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INFLUENCE OF AMBIENT TEMPERATURE ON PROLIFERATION RATE OF THE SAND DOLLAR EMBRYO *Echinarachnius parma*

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Fertilized sand dollar eggs have been used for some time as a model for studies of differentiation. In addition, previous work by Karnofsky, et al (Progr. Exp. Tumor Res., 3:254, 1963) has demonstrated the utility of this system in detecting chemical agents which exert metabolic effects on the sand dollar embryo and have important parallels for human proliferating malignant cells. Most of these latter studies have been more concerned with the effects of drugs in the later stages of embryo development than on the initial fertilized egg cleavage rates. These early stages of embryo development are of some broad interest in that direct visual measurement of cleavage rates can be readily made between the 2 and 16 cell stage on fixed embryos, making unnecessary the usually derived biochemical or metabolic indicators of cell division.

One of the prime concerns of modern drug therapy for malignant disease is the relationship of cellular proliferation to the cytotoxic effects of drugs. This has led many drugs to be classified as cell-cycle-active or cell-cycle-inactive with respect to their cytotoxicity on a proliferating or a resting cell population. A resultant but as yet unresolved problem is the development of techniques which can modulate cell proliferation rates so as to render such cells increasingly sensitive to drugs which require a proliferating cell pool to demonstrate major cytotoxic effects. Towards this end therefore, the influence of temperature on sand dollar egg proliferation was studied, as this system, because of its visually observable and slow cell (embryo) proliferation rate, is particularly suited to the investigation of potential modulators of proliferation.

Mature *Echinarachnius parma* were harvested from Frenchman Bay and maintained in sea water at ambient (15° C) temperature prior to study. All sand dollars were studied within three days of collection. Ovulation and spermiation were induced employing customary techniques of injecting 0.5 molar potassium chloride into the anal pore. Fertilization was effected at ambient sea water temperature. After appropriate mixing and diluting with sea water the fertilized egg population samples were divided and sequentially observed at differing sea water temperatures. Temperature ranges studied were between 11° and 28° C and were maintained constant throughout the period of experimentation by placing the incubation vessels in constant temperature baths or in monitored ice water pans. At appropriate time intervals following fertilization (½ to 12 hours) aliquots of the fertilized eggs were removed from the individual containers fixed with a tenfold dilution of 25% formaline and observed under the microscope with visible light for morphologic integrity and stage of cleavage. All experiments where fertilization rate was less than 95% were discarded. Cleavage stage was characterized as complete when at least 50% of morphologically intact embryos had undergone appropriate division, i.e. the two cell stage (first cleavage) preparation had less than 50% of cells uncleaved and more than 50% completely cleaved.

The results of these studies indicated that time from fertilization to the first cleavage (2 cell) stage was markedly temperature dependent. From 10°C to 23°C time of fertilization ranged from 255 minutes to 73 minutes respectively. The kinetic relationship between ambient temperature and time to first fertilization was non-linear and indirect. For 23°C time to first cleavage was 73 minutes (range 80-65) for 19°C, 80 minutes (range 75-90), 16.5°C, 96 minutes (range 90-105), 11.5°C, 170 minutes (range 145-190). Although there was variation between experiments on different days in six studies these relationships were qualitatively similar. When these studies were evaluated with regard to time required to reach second, third, and fourth cleavage stages (4 cell, 8 cell, 16 cell, respectively) a similar temperature effect was seen. Decreasing temperature prolonged the time required for successive fertilizations. When a doubling time for cleavage was derived for the proliferation rate from the 2 to the 16 cell stage, the following figures were obtained: 23°C, 30 minutes; 19°C, 38 minutes; 16.5°C, 47 minutes; 14°C, 60 minutes; 11°C, 76 minutes. These data indicate that temperature effects the proliferating fertilized sand dollar embryo in two important ways: 1) to prolong the duration of the one cell stage or the time to initial cleavage, and 2) influences the rate of subsequent cell cleavages in a reciprocal non-linear fashion.

These physical effects of temperature on proliferation rates were then evaluated to assess the influences of such changes on the cytotoxicity by the agents cyclophosphamide and methotrexate. At drug dosages of 0.1 to 10 gamma per ml of incubation mixture no drug effects on proliferation rate or on direct cytotoxicity were visualized on the formalin fixed specimens at any temperature. These studies, which confirm and extend an early observation by Rieck, et al (Bull. Mt. Des. Is. Biol. Lab., 4:75, 1959) indicate that in a suitably slow proliferating system, ambient temperature variations from approximately 5°C below normal growth temperatures to 10°C above normal growth temperature exert important and discernible differences in rates of cell division. These differences can be evaluated with regard to drug incorporation and cytotoxicity using appropriate systems, and will form the basis of future studies.

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THE BILIARY EXCRETION OF THE ORGANIC ANIONS, SULFOBROMOPHTHALEIN (BSP) AND PHENOLDIBROMOPHTHALEIN (DBSP) BY *Squalus acanthias*

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The excretion of many organic anions in mammals is dependent on hepatic uptake from plasma, conjugation to water soluble metabolites and transport into bile against a high concentration gradient. Previous studies indicate that the liver of the dogfish shark is also capable of excreting organic anions such as BSP into bile (Bradley, et al. Bull. MDIBL 5:3, 1965). However, the importance of the bile as the major route of excretion of these compounds is in doubt, not only because dog shark livers lack organic anion binding proteins (Levine, R.I. et al. Nature New Biology 231:277-279, 1971) but presumably because these compounds may be eliminated by the gills or kidneys (Brodie, B.B. Pharmacologist 6:12, 1964).