CYTOPHOTOMETRIC ANALYSIS OF CORRELATION BETWEEN CHROMATIN STRUCTURE OF INTERPHASE NUCLEI REVEALED IN UV LIGHT AND ON STAINING WITH GALLOCYANIN

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An interesting problem in the study of the suprachromosomal organization of chromatin is the extent to which the structure of the stained nuclei corresponds to the structure observed in UV-light, which more adequately reflects the distribution of material in the nucleus. The quantity of a nuclear stain such as gallocyanin and chrome alum (GCA) is known to correlate well with the number of phosphate groups of nucleic acids [4, 7]. Kiefer [8], who undertook comparative measurement of nuclear staining in UV light and after staining with GCA, showed that stoichiometric correlation exists between the quantity of dye and the nucleic acid content. However, no quantitative data could be found in the literature on the extent to which the distribution of dye corresponds to the structure of the nuclear chromatin observed in UV light. In the present investigation structures of the same interphase hepatocyte nuclei in mice and susliks were investigated before and after staining with GCA.

EXPERIMENTAL METHOD

Squash films were prepared from liver tissue and stained by Einarson's method [2]. Cytophotometric analysis was carried out on an SMP-05 scanning microscope – photometer (Opton, West Germany) in UV light at $\lambda = 265$ nm and at half the maximum of absorption of GCA, namely $\lambda = 497$ nm [8]. Memorizing of the coordinates and automatic discovery of the same nuclei (volume of sample 196) were done by computer on the PDP-12/20 program.

To identify the nuclear structure a set of criteria, shown below in Table 1, was drawn up and used. Geometric (Nos. 1 and 2), optical (No. 3), and combined (No. 4) characteristics of the chromatin structure of the interphase nuclei were compared. Coincidence of the coordinates of the centers of the granules (the center was taken to the point of maximal optical density) was evaluated by the criterion $t = \Delta Z / \Delta Z_{st}$, where $\Delta Z = (\Delta X^2 + \Delta I^2)^{1/2}$; ΔX, ΔI denote noncoincidence of coordinates along the X and Y axes in the same granule before and after staining; $\Delta Z_{st} = (\Delta X_{st}^2 + \Delta I_{st}^2)^{1/2}$ denotes noncoincidence of coordinates due to error of the scanning stage, equal to one scanning step ($\Delta X_{st} = \Delta Y_{st} = 1$).

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the geometric characteristics of the granules, the coordinates of their centers, and their area were identical.

This comparison of the structural elements identified with the aid of GCA and UV light showed that staining the nuclei with GCA reveals the real structure to a greater degree, and not only the heterogeneity (mosaic character) of the tinctorial properties, which at the same time must also make a definite contribution to the distribution of optical densities of the stained nucleus. However, this is a problem which requires special study.

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TABLE 1. Correlation between Chromatin Structure of Interphase Hepatocyte Nuclei Revealed by Staining with GCA (λ = 497 nm) and UV Light (λ = 265 nm)

Serial No.	Parameter	Criterion of correspondence of structures	Numerical values of criterion	p	Volume of sample (number of granules)
1	Coincidence of coordinates of granule centers	$t=\Delta Z:\Delta Z_{st}$	—	$\geqslant 0{,}05$*	568
2	Mean area of granule in UV light and on staining with GCA	t'	—	$\geqslant 0{,}01$	600
3	Coefficient of correlation between mean optical density of the same granule in UV light and on staining with GCA	r	0,84	$\geqslant 0{,}99$	586
4	Coefficient of correlation of weight of same granule in UV light and on staining with GCA	r'	0,65	$\geqslant 0{,}95$	600

Legend. Asterisk indicates null hypothesis untrue at the $P \leq 0.01$ level.

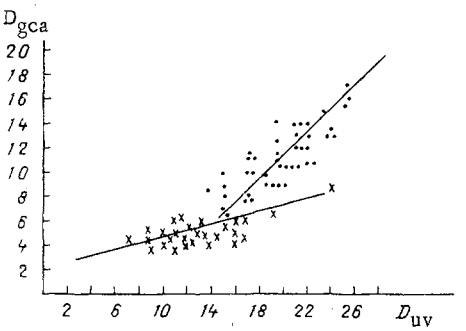

Fig. 1. Correspondence of optical density of same regions of nuclear chromatin of susliks before (D_{uv}) and after staining with GCA (D_{gca}). Abscissa and ordinate: optical density (in conventional units in UV light and on staining with GCA respectively. Dots indicate granular, crosses nongranular component of nucleus.

To determine differences in the staining properties of elements of chromatin structure such as the granular and nongranular (matrix) components, regression analysis was carried out and the degree of correlation determined between the same areas of these structural elements of the nucleus in GCA and UV light. It will be clear from Fig. 1 that linear correlation exists for optical density between the identical regions (see Table 1: 3 and 4). Comparison of the parameters of the regression equations for granules ($D_{gca} = 0.8$, $D_{uv} - 8.8$; $r = 0.7$) and the matrix ($D_{gca} = 0.28D_{uv} - 2$; $r = 0.65$) showed significant twofold differences between them ($P \leq 0.01$). This proves a qualitative difference between the granular and nongranular components (phases) of chromatin, i.e., they differ not only in the level of their optical density, but also in their tinctorial properties, which undoubtedly indicates physicochemical and functional differences between them [1, 3, 6].

Investigation of the degree to which the tinctorial properties are sensitive to changes in the functional state of the cells and, in particular, of the chromatin is of great interest. Analysis of the hepatocyte nuclei of the hibernating suslik in different physiological states (hibernation, in spring, in summer) showed that the ability of the matrix to take up the dye does not change significantly during transition of the animals from hibernation to active waking, whereas the tinctorial properties of the granules changed significantly and twofold ($P \leq 0.05$). We analyzed this fact on a model of activation in rats receiving phenobarbital [5]. The regression line "straightened out" 30 min after administration of the inducer: the angle of slope of the regression line for granules came close to that of the regression line for the matrix. These differences may perhaps be one component in the as yet unknown mechanism which determines differential condensation-decondensation of the two types of chromatin, which constantly takes place in individual phases of the cell cycle under natural conditions.

On the basis of the facts described above it can accordingly be concluded that the quantitative parameters of chromatin structure revealed with the aid of the basic nuclear stain GCA are identical with the distribution of

nuclear material observed in UV light. The tinctorial properties of different structural elements of the interphase nuclei, of the granular and nongranular components, differ qualitatively, and this indicates not only optical, but also physicochemical differences between them. The granular component is more sensitive to the functional states of the chromatin, and this may be an argument in support of its use in the quantitative evaluation of the functional state of the nucleus and the cell as a whole.

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