It can be concluded that the decrease in the cAMP level in the brain after extremal stimulation was not associated with a disturbance of energy metabolism or with inadequacy of ATP synthesis. The main cause of the decrease in the cAMP concentration in the brain during extremal stimulation was the decrease in the level of NA and, perhaps, of other biologically active amines. In the writers' laboratory a sharp decrease in the NA level in the brain tissue was observed after electrical stimulation of the immobilized rats for 3 h, when a marked decrease in the NA concentration is observed in the hypothalamic region [3].

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EFFECT OF CARBON TETRACHLORIDE ON RNA METABOLISM IN THE RAT LIVER

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The effect of systematic administration of carbon tetrachloride (CCl_4) to rats on the RNA content in the liver and the intensity of incorporation of the labeled precursor (uridine-H^3) into it was investigated. Comparison of the results of morphological and biochemical studies revealed two consecutive stages of the toxic process, terminating in the formation of septal fibrosis. The sharpest changes in rapid RNA turnover in the rat liver were observed during the first 3 months of action of the toxic agent. The disturbance of metabolism also was reflected in a lowered RNA level and changes in the nucleo-cytoplasmic ratio in the tissue of the affected liver.

KEY WORDS: carbon tetrachloride; liver; total, nuclear, and cytoplasmic RNA; RNA turnover.

In experimental models of nonspecific liver damage administration of carbon tetrachloride (CCl_4) to animals is widely used [3, 11]. Resting on a firmly established morphological basis [9], these models have nevertheless been inadequately studied as regards the metabolism of nucleic acids, the most important class of biological compounds.

The object of this investigation was to study the effect of prolonged systematic administration of CCl_4 on the content and intensity of incorporation of labeled precursor in RNA and its various fractions in the tissues of rat liver, with an accompanying morphological control of the stages of the toxic changes.

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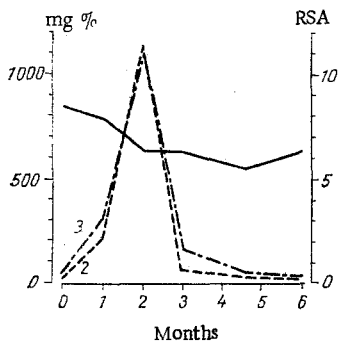

Fig. 1

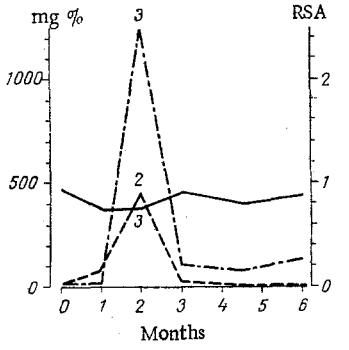

Fig. 2

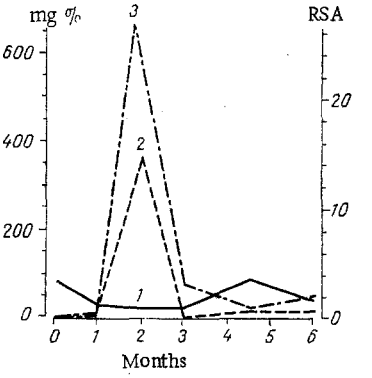

Fig. 3

Fig. 1. RNA of rat liver during prolonged toxic action of CCl_4. Here and in Figs. 2 and 3, abscissa: duration of CCl_4 administration to rats (in months); ordinate: on left, RNA content (in mg %), on right, RSA of RNA. 1) RNA content; 2 and 3) RSA of RNA after exposure for 20 and 60 min with uridine-5-H^3 respectively.

Fig. 2. Intensity of incorporation of uridine-5-H^3 into cRNA of liver and content of this fraction during prolonged administration of CCl_4: 1) cRNA content; 2 and 3) RSA of cRNA after exposure for 20 and 60 min respectively to uridine-5-H^3. Ordinate: on left, cRNA content (in mg %), on right, RSA of cRNA.

