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-

obic production of ATP is sufficient for the processes preceding gastrulation and that either the quantity or source of ATP from aerobic metabolism is required for the development at all stages beyond gastrulation. It was shown that lactic acid accumulates in large quantities in embryos subjected to cyanide, thus indicating that the metabolic apparatus for anaerobic ATP production is present. Furthermore, the resumption of oxygen consumption and the diminution of accumulated lactic acid following removal of embryos from the cyanide demonstrated an approximate one day lag corresponding to the one day lag in the resumption of morphogenesis. Thus, it is concluded that aerobic sources of ATP production are required for embryo development from gastrulation on and are not required for the minimal conditions of embryo vitality.

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1963 #37

THE EFFECT OF LIPOIC ACID ON THE DEVELOPING EGGS OF THE SAND DOLLAR

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In addition to its activity as a co-factor in oxidative decarboxylation, lipoic acid disturbs normal morphogenetic and regenerative processes when applied exogenously to several invertebrates. To distinguish between possible effects on cell division and on differentiation, we have applied lipoic acid to the embryos of the sand dollar Echinarachnius parma during several developmental stages.

We observed that the rate and form of cell division were undisturbed by concentrations of lipoic acid which induce marked changes in differentiating embryos. Embryos remaining in 10^{-5} M lipoic acid are arrested as spinning blastulae.

To define the period of maximum sensitivity of the embryos to lipoic acid we used short exposures at various times during development, and found that the early cleavage stages were sensitive to the deleterious action of the lipoic whereas blastulae and subsequent stages were not. We conclude that the cellular components sensitive to the action of lipoic acid are present during early cleavage but presumably remain inactive until gastrulation.

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