CB 23

ULTRACYTOCHEMICAL STUDIES OF CERIUM PRECIPITATION IN THE TADPOLE TAIL OF RANA JAPONICA DURING METAMORPHOSIS.

F.Sasaki, T.Kinoshita, H.Takahama and K. Watanabe. Dept. of Biology, School of Dental Medicine, Tsurumi University, Yokohama.

Degeneration of tadpole tail tissue was investigated cytochemically by the localization of hydogen peroxide production using the cerium-method (Briggs et al. '75; Ohno et al. '82). No reaction product was found in resting macrophages and intact muscle cells during premetamorphosis. During metamorphosis, extensive cerium precipitations were observed on the outer surface of the plasma membrane of phagocytotic macrophages, fibroblasts, neutrophils, epidermal cells, muscle cells, notochordal cells, nerve cells and capillary endothelial cells. Reaction product was observed on the surface membrane of processes of macrophage and facing neighbouring cells. The addition of catalase, the amount of deposits was decreased. α-tocopherol and indomethacin, but not dexamethasone significantly inhibited the formation of the reaction product. These findings indicate that the active oxygen production sites were on the plasma membranes, the phagosomal membrane in macrophages, and on the plasma membrane in degenerating myelin sheath and notochordal cell.

CB 24

EFFECTS OF UV AND X-RAYS ON THE CULTURED CHROMATOBLASTOMA CELLS.

H,Etoh[1] and I. Suyama[2], [1]Div. of Biol., [2]Div. of Environ. Health, Natl. Inst. Radiol. Sci., Chiba.

Chromatoblastoma, pigment cell tumor induced spontaneously in aged goldfish, was transferred into in vitro culture system, and a cell line, CAEP, was established. Doubling time of CAEP cells (38h) estimated from growth curve was shorter than that of CAF cells (48h). Measurement of DNA/cell by cytofluorometry showed tetraploid amount of DNA for CAEP cells but diploid amount for CAF cells. The values of D_0, D_q and extrapolation number estimated from dose-survival curves were 660 rad, 930 rad and 4 for X-rays and 1.3 J/m^2, 2.8 J/m^2 and 10 for UV, respectively. On the appolication of split-dose of X-rays, recovery in colony forming ability was obtained. When photoreactivating light was administered immediately after UV-irradiation, photoreacivation was found (1.7 J/m^2 for D_0, 8.1 J/m^2 for D_q and 105 for n). When UV-irradiated cells were kept in caffeine containing medium (0.1mg/ml) until the termination of the experiment, diminished survivals were obtained (1.0 J/m^2 for D_0, 1.0 J/m^2 for D_q and 2.5 for n). From the present results, it seems that the CAEP cells are more sensitive to UV but more resistant to X-rays and that the nature of recovery from UV-induced damage may be different between the CAF and CAEP cells.

CB 25

AN ATTEMPT TO OBTAIN PURELY HAPLOID CELL LINES OF THE MEDAKA (Oryzias latipes).

J.Komura, K.Naruse, A.Shima. Zool. Inst., Fac. Sci., Univ. of Tokyo, Tokyo.

In cultured cell lines derived from gynogenetic haploid embryos of the Medaka, haploid population decreased, and diploid variants which seemed to arise by endomitosis increased with advancing subculture. We tried to obtain purely haploid population from the mixture of haploid and diploid cells, because the haploid cells would be useful for mutation research. Each cell line was originated from 12-24 gynogenetic embryos (4 days, stage 26-27), and cultivated in L-15 medium supplemented with 20% FBS at 27°C. Cellular DNA content was examined by DAPI-DNA microfluorometry. Despite extensive attempts, cloning of the haploid cells has, to date, been unsuccessful due to very low colony forming ability. Cells did not form any colonies irrespective of presence or absence of the conditioned medium. Further, the cells on feeder layer showed low plating efficiency (0.10-1.02%). The fractionation of the haploid cells, which seemed to be smaller in size than the diploid cells, by gravitation through 1-3% BSA gradient, was not effective enough to exclude the diploid cells.

CB 26

A CLONAL CELL LINE(TN-1) DERIVED FROM A SCORPION FISH.

T.KUBO[1]and N.EBITANI[2] [1]Biol.Lab.,Sophia Univ. ,Tokyo and[2]Biol.Lab.,Shohoku Coll.,Atsugi.

A clonal cell line(TN-1) with 50% plating efficiency was established by a single-cell method. This cell line was isolated from SMF cells(275 PDN) derived from normal fins of a scorpion fish, *Sebasticus marmoratus*. A single-cell method applied in this study is as follows: 1)Culture medium:TC-199 supplemented with 15% FBS, 2.28g/l NaCl and antibiotics.2)10-12 plastic sheets for tissue culture(14mmφ,Wako) were scattered in a 10cm petri dish.3)50-100 SMF cells suspended in 15ml of the conditioned medium were inoculated in the petri dish and kept at 25°C. 4)On the second day, the plastic sheet covered with only one cell was transferred to a 25cm^2 culture flask containing 5ml of conditioned medium.5)Confirmation of the cells to make up only one colony was performed several times. Chromosome number is 98 (4 metacentric and 94 acrocentric). Population doubling time is about 37 hours. Plating efficiency is 51-57% when 100-400 cells/25cm^2 were cultured in conditioned medium. When TN-1 cells were treated with various concentration of 4NQO and MNNG for 90 minutes at 25°C, the D_{37} values were 4μM and 1.2μg/ml respectively. TN-1 cells is a very useful material for *in vitro* study of the effects of radiations, chemicals and other environmental agents on fish cells.