THE EFFECT OF DESOXYRIBONUCLEOPROTEINS OF THE REGENERATING RABBIT'S LIVER ON THE REGENERATION OF MOUSE LIVER

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Previous investigations have shown that during thermal fractionation of nucleoproteins isolated from the liver of the rabbit or ox, fractions are obtained which differ in their relative proportions of nitrogenous bases [1]. These proportions differ in the desoxyribonucleoprotein (DNP) fractions isolated from the normal and regenerating liver of the rabbit; these fractions differ in their action on the regeneration of mouse liver [2]. After intravenous injection of the 1st fraction of the DNP isolated from the regenerating liver of rabbits into mice from which the left lateral lobe of the liver had been removed, the weight of the regenerating liver on the 3rd day after the injection was 15% greater than the corresponding weight in control animals. This difference was due to hypertrophy of the liver cells and of their nuclei. Injection of the 1st fraction of DNP from normal rabbit's liver had no such effect.

In the present research we investigated the effect of the 1st fraction of the DNP isolated from the regenerating liver of a rabbit on the distribution of the nucleic acids in the cells of the regenerating liver of mice, on the dimensions of the cell and its nucleus and cytoplasm, and on the size and number of nucleoli.

EXPERIMENTAL METHOD

The method of obtaining DNP was described in previous papers [1, 2]. Experiments were carried out on 30 mice weighing 18-20 g, from which the left lateral lobe of the liver was removed. The animals were divided into three groups. The mice of the first group received, on the 2nd day after the operation, an injection of 0.1 ml of a solution of the 1st fraction of DNP from regenerating liver (DRL), containing 17 μg of DNA, into the caudal vein. The mice of the second group received an injection of the same volume of a solution of the 1st fraction of DNP from normal rabbit's liver (DNL). The third group of animals (controls) received an injection of saline solution of the same concentration as that containing the DNP fractions. The mice were sacrificed 4 days after the operation. The liver was fixed in cold 80% ethyl alcohol and cut into sections 7-8 μ thick. The sections were stained with azure-eosin, with methyl green–pyronine, and by Feulgen's method.

Measurements were made by means of a screw ocular micrometer, using a 15× ocular and a 120× objective. Mononuclear liver cells of mice receiving DRL injections (7) and of control animals (5) were measured. In each case 100 cells were measured, in two diameters, and also their nuclei and nucleoli in two diameters, and the nucleoli were counted. The area (S) of the cell, nucleus, and nucleolus was calculated. The area of the cell was taken to be the product of the two diameters. The area of the nucleus was calculated by the formula for the area of an ellipse, and the area of the nucleolus by the formula for the area of a circle.

EXPERIMENTAL RESULTS

Two types of distribution of RNA in the liver cells during the interphase were observed in the liver sections from the control animals (Fig. 1, a): in the first, RNA granules filled the whole cytoplasm of the liver cell uniformly; in the second, the RNA granules formed a "belt" around the nucelus, rather like a string of beads in appearance. In the second type, the rest of the cytoplasm remained free and the granules were large.

After injection of the DRL, only the first type of distribution of RNA in the cytoplasm of the liver cells was observed (Fig. 1, b). The RNA granules were of various sizes – from very large to very small, – giving the impression

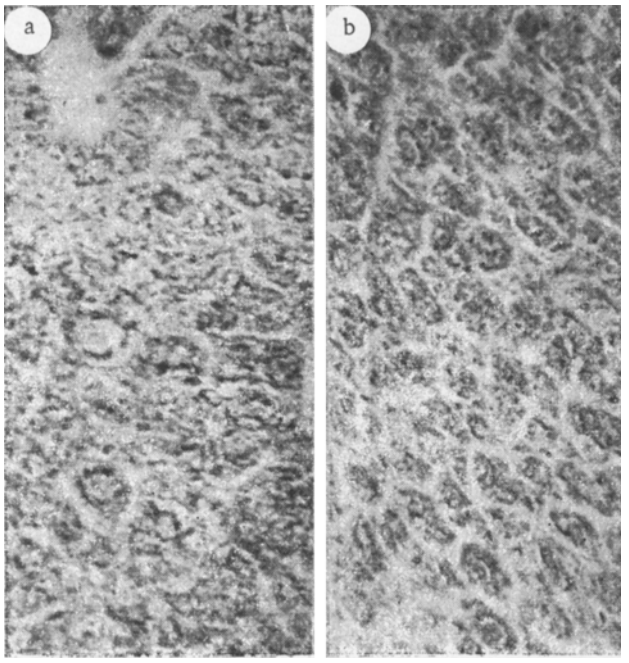

Fig. 1. RNA granules in cells of the regenerating liver of a control mouse (a) and after injection of DRL (b). Ocular 10×, objective 50×. Methyl green – pyronine.

of a "washed-out" basophilia. The granules were irregular in shape. The amount of RNA in both the cytoplasm and the nucleoli increased sharply over that present in the controls.

