transport) are much higher than in mammalian erythrocytes. Cyanide, antimycin-A, and 2,4 dinitrophenol did not alter the active transport of Na^+. Active transport continued, even though cyanide and antimycin-A reduced oxygen consumption by 70%, and 2,4, dinitrophenol, a classic uncoupler of oxidative phosphorylation, stimulated oxygen consumption by 34%. Ouabain, which does not significantly alter O_2 consumption in the dogfish erythrocyte does markedly inhibit Na^+ transport.

It would thus appear that Na^+ transport in this sytem is not dependent upon energy derived from the cytochromechain. Whether anaerobic glycolysis or non-cytochrome mediated oxidative metabolism represents the energy source for cation transport remains to be elucidated.

1965 #40

CHARACTERISTICS OF OXYGEN CONSUMPTION OF THE DOGFISH ERYTHROCYTE

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The metabolic characteristics of the dogfish erythrocyte are of interest because they are nucleated and maintain active metabolic processes at low ambient temperatures. Oxygen consumption of dogfish erythrocytes was studied in a Gilson differential respirometer. The erythrocytes were separated by centrifugation and washed twice with cold dogfish Ringers. Cells from different fish were not mixed since such mixing causes hemolysis. After gassing with O_2, the flasks were isolated and readings taken at 15 minute intervals. Because of the low HCO_3^- in dogfish plasma, a post-gassing technique was developed for adding HCO_3^- in to the Ringers. Cells were studied in oxygen with and without metabolic inhibitors. After 15-30 minutes the vessels stabilized and serial 15 minute readings gave close agreement. The data are summarized in the table.

Table

OXYGEN CONSUMPTION IN μl/hr/gm ERYTHROCYTES

Experimental conditions	30°	12-14°C
Control	49.9 ± 12.0 (22)*	15.4 ± 5.5 (11)
Cyanide, 10^{-3}M	17.1 ± 3.9 (8)	15.5 ___ (2)
DNP, 10^{-5}M	68.9 ± 23.5 (16)	16.8 ± 5.3 (12)
Ouabain, 10^{-4}M	44.3 ± 8.5 (11)	15.0 ± 6.1 (10)
Antimycin-A, 50 pgm/flask	17.8 ± 3.6 (11)	15.9 ___ (2)

*( ) = number of studies performed.

Unlike mammalian erythrocytes there is distinct and measurable O_2 consumption by these erythrocytes. In addition, a cytochrome system is present in dogfish erythrocytes as evidenced by the partial inhibition of O_2 consumption by cyanide and antimycin-A. The increase in O_2 induced by dinitrophenol at 30°C, indicates that oxidative phosphorylation in this system resembles that found in many mammalian systems. Of great interest was the demonstration that both

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.

1965 #41

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

*Supported by Grants from American Cancer Society and National Cancer Institute.