ALKALINE RIBONUCLEASE ACTIVITY IN THE SERUM OF RATS POISONED WITH CARBON TETRACHLORIDE AND THE EFFECT OF HEPATIC INHIBITOR IN VITRO

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Activity of free alkaline RNase in the blood serum was increased 3 h after intraperitoneal injection of CCl_4 (0.3 ml/100 g body weight, in vegetable oil) into rats. Addition of partially purified inhibitor from the liver of intact rats to the sera considerably inhibited activity of the enzyme in the sera of the poisoned and control animals. Activity of the free enzymes in the serum fell after 3 h, then began to rise again to normal. The role of changes in free alkaline RNase activity in the blood serum during CCl_4 poisoning in the transport of exogenous polymer RNA to the liver is discussed.

An important role in the investigation of the possible therapeutic use of exogenous hepatic RNAs in pathological conditions of the liver is evidently played by the transport of RNA in a polymer state to the liver cells. In previous investigations the writers observed an effect from administration of small doses of polymerized hepatic RNA in rats with chronic CCl_4 poisoning, but only if this RNA was injected during the period of regeneration after administration of the poison had ceased [2, 3]. RNA injected in the course of poisoning evidently undergoes rapid degradation through the activity of RNases in the blood or in the liver cells. This phenomenon considerably reduced the prospective value of therapeutic use of exogenous RNAs. It was decided to investigate which RNases are activated in CCl_4 poisoning and where this activation takes place.

Acid RNase of the lysosomes is found relatively soon after administration of CCl_4 in the cytoplasm of the liver cells [4] and in the blood serum [1]. It is difficult to imagine that the acid optimum of action could be responsible for degradation of the exogenous RNA in the blood or cytoplasm of the liver cells in the absence of any massive necrosis of the cells. Moreover, the formation of lysosomal RNase, like that of other proteins, may be distrubed in CCl_4 poisoning with a simultaneous increase in its liberation from the lysosomes, as a result of which the breakdown of exogenous RNA in the phagosomes by pinocytosis may actually be reduced.

The suggestion has been made [2, 3] that one of the alkaline RNases of the liver may participate in the degradation of exogenous RNA in liver damage by CCl_4, namely RNase II, with an optimum of action at pH 7-8. It and its inhibitor evidently control intracellular breakdown of RNA [7]. The inhibitor is present in excess relative to alkaline RNase II in the liver cells of intact rats, as a result of which the activity of the free enzyme in the liver is very low [7, 9]. However, free alkaline RNase II is present in the blood of intact rats in large quantities comparable with those of the bound enzyme [12]. The protein nature of the inhibitor has now been demonstrated [6-9, 13]. After partial hepatectomy a decrease in the activity of the free enzyme is observed in the blood serum on account of an increase in the quantity of enzyme bound with the inhibitor [12].

Considering that the permeability of the liver cells is increased during the action of CCl_4 and that synthesis of the inhibitor protein may be disturbed, an increase in the concentration of free RNase II in the

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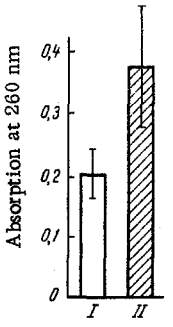

Fig. 1

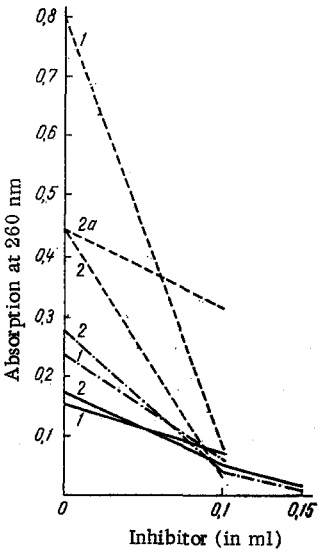

Fig. 2

Fig. 1. Effect of acute CCl_4 poisoning on RNase II activity in blood serum of rats: I) serum of control rats; II) serum of poisoned rats.

Fig. 2. Effect of hepatic inhibitor in vitro on activity of pancreatic RNase II in sera of rats. Broken line shows pancreatic RNase; continuous line, serum of control rats; line of dots and dashes represents serum of poisoned rats; 1) inhibitor 1; 2) inhibitor 2 (independent experiments); 2a) inhibitor 2 heated for 7 min at 65°C.

