DNA CONTENT AND SIZE OF CELL NUCLEI IN THE REGENERATING RAT LIVER

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The DNA content in single nuclei and the size of the nuclei were investigated in the intact and regenerating rat liver from 18 h to 21 days after partial hepatectomy. The results of the measurements show that the mean DNA content per nucleus in the intact rat liver is 6.5 pg, and that most nuclei are about equal in size to the diploid nucleus (42.5 μ^2). DNA synthesis began in the regenerating liver before 18 h after the operation. By 24 h the DNA content in most nuclei of the experimental animals was twice that in the intact rats. This shows that the first wave of synthesis involved 85-90% of the liver cells. After mitosis, which in most cells took place before 36 h after partial hepatectomy, weaker waves of DNA synthesis followed, after approximately 42 and 60 h.

KEY WORDS: *regeneration of the liver; DNA content; size of nuclei of hepatocytes.*

Existing data in the literature are concerned chiefly with changes in nucleic acids in the first stages of regeneration of the liver, i.e., before the second to third day [4-7, 12]. The object of this investigation was to make a cytophotometric study of the changes in the DNA content and size of the nuclei in the liver of rats in the course of 21 days after partial hepatectomy, i.e., for the whole of the period of regeneration.

EXPERIMENTAL METHOD

Experiments were carried out on 65 adult male Wistar albino rats with a mean weight of 200-220 g. Partial hepatectomy was performed on the animals [8] which were killed at various intervals from 18 h to 21 days after the operation, simultaneously with intact control animals. Pieces of liver were ground in a mortar and films were stained by Feulgen's method. Preparations were made in the same way from bovine spermatozoa, which were used as the standard for calculating the DNA content. The microscopic preparations were evaluated cytophotometrically. In each group of five animals 250 measurements of extinction were made; the dimensions of the nuclei, i.e., their length and width, were measured with an ocular micrometer. Depending on their DNA content and area the individual nuclei were divided into 25 classes and displayed graphically on histograms, and the mean DNA content and mean area of the nuclei were calculated for the different classes. Parallel with the cytophotometric measurements, the mitotic index (MI) was determined in the same animals and expressed in promille.

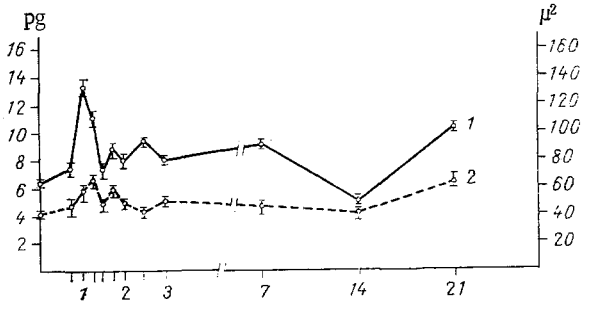

Fig. 1. Changes in mean DNA content in nucleus (1) and mean area of nuclei (2) in regenerating rat liver from 18 h to 21 days after partial hepatectomy. Starting point corresponds to mean value of these indices in intact animals. Ordinate, DNA content in nucleus (in pg) and area of nucleus (in μ^2) respectively; abscissa, days after hepatectomy.

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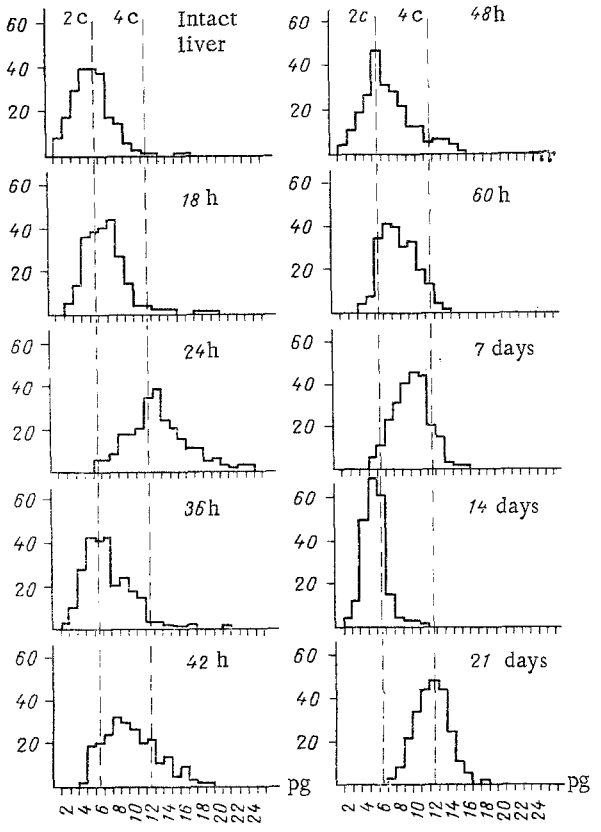

