## Research Reports: 1956

# Comparative Study of the Ribonucleases of Several Marine Species

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As a part of a general program of study of the molecular basis of enzymatic function, it was of interest to examine the pancreas of pyloric caecal glands of several marine animals in regard to their content of ribonuclease activities and to their comparative biochemistry. Acetone powders were prepared from the pancreas of the dogfish, from hepatopancreas of the common mussel and from the pyloric cecum of the starfish. Extracts prepared from these powders by the method described originally by Kunitz for bovine pancreatic ribonuclease were studied with respect to the stability of the ribonuclease activity to heat, hydrogen ion concentration and high concentrations of urea. Most of the work was carried out using the starfish material. The ribonuclease present in this tissue was relatively heat-stable although not as stable as the enzyme from bovine pancreas. Its activity was decreased to about 25% of the control value when assayed in the presence of 8 M urea. Preliminary experiments on ion exchange resin columns and by ammonium sulfate fractionation suggested that the general properties of the starfish enzyme in terms of its chemical behavior might not be too dissimilar from those of the beef enzyme. Further studies on the purification and characterization of the enzyme from this source will be required before adequate comparison can be made.

## Urinary Excretion of Divalent Ions and Organic Bases in the Aglomerular Goosefish, Lophius americanus

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### **Divalent** ions

Earlier studies have demonstrated that divalent, i. e.  $Mg^{++}$ ,  $Ca^{++}$ , and  $SO_{4}^{-}$ , are actively secreted by renal tubules of the aglomerular goosefish; the concentration of these ions in blood and urine rises after capture when the animals are kept in tubs with running sea water. Thus maximal tubular secretory rates are often reached spontaneously and these vary widely from one animal to another.

The present work was concerned with the specificity of the transport mechanisms for these ions, particularly with respect to

competitive inhibition. After 2 - 3 control periods, which included taking quantitative urine collections and blood samples, various salts were given i.v. Administration of sodium thiosulfate depressed sulfate excretion to one-fourth of control values in the two fish examined, and thiosulfate replaced sulfate in the urine. Thiosulfate also markedly depresses the tubular reabsorption of sulfate in the dog (Berglund, unpublished). Thiosulfate and sulfate therefore seem to share the same transport mechanism in the renal tubules, both when sulfate is transported from tubular lumen to blood, as in the dog, or in the opposite direction, as in the goosefish.

Competition between calcium and magnesium was studied in a similar manner. Injection of  $MgCl_2$  almost completely inhibited the tubular excretion of calcium. CaCl2, however, did not seem to have any effect on magnesium excretion when its plasma concentration was elevated in a few experiments. This is not surprising in view of the fact that magnesium normally is excreted more actively, appearing in 5-10 times as high concentration as calcium in goosefish urine.

#### Organic basses

Creatine and trimethylamine-oxide occur in considerable concentration in urine of freshly caught goosefish. Under laboratory conditions trimethylamine-oxide excretion rapidly diminishes, whereas creatine excretion remains relatively constant. Injections of glycine or creatinine did not affect the excretion of creatine and trimethylamine-oxide. Injection of tetraethylammonium bromide had no effect on creatine excretion but completely inhibited trimethylamine-oxide excretion.

Trimethylamine-oxide and tetraethylammonium seem to be excreted by a common mechanism distinct from that which transports creatine and creatinine.

## Experimental Study of Calcification In Sand Dollar Embryos

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This report deals with an experimental study of the formation of the skeletal system in the embryos of *Echinorachnius parma* with particular reference to the possible role acid polysaccharides may play in the process.

Embryos exhibit metachromasia when treated with methylene blue and toludin blue in the periphally located epithelium of the blastula, gastrula and pluteus larvae. Radio autographs of comparable stages reared in the presence of S-35 shows that S-35 is localized in the epithelium of these embryos exclusively. The combined techniques demonstrate fairly conclusively the presence of a sulphated polysaccharide localized in the surface epithelium of the developing embryo.