of the spleen and all colonies of granulocytic hematopoiesis were present in the subcapsular zone. The number of megakaryocytes was very considerable: up to 20 per field of vision in the sections (140×).

After injection of AES followed by bone marrow, proliferation of cells of the erythroid series was more marked and that of the megakaryocytic series less marked than after injection of ATS. Foci of hematopoiesis consisting of cells of all three series (colonies of mixed type) were found in the subcapsular zone.

The results of these investigations show that foci of hematopoiesis (microcolonies) may be formed in the spleen of unirradiated mice after repeated injections of immune cytotoxic sera. The formation of microcolonies connected with the functioning of hematopoietic stem cells is evidently not specific for the irradiated organism.

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ULTRASTRUCTURAL AND FUNCTIONAL REVERSIBILITY OF SCLEROTIC CHANGES IN THE RAT LIVER CAUSED BY EXOGENOUS ORGAN-SPECIFIC RNA

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The ultrastructure of parenchymatous and stromal cells was studied in relation to reversibility of experimental cirrhosis of the liver in rats under ordinary conditions and also under the influence of exogenous RNA administered in various ways. The dynamics of the changes in cell ultrastructure correlated with the dynamics of the quantitative indices. A short course of RNA was found to have a beneficial role on intracellular reparative regeneration of the hepatocytes, but a long course had an adverse effect. Both parenchymatous and stromal cells take part in the resorption of collagen.

KEY WORDS: reversibility of cirrhosis of the liver; effect of exogenous RNA; ultrastructure and function; parenchyma and stroma; resorption of collagen.

Several workers have demonstrated the reversibility of sclerotic changes in the liver [8, 12, 14]. An important role in the resorption of collagen is ascribed to the cells both of the parenchyma and of the stroma of an organ, including Kupffer cells [6, 8, 10, 14, 15]. However, the mechanism of resorption of the excess of fibrous tissue has not been finally elucidated.

One of the main metabolic processes concerned in the regeneration of the pathologically changed organ is protein biosynthesis programmed by nucleic acids. The ability of exogenous nucleic acids or their hydrolysis products to stimulate regeneration has been demonstrated [5, 9-11]. Nevertheless, this problem continues to provoke discussion. The effect of nucleic acids on the subcellular manifestations of reparative regeneration of the liver has not been studied at all.

In this investigation the reversibility of the subcellular changes and indices of the protein-synthesizing function of the cirrhotic liver were studied at different stages of regeneration of the organ under ordinary conditions and under the influence of exogenous organ-specific RNA.

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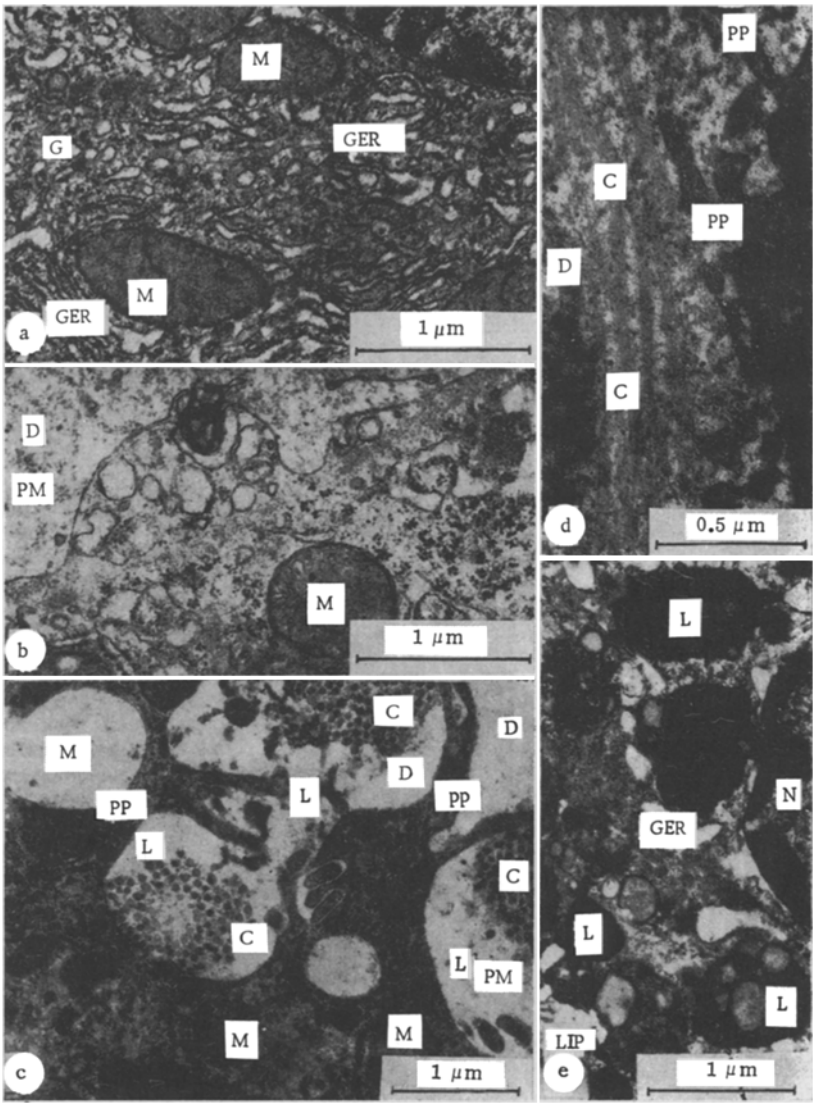

