lar ultrastructure is stronger than that with concentrations of hypophyseal gonadotropins. This suggests that prolactin is a very important regulator in the developing testis.

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Morphofunctional Aspects of Liver Regeneration in Experimental Correction of Toxic Hepatitis

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It is demonstrated that cytoplasmic extract of rat pup liver stimulates cellular and intracellular regeneration of hepatocytes exposed to the toxic agent CCl_4. Injection of liver cytosol stabilizes lysosomal membranes, increases the coefficient of energy efficiency of mitochondria, and activates proliferation of polyploid hepatocytes.

Key Words: *toxic hepatitis; hepatocytes; regeneration; polyploidization; liver cytosol*

In the search for new drugs effective against liver diseases considerable attention has been paid to natural metabolites which can be used for the prevention and correction of toxic damage to the liver [2,4,5]. A detailed analysis of cellular and intracellular mechanisms of liver regeneration exposed to toxic agents [9-11] makes it possible to define key events in the transformation of the organ and to select such methods of correction that maximally approximate the processes to physiological regeneration. For example, it has been demonstrated that under the action of CCl_4 the mass of hepatocytes and, consequently, of the liver increases predominantly due to polyploidization of cells at a low proliferative activity [8,13] and reduced percentage of binuclear hepatocytes [1]. It has been assumed that these changes in the hepatocyte population promote premature aging of the organ [3], since the number of cells never reaches the initial value.

We studied regeneration of the liver after acute damage caused by CCl_4 against the background of cytoplasmic extract prepared from rat pup livers (liver cytosol, LC) by monitoring intracellular and cellular mechanisms of hepatocyte regeneration.

MATERIALS AND METHODS

Experiments were performed on 50 male Wistar rats weighing 150-200 g. The animals were divided into four groups. Group I rats were given CCl_4 in olive oil (0.1 ml/100 g body weight) via a gastral tube three times at 48-h intervals. Group II animals were administered the same dose of CCl_4 against the background of a 7-day administration of LC. Groups III and IV animals served as controls; they were administered the extract solvent (0.6% NaCl, 0.5 ml) and LC, respectively, during a 7-day period. Material for investigation was collected 24 h after the last administration of solvent or extract.

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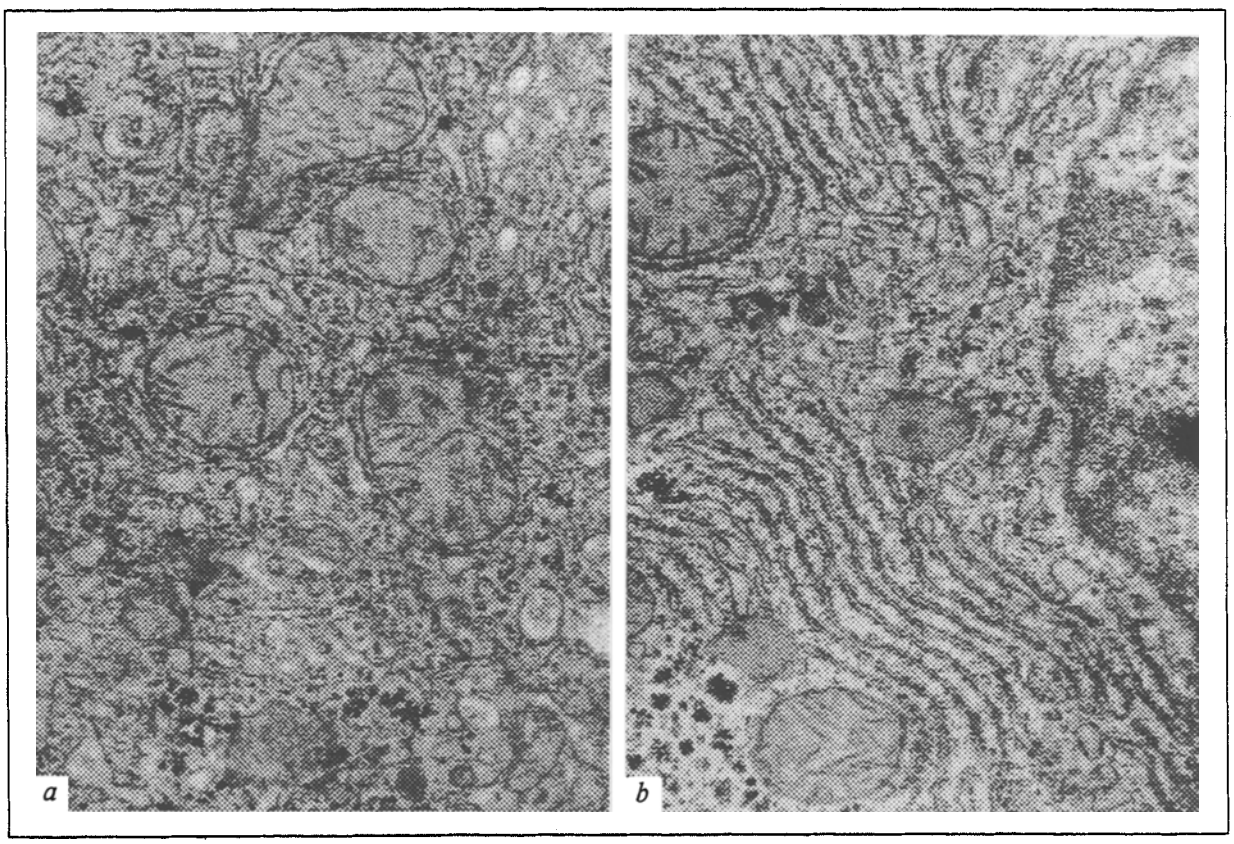

