tion is evidently different. Elevation of the cAMP level in the mucosa through the action of cholera toxin does not correlate with intensification of glucose synthesis in that organ.

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REPAIR OF HEPATOCYTE MEMBRANES BY PHOSPHATIDYLCHOLINE AFTER HELIOTRINE POISONING

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KEY WORDS: heliotrine; aldolase; cytochrome P-450; glucose-6-phosphatase; liposomes.

Toxic hepatitis induced by various compounds (CCl_4, heliotrine, etc.) is accompanied by damage to hepatocyte membrane systems. Evidence of this is given by distrubances of the enzyme systems of the endoplasmic reticulum, which metabolize toxic substances entering the body [2, 3], and the marked elevation of the blood enzyme levels, reflecting disturbances of the integrity of the plasma membrane [8]. These lesions are partly abolished by the use of phosphatidylcholine liposomes [4, 7], as has been shown in experimental toxic hepatitis due to CCl_4.

The aim of this investigation was to study the reparative action of phosphatidylcholine liposomes in experimental toxic hepatitis caused by heliotrine.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 100-150 g. A heliotrine model of toxic hepatitis was used. Heliotrine was injected subcutaneously or intraperitoneally (in the form of a neutral aqueous solution) in doses of 10, 20, and 30 mg/100 g body weight. The animals were decapitated 24 h later, blood was collected, and fructose-1-monophosphate aldolase activity in the serum obtained from it was determined by Shapiro's method in Baginski's modification [9]. Egg phosphatidylcholine was isolated by the method in [6]. Dilinoleylphosphatidylcholine (DLPC) and dipalmitoylphosphatidylcholine (DPPC) (both from Sigma, USA), and preparations of Lipostabil and Essentiale (Nattermann, West Germany) were used. Multilayered

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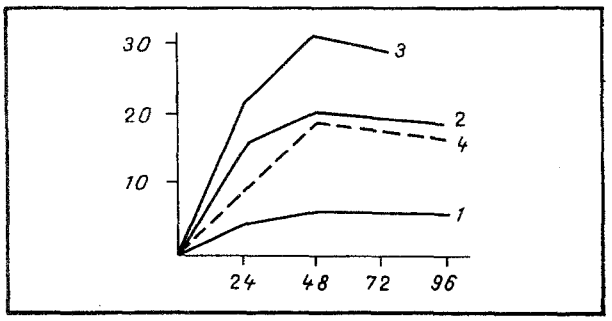

Fig. 1

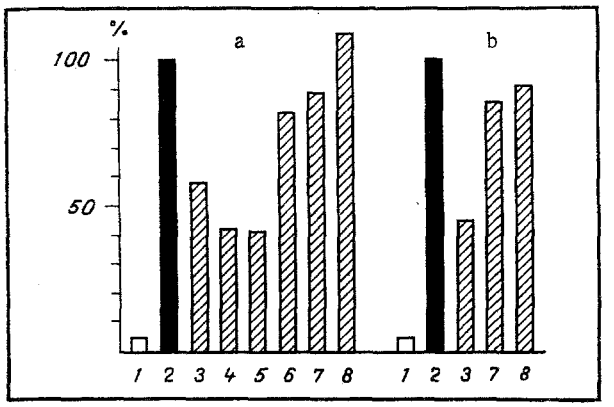

Fig. 2

Fig. 1. Blood serum aldolase activity after injection of heliotrine. Abscissa, time (in h); ordinate, activity (in relative units). 1, 2, 3) Intraperitoneal injection of heliotrine in doses of 10, 20, and 30 mg, respectively; 4) subcutaneous injection of heliotrine in a dose of 25 mg.

