cytochrome inhibition and uncoupling were lost at 13°. Whether this is a manifestation of a specific temperature effect on metabolic processes or whether this is produced by decreased diffusion of inhibitors to critical intercellular sites remains to be elucidated.

Use of hemolyzed cells showed no significant decrease of O_2 consumption at 13° as compared to intact cells, indicating the possibility of studying metabolic events in this unit in a cell free system.

Aerobic glycolysis as indicated by lactate production was markedly reduced, or absent, at 13°C suggesting that at ambient fish temperature oxydative dependent metabolism is the dominant mode of energy generation.

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PROTEIN SYNTHESIS IN SAND DOLLAR EMBRYOS

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This investigational program is directed toward increasing our understanding of events which occur in the course of embryogenesis and of the mechanisms by which chemical agents alter normal embryonic development. In previous years, Dr. Karnofsky has employed microscopic observation of living embryos supplemented by histologic and radioautographic examination of fixed and sectioned materials to analyze drug effects upon developing embryos of Echinarachinus parma, a sand dollar species common along the coast of Maine. This summer we have developed techniques to utilize bulk incorporation of radioactive materials into protein and nucleic acids of populations of embryos in order to examine pharmacologic effects in biochemical terms. The radioactive compounds we have used include: thymidine, cytidine, 5 bromodeoxyuridine and leucine, labeled either with carbon-14 or tritium. The following discussion is a preliminary analysis of the data presently available.

1. The hydroxamic acids N. hydroxyurea and N. hydroxyurethane inhibit synthesis of DNA in mammalian cells and in some bacteria. They interfere with the reduction of ribonucleotide diphosphates to deoxyribonucleotide diphosphates in cell free mammalian systems. In the developing sand dollar embryo treated with hydroxyurea or hydroxyurethane, thymidine is incorporated normally into DNA of the embryos for the first four (DNA) synthesis periods following fertilization; drug induced-inhibition of thymidine uptake is detectable in the fifth synthesis period. These observations are consistent with the concept, suggested by chemical analysis of sea urchin ova, that Echinoderm ova contain "stored" deoxynucleotide materials which are sufficient to supply their needs for the first few periods of DNA synthesis. When these "stored" materials are exhausted the embryo must use the reductive pathways from the ribonucleotides in order to continue replication of DNA. If these pathways are (chemically) blocked normal replication cannot be carried out, the embryo becomes visibly abnormal, and undergoes death and cytolysis.

2. Two purine ribosides (adenosine and guanosine) and two pyrimidine ribosides (5-fluorouridine and 5-bromouridine) inhibited incorporation of a radioactive pyrimidinecleoxyriboside (5-bromodeoxyuridine-^{14}C) into DNA of sand dollar embryos in the first two hours following

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fertilization. Between two and four hours following fertilization the purine ribosides were not inhibitory and the degree of inhibition induced by the pyrimidine ribosides decreased greatly. These experiments suggest that the kinases initially present in the sand dollar embryo phosphorylate multiple substrates but that as development proceeds new enzymes appear which possess greater substrate specificity. Detailed exploration of the changing enzyme characteristics may be undertaken in subsequent years.

3. In an effort to examine the relationships between synthesis of protein, replication of DNA and cell division we have continued studies on the effects of cycloheximide, pactamycin and puromycin upon Echinarachinus embryos. This year we demonstrated that each of the three drugs rapidly inhibits incorporation of leucine into the developing embryo. Depending upon time of drug addition and drug concentration these drugs prevent successful cell division. Both in inducing gross morphologic damage and in its inhibitory effects on leucine uptake cycloheximide is by far the most potent. We plan to pursue this line of investigation further in subsequent seasons.