experiments. It is seen in Table 2 that absorption from both water and food did, indeed, occur; and furthermore, the tissue distribution is essentially the same as after injection into the pericardial sinus. As expected, there was quite a range in the tissue values from these modes of administration, but nontheless, the amounts of radioactivity residing in the hepatopancreas from either water or food is more than 95% of the absorbed dose.

1971 #14

THE ROLE OF NA-K-ATPase IN THE RENAL REABSORPTION OF SODIUM IN *Squalus acanthias*

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Previous studies have demonstrated that Na-K-ATPase plays an important role in net sodium transport across several epithelial membranes. Increased specific activity of this enzyme in the gill of lower vertebrates and in the kidney of mammals has been shown to parallel rises in active sodium transport under a variety of experimental and environmental conditions. Conversely, in the dog, inhibition of enzymatic activity in the kidney with ouabain is associated with a marked decrease in the tubular reabsorption of sodium and a natriuresis. In the present experiments the relationship of Na-K-ATPase to renal sodium metabolism was studied in the elasmobranch, since in this unique species the tubular transport of sodium is of lesser importance in osmoregulation.

Studies were performed in unanesthetized dogfish, maintained at 13°C in running seawater. All injections were administered through an intraaortic catheter with the tip placed proximal to the kidneys. Measurements of renal function were performed during control periods lasting 2 to 16 hours, and for four hours following the injection of ouabain.

In preliminary experiments it was determined that a dose of ouabain exceeding 75 μg/kg resulted in death within 4 hours. Attempts to increase the amount of this agent reaching renal tissue, by infusing solutions into the tail vein, did not alter these results. In 9 normal fish the renal specific activity of Na-K-ATPase was 7.30 ± 0.34 (mean ± S.E.) and of Mg-ATPase was 17.29 ± 3.53 Pi μM/mg protein per hour. One hour following the injection of ouabain, in a dose which varied from 100 to 250 μg/kg, the mean Na-K-ATPase level was 2.81 ± 0.18 (a reduction of 61.6 ± 2.5 per cent) and the Mg/ATPase level was 17.29 ± 3.5 Pi μM/mg protein per hour. There was no apparent relationship between dose and the degree of enzyme inhibition.

In 4 fish measurements of GFR, urine volume, sodium and potassium excretion were made before and during four hours following the injection of ouabain (average dose - 57 μg/kg). The specific activity of Na-K-ATPase was measured at the termination of the experiment in each fish and compared to control fish studied on the same day. As shown in Table 1 the level of Na-K-ATPase was reduced to a mean of 64.0 ± 9.3 per cent in the experimental fish, a value similar to that obtained after giving larger doses. Ouabain caused a slight increase in GFR which rose from 1.26 ± 0.20 to 1.89 ± 0.16 ml/kg · hr and in V which rose from 0.52 ± 0.10 to 0.64 ± 0.05 ml/kg · hr. Both the fraction and

TABLE I

Effect of ouabain on renal function and Na-K-ATPase levels

	CONTROL					EXPERIMENTAL					CONTROL		EXPERIMENTAL	
Experiment	GFR[+]	V[+]	Fractional Excretion Na	Fractional Excretion K	$U_{Na}V$ µEq/hr	GFR	V	Fractional Excretion Na	Fractional Excretion K	$U_{Na}V$ µEq/hr	Na-K-ATPase Pi µM/mg protein/hr	Mg-ATPase Pi µM/mg protein/hr	Na-K-ATPase Pi µM/mg protein/hr	Mg-ATPase Pi µM/mg protein/hr
13	0.93	0.29	0.32	2.80	309.8	1.7	0.55	0.25	4.48	271.2	7.72	10.30	2.33 (70.0%)	18.10
19	0.82	0.58	0.45	8.16	600.6	1.48	0.67	0.40	9.00	581.8	6.44	18.73	1.02 (84.2%)	20.29
23	1.8	0.82	0.52	5.89	849.6	2.2	0.79	0.29	6.74	612.1	8.74	23.15	3.64 (69.0%)	24.24
25	1.5	0.39	0.28	11.11	504.0	2.2	0.53	0.19	13.09	498.4	7.05	16.00	4.75 (33.7%)	14.00
Mean ± S.E.	1.26 ± 0.20	0.52 ± 0.10	0.39 ± 0.05	6.99 ± 1.52	566.0 ± 97.2	1.89 ± 0.16	0.64 ± 0.05	0.28 ± 0.04	8.33 ± 1.59	490.9 ± 66.7	7.49 ± 0.43	17.05 ± 2.33	2.93 ± 0.69	19.16 ± 1.85
P value						<0.05	<0.05	<0.05	<0.05	N.S.				

[+] vales for GFR and V are expressed as ml/kg · hr; number in parenthesis is the per cent reduction in specific activity of Na-K-ATPase compared to control.

absolute excretion of sodium fell, however, despite marked inhibition of Na-K-ATPase, while fractional excretion of potassium rose. The failure of ouabain to cause a natriuresis was not due to an inability of tubular epithelium cells to respond to a blocking agent since under similar experimental conditions ethacrynic acid and furosemide (Bull. MDIBL 10:56, 1970) caused a marked natriuresis. The action of these diuretic agents apparently was not associated with inhibition of Na-K-ATPase since 4 hours following the administration of furosemide (40 mg) to 2 fish, the specific activity of the enzyme (7.21 Pi μM/mg protein per hour) was unchanged from control values.

In dogs a marked natriuresis is caused by inhibition of the specific activity of Na-K-ATPase to levels one-third or less than control values (Amer. J. Physiol., in press). The lack of an effect in *S. Acanthias*, despite a similar reduction in specific activity suggests either a greater excess of Na-K-ATPase in the shark kidney, so that this degree of inhibition is insufficient to produce detectable changes in net sodium transport or that the ouabain sensitive sodium pump is relatively unimportant in net reabsorption of sodium chloride. Whittembury (Pflugers Arch. 307:138, 1969) has postulated two separate pumps in tubular epithelial cells; 1) mode A is electrogenic, ouabain insensitive and is important in net NaCl efflux, while 2) mode B is sensitive to ouabain and is the principal factor involved in the reciprocal transfer of sodium and potassium ions. Our data suggest that in the marine elasmobranch in which osmoregulation is not dependent upon sodium conservation by the kidney, the renal specific activity of Na-K-ATPase does not play an important role in sodium reabsorption.

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EFFECT OF CALCITONIN ON SODIUM METABOLISM IN *Squalus acanthias* AND *Anguilla Rostrata*

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Calcitonin is present in most primitive (elasmobranchs) and higher (birds and mammals) vertebrates possessing ultimobrachial tissue. In mammals, in addition to acting on bone to inhibit osteolysis, this hormone causes a reduction in the renal tubular reabsorption of sodium, calcium and phosphate. In the present experiments the effect of calcitonin on renal function was examined in *Squalus acanthias*, which possesses no bony skeleton, in order to determine its possible importance in volume homeostasis. The influence of calcitonin on gill transport of Na^+ in *Anguilla rostrata* was also studied since a hypocalcemic effect of the hormone has been demonstrated in this species.

Studies of renal function were performed in 6 dogfish sharks *(S. acanthias)*, maintained in running seawater at 13°C. All injections were administered through an intraaortic catheter with the tip placed cepholad to the kidneys. Following control measurements, observations were continued during periods 1 (5 hours), 2 (10 hours), and 3 (10 hours). Salmon calcitonin (4.4 MRC units/kg) was injected at the beginning of each period in 4 fish and 1 percent gelatin in the remaining 2 animals.