Fig. 1. Ultrastructure of hepatocytes from rats administered CCl_4 and rat pup LC. ×45,000. *a*) reduction and vacuolization of granular endoplasmic reticulum and clearing of mitochondrial matrix after three administrations of CCl_4; *b*) preservation of fine structure of mitochondria in endoplasmic reticulum of rats given CCl_4 against the background of LC.

LC was prepared by homogenization in normal saline (1:3) with subsequent centrifugation at 3000 rpm for 20 min. The supernatant was used on day 2 after preparation; it contained up to 80% glucosaminoglycans and up to 8% nucleotides, the protein concentration being 88 mg/ml of the baseline value. The LC doses used in this study had no cytotoxic effect (LD_{50}=172.3 mg/kg).

For light microscopy the material was fixed in 10% formaldehyde, dehydrated, and embedded in paraffin. Sections were stained with hematoxylin and eosin. For electron microscopy liver specimens were fixed for 1.5 h in 1% OsO_4 in phosphate buffer (pH 7.2), dehydrated, and embedded in Araldite M. Ultrathin sections were cut in an LKB-8800 ultratome, stained with lead citrate, and

TABLE 1. Quantitative Analysis of Electronograms of Rat Hepatocytes after Intoxication with CCl_4 against the Background of Rat Pup LC ($M \pm m$)

Parameter	Control rats	CCl_4	CCl_4+LC	LC
Number of mitochondria in an electronogram	6.00±1.05	4.10±0.60	6.30±0.30	9.20±0.45
Number of cristae per mitochondrion	10.10±0.80	7.20±0.57	12.00±1.37	14.30±1.60
Area of occupied by a mitochondrion, μ^2	0.19±0.06	0.22±0.01	0.23±0.07	0.14±0.04
Coefficient of energy efficiency of mitochondria, %	100	34.9	140.8	225.0
Coefficient of rate of division of mitochondria	0.027	0.041	0.083	0.074
Number of lysosomes in an electronogram	3.20±0.60	12.00±2.40	8.00±1.90	5.10±0.90

TABLE 2. Percent Ratio of Diploid, Tetraploid, and Octaploid Nuclei in Rat Liver after Administration of CCl_4 and Rat Pup LC ($M \pm m$)

Ploidy	Control rats	CCl_4	CCl_4+LC
Diploid	9.9±1.2	3.2±0.8*	49.7±7.3
Tetraploid	84.0±5.3	71.4±6.4	34.6±3.8**
Octaploid	6.1±1.4	25.4±2.3*	15.7±1.5*

Note. $p<0.05$: one asterisk indicates values in comparison with the control, two asterisks indicate values in comparison with CCl_4 – treated rats.

viewed in a JEM-7A electron microscope at a 45,000-fold magnification.

