

Experiments Concerning the Cleavage Stimulus in Sand Dollar Eggs

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When the form of a sand dollar egg is modified to that of a torus before the cleavage furrow is determined, it divides only in the region of the spindle. The torus is thus converted into a binucleate, horseshoe-shaped cell. The furrow is temporally and morphologically normal. In this experiment, a large portion of the cell was at a considerable distance from the asters but did not exhibit any signs of furrowing. This observation cannot be reconciled with any hypothesis in which the position of the furrow is established by the absence of stimulus.

The second division of the torus-shaped cell occurred in concert with the controls and resulted in isolation of two uninucleate cells from the free ends of the horseshoe. The bend of the horseshoe was binucleate but within five minutes of the completion of the two normal divisions, a furrow appeared between the polar regions of the two asters in the binucleate cell. A furrow was thus completed in a region that was never in close proximity to a spindle or chromosomes. The non spindle division could be delayed until the third or fourth cleavage. It appeared normal; it was completed in normal time and in concert with the other cells of the embryo. Since normal division has thus been demonstrated to occur in the absence of the spindle and its contents, it is proposed that neither of these entities play any necessary role in cytokinesis of sand dollar eggs.

Following frequent kneading, accomplished by compression and release and by extrusion, temporally and morphologically normal division occurs. The mechanism whereby the stimulus is delivered is therefore either very resilient or very rapid.

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Effect of Carbonic Anhydrase Inhibition on Urine of a Marine Teleost, *Pseudopleuronectes Americanus*

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Heinemann and Hodler (Bull. Mount Desert Island Biol. Lab. IV, part 1. p. 42, 1953) found that in general marine teleosts, like marine elasmobranchs, could not alkalize their urine following the administration of acetazolamide (6063). Their data, however, indicated that in the special case of the flounder, *P. Americanus*, acetazolamide caused a rise in urine pH, Na⁺ and K⁺. Since we have found carbonic anhydrase in the kidney

of this species (Bull. Mount Desert Island Biol. Lab. IV, part 3, p. 72, 1959) it seemed worthwhile to re-investigate the problem.

Five flounder were catheterized and urine collected for 24 hours in a balloon sewed to the catheter. Two fish were untreated; three received 25 mg/kg of acetazolamide intramuscularly at zero time. In all five fish the 24-hour urine was pH 6.70-6.95. Total CO_2 , however, was 0.9 mM/L in the control fish and 4.7 mM/L in the treated fish. This corresponds to pCO_2 of 7.5 mm. Hg in the control, and 22 mm. Hg in the treated animals. Parallel changes in pCO_2 were found in the plasma, in accord with the general pattern of respiratory acidosis which is found in all fish following carbonic anhydrase inhibition (Hodler *et al.*, Am. J. Physiol. 183, p. 155, 1955).

It therefore appears that the urinary changes are accountable to the systemic effects of the drug, and it may be said that no marine fish yet examined has shown a renal response to acetazolamide, or to sodium bicarbonate. The presence of carbonic anhydrase in the kidneys of marine teleosts remains unexplained.

Carbonic Anhydrase Inhibition In The Rectal Gland Of *Squalus Acanthias*

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Dr Wendell Burger (Science, 131: 670, 1960) reported on the function of the rectal gland of the spiny dogfish *Squalus acanthias*, showing it to secrete Na^+ and Cl^- in relatively high concentrations. He demonstrated a method of continuous sampling of the secretion practical over 3 - 4 day periods and showed a secretory response of the gland to small intramuscular injections of saline solutions. Previously unreported observations by Dr. Thomas Maren (MDIBL 1959-1960) show the gland to contain carbonic anhydrase (C.A.). Concentration was (mean \pm S.E.) 92 ± 14 units/gm. of tissue. This is the highest C.A. concentration of any tissue in this species.

The effects of inhibition of this enzyme in relation to rectal gland secretion were studied. Burger's method of collection, and specific inhibition with methazolamide were employed. Methazolamide is a carbonic anhydrase inhibitor with activity and effects similar to those of acetazolamide, but with the advantage of better penetration into cells, and a lower pK, so that its sodium salt, used for injection, is close to neutral.

Two protocols were used. In the first, three fish were prepared according to Burger. A baseline flow of 0.2 ml/kg/hr or more was established; if this did not occur the fish was not used. The response to three successive intravenous injections (approximately 4 hours apart) of Molar NaCl (2 meq/Kg) was then determined. There is no apparent difference between intramuscular and intravenous injections of NaCl. Typically the gland responds with increase in flow in 30-90 minutes. The flow increases to 1.2 - 1.8 ml/Kg/hr, is maintained for 60 - 120 minutes and then falls