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EFFECT OF CHRONIC CARBON TETRACHLORIDE POISONING ON THE TURNOVER OF RNA FRACTIONS IN RAT LIVER TISSUE

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Changes in the content and incorporation of 5-^{3}H-uridine after brief exposure to its labeled precursor were studied in the individual liver RNA fractions of rats during administration of carbon tetrachloride for 24 weeks. These fractions were obtained by preparative electrophoresis in 2.5% polyacrylamide gel from previously isolated nuclear and cytoplasmic RNA. Administration of CCl_4 to rats was shown to reduce the quantity of transfer and ribosomal RNA in the liver tissue. Chronic CCl_4 poisoning also disturbs the synchronization of the turnover of the individual components of fast-labeled RNA.

KEY WORDS: *carbon tetrachloride; liver; RNA metabolism.*

Administration of carbon tetrachloride (CCl_4) to rats is a widely used model of nonspecific toxic injury to the liver [6-8]. During the chronic action of this compound septal fibrosis and cirrhosis of the liver develop in animals of several species [1, 6]. However, the mechanism of action of CCl_4 is not clear and the biochemical changes taking place under these circumstances have been inadequately studied.

The object of this investigation was to study changes in the concentration and intensity of incorporation of a labeled precursor into individual RNA fractions of rat liver tissue during chronic administration of CCl_4.

EXPERIMENTAL METHOD

A subcutaneous injection of CCl_4 in a dose of 1 ml/kg body weight was given twice a week to noninbred male albino rats weighing 120-150 g. The liver of these animals was investigated between 4 and 24 weeks after the beginning of CCl_4 poisoning. At the end of

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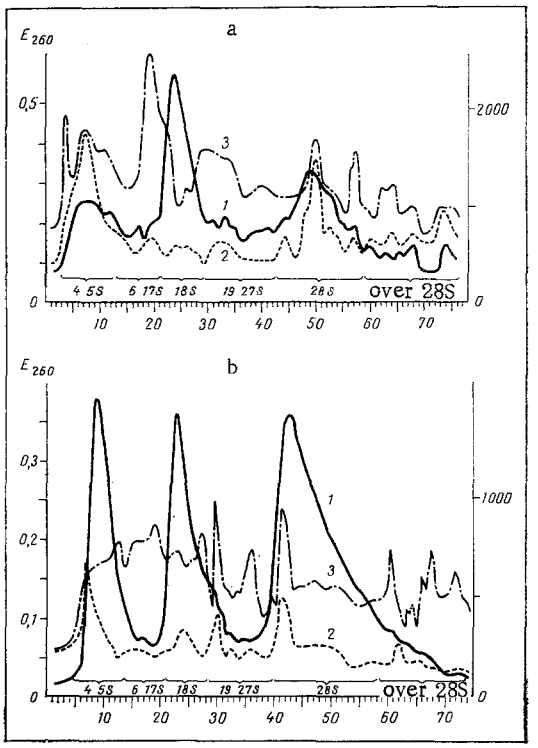

Fig. 1. Elution profile of nuclear (a) and cytoplasmic (b) liver RNA obtained by preparative electrophoresis in 2.5% polyacrylamide gel (mean results of five experiments). 1) RNA concentration; 2) radioactivity or fraction after exposure for 20 min, and 3) for 60 min with 5-^{3}H-uridine. Abscissa, braces mark boundaries of individual components of cRNA and nRNA obtained by pooling eluted fractions; bottom row of figures give fractions. Ordinate: right, radioactivity of fraction (in cpm); left, absorption at 260 nm.

this period septal fibrosis had developed in the liver tissue. The liver of healthy rats, held for the same length of time, served as the control. To study the rate of RNA turnover. 5-^{3}H-uridine (5 mCi/kg body weight, specific radioactivity 18-22 Ci/mmole) was injected intraperitoneally into the animals 20 and 60 min before sacrifice. Cytoplasmic (cRNA) and nuclear (nRNA) RNA were isolated by thermal phenolic fractionation [1, 3]. Individual fractions of cRNA and nRNA were obtained by preparative electrophoresis of the biopolymers in 2.5% polyacrylamide gel (PAG) [2]. The radioactivity of the samples was measured in a Mark-2 liquid scintillation counter in dioxane scintillator [1]. The specific radioactivity (SR) of the RNA (in counts/min/mg) and the relative specific radioactivity (RSR), as the ratio between SR of the RNA fraction and SR of the corresponding liver tissue, were calculated. The value of the sedimentation coefficient (K_S) was determined on the basis of the results of analytical electrophoresis in 2.5% PAG [4].

The Pearson—Bravais coefficient of correlation (r) and the criterion of its significance [5] were calculated on the Minsk-22 computer. Numerical values of r between significantly correlating parameters only are given below ($P < 0.05$).

