titative description of embryo development not previously available. In embryos grown in seawater at 15°C in fluorescent light nuclear number increases from one (the fertilized egg) to a mean value of 42 by 4 days and to 155 by 10 days. At this stage a single rhizoid has developed and a multicellular thallus body with one to several apical hairs has formed.

When fertilized eggs are placed in seawater supplemented with 0.6M sucrose, there occurs an initial rapid plasmolysis of the cell but the embryos soon deplasmolyze and proceed to develop, lacking, however, normal polarization or rhizoid formation. Embryos can be maintained for up to 10 days in sucrose-seawater as apolar structures, developing as slowly growing, symmetrical, multicellular spheres. Mitoses continue, but at a much reduced rate compared to the seawater controls; embryos in seawater containing 0.6M sucrose showed a nuclear count of about 10-11 at 4 days. If such embryos were transferred after 4 days to seawater, they resumed the normal rate of cell division. Instead of forming a single rhizoid, the released spherical embryos developed multiple rhizoids, each rhizoid arising from one of the small peripheral cells of the sphere, usually all located together in one quadrant of the embryo. As many as 7 rhizoids were observed developing from sucrose-treated embryos, instead of the single rhizoid typical of normal embryos.

In normal embryo development in seawater, the single rhizoid cell typically develops in that embryo-half away from low-intensity unilateral illumination. A preliminary analysis of the influence of unilateral illumination on sucrose-treated apolar embryos indicated that polarization could be imposed on the multicellular spherical embryo either early or late in its development before release from sucrose inhibition of rhizoid formation. The implications of these results on the concept of a rhizoid-forming stimulus subject to environmental orientation are still being explored.

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1968 #37

STUDIES ON THE CLEAVAGE CYCLE IN THE SAND DOLLAR EMBRYO: EFFECTS OF PROTEIN SYNTHESIS INHIBITION AND OF ADENINE DERIVATIVES

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We have previously observed that proteins required for S_2 and for the first cleavage of fertilized ova of Echinarachinius parma were synthesized between 20 and 35 minutes following fertilization (Fed. Proc. 27:366, 1968). Therefore, synthesis of these proteins was coincident with the initial DNA replication or S phase. Protein synthesis was not required for extensive replication of DNA present in pronuclei but was necessary for a second replication. To determine the time of synthesis of the proteins which mediate S_3 and the second cleavage (Cl_2) similar studies have been undertaken. Aliquots from a population of simultaneously fertilized ova were added to cycloheximide (1μg/ml) at 10 minute intervals from 60 to 130 minutes following

fertilization. This drug concentration inhibits protein synthesis by greater than 97%. Synthesis of DNA in treated and control groups was evaluated by measuring incorporation of ^{3}H-thymidine into an acid-insoluble fraction during S_3, which occurs between 135 and 150 minutes following fertilization. Bulk incorporation and radioautographic studies demonstrated that proteins required for S_3 and Cl_2 were made between 90 and 110 minutes after fertilization, i.e. during the latter half of S_2.

An attempt was made to dissociate S_2 from the synthesis of proteins for S_3 and Cl_2 by using arabinosyl cytosine (AraC), an abnormal nucleoside that delays the first karyokinetic events and cleavage by 20 to 30 minutes when present at a concentration of 10 to 30 μg/ml (Bull. MDIBL 5 (1):36, 1965). Under these conditions much of S_2 was observed to be similarly delayed. Although AraC had no effect on the rate of synthesis of proteins measured in the whole embryo, it delayed synthesis of those proteins required for S_3 and Cl_2 to the same extent that it had delayed S_2. These observations suggest that existence of an obligatory relationship between replication of DNA and the synthesis of proteins that will distribute the replicated material into daughter cells and permit further replication.

Studies on 8-mercapto-2-piperdinoadenine (MPA) and related compounds, known to be potent cleavage inhibitors in the echinoderm embryo (Ibid.), revealed that the drug did not inhibit protein synthesis. Although thc cyclic nature of DNA synthesis was upset by the drug, incorporation of ^{3}H-thymidine continued for at least four hours in the absence of cell cleavage. Since synthesis of protein and DNA continued in the presence of MPA but was profoundly inhibited by dinitrophenol at concentrations preventing mitosis, it is probable that the MPA-induced cleavage inhibition is not occasioned by a generalized depression of ATP formation or utilization. Since β-mercaptoethanol and dithiothreitol profoundly inhibited protein synthesis at concentrations which prevented cleavage, the selectivity of the MPA-induced inhibition cannot be reproduced ty potent sylfhydryl-containing reducing agents. Thus, although the 8-SH substituant may be required for the drug's inhibitory effect, MPA did not produce generalized disorganization of the embryo's sulfhydryl metabolism.