Quantitative analysis of hepatocyte ultrastructure was performed with the use of electronograms. The mean number of mitochondria, the number of cristae in a mitochondrion, the total area occupied by mitochondria in an electronogram, and the coefficient of energy efficiency of mitochondria (CEEM) [6] were calculated. The coefficient of intensity of mitochondrial division (CIMD) was determined as the ratio between the number of dividing mitochondria and their total content. Correlation analysis of the number of lysosomes, CEEM, and CIMD was performed by computer.

For cytophotometric determination of the DNA content in hepatocyte nuclei [7,12,14] 72 h after the last administration of LC the liver tissue was hydrolyzed in 5 N HCl for 8 min at 37°C. Smears of isolated cells were stained by the method of Feulgen. Photometry was performed in a one-beam photometer by the double-wave method [12]; 1000 nuclei were analyzed.

RESULTS

Administration of CCl_4 induced marked changes in the ultrastructure of hepatocytes. Mitochondria became enlarged as a result of swelling, other changes being disorganization and a decrease in the number of cristae. The granular endoplasmic reticulum was shrunken in most hepatocytes (Fig. 1, *a*), and this was accompanied by vacuolization

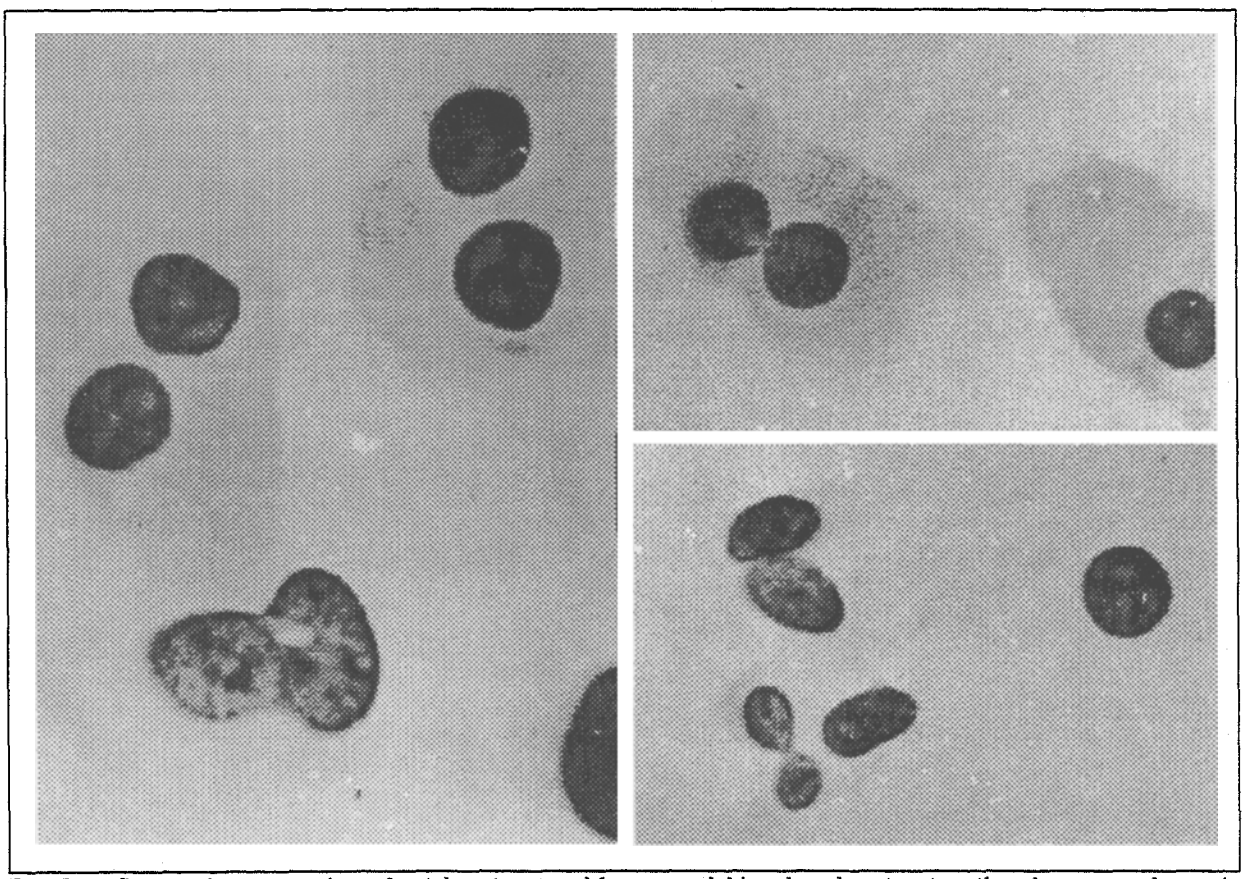

Fig. 2. Smear of a suspension of rat hepatocytes. Mono– and binuclear hepatocytes: the plasma membrane is preserved. Feulgen staining. ×400.

and partial degranulation of the endoplasmic reticulum. On the whole, the ultrastructural changes in hepatocytes were indicative of disorders in protein synthesis and transition of cell metabolism to the intracellular type. CEEM decreased considerably against the background of pronounced alterations in the mitochondria, despite a significant increase in CIMD compared with the control (Table 1). The number of primary lysosomes increased simultaneously with the CCl_4-induced alterations in the mitochondria, the lysosomes being localized predominantly around the Golgi apparatus. An inverse proportionality between the number of mitochondria and of lysosomes in hepatocytes was recorded.

