

The lateral surface of the absorptive cell is amplified by surface infolding, rather than by the lateral interdigitations found in mammalian intestine. Connections between the lamellar structures in the basal region and the basal plasma membrane were not observed, but more extensive search of thin sections may have revealed such connections. Further evidence that these lamellar structures represent extensions of lateral (and possibly the basal) cell surface was obtained with the electron-opaque extracellular marker, lanthanum, which penetrated into the folds at all levels of the cytoplasm.

Physiological data suggest that in flounder intestine resistance to intercellular Na diffusion is greater in the lateral space than in the tight junction (see Field, elsewhere in this bulletin and also in *Coupled Transport Phenomena in Cells and Tissues*. J. F. Hoffman, Ed., Raven Press, New York, in press). The close apposition of cells at their apical ends may provide a morphological basis for this relatively high resistance to Na diffusion.

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AUTORADIOGRAPHIC LOCALIZATION OF ³H-OUABAIN BINDING IN DOGFISH (*Squalus acanthias*) RECTAL GLAND

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One of the most intensely investigated transport enzymes is the ouabain-sensitive, sodium- and-potassium-dependent adenosine triphosphatase (Na,K-ATPase). Associated with most animal cells, the main role of this membrane-bound enzyme is thought to be the active cation transport (Na pump) involved in maintenance of ion gradients at the single cell level, and movement of salt and water across epithelial structures. Na,K-ATPase activity is especially high in a number of osmoregulatory epithelia and appears to be correlated with the Na transport rate. For example, when euryhaline teleosts are adapted to salinities ranging from 50% to 200% seawater (SW), the gill responds with parallel increases in enzyme activity and NaCl secretion. Using ³H-ouabain autoradiography it has recently been demonstrated that the adaptive Na,K-ATPase in teleost gills is located not on the apical membrane of the chloride cells as expected, but on the amplified basal-lateral membrane (Karnaky et al., *J. Cell Biol.*, 70:157-177, 1976). This finding, which indicates that Na is pumped toward the blood side of the transporting cell, poses a major enigma concerning the role of the Na pump in salt secretion by teleost gills. The present investigation with the elasmobranch rectal gland establishes that this enigma extends to an additional osmoregulatory epithelium responsible for secreting NaCl from blood to SW.

In order to establish the subcellular location of the Na,K-ATPase by means of radiolabeled inhibitor bound specifically to the enzyme, both the luminal and basal-lateral cell surfaces must be exposed. Stadie-Riggs slices (200-500 μm thick) of dogfish rectal gland were used to insure that both the lumens of the secretory tubules and the interstitial spaces would be in communication with the in vitro incubation medium containing ³H-ouabain. Furthermore, for autoradiographic localization to be meaningful, ouabain binding by rectal gland slices must be characterized as to rate, specificity, etc. As shown in Figure 1, the binding rate was markedly reduced by high K