Fig. 2

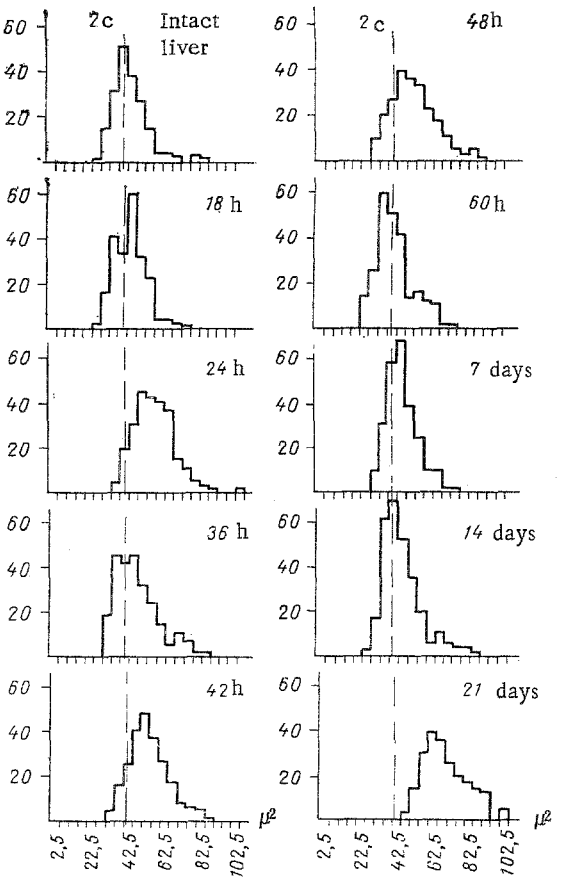

Fig. 3

Fig. 2. Histogram of distribution of nuclei by DNA content per nucleus in intact and regenerating rat liver 18 h–21 days after hepatectomy. Ordinate, number of nuclei in corresponding class; abscissa, DNA content per nucleus (in pg); nuclei divided into classes at 1 pg intervals.

Fig. 3. Histograms of distribution of nuclei by area in intact and regenerating rat liver 18 h–21 days after hepatectomy. Ordinate, number of nuclei in corresponding class; abscissa, area of nucleus (in μ^2); nuclei divided into classes at 5 μ^2 intervals.

EXPERIMENTAL RESULTS

The mean DNA content in the nucleus in the intact rat liver was 6.5 pg (Fig. 1). This amount reflects polyploidy of the hepatocytes, and it is therefore a little higher than the mean DNA content in the nuclei of rat somatic cells, namely 5.7 pg [11]. The mean area of the nuclei (45.4 μ^2) was close to that given by other workers [2]. Most of the nuclei of the intact liver corresponded approximately in size to the diploid nucleus (Figs. 2 and 3). A similar distribution of nuclei in rats of the same age as those used in the present experiments was obtained by Ryabinina [3], who states that 70–80% of hepatocytes are diploid and 20–30% are tetraploid. According to figures given by other workers [10], the number of polyploid cells in the liver of adult rats is much greater.

The DNA content in some nuclei 18 h after the operation was increased as a result of premitotic DNA synthesis, which begins after 14–18 h [4–6, 12]. By 24 h the DNA content in the nuclei had increased to a maximum, and this was accompanied by a smaller increase in size of the nuclei. On the basis of the mean DNA content per nucleus, the distribution of the nuclei by size, when most were approximately the same size as the tetraploid nucleus, and the low MI (Table 1) it can be postulated that at this time most of the cells were in the late (S) phase or in the postsynthetic phase of interkinesis. The mean DNA content per nucleus at this time was 13.4 pg, or twice that in the cell nuclei of the intact liver. This increase in the total DNA content in the regenerating liver 24 h after partial hepatectomy [9] can be explained on the grounds that the DNA content was doubled in nearly all the cells and was less

TABLE 1. MI (in ‰) in Regenerating Rat Liver 18 h-21 days after Partial Hepatectomy (M ± m)

Time	MI
Control (intact animals)	0,04±0,02
18 h	0,06±0,04
24 h	0,17±0,03
30 h	27,15±0,57
36 h	16,24±0,24
42 h	13,55±0,46
48 h	12,64±0,27
60 h	9,03±0,77
72 h	9,12±0,46
7 days	0,74±0,13
14 days	0,87±0,24
21 days	0,25±0,09

than doubled in only a small proportion of the cells. The histogram of distribution of the nuclei 24 h after the operation shows that most nuclei, approximately 85-90%, were involved in the first wave of DNA synthesis. Similar results based on the investigation of DNA synthesis were obtained by Grigor'ev et al. [1].

The many small nuclei with a diploid DNA content and the decrease in the mean DNA content per nucleus almost to the control level 36 h after the operation were the result of intensive mitotic division, which affected most cells with the maximal MI 30 h after partial hepatectomy. The increase in the DNA content and size of the nuclei 42 and 60 h after the operation was the result of the second and third waves of DNA synthesis, respectively. Data in the literature [6, 7] confirm the presence of only the second wave of DNA synthesis, observed after about 36 or 44-52 h.

After the first wave of DNA synthesis synchronization of the entry of the hepatocytes into the various phases of the cell cycle is reduced [6, 7], and fewer cells commence the second and third waves of DNA synthesis. This was shown by the fairly high mitotic activity and the increase in the DNA content in only some of the nuclei at these times.

During the second week after the operation the size of the nuclei became stabilized; the mean size of the nuclei and their distribution by DNA content and area were the same until the 14th day as in the intact animals. The histogram obtained on the 14th day after the operation shows that in most nuclei (96%) the DNA content was back to its initial level. This reflects the ending of regeneration in the liver. An unexpected finding was the increase in the DNA content in the nuclei and in the size of the nuclei on the 21st day after partial hepatectomy. The DNA content in the nuclei at this time was 65% greater and their area 52% greater than before the operation. This phenomenon cannot at present be explained. It may perhaps be the result of fusion of the nuclei or changes in DNA metabolism.

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