Thus after 6 h of ischemia differentiated MF of the lumbrical muscle, transplanted into ACE, are still capable of recovery, but at the later stages of ischemia, regeneration of the muscle probably takes place through the activity of satellite cells.

LITERATURE CITED

1. M. G. Gonchar' and E. P. Mel'man, Arkh. Anat. Gistol. Émbriol., No. 6, 13 (1990).
2. A. Cervu, W. S. Moore, E. Homsher, et al., J. Surg. Res., **47**, 12 (1989).
3. E. Jennische and H. Hansson, Exp. Molec. Path., **44**, 272 (1986).
4. H. U. Steinau, Major Limb Replantation and Postischemia Syndrome: Investigation of Acute Ischemia-Induced Myopathy and Reperfusion Injury, New York (1987).
5. L. A. Strenberger, Immunochemistry, New York (1979).
6. H. Yellin, Exp. Neurol., **51**, 579 (1976).

MORPHOLOGICAL EVIDENCE OF A POSSIBLE ROLE FOR CYTOCHROME P-450 IN THE DEVELOPMENT OF AUTOIMMUNITY IN THE LIVER

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In recent years there has been a marked increase in the number of patients with various forms of autoimmune pathology. Although significant progress has been achieved in the study of the pathogenesis of many autoimmune diseases [6], nevertheless the data on the etiology of these diseases are often extremely contradictory. In particular, great importance has been attached to the role of circulating immune complexes and autoantibodies in these processes [14]. As yet, however, no sufficiently convincing explanations of the causes of these widespread diseases have been established. Many previous investigations have shown that different pathological processes in the liver lead to a multicomponent disturbance of the integrity of its cells [13]. There is every reason to suppose that many acute diseases are transformed into chronic as a result of the addition of an autoimmune component, which thereafter plays an important role in the maintenance of inflammation. However, their probable trigger factors have not yet been explained. because we have no adequate model to reflect the mechanisms of onset of autoimmune diseases.

We accordingly decided to attempt to produce a model of autoimmune liver damage by injecting animals with paraquat, a herbicide which specifically activates lipid peroxidation (LPO) and which, because of this mechanism, induces cirrhosis of the liver [4].

EXPERIMENTAL METHOD

Mature male Wistar rats weighing 180-200 g were used. The animals received paraquat intraperitoneally in a dose of 60 mg/100 g body weight; control male rats received injections of sterile physiological saline. Liver samples from the

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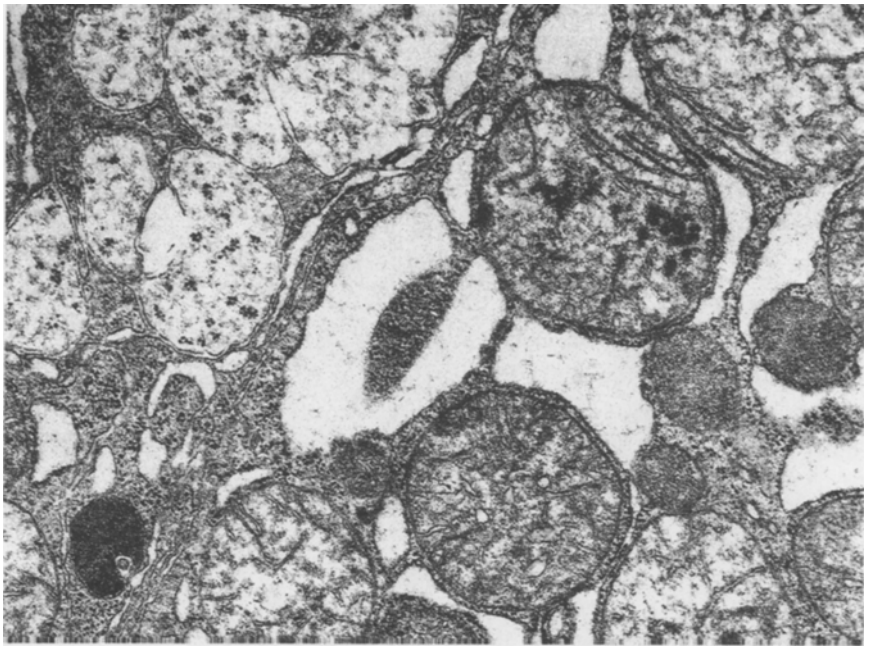

Fig. 1. Swelling of a mitochondrion with homogeneous-granular matrix.

