PLASMA MEMBRANE ISOLATION FROM THE RETINAL PIGMENT EPITHELIUM OF SQUALUS ACANTHIAS

E. Kinne-Saffran*, R. Kinne*, M. Rubinstein**, J. Zadunaisky+
* MPI für Systemphysiologie, Dortmund, West-Germany
** La Jolla, California, USA
+ New York University Medical Center, N.Y., USA

The retina is a projection of the brain and consists of various cell types which are traditionally arranged in eight 'layers' and two 'membranes'. The tissue of interest in this study is the so-called 'epithelial layer' or retinal pigment epithelium (RPE). The RPE is a monolayer of cells interposed between the choroid capillaries and the retinal photoreceptors. It plays a vital role in the transport of metabolites and ions between the visual cells of the retina and their choroidal blood supply. The hexagonal shaped cells of the RPE show differentiation of their apical cell membrane in form of microvilli.

In isolated pigment epithelium-choroid preparations obtained after detaching the retina J. Zadunaisky and coworkers (Exp. Eye Res. 37:409-420, 1983) could show in electrophysiological studies that the cells of the RPE are able to transport chloride from the apical (retinal) to the basal-lateral (choroidal) cell side by means of an active transport mechanism. It was also found that the intracellular chloride activity was about 3 times higher than predicted from a potential difference of about -65 mV over the apical cell membrane (Wiederholt et al., Curr. Eye Res. 3:673-675, 1984). The active transport of chloride is sodium dependent and inhibited by ouabain, but only when applied to the retinal cell side of the RPE preparation (Miller, et al., J. Membr. Biol. 44:259-279, 1978. J. Zadunaisky et al., as above). This fact led the authors to postulate that a Na-K-ATPase located in the apical cell membrane provides the driving force for sodium-chloride cotransport across the same membrane.

In order to elucidate further the mechanism of active chloride transport we attempted to prepare luminal membranes from isolated RPE. For this purpose eyes of freshly sacrificed male and female dogfish (Squalus acanthias) were enucleated and kept in the dark for 30 minutes to contract the microvilli of the RPE. Then, the eyes were hemidissected, the retinas were removed and the RPE scraped from the sclera. Tissue from several fish were combined and stored at -20°C until use. For a typical membrane isolation about 4.0 g of material was needed. The frozen tissue was minced with scissors and homogenized in a Waring Blendor for 60 s at full speed. Similar to the method described by Booth and Kenny (Biochem. J. 142:575-581, 1974) luminal membranes were prepared by Mg^{2+} precipitation (20 mM final concentration), followed by several differential centrifugation steps. The final (white) pellet was analysed for alkaline phosphatase, 5'-nucleotidase, Na-K-ATPase and protein as described previously (Kinne et al., Pflügers Arch., 329:191-206, 1971).

Table 1 shows the enrichment factors and recoveries of alkaline phosphatase, 5'-nucleotidase and Na-K-ATPase in the membrane fraction. Alkaline phosphatase is enriched about 10 times. In one experiment the Mg^{2+} precipitation step was repeated but did not lead to a further increase in enzyme activity. 5'-Nucleotidase, also regarded as a marker enzyme for apical membranes, shows

the same enrichment as alkaline phosphatase. Na-K-ATPase, usually used as marker enzyme for basal-lateral membranes, is enriched about 6 times.

Table 1.-- Enrichment and recovery of enzyme activities found in a luminal membrane preperation from retinal pigment epithelium

Enzyme	Enrichment	Yield
Alkaline Phosphatase	9.4	9.4%
5'-Nucleotidase	9.5	6.8%
Na-K-ATPase	6.8	3.9%

Mean values of four experiments are given. Enrichment represents the ratio between the specific activity found in the membrane fraction and the specific activity found in the homogenate. The latter were 0.39 moles/hr/mg protein for alkaline phosphatase, 0.62 moles/hr/mg protein for 5'-nucleotidase and 0.15 moles/hr/mg protein for Na-K-ATPase. The total recovery for the enzymes was 125%, 102%, and 127%, recovery of protein was 95%.

