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LACK OF INFLUENCE OF K-FREE SEAWATER ON PLASMA Na IN SEAWATER EELS *Anguilla rostrata*

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It has been proposed (Maetz, J., Science 166:613, 1969) that sodium extrusion by teleost gills in seawater is accomplished by exchange with external potassium in a reaction facilitated by Na-K-ATPase. A corollary of this hypothesis is that serum Na^+ should rise in seawater-adapted fish if K^+ is removed from the external medium.

Fully adapted saltwater eels (*Anguilla rostrata*) were immersed in aerated artificial seawater containing normal quantities of Na^+, Cl^+, HCO_3^-, Mg^{++}, and Ca^{++}, but without K^+. Control animals were placed in a similar artificial solution containing 10mM K^+. Each bath was changed daily so that the external potassium concentration remained less that 0.4 meq/L. In three eels placed in K-free seawater serum Na did not change importantly in the course of 72 hours (150-152; 151-153; 149-159). Changes in two control eels were likewise negligible (143-148; 148-152). Eels kept in K-free seawater were able to adjust rapidly to injections of hypertonic saline designed to elevate their serum Na by 30 meq/L (3 ml/100 gm of 0.7 N NaCl). Twenty-four hours after injection their serum Na had returned to normal

These data do not support the notion of external-K-for-internal-Na exchange across the gill (though they do not necessarily exclude it). The "potassium effect" described by Maetz may vary in different species and some mechanism for the active extrusion of sodium chloride in seawater appears to operate regardless of the concentration of external potassium.

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OSMOREGULATORY ROLE OF THE URINARY BLADDER IN THE STENOHALINE MARINE TELEOSTS, *Lophius americanus* and *Hemitripterus americanus*

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These *in situ* observations reveal the ability of the urinary bladder to alter markedly the composition and volume of urine formed both by the aglomerular kidneys of the goosefish *(Lophius americanus)* and the glomerular kidneys of the sea raven *(Hemitripterus americanus).* This is a preliminary report of a study aimed at characterizing secretory mechanisms which underlie the formation of urine by renal tubules in the absence of glomeruli, and in the production of urine flows that are significantly higher than simultaneous glomerular filtration rates under certain conditions in glomerular teleosts (J. Cell. Comp. Physiol., 42: 487-510, 1953). In comparing our current data on urine collected by ureteral catheterization we found discrepancies with earlier studies on residual bladder urine taken

from fish at the time of capture or on urine samples that were obtained during experiments by periodically emptying the bladder of urine stored for collection periods of 12 hours or so (J. Gen. Physiol. 39: 349-359, 1956). The brisk transfer particularly of Na from bladder urine to plasma and the accompanying marked reduction in urine volume that we note in current studies support the hypothesis that these stenohaline teleosts living in a strongly hypertonic external environment may generate free water from urine in a two-step process; first, by a coupling between water transport and the active transport of solute in urinary bladder epithelium, and then subsequently by the active extrusion of univalent ions from plasma in the relatively water-impermeable branchial membrane of these marine teleosts.

Two kinds of experimental procedures were followed for these *in vivo* observations on bladder function. First, 26 "by-pass" urine collections were made of five goosefish weighing between 1.25 and 9.6 kg. In this procedure urine from one kidney was led directly to a rubber balloon via an indwelling polyethylene ureteral catheter. The ureter leading to the bladder from the other kidney was left intact, and the urinary papilla cannulated so that at intervals of 12-18 hours it could be unplugged and "bladder urine" so collected compared with "ureteral urine" from the contralateral kidney that had bypassed the bladder. Plasma samples were taken from caudal vessels at the beginning and end of the urine collection series. In a second procedure on five sea ravens weighing between 750 and 1250 grams, urine of known composition was inserted into the emptied bladders after the ureters had been separately cannulated to the outside and their distal ends tied near the bladder. In these experiments 5 ml of previously collected ureteral urine from other marine teleosts was substituted for endogenously formed urine, and the bladder was untied and emptied 12-14 hours later. Plasma samples were taken when the bladders were emptied at the end of the experiment.

Na and K determinations were done by flame photometry, Mg, Ca, and SO_4 by atomic absorption spectrophotometry, Cl by coulombmetric titration, and osmometry by freezing point depression. Fish were maintained in a small wading pool with rapid change-over of cold circulating sea water.

The reabsorption of Na from bladder urine into plasma against steep chemical activity gradients, and the linked isosmotic absorption of fluid were found to be the main operations carried on by urinary bladder epithelium in both of these *in situ* experimental preparations. In a typical by-pass experiment on a 4.15 kg *Lophius*, for example, urine flow rates for two successive periods were 1.74 and 2.80 ml per kg B.W. hr for ureteral urine, and for bladder urine they were 0.90 and 0.59 respectively. Sodium concentrations in these same successive collections were 94.6 and 78.7 meq/L for ureteral urine, and for bladder urine they were 15.9 and 26.9 respectively. The simultaneous concentration of Na in plasma was 180 meq/L. The concentrations of the divalent cations, Ca and Mg, were higher in bladder urine than in ureteral, approximately in proporation to the fractional volume of fluid taken up from the bladder. It is not clear which anions cover the Na+ taken up. Some chloride is extracted, but not to the degree that Na is removed. Ureteral chlorides were 210 and 181 meq/L, and the concentrations in bladder urine were 170 and 167 respectively. Sulfate concentrations rose somewhat in bladder urine, but not to the degree the Ca and Mg did, so the possibility exists that some SO_4 is taken up as well as other anions not identified in these preliminary studies.

The "substitution" experiments on the sea raven, although somewhat imperfect technically, point to essentially the same bladder functions. Again, the active uptake of Na from bladder urine to plasma along with volume reduction were found to be the main features. Urine containing 55.2

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.

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

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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