THE NUCLEAR COMPOSITION OF UNINJURED AND REGENERATING RAT LIVER CELLS

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Liver parenchyma contains nuclei exhibiting various degrees of polyploidy, as revealed by a definite variation in the nuclear volume [1, 4, 2, 12, 14, 17, 19] and by their DNA content [15, 16, 18]. With increasing ploidy the nuclei undergo a rhythmical doubling of their volume [9, 12, 15]; there is some evidence to suggest that the DNA content undergoes the same consecutive rhythm of doubling with increasing polyploidy [15]. It is possible to find indications in the literature that the polyploidy of the liver cells in rats [4, 6, 15], mice [13] and man [18] increases during the period of growth and at the time of regeneration [4, 2, 8, 17]. However, data about the changes in polyploidy of the parenchyma cells in the undamaged liver during the aging process of the organism is scanty and contradictory [3, 6, 18]; in general, no attempt has been made to study polyploidy in regenerating liver tissue following an operation.

We have investigated polyploidy in the nuclei of mononuclear cells of undamaged and regenerating livers from rats of various ages. We used the dimensions of the nucleus as a criterion of the degree of ploidy in all cases.

EXPERIMENTAL METHODS

The diameters of mononuclear cells in undamaged and regenerating liver tissue from rats of various ages were measured by means of an ocular micrometer. Measurements were made on preparations stained with Feulgen after fixation in Carnoy's fluid. Measurements were made of 500 nuclei in the liver of each animal. The volume of the nucleus was calculated from the formula for the volume of a sphere. The numerical data was split up into classes [10]. The polyploidy of the nuclei was estimated from their size. The following 6 groups of animals with undamaged livers were used in our experiments: new born rats (weight 65 g), adult rats (weight 220-250 g), rats during period of sensescence (weight 340 g, age about 16 months), senile rats (weight 280 g, age 20-24 months). The last four groups of animals served as controls for rats of the same age with regenerating livers. Rats with regenerating livers were studied 2, 5, 11 and 17 months after removal of 70% of their liver tissue using the technique of Higgins and Anderson [11]. Each control and experimental groups consisted of 5-7 animals.

EXPERIMENTAL RESULTS

Three types of nuclei were found in the livers of young rats (65 g); the most frequently encountered type in these and other animals was the diploid nucleus, which comprised 80.3% of the total in newborn rats and 70.8% in young rats. These findings agree with those recorded in the literature [12, 15]. In young rats (65 g) tetraploid nuclei made up 20.1% of the total. Furthermore, a small number of nuclei occupied an intermediate position between the two main types as judged by their dimensions; the livers of young rats before sexual maturity contained 3 such intermediate types of nuclei.

In the livers of newborn and young rats we have observed small nuclei, which according to their size must be considered as possessing a haploid complement of chromosomes. The chromosomal complement of such small nuclei cannot however be finally decided without actually counting the number of chromosomes in them and carrying out a quantitative estimation of their DNA contents (Fig. 1a, b).

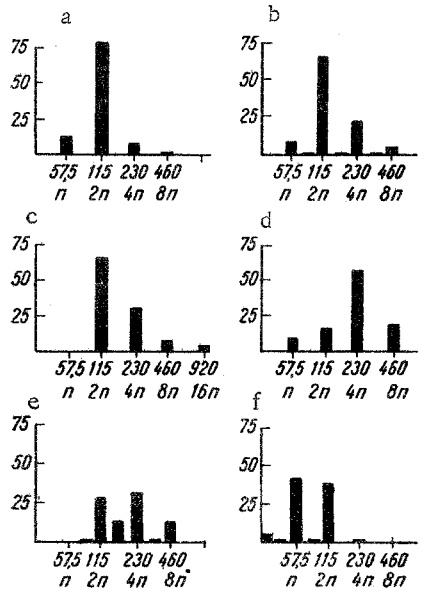

Fig. 1. Composition of nuclei in mononuclear liver cells of rats of various ages which had not been operated on (as %): a) newborn rats; b) young rats weighing 65 g; c) sexually mature rats weighing 220 g; d) sexually mature rats weighing 250 g; e) senescent rats (aged 16 months) weighing 340 g; f) senile rats (aged 24 months) weighing 280 g.

In the livers of adult rats (220 g) most of the nuclei (62.7%) are diploid, although tetraploid nuclei are quite well represented (28.8%). The very small nuclei and those of an intermediate type are absent. In rats weighing 250 g the liver nuclei are mainly of the tetraploid type (63%). The diploid nuclei comprise 13.0% of the total; octoploid nuclei 16.6% and small nuclei 6.6%.

Intermediate types of nuclei are absent (Fig. 1c, d).

During the period of senescence (age about 16 months) there is a gradual reduction in the number of cells with large nuclei (tetraploids and octoploids); the tetraploid nuclei comprise only 38.5% and the octoploid only 13% of the total, whereas the diploid nuclei increase to 32.5%. Nuclei of the intermediate classes appear, one of which occupies a position between the diploid and tetraploid nuclei and comprises 13% of the total number (Fig. 1e).