Fig. 1. Ultrastructure of hepatocytes, Kupffer cells, and collagen fibrils during post-toxic spontaneous regeneration of cirrhotic rat liver; a, b, c) fragments of hepatocytes 60, 8, and 40 days, respectively, after end of exposure to CCl_4 (magnification: a, b – 30,000×, c – 22,500×); d) fibrils showing loss of contrast of periodic striation, and floccules of lysed collagen in Desse's space 40 days after end of exposure to CCl_4 (50,000×); e) part of a Kupffer cell 60 days after end of exposure to CCl_4 (25,000×). GER) Granular endoplasmic reticulum; D) Desse's space; C) collagen; L) lysosome; M) mitochondrion; PM) plasma membrane; PP) pseudopodium; N) nucleus; G) glycogen; LIP) lipid drop. Arrow points to classmatosis of part of the cytoplasm of a hepatocyte.

EXPERIMENTAL METHOD

Cirrhosis of the liver was induced in male Wistar rats weighing 180-230 g by inhalation of CCl_4 for 4 h at a time, twice a week for 6 weeks [14]. Samples of liver and blood were taken from decapitated animals 8, 20, 40, and 60 days after the last exposure to CCl_4 and also after intraperitoneal or intramuscular injection of high-polymer total RNA from bovine liver, purified by the phenolic method (a product of the Special Design and Technology Bureau for Biologically Active Substances, Siberian Branch, Academy of Sciences of the USSR), on the 20th, 40th, and 60th days of regeneration starting from the 8th day after the last inhalation of CCl_4. RNA in Ringer's solution was injected on alternate days in a dose of 5 mg/100 g body weight. The solvent was injected into control rats. Samples of liver were studied in the IEM-7A electron microscope. The total blood protein was determined refractometrically. Serum proteins were fractionated by Gurvich's method [4] and

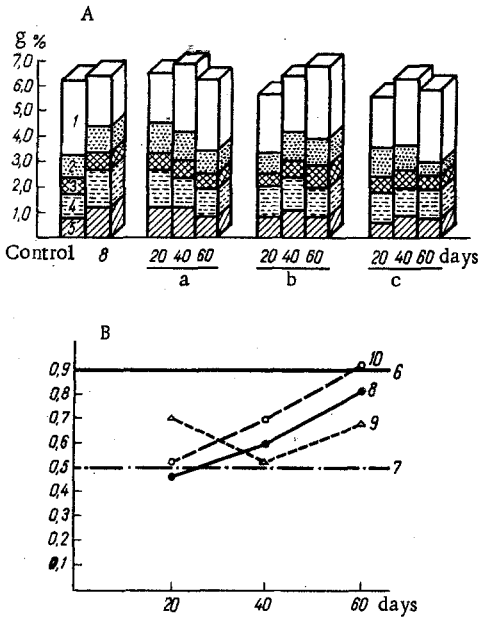

Fig. 2. Distribution of rat serum protein fractions (A) and change in albumin–globulin ratio (B) at different stages of spontaneous posttoxic reparative regeneration of the liver, and also during intraperitoneal and intramuscular injection of organ-specific RNA. A: 1) albumin; 2) α_1-globulin; 3) α_2-globulin; a) spontaneous regeneration; b) intraperitoneal injection of RNA; c) intramuscular injection of RNA. Abscissa, control and days after end of exposure to CCl_4; ordinate, quantitative indices of fractions (in g%). B: 6) control; 7) 8 days after last inhalation of CCl_4; 8) spontaneous regeneration; 9) intraperitoneal injection of RNA; 10) intramuscular injection of RNA. Abscissa, days after end of exposure to CCl_4; ordinate, albumin–globulin ratio.

were then estimated colorimetrically. The results were subjected to statistical analysis. The probability of significance of differences between means was estimated by Student's test. Differences were regarded as significant when $P < 0.05$.

