CHANGES IN SIZE AND MITOTIC ACTIVITY OF REGENERATING MOUSE LIVER CELLS AFTER ADMINISTRATION OF DESOXYRIBONUCLEOPROTEINS AND DNA ISOLATED FROM REGENERATING RABBIT LIVER

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Previous investigations showed that desoxyribonucleoproteins (DNP) isolated from the normal and regenerating rabbit's liver differ in their action on the regenerating mouse liver. DNP from the regenerating liver (rDNP) stimulates regeneration of the liver, whereas DNP from the normal liver (nDNP) does not possess this property [2-4]. It was further discovered that if DNA from a regenerating rabbit's liver (rDNA) is administered to mice with a regenerating liver, the weight and the RNA content of the regenerating content both increase; DNA from the normal liver (nDNA) has no such action [1].

In the present investigation the effect of rDNP and rDNA isolated from the regenerating liver of a rabbit was compared with the effect of fractions obtained from a normal liver (nDNP and nDNA) on the dimensions of the hepatic cells, on their mitotic activity, and also on the mitotic activity of the nonparenchymatous cells in the regenerating liver. The effect of the DNP and DNA on the number of binuclear hepatic cells was also investigated.

EXPERIMENTAL METHOD

The method of isolation of the DNP and DNA, the planning of the experiments, and the results obtained from determination of the weight of the liver in the mice have been described previously [1]. The DNP and DNA were isolated by Leukina. Non-inbred albino mice weighing 20-29 g, from which the left lateral lobe of the liver had been removed, were used in the experiment. The DNP and DNA were injected on the second day after the operation in a dose of 1 μg/g body weight in 0.35 M and 0.14 M NaCl solutions, respectively. The mice were decapitated on the fourth day after the operation. The liver was fixed in Carnoy's fluid and the sections were stained with hematoxylin-eosin. The liver cells (100 cells per mouse) and their nuclei and nucleoli were measured by means of a screw-adjusted ocular micrometer (objective 90, ocular 15). The area of the cells was determined as the product of two diameters, and the area of the nuclei and nucleoli by the formula for the area of a circle. The number of nucleoli in the nucleus, the number of binuclear hepatic cells (per 1000 cells), and the number of mitoses among the parenchymatous and nonparenchymatous cells (per 6000 cells) were counted.

EXPERIMENTAL RESULTS

When fractions isolated from the regenerating rabbit's liver were injected into mice from which a lobe of the liver had been removed, hypertrophy of the liver cells was observed, and the area of these cells in the mice receiving rDNP was 54% greater than their area in the mice receiving nDNP (see table). The difference in the areas of the liver cells of the mice receiving rDNA and nDNA was 11% ($P < 0.001$ and $P = 0.001$, respectively).

In the mice receiving rDNP, the dimensions of the nuclei of the hepatic cells were increased by about 30% over their dimensions in the mice of the other groups ($P = 0.012$). The nuclei of the hepatic cells were the same size in the mice receiving rDNA and in those receiving nDNA; the dimensions of the

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Table 1. Effect of DNP and DNA from the Regenerating Rabbit's Liver on Dimensions of Cells, Nuclei, and Nucleoli in the Regenerating Mouse Liver

Index	Fraction			
	rDNP	rDNA	nDNP	nDNA
Area of cell (μ^2)	360±41	317±34	234±16	269±29
Area of nucleus (μ^2)	40±5	35±3	31±2	34±2
Area of Nucleolus (μ^2)	2,7±0,3	2,7±0,2	2,2±0,3	2,4±0,2
No. of nucleoli in nucleus	3,17±0,39	3,09±0,54	3,12±0,43	2,67±0,24
Total area occupied by nucleoli (μ^2)	8,6±1,7	8,3±1,8	6,1±0,4	6,3±0,8
Nucleo-cytoplasmic ratio	1 : 8	1 : 8	1 : 6	1 : 7
Nucleolo-nuclear ratio	1 : 4	1 : 3	1 : 4	1 : 4

nucleus of the hepatic cell were influenced by rDNP, i.e., by the DNA-histone complex isolated from the regenerating liver. Meanwhile, injection of the fractions isolated from the regenerating liver led to an increase in the mass of nucleolar material in the nuclei of the hepatic cells by comparison with that in the mice receiving the fractions from the normal liver. The action of DNP and of the DNA isolated from it was identical. As the table shows, after injection of rDNP or rDNA, the total area occupied in the nucleus by the nucleoli increased by about 40% over their area in the mice receiving nDNP or nDNA ($P = 0.03$). This increase was due to an increase in the dimensions of the nucleoli.

