1963 #25

HISTOCHEMICAL STUDIES OF LYSOSOME ACTIVITY IN HYDROID REGRESSION-REPLACEMENT CYCLES

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The regression-replacement cycle of Campanularia flexuosa, a calyptoblastic hydroid, was studied histochemically to determine the possible relationship between lysosome activity and this remarkable senescence cycle. The distribution and fluctuation of acid phosphatase, cathepsin and aryl sulfatase were observed as an index of lysosome action.

The individual hydranth was a longevity of 3-7 days. During this period the hydranth can capture food, digest the food and supply the adjacent members of the colony with the captured nutrients. At the first visible onset of senescence, the tentacles withdraw into the hypostome and the individual undergoes an autolytic digestion. Strehler has studied the acid phosphatase activity (Gomori) during the cycle using this enzyme as an index to lysosome activity and areas of cellular death. Using Burstone techniques for acid phosphatase, our data corroborates Strehler's findings that acid phosphatase activity is associated with regression and lysis. The aryl-sulfatase data indicates a sudden increase in lysosome activity paralleling senescence and increasing progressively over the period of regression. Preliminary analysis of cathepsin B data have not agreed with the previous observations; there not being a significant increase in activity during senescence. This is being checked. High acid phosphatase, aryl sulfatase and cathepsin B are found in regions of new growth and in regions of digestive activity. The relationship of lysosome activity and phagocytic activity is being investigated.

Electron microscope studies of the regressing hydranth have revealed a structure in tentacle cells which might be identified as a lysosome. The relationship between stage of regression and lysosome fragility is now being investigated.

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1963 #26

POTENTIAL DIFFERENCE OF THE VENTRICULAR FLUID *in vivo* AND *in vitro* IN THE DOGFISH

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The mechanism of secretion of cerebrospinal fluid (CSF) by the choroid plexus (CP) is unknown and attempts to analyze the mechanism *in vivo* are difficult. The technical advantages of an *in vitro* system led us to explore the CP of the dogfish, S. acanthias. The fourth ventricular CP of this elasmobranch is a 4 x 7 mm sheet which separates ventricular CSF (VF) from the non-neural extradural fluid (EDF) which freely communicates with the general extra-cellular space of the body. This membrane was placed in a Ussing type chamber (aperture, 10 mm^2) and bathed in aerated elasmobranch ringers. The electric potential difference varied from 1-3 mv, with VP positive in all of the experiments. This p.d. was independent of the pH of the Ringers solution and stable for at least 3 hours. The membrane was viable after mounting as evidenced by

its ability to concentrate chlorephenol red within the capillaries. The specific resistance was 100-200 ohm cm^2, the short circuit current 1-3 μamps. Addition of 10^{-3} or 10^{-4} molar ouabain or 10^{-4} molar KCN to both sides caused a steady decrease so that the p.d. was less than 0.5 mv after 30 min. In contrast, the in vivo p.d. between VF and general extracellular space of the fish was 5-15 mv, VF negative. This p.d. was stable even after the fish was decapitated. In 4 experiments the thin neural tissue of the floor of the fourth ventricle was mounted in the Ussing chamber and for the first 15 min the p.d. was 1-2 mv, VF negative. In 3 of these preparations the p.d. reversed with time. These preliminary experiments suggest the possibility that the in vivo p.d. between VF and blood or EDF may, in some circumstances, be the sum of the p.d. across the CP and that generated by neural tissue.

1963 #27

EXPERIMENTS CONCERNING THE CLEAVAGE STIMULUS AND FURROW IN INVERTEBRATE EGGS

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The geometrical relations of the cleavage stimulus in the egg of the sand dollar (Echinorachnius parma) were determined by constricting uncleaved eggs and changing the normally spherical form to that of a modified dumbbell before the position of the furrow was determined.

When the mitotic apparatus of such cells lay with an aster on either side of the constriction, the distance from aster to cell surface was virtually uniform throughout the cell. Since subsequent cleavage of such cells is temporally and morphologically normal, the position of the furrow cannot be determined by absence of stimulus occasioned by a greater distance from aster to presumptive furrow than from aster to polar region. The furrow appeared adjacent to the zone between the asters in all cases. A portion of the surface in intimate contact with an aster could produce a furrow a few minutes after relocation of the mitotic apparatus.

In cleaving eggs of Echinorachnius, Cerebratulus fuscus and Hydractinia echinata paraxially oriented needles were placed in the path of the deepening furrow on diametrically opposed sides of the cell. The furrow progressed until it contacted the needles at which time progress ceased. In no case did a furrow sever itself upon a needle.

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EFFECT OF CARBACHOL AND THIOCYANATE ON POTENTIAL, RESISTANCE AND H^+ SECRETION OF THE GASTRIC MUCOSA OF THE DOGFISH

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Experiments were performed at room temperature (17 to 24 C) with an in vitro method. The nutrient solution contained in mM: 252 Na^+, 10 K^+, 5 Ca^{++}, 2 Mg^{++}, 240 Cl^-, 30 HCO_3^-, 2 SO_4^{--}, 1 P, 10 glucose (secretory solution same cation content but Cl^- only anion). The H^+ rate was determined by the pH stat method and the electrical resistance as the change in PD per unit of applied current. Carbachol (10^{-6} M) to the nutrient usually produced a marked increase in the H^+