CYTOPHOTOMETRIC DETERMINATION OF DNA IN CELL NUCLEI

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The laboriousness of cytophotometric analysis has considerably restricted its potential use in the investigation of many objects. This problem was encountered in the course of the study of the degree of polyploidization of the nuclei in the regenerating liver of animals of different ages and at different times after resection. It was necessary to make several tens of thousands of measurements. An attempt was, therefore, made to speed up the fundamental processes of cytophotometric analysis: photometry, measurement of the area of the nuclei, and analysis of the results.

The liver of albino rats of different ages was used in the experiments. The specimens for photometry were prepared by compressing a small piece of the organ between two glass slides and then treating the resulting smears. In such preparations, the cell nuclei were flattened, their optical density was reduced, and their visible dimensions were increased, so that the accuracy of the measurements was enhanced. Cytophotometry was carried out on the MUF-5 apparatus in visible light after histochemical staining for DNA by the Fuelgen method.

The single-wave probe method of photometry was chosen as being the least laborious. Its disadvantage is the error caused by the uneven distribution of the light-absorbing matter, reaching tens or even hundreds per cent in some cases [1-4]. For this reason, the degree of unevenness of distribution of the optical density in the preparations used was studied carefully by threefold scanning of randomly chosen nuclei by means of a small probe (area of the probe in the plane of the object about 0.6 μ^2). These measurements showed that the mean optical density of the nuclei was 0.15-0.2, the minimal density never reached zero within the limits of the nucleus, and the ratio between the maximal and minimal densities (K), characterizing the unevenness of distribution, did not exceed 3. These results were used to compose an analytical model for assessing the possible error in photometry, by analogy with the models describing extreme cases of uneven distribution [1, 2]. If the minimal optical density in the photometric field is designated D', and the density of a certain part of the field β is expressed by D'k (D' > 0; $0 < \beta < 1$; $k > 1$), the density which would correspond to an even distribution of the matter may be calculated by the expression:

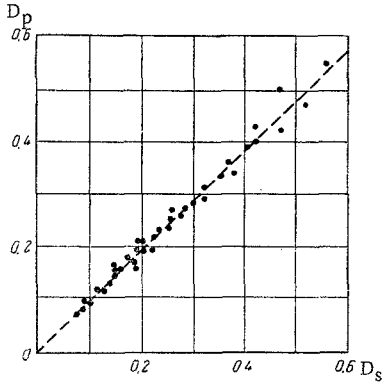

Fig. 1. Results of comparative measurement of optical density of rat liver cell nuclei by the probe method (D_p) and the scanning method (D_S).

$$\bar{D} = D'(1-\beta) + D'\beta\kappa = D'[1+\beta(\kappa-1)]. \quad (1)$$

The density measured by the photometer D is equal to:

$$D = \log\frac{\Phi_0}{\Phi'} = \log\left(\frac{1-\beta}{10^{D'}} + \frac{\beta}{10^{D'\kappa}}\right), \quad (2)$$

where φ_0 is the incident light flux, φ' is the total transmitted flux, corresponding to the sum of fluxes passing through areas with density D' and D'K.

$$\Phi' = \Phi_0(1-\beta)10^{-D'} + \Phi_0\beta 10^{-D'\kappa}. \quad (3)$$

Since $\bar{D}$ characterizes the amount of absorbing matter in the photometric field, the relative error of photometry $\Delta D/\bar{D}$ may be

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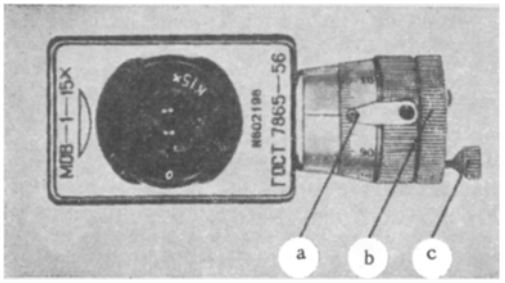

Fig. 2. Modified MOV-1-15 ocular micrometer. a — Indicator; b — additional knob; c — locking screw.

