CHANGE IN THE CONCENTRATION OF NUCLEIC ACIDS IN REGENERATING MOUSE LIVER UNDER THE INFLUENCE OF RABBIT-LIVER DNA

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The results obtained [3,7,9,12] on the stimulation of regeneration of various organs by preparations of nucleoproteins or nucleic acids are widely at variance. Also it is not clear what biochemical processes occur on stimulation of regeneration by these substances.

Previously it was shown [2] that if mice are injected one day after partial hepatectomy with a preparation of desoxyribonucleo protein (DNP), obtained from regenerating rabbit liver, by the fourth day after the operation there is a marked increase in liver weight due to an increase in the size of the cells and of their nuclei. Histological study has shown that together with the increase in liver weight the RNA content of the cells also increases. DNP obtained from undamaged rabbit liver produced no such effect. We do not know whether only DNP from regenerating liver exerts such an effect, or whether it is the desoxyribonucleic acid (DNA) which enters into its composition.

In the present work we have attempted to determine how the injection of DNA obtained from normal and from regenerating rabbit liver influences the concentration of nucleic acids in the tissue of regenerating mouse liver.

METHOD

In the experiment we used preparations of DNA separated from DNP of normal or of regenerating rabbit liver.

To obtain the nucleo proteins we used the liver of male rabbits weighing 2-2.5 kg. Usually for the experiment we used the livers taken from five animals. To produce regenerating liver we removed two thirds, always the right and left lobes, and ligated their base. The extirpated portions were washed with cold 0.14 M NaCl solution and were immediately frozen with solid CO_2. On the next day, from the normal portion of the liver taken from all rabbits we extracted DNP by the method of Mirsky and Pollister [8]. The rabbits were killed by decapitation 68-70 hours after hepatectomy. This period of regeneration was chosen because according to published results [4] in rabbits, 72 hours after excision of $^2/_3$ of the liver numerous mitoses are observed in the hepatic cells, and after 68-70 hours a considerable increase in the size of the organ has occurred and the maximum increase in the concentration of DNA has been reached.

The liver was removed, and after it had been washed in a cold solution of 0.14 M NaCl it was frozen in solid CO_2. On the day after the animals had been killed DNP was extracted from the regenerating liver.

The DNA was extracted from the DNP by the phenol method [1]. To remove the phenol the DNA solutions in 0.14 M NaCl were dialized in the cold for three days against 0.14 M NaCl solution, and the N/P ratio determined (nitrogen was determined by the Kjeldahl-Conway method, and phosphorous was determined spectrophotometrically [5], and also in terms of the molecular weight [6]). In the preparations we tested for protein by the microburet reaction [7]. For the experiment we took only the preparation of DNA having a N/P ratio of 1.76-1.78, with a

TABLE 1. Weight of regenerated mouse liver on the 4th day after partial hepatectomy and intravenous injection of n-DNA, r-DNA, and 0.14 M NaCl solution.

Injected substance	No. of animals	M
n-DNA	19	4.67
r-DNA	24	5.39
0.14 M NaCl solution	20	4.26

Note. D = 0.72; t = 4; P = 0.001.

TABLE 2. Effect of Intravenous Injection of n-DNA, r-DNA, and 0.14 M NaCl Solution on the Concentration of Nucleic Acids in Regenerating Mouse Liver

Injected substance	No. of rats	Concentration		RNA/DNA
		RNA	DNA	
		In mg per g raw liver weight		
n-DNA	1	10.4	4.2	2.47
	2	9.8	3.8	2.57
	3	9.6	3.6	2.66
	4	10.2	4.2	2.42
	5	10.2	4.2	2.42
	Mean	10.0	4.0	2.50
r-DNA	6	13.2	3.8	3.47
	7	12.8	4.2	3.04
	8	13.4	4.4	3.04
	9	12.6	4.4	2.86
	10	13.2	4.2	3.14
	Mean	13.0	4.2	3.11
0.14 M NaCl solution	11	9.6	3.6	2.66
	12	9.8	4.0	2.45
	13	10.0	4.0	2.50
	14	10.0	4.2	2.38
	15	9.8	3.6	2.72
	Mean	9.8	3.7	2.54

a molecular weight of 4-5 million, and containing no protein.

The work was carried out on sixty white male rats weighing 18-20 g. We removed the left lobe of the liver from all the animals. The mice were divided into three groups of twenty animals each. In the first group, on the day after the operation the DNA solution obtained from normal rabbit liver (n-DNA), in a 0.14 M NaCl solution was injected into the tail vein; mice of the second group received DNA obtained from regenerating liver (r-DNA) in 0.14 M NaCl solution; mice of the third group (controls) received a 0.14 M NaCl solution. The amount of the preparation given was 20 μg of DNA (0.25 ml) per 20 g weight of the animal. On the fourth day after hepatectomy the mice were killed by decapitation.

In order to determine the extent of the regeneration, in each animal the liver weight was determined (results treated statistically), and in five of each group the concentration in the liver of RNA and DNA was determined by the Schmidt-Thannhauser method [10]. The whole analysis was carried out in a cold room. For this purpose from each animal we took two weighed portions of 100 mg of raw liver, and the estimation was carried out on four parallel samples. For the analysis we used chloric acid. RNA and DNA were determined spectrophotometrically [5].

RESULTS

The results obtained are shown in Tables 1 and 2.

There is no overlap between the figures for the amounts of RNA and DNA, and therefore no statistical treatment is required.

As can be seen from the results given, under the influence of injected r-DNA the amount of RNA increases and at the same time the weight of regenerated tissue also rises; n-DNA shows no influence of this kind. The problem of the nature of the activity of the DNA liberated from regenerating rabbit liver and stimulating regeneration of mouse liver, and therefore having no species-specificity, requires a further study.

LITERATURE CITED

1. G. P. Georgiev. Biokhimiya, No. 3, (1959), p. 472.
2. E. M. Leikina, V. S. Tongur, and L. D. Liozner. et al. Biokhimiya, No. 1, (1960), p. 96.
3. L. V. Polezhaev, L. V. Akhabadze, and N. A. Muzlaeva. et al. Dokl. AN SSSR 145, No. 5, (1962), p. 1180.
4. B. P. Solopaev. Relationship Between Repair Processes in the Liver and the Function of Biliary Secretion. Candidates' dissertation. Moscow, (1956).
5. A. S. Spirin. Biokhimiya, No. 5, (1958), p. 656.
6. D. M. Spitkovskii. Bio. No. 4, (1958), p. 396.

7. G. D. Tumanishvili. J. Embryol. exp. Morphol., 8, (1960), p. 226.
8. A. E. Mirsky and A. W. Pollister. J. gen. Physiol., 30, (1946), p. 117.
9. J. Mortelmans. Scalpel (Brur.) 113, (1960), p. 409.
10. G. Schmidt and S. J. Thannhauser. J. biol. Chem., 161, (1945), p. 83.
11. J. D. Smith and R. Markham. Biochem. J. 46, (1950), p. 509.
12. V. Vasilescu, V. Niculescu and S. Capilna. et. al. Stud. Cercet. Fiziol., 6, No. 2, 229 (1961).

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