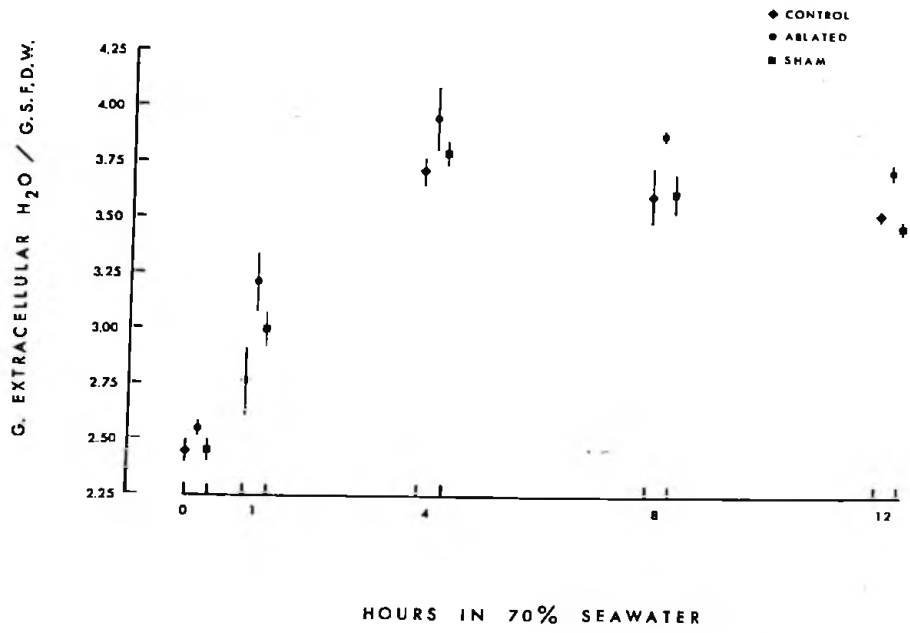

Figure 2. Mean extracellular water (grams/gram solute free dry weight) ± S.E. during acclimation to 70% seawater. n as in Table 1.

ACIDIFICATION OF THE URINE BY THE URINARY BLADDER OF THE FEMALE LITTLE SKATE, RAJA ERINACEA

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We report here the results of preliminary experiments to determine the function of the urinary bladder of the mature female skate. Our results show that the bladder participates in acidification of the urine.

Although the morphology of the female skate urinary bladder is well described in both the older (Borcea, Arch. Sool. Exp. et Gen. 4: 199-484, 1906) and recent (Lacy et al., Bull. Mt. Des. Is. Biol. Lab. 15: 56-58, 1975) literature, no published values for the ionic composition of skate bladder urine exist. Shown in Table 1 are the results of ion analyses performed on serum and bladder urine obtained from mature female skates (800-1300 g)

Table 1. Composition of serum, collecting duct urine and bladder urine in the little skate, Raja erinacea. Mean values ± S.E.; number below mean value is n.
All values, except pH, are in mmoles/l

	pH	Na^+	K^+	Cl^-	Mg^{2+}	Ca^+	Urea
Serum	7.46(0.05) 5	253(4) 18	4.5(0.2) 18	285(15) 15	1.9(0.3) 9	5.6(0.4) 11	347(5) 3
Collecting Duct Urine*	--	180(16) 23	31.6(8.8) 23	209(16) 9	154(29) 9	10.4(1.6) 9	--
Bladder Urine	5.01(0.07) 12	187(20) 19	110(18) 17	286(27) 15	238(53) 3	11.6(1.8) 12	40.0(14.4) 3

*Data from Stolte, et al., (J. Exp. Zool. 199: 403-410, 1977).

anesthetized with MS 222. Also shown are comparable values for skate urine obtained by micropuncture from the end of the renal collecting duct (Stolte et al., J. Exp. Zool., 199: 403-410, 1977). The most striking difference between collecting duct and bladder urine is the greatly increased K^+ concentration in bladder urine. Both Na^+ and K^+ concentrations were quite variable in bladder urine; Na^+ ranged from 103-348 mmole/l and K^+ from 17.6-210 mmole/l. There was no significant correlation between Na^+ and K^+ concentrations.

Because skate bladder urine has a six-fold higher H^+ concentration than the urine of the spiny dogfish, which has no bladder, Lacy et al., (1975) have suggested that the skate bladder functions in acidification of the urine. A test of this hypothesis was conducted as follows: female skates were anesthetized with MS 222 (0.25 g/l sea water) and maintained in a closed-circuit gill superfusion apparatus with MS 222 at 0.01-0.05 g/l sea water. The peritoneal cavity and urogenital sinus were opened by incision and a catheter was sutured in place in the opening of the urethra. The bladder was drained and rinsed three times with 3 ml of Forster's skate Ringer (FSR) and 3 ml of FSR were left in the bladder. At time zero and at 2h, 1 ml of FSR was removed from the bladder. In a second series of experiments the skates were similarly prepared, but, in addition, the ureters leading into the bladder from the kidneys were cut. The results of these experiments are shown in Table 2. When the ureters are intact the pH of the FSR in the bladder

Table 2. Ionic composition Mmoles/l of Forster's skate Ringer as a function of residence time in the bladder. Mean values ± S.E.

	pH	Na^+	K^+	Cl^-	Mg^{2+}	Ca^{2+}
Ureters Intact						
n = 3						
T = 0	7.34(1.10)	274(7)	7.0(0.5)	314(6)	7.6(1.6)	3.8(0.6)
T = 2h	6.11(0.20)	235(16)	39.8(10.0)	314(21)	55.6(21.9)	36 (0.6)
Ureters Cut						
n = 3						
T = 0	7.48(0.03)	288(1)	6.8(0.1)	345(27)	3.8(0.1)	4.5(0.2)
T = 2h	7.33(0.03)	296(9)	7.2(0.9)	360(30)	3.9(0.3)	4.8(0.8)

rapidly drops. However, when the ureters are cut the rate of acidification is much slower, indicating that newly formed, acidic urine entering the bladder from the ureters is responsible for the greater part of the observed drop in pH. In addition, in skates with intact ureters the concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} in the FSR held in the bladder for 2 h approach values typical of those in bladder urine (Table 1), while the ionic composition of FSR in skates with cut ureters was nearly unchanged. The pH of the FSR in these skates did show a significant decrease over a 2 h period. FSR was titrated with 0.01N HCl and the results were used to calculate the amount of H^+ added to the bladders of the skates with cut ureters; the value is 0.37 ± 0.04 (s.e.) μmale H^+/h. Thus, these preliminary experiments show that the skate bladder can acidify the urine.

Lacy et al (1975) also reported that measurements of carbonic anhydrase activity in skate bladder showed small amounts of activity. Using a modified assay system (Maren, J. Pharm. & Exp. Therap. 130: 26, 1960; 1 ml reaction vessel and barbital buffer) we have again found small amounts of activity; bladder tissue had about 25 enzyme units (4 determinations, tissue perfused with FSR via vasculature to remove blood). Skate red blood corpuscles (RBC) showed about 6500 units of activity/ml packed cells in this assay system. Further, the bladder carbonic anhydrase was only slightly inhibited by 0.8 M NaCl, while the skate RBC activity was strongly inhibited by 0.15 M NaCl (I_{50} = 0.15 M), indicating that the bladder tissue activity is not due to contamination of the tissue with RBC.

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