After injection of DNL the distribution of RNA granules in the cytoplasm of the liver cells was largely the same as in the controls. However, the number of cells with a bead-like distribution of RNA was greatly increased. The RNA granules were larger and stained more intensively with pyronine than in the controls, and the appearance of "washed-out" basophilia was not observed. The nucleoli stained more intensively with pyronine than in the controls. The cells contained more RNA than in the control animals, but significantly less than after injection of DRL (see table). Both in the control preparations and in the liver of the mice receiving injections of DNL and DRL, variations in the degree of basophilia occurred. In all cases the more strongly basophilic areas were mainly concentrated around the blood vessels (around the central veins and at the periphery of the lobules), but after injection of DRL they occupied a much larger area than after injection of DNL or physiological saline.

The RNA granules in the cells in a stage of mitosis in all three groups of mice were fine and gelatinous, and were uniformly situated in the cytoplasm at a short distance from the nucelus.

Hence, the injection of the first fraction of DRL affected both the character of the distribution of RNA and its amount in the cells of the regenerating liver. This indicates changes in the metabolism and mobilization of the RNA in the cells and, possibly, an increase in protein synthesis in the liver.

The DNA content in the nuclei of the liver cells was low in all three groups of animals. It was slightly greater after injection of DRL than in the controls. Previous investigations [2] also showed that injection of DRL and DNL does not affect the mitotic activity of the regenerating liver.

Effect of Desoxyribonucleoproteins on the RNA Content in the Cells of the Regenerating Mouse Liver

Injection of DRL		Injection of DNL		Injection of physiological saline (control)	
Mouse No.	Amount of RNA	Mouse No.	Amount of RNA	Mouse No.	Amount of RNA
1	+++++	1	+++	1	+++
2	++++	2	+++	2	++
3	++++	3	+++	3	+++
4	+++++	4	+++	4	++
5	++++	5	+++	5	++
6	++++	6	+++	6	++
7	+++++	7	+++	7	++
Mean	++++		+++		++

Legend: ++ very small amount of RNA; +++ moderate; +++++ large amount of RNA

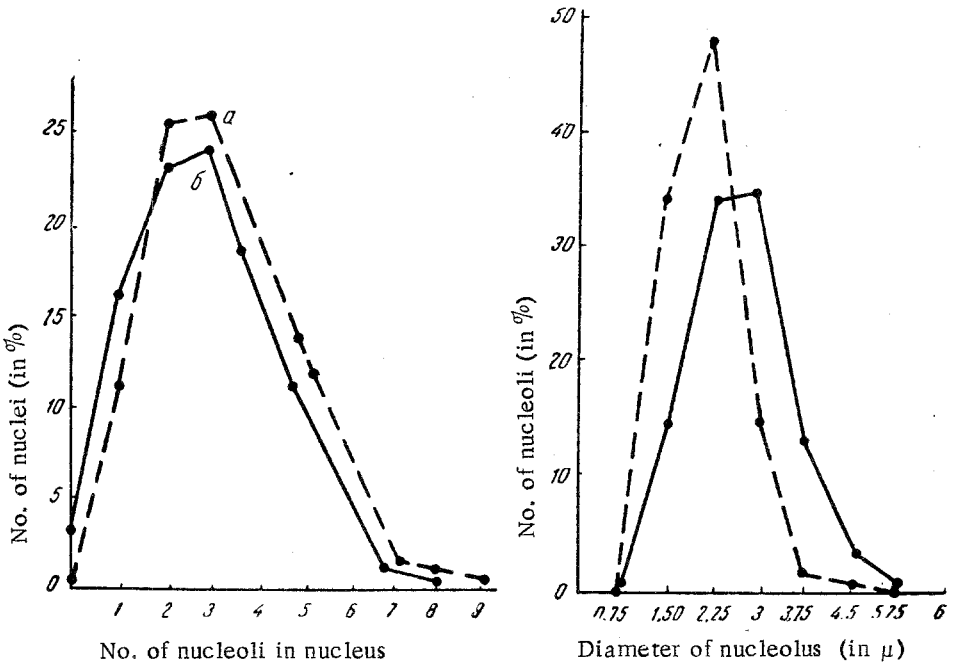

Fig. 2. Number of nucleoli in the nuclei of the regenerating liver cells in the control (a) and experimental (b) animals.

