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.

This study was supported by USPHS grants TIAM 5015 and HE 13647-01A1 and the American Heart Association.

1971 #15

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

John P. Hayslett, Mark Epstein, David Spector, Jack D. Myers, H. Victor Murdaugh and Franklin H. Epstein; Yale University School of Medicine, New Haven, Connecticut, and University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania

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.

TABLE I

Effect of calcitonin on renal tubular function in S. acanthias+

CONTROL				CALCITONIN											
				Period 1				Period 2				Period 3			
GFR‡	V‡	Fractional Excretion		GFR	V	Fractional Excretion		GFR	V	Fractional Excretion		GFR	V	Fractional Excretion	
		Na	K			Na	K			Na	K			Na	K
1.43	0.45	0.33	9.14	1.55	0.43	0.37	8.24	1.78	0.47	0.21	6.90	1.77	0.41	0.19	6.45
±0.19 (4)	±0.02 (4)	±0.04	±2.21	±0.24 (4)	±0.06 (4)	±0.12	±2.29	±0.12 (4)	±0.07 (4)	±0.02	±2.82	±0.30 (4)	±0.08 (4)	±0.02	±2.49
P value				NS	NS	NS	<0.05	<0.05	NS	<0.05	<0.05	NS	NS	<0.05	<0.05

CONTROL				GELATIN 1%											
				Period 1				Period 2				Period 3			
GFR	V	Fractional Excretion		GFR	V	Fractional Excretion		GFR	V	Fractional Excretion		GFR	V	Fractional Excretion	
		Na	K			Na	K			Na	K			Na	K
1.49	0.47	0.32	0.77	1.17	0.33	0.29	2.61	1.45	0.33	0.27	2.06	1.45	0.33	0.27	2.06
±0.03 (2)	±0.16 (2)	±0.08	±0.38	±0.10 (2)	±0.09 (2)	±0.02	±1.05	(1)	(1)			(1)	(1)		
P value				NS	NS	NS	NS								

+ values expressed as mean ± S.E.

‡ values for GFR and V expressed as ml/kg. hr.

value in parenthesis represents number of animals studied in each period

P represents comparison of each experimental period with control period

There was no effect of calcitonin on the level of serum calcium. The control value of 15.3 ± 1.2 (mean ± S.E.) mg per cent was not different from the value at the end of each experimental period. Parameters of renal function measured in these studies are shown in Table 1. Injections of calcitonin did not alter GFR, urine volume (V) or the fractional excretion of calcium or urea, which average 0.58 ± 0.10 and 0.07 ± 0.01, respectively, during control periods. During the last two experimental periods the fractional excretion of sodium fell slightly, while the excretion of potassium was reduced in all 3 periods.

Since cortisol has been shown to enhance sodium outflux across the gill of intact eels, calcitonin was administered intraperitoneally (24-40 MRC units/kg) to 3 groups of eels. The Na^{22} outflux was 56 (freshwater adapted), 36 (transfer to seawater for 24 hours) and 515 μEq/100 gm . hr (seawater adapted). These fluxes were similar to values measured in normal eels.

These studies show that, in contrast to the mammal, calcitonin has no effect on tubular function in the elasmobranch and no influence on gill transport in a teleost, in which an effect on calcium metabolism is demonstrable. There is no evidence, therefore, that this hormone is important as an osmoregulator in these lower vertebrates.

This study was supported by USPHS grants TIAM 5015, HE 13647-01A1 and the American Heart Association.

1971 #16

EFFECT OF THREE NEW ANTITUMOR AGENTS ON DEVELOPING EMBRYOS OF THE SAND DOLLAR, *Echinarachnius parma*

F.J. Hendler and R.H. Adamson; University of Chicago, Chicago, Illinois and National Cancer Institute, Bethesda, Maryland

Gallium nitrate, rifamycin SV, and camptothecin are three drugs of diverse structure which have recently been reported to have antitumor activity in experimental animals. As the mechanism of antitumor activity of these agents is unknown, we have studied the effects of these drugs on early embryologic development in the sand dollar, *Echinarachnius parma*.

Camptothecin was dissolved in millipore filtered sea water (MFSW) while rifamycin SV and gallium nitrate were dissolved in distilled water. Fertilized ova were added to MFSW containing drug concentrations ranging from 0.1 to 100 μg/ml three minutes after fertilization. Embryos were observed at 2, 5, 12, and 24 hours. Gallium nitrate had no effect on development in the time period studied; rifamycin SV had no effect on the first cell division at all concentrations. At 100 μg/ml rifamycin SV further cell division did not occur, whereas at all other concentrations development through the first 24 hours proceeded normally. Camptothecin inhibited the first cell division at concentrations greater than 0.1 μg/ml; at concentrations of 1 - 100 μg/ml the cells eventually divided and reached blastula but developed no further. In addition the number of minutes which cleavage was delayed appeared to be dose related.

The ability of the embryos to overcome an initial effect on cleavage and then to develop only to blastula resembled the effects seen in the sand dollar embryo with cytosine arabinoside (Exptl Cell