The fractions isolated from the regenerating liver definitely stimulated the proliferation of the parenchymatous cells of the regenerating liver. The number of mitoses among the parenchymatous cells of the mice receiving rDNP and rDNA was 1.6 ± 0.9 and $2.5 \pm 1.1‰$, respectively, whereas, after injection of nDNP and nDNA the level of mitotic activity of the cells was 0 and $0.3 \pm 0.2‰$, respectively. The action of DNP and DNA was identical, i.e., the number of mitoses in the hepatic cells of the mice receiving the fractions from the regenerating rabbit's liver was greater than the number in the mice receiving the fractions from normal liver, and the increase was independent of the composition of the fractions. The number of binuclear hepatic cells in the regenerating liver likewise was independent of the composition of the fraction injected. After administration of rDNP it was $10.5 \pm 1.1\%$ and after administration of nDNA it was $15.7 \pm 3.8\%$.

According to the earlier findings, the normal liver contains 28.9% of nonparenchymatous cells, while the regenerating liver on the fourth day of regeneration shows an increase in the proportion of nonparenchymatous cells to 36.6% [5]. After injection of nDNP and rDNP the number of nonparenchymatous cells in the regenerating liver was 39.0 ± 1.7 and $39.7 \pm 2.4\%$, respectively, i.e., it was the same as the number in the regenerating liver without injection of DNP as found previously. After injection of rDNA the number of nonparenchymatous cells in the liver was $34.9 \pm 2.2\%$, which again was indistinguishable from their number on the regenerating liver of the mice not receiving injections. After injection of nDNA the number of nonparenchymatous cells in the liver of the mice increased to $43.5 \pm 3.0\%$ ($P < 0.001$).

The number of mitoses among the nonparenchymatous cells of the regenerating liver of the mice receiving rDNP, rDNA, or nDNA was the same, namely 2.6 ± 1.5, 2.6 ± 1.6, and $2.2 \pm 0.6‰$, respectively, and it differed from the level of mitotic activity of the nonparenchymatous cells of the regenerating liver in the mice receiving nDNP ($0.5 \pm 0.3‰$).

The nonparenchymatous cells were thus influenced by the fractions isolated, not from the regenerating, but from the normal liver. It is difficult to decide whether this action is one of stimulation, for at this stage of regeneration the number of mitoses among the nonparenchymatous cells was increased after injection of nDNA, while the mitotic activity was depressed under the influence of injection of nDNP. It may be postulated that the number of mitoses among the nonparenchymatous cells reached a maximum at an earlier time. The mechanism of action of these substances on the parenchymatous and nonparenchymatous cells of the liver is evidently different.

After assessing the results obtained and comparing them with previous findings, it may be concluded that DNP and DNA isolated from the regenerating rabbit's liver stimulate regeneration of the mouse's liver, and that the clearest stimulation is observed after injection of DNP. So far as the fractions from

normal liver are concerned, without affecting the parenchymatous cells, they stimulate proliferation of the nonparenchymatous cells.

LITERATURE CITED

1. M. A. Guberniev, E. M. Leikina, L. D. Liozner, et al., Byull. éksp. Biol., No. 6, 88 (1964).
2. E. M. Leikina, V. S. Tongur, L. D. Liozner, et al., Biokhimiya, No. 1, 96 (1960).
3. V. S. Tongur, E. M. Leikina, and L. G. Kulikova, Byull. éksp. Biol., No. 4, 66 (1959).
4. I. N. Yashina, Byull. éksp. Biol, No. 3, 101 (1963).
5. I. N. Yashina, Z. A. Ryabinina, and B. N. Gladyshev, Byull. éksp. Biol., No. 9, 116 (1964).