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PROPERTIES OF THE ADENOSINE TRIPHOSPHATASE SYSTEM OF THE CELL MEMBRANES OF RED BLOOD CELLS OF THE DOGFISH SHARK (Squalus acanthias)

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Cell membrane rich fractions of hemolyzed dogfish erythrocytes have been prepared by repeated washing and centrifugation with hypotonic solutions of 100 mM tris buffer and 1 mM EDTA. Nuclei were removed by differential centrifugation. The membrane rich pellet was homogenized in the cold and diluted with tris buffer to a final concentration of 5 mg protein/ml. Some preparations were dialyzed for six hours against a 100 mM tris solution containing 1 mM EDTA. ATPase activity was assayed generally in the presence of 2.5 millimolar concentrations of ATP. Two reactions were used in the assay system. In the initial one, .75 mg of enzyme preparation was incubated in a buffered solution in the presence of ATP for twenty minutes. The reaction was stopped with 20% perchloric acid and neutralized with NaOH. The activity of the enzyme, measured in mmoles of ATP transformed, per hour per mg of protein, was assayed by the amount of ADP generated in the initial reaction. The test system consisted of phosphoenol pyruvate, ADP (the unknown), DPNH and lactic dehydrogenase. The reaction was started by the addition of pyruvate kinase, and the ADP was measured fluorometrically by the disappearance of DPNH. ATPase activity was found to be as much as 1000 times as great as values recorded for mammalian red blood cell systems. Magnesium increased the rate of the reaction by approximately twofold and the reaction was increased by an additional 50% when sodium and potassium were added to the reaction mixture. Oligomycin inhibited the NA + K + magnesium reaction, but it also exhibited some inhibitory effect with magnesium alone. Ouabain also produced some inhibition but this was less than that observed with oligomycin. 2 - 4 Dinitrophenol variously inhibited and stimulated the ATPase activity. Studies are currently in progress to further define the characteristics of the stimulatory effect of cations and of the inhibitory effects of oligomycin and ouabain.

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THE DEVELOPMENT OF NEURAL CONTROL OF ALIMENTARY MOTOR FUNCTION IN THE VERTEBRATES - A COMPARATIVE STUDY

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Representatives of three classes of vertebrates were studied as to their neurophysiological mechanisms that control gastrointestinal motility. Serotonin, acetylcholine, and epinephrine given intravenously (I.V.), intraarterially (I.A.) and intraluminally (I.L.) were used to determine

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the presence or absence of specific receptors. In addition pressure receptors in the gastrointestinal wall were stimulated mechanically. Selected areas of the alimentary tract were denervated and alterations in function noted.

The phylogenetically primitive hagfish (Myxine glutinosa) was demonstrated to have no neural control of its intestinal tract and no intrinsic propulsive mechanism. Ingested material passes through the alimentary tract by hydraulic pressure from ingested water and cephalo-caudad contractions of the body wall.

The spiny dogfish, Squalus acanthias, has developed two neuronally controlled propulsive mechanisms. One is a fast acting vomiting mechanism which is confined to the proximal stomach and the other is a slow reacting peristaltic mechanism largely limited to the distal stomach and pylorus. The ganglion cell that controls the peristaltic capacity of the distal stomach is predominantly confined to the C.N.S. However, continued minimal reactivity to serotonin in the chronically denervated preparation suggest the beginnings of a myenteric plexus in the wall of the distal stomach only. The dogfish intestine has peristaltic activity but there is no evidence of neuronal control. This suggests that the primitive smooth muscle is capable of some organized motor activity prior to the establishment of nervous connections.

In the frog, Rana catesbiana, the intestine has evolved serotonin and acetylcholine receptors which continue to function in spite of external denervation. This suggests that the ganglion cell has migrated to the intestinal wall and can function independent of C.N.S. connections.

These results would suggest that the primitive vertebrate intestine functions predominantly through its qualities of elasticity and tone. As neural control evolves there is a coincidental appearance of sensory and motor nerve fibers with receptors for acetylcholine and serotonin but the ganglion cell still remains in the C.N.S. In the higher vertebrates the ganglion cell migrates to the intestinal wall endowing it with neural excitatory control largely independent of C.N.S. connections.

Epinephrine is excitatory in the dogfish distal stomach but plays no apparent role in motor function either excitatory or inhibitory in the frog.

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THE EFFECTS OF MERSALYL ON THE FORMATION OF THE TELEOST BLASTODISC

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In a previous study (Huver, 1962 Doctoral Dissertation, Yale University) time-lapse cinephotomicrographic films revealed contractile waves occurring in the cortex of the egg of Fundulus heteroclitus during blastodisc formation. The SH-binding agent mersalyl which has been frequently used to inhibit contractility (The Motility of Muscle and Cells, Harvard University Press, 1958) was applied to the Fundulus egg in order to test the hypothesis that contractile waves play an important role in the formation of the blastodisc.

The fish were segregated according to sex upon delivery by the M.D.I.B.L. collectors to prevent spawning. Eggs were available from time of arrival, June 18, until July 12, when experiments