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## STUDIES ON THE ROLE OF PROTEIN SYNTHESIS IN GENETIC REPLICATION, KARYOKINESIS AND CYTOKINESIS DURING THE FIRST CLEAVAGE CYCLE IN THE SAND-DOLLAR EMBRYO

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Ova of the sand-dollar species Echinarachnius parma were added to cycloheximide (1 $\mu$g/ml) at varying times before and following fertilization; this concentration of cycloheximide inhibits synthesis of protein in these animals in excess of 97%. Effects of drug exposure upon replication of DNA were evaluated by measuring incorporation of $^{3}$H-TdR† into an acid-insoluble fraction. Intracellular localization of the isotope and morphologic alterations associated with drug treatment were monitored by histologic and autoradiographic examination.

Exposure to cycloheximide prior to fertilization did not alter synthesis of DNA during the first replication (or "S") period; however, exposure to the drug within the first 15 minutes following fertilization produced greater than 90% inhibition of DNA synthesis during the second "S" phase. Cytocleavage in these animals was similarly prevented. When embryos were first exposed to cycloheximide 30 minutes following fertilization, the second "S" period was accomplished normally although the majority of animals did not cleave. Embryos exposed to the drug within 15 minutes of fertilization appeared to be arrested in an interphase nuclear configuration, pro-nuclear fusion having been accomplished normally. With later exposures to the drug, developmental arrest at metaphase, anaphase and telophase was observed. In view of the 40-minute time lag between the synthesis of the requisite proteins and evidence of their morphologic effect, control mechanisms other than protein synthesis are involved in the timing of the cleavage events. By inducing polyspermy within fertilized embryos which had been rendered incapable of replicating their own nuclear DNA by exposure to cycloheximide, we investigated the nature of a protein required for normal synthesis of DNA during the second "S" period. These embryos were still capable of synthesizing DNA on templates supplied by the newly introduced sperm. We suggest that among the enzymes synthesized following fertilization are one or more which carry out the nuclear transformations grouped under the term karyokinesis and that embryos which lack these enzymes can carry out but a single replication of their genetic material.

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## STUDIES ON NUCLEIC ACID AND PROTEIN METABOLISM ON SAND-DOLLAR EMBRYOS

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As a part of a study on the role of preformed deoxyribonucleosidic materials present in embryonic cells, we injected $^{3}$H-TdR† into females of the sand-dollar species Echinarachnius parma during oogenesis. The injections were given daily over a ten-day period commencing shortly following KCl-induced shedding. Four days following the last injection the prelabeled

† = $^{3}$H-thymidine

female was again induced to shed; the eggs so obtained developed normally to the pluteus stage. Samples of maternal tissues, ova, and developing embryos at various stages were fixed in formalin and prepared for autoradiography which disclosed that all ova contained acid-insoluble radioactivity; approximately 20% being very heavily labeled. Radioactivity was present only over the cytoplasm; minimal or no labeling was observed over pronuclei or polar bodies. Within an hour following fertilization, nuclei became intensely labeled, due in part to transfer of labeled material from cytoplasm to nucleus. In division figures grains were seen over the condensed chromosomes. This type of chase experiment conclusively demonstrates the existence and functional significance of a precursor pool of deoxyribonucleosidic or -tidic material that becomes associated with the chromosomes as genetic replication occurs. Examination of the maternal tissues disclosed extensive incorporation of radioactivity into the nuclei of the so-called visceral-lining layer over the ovaries; label was also present over nuclei of early phase oocytes. Nuclear labeling over more mature forms decreased progressively; however, mature oocytes contained considerable label over the cytoplasm.

Studies on the role of the synthesis of protein in the initial cleavage cycles of the sand dollar embryo were carried out by placing the embryos in cycloheximide at a drug concentration (1 $\mu$g/ml) which inhibited synthesis of protein in excess of 97%. Cleavage did not occur when embryos were added to the drug within 20 minutes following fertilization. When embryos were so treated at 25, 30, and 35 minutes respectively following fertilization, cleavage figures were seen in progressively increasing numbers when examined at 120 minutes. This data indicates that the developing embryo synthesizes those proteins required for a successful first cleavage within the first 35 minutes following fertilization; that cleavage actually occurs at 90 minutes following fertilization.