EFFECT OF LIVER RNA ON THE COURSE OF EXPERIMENTAL CIRRHOSIS OF THE LIVER

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Injection of cytoplasmic RNA from rat liver into mice during chronic poisoning with CCl_4 reduced the mortality among the animals, reduced the number of foci of necrosis, and increased the quantity of interlobular connective-tissue fibers in the liver. An increase in mitotic activity of the liver cells also was observed.

In connection with investigation of the possible therapeutic use of nucleic acids, an increasing number of papers describing the study of the nonspecific action of exogenous yeast RNA and its hydrolysates and also the action of organ-specific RNA in vivo have appeared in the literature [6, 8].

In a series of investigations by Belous et al. [2, 3], the specificity of the effect of exogenous bone RNA on regeneration of injured bones, on the synthesis of collagen in them, and on the intensification of synthesis of endogenous RNA was demonstrated. Alekseev and Konyshev [1] found an increase in the rate of growth of the liver in chick embryos and acceleration of incorporation of label and of their RNA under the influence of RNA from cock liver.

Although the mechanism of the observed phenomena cannot yet be regarded as fully explained, their practical significance is so important that attempts to reproduce these effects of organ-specific RNA on other models are completely justified.

Several workers have shown [5] that in cirrhosis of the liver produced by CCl_4, partial hepatectomy leads to regression of connective tissue in the liver. It is natural to assume that the formation of new RNA during regeneration provoked by hepatectomy plays an important role in this process.

In the investigation described below, the effect of exogenous liver RNA on the survival rate and the histological picture of the liver was studied during chronic CCl_4 poisoning.

TABLE 1. Effect of RNA on Content of Total Lipids and Collagen in Liver of Mice During Poisoning with CCl_4 for 2 Months

Group of mice	Survival rate				Lipids, % of dry weight	P	Collagen, % of dry weight	P
	total no.	no. of survivors	no. dying	no. used for analysis				
1-st	76	10	66	10	12—3,8	>0,05	0,95—0,13	0,02
2-nd	76	52	24	10	16—3,4		1,15—0,11	
3-rd	16	16	0	5	18—3,0		0,45—0,21	

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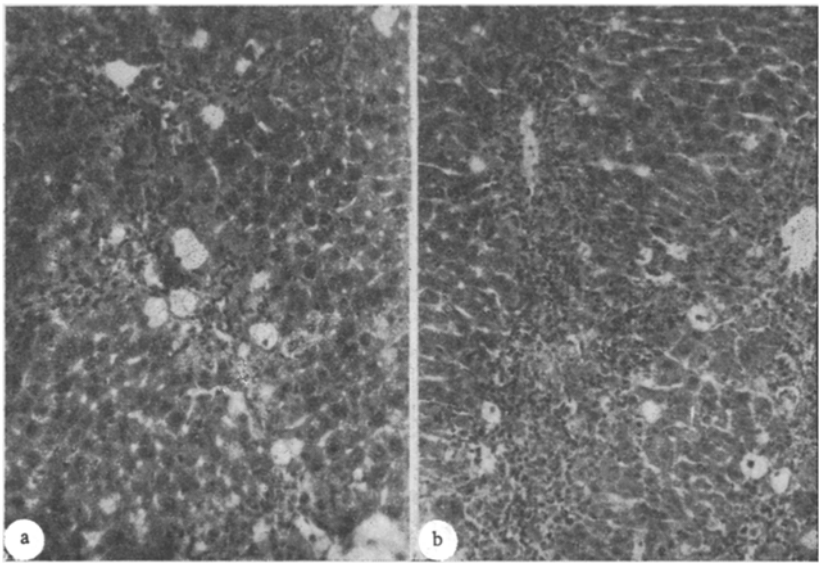

Fig. 1. Proliferation of interlobular connective tissue in mouse liver: a) CCl_4 poisoning; b) CCl_4 poisoning and RNA. Van Gieson, 100 ×.

