Computer Analysis of Sub-parameters of Depurination and Depolymerization Rate Constants in Feulgen DNA Hydrolysis

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Feulgen DNA hydrolysis curves derived from cytofluorometry at various temperatures and HCl-concentrations were analysed by computer with least squares fit to Bateman function. By comparing the depurination(k_1) and depolymerization (k_2) rate constants at different hydrolysis conditions, it was found that the two parameters can be expressed as a form $k=AN^2.\exp(-B/T)=AN^2.\exp(-E/RT)$, where A and B are constants, N is the HCl-concentration in normal, T is its absolute temperature, E is the activation energy, and R is the gas constant. For 4C mouse hepatocytes fixed with methanol, it was calculated that $A_1=5.4\times10^{14}$, $B_1=12133.5$, $A_2=6.2\times10^{14}$, $B_2=12181.7$, $E_1=24.097$Kcal, $E_2=24.194$Kcal. Theoretical hydrolysis curves synthesized by computer with the calculated values coincided with those obtained by experiment satisfactorily. The peak time of a hydrolysis curve is found to be expressed in a form, $t_p=DN^2.\exp(B_1/T)$, and it was calculated, $D=1.8\times10^{-15}$, $B_1=12149.4$. The peak times of hydrolysis curves determined by experimental analyses and theoretical estimations also coincided with each others. k_1 or k_2 is a probablity of HCl to be adsorbed(AN^2) by DNA after collision, $\exp(-B/T)$.

QUANTITATIVE HISTOCHEMICAL STUDIES OF THE RELATION BETWEEN CELL PROLIFERATION AND DIFFERENTIATION IN BONE TUMORS

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In this paper, we have attempted to analyze the cell kinetics of the bone tumors using DNA-RNA cytofluorometry (with NIKON SPM RF1-D). One case of fibrosarcoma of the bone was composed of both the intraosseous regions consisting of fibrocytic tumor cells and the invasive, extraosseous regions of remarkablly pleomorphic fibroblastic tumor cells. It was cytofluorometrically found that diploid or aneuploid cell proliferation was more active in the latter regions than the formers. In one case of osteosarcoma showing notable, extraosseous growth, polyploid cell proliferations were also more active in the regions consisting of many pleomorphic histiocyte-like cells than in the regions of well differentiated cells with abundant osteoid or cartilage matrix. And from the same studies of three cases of giant cell tumor of the bone, it was shown that diploid cell proliferation was more active in histiocytic or fibroblastic cell regions than in fibrocytic cell regions. It was therefore suggested that in more invasive growth regions of bone tumors, cell proliferation was very active, depressing cell differentiation.

Cytofluorometric Nuclear DNA-determinations in Human Corneal Endothelial Cells

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In order to investigate cell kinetics of human corneal endothelium, nuclear DNA-cytofluorometry was carried out.

Cell preparation was performed with two methods. One method was soft touch smears of 31 human corneal endothelium taken from 21 autopsies and 10 donor eyes. By method of Fujita(1973), pararosaniline-Feulgen nuclear staining and DNA-cytofluorometry were performed. The other was preparation of corneal endothelial layer obtained by mechanical separation in distilled water after treatment in 0.15M PBS containing 17mM EDTA and fixation with absolute methanol.

Polyploid nuclei which demonstrate abortive cell division after DNA-synthesis appeared with DNA-contents of tetraploid(19 cases) and octaploid(2 cases). All other nuclei had DNA levels within the range of diploid class and neither nuclei of intermediate DNA-values between 2C and 4C, nor ones between 4C and 8C were found. Many enlarged cells with diploid nuclei also observed on corneal endothelial sheets of aged cases.

It was concluded that almost human corneal endothelial cells are static cell population but that potentially a few cells are in renewing stage.

Combined Staining with DAPI and Rhodamine-labeled Lectin for DNA-cytofluorometry

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In order to differentiate mucin-containing cancer cells from interstitial cells in DNA determination of signet-ring cell carcinoma, we have developed a method for combined staining of DNA and mucin.

Specimens were surgically obtained signet-ring cell carcinoma fixed in Carnoy's solution and embedded in paraffin. The carcinoma tissue was separated from 100μm-thick section under a stereomicroscope. After isolation with EDTA, collagenase and ultrasonication the cells were smeared and fixed in absolute methanol. DAPI(4',6-diamidino-2-phenylindole) was chosen for DNA determination instead of Feulgen reaction to avoid modifying binding sites of lectin. To avoid reabsorbing the emission peak of DAPI, Rhodamine-labeled lectin was used instead of FITC. The smears were incubated with the labeled peanut lectin for 30 min at 37°C and subsequently with DAPI after the method of Hamada et al.(This meeting II-C-39).

In this method, accurate DNA-cytofluorometric determinations could be carried out for a fraction of cancer cell population isolated from human material by lectin-binding specificity.