A Trial on the Computer Analysis to the Intensity of Cytochemical Reaction Products in the Mitochondria

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Expression of the intensity of cytochemical reaction products has generally been used with signs, +,++, or strong and weak. But there is some unscientific sence in the expression. We have tried to express as the median filtrated gray values(GV) of the electron microscopic cytochemical image related reaction products of mitochondrial(M1,M2, M3) adenosinetriphosphatase(ATPase) activity by the aid of Zeiss image analyzing computer system, IBAS. The hypodermal epithelial mitochondria in the molting crayfish was used as a material. Prefixed tissue blocks were examined with Mg- Ca-ATPase activities and compared with the intensity to that of the control(C). The EM-photograms were analyzed under IBAS system in the same experimental conditions after two times filtration with" 11 x 11 "picture poins (homogenizing the picture intensity).

Three steps GV(M1,M2,M3) against the control (C) were:

82.38(M1):80.77(M2):45.82(M3)/103.34(C)

and the maxinum channel value in their histograms were:

81(M1):77(M2):33(M3)/101(C).

They can also be demonstrated in the histograms and the "profile" which is a line intensity analysis after with or without filtrations.

Nuclear DNA determination for the cells of the atrioventricular (AV) node.

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There is no attempt to investigate the process of the polyploidization of the cells of the cardiac conduction system. Our study was aimed to measure the DNA content of the AV node cell by cytofluorometry. The tissue sample is taken from the autopsied heart by the serial sectioning method devised by Lev et al. Thin section are made by 4-5μm and stained with H-E, Masson Trichrome, and Elastica Domagk for microscopic study. Every 10 slice, 150μm section is made for application to DNA cytofluorometry. After recognizing AV node in the thin section, subsequent thick section is observed by the stereoscopic microscope which enables us easily to recognize and pick up AV node from it. The cell isolation and staining were performed by the method described by Takamatsu et al (1980). DNA determination for the AV node cell can be carried out selectively because of the presence of its striated cytoplasm. The result of our study was the followings. Tere was no tendency to increase the polyploidization, irrespective of the heart weight. The DNA-ploidy pattern consisted of diploidy and tetraploidy. Almost all of AV node cells (89~95%) were in diploidy.

New Quantitative Double-staining Methods for Tryptophyl and Cysteinyl Residues and Protein

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The reliability of three new staining methods developed by the authors (Nakae et al., J. Histochem. Cytochem. 31, 967 (1983)) were examined with polyacrylamide gel films containing concanavalin A and ovalbumin. The methods were a 2-nitrophenylsulfenyl chloride technique for staining tryptophyl and cysteinyl residues together, a nitrophenylsulfenyl-2-mercaptoethanol method for tryptophyl only, and the combination of both these techniques with Coomassie Brilliant Blue for staining total protein. The relative contents of tryptophyl and cysteinyl residues of concanavalin A and ovalbumin in the films, calculated from absorbance values at 370 and 650 nm of double-stained films, were in good agreement with the theoretical values calculated from their amino acid compositions. Thus, these quantitative double-staining methods are reliable and should be useful for histochemical investigations of changes in the amount and composition of tissue proteins.

The method of Combined Cytofluorometric Determination of DNA and Sulfhydryl Groups of Nucleoprotein of Adult Mouse Hepatocytes

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In order to investigate the role of nucleoprotein in the cell cycle phase, SH groups including those produced by reduction of SS groups were measured cytofluorometrically using N-(7-dimethyl aminocoumarinyl)maleimide(DACM), simultaneously combined with DNA quantitation by propidium iodide(PI). Using albumin slides prepared by dipping in 10% bovine albumin solution, varying effects on DACM fluorescent intensity by reduction with dithiothreitol(DTT) and by blocking with N-ethylmaleimide(NEM) were studied, and quantitative nature of DACM fuorescence was tested by using polyacrylamide film which finally contained 0.15% to 2.38% of bovine albumin. Optimal conditions were applied for denuded nuclei of adult mouse hepatocytes fixed with 10% neutral buffered formalin, and then the fluorescence of DACM and further combined PI on each nuceli were measured. The ratio of SH+SS/DNA of 4C nuclei was equal to that of 8C nuclei, and larger than that of 2C ones.

Thus, the possibility of quantification of nucleoprotein was pointed out.