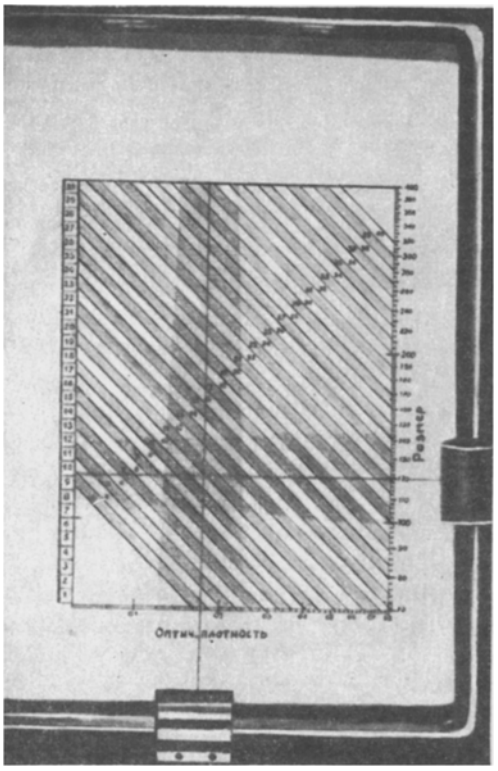

Fig. 3. Computer. Explanation in text.

expressed in terms of β, K, and D, by using Eqs. (1) and (2):

$$\frac{\Delta D}{\bar{D}} = \frac{D-\bar{D}}{\bar{D}} = -\frac{1}{\bar{D}} \log \left[\frac{1-\beta}{10^{\bar{D}}/1+\beta(\kappa-1)} + \frac{\beta}{10^{\bar{D}\kappa}/1+\beta(\kappa-1)} \right] - 1. \quad (4)$$

Substituting in this equation values of K and D obtained in the actual experiments showed that the error ranges from 0 to — 12%. Verification by comparative measurements of the optical density of the nuclei by scanning and probe methods simultaneously confirmed the results of the calculation (Fig. 1). On the graph given, the gradient of the regress line (broken line) calculated by statistical analysis of the experimental data is 0.97; the standard error is about 6%, and this figure includes the intrinsic error of the scanning method itself. The deviation of the gradient of the regression line from unity is not reflected in the accuracy of the relative measurements, because it is a systematic error.

To calculate the DNA content in the nucleus, besides measuring the optical density, the area of the nucleus must be determined. This area (in relative units) was calculated by squaring the mean value of the diameters of the nucleus measured with a screw ocular micrometer. Since the liver nuclei in the preparations were almost elliptical in shape, with a ratio between their diameters not exceeding 1.5 : 1.0, this method of determination givs an accuracy of ±5%.

To speed up the process of measuring the size of the nuclei, the MOV-1-15 ocular micrometer was reconstructed (Fig. 2). Its cross wire was turned through 45° so that the nuclei could be measured by a reference line perpendicular to the direction of movement of the cross wire. This made it unnecessary to place the object at the center of the cross wire. The vernier dial of the micrometer was freed from its attachment to the micrometer screw and fitted with a spring indicator marking coincidences of the zero scale division with the indicator line. An additional know with a locking screw was connected to the micrometer screw. The locking screw was released during the measurement, the reference line brought up to the edge of the object, the vernier scale set at 0 by the click of the indicator, and the locking screw again tightened. These operations can be performed without losing sight of the object, and there is no need to record the initial position of the scale and to subtract its reading from the final result. Each scale division (100 divisions) is marked by click of the indicator.

The results obtained for the DNA content in the nuclei were represented by distribution variance curves. The DNA content was plotted on a logarithmic scale, in which each main class was subdivided into 6 intervals. This scale made it clearer to visualize the material, facilitated equivalence of class groupings and, in addition, helped to rationalize the analysis of the results of the measurements with the aid of simple methods. Since the DNA content in the nucleus in relative units is determined by the product of optical density and area, by taking logarithms of this relationship we obtain:

$$\log S = \log Q - \log D. \quad (5)$$

where Q is the DNA content, D the optical density, and S the area of the nucleus. In a rectangular system of coordinates (log D; log S) a constant value of log Q is represented by a straight line, and the whole scale of DNA content adopted in this method is described by a family of parallel straight lines, separated

from each other by the magnitude of the class interval. This sytem was used as the basis for the nomogram of the computer (Fig. 3), in which the units of optical density are plotted on the abscissa on a logarithmic scale, and the diameters in ocular micrometer units are plotted on the ordinate, the scale of area, on a double logarithmic scale. The nomogram was mounted on a photographic printing frame, along the perpendicular axes of which moved the riders of the indicator lines. The desired class interval was determined by the intersection of the reference rulers placed on the corresponding values of the density and diameter of the nucleus. By means of this device the results of the measurements may be analyzed quickly, a clearer picture of the analysis is obtained, and the situation may be kept under immediate control.

By using the system of measures described above, without reducing the quality of the measurements, it was possible to diminish very considerably the laboriousness of the cytophotometric analysis and to extend the field of its application.

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