Fig. 2. Action of phospholipids on blood serum aldolase activity: a) intraperitoneal injection of heliotrine; b) subcutaneous injection of heliotrine. 1) Control; 2) heliotrine; 3) heliotrine + phosphatidylcholine, one injection; 4) heliotrine + phosphatidylcholine, two injections; 5) heliotrine + DLPC; 6) heliotrine + DPPC; 7) heliotrine + Lipostabil; 8) heliotrine + Essentiale. Blood serum aldolase activity in heliotrine poisoning taken as 100%.

liposomes were obtained by the method in [10] in 0.1 M Tris-HCl, pH 7.4. Liver microsomes were obtained by the method described previously [1]. The cytochrome P-450 concentration was determined spectrophotometrically [12], glucose-6-phosphatase (G-6-P) activity by the change in inorganic phosphorus concentration (P_i) [5], and protein by Lowry's method [11].

EXPERIMENTAL RESULTS

Dose-dependence of the poison and the course of hepatocyte membrane damage were studied at different times after poisoning. Heliotrine was injected into the animals in the above-mentioned doses and the serum aldolase activity, G-6-P activity, the concentration of cytochrome P-450, and the rate of its inactivation in membranes of the endoplasmic reticulum were determined 24, 48, 72, and 96 h later. Blood enzyme levels were found to depend on the dose of heliotrine injected. For instance, when intraperitoneal injections in a dose of 10 mg was used, it caused a very small change in blood aldolase activity. It will be clear from Fig. 1 that activity of this enzyme, which is virtually absent under normal conditions, increased a little 48 h after poisoning, and remained thereafter at the same level. Increasing the dose of heliotrine to 20 and 30 mg raised the blood enzyme levels still more. Aldolase activity reached a peak 48 h after injection of the poison and it remained at this level until the 4th day. Similar changes were observed after subcutaneous injection of heliotrine (Fig. 1: 4). When heliotrine was injected by different methods, the time course of the changes in blood aldolase activity was thus similar.

Injection of heliotrine was followed by a fall in the cytochrome P-450 concentration in membranes of the endoplasmic reticulum. The cytochrome P-450 concentration fell 48 h after injection of the poison in doses of 10 to 30 mg from 1.05 nmole (control) to 0.45 nmole/mg protein and remained at that level for 3 days. An increase in the dose of intrapertioneally injected heliotrine (10, 20, and 30 mg) caused an increase in the coefficient of inactivation of cytochrome P-450 (54, 72, and 100, respectively) compared with the control (44). G-6-P activity fell after injection of heliotrine in a dose of 30 mg to 65% of normal. Changes in the aldolase level and activity of microsomal marker enzymes are evidence of marked damage to hepatocyte membrane structures in toxic hepatitis due to heliotrine. Since injection of heliotrine into rats in a dose of 30 mg/kg caused death of 50% of the experimental animals, later heliotrine was used in a dose of 25 mg.

The reparative action of phosphatidylcholine liposomes in the case of liver damage by CCl_4 was demonstrated previously [4]. In the present investigation phospholipids were injected into animals during the development of heliotrine poisoning, 24 h after injection of

TABLE 1. Effect of Phospholipid Therapy on Enzyme Systems of Endoplasmic Reticulum after Heliotrine Poisoning

Experimental conditions	Concentration of cytochrome P-450, nmoles/mg protein	Coefficient of inactivation	G-6-P activity, μmoles P_i/mg protein/min
Control	1,05	44	4,6 (100%)
Heliotrine	0,45	100	3,1 (68%)
Heliotrine + phosphatidylcholine:			
one injection	0,49	78	4,7 (102%)
two injections	0,46	81	4,7 (102%)
Heliotrine + DPPC	0,55	80	3,4 (72%)
Heliotrine + DLPC	0,58	77	4,0 (85%)

Legend. The coefficient of inactivation of cytochrome P-450 was calculated by the ratio:

$$\frac{A_{420-450} \text{ during incubation for 30 min}}{A_{420-450} \text{ at zero time}} \cdot 100$$

according to the differential spectrum of the reduced CO-complex of cytochrome P-450. Samples were incubated in 100 mM Tris-HCl, microsomal protein 1-3 mg/ml.