EXPERIMENTAL RESULTS AND DISCUSSION

The results of fractionation of the liver nRNA and cRNA of the control rats by preparative electrophoresis are illustrated in Fig. 1. The principal components in the fractions were as follows: fractions with K_S 18 and 28S, ribosomal RNA (rRNA); fractions with 4-5S,

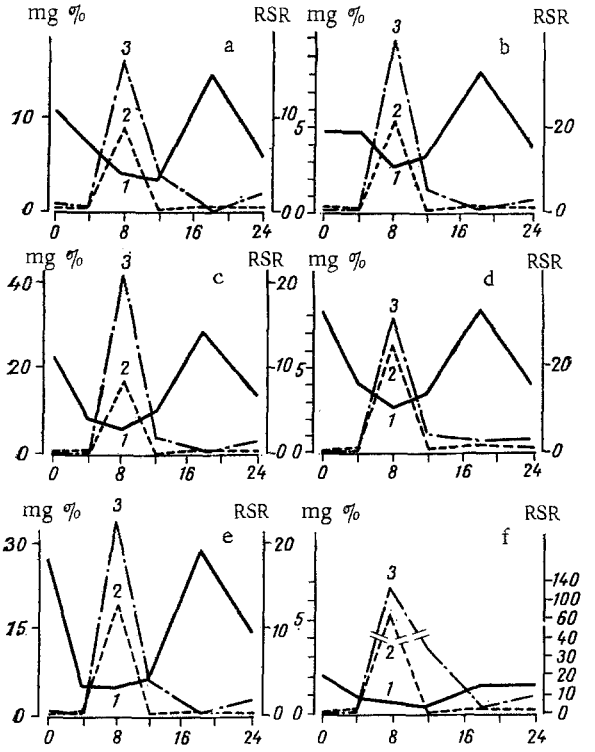

Fig. 2. Changes in concentration and incorporation of 5-^{3}H-uridine into individual components of rat liver nRNA during CCl_4 administration for 24 weeks: a) K_S of nRNA; b) 6-17S; c) 18S; d) 19-27S; e) 28S; f) over 28S. 1) RNA content in liver tissue; 2) RSR of components of nRNA after exposure to 5-^{3}H-uridine for 20 min, and 3) for 60 min. Abscissa, time (in weeks) after beginning of chronic CCl_4 poisoning. Ordinate: left, RNA concentration (in mg %) in liver tissue; right, RSR of RNA components.

mainly transfer RNA (tRNA) or its precursors; and RNA fractions with K_S values over 28, 19-27, and 6-17S, rRNA precursors and their degradation products. Low-molecular-weight RNA [10] made a small contribution to the 6-17S fraction of nuclear RNA [10]. Messenger RNA (mRNA) and its precursors were widely dispersed in the region of K_S from 9-10 to 35S and over [4]. These classes of macromolecules were characterized by a high rate of turnover, as reflected in incorporation of 5-^{3}H-uridine into the fractions during short exposures (20 and 60 min in the present experiments).

Changes in the individual components of liver nRNA of rats during CCl_4 poisoning for 24 weeks were analyzed. In the first 4-12 weeks of the experiment the content of all fractions of nRNA was reduced, but from the 12th to the 18th weeks it increased to values close to the control. From the 18th to the 24th week of the experiment the nRNA content fell in fractions with K_S of 4 to 28S (Fig. 2a-e). The turnover of all components of nRNA increased from the 4th to the 8th week of experiment, after which RSR fell sharply to the 12th week of the experiment (Fig. 2a-f). Later, RSR of the individual fractions of nRNA showed negligible changes with the exception of nRNA with K_S over 28S after exposure to the labeled precursor for 60 min.

The components of liver cRNA during administration of CCl_4 for 24 weeks underwent the following changes. The content of tRNA and 28S rRNA fell during the first 4 weeks of the experiment and thereafter remained below the control level (Fig. 3a, e). The content of the remaining components of cRNA did not differ significantly from normal during the 24 weeks of the experiment, with the exception of RNA with K_S over 28S, the content of which

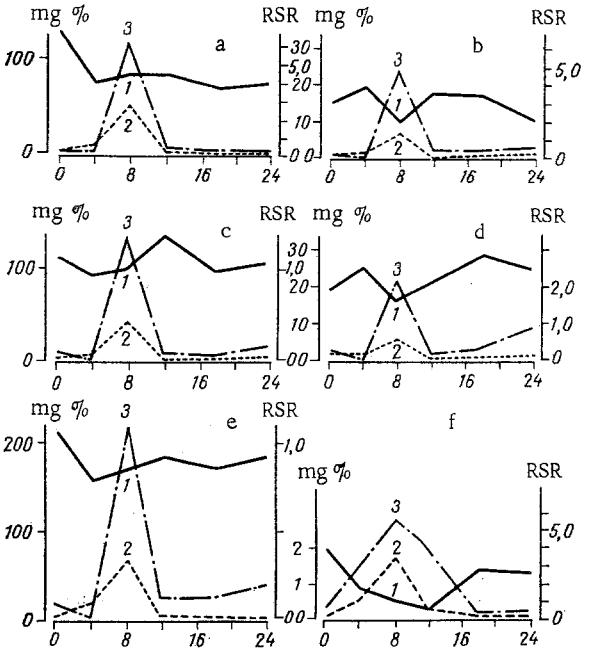

Fig. 3. Changes in concentration and incorporation of 5-^{3}H-uridine into individual components of cytoplasmic RNA of rat liver during CCl_4 administration for 24 weeks. Legend as in Fig. 2.