blood serum of the poisoned rats may be expected. On this account it was decided to investigate the activity of free RNase II in the serum of rats during acute CCl_4 poisoning and the effect of partially purified inhibitor from the liver of intact rats on this enzyme in vitro.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 200-300 g were used and for the 18 h before the experiment they were given water only. The control animals received vegetable oil (apricot or sunflower) intraperitoneally, while the experimental rats received a mixture of CCl_4 with the same oil (1:1) in a dose of 0.3 ml CCl_4/100 g body weight. Blood was taken from the jugular vein 3 h later under ether anesthesia. Two groups of animals were used to investigate the dynamics of RNase II activity: the rats of group 1 received CCl_4 by subcutaneous injection in a dose of 0.125 ml/100 g body weight twice a week for 1.5 months; the intact rats of group 2 received CCl_4 intraperitoneally in oil by a single injection in the same dose. The blood was immediately centrifuged and activity of RNase II determined in the serum [12, 13]. The samples contained 0.1 ml serum, diluted 1:5 with physiological saline, 0.1 ml water, 0.1 ml 0.2 M Tris-HCl buffer, pH 7.8, and 0.2 ml 1% solution of lyophilized rat hepatic RNA. The RNA was obtained by Georgiev's method [1] and reprecipitated with 2.5 M NaCl solution and ethanol. After incubation (20 min at 37°C) samples were quickly cooled and the RNA not yet broken down was precipitated with 0.5 ml 1 M HCl solution in 76% ethanol. The optical density of the supernatant, diluted 6 times with water, was measured at 260 nm. To determine the action of the inhibitor, it was added to the samples in a volume of 0.1 ml instead of bidistilled water. The inhibitor was obtained in a partially purified form by Roth's method [8]. Its activity was determined against crystalline pancreatic RNase diluted in 0.1% gelatin [5].

Nucleotides preformed in the serum were determined in 0.05 ml after precipitation with acid ethanol or with uranyl reagent (to determine the content of small nucleotides formed by the action of acid RNase). The preformed nucleotides in the liver tissue were also determined in samples of liver kept at −20°C, after freezing and thawing 3 times and homogenization in a glass homogenizer with glass pestle. The nucleotide concentration was expressed relative to fresh and dry weight of the liver.

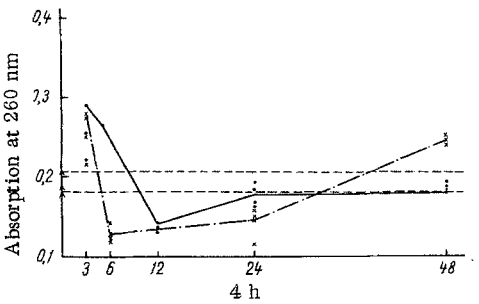

Fig. 3. Dynamics of changes in RNase II activity in blood serum of rats after injection of CCl_4. Continuous line represents serum of rats after chronic subcutaneous injection of CCl_4; dots indicate individual measurements. Line of dots and dashes represents serum of rats after a single intraperitoneal injection of CCl_4; crosses represent individual measurements. Broken lines show limits of enzyme activity in serum of intact rats; triangles show individual measurement.

EXPERIMENTAL RESULTS AND DISCUSSION

Activity of free RNase II in the blood serum of the rats was increased 3 h after intraperitoneal injection of CCl_4 by comparison with its activity in the serum of the control rats by 30-120% in individual experiments. The mean results from 10 experiments are given in Fig. 1.

After this time no statistically significant increase in the content of preformed nucleotides could be found in the serum of the poisoned rats when acid ethanol or uranyl reagent was used to precipitate the nucleic acids. A slight decrease in the nucleotide level in the liver tissue of the poisoned rats was evidently due to an increase in the moisture content in the organ. No marked breakdown of endogenous RNA in the liver had thus yet taken place under the action of acid or alkaline RNases.

The results of 4 experiments demonstrating the action of preparations of hepatic inhibitor are given in Fig. 2. Identical quantities of the same preparation of inhibitor inhibited the activity of both pancreatic crystalline RNase and of RNase II in the sera of the control and poisoned rats. Heating the inhibitor considerably reduced its activity. An increase in the activity of free RNase II in the serum, capable of being bound with the added hepatic inhibitor, could be taken to indicate a decrease in the quantity of inhibitor entering the blood stream from the liver. It is not yet clear whether this is the result of a decrease in its synthesis or a disturbance of its binding with RNase under the influence of CCl_4.

These results suggest that the increase in the blood level of RNase II could be the reason for rapid degradation of the exogenous polymerized RNA if it was administered 3 h after the CCl_4. However, as Fig. 3 shows, the increase in activity of the free enzyme in the serum caused by administration of CCl_4 was quickly followed by a decrease in its activity. As a result, the level of activity for some time could actually be lower than the lower limit of activity of the enzyme in the serum of the intact rats. Despite certain differences in the dynamics of the changes in the rats as a result of chronic poisoning by subcutaneous injection of CCl_4 and in the intact rats after a single intraperitoneal injection of the poison, the trend of the changes was identical.

After damage to the liver cells by CCl_4, when the administration of the poison had ceased, formation of the inhibitor of alkaline RNase in the liver thus increased just as was observed after partial hepatectomy and acute stress [12]. Probably the increase in the formation of this inhibitor during regeneration may explain the fact which the writers observed previously, that rats previously poisoned with CCl_4 are more "suitable" recipients for detection of the effect of polymerized exogenous RNA than intact rats [3].

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