

comparison with material fixed in the antimoniate reagent.

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1966 #14

FINE STRUCTURE OF THE RECTAL GLAND OF Squalus acanthias AFTER INCUBATION OF TISSUE SLICES IN ISOTIC AND HYPEROSMOTIC SOLUTIONS

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Slices of the rectal gland of Squalus acanthias were incubated for periods up to 12 hours in chilled Hogben's solution and in solutions with added sucrose or sodium chloride. Experiments on indirect osmometry of the slices done in 1964 showed characteristic shrinkage curves in sucrose solutions and shrinkage followed by swelling in strong sodium chloride solutions. During 1965 similarly treated slices were fixed for electron microscopy after appropriate periods of incubation in chilled solutions gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Subsequent examination revealed that essentially intact fine structure was maintained for several hours with evidence of continuing secretory activity in the control Hogben's solution.

With addition of 0.6 M sucrose the slices shrank to 74% of initial volume after 24 hours. Despite prolonged incubation the fine structure remained intact. There was reduced cell volume and generally increased electron density but evidence of continuing vacuole formation at cell bases.

With addition of sodium chloride two morphologies result, one during shrinkage and another during swelling. During shrinkage there is dislocation and clumping of chromatin granules, clumped cytoplasmic organelles, shrunken and dense mitochondria and decreased cell volume. The lateral cell surfaces become attenuated and narrow and there are changes in the apical terminal web.

Slices immersed in 1.0 M sodium chloride begin to swell 2-5 hours after incubation with deterioration of fine structure. It is clear that previous reports by others of anomalous swelling in such solutions is not an osmotic response of viable cells.

In suitable media, slices of the rectal gland provide favorable material for correlation of fine structure with secretory activity maintaining structural integrity for long periods under a broad range of conditions.

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NEUROSECRETION IN Cucumaria

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Electron microscopic examination of the haemal vessels of this holothurian reveals the presence of nerve strands with axons containing large dense membrane-bounded granules. These are of particular interest since there is only fragmentary evidence of neurosecretion in echinoderms. The nerve strands pass between the cell processes of epithelial cells and are distributed

to the non-striated muscle cells of the vessel wall. The axons terminate in channels or invaginations of the muscle cell without specialized junction areas. Microtubules 260 Å in diameter are present in the axons along with a variety of sizes (0.2 to 0.3 microns in diameter) of dense cored vesicles. The dense cores are aggregates of smaller (250 Å) dense particles. In the occasional cell bodies of neurones which are encountered the cytoplasm contains large numbers of the characteristic vesicles in close association with an elaborate Golgi region from which the vesicles appear to arise.

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#### THE OCCURRENCE OF MITOCHONDRIA IN MATURE ERYTHROCYTES OF Myxine glutinosa

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In the circulating blood in the gills of the hagfish the erythrocytes have normal mitochondria as identified in the electron microscope. Not all sections passing through the nucleus show mitochondria but most show one and about 10% show 3-5 mitochondria per section. No ribosomes were observed.

In contrast the mature erythrocytes of Squalus acanthias show polyribosomes but no mitochondria.

In both species there are other vesicles in the erythrocyte cytoplasm and in some of these myelin forms have a superficial resemblance to mitochondrial cristae.

In Myxine pinocytotic vacuoles occur at the cell membrane.

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#### ON THE ORIGIN OF TRIMETHYLAMINE OXIDE (TMAO) IN THE SPINY DOGFISH, Squalus acanthias

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This investigation is part of a general study on the comparative biochemistry of nitrogen metabolism, especially as related to environment. Trimethylamine oxide (TMAO), which appears to play an osmoregulatory role in elasmobranchs, is found in high concentrations in the body fluids of the dogfish, Squalus acanthias. The source (endogenous vs. exogenous) of this nitrogenous compound is unknown.

The level of TMAO (approximately 70 μmoles/ml) in the plasma of dogfish maintained in live cars remained relatively constant for weeks even though the fish were not fed and stomachs were found to be empty (this study and Cohen, Krupp and Chidsey, Am. J. Physiol. 194:229, 1958). The ability of whole dogfish and isolated liver preparations to synthesize TMAO from radioisotopically labeled precursors was tested. No counts were detected in TMAO after incubating either liver homogenates or liver slices with C<sup>14</sup>-trimethylamine for 1-3 hours at room temperature under conditions permitting the detection of as little as 1% of the counts incorporated into TMAO.