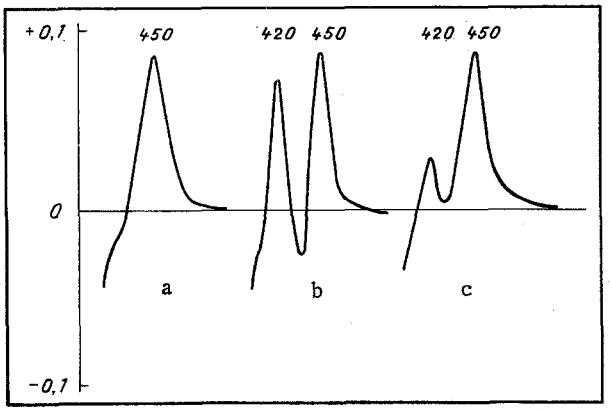

Fig. 3. Differential absorption spectra of reduced CO-complex of cytochrome P-450 in control microsomes (a), in microsomes damaged by heliotrine (b), and after their reconstitution by phosphatidylcholine (c). Spectra were recorded at zero time. Ordinate, optical density (in relative units).

the poison. The effectiveness of their action was assessed in the course of 2 days. A single injection of phosphatidylcholine, after intraperitoneal injection of heliotrine, lowered the raised blood enzyme levels; the effect of the phospholipids was observed, moreover, for 2 days (Fig. 2). A second injection of phosphatidylcholine had a stronger protective effect. The protective action of multilayered phosphatidylcholine liposomes also was manifested after subcutaneous injection of the poison: aldolase activity was reduced by half and kept at that level for 2 days. Normalization of the aldolase level was not observed after administration of Essentiale and Lipostabil. DLPC liposomes possessed egg phosphatidylcholine activity, but DPPC had no reparative action. The effectiveness of action of phosphatidylcholine on the enzyme systems of the endoplasmic reticulum of the liver was assessed from the degree of conversion of the active form or reduced cytochrome P-450 into its inactive form — cytochrome P-450. Microsomes isolated isolated from the liver of the poisoned animals were characterized by a distinct peak in the 420 nm region (Fig. 3b), whereas in control microsomes the initial state was represented only by cytochrome in the P-450 form (Fig. 3a). As the differential absorption spectra show, after injection of phosphatidylcholine the concentration of the inac-

tive form of cytochrome P-420 fell in microsomes from the liver of the poisoned animals (Fig. 3c). At the same time the coefficient of inactivation of cytochrome P-450 fell (Table 1). However, the concentration of microsomal cytochrome P-450 did not return to normal during phospholipid therapy. An increase in the degree of unsaturation of the phosphatidylcholine did not increase the effectiveness of phospholipid therapy. Activity of the other microsomal marker enzyme — G-6-P — was restored to the control level by means of phosphatidylcholine (Table 1).

The results demonstrate the possibility of repair of damaged liver cell membranes by injection of phospholipids in the form of multilayered liposomes. The reparative action may be based on their interaction with injured biological membranes by the principle of fusion or exchange of lipids.

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LIPID PEROXIDATION AS THE MAIN CAUSE OF MODIFICATION OF THE CATALYTIC FUNCTION OF SARCOPLASMIC RETICULUM Ca-ATPase IN HYPERCHOLESTEROLEMIA

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In some pathological states such as atherosclerosis, ischemia, myopathy, and malignant degeneration of tissue cholesterol (ChS) metabolism is disturbed and, in particular, an excess of ChS accumulates in the tissues. This process can be simulated by experimental hypercholesterolemia (HChE) [6]. It has been shown that in experimental HChE additional accumulation of ChS takes place in the plasma membranes, which normally contain about 30% of the total content of lipids. Elevation of the ChS level in the plasma membranes is evidently the primary cause of the change in activity of membrane-bound enzymes and of membrane permeability [11]. In HChE the absolute ChS content in the intracellular membrane is not significantly

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