Fig. 3. Intensity of incorporation of uridine-5-H^3 into nRNA of liver and content of this fraction during toxic action of CCl_4: 1) content of nRNA; 2 and 3) RSA of nRNA after exposure for 20 and 60 min respectively with uridine-5-H^3. Ordinate: on left, nRNA content (in mg %), on right, RSA of nRNA.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 120-150 g were used. The animals received CCl_4 by subcutaneous injections twice a week in a dose of 1 ml/kg body weight. In acute experiments the liver was taken 1-6 months after the beginning of CCl_4 administration. The liver of healthy rats acted as the control. Radioactive precursor of RNA, namely uridine-5-H^3 (5 mCi/kg), was injected intraperitoneally into each rat after 20 or 60 min. The animals were decapitated between 10 a.m. and noon, to allow for the effect of diurnal rhythm on the content and biosynthesis of RNA [6]. The liver was perfused with cold physiological saline. Both RNA and DNA were isolated from a 10% liver tissue homogenate in physiological saline by the method of Schmidt and Thannhauser [12]. The native fractions of cytoplasmic and nuclear RNA (cRNA, nRNA) were obtained by temperature phenol fractionation [1] with certain modifications, as described by the writers previously [2]. The nucleic acid content in the samples was measured spectrophotometrically. Radioactivity in the test samples was determined on the Mark-2 automatic scintillation counter in dioxan scintillator. The radioactivity in the liver tissue was determined after "digestion" of the tissue in a solution of thiamine with methanol [7].

The radioactivity of the samples (in counts/min) was calculated per unit RNA contained in the sample and per unit of liver tissue, so that in this way the specific radioactivity (SR) of the RNA and SR of the liver tissue were determined. Next, by dividing the first value by the second, the relative specific radioactivity (RSR) was obtained.

Simultaneously with the biochemical analysis, macroscopic and microscopic investigations of the liver tissue were undertaken. The material was fixed in 10% neutral formalin and embedded in paraffin wax. Sections were stained with hematoxylin–eosin and by Van Fieson's method, with the use of Saturn Red instead of fuchsin. After fixation in formalin, some of the sections were stained for fat with Oil Red O.

EXPERIMENTAL RESULTS

Analysis of the morphological and biochemical changes arising in the liver tissue as a result of administration of CCl_4 revealed two principal stages of the process, the first of which corresponded to the time from 1 to 3 months, and the second from 3 to 6 months, after the beginning of administration of the agent.

TABLE 1. Changes in DNA Content and RNA/DNA and cRNA/nRNA Ratios in Liver of Rats Treated with CCl_4

Period of CCl_4 administr. (in mos.)		DNA conc. (in mg %)	RNA/DNA	cRNA/nRNA
Control		294,6±24,8	3,28±0,36	7,18±0,88
1		285,0±57,3	3,46±1,15	12,55±1,61
	P	>0,05	>0,05	<0,05
2		325,0±48,9	1,98±0,27	21,1±10,75
	P	>0,05	<0,05	>0,05
3		191,5±39,6	3,57±0,7	25,0±16,68
	P	<0,05	>0,05	>0,05
4		390,0±29,1	1,41±0,07	6,6±3,81
	P	<0,05	<0,01	>0,05
6		324,8±33,9	2,05±0,24	10,13±0,27
	P	>0,05	<0,05	<0,05

The first stage was characterized by fatty degeneration, necrosis of some hepatocytes, and proliferation of the epithelium of the small bile ducts. Compared with the corresponding control there was a sharp increase in the intensity of incorporation of radioactive label into RNA ($P < 0.01$), cRNA, and nRNA ($P < 0.01$), followed by an equally sharp decrease (Figs. 1-3). Meanwhile there was a decrease in the content of RNA ($P < 0.05$), nRNA ($P < 0.01$), and DNA, a decrease in the RNA/DNA ratio, and an increase in the cRNA/nRNA ratio (Table 1).

In the second stage of the toxic process (3-6 months) progressive proliferation of reticulin fibers became the predominant feature, with the appearance of collagen fibers and septal fibrosis. During this period the intensity of incorporation of label into RNA and its fractions did not change as sharply as in the early stages of action of CCl_4 (Figs. 1-3). The intensity of incorporation of label into RNA in the final stage of the process did not exceed the control, whereas the turnover of nRNA was increased after exposures for 20 and 60 min. The RNA content in the liver tissue remained low ($P < 0.05$), whereas the DNA content rose, so that the RNA/DNA ratio fell correspondingly (Table 1, Fig. 1). The cRNA/nRNA ratio was significantly increased at the beginning and end of the time of action of CCl_4 compared with the control (Table 1), apparent evidence of a disturbance of the transport system characterizing the transfer of RNA from nucleus into cytoplasm.

