1974 #33

INTRACELLULAR IONIC COMPOSITION OF THE RECTAL GLAND OF THE SPINY DOGFISH *Squalus acanthias*

PATRICIO SILVA, FRANKLIN H. EPSTEIN, KATHERINE SPOKES AND SARA EPSTEIN.
DEPARTMENT OF MEDICINE AND THORNDIKE LABORATORY, HARVARD MEDICAL SCHOOL, BETH ISRAEL HOSPITAL, BOSTON, MASSACHUSETTS

The intracellular concentration of sodium, potassium, and chloride in rectal gland tissue has special interest and importance because of the high concentration of sodium in the plasma of elasmobranchs and the even higher concentration in rectal gland secretion. Published results vary widely and it is not clear whether the intracellular ionic composition of the rectal gland resembles or differs greatly from that of other vertebrate organs that are not habitually in contact with hyperosmotic fluids.

A total of 11 male dogfish weighing 1.5 to 2.3 kg were used for these experiments. They were divided into two groups. The five fish in the first group were injected with a single intravenous bolus of 20 μC of ^{14}C-inulin as a marker of extracellular space, and after an interval of eight hours a blood sample was collected from the dorsal aorta and the animals sacrificed. The rectal gland was then extirpated, dissected free of connective tissue, weighed, divided into 100 to 200 mg pieces, and placed in tarred glass vials. (The tarred vials were weighed to determine wet tissue weight.) The vials were then placed in 100°C oven for 24 hours and reweighed to obtain tissue water content. The tissues were then digested in hot concentrated nitric acid in the same glass-stoppered vials, brought up to known volume, and appropriate dilutions made for Na and K measurements. Separate tissue and plasma samples were placed in glass scintillation vials digested with 0.4 ml

TABLE 1

INTRACELLULAR SODIUM, POTASSIUM AND CHLORIDE CONTENT OF THE

RECTAL GLAND OF THE SPINY DOGFISH *Squalus acanthias*

	PLASMA mEq/Liter			INTRACELLULAR mEq/Liter of tissue water			INULIN SPACE
	Na	K	Cl	Na	K	Cl	x 100
Nitric acid digest (5)	269.3±4.9	4.0±0.3		24.8±13.3	156.3±3.5		26.67±1.2
Leaching (6)	274.3±10.0	4.8±0.3	249.9±9.6	17.9±6.0	147.7±9.5	78.9±17.5	

of Nuclear Chicago solubilizer for 24 hours in a 40°C water bath.. Fifteen ml of scintillation fluid were then added, the samples were counted in a constant temperature scintillation spectrometer, and the extracellular space was calculated from these values. Sodium and potassium content of the plasma and nitric acid digests of gland tissue were measured in IL 113 flame photometer. Results are expressed as mEq per Liter of tissue water.

A second group of six dogfish were sacrificed after sampling the plasma. The rectal gland was divided into 100 to 200 mg pieces which were then divided into two groups. Half were placed in tarred glass vials, weighed, desiccated, and reweighed to obtain wet and dry weight for each rectal gland. The other half were weighed, placed in 4 ml of distilled water in glass homogenizing tubes, heated to 100°C in a boiling water bath, homogenized with a teflon pestle, centrifuged, and the supernatant assayed for sodium, potassium, and chloride. The results are expressed as mEq per Liter of tissue water. All values are means ± SD.

The results are shown in Table 1. Both methods of measuring intracellular cations are in reasonably good agreement without significant differences in the values found.

A large difference exists between the sum of intracellular sodium and potassium and that of extracellular cations, implying an unmeasured gap in osmolality of approximately 200 mOsm/L. Presumably another inorganic or organic substance must be accumulated intracellularly in order to satisfy the requirement for osmotic equilibrium. Urea is probably present at the same concentration in intracellular and extracellular water so that the unidentified material is more likely to be trimethylamine oxide or an amino acid. It is especially notable that intracellular potassium does not exceed the value found in mammalian tissues despite the high ionic concentration of shark plasma and rectal gland secretion and that intracellular sodium and chloride are both low, though chloride is higher than sodium. The active step in the transport of sodium chloride from plasma to rectal gland duct is likely to be at the luminal border of the cell rather than at the basal border since diffusion of both sodium and chloride from intracellular fluid into duct fluid against a large chemical gradient seems improbable.

1974 #34

QUANTITATIVE ASPECTS OF THE INHIBITION OF GILL Na-K-ATPase BY INTERNAL AND EXTERNAL OUABAIN IN SEAWATER EELS, *Anguilla rostrata*

PATRICIO SILVA, FRANKLIN H. EPSTEIN, KATHERINE SPOKES, SARA EPSTEIN AND RICHARD SOLOMON. DEPARTMENT OF MEDICINE AND THORNDIKE LABORATORY, HARVARD MEDICAL SCHOOL, BETH ISRAEL HOSPITAL, BOSTON, MASSACHUSETTS

A critical question in the physiology of ion transport by teleost gills is the anatomical position of the sodium pump. Much evidence favors an important role for Na-K-ATPase located in chloride cells in the active extrusion of sodium across the gill in fish adapted to seawater. It is not clear however whether the Na-K-ATPase of gill chloride cells "faces" outward so as to transport Na^+ from the cell to the sea or inward so as to transfer Na^+ from chloride cell cytoplasm to the intracytoplasmic tubules which are believed to be continuous with extracellular fluid. Since ouabain binds to Na-K-ATPase only on that side of the membrane toward which Na^+ is transported, one way to approach the problem is to see whether ouabain inhibits gill Na-K-ATPase when applied from the blood side or the sea side of the gill.

Eels (*Anguilla rostrata*) adapted to seawater for at least three weeks were used. Varying amounts of ouabain were placed in the external aerated