or skate IEA pattern showed removal by cross-reaction in these analyses. Such procedures also failed to show more than a slight cross-reaction between the shark and skate antigens.

It appears that considerable immunologic specificity distinguishes the elasmobranch pituitary hormones from those of the mammalian pituitary, at least in so far as recognition by rabbit antibody is concerned. Furthermore, only slight antigenic similarity seems to relate the two elasmobranch species to one another.

Adjunct studies were carried out on the shark antibody response to subcutaneous injection of ovine prolactin. It had been reported by other workers that shark antibodies failed to act as precipitins in solution. Attempts to obtain precipitate in gel were unsuccessful. An incidental finding is of significance in serology of the dogfish. It was found that clotting of whole dogfish blood can be obtained consistently if the drawn sample is allowed to incubate at a cool temperature (10°C) for ten minutes immediately following withdrawal.

In collaboration with Dr. Leon Goldstein, preliminary observations were made on the effect of ovine prolactin on the skate osmoregulatory response to fresh water (50% sea water). Weight-gain in hypophysectomized skates was diminished by prolactin supplement. Promotion of NaCl elimination from the body is a postulated mechanism of action.

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1968 #18

## ROLE OF Na-K-ATPase IN OSMOTIC REGULATION BY MARINE VERTEBRATES

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The exact role of sodium-potassium activated adenosine triphosphatase in the active transport of sodium across epithelial surfaces is not clear. The case for its intimate involvement in sodium transport would be strengthened if the enzyme were shown to change in an adaptive way when the rate of sodium transport was varied. Accordingly, enzyme activity was measured in the gills, intestinal mucosa, and kidneys of several species of fish in both fresh and salt water since in these situations transport activity is known to vary widely.

Gill epithelium and filaments, intestinal mucosa scraped from the entire intestine, and both kidneys in toto, were homogenized with a Teflon pestle in the ratio of 100 mg of tissue to 2 ml of an ice-cold solution at pH 6.8 containing 0.25 M sucrose, 5 mM EDTA, 30 mM histidine and 1 g sodium deoxycholate per liter. The homogenate was filtered through a single layer of gauze and immediately assayed for ATPase activity with an incubation time of 15 minutes at 37°C (J. Clin. Invest. 46:1999, 1967). Results are listed in Table 1 (mean ± standard deviation).

Extremely low values for gill Na-K-ATPase were seen in Squalus acanthias, corresponding to the absence of active sodium transfer by the gills of elasmobranchs. Two stenohaline freshwater species (Micropterus dolomieui and Notropicus) also had low gill levels of Na-K-ATPase, as did the euryhaline eel, Anguilla rostrata, trapped and maintained in fresh water. All salt-water teleosts studied had specific activities of gill enzyme several times higher than these fresh water species, in keeping with the much higher rate of active sodium extrusion across the gill in salt water.

Table 1

| Species | Environment | Number | Organ | μMPi/mg protein/hour | |
|---|---|---|---|---|---|
| | | | | Mg-ATPase | Na-K-ATPase |
| Squalus acanthias | Salt | 7 | gill | 14.3 ± 2.8 | 2.7 ± 1.0 |
| Micropterus dolomieui | Fresh | 5 | gill | 15.4 ± 1.4 | 4.4 ± 2.0 |
| Notropicus | Fresh | 1 | gill | 15.4 | 1.0 |
| Hemitripterus Americanus | Salt | 6 | gill | 10.8 ± 3.1 | 13.8 ± 3.3 |
| Pseudopleuronectes Americanus | Salt | 9 | gill | 15.9 ± 4.7 | 16.0 ± 5.6 |
| Fundulus heteroclitus | Salt | 2 | gill | 24.8 | 11.1 |
| Myxocephalus octodecimspinosus | Salt | 1 | gill | 7.6 | 9.4 |
| Lophius Americanus | Salt | 2 | gill | 20.5 | 21.4 |
| Anguilla rostrata | Fresh | 6 | gill | 11.5 ± 1.5 | 6.0 ± 1.5 |
| Anguilla rostrata | Salt (2 wks) | 5 | gill | 11.4 ± 1.6 | 11.4 ± 2.4 |
| Anguilla rostrata | Fresh | 5 | intestine | 20.9 ± 10.4 | 9.0 ± 2.8 |
| Anguilla rostrata | Salt (2 wks) | 6 | intestine | 29.9 ± 8.8 | 17.8 ± 4.3 |
| Micropterus dolomieui | Fresh | 6 | kidney | 26.3 ± 5.2 | 21.0 ± 4.9 |
| Hemitripterus Americanus | Salt | 3 | kidney | 19.5 ± 5.4 | 13.1 ± 1.1 |
| Pseudopleuronectes Americanus | Salt | 5 | kidney | 18.8 ± 3.4 | 10.5 ± 4.5 |
| Lophius Americanus | Salt | 2 | kidney | 24.6 | 26.0 |
| Squalus acanthias | Salt | 1 | kidney | 19.9 | 8.8 |
| Squalus acanthias | Salt | 2 | rectal gland | 40.9 | 45.1 |