EXPERIMENTAL RESULTS

At all times of regeneration studied no clearly defined changes in the ultrastructure of the hepatocytes could be found, such as were observed immediately after the end of a similar course of CCl_4 poisoning [2]. At the times of observation a progressive normalization of hepatocyte ultrastructure was noted, but even 60 days after the last dose of CCl_4 it was not completely restored (Fig. 1a). Besides a decrease in the number of lipid drops and the appearance of glycogen granules in the cells, the tubules of the granular endoplasmic reticulum (GER) remained unequally dilated; the number of ribosomes was reduced; polysomes were particularly few in number. Condensation of the basic material of the cell cytoplasm was observed. The matrix of the mitochondria in the late stages of regeneration was condensed and the normal orderly arrangement of the cristae was restored. The most marked changes in GER and the most marked dysproteinemia, manifested as a considerable reduction in the albumin concentration and an increase in the coarsely dispersed globulin fractions, were found on the 8th day after the last inhalation of CCl_4 (Fig. 2A). This was evidence of depression of the protein-synthesizing function of the hepatocytes and, conversely, activation of cells of the reticuloendothelial system (RES). Albumin is known to be synthesized only by hepatocytes, whereas γ-globulin is synthesized by RES cells [13], which include Kupffer cells. In the next stages of regeneration the relative normalization of GER was ac-