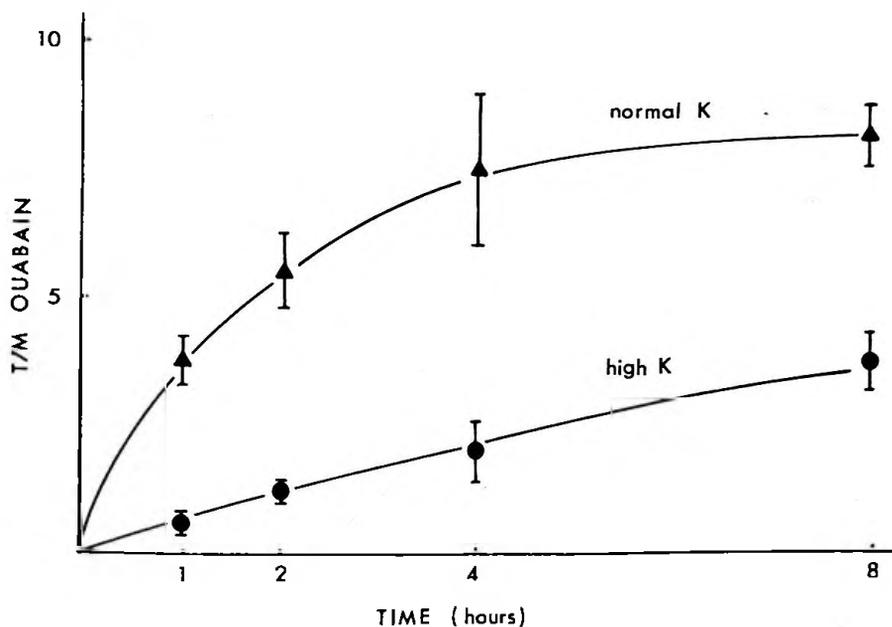


Figure 1. ^3H -ouabain binding (tissue/medium ratios after washing) by slices of *Squalus acanthias* rectal gland. Stadie-Riggs slices of glands from SW maintained dogfish were incubated for times indicated at 18°C in shark Ringer's medium containing $5\ \mu\text{M}$ ^3H -ouabain ($1\ \mu\text{Ci}/\text{ml}$) and either $6\ \text{mM}$ (normal) or $65\ \text{mM}$ potassium (high). Following incubation, slices were washed 30 min in large volumes of ouabain-free Ringer's (same K conc. as incubated with), weighed, digested, and counted along with aliquots of the medium. Ratios shown as mean points \pm SE bars ($n=4$ fish).

concentration, demonstrating specific binding to Na,K-ATPase. Freeze-dry, plastic-section auto-radiographs (Karnaky et al., *J. Cell Biol.*, 70:157-177, 1976) of slices incubated 2-4 h in ^3H -ouabain show binding only by basal infolds and lateral membranes between cells (Figure 2). The absence of ouabain binding at the luminal membrane indicates that the enigma concerning the Na pump in gill chloride cells also pertains to rectal gland cells. Additionally, investigators employing a ouabain-sensitive cytochemical reaction (p-nitrophenyl phosphate hydrolysis), have shown the basal-lateral surface to be the major site of Na,K-ATPase activity in nasal salt glands of both a bird (Ernest. *J. Histochem. Cytochem.*, 20:23-38, 1972) and a lizard (Ellis and Goertemiller, *Anat. Rec.*, 180:285-297, 1974). Thus large quantities of basal-lateral Na,K-ATPase appear to be a general characteristic of salt secretory cells. Future elucidation of the role of basal-lateral Na,K-ATPase should clarify our understanding of the basic mechanism(s) of NaCl transport by secretory cells.

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Figure 2. ^3H -ouabain autoradiograph of dogfish rectal gland (incubated 2 h in medium containing 5 μM radiolabeled ouabain, 60 $\mu\text{Ci/ml}$) showing good morphological preservation of a secretory tubule in cross-section. Many silver grains (black dots) are located over the regions occupied by basal infolds or lateral plasma membranes and essentially no grains over the membrane bounding the tubule lumen (L), the cell nuclei (N), or the mitochondria (hazy gray dots) packed between basal folds. X 2000.

EFFECT OF PARASYMPATHECTOMY ON THE RESPONSE TO HYPOXIA IN *Squalus acanthias*

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Neural control of the cardiovascular system in the dogfish *S. acanthias* is almost completely by way of the vagus nerve (Satchell, *Circulation in Fishes*, Cambridge Univ. Press, 1971; Johansen K, *Ann. Rev. Physiol.*, 1971). The vagus is the efferent pathway of a reflex bradycardia and increased resistance to blood flow across the gills induced by an increase in CO_2 in the sea water perfusing the gills (*Bull. MDIBL* 9:13, 1969). When O_2 tension in sea water is lowered there is also a decrease in heart rate and cardiac output accompanied by an increase in gill resistance (*Bull. MDIBL*, Vol. 15; Kent et al., *Cardiovascular responses to hypoxia in *S. acanthias**, 1975). In the present study the role of the parasympathetic nervous system in the response to hypoxia was assessed.

Thirteen fish of either sex ranging in weight from 1.7 to 7.0 kg were used. They were surgically prepared for measurement of cardiac output (\dot{Q}_B) by an electromagnetic flow probe (18-25 mm circumference, Carolina Medical) placed on the conus arteriosus (*Bull. MDIBL* 8:20, 1968). Catheters were placed in the ventral aorta and dorsal aorta for pressure recording (VAP and DAP) and for drawing venous and arterial blood samples. Pressures and flow were recorded simultaneously on a