While transport of sodium by the gills of salt water teleosts is much higher than that of fresh water fish, the opposite is generally true of their kidneys. This is because glomerular filtration is higher in fresh water than in seawater, while reabsorption of filtered sodium by renal tubules is almost complete. It was therefore interesting that the Na-K-ATPase activity of kidneys of fresh-water Micropterus was significantly higher than salt-water Hemitripterus and Pleuronectes. A paradoxical finding was the high specific activity of Na-K-ATPase in the aglomerular kidney of Lophius Americanus, reminiscent of the ATPase activity in certain secretory organs. Na-K-ATPase in rectal gland of Squalus acanthias was extremely high.

After 2-3 weeks of adaptation in seawater, Na-K-ATPase content of gill and intestinal mucosa of Anguilla rostrata doubled, while there was no significant change in magnesium-activated ATPase. This adaptive change in the enzyme activated by sodium plus potassium is consistent with the considerable increase in sodium transport by intestine and gill demonstrated by others when Anguilla is transferred from fresh to salt water.

The data indicate that Na-K-ATPase activity varies with sodium transport in several or-

gans of teleost and elasmobranch under differing circumstances of osmotic stress. The enzyme is likely, therefore, to play an important role in the active transport of sodium across epithelial membranes and the adjustment of marine animals to their environment.

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1968 #19

## UREA: EXPERIMENTS DEMONSTRATING ITS INERT CHARACTER IN TWO PHYSIOLOGICAL SYSTEMS OF THE SPINY DOGFISH, Squalus acanthias

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The high concentration of urea (approximately 350 mM) in the tissues of elasmobranchs has been considered to be of physiological importance only in relation to its osmotic effect. Two test systems, the accumulation of p-aminohippurate (PAH) in kidney slices and oxygen ($O_2$) consumption in isolated liver mitochondria were used to examine this view. The uptake of PAH by kidney slices of Squalus was measured according to the procedure of Taggart and Forster (Meth. Med. Res. 5:228, 1952) at 25°C. Urea failed to effect the PAH S/M (slice/medium) ratio in eight assays. When the Ringers solution contained 360 mM urea the S/M ratio was 8.6 ± 1.1, whereas in Ringers solution without urea the S.M. ratio was 8.8 ± 1.2. Similarly urea failed to affect the uptake of PAH by slices prepared from the kidney of a teleost (Pseudopleuronectes americanus).

For studies on $O_2$ consumption, intact mitochondria were prepared from Squalus liver in 0.88 M sucrose according to the procedure of Massey and Smith (Comp. Biochem. Physiol. 25: 241, 1968) with the addition of enough mannitol, or mannitol and urea, to bring the final osmolarity to 1.0 molar. Succinate (9 mM) served as substrate and was added from the sidearm to initiate the reactions which were run in duplicate, at 25°C, in a Warburg apparatus. Oxygen consumption ($\mu$l $O_2$ consumed by mitochondria from one gram liver per hour) in the presence of 360 mM urea was 84 ± 3.7 and without urea 82.0 ± 6.4 (five assays each).

These observations support the thesis that marine elasmobranchs are not dependent upon urea for reasons other than maintaining an osmolarity slightly above that of their external environment.

It was noted, incidentally, that sucrose inhibits $O_2$ consumption in isolated liver mitochondria of Squalus. Sucrose concentrations as low as 0.12 M completely inhibited $O_2$ consumption. The "sucrose-effect" was reversible since mitochondria isolated in sucrose and resuspended in 1.0 M mannitol consumed oxygen at the normal rate. Liver mitochondria prepared from the short horned sculpin (Myosocephalus scorpius) readily consumed $O_2$ in the presence of sucrose indicating the inhibitory effect of sucrose may be peculiar to the elasmobranchs.

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