Electromicroscopical studies of the membrane fraction (Fig. 1) reveal mostly vesicular structures. Preliminary uptake studies with radioactively label led D-glucose have, however, so far not demonstrated transport activity of these membrane vesicles. We have also not yet determined the sidedness of the membrane vesicles.

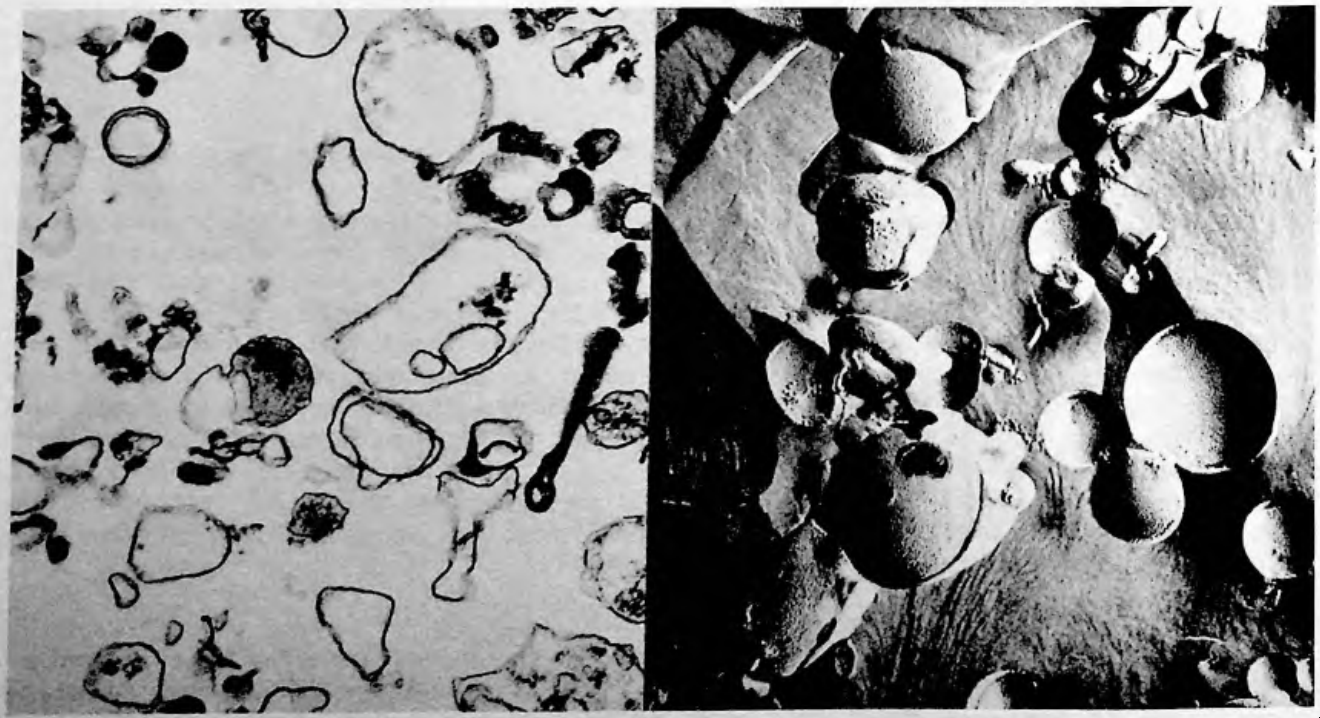

Figure 1.-- Electron micrographs of luminal membranes isolated from retinal pigment epithelium. Left panel: thin section, right panel: freeze-fracture.

The enrichment for Na-K-ATPase in the membrane fraction of 6 is lower than that found for alkaline phosphatase and for 5'-nucleotidase but unusual high compared to apical membrane fractions prepared from other epithelia using the Mg^{2+} precipitation method. This result might suggest that in the RPE a large portion of the Na-K-ATPase is located in the luminal membrane, a localisation consistent with the luminal action of ouabain on transport. If this were the case the Na-K-ATPase of the RPE could also contribute to the K siphoning that controls extracellular K levels in the retina.