There were no significant changes in the hepatocyte ultrastructure in rats administered CCl_4 against the background of LC. The fine structure of the mitochondria was preserved, and the cisternae of granular endoplasmic reticulum were densely packed around the nucleus (Fig. 1, *b*). Administration of LC markedly increased CEEM and CIMD. The number of cristae in a mitochondrion increased. The number of primary lysosomes was lower than in CCl_4-treated animals, but still higher than in the controls (Table 1).

In hepatocytes of rats given only LC the number of mitochondria and the number of cristae in a mitochondrion markedly increased compared with control rats administered normal saline. In these animals, CEEM and CIMD increased 2.2- and 2.7-fold, respectively, which may account for the smaller area occupied by a mitochondrion. The number of primary lysosomes in an electronogram did not differ significantly from the control (normal saline). It is noteworthy that a negative correlation between the number of lysosomes, CEEM, and CIMD was established in both experimental groups. Presumably, lysosomes are activated by CCl_4 in response to the suppression of biosynthetic processes and intensified degradation of hepatocyte ultrastructure. The protective and stimulatory effects of LC can manifest themselves not only in the normalization of biosynthesis but also in a possible stabilization of lysosomal membranes and lowered intensity of ultrastructural degradation.

Cytophotometry of the suspension of isolated hepatocytes (Fig. 2) revealed a decrease in the proportion of diploid and an increase in the proportion of octaploid cells under the action CCl_4 (Table 2). In group II animals (simultaneous administration of CCl_4 and LC), the number of diploid hepatocytes markedly increased (5-fold) predominantly due to the division of tetraploid cells. This specific feature of regeneration abolished the consequences of the pathological process at the cell level. In response to administration of LC the hepatocytes became functionally transformed and acquired a higher proliferative potential.

Thus, the stimulatory effect of rat pup LC on liver exposed to CCl_4 manifests itself primarily in activation of the mitochondria and proliferation of polyploid cells. The number of lysosomes in a hepatocyte is inversely proportional to the coefficient of energy efficiency of mitochondria. Administration of rat pup LC results in stabilization of lysosomal membranes and, consequently, in intracellular and cellular regeneration of hepatocytes.

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