cally, $P < .2$) in skates maintained in 50% seawater as compared to the values obtained in 100% seawater (Table 1). The fractions of filtered urea and chloride reabsorbed in the renal tubules of skates were reduced 44 and 42% respectively by environmental dilution (Table 1). The fact that TMAO reabsorption was not similarly altered in skates kept in diluted seawater argues against the possibility that the decrease in reabsorption of urea and chloride was due to generalized renal tubular failure or to some non-specific "flushing-out" of urinary solute during diuresis resulting from a greater flux of water through the fish maintained in the dilute medium. Although the renal clearance of urea was elevated in skates maintained in dilute seawater, the total clearances of urea from the body fluids of skates (calculated from total urea appearing in seawater bath) kept in diluted and undiluted seawater were similar (0.72 ± 0.20 and 0.83 ± 0.15 ml/Kg/hr respectively).

This study indicates that as in other elasmobranchs urea plays an important role in the adaptation of the skate, Raja erinacea, to alterations of the salinity of the medium. The adjustment of urea concentration in the body fluids is achieved mainly by altering rates of biosynthesis rather than total excretion of the end-product. However, the kidneys do play an important role in this adaptation by regulating the fraction of filtered urea that is reabsorbed by the renal tubules.

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1968 #17

IMMUNOLOGIC INVESTIGATION OF ELASMOBRANCH PITUITARY HORMONES

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As an approach toward elucidation of phylogenetic variation of pituitary hormone principles immunologic techniques have recently been applied to advantage. On the basis of bioassays, endocrine principles corresponding to mammalian prolactin appear throughout the vertebrate series. The present investigation consisted of an attempt to demonstrate and characterize the prolactin form in the anterior pituitaries of the dogfish (Squalus acanthias) and skate (Raja erinacea) by immunologic means.

Potent rabbit antisera were developed individually with regularly scheduled injections (w/ adjuvant, Freund's) of purified ovine prolactin (NIH-P-S8), shark anterior pituitary brei, or skate pituitary brei. In-gel immunoelectrophoretic analysis (IEA) presented consistent, multiple-antigen patterns for both elasmobranch pituitaries. A combination organ culture/IEA system for the shark pituitary indicated that the IEA pattern represented soluble products of the intact, isolated anterior lobe.

Numerous double diffusion (Ouchterlony) and IEA systems were designed to show cross-reactivity among the several antigens and antisera available. No indications of any common immunologic affinity were obtained. More sensitive analyses were then employed, based on neutralization of antigens, or on absorption of antibodies, before their entry into IEA or diffusion. In addition, to enhance antibody potency, globulin fractions were isolated (salt precipitation) and used as antibody agent. Absorbants included mammalian (ovine) prolactin, (bovine) STH, and synthetic ACTH, as well as elasmobranch pituitary brei. No participant antigen of either the shark

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.