DETERMINATION OF DNA CONTENT IN NUCLEI OF HEPATOCYTES OF NORMAL AND REGENERATING RAT LIVER

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The ploidy of rat liver cell nuclei increases with age of the animals (the proportion of tetraploid nuclei becomes greater and octaploid nuclei appear). In the regenerating liver of rats of all age groups the ploidy increases, and in old animals nuclei with a DNA content corresponding to 16n and 32n appear.

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The number of polyploid cells and the degree of their ploidy increase in the liver of rats, mice, and man in the period of growth [2, 3, 8, 10, 11, 14]. Polyploidization is also found in the regenerating rat liver [7, 9, 13]. However, the relationship between processes of polyploidization in the regenerating liver and age has received little study. Only isolated data concerning the formation of polyploid cells in the liver of aging rats have been published [6, 7, 12].

In the present investigation the ploidy of the nuclei of hepatocytes in the undamaged and regenerating liver was studied in rats of different ages.

EXPERIMENTAL METHOD

Experiments were carried out on 120 noninbred rats of various ages: group 1 consisted of sexually immature animals weighing 40-50 g (age 1-1.5 months), group 2 of young, sexually mature rats weighing 170-200 g (age 5-6 months), and group 3 of aging animals weighing 300-380 g (12-15 months). About 65% of the liver tissue was removed from the experimental rats by the method of Higgins and Anderson [9]. A mock operation was performed on the control animals. Experimental and control rats were sacrificed on the 3rd, 7th, 14th, and 30th days after partial hepatectomy, five animals at each time. The animals and their liver were weighed. For cytophotometric analysis, impression preparations were made from the intact and regenerating liver, fixed for 60 min in Carnoy's fluid, and stained by the Feulgen method under standard conditions. The DNA content (in relative units) was determined with a type MUF-5 apparatus at a wave length of 546 mμ. In each preparation 100 nuclei were examined. Two perpendicular diameters of the nucleus were measured with a screw-adjusting ocular micrometer in our modification [1]. The results of cytophotometric analysis were presented as variants curves with logarithmic division of the scale of ploidy into class intervals. The scale of ploidy was graduated in mean values of DNA content in the nuclei of the diploid class of group 1 control rats. To facilitate and speed up the calculations, a graphic method of analysis of the results was developed, using a special nomogram fixed to a board with moving indicators [1].

EXPERIMENTAL RESULTS

The weights of the uninjured and regenerating rat livers are given in Table 1, which shows that restoration of the weight of the regenerating liver took place differently in animals of different ages: in sexually immature rats it was observed on the 3rd day after the operation, in sexually mature rats on the 14th day, and in aging rats complete restoration of the weight of the regenerating liver was not observed, even one month after resection. During the experiment (one month) a rapid change in the relative percentages of the nuclear classes was observed in the intact liver of the group 1 rats. Whereas on the 3rd day after the beginning of the experiment the majority of liver nuclei were diploid (89%) and only 7.6% were tetraploid, by the 30th day this ratio had changed and the percentages were now 41 and 56% respectively.

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TABLE 1. Changes in Body Weight and Relative Weight of Liver in Control and Hepatectomized Animals

Group 1					
Time of experiment (in days)		3	7	14	30
Weight of animal (in g)	control experiment	52,1 53,4	61,4 60,8	74,3 78,1	124 95,6
Relative weight of liver (in %)	control experiment	4,81 4,73	5,18 5,82	5,03 6,11	4,92 5,60
Relative restoration of weight of regenerating liver (in %)		98	112	121	116
Group 2					
Time of experiment (in days)		3	7	14	30
Weight of animal (in g)	control experiment	192 175	205 192	207 210	236 218
Relative weight of liver (in %)	control experiment	3,63 2,92	3,41 3,03	3,72 3,79	3,60 3,35
Relative restoration of weight of regenerating liver (in %)		80	89	102	93
Group 3					
Time of experiment (in days)		3	7	14	30
Weight of animal (in g)	control experiment	350 301	323 280	371 355	393 341
Relative weight of liver (in %)	control experiment	3,10 2,24	3,25 2,27	3,05 2,35	3,18 2,71
Relative restoration of weight of regenerating liver (in %)		72	70	77	85

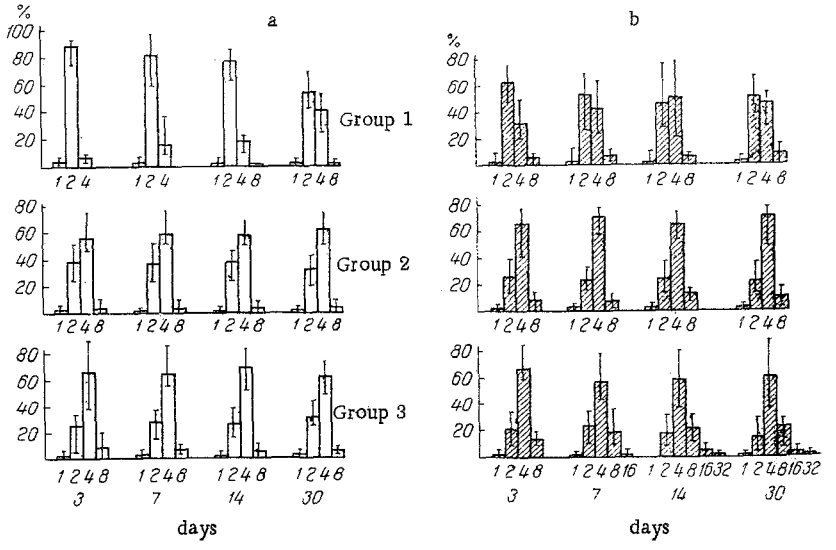

Fig. 1. Percentage distribution of nuclei of hepatocytes by ploidy classes in normal and regenerating liver of rats of different ages. a) Control; b) regeneration. Numbers below columns denote classes of ploidy.