was lowered (Fig. 3b-f). Incorporation of 5-^{3}H-uridine into individual fractions of cytoplasmic RNA was similar during the first 12 weeks of the experiment: It rose sharply from the 4th to the 8th weeks of the experiment, but fell toward the 12th week. From the 12th to the 24th week of the experiment 20- and 60-min labeling of cRNA with K_s over 28S decreased, whereas RSR of the 18, 19-27, and 28S fractions of cytoplasmic RNA increased a little during labeling for 60 min.

The results of the statistical analysis of the biochemical data showed that throughout the 24 weeks of CCl_4 poisoning changes in the concentration of 18 and 28S nuclear rRNA correlated with each other (r = 0.988-0.998), whereas no such correlation was observed in the cytoplasm. This could be evidence of differences in the metabolism of rRNA in the small and large subunits of the ribosomes. Changes in RSR of the various RNA fractions during the first 12 weeks of the experiment were similar for all individual components of cRNA or nRNA (r = 0.999-0.951) for exposures of both 20 and 60 min to 5-^{3}H-uridine. From the 12th to the 24th week correlation was observed between 20-min labeling of nRNA of the 4-5 and 28S nRNA fractions (r = 0.999), 18 and 28S (r = 0.994) fractions, and fractions with K_s over 28 and 19-27S (r = 0.999), whereas after exposure to the labeled precursor for 60 min, a similar degree of labeling was observed for 4-5 and 6-17S nRNA (r = 0.999) and 19-27 and 6-17 cRNA (r = 0.999). Changes in the remaining parameters of the individual RNA components did not correlate with each other. Whereas in the first stages of CCl_4 poisoning the turnover of all the individual RNA fractions thus was synchronized, during prolonged administration of the hepatotoxic agent the synchronization of the turnover of several RNA components was disturbed.

The results suggest that in the initial period CCl_4 poisoning leads to a decrease in the content of rRNA and cRNA in the liver. The increase in RSR of RNA fractions with K_s over 28S after labeling for 60 min may indicate compensatory synthesis of the rRNA precursors. Probably as a result of this synthesis the content of RNA ribosomes showed a general tendency to increase from the 4th to the 24th weeks of the experiment (Fig. 3c, e). The tRNA content remained low until the very last stages of CCl_4 administration. The changes observed in the rRNA and tRNA content could be the cause of the decrease in the rate of translation in the liver tissue after administration of a hepatotropic poison [9].

The increase in the turnover of individual fractions of cRNA and nRNA within the range of K_s from 4-5 to 28S and over from the 4th to the 8th weeks of the experiment could

indicate an increase in the rate of turnover of mRNA and of heterogeneous nRNA. In the case of heterogeneous nRNA, by the 24th week of the experiment the increase in the rate of incorporation of 5-^{3}H-uridine compared with normal was considerable (Fig. 2a-f). As the results of correlation analysis show, from the 12th to the 24th weeks of the experiment synchronization of metabolism between individual fractions of heterogeneous nRNA also was disturbed. The results described above are evidence of profound changes in transcription in the liver as a result of chronic CCl_4 poisoning.

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INVESTIGATION OF THE POSSIBLE USE OF IMMOBILIZED UREASE FOR DECOMPOSING UREA IN BLOOD PLASMA

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The rate of decomposition of urea in citrated donors' plasma by soluble urease and by urease immobilized by addition to the carboxymethyl ester of cellulose, the 2-(3'-amino-4'-methoxyphenyl)sulfonylethyl ester of cellulose, the diethylaminoethyl ester of cellulose, stained with dichlorotriazine dye, or the grafted copolymer of cellulose and polyglycidylmethacrylate was found to be closely similar. Preparations of immobilized urease can be used repeatedly to decompose urea in citrated donors' plasma. Periodic treatment of these preparations with cysteine solution resulted in a smaller degree of decrease in the enzyme activity of the immobilized urease during repeated use.

KEY WORDS: *urea, urease; immobilization of enzymes; blood plasma.*

A basically new method of purifying blood from toxic substances has begun to be developed in recent years, namely the hemosorption method. This method is based on the use of "sorbents," capable of selectively removing certain toxic substances from the blood, or specific catalysts which convert toxic into less toxic substances. In 1971, Lopukhin et al.

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