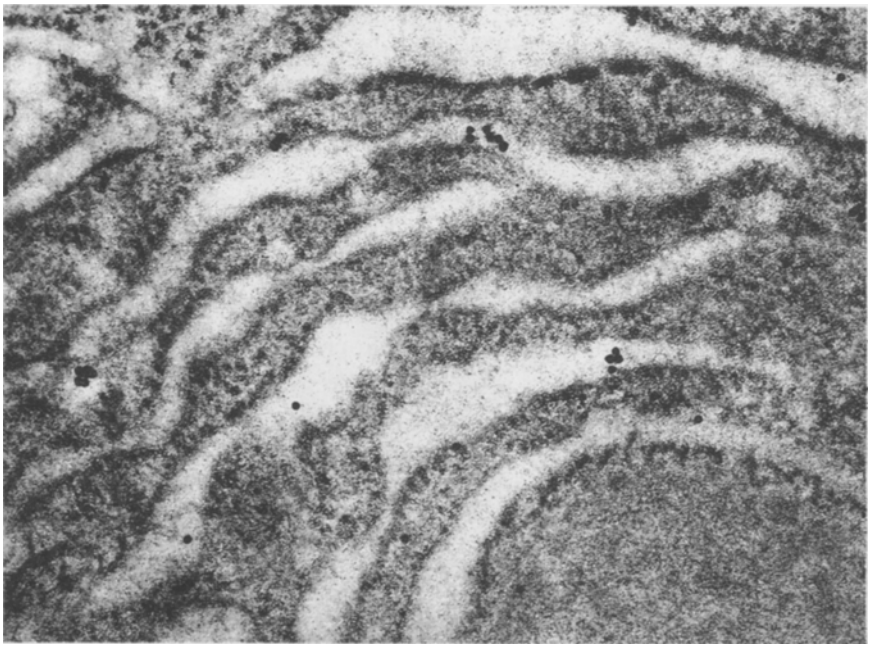

Fig. 2. Colloidal gold particles on membranes of rough endoplasmic reticulum and cisterns from liver specimens.

experimental and control animals were taken two days after injection of paraquat and of physiological saline. Pieces of liver were fixed in a 4% solution of paraformaldehyde in 0.1 M phosphate buffer for 4 h, and these were subsequently dehydrated and embedded in Epon 812. Ulrathin sections were cut on an LKB Ultrotome (Sweden) and examined in a "Philips" electron microscope.