TABLE 2. Effect of RNA on Mitotic Activity of Liver Cells in Mice Poisoned with CCl_4

Experimental conditions	No. of mice taken for analysis	Mitotic index
CCl_4 (2 months)	10	12,9± 1,0
The same + RNA	10	23,0± 3,15 P=0,05
CCl_4 (1 month)	7	5,2± 1,9
The same + RNA	7	22,3± 6,16 P=0,05
The same + heated RNA	5	2,63± 0,59 P=0,05

EXPERIMENTAL METHOD

Experiments were carried out on albino mice weighing 24-28 g. In the first series of experiments mice of group 1 received a subcutaneous injection of 0.04 ml CCl_4 twice a week; mice of group 2 also received injections of CCl_4 and, on the same days, received intraperitoneal injections of freshly prepared cytoplasmic RNA from rat liver in doses of 60-80 μg per mouse; the mice of group 3 received RNA only. The experiment continued for 2 months.

In the second series of experiments, mice of both groups were injected with CCl_4 only for 1 or 2 months, and subsequently the mice of group 1 received intraperitoneal injections of physiological saline while the mice of group 2 received RNA without stopping the administration of CCl_4.

RNA was obtained by the method of Georgiev and Mant'eva [4] and was used on the day of its preparation. Pieces of liver for histological examination were fixed in 12% formalin and in Carnoy's solution. The material was stained by the methods of Deddi and Van Gieson and with hematoxylin and eosin.

Total lipids were determined gravimetrically after extraction with dichloroethane. Collagen was extracted from the defatted dry tissue with trichloroacetic acid [7], and hydroxyproline was determined by the method of Neman et al. [10] as modified by Martin and Axelrod [9].

RESULTS

Because of the concept of species nonspecificity of messenger RNA, mice were injected with RNA obtained from rat liver. After 1 month, differences between the mortality of mice of groups 1 and 2 began to be observed. This difference increased sharply, and after 2 months, when of the 76 mice of group 1 only 10 had survived, all the experimental animals were sacrificed (Table 1).

Histological investigation of the liver from the mice of group 1 revealed degenerative changes in the liver tissue in the form of homogenization of the cytoplasm and pycnosis of the nuclei of the liver cells with the appearance of optically empty and fat-loaded cells at the periphery of the lobules and marked

hypertrophy of the interlobular connective tissue (Fig. 1a). In most animals, in addition, small foci of necrosis were observed at the periphery of the lobules, while in three of the 10 animals the foci were massive. Diffuse infiltration with tiny droplets of fat and marked proliferation of Kupffer cells were observed. The hepatic vessels were frequently hyperemic, and stasis was found here and there.

Optically empty cells at the periphery of the lobules were more frequently found in the liver of mice belonging to group 2, the quantity of interlobular connective tissue was twice as great (Fig. 1b), but foci of necrosis were found only in isolated cases. The quantity and distribution of fat did not differ significantly from those in the liver of the mice of group 1. Hyperemia of the vessels, stasis, and sometimes thrombi in the lumen of the vessels, with small hemorrhages around them, were observed. No significant difference was observed in the liver of the mice of group 3 compared with that of intact mice. Quantitative estimation of total lipids and collagen was obviously important. Injection of RNA into the animals poisoned with CCl_4 had no appreciable effect on the total lipid content in the liver (Table 1). In all poisoned animals the collagen content was increased, but this increase was more marked in the group of mice receiving RNA. The difference is statistically significant.

In another series of experiments, the beneficial effect of RNA on mitotic activity of the cells was demonstrated, while injection of these same RNA preparations, if heated on a boiling water bath for 30 min, actually produced a statistically significant decrease in the mitotic index (Table 2).

It is thus possible that the effect of RNA on the survival of mice may be associated with its effect on mitotic activity. However, comparison of the decrease in mortality among animals receiving RNA during CCl_4 poisoning with the acceleration of proliferation of connective tissue still does not allow the possibility to be excluded that the more rapid replacement of necrotic liver cells by connective tissue could have reduced the intensity of poisoning at that stage of CCl_4 administration when toxic effects were mainly responsible for death of the animals.

Investigation of the cause of the increased collagen synthesis following administration of RNA to poisoned animals is being continued. However, since injection of RNA into intact mice did not change the collagen content in the liver, the impression is gained that the RNA did not carry information for the synthesis of this protein and that most probably RNA nonspecifically increased protein synthesis in fibroblasts, which are probably more resistant to the action of CCl_4 than liver cells.

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