meq/L of Na inserted into bladders of 3 fish was reduced to 28.8, 4.5 and 23.3 meq/L when their respective plasma concentrations were 177.9, 178.9 and 184.4 meq/L. The original 5 ml volumes of ureteral urine inserted were reduced to 2.25, 3.2 and 3.25 ml respectively. Chloride concentrations were reduced slightly in each instance, and Ca and Mg concentrations rose significantly in all three. The sea raven is poorly suited for this experimental preparation because it is difficult to catheterize the very short and relatively inaccessible ureters in these animals. Two of the five preparations were technically faulty. These preliminary substitution experiments will be repeated on the goosefish where the ureters are very long and can be easily catheterized and exteriorized to by-pass the bladder in which substituted urine is being modified.

These experiments suggest that ionic regulation by gut, kidneys, and gills in stenohaline teleosts is assisted by the active extraction of Na from bladder urine, coupled with a solute-linked extraction of fluid. Marine teleosts maintain water balance in their strongly hyperosmotic environment by drinking seawater, absorbing salt and water in the gut, and then excreting divalent ions preferentially from plasma through the kidney and univalent ions through the gills. Now it appears the so-called "chloride-free" urine that traditionally was thought to be characteristic of freshly captured "non-diuretic" marine fishes is residual bladder urine from which NaCl and bulk fluid have been removed by bladder epithelium. The "laboratory diuresis" characteristic of marine fish following capture is due in part to the artifactual collection of urine exposed relatively briefly to the bladder epithelium as well as resulting from the demonstrably higher drinking rate that marine teleosts exhibit following handling. The presence of considerable Na and Cl, in addition to the actively secreted divalent ions, is characteristic of "ureteral urine" even in that of the aglomerular marine fishes. A salt-saving operation by bladder epithelium makes sense for frogs, toads, and turtles with respect to its adaptive value for an aquatic animal in a dilute environment and also in the euryhaline flounder or toadfish. However in stenohaline teleosts restricted to a strongly hypertonic saline environment, it could have no value as an osmoregulatory device unless it were coupled with a highly selective solute extrusion function capable of generating free water such as that performed by the branchial epithelium of the marine teleosts. The latter is well documented and it appears that the urinary bladder epithelium of these forms which is merely an expansion of the primitive archinephric duct, and therefore embryologically of the same origin is the kidney itself, is well suited to carry on the initial solute-linked isosmotic transport process that presumably results eventually in the generation of free water at the gills.

This work was supported by USPHS Grant HL-04457.

1972 #18

THE ROLE OF INTESTINAL BACTERIA IN UREA METABOLISM BY THE SKATE, *Raja erinacea*

Leon Goldstein and Susan Dewitt-Harley, Brown University, Providence, Rhode Island

This study was undertaken to determine whether urea degradation by intestinal bacteria plays a significant role in the metabolism of urea in elasmobranchs and whether this process contributes

to the reduction in urea concentration that occurs in these fish during environmental dilution. First we assessed the effects of reduction in intestinal urease activity on plasma urea concentration and rates of urea excretion in skates maintained in 100 percent and 50 percent seawater.

Skates averaging 600 g were bled and then placed in 3L of aerated 100 percent seawater or 50 percent seawater for one hour to determine urea excretion rates. The fish were then given Neomycin (250 mg/kg x day) for two days by stomach tube. On the third day Neomycin was administered along with a bacterial urease inhibitor, acetohydroxamic acid (250 mg/kg). One hour later the fish were injected i.m. with a similar dose of acetohydroxamic acid and placed in 3 L 100 percent or 50 percent seawater. Urea excretion rates were determined and blood was withdrawn for urea analyses at the end of the experiment. The effectiveness of the antibiotic treatment in reducing intestinal urease activity was tested by assaying intestinal fluid from control and treated fish for urea splitting activity. Samples were incubated at 18°-20°C for four hours in elasmobranch saline-urea solution (Comp. Biochem. Physiol., 42A, 3-12, 1972) and assayed for ammonia concentration (urease activity). Ammonia production by samples of intestinal fluid from treated fish averaged less than one percent of those from untreated fish, indicating the effectiveness of the antibiotic treatment in reducing intestinal urease activity.

