pass these viruses in embryo dogfish tissue cultures were unsuccessful.

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1963 #34

EARLY EMBRYONIC DEVELOPMENT IN Fucus

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Although classic material for the study of early embryological development in plants, the brown alga, Fucus, has been little studied with respect to nuclear and cytoplasmic cytology or cytoplasmic events associated with fertilization, polarization and embryonic cleavage. We have explored appropriate technical procedures for fixation and staining of the unfertilized egg, the zygote and early embryonic stages for use in studies ranging from macroscopic morphology to ultrastructure. Effective fixation was achieved with 10% formalin in sea water or 2-1/2% glutaraldehyde in sea water. A number of routine nuclear stains proved ineffective; acetocarmine, alum cochineal, azure B, and toluidine blue all proved of some value in staining nuclear, cytoplasmic or wall structures in squash preparations. Some success in obtaining adequate fixation for electron microscopy was achieved. We gained sufficient experience in collecting reproductive plants and obtaining gamete release and fertilization in vitro to allow preliminary experiments on two specific problems: a) the distribution of nuclear and cytoplasmic structures within the zygote associated with the polarization of the embryo and the determination of unequal cytoplasmic division at the first cell division, and b) the use of raised temperature in the production of abnormal eggs which should make possible a study of the role of the nucleus in development and the importance of the nuclear-cytoplasmic ratio in normal embryogenesis.

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1963 #35

EFFECT OF POLYURIDYLIC ACID ON DEVELOPMENT AND PROTEIN SYNTHESIS OF INTACT SAND DOLLAR EMBRYOS

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Previous studies on polyuridylic acid (polyU) in subcellular systems suggest that if messenger RNAs can cross the cell membrane, they should redirect the pattern of protein synthesis of intact cells. This study describes the effect of polyU on the pattern of development and protein synthesis by intact sand dollar embryos (Echinarachnius parma). Embryos incubated in sea water containing 500 to 1000 μg/ml of polyU developed normally to the early blastula stage. Thereafter the central cavity became filled with cells producing a multicellular mass which underwent subsequent opacification and degeneration. External cell layers did not develop ciliation. We have

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termed this phenomenon "blastula arrest." A study of the effects of concentration and time of addition of polyU indicated that despite the fact that polyU does not produce its effect until blastulation, the degree of effect is most pronounced by initiation of treatment just after fertilization. From gastrulation onward, polyU had little effect on morphogenesis. When equimolar amounts of uracil or uridine were substituted for polyU, embryos developed normally. Unfertilized eggs incorporated small amounts of C^{14}-phenylalanine into protein. Fertilization resulted in a sevenfold increase in this incorporation. PolyU also caused a nonspecific stimulus to amino acid incorporation by unfertilized eggs, perhaps by mimicking the effects of parthenogenetic agents. On the other hand, polyU added 30 seconds after fertilization specifically stimulated phenylalanine incorporation and inhibited leucine and histidine incorporation. Taken together, these findings suggest that when added to developing embryos polyU induces the synthesis of a "nonsense protein" at the expense of protein(s) necessary for gastrulation.

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1963 #36

ANTIMORPHOGENETIC ACTIVITY OF ACTINOMYCIN D

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Actinomycin D acts to prevent the DNA-dependent formation of RNA in a variety of animal and microbial systems. The primary effect is on the inhibition of synthesis of messenger RNA. It is to be presumed that morphogenesis and cellular differentiation in embryogenesis is directed by a timed release of cogent information contained in RNA 'transcribed' from DNA and expressed in specific protein synthesis.

Actinomycin D was presented to freshly fertilized eggs of Fundulus heteroclitis in varying concentrations under controlled conditions and the development of the eggs and the embryos studied in comparison with controls. Cell division is not impeded by the concentrations used nor is oxygen uptake altered when studied polarographically. Blastodisc formation is irregular and no axiation supervenes. The cells of the blastodisc migrate irregularly over the yolk in a manner similar to the growth of a tissue culture. The result is a uniform sphere of cells covering the yolk. Cell differentiation and morphogenesis do not take place. Upon removal of Actinomycin D from the embryos, growth and cell division continues. Cellular differentiation takes place at random in the surface sheet expressed as blood islands and pigment cells. Morphogenetic activity is not resumed and no recognizable morphogenesis occurs up until hatching of the controls. It is concluded that morphogenesis is dependent upon information carried from the nucleus via messenger RNA. Furthermore, morphogenetic information is essential for development since following default morphogenesis cannot be resumed whereas RNA mediated information for cellular differentiation can be reconstituted.

A large number of embryos were subjected to incubation in 2×10^{-3} M NaCN for various periods of time, starting at various stages in development. It was found that until stages of late blastula or early gastrula were reached, there was no effect of the cyanide on embryo development. Once the gastrula stage was reached, the presence of cyanide was completely inhibitory to further development and the inhibition was reversible. In other words, it appears that anaer-