(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.

*Two separate NIH Grants, one research grant and one training grant.

Brodie suggests that teleologically these metabolic transformations are unnecessary as the gills act as a dialyzing membrane allowing foreign compounds to diffuse from plasma to an almost infinite seawater environment. We investigated plasma half times of various agents and studied oxidative and reductive drug metabolic pathways in vitro. The following points summarize our findings:

1. Plasma half-times of very lipid soluble and readily diffusible substances did not suggest a rapid diffusion of these substances from dogfish (Squalus acanthias) plasma to seawater. The plasma half-time of 4-aminoantipyrine and sulfanilamide after intraarterial injection was approximately 4 and 6 hours.

2. Dogfish liver homogenate fortified with a proven TPNH ($NADPH_2$) generating system was unable to oxidize hexobarbital or chlorpromazine and was also unable to convert aminopyrine to 4-aminoantipyrine.

3. Reductive metabolic pathways were investigated using either tissue homogenate of 9000 x g supernatant fortified with a TPNH generating system and incubated under nitrogen for one hour at 37°C. There was no difference in results obtained with homogenate when compared to 9000 x g supernatant. Homogenates of dogfish liver, spleen and testes were able to reduce the azo linkage of neoprontosil yielding the metabolite sulfanilamide. None of these tissues were able to reduce the aromatic nitro group of p-nitrobenzoic acid to produce p-aminobenzoic acid. Homogenates of livers from embryonic, male (adult), and adult female dogfish showed no difference in their capacity to reduce neoprontosil. Skate, lungfish (Lepidosiren), and hagfish (Myxine glutinosa) liver homogenates were also able to reduce neoprontosil. However, only skate liver homogenate was able to reduce the aromatic nitro group of p-nitrobenzoic acid.

Azo-reductase activity of dogfish liver homogenate was shown: a) to be TPNH dependent; b) to increase with increasing temperature from 5 to 37°C; c) to increase with increasing levels of TPN; d) to be stimulated fivefold by flavin-adenine dinucleotide (FAD) 10^{-3}M; e) to be inhibited only 50% by an atmosphere of oxygen; f) to be relatively unaffected by SKF-525a, a drug metabolizing enzyme inhibitor, whether added directly to the incubate; preincubated; or injected into the animal 30 min prior to sacrifice; g) to increase with increasing pH to an optimum at 7.4 - 8.0; h) to increase with increasing levels of substrate as high as 10^{-2}M; and i) to indicate a very low affinity of the enzyme for the substrate.

The authors wish to acknowledge and thank Mr. Edgar A. Bering, III for his fine technical assistance on this project.

1965 #34

ULTRA STRUCTURE OF 1) FISH KIDNEYS, 2) THE URINARY BLADDER OF THE FROG

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During the summer of 1964, two projects were carried out, one dealing with the fixation of fish kidneys, the second with the study of the microcirculation in the frog's urinary bladder. Primarily, the kidneys of the goosefish, Lophius piscatorius, were fixed with a variety of fixatives, since earlier studies of this kidney had demonstrated a poor preservation of some cell types. The fish were obtained by deep sea dragging, and upon capture the kidneys were removed imme-