DNA cytofluorometry combined with ^{3}H-TdR ARG of the cultured cells from 4-HAQO induced sarcoma of the rat

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The present study was undertaken for the detailed analysis of cell kinetics of the cultured 4-HAQO induced sarcoma showing active polyploid and aneuploid cell proliferation, using DNA cytofluorometry combined with ^{3}H-TdR ARG.

The cultured cells were smeared onto the cover-glass 30 mins after labeling with ^{3}H-TdR (1 μCi/ml in medium), and were processed for ARG by a dipping method, followed by developing and nuclear DNA stain with PI. This smeared coverglass was then mounted with balsam on the slide-glass with the emulsion-side facing downward. Both the red fluorescence from nuclear DNA and a part of green excitation light reflected on the silver grains of ^{3}H-TdR were simultaneously measured by an epi-illumination cytofluorometer (NIKON SPM-RF1-D).

From this analysis, we could clearly distinguish indivisual, proliferative populations involving aneuploid cells, and also compare DNA synthesis rate of each cell by its incorporation activity of ^{3}H-TdR. These informations indicated that the DNA synthesis rate was different in each cell and increased in proportion to nuclear DNA contents.

Studies on Heterogeneity of Fluorescence Labeled Chromatin

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We studied on differences between the euchromatin and heterochromatin by determining the fluorescence and optical density with fluorochromes. The euchromatin and heterochromatin were extracted from rat river nuclei, and labeled with fluorochromes before fractionation. The fluorochromes, able to interact to DNA or chromatin, such as ethidium bromide, Hoechst 33258, mithramycin or chloro tetracycline was employed to this work. The labeled chromatin was fractionated on a gel filtration of Sephadex G-25 or a sucrose density gradient centrifugation.

Differences were found in the profiles of euchromatin-fluorochrome complexes, and more prominent in those combined with the heterochromatin. The heterogeneity of the chromatin was amplified with fluorescence labeling in the fraction. The difference can be characterized between the subfractions of the euchromatin and heterochromatin in which the fluorochrome is located or not.

A new color-modified DAB reaction for cytochrome oxidase and its use for microspectrophotometry

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1) A new color-modified diaminobenzidine (DAB) reaction for cytochrome oxidase has been developed. The new reagent consisted of 2.5mM cobalt acetylacetonate, 2.5mM DAB·4HCl, 0.25 mg/ml cytochrome c and 0.1 mg/ml catalase dissolved in 0.1M cacodylate-HCl buffer of pH 7.4. 2) On treating rat liver specimens with the reagent for 10 min at 37°C, mitochondria turned blue. They were the only site of positive reaction in liver cells. 3) The reaction was inhibited by heating the specimens at 80°C prior to reaction or by adding KCN to the reagent. 4) The reaction product showed a sharp absorption peak at 600nm. The peak shifted to shorter wave lengths as the staining was weaker. 5) A standard step wedge containing known amounts of reaction product was prepared. This wedge permitted to establish the conformity to Beer's law and an absolute quantification of the product in terms of g DAB equivalent per unit area. 6) The reaction conformed to Michaelis-Menten's formula, implying its enzymatic nature. 7) A microspectrophotometric quantification of cytochrome oxidase was carried out by scanning at 600nm. A local difference in activity with respect to the liver lobule was found.

Hematoporphyrin Derivative (HpD) Uptake and Photocytotoxity of Cultivated Human Bladder Cancer Cell Line: KK-47.

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Asynchronous KK-47 cells were incubated in serum-free Eagle MEM containing various concentrations of hematoporphyrin derivative (HpD) for 30 min at 37 °C. After washing out free HpD molecules, cultivation was continued in HpD-free medium for 0, 2, 4 and 6 hr. The HpD containing cells were irradiated by argon-dye laser light (635±5 nm, 0.26 mW/cm^2).

The relationship between the mode of cellular HpD uptake and photodyenamic cellular inactivation by the laser light was studied using a clonogenic assay system. Cellular photoinactivation increased with increasing concentration of intracellular HpD and increasing incubation times after the washing. The photodynamic action on the cells incorporated HpD aggregate at long incubation was significantly larger than that on the cells to which HpD will be weakly bound within 2 hr incubation.

Morphological changes in the cells treated with the irradiation were observed under a phase contrast microscope and a light microscope.