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LACK OF EFFECT OF ALDOSTERONE¹ ON THE RECTAL GLAND OF THE SPINY DOGFISH, Squalus acanthias

J. W. Burger,² Trinity College, Hartford, Conn.

The mechanism whereby the rectal gland is stimulated to secrete is unknown, but apparently is not nervous. Single intra-arterial doses of 200 and 800 micrograms of aldosterone in a 5 kg dogfish did not stimulate a rectal gland secreting at a low rate, and did not inhibit a rectal gland secreting at a high rate. Injected sodium chloride, however, produced a characteristic increase in secretion, showing that failure of the gland to respond to aldosterone was not due to a debility within the gland itself.

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CERTAIN ASPECTS OF THE EXCRETION OF PHENOL RED, INULIN Mg^{++} AND Ca^{++} BY THE HAGFISH, M. glutinosa*

J. W. Burger and D. P. Rall, Trinity College, Hartford, Conn., and National Cancer Institute, Bethesda, Md.

Little is known concerning excretory patterns of the primitive marine cyclostome M. glutinosa. We have studied the excretion of inulin, phenol red, Mg^{++} and Ca^{++} into the urine and, of the last 3 ions, into the bile of the hagfish. The results clearly indicate that the bile may be an important excretory pathway in this animal.

MATERIALS AND METHODS

Hagfish were caught in Frenchmen Bay in approximately 200 ft. of water in traps. They were then transferred to aquaria with rapidly running sea water (temp. 13-15°C) and used as soon as possible.

The fish were anesthetized with Tricaine[®] (MS222) 2 g/liter in sea water, and the following samples taken in rapid sequence before the animal was sacrificed. A ventral abdominal incision was first made and the gut was ligated at the cloaca. Urine was obtained by aspiration from the cloaca after gentle stripping of both ureteral ducts. Blood was obtained by direct puncture, with a sharp needle and heparinized syringe, of the dorsal aorta. With patience, 2.5 ml of hemolysis free blood could be obtained from 100 gm fish. Bile was taken by puncture of the gall bladder.

Phenol red in sea water (2-4 mgm in 1 ml) was given subcutaneously. Phenol red in urine, bile, and plasma was estimated by a spectrophotometer or by direct comparison of standards of known concentration. Tris buffer, pH 8.4, was used to dilute all samples for the spectrophotometer. NH_4OH vapors were blown across the surface of certain samples which were too small and

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dilute to allow further dilution to achieve alkalinization. Appropriate plasma, urine, and bile blanks and standards were utilized. Tracer quantities (2-4 μc) of $^{14}\text{COOH}$ Inulin (New England Nuclear) were given subcutaneously. Inulin ^{14}C was assayed in a liquid scintillation counter. Plasma and urine samples of 10-100 μl were added directly to Gjones solution for counting. Ca^{++} and Mg^{++} were measured as previously reported. Plasma ultrafiltrates were obtained by gentle centrifugation across a Visking dialysing tubing.

RESULTS

Inulin - Results of 8 fish given Inulin 24-48 hours prior to sampling are given in Table 1.

Table 1
C/M/ml INULIN (X1000)

Fish #	Plasma	Urine	U/P
1	62	50	0.81
2	62	59	0.95
3	63	20	0.32
4	40	36	0.90
5	46	30	0.65
6	76	38	0.50
7	93	126	1.35
8	69	67	0.97
			Mean = 0.85

It is clear that the U/P ratios, with one exception, were below 1, and therefore, hagfish urine is reminiscent of mammalian glomerular filtrate.

Phenol Red - The results showing the excretory patterns of phenol red are in Table 2.

Table 2
PHENOL RED (gm/ml) CONCENTRATION

Fish #	Plasma	Urine	Bile (mgm/ml)	U/P
10	42	22	26.	-
11	48 (9.6)	8	2.7	0.8
12	48 (9.6)	8	3.0	0.8
13	54 (5.3)	9	2.2	1.7
14	12 (4.8)	2	3.0	0.5
15	1.5 (0.6)	1	1.6	1.7
16	6.8	1	0.6	-
17	40	6	2.6	-
18	1 (0.3)	0.1	0.7	0.3
				Mean = 0.98

Figures in parenthesis are the ultrafilterable fractions as determined by estimating the percentage ultrafilterable in each plasma sample and multiplying this by the plasma concentration. Phenol red given 24-72 hours before sampling.

The mean U/P ratio (using the estimated ultrafilterable phenol red concentration) was 0.98, and this provides no evidence for secretion of phenol red by this kidney and provides an interesting parallel to the observations concerning U/P ratio for Inulin. The concentration of phenol red in the bile varied from 0.7-26 mg/ml while the plasma concentration varied from 0.001-0.054 mg/ml. In fact, often about half the injected dose could be found in the gall bladder bile.

Magnesium and Calcium - These results are in Table 3.

Table 3

Fish #	Mg ⁺⁺ (mM/L)			Ca ⁺⁺ (mM/L)		
	Plasma	Urine	Bile	Plasma	Urine	Bile
1	10.	-	120.	3.8	-	46.
2	8	-	55.	4.5	-	39.
3	16.	12.	98.	3.3	1.2	26.
4	32.	-	83.	4.9	-	34
5	12.	14.	76.	1.7	0	40.
6	44	30	75	1.2	2.5	32
7	17.	-	116	10.	-	0.8
8	24.	-	125	4.9	-	29.
9	36	20	-	2.5	0	-
10	14.	17	-	4.6	7.5	-
11	31	-	105	8.3	-	41

Fish 1 and 2 were sample 3 hours after capture, mean Pl Mg = 9.

Fish 3-5 were sampled 7 days after capture, mean Pl Mg = 20.

Fish 6-11 were sampled 12 days after capture, mean Pl Mg = 27.

It is clear that the mean U/P ratio for Mg⁺⁺ in the 4 fish studied was essentially 1.

The plasma Mg⁺⁺ concentration appeared to increase as the animals were kept for longer periods of time. The concentration of Mg⁺⁺ (and Ca⁺⁺) in the bile is striking.

DISCUSSION AND CONCLUSIONS

These observations that the U/P ratios for inulin, phenol red and Mg⁺⁺ in the hagfish all approximate unity, strongly suggest that there is little tubular function in this cyclostome. The hagfish may be considered as having an atubular kidney in respect to a variety of substances. The main excretory pathways for organic acids and divalent cations may be via the liver and the bile.