Pharmacologic doses of ouabain (2.5×10^{-4} M) or furosemide (5×10^{-4} M) were added to the recirculating perfusion fluid after 30 minutes of control perfusion. Rectal gland fluid collections were made for 15 minutes prior to giving the drug (control period) and for two consecutive 15-minute periods thereafter (experimental period I and II). No significant effect on sodium or potassium concentration in gland fluid, gland fluid flow rate or rate of sodium excretion resulted from the addition of either ouabain or furosemide (Table 2).

The results of both the in vivo and in vitro studies indicate that the active secretion of sodium is not inhibited by ouabain in concentrations which are known to be inhibitory in other marine organs to active sodium transport. Inhibition of Na-K-ATPase activity with ouabain was not demonstrated in vivo. This may have resulted from insufficient amounts of ouabain reaching the rectal gland. Therefore a dose of ouabain sufficient to inhibit 100 percent of Na-K-ATPase activity in homogenized rectal gland was given to the perfused rectal gland. The failure of ouabain to result in inhibition of sodium transport in the rectal gland is interesting in view of the fact that ouabain has no effect on urinary excretion of sodium in this species. The rectal gland also appears to be insensitive to furosemide both in vivo and in vitro.

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ALTERATIONS IN RENAL CARBONIC ANHYDRASE IN RESPONSE TO SALINITY IN THE EURYHALINE EEL, *Anguilla rostrata*

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The absence of renal carbonic anhydrase activity appears to be a general property of marine fish, while its presence is characteristic of freshwater

fish and the other vertebrate phyla (Fed. Proc. 26, 1097, 1967). This conclusion has been based largely on the observation that carbonic anhydrase inhibitors raise the urinary pH of freshwater fish, (as for virtually all vertebrates) while they have no effect on the urine pH of marine fish (Hodler, Heinemann, Fishman and Smith, Amer. J. Physiol. 183: 155, 1955; Rawls and Maren, Bull. MDIBL 4: 57, 1962). If such a distinction is a fundamental difference in the renal function of these two groups and represents an adaptation to the two different environments then a euryhaline fish might be expected to show either character depending upon the environment to which it is acclimated.

To establish the presence or absence of carbonic anhydrase in the renal tubules of a euryhaline fish we studied the kidneys of the American eel, *Anguilla rostrata*. The eels, weighing 200-300 g, were caught in brackish water by baited traps. Immediately after capture they were placed either in freshwater or seawater aquaria. The animals were acclimated to each environment at least three weeks prior to experimentation. Water temperature was constant at 10°C.

Direct assay of renal carbonic anhydrase was performed on freshly caught, brackish water eels as well as animals acclimated to freshwater and seawater. The animals were decapitated and the kidneys were removed and prepared for assay by homogenization. The homogenate was diluted 1:50 with distilled water. The micro-method of Maren (J. PHarm. Exp. Therap. 130: 26, 1960), altered to use a barbital buffer, was employed to measure carbonic anhydrase activity. In some cases the kidneys were perfused with fish Ringer's solution through the renal arteries and renal portal veins to remove excess blood. The non-perfused kidneys of all three types of eels contained 7-19 units of carbonic anhydrase per gram of kidney tissue. Blood contained 70-190 units

per gram. The benzidine method was then used to estimate the blood content of the kidneys, and showed that 10 percent of the kidney tissue was blood. This could be interpreted to mean that all of the enzyme activity in the renal tissue was due to the presence of blood; however, substantial individual variability and the comparatively high enzyme concentration in the blood could have left a small amount of renal enzyme undetected. Perfused kidneys, in which the blood concentration was reduced to 1 percent, showed an enzyme activity of four to five units per gram of tissue. Thus it appeared that some carbonic anhydrase activity was present in renal tissue; this was true regardless of the medium to which the eels were acclimated. The lack of a significant difference in enzyme activity among the three groups could be due to the fact that in fish hemopoietic tissue is interspersed with renal tubules and the late stages of immature red blood cells may contain carbonic anhydrase. Thus variations in true renal enzyme could readily be obscured. Additionally these studies were performed at the lower limit of sensitivity of the analytical method for this enzyme, making it unlikely that the problem could be solved in this fashion. Therefore we turned to pharmacological experiments and used the renal response to methazolamide as an indicator for the presence of carbonic anhydrase in the kidneys. By inhibiting the enzyme, methazolamide reduces bicarbonate reabsorption. Bicarbonate reabsorption is in part dependent upon the rate at which the kidney proximal tubules can produce H^+ to react with bicarbonate in the glomerular filtrate.

