

## TISSUE CULTURE AND VIROLOGICAL STUDIES ON MARINE ORGANISMS

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Systematic studies on marine organisms as possible reservoirs of viruses pathogenic for terrestrial or fresh water animals or of indigenous viruses were initiated. A tissue culture laboratory was equipped so as to allow the handling of mammalian cell cultures by advanced methods for virological studies. Such cultures are fastidious, and adjustments to new locales have to be made. The test system consisted of the human carcinoma-derived KB cell line and several antigenic types of dengue virus (arthropod-borne viruses). The latter have been attenuated by passage through laboratory animals and are harmless for the human population. Their physical-chemical characteristics, antigenic relationships, and replication in cultured cells are under study in our St. Louis laboratory. A few critical experiments satisfied us that conditions at the M.D.I.B.L. were excellent (1) for establishment of KB cells in monolayers or in exponentially growing suspension cultures, (2) for productive infection of these cells with dengue viruses.

These results encouraged attempts at in vitro cultivation of dogfish embryo cells. Embryos from first year candles were minced and trypsinized, and the resulting single cell suspensions were seeded in medium consisting of salts (Earle's saline) at 2 x the conc. used for mammalian cells, vitamins, glucose, chick embryo extract, and various sera. 10% human plus 10% calf serum favored attachment and spreading of cells on plastic Petri dishes. Dogfish serum was unfavorable. Cultures were incubated at sea water temp. (13-17°C) in a humidified 4-5% CO<sub>2</sub> air atmosphere. With 2-4 x 10<sup>6</sup> cells/ml seeded, confluent monolayers formed which survived for 2 weeks. Mitosis or net increase in cell number was not observed, but radioautography with H<sup>3</sup> thymidine (30-45 minute bursts) revealed active incorporation into nuclei. Prelim. attempts to "infect" cultures with phenol-extracted dengue RNA gave inconclusive results.

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## STUDIES OF DIFFERENTIATION IN CELLULAR SYSTEMS

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Preliminary investigations were conducted to establish qualitative and quantitative aspects of energy production and utilization during the embryonic development of the teleost Fundulus heteroclitis. Employing polarographic and spectrophotometric techniques it was established that these embryos exhibit active DPNH- and succinic-oxidase, DPNH- and succinic-cytochrome c reductase, and lactic dehydrogenase. Cyanide was found to completely inhibit oxygen consumption and morphogenesis with little or not apparent lethal effect on the embryos. Removal of this inhibitor allowed the embryos to continue their development. It was also noted that whereas an anti-mycin insensitive system is present for oxidation of pyridine nucleotides at early stages, an anti-mycin sensitive system appears with further development.

One of us carried out preliminary investigations on the cytological and metabolic aspects of