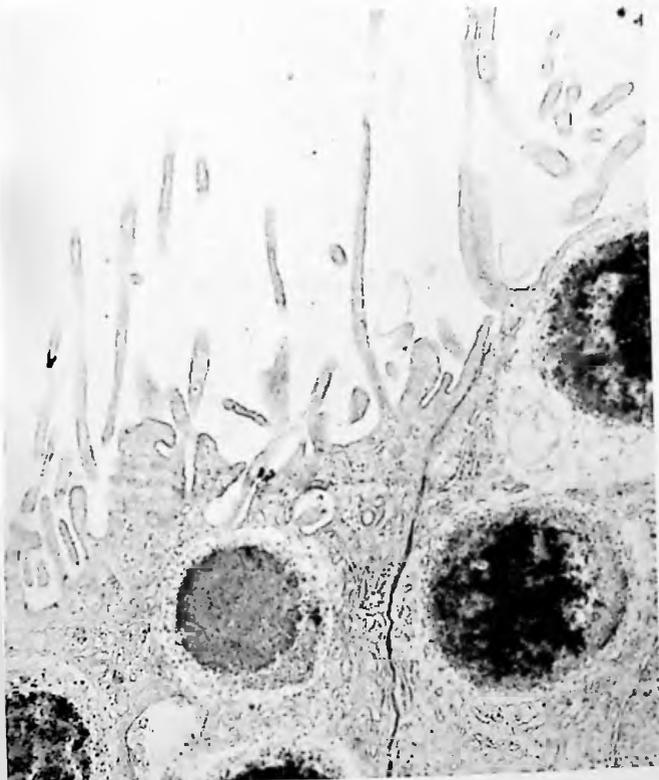


Figure 2

14 • 1975

#### 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

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

Data for electrolyte concentration 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 octodecspinosum* 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 catesbeiana*) 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 paracentesis. 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% lanthanum 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 *Aplysia 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 reservoir 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