TABLE 1

EFFECT OF NEOMYCIN & ACETOHYDROXAMIC ACID
ON UREA METABOLISM IN *Raja erinacea*

Medium (no. fish)	Plasma Urea (μmoles/ml)			Urea Excretion (μmoles/kg x hr)		
	Before*	After	Δ	Before	After	Δ
Seawater 100% (6)	353±19.5	367±12.9	14±22	347±46	286±30	-61±28
Seawater 50% (6)	204±11.5	219±6.0	15±6	190±48	254±45	+64±60

* Values are means ± S.E. before and after treatment of the fish with Neomycin and Acetohydroxamic acid.

As shown in Table 1 antibiotic treatment (and therefore reduction in intestinal urease activity) had no significant effect on urea metabolism in skates maintained in 100 percent seawater. Plasma urea concentration and urea excretion rates were similar in treated and untreated fish. These results are consistent with those obtained in an earlier study by Lloyd and Goldstein (The Bulletin, 9, 22-23, 1969) who estimated that the rate of urea degradation by intestinal bacteria in the dogfish *(Squalus acanthias)* was less than one percent of the total urea turnover (excretion) in this fish.

Table 1 also shows that antibiotic treatment had no significant effect on the reduction in plasma urea concentration following environmental dilution. There was a tendency for urea excretion rates to be higher in treated fish than in controls adapted to 50 percent seawater, but there

was a great deal of individual variation and the differences before and after antibiotic treatment were not significant ($P > .05$). Thus intestinal bacteria do not appear to play a significant role in the adaptation of urea metabolism to environmental dilution.

This work was supported by NSF grant GB 30357X.

1972 #19

OBSERVATIONS ON THE EFFECT OF DDT IN HERMIT CRABS

W.C. Grant, Jr., Williams College, Williamstown, Massachusetts

Studies by Gaurino *et al.*, 1971 (Bull. MDIBL 11: 29-31) and Kinter *et al.*, 1972 (Environ. Health Perspec. 169-173) have shown that lobsters and selected teleosts concentrate DDT directly from seawater. Similar studies were conducted on hermit crabs in order to judge their possible use as indicator organisms of pesticide contamination. Animals of two species, *P. acadianus* and *P. pubescens* were placed for six hours in 750 ml of aerated seawater at 10-11°C containing 1.0 ppm, 0.5 ppm and 0.1 ppm DDT (Nutritional Biochemicals Corp.) made up in a 0.2 percent solution of ethanol in seawater. The number of animals surviving at six, 12, 36, and 72 hours following initial exposure was recorded. Crabs surviving for 12 hours, which had been previously evicted from their domicile shells, were placed in the center of a water table containing two empty mollusc shells of appropriate size. The time taken for an individual to orient to one of the shells and to effect entry was noted. The results of these experiments are shown in Tables 1 and 2.

TABLE 1

Differential survivorship of hermit crabs exposed to varying levels of DDT contamination in seawater for six hours. *Average crab weight in mgms. per ml. of water.

Species	DDT Concentration	Total number of crabs	*mg/ml	Percent surviving in hours 6	12	36	72
P. acadianus	1.0 ppm	15	9.2	40	0		
	0.5 ppm	15	8.8	87.7	37.3	0	
	0.1 ppm	15	9.0	100	87.7	46.6	46.6
	controls (seawater)	10	8.7	100	93.4	93.4	93.4
P. pubescens	1.0 ppm	10	3.8	0			
	0.5 ppm	10	4.6	80.0	20.0	10.0	10.0
	0.1 ppm	10	3.6	100	80.0	60.0	60.0
	controls (sea water)	10	3.9	100	100	100	100