The bladders of freshwater and seawater eels were catheterized while the eels were sedated by partial immersion in water containing MS-222. Each animal was placed inside a container made of rubberized webbing that afforded the fish adequate contact with the water but restricted its movement. Twenty-four hours were allowed for recovery. Small balloons attached to the end of

TABLE 1

THE EFFECT OF METHAZOLAMIDE ON RENAL BICARBONATE EXCRETION, IN *Anguilla rostrata* ACCLIMATED TO FRESHWATER AND SEAWATER

	pH	Total CO_2 (mM)	Flow ml/hr	HCO_3^- Excretion (mequiv/kg/hr)
		Freshwater-acclimated		
Control (n = 6)	7.51±0.25	8.9±4.5	0.18±0.10	8.9±3.20
Methazolamide (n = 2)	7.90	16.1	0.29	24.2
Acid-loaded (n = 6)	6.91±0.29	1.2±0.9	0.17±0.12	1.1±0.82
Acid-loaded + Methazolamide (n = 4)	7.45±0.18	4.4±2.1	0.29±0.21	5.7±1.80
		Seawater-acclimated		
Control (n = 4)	7.86±0.53	11.7±2.3	0.05±.004	2.7±2.1
Acid-loaded (n = 6)	6.66±0.38	2.5±2.5	0.04±.003	0.50±0.46
Acid-loaded + Methazolamide (n = 4)	6.98±0.48	2.7±3.0	0.03±.03	0.47±0.62

All values are mean ± standard error. The dosage of methazolamide was 30 mg/kg body weight. The acid-loaded animals received 1 mequiv of HCl per kg body weight, intraperitoneally.

the catheters were used to collect urine. Samples were analyzed for total CO_2 (micromanometrically), pH, and volume. Collection periods varied from six to 48 hours. Methazolamide was given intramuscularly at a dosage of 30 mg/kg. Blood samples were withdrawn from the caudal vein. Because of variation in the pH of control urine and initial alkalinity, especially in the seawater-acclimated group, the effect of methazolamide was not distinct. In control seawater-acclimated eels, urine pH varied from 6.72 to 8.63. This was similar to the range seen in the seawater-acclimated southern flounder (Hickman and Trump, in Fish Physiology, vol. 1, eds. W.S. Hoar and D.J. Randal, Academic Press, 1969). Therefore we stabilized control urine pH in both fresh and seawater experiments by injecting HCl intraperitoneally (1 mequiv. per kg body weight) and subsequently observed the effect of the drug.

The data show a substantial effect of carbonic anhydrase inhibition in freshwater-acclimated fish (Table 1). On the other hand there was no effect in the seawater eels; inhibition did not significantly alter urinary pH, CO_2 concentration or flow (Table 1).

The lack of response to carbonic anhydrase inhibition in seawater eels suggests absence of the renal enzyme, while response in freshwater eels substantiates its presence. Because direct assay had indicated carbonic anhydrase in the kidneys of seawater eels, the enzyme activity must be relegated to cells with other than renal function, probably hemopoietic tissue. This situation has been encountered previously, in *Pseudopleuronectes americanus* (Rawls and Maren, op. cit.) and even, in traces, in *Squalus acanthias*. The positive findings in the freshwater eels may therefore be due to a combination of hemopoietic stem cell enzyme and a low level of true renal carbonic anhydrase.

In conclusion, renal carbonic anhydrase activity in the euryhaline eel appears upon acclimation of the animal to freshwater, and is absent upon acclimation to seawater. It also appears that the lack of renal carbonic anhydrase activity in the seawater-acclimated teleost does not necessarily coincide with fixed acid urine. The suggestion, originally from Homer Smith, that the urine pH of marine fish is fixed on the acid side apparently does not apply to euryhaline seawater-acclimated fish such as the American eel and southern flounder (Hickman and Trump, op. cit.). This fact tends to weaken the argument that the acid urine of marine fish is necessary to prevent precipitation of Ca^{++} and Mg^{++} salts in the renal tubules. It is perhaps more likely that the presence or absence of renal carbonic anhydrase is related to efficient maintenance of acid-base balance. It is clear that in stenohaline marine elasmobranchs, fixed urinary pH (5.8) is associated with lack of renal carbonic anhydrase (Hodler et al., op. cit.; See also Maren, in Drugs and Transport Processes, ed. B.A. Callingham, pp. 73-93. University Park Press, Baltimore, 1974). The same appears true for one marine teleost, the winter flounder, *P. americanus*, at urine pH 6.7 (Rawls and Maren, op. cit.). However, the general situation in marine teleosts with respect to urinary pH regulation surely merits further work, since it appears that in some (euryhaline) situations regulation is accomplished without carbonic anhydrase. Finally, these data show one of the few examples of generation or repression of carbonic anhydrase; discovery of the underlying mechanism for such changes is an important goal.

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