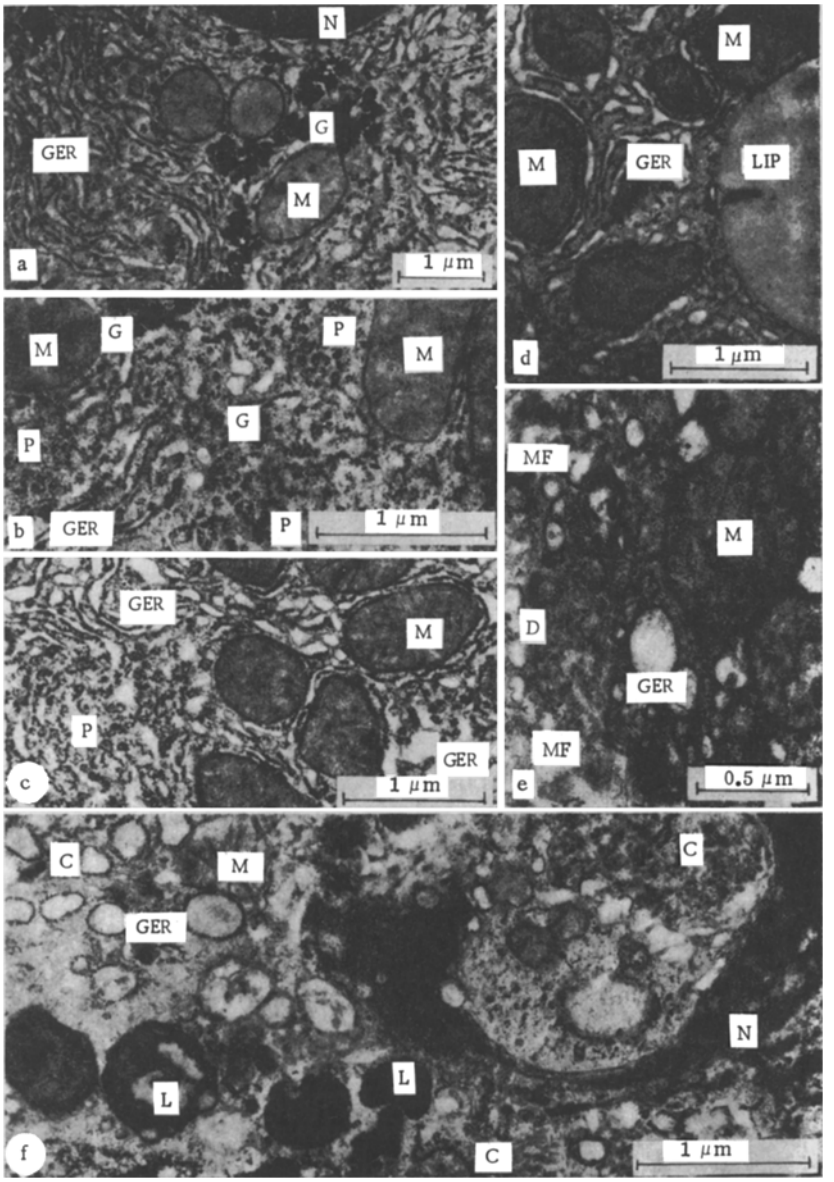

Fig. 3. Ultrastructure of hepatocytes, Kupffer cells, and collagen fibrils following injection of organ-specific RNA at different stages of posttoxic regeneration of the cirrhotic rat liver: a, b) fragments of hepatocytes of rats receiving RNA intraperitoneally during 20 days after end of exposure to CCl_4 (magnification: a) 15,000×, b) 30,000×); c) part of hepatocyte of rat receiving RNA intramuscularly for 20 days after end of exposure to CCl_4 (25,000×); d) fragment of hepatocyte of rat receiving RNA intraperitoneally for 60 days after end of exposure to CCl_4 (25,000×); e) part of hepatocyte and Desse's space with fragmented and lysed collagen fibrils after 60-day course of intraperitoneal injections of RNA in posttoxic period (40,000×); f) part of Kupffer cell with phagocytosed collagen after intraperitoneal injection of RNA for 40 days after end of exposure to CCl_4 (20,000×). MF) Microfibrils of lysed collagen; P) polysomes. Remainder of legend as in Fig. 1.

companied by an increase in the albumin concentration approximately to the control level and by a decrease in the coarsely dispersed globulin fractions (Fig. 2A, a), and these changes were particularly marked by the 60th day. The albumin-globulin ratio accordingly returned almost to normal (Fig. 2B).

In the course of the investigation the signs of "self-purification" of the hepatocytes continued to develop, as shown by an increase in the number of autophagic vacuoles containing altered mitochondria. The manifestations of clasmatosis were correspondingly intensified. The part separated from the hepatocyte usually contained osmiophilic membranous formations (Fig. 1b) or lipid drops, which indicated the removal of metabolites and excess lipids in this way during posttoxic intracellular regeneration.

A very characteristic feature was the increased ability of the hepatocytes to form pseudopodia (Fig. 1c, d). Often the pseudopodia could be seen to "seize" bundles of collagen fibrils, as described previously [6], when as a rule mitochondria and also lysosomes, some of them lying extracellularly, among the swollen and partially lysed collagen fibers, could be seen in the digitiform processes of cytoplasm (Fig. 1c). This localization of the mitochondria and lysosomes could be connected with increased expenditure of energy on the phagocytic function of the hepatocytes. Considering the indices for the protein spectrum (Fig. 2A, a) the manifestation of the phagocytic function of the parenchymatous cells was presumably accompanied by a decrease in albumin synthesis.

At all periods of regeneration studied signs of increased activity of phagocytes and Kupffer cells were observed. The latter were unusually large and contained many secondary lysosomes with osmiophilic material and lipid drops (Fig. 1e). These phenomena were particularly well marked 8 days after the end of exposure to CCl_4, when an increase in the content of coarsely dispersed globulin fractions was found (Fig. 2A); this was evidently connected with certain special features of the functional manifestations of the Kupffer cells.

Contrast of the periodic striation of some of the collagen fibrils in the Desse's spaces was reduced at the various times of the investigation and swollen fibrils and floccular masses were frequently visible (Fig. 1d), pointing to lysis of the excessively growing fibers. The latter also were frequently found in the phagocytic vacuoles of the Kupffer cells; the fibrils could be observed to have been "seized" by the digitiform processes of the cells.

During regression of the cirrhotic changes in the liver both hepatocytes and Kupffer cells thus took part in the resorption of the excess of collagen, in confirmation of the data obtained by Kalashnikova and Rubetskii [6]. The protein-synthesizing function of the parenchyma gradually returned to normal, although the initial values had not yet been restored 2 months after the end of exposure to CCl_4 (Fig. 2A, a).