In livers of senile rats (aged 20-24 months) there is an even more definite shift in the clases of nuclei; the majority classes consist of diploid nuclei (48%) and tetraploid nuclei (43.4%). Octoploids comprise only 3%. There are very few nuclei of an intermediate type. It is interesting to note that a few (0.1%) very small nuclei having a volume of 14.2 μ^3 sometimes occur in the nuclei of senile rats; this type was observed in one of the previously investigated groups of animals (Fig. 1f).

Two months after removal of 70% of the liver tissue, three classes of nuclei were found in the regenerating livers; the majority class consisted of tetraploid nuclei (65%); a large number of octoploid nuclei occurred (26.3%) and diploid nuclei were few (5.8%). Very small nuclei and those of an intermediate type were absent. At this period of regeneration, the nuclear picture differed markedly from that shown by the intact rats of the same age which were used as controls and in which dipolid nuclei predominated (Fig. 2a).

Five months after the operation four classes of nuclei were found in regenerating liver tissue; the majority class consisted of tetraploid nuclei (65.2%) but in contrast to the situation in the previous group of animals this category had increased by 19.4% whereas the cotoploid nuclei had decreased to 13.6%. A few very small nuclei (1.2%) were recorded. Nuclei of the intermeidate type were absent. The nuclear picture at this stage in the regenerative process was very like that in control animals of the same age (Fig. 2b).

Eleven months after the operation, i.e., during the period of senescence, four classes of nuclei were again found to occur in the regenerating liver tissue. However, their percentage distribution differed from that found in the experimental animals of the previous group with a trend towards nuclei of smaller dimensions. The nost numerous category was that of diploid nuclei (43.3%), followed by tetraploid nuclei (28.6%) and octoploids (10%); the percentage of very small nuclei had increased to 2.2%. At this period of regeneration the 3 intermediate classes of nuclei were noticed for the first time and these made up 14.3% of the total. These changes in nuclear distribution, 11 months after the operation, are similar to those which have occurred in control rats of the same age; the only difference is that in the group with regenerating livers the trend towards nuclei with a lower level of polyploidy is more sharply expressed (Fig. 2c).

In rats examined 17 months after operation the number of dipolid nuclei in the liver tissue had increased to 53.7% of the total and the number of small nuclei to 18.8%. As in the case of control animals of the same age, nuclei were found with a very small volume (14.2 μ^3); these comprised 0.3% of the total. The rats which had been operated on possessed a small proportion of nuclei belonging to 2 intermediate classes. Once again, it was possible to see that the changes in nuclear composition of the experimental rats 17 months after operation closely paralleled those shown by the controls of the same age; however, these changes were expressed more sharply in the former than in the latter group (Fig. 2d).

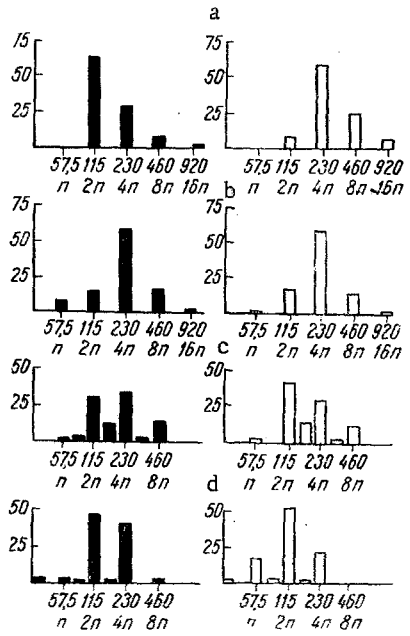

Fig. 2. Composition of nuclei in mononuclear liver cells of control (black histograms) rats and those with regenerating livers (white histograms) at different periods (as %). a) Sexually mature rats 2 months after partial resection of liver; b) sexually mature rats 5 months after partial resection of liver; c) sexually mature rats during period of senescence 1 month after partial resection of liver; d) senile rats 17 months after partial resection of liver.

Certain conclusions may be drawn from the findings described above. The nuclear composition of liver tissue does not remain constant throughout the ontogeny of the animal. Initially, there is a trend towards an increase in the number of polyploid cells and an increase in their ploidy. During the period of senescence this trend is reversed; the percentage of cells with a high polyploid number diminishes and the percentage of diploid nuclei increases.

The biological rhythm of nuclear change found in intact livers, is preserved in livers undergoing regeneration in the later stages after trauma. However the changes in regenerating liver tissue are expressed more clearly. These characteristics of change in the ploidy of nuclei in normal and regenerating livers lead us to suppose that the phenomenon of polyploidy is not merely related to the growth of an organ, but it also has a definite functional significance.

SUMMARY

The paper treats of the changes in the nuclear composition of mononuclear hepatic cells in the normal and regenerated liver of rats of different age. In the liver of neonatal rats 80.8% of the nuclei are diploid. In rats, weighing 65 gm, prior to maturation, there is an increase in the number of tetraploid nuclei with the appearance of intermediate classes. In the animals aged 20-24 months the bulk of the nuclei is made up of diploid, whereas the number of tetraploid and octoploid is down. At late postoperative periods (in 2, 5, 11 and 16 months) biological regularity of the changes in the nuclear composition during ontogenesis continues in the regenerated liver; these changes are more pronounced than in the intact liver of rats of the same age.

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