These findings suggest that the sharply increased incorporation of labeled precursor into RNA of the liver during the first stage of the toxic action of CCl_4 reflects the compensatory character of changes in nucleic acid metabolism in that period aimed at restoring the disturbed equilibrium of metabolism. In the second stage, during the final formation of septal fibrosis, the level of biosynthesis falls and nucleic acid metabolism becomes relatively stabilized at a new level.

The mechanism of action of CCl_4 as a toxic agent and also as a carcinogen for mice [4, 5] and for rats of certain strains [9, 10] has not yet been explained. The reduced concentration of RNA and its fractions throughout the period of the toxic process may be the result both of the direct destructive action of CCl_4 on RNA molecules and of its effect on the enzymes of metabolism of this nucleic acid and a corresponding change in the relative intensities of the processes of synthesis and breakdown. Incorporation of the labeled RNA precursor after short exposures reflects the rapid turnover of messenger RNA, whereas the RNA concentration in the cell is determined chiefly by transfer and ribosomal RNA; there is thus a need for further study of the fractional composition of RNA during pathological changes in the liver tissue.

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LIBERATION OF β-LIPOPROTEIN, ALKALINE PHOSPHATASE, AND 5'-NUCLEOTIDASE FROM PLATELETS IN RESPONSE TO AGGREGATION INDUCED BY ADRENALIN

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During aggregation induced by adrenalin, β-lipoprotein, alkaline phosphatase, 5'-nucleotidase, and factor 3 are liberated from the platelets. The electrophoretic mobility of platelet alkaline phosphatase is the same as that of the β-lipoprotein. This suggests that the β-lipoprotein, alkaline phosphatase, and 5'-nucleotidase are structural components of platelet material carrying the factor 3 activity.

KEY WORDS: platelets; adrenalin; β-lipoprotein; alkaline phosphatase; 5'-nucleotidase.

One of the most important consequences of reactions associated with emergence in neurohumoral regulation of blood clotting is the secretion of adrenalin by the adrenals [4]. The increased liberation of adrenalin into the blood stream causes the development of a hypercoagulemic reaction, the time of which coincides with that of increased activity of alkaline phosphatase and 5'-nucleotidase in the blood plasma [2, 3]. During adrenalin-induced aggregation of platelets, platelet factor 3 is liberated [13], whereas 5'-nucleotidase and 80% of the alkaline phosphatase are bound with membrane structures which possess factor 3 activity [7].

It is possible that 5'-nucleotidase and alkaline phosphatase are indicators of the appearance of fragments of platelet membranes in the blood stream during the reaction of adrenalin-induced aggregation of platelets. This paper describes an attempt to verify this hypothesis experimentally.

EXPERIMENTAL METHOD

Blood taken from the femoral artery of dogs was stabilized with 3.8% of sodium citrate (9 : 1) to study the platelet release reaction in the presence of plasma, or with EDTA solution (7.5 ml of 0.077 M EDTA + 92.5 ml blood) to study the reaction in buffer solution, pH 7.4. Platelet-rich plasma was obtained from blood by centrifugation at 12°C (280 g, 25 min). A suspension of washed platelets was prepared by Dechavanne's method [11]. Platelet-rich plasma, stabilized with EDTA, was centrifuged at 12°C (500 g, 30 min) and the residue of platelets was rinsed twice with a solution containing 139 mM NaCl, 5 mM KCl, 5 mM glucose, 15 mM Tris-HCl, pH 7.4, and 0.5% bovine albumin, and mixed with 77 mM EDTA in the ratio of 49 : 1. The washed platelets were suspended in the same buffer without EDTA. The morphological control and counting the platelets in the suspensions were carried out by phase-contrast microscopy in a Goryaev's counting chamber.

The platelet release reaction was induced by a $5.4 \cdot 10^{-4}$ M (final concentration) solution of adrenalin hydrochloride in the cell of an aggregometer at 37°C, with constant mixing for 15 min. To reproduce the release reaction in buffer at pH 7.4, fibrinogen and $CaCl_2$ up to final concentrations of 3.75 mg/ml and $4 \cdot 10^{-4}$ M respectively were added to the platelet suspension in addition to adrenalin. Aggregation was recorded automatically by the nephelometric aggregometer. At the end of 15 min the contents of the cells were centrifuged at 4°C (5000g, 30 min). Activity of alkaline p-nitrophenylphosphatase [10], alkaline β-glycerophosphatase [1], 5'-nucleotidase, and platelet factor 3 [5] was determined in the supernatant. The results were subjected to statistical analysis [8].

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