On the 20th day of regeneration after intraperitoneal injection of RNA marked normalization of the hepatocyte organoids was observed. Close to the normal numbers of glycogen granules and large areas of GER appeared in the cells (Fig. 3a). Unusually extensive areas of polysomes were visible in some hepatocytes (Fig. 3b). These distinguishing features of the cells, taking into account the data of the protein spectrum (Fig. 2A, b), are evidence of the stimulating effect of exogenous RNA on albumin synthesis and its inhibitory effect on production of globulin fractions of protein at this stage of regeneration.

After intramuscular injection of RNA the signs of restoration of the ultrastructure of the hepatocytes at the same period were much less marked (Fig. 3c). The protein spectrum showed only a decrease in the proportions of γ- and β-globulin fractions (Fig. 2A, c) compared with the corresponding period of spontaneous regeneration (Fig. 2A, a) and the other indices of the protein spectrum were identical. By whatever method the RNA was injected for 20 days, autophagosomes with changed mitochondria and membranous formations, signs of clasmatosis, and also phagocytosis of collagen fibers were seen more frequently in the hepatocytes than in the control.

Prolonged intraperitoneal and intramuscular injection of RNA (for 40 and 60 days) led to changes in the ultrastructure of the hepatocytes that were similar to toxic in character (Fig. 3d). Lipid infiltration increased, the glycogen content and number of ribosomes fell sharply, and fragmentation and vacuolation of GER were intensified. Organoids of destroyed hepatocytes were often visible in Desse's spaces. These phenomena were less marked after prolonged intramuscular injection of RNA. The plasmalemma of the hepatocytes was highly tortuous, and as a result it was frequently cut tangentially by the plane of the section (Fig. 3e). Lysis of collagen fibrils in Desse's spaces was increased (Fig. 3e).

Intraperitoneal injection of RNA for 40 days led to depression of albumin synthesis and an increase in the α-globulin fraction (Fig. 2A, b) compared with the previous period of RNA treatment and with spontaneous regeneration after 40 days. Intraperitoneal injection of RNA for 60 days gave indices of albumin synthesis that were identical with those at the same period of spontaneous regeneration, but an increase in the α-globulin fractions was observed. The increase in the latter was associated with degenerative changes developing in the parenchymatous cells of the liver [1, 3], as the electron-microscopic data confirmed. Since considerable

breakdown of its molecules under the influence of hydrolysis evidently takes place in the muscle during intramuscular injection of RNA for 60 days, the degenerative changes in the hepatocytes were less marked than after intraperitoneal injection of RNA. The albumin–globulin ratio therefore returned close to the control level (Fig. 2B).

Hypertrophy and activation of phagocytosis of Kupffer cells increased considerably during prolonged administration of RNA (Fig. 3f), whether injected intraperitoneally or intramuscularly. The phenomena of "seizure" of large bundles of collagen fibers were distinctly seen (Fig. 3f). The structural manifestations of activation of phagocytosis were probably interconnected also with changes in the protein-synthesizing function of the RES cells of the liver. Evidence in support of this view was given by the decrease in the γ-globulin fraction after intramuscular injection of RNA for 40 days (Fig. 2A, c) compared with its value at the same time of regeneration under ordinary conditions (Fig. 2A, a). As a result, the albumin-globulin ratio was higher than during spontaneous regeneration (Fig. 2B).

It can accordingly be concluded from these results that short-term intraperitoneal injection of RNA stimulate intracellular reparative regeneration of hepatocytes. Such regeneration is less marked after intramuscular injection of RNA. Prolonged administration of RNA inhibits protein synthesis destined for intracellular reparative regeneration of hepatocytes, thus giving rise to destructive changes in them. The reversibility of the subcellular changes in the liver after posttoxic cirrhosis is accompanied by resorption of excessively grown collagen fibers, and cells of both parenchyma and stroma take part in this process by virtue of their increased phagocytic and lytic ability. The latter is considerably intensified after RNA injection. The effect of RNA on the reversibility of the subcellular changes in the 20-day period of posttoxic regeneration after intraperitoneal injection is evidently connected with the specific effect of the biopolymers entering the liver instantaneously, whereas intramuscular injection of RNA was less effective because of its preliminary hydrolysis in the muscle and the more prolonged action of the blood ribonucleases on the way to the liver. In the late stages adaptive mechanisms of RNA inactivation are evidently brought into play. The possibility of discoordination of the biorhythms of the parenchymatous cells and the rhythm of prolonged administration of RNA, in accordance with Sarkisov's concept [7], likewise cannot be ruled out, whereas this is probably not particularly important for stromal cells, whose collagenolytic function is activated during prolonged RNA administration also.

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