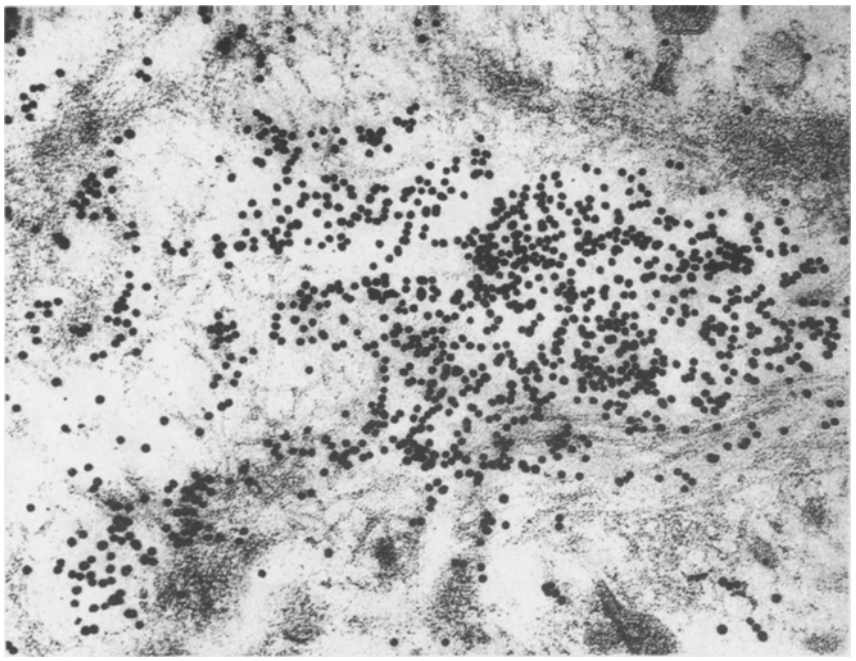

Fig. 3. Colloidal gold particles in widened Disse's spaces and on fibrin fibers.

Specific antibodies against cytochrome P-450 were obtained from the Department of Cell Physiology and Pathology, Institute of Clinical and Experimental Medicine, Siberian Branch, Academy of Medical Sciences of the USSR. Colloidal gold particles measuring 20-30 mm were prepared by Frens' method [5] (1977), using sodium citrate. The optimal quantity of antibodies (determined by Zsygmondi's reaction) was mixed with the gold sol, previously adjusted to pH 8.0 with 0.2 M K_2CO_3 solution, and this complex with antibodies was centrifuged at 25,000*g* for 40 min; the residue was resuspended in 1 ml phosphate buffer, containing polyethylene-glycol 20,000 in a concentration of 0.2 mg/ml.

Ultrathin sections were incubated for 30 min in a 1% solution of sodium metaperiodate, and added to phosphate buffer through descending concentrations of ethanol. The grids were then incubated for 30 min in a 1% solution of bovine serum albumin at room temperature in a humid chamber with rabbit antibodies in a dilution of 1:1000 at 37°C. After careful washing for 20 min the sections were incubated with antirabbit antibodies, conjugated with colloidal gold particles, for 30 min. After rinsing, the sections were counterstained with a 1% aqueous solution of uranyl acetate. Sections not incubated with specific antibodies and also sections treated with secondary antibodies only served as the control.

EXPERIMENTAL RESULTS

Intralobular solitary foci of necrosis of the hepatocytes, focal dystrophy of the parenchymatous cells, and accumulations of lymphocytes, macrophages, and plasma cells in these areas were discovered at the light-optical level two days after injection of paraquat. Electron-microscopic investigation of the liver showed that the cytoplasm of individual hepatocytes was partly translucent and contained fragments of the rough endoplasmic reticulum and mitochondria. Areas of cytoplasm completely free from all organelles were seen. In cells which remained intact numerous swollen mitochondria with a homogeneous granular matrix were observed (Fig. 1). Membranes of the rough endoplasmic reticulum were mainly considerably widened, and in certain cases they were totally free from ribosomes. The nuclear chromatin was in a dispersed state. Biliary capillaries were greatly dilated, and sometimes the microvilli had almost completely disappeared.

After incubation of the sections with specific antibodies, particles of colloidal gold in the control specimens were located on the membranes of the rough reticulum and also inside the cisterns (Fig. 2). Disse's space and the collagen fibers did not contain the immune label. Colloidal gold particles were found in specimens of liver from the experimental animals mainly inside the dilated cisterns and on membranes of the rough reticulum. However, the label was particularly abundant in the widened Disse's spaces, on fibrin fibers (Fig. 3).

Among the many enzymes located in hepatocytes, an important role is played by the system of mixed liver monooxygenases, which are one of the basic proteins of the microsomal fraction of the liver and occupy an important place in the metabolism of endogenous substrates and xenobiotics. It has been shown immunohistochemically that the cytochrome system is located on membranes of the rough and smooth endoplasmic reticulum [3]. The LPO level in the body plays an essential role in the functioning of the mixed monooxygense system of the liver [10]. However, despite the many publications devoted to the study of the effect of activation of LPO on function of the liver monooxygenases, the structural aspects of this problem still remained unsolved. There have been very few studies of structural changes in the liver during activation of LPO without any accompanying toxic manifestations.

As several investigators have shown, in certain types of liver pathology autoantibodies to various hepatocyