NONUNIFORMITY OF PERMITOTIC DNA REDUPLICATION IN THE CELLS OF MAMMALS (ACCORDING TO THE DATA OF CYTOSPECTROPHOTOMETRY)

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Indirect cell division (mitosis) is preceded, as is well known, by reduplication of the amount of DNA in the nucleus. In connection with a study of the premitotic DNA synthesis, an investigation of the rate of increase in the amount of this substance in the nucleus is of definite interest. Some information on this question can be found in the works of a number authors [3-7], who have investigated the principles of the increase in the DNA content during the division of plant cells, cells of unicellular organisms, and cells in tissue culture.

This reports presents a new variation of an approximate solution of the problem, suitable for the study of cells of any tissue, including the tissue cells of higher animals.

EXPERIMENTAL METHODS

Histograms of the DNA content in the spermatogonia and fibroblasts of the areolar tissue (normal and from a focus of inflammation) of adult mice, and in the cells of the liver and pancreas of rats 10-30 days of age was used as the material for the investigation [1, 2]. After the Feulgen reaction, the amount of DNA in individual nuclei was determined by a cytospectrophotometric method and expressed in units of ploidy, which was possible thanks to a comparison of the results of a measurement of the investigated nuclei with known haploid sperms and spermatids.

EXPERIMENTAL RESULTS

The curves (Fig. 1) have the form typical of histograms of the DNA content in the nuclei of cells of mitotically dividing tissues. In a considerable portion of the nuclei, the amount of DNA corresponds to the diploid set of chromosomes (2n) or close to it. The fraction of the nuclei containing DNA in amounts between 2n and 4n is apparently preparing for mitosis; DNA reduplication is occurring in these nuclei, while those containing DNA in amounts of 4n are just about to divide. Nuclei are also encountered with amounts of DNA less than 2n and greater than 4n. Usually the deviations did not exceed 2-3 σ, i.e., did not differ statistically from the real values of 2n or 4n (the root-mean-square deviation, equal to 0.1-0.15n, was determined in a special investigation). Among the fibroblasts, and to a lesser degree in the cells of the liver and pancreas, nuclei were observed with amounts of DNA less than 1.7n and more than 4.3n. This permits us to assume the presence of cells that had divided by amitosis [2].

We should mention that, according to the data of cytophotometry, the number of nuclei preparing for mitosis is usually greater than that which might be assumed on the basis of a histoautoradiographic analysis. These discrepancies are understandable if we consider the fact that labeled thymidine introduced into the organism competes with unlabeled thymidine synthesized in the organism itself for incorporation into DNA, the intrinsic thymidine being quite sufficient to provide for the DNA reduplication. From this it is clear that labeled thymidine cannot be

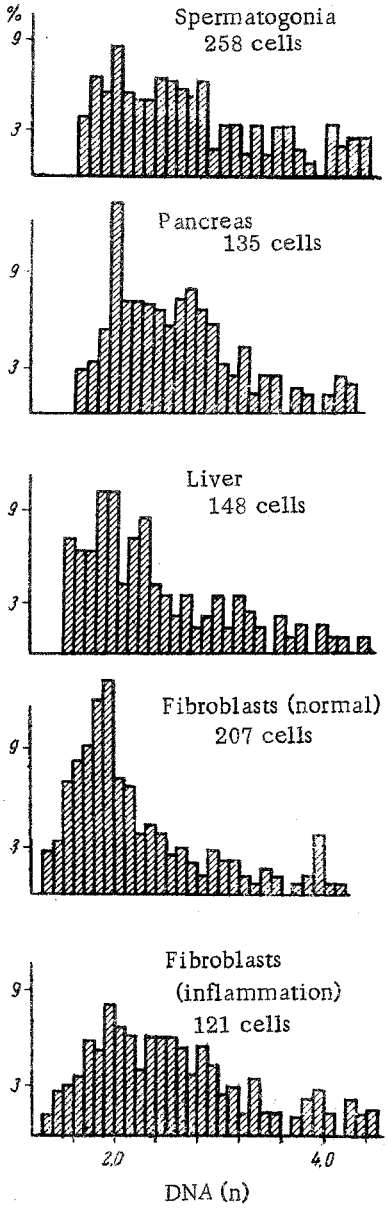

Fig. 1. Histograms of the DNA content in the nuclei of cells of the investigated tissues.

incorporated into all the nuclei synthesizing DNA, and the label will be so little incorporated into some nuclei that it will practically not be detected.

In analyzing the histograms, we assumed that DNA synthesis that had begun before the end of reduplication proceeds without any stoppages, while the degree of preparation of the nucleus for mitosis is characterized by its DNA content. For example, nuclei containing 3.5n DNA are closer to division than nuclei with 2.3n DNA.

Figure 2 presents cumulative curves calculated from the histograms (see Fig. 1). (Let us recall that to compare a series of cumulative frequencies, one must add the frequency of the second class to the frequency of the first, smallest class. The sum obtained will be the cumulative frequency of the second class. By adding the frequency of the third class to the latter, we obtain the cumulative frequency of the third class, etc.). The curves cited in Fig. 2 are similar in form and give no basis for speaking of any differences. At least three portions, characterized by different slopes to the X-axis, can be distinguished on each curve. The first portion is a slow rise of the curves; it includes most of the nuclei (about 60%); the second portion represents a sharp rise (12-15% of the nuclei), and, finally, the third portion represents a certain deceleration of the rise of the curves (5-7% of the nuclei).

Fixation freezes the cells at various phases of preparation for mitosis, which are characterized by different DNA contents. The longer the cell is kept in a definite state, the greater the frequency of nuclei with the corresponding amount of DNA (class frequency). On the contrary, the smaller the frequency of the class, the more rapidly the cells pass through this phase of reduplication, i.e., under the condition of continuity of DNA synthesis, the frequency of nuclei with a definite DNA content (class frequency) is proportional to the time of stay of the nuclei in the given state. In this case, the slope of the curve (see Fig. 2) should give evidence of the rate of passage of the nucleus from one state (with respect to DNA level) into the following, i.e., should indicate the rates of increase in the DNA content during the process of reduplication.

Considering the above, an analysis of the curves (see Fig. 2) gives us a basis for concluding that the first half of the process of DNA reduplication (up to 3n) proceeds at only one-fourth the rate of the second, as well as the fact that by the end of the process the intensity of the synthesis has somewhat slowed down. However, the point of inflection on the curves may be related to an increase in the number of tetraploid cells, existing in the permitotic period (G_2).

Noteworthy is the fact that all the curves intersect the X-axis at some distance from zero. This is apparently due to the fact that the cells exist in the diploid state for some time after division (period G_1) before they begin DNA synthesis.

The assumptions made permit us to give another graphical expression of the process of DNA reduplication. Thus, if the frequency of each class (m_i) is proportional to the time of stay of the nucleus in the state n_i, then the ratio

$$\frac{n_i - n_{i-1}}{m_i} = V_i$$

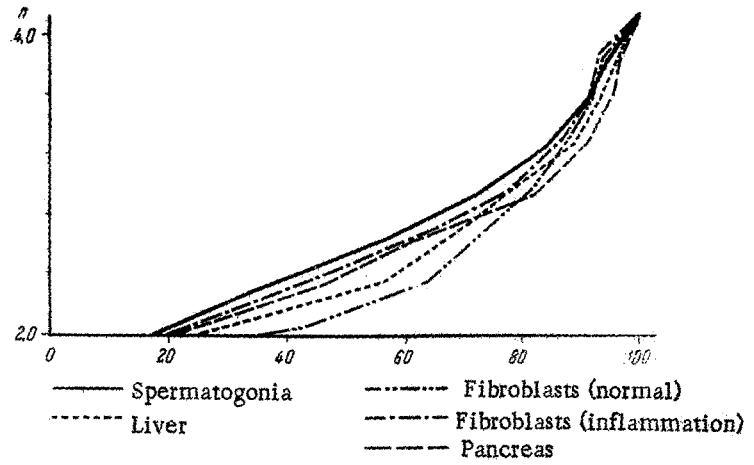

Fig. 2. Cumulative curves calculated from the histograms (Fig. 1). Along X-axis – cumulative frequencies; along Y-axis – amount of DNA in the nuclei in units of ploidy (n).

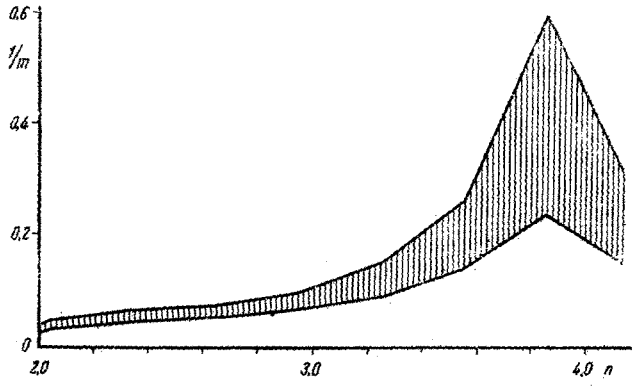

Fig. 3. Rate of DNA synthesis (1/m) during various periods of reduplication. The limits of the zone of confidence ($m \pm 2\sqrt{m}$) were calculated from data summarizing all the material (869 nuclei).

gives an idea of the rate of the process (V_i) between the points n_i and n_{i-1}. The distance between n_i and n_{i-1} is no other than the interval between definite classes, i.e., a constant. Hence the rate of the process can be taken as the reciprocal of the frequency:

$$V_i = \frac{1}{m_i} .$$

Figure 3, summarizing the results of an analysis of all our material (869 nuclei), represented by the zone of confidence, shows still more convincingly that the rate of DNA synthesis ($1/m_i$) differs at various stages of reduplication. The point of inflection of the curves before 4n is apparently due not only to a slowdown of the synthesis, but also to the premitotic period of the cells (G_2), which gives an increase in the frequency of the class including nuclei with the tetraploid amount of DNA.

Thus, an analysis of the histograms of DNA content in the nuclei of cells of a number of mitotically dividing tissues shows that the process of DNA reduplication is completely nonuniformly. Each successive stage of DNA synthesis proceeds more intensively than the preceding. Moreover, the rate of DNA synthesis increases sharply in the second half of the process, reaching a maximum in the region close to the end of reduplication.

SUMMARY

The DNA content was studied histographically in the nuclei of the cells of certain mitotically dividing tissues (spermatogonium, fibroblasts of albino mice, liver cells and those of the pancreas in young albino rats). The authors came to a conclusion, that the process of DNA reduplication is nonuniform. Each subsequent stage of the DNA synthesis is more intense than the preceeding one; the rate of synthesis increases during the second half of the process, reaching the maximum near the end of reduplication.

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