Fig. 3. Dimensions of nucleoli in the nuclei of the regenerating liver cells in the control (a) and experimental (b) animals.

After injection of DRL, the value of S of the liver cell increased by 25% over the control (S in the experimental animals was 825 μ^2 and in the controls 670 μ^2; P = 0.005). These results agreed with the previous findings [2], when the size of the cells was estimated from their number in a field of vision.

The difference between the area of the nuclei in the liver of the mice receiving DRL and of the control animals was about 33% (S in the experimental animals was 126 μ^2 and in the controls, 94 μ^2). The results were close to statistically significant (P = 0.044). S of the cytoplasm in the experimental series was 698 μ^2 and in the controls, 505 μ^2, the difference being 30%. The plasmonuclear ratio in both the experimental and the control series was 6:1.

After injection of DRL the number of nucleoli in the nuclei of the regenerating liver cells decreased. In the experimental series the number of nucleoli in the nucleus averaged 2.88, and in the controls, 3.13. We counted the nuclei with 1, 2, 3 and more nucleoli in the control and experimental animals. The curves reflecting the number of nucleoli in the nucleus in the experimental and control series were found to coincide (Fig. 2). The decrease in the mean number of nucleoli after injection of DRL was the result of a decrease in the number of cells with 2 and 3 nucleoli by comparison with the controls. However, the dimensions of the nucleoli in the experimental animals were increased: their average area was 4.19 μ^2 compared with 2.84 μ^2 in the controls; P = 0.007. After injection of DRL there was an increase in the number of large nucleoli, the diameter of which exceeded 3 μ (Fig. 3). The total area of the nucleus occupied by the nucleoli averaged 12 μ^2 in the experimental animals and 9 μ^2 in the controls. The nucleolo-nuclear ratio in both the experimental and the control animals was 1:10.

The increase in the size of the nucleoli indicated an increase in the RNA synthesis in the nucleus. We conclude from our results that injection of the first fraction of DRL affected the formation of RNA in the cells of the regenerating liver and caused RNA to accumulate in the cytoplasm. The nature of the biological activity of the first fraction of DRL is still uncertain.

We also performed one variant of this experiment, in which the mice were sacrificed on the 4th day after operation. The effect of the first fraction of DRL in stimulating the process of regeneration in this case took the form of hypertrophy of the cells, nuclei, and nucleoli, and of the accumulation of RNA in the cells. In the later stages of regeneration the effect of DRL would possibly be shown more completely.

We may thus conclude that the intravenous injection of the first fraction of desoxyribonucleoproteins isolated from the regenerating liver of a rabbit into mice from which part of the liver had been removed led to hypertrophy of the cells of the residual part of the liver, and also of their nuclei and nucleoli. The injection of DNP from the regenerating liver and from normal liver caused an increase in the RNA concentration in the cells of the regenerating mouse liver. The DRL had the stronger action in this respect. As a result of its administration the number of nucleoli in the nuclei of the liver cells decreased, but the size of the nucleoli and the total amount of RNA in the nucleus increased. The plasmonuclear and nucleolo-nuclear ratios were unchanged.

SUMMARY

A study was made of the effect produced by the I desoxyribonucleoprotein fraction obtained by thermal fractionation from the rabbit liver (both normal and regenerating) on the regenerative process of the mouse liver.

The I DNP fraction of the regenerating rabbit liver was injected intravenously to mice with a removed part of the liver; on the 4th day of regeneration this led to hypertrophy of the cells of the remaining part of the liver, as well as to hypertrophy of their nuclei and nucleoli. Administration of DNP both from the regenerating and from the normal liver provoked a rise of the RNA content in the cells of the regenerating liver of mice. The effect of DNP from the regenerating liver proved to be stronger. As a result of the DNP administration from the regenerating liver there was a decrease in the number of nucleoli in the nuclei of hepatic cells, but the sizes of the nucleoli and the total amount of RNA in the nucleus increased. The nuclear-plasma ratio and the nucleolar-nuclear ratio exhibit no changes.

LITERATURE CITED

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2. V. S. Tongur, E. M. Leikina, and L. G. Kulikova. Byull. éksper. biol., 4, 66 (1959).

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