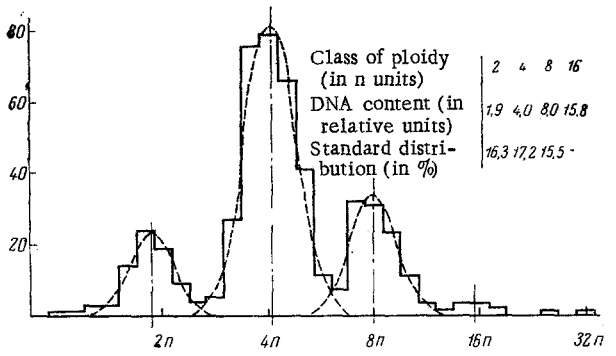

Fig. 2. Statistical distribution of liver nuclei in regenerating liver of group 3 rats. Ordinate—number of nuclei (in n units), abscissa—classes of ploidy. Continuous line represents experimental distribution, broken line approximation of experimental distribution by Gaussian curve.

In the animals of group 2 (young, sexually mature rats) the nuclei of the liver cells were mainly tetraploid, their percentage increasing from 56 (on the 3rd day) to 63 (by the 30th day). In addition, 3-4% of the nuclei were octaploid.

In the liver of the aging animals of group 3 the percentage of octaploid nuclei increased to nine. Most nuclei, as in the young, sexually mature rats, were tetraploid, although toward the end of the experiment their number diminished somewhat. Results of measurement showed that toward the end of the experiment the number of diploid nuclei showed a tendency to increase, although no statistically significant difference was observed because of the considerable individual variations (Fig. 1, a).

In the regenerating liver of the group 1 rats the number of tetraploid nuclei showed a sharp increase on the 3rd day after the operation—from 7.6% in the control to 31% in the experimental animals, and this increase continued until the 30th day. By the end of the period of investigation, differences between the proportions of diploid and tetraploid classes in the experimental and control animals had become very slight. Meanwhile, in the regenerating liver at all times of the investigation a new class of octaploid nuclei had appeared, numbering 5-7% but nuclei of this class were absent from the control.

In young, sexually mature animals of group 2 polyploidization was also observed during regeneration, in the form of an increase in the percentages of tetraploid and octaploid nuclei by comparison with this index in the controls.

The pattern of liver regeneration in the aging rats (group 3) differed in the appearance of classes of nuclei with high ploidy values (16n and 32n) toward the end of the 30th day. Meanwhile, the percentage of tetraploid nuclei fell somewhat compared with that in the control, whereas in the regenerating liver of the rats of the first two groups an increase in the number of these nuclei was found (Fig. 1, b).

The single-wave method used enabled cytophotometric analysis of an extensive material to be carried out quickly and relatively accurately. In our experiments the standard error during photometry of stained liver cell nuclei in impression preparations was within the range ±6%, as was determined experimentally by comparing the results of parallel measurements by the single-wave method and by scanning. An experimental error of this magnitude had practically no effect on the shape of the variants curves of distribution of the nuclei by DNA content, for as the calculations showed, the true coefficients of variability of the DNA content in the nuclei of the principal ploidy classes were about ±15-18%.

A general histogram, plotted by summation of the results of measurements of the liver nuclei of five animals (group 3; 30th day of regeneration) is given in Fig. 2. In most other cases the distribution was similar in type, differing only in the relative percentages of the ploidy classes. Statistically analysis of the histogram by the method suggests that the experimental distribution is a sample from the normal set. If the experimental distribution is approximated by Gaussian curves with corresponding values of Σn, M_{mean}, and σ, the presence of a certain percentage of nuclei in intervals occupying an intermediate position between the maxima of two neighboring classes of ploidy may easily be explained by the normal distribution of the variants. For this reason, we did not separate independent intermediate classes. In the analysis of the results obtained it must also be pointed out that since in most cases a few (0.5-3%) of the nuclei in both the intact and regenerating liver of animals of all age groups had a DNA content below the minimal limit of the diploid class. However, no characteristic maximum corresponding to the mean value characteristic of haploid nuclei could be found for this group of nuclei. The percentage of such nuclei fell in the old animals, and also in the late periods of regeneration, but these changes cannot be taken as statistically siginificant because the absolute number of these nuclei was extremely small in our experiments. Such nuclei perhaps arise in the process of degeneration, of irregular distribution of the chromosomes in mitosis, or by amitosis.

Polyploidy thus increased with the age of the animal in the liver of all the investigated groups.

In the regenerating rat liver a progressive increase of ploidy was observed. In the young animals, a class of octaploid nuclei appeared, in the sexually mature animals only the relative percentages of existing nuclear classes was changed, while in the aging animals the formation of polyploid nuclei with 16n and 32n was observed. The rat liver contains a few nuclei (.05-3%) with a DNA content below that characteristic of the diploid class.

The results of this investigation, undertaken by cytophotometric analysis, confirm the basic conclusions drawn previously by a karyometric method [4, 5].

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