

movement of Cl into the cell and its net secretion depend upon the maintenance of a low intracellular concentration of Na by the action of the NaK-ATPase pump. Chloride would then be extruded from the cell across the luminal cell membrane by electrical forces. A chloride carrier (as suggested by Dr. Michael Field) analogous to those responsible for the linked transport of glucose or amino acids into cells together with Na, is hypothesized to be present in the basal plasma membranes of rectal gland cells.

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Single Nephron Handling of Electrolytes (Na, K, Mg, Ca, Cl, P, S) in the Little Skate, *Raja erinacea*

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The kidneys of elasmobranchs regulate the excretion of electrolytes in the urine by tubular reabsorption and secretion. The present study was undertaken to obtain information on the handling of electrolytes by the individual segments of the nephron in the kidney of the little skate, *Raja erinacea*.

Experiments were performed on 16 female skates, weighing from 600 to 3,100 g. Each fish was anesthetized by i.v. injection of 1.5 mgs/kg sodium pento-barbital (Diabotal) and 1 mg/kg curare in the lateral tail veins. The fish was placed either on the ventral or dorsal side depending on which side of the kidney was used for micropuncture. The spiracles were perfused with un-aerated seawater at 1 - 1.5 ml/min at 10°C (Bull. MDIBL 11: 91, 1971).

Tubular fluid was taken by micro glass capillaries (O.D. 8 - 15 μ) from the proximal tubular segment I (PTS I) from the dorsal surface of the skate kidney, and from the proximal tubular segment II (PTS II) from the ventral surface. (PTS I probably corresponds to segment III, and PTS II to segment IV described by Deetjen and Antkowiak, Bull. MDIBL 10: 5, 1970). For further details see also Lacy, Schmidt-Nielsen, Galaske and Stolte, this volume. To collect fluid from the end of the collecting duct (ECD) small catheters of PVC-tubing (O.D. 150 μ) were inserted.

The collected fluid, as well as plasma and seawater, was analyzed for osmolality by the freezing point depression method of Ramsay-Brown and for electrolytes by electron probe microanalysis (Lechene, Proc. Fifth National Conference on Electron Probe Analysis, New York, 32A-32C, 1970; Lechene, in: *Microprobe Analysis as Applied to Cells and Tissues*, ed. by Theodore Hall, Patrick Echlin and Rudolf Kaufmann, pp. 351-368, Academic Press, London, 1974).

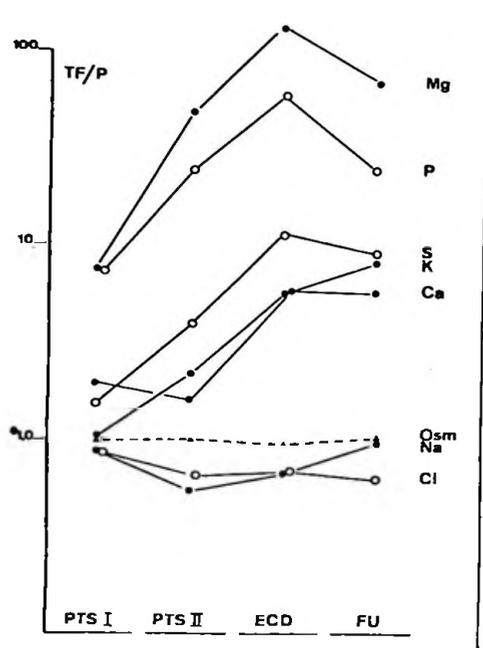
Figure 1 summarizes the tubular fluid to plasma ratio (TF/P) for osmolality and electrolyte concentrations. The osmolality in the individual segments was not significantly different from that of plasma. Sodium concentration was already below that of plasma in the PTS I and declined further in the PTS II (TF/P 0.86 ± 0.05 , n = 16 and 0.52 ± 0.04 , n = 22). Chloride showed a similar transtubular concentration ratio. In the final urine (FU), chloride concentration was lower than that of Na. There was no change in the TF/P ratio for potassium in the PTS I whereas it increased significantly in the PTS II and increased further until the end of the collecting duct (ECD). The major finding is the high transtubular concentration difference for magnesium and phosphate in the PTS II with a ratio of tubular fluid to plasma with $47.29 \pm$

TABLE I

	HCT %	Osm mOsm	Na mM	K mM	Mg mM	Ca mM	Cl mM	P mM	S mM
SW	-	858	425	9.06	46.9	10.3	503	1.55	25.7
		± 24	± 7	± 0.23	± 0.6	± 0.4	± 6	± 0.21	± 1.7
		(9)	(7)	(7)	(6)	(6)	(6)	(9)	(9)
PLASMA	19.6	968	267	5.40	1.19	1.74	301	1.86	5.36
	± 1.1	± 16	± 6	± 0.51	± 0.19	± 0.24	± 13	± 0.33	± 0.45
	(16)	(16)	(12)	(12)	(9)	(9)	(9)	(9)	(9)

Osmolalities and electrolyte concentrations in seawater in comparison to plasma in the little skate, *Raja erinacea*, and the mean hematocrit (HCT).

FIGURE 1



7.43, $n = 22$ and 24.04 ± 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.

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

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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 choriocappilaris 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 choriocappilaris 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 follow by horseradish peroxidase (Sigma Chemical, St. Louis type II) as 0.25 mg HRPO/g body weight from a prepa 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, 1:1 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 μ m were made without freezing and were incubated with 3.3' diamino-benzidine for visualization of the exogenous peroxidase according to the method of Graham and Karnovsky (J. Histochem. Cytochem. 14: 297, 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.

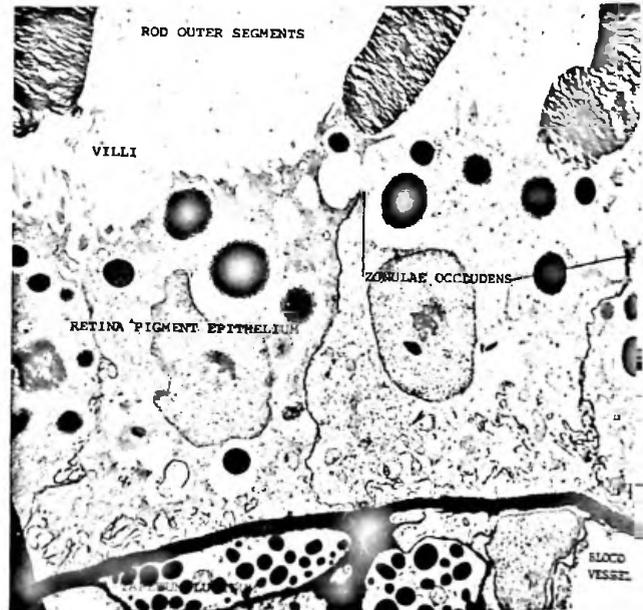


Figure 1

Figure 1 in 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 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