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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 indiginous 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 cultuvation 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_2 air atmosphere. With 2-4 x 10^6 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^3 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 antimycin insensitive system is present for oxidation of pyridine nucleotides at early stages, an antimycin sensitive system appears with further development.

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

limb regeneration in the crustacean hermit crabs. The whole animal respiration rate was found to increase over the normal level following autotomy of the legs, the more legs removed the greater the increase in respiratory rate. The period of increased respiratory rate coincides with the period of growth and differentiation of the limb regenerates. The adult muscle structure consists of bundles of myofibrils interspersed with mitochondria arranged in a multinucleate syncytium. Light and electron microscopic studies of fixed material are presently being carried out to elucidate the details of muscle development.

In conjunction with these problems we applied and modified the disc electrophoresis and paper chromotographic techniques to the study of protein components in developing cellular systems. Preliminary data indicate changes in the number of electrophoretic mobility of components during development.

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A DIFFERENCE BETWEEN TUMOR CELLS OF THE LOCAL OR PRIMARY TRANSPLANTED GROWTH AND TUMOR CELLS OF THE METASTASES

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Existing observations indicate that many tumor cell emboli, released by the primary cancer into veins, never develop into metastases. The following hypothesis was erected to explain this phenomenon. The cells of a primary tumor must change to produce new cells capable of growing out of a blood vessel into a new organ; since this change is in the nature of a mutation, the incidence of change is low. Hence, very few tumor cell emboli can yield metastases.

If this hypothesis were true, then cells of the metastasis should yield more metastases than cells of the primary tumor. Experiments were designed to test this hypothesis, using the B16 melanoma in C57 mice. Metastases were produced by the intravenous injection of a cell suspension made from a stock tumor. Then cells of the metastases were injected subcutaneously into a normal mouse to make a "metastasis stock" tumor. This tumor was to be compared with the "regular stock" tumor, the tumor which was always passed subcutaneously. Suspensions of tumor cells were made from "metastasis stock" and "regular stock" tumors. Equal numbers of tumor cells were injected intravenously into corresponding series of mice. All mice were sacrificed one month later, and numbers of lung metastases were counted. Mice receiving cells from the "metastasis stock" tumor developed about 50 times as many lung tumors as did recipients of cells from "regular stock" tumors. This experiment indicates that cells of a metastasis differ from those of the primary tumor.

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