composition of the two classes. The Na^+ and K^+ stimulated ATP-ase activity was inhibited by ouabain ($I_{50} = 10^{-6}$ M), para chloromercuribenzoate ($I_{50} = 3 \times 10^{-6}$ M), chlormerodrin ($I_{50} = 3 \times 10^{-6}$ M), and 20% glycerol. Previous work on enzyme from nerve tissue in the blue crab Callinectes sapidus showed that in the presence of 350 mM KCl (approximate intracellular concentration in axoplasm) addition of Na^+ ion gave maximal activation at 50 mM Na^+. This was not true in the rectal gland. Maximal activation (in the presence of 350 mM KCl) was not approached even at 250 mM Na^+. Thus a minor but perhaps important distinction has been made in ion activation properties between the two species.

1965 #31

IN VITRO PERFUSION OF THE ISOLATED RECTAL GLAND OF Squalus acanthias*

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In vivo the rectal gland secretes a neutral solution of NaCl containing very little urea and essentially no Mg^{++} or Ca^{++}. The anatomy of the gland, with a single afferent artery and a single excretory stoma, lends itself to available techniques for arterial perfusion and collection of the secretion in vitro. Glands obtained from freshly killed dogfish were placed in cold (10°C) perfusion medium. The artery was cannulated with P.E. #50 tubing attached to a #22 needle. The stoma was cannulated with P.E. #100 tubing and perfusion begun from a reservoir 10-50 cm above the gland. The perfusion fluid contained in meq/L NaCl 250, KCl 5, $CaCl_2$ 6, $MgCl_2$ 3, $NaHCO_3$ 12, Urea 350 mM, glucose 10 mM. The perfusion gluid was gassed with 95% O_2 and 5% CO_2, and maintained at 10-15°C. Perfusion rates varied from 25 to 150 ml/hr. The technique was developed to the extent that about three minutes elapsed from the time of removal to the time when perfusion was begun. Functional preparations began to secrete a fluid immediately and continued to do so for 3-4 hours. If the initial secretion contained Mg^{++} or Ca^{++} greater than 1 meq/L the preparation was discarded. Altogether ten glands had no Mg^{++} or Ca^{++} in the secretion during the observation periods. Secretory flows ranged between 0.2 and 2.0 ml/hr. Chloride concentrations were constant for any single gland and ranged from 250 to 520 meq/L. In six cases the secretory fluid had chloride significantly higher than that of the perfusate. It was the impression, although not systematically investigated, that glands being perfused at faster rates tended to have lower secretory chlorides, although Ca^{++} and Mg^{++} were excluded from the secretion at these faster rates. Acetazolamide (4×10^{-4} M) stopped secretion immediately and completely in the three preparations where it was tested. Ouabain (10^{-4} M) initially increased secretory flow 2-5-fold, then completely stopped secretion in three glands where it was given. Neither drug altered the arterial perfusion rate.

In this preliminary investigation the following conclusions were reached:

(1) The rectal gland is technically approachable for isolated perfusion, and gradients from perfusion fluid to secretion fluid for Cl^-, Mg^{++} and Ca^{++} can be achieved.

(2) The rate of perfusion and the perfusion pressure are critical determinants of gland function in vitro, and require further investigation and better control.

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(3) The isolated perfused gland can be used to study various pharmacological and hormonal effects on electrolyte secretion in the absence of complicating diverse physiologic change in the intact animal.

1965 #32

ULTRASTRUCTURE OF MARINE CALCIFICATION*

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The technique of x-ray diffraction was applied to the study of marine calcification. It was shown that the mineralized spine of the sea urchin was composed of a single crystal of the calcium carbonate mineral calcite. Similar studies on the plates of the sand dollar test showed them to be single crystals of calcite. Studies are now in progress to see if the earliest development of the sea urchin spine is as a single or multiple crystal system. A similar study is planned on the sand dollar calcification history.

In collaboration with Drs. David Karnofsky and Charles Young an x-ray diffraction study is being run on the development of calcite crystals in the embryo of the sand dollar. Preliminary results have shown that both large crystals (no doubt the trigonal spicules reported in the literature) and smaller crystals can exist in the early pluteus stage. Further experiments are under way to determine the earliest stage of mineral development.

X-ray diffraction studies on the operculum bone of the pollock and cod show that this tissue contains the typical poorly crystallized bone apatite (i.e., the calcium phosphate mineral of bone) as seen in other bones. Earlier work by these investigators showed the mineral of carp operculum to be non-crystalline, or amorphous. (Care was taken with the present work to study a freshly excised bone which was kept moist with Fish Ringer's Solution while under study. This was done to avoid crystallization of a possible amorphous phase.) Additional x-ray studies of freshly excized, lyophilized dogfish vertebrae showed this tissue to contain bone apatite as in teleost fish.

It is planned to continue this project concentrating on the earliest stages of hard tissue development.

1965 #33

DRUG METABOLISM BY VARIOUS MARINE ANIMALS

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Very little is known concerning the metabolism of foreign compounds by marine animals. In the studies to be described it was our purpose to investigate further the hypothesis of Dr. Brodie that fish lack the capacity to metabolize foreign compounds. These metabolic transformations usually convert the substrate to a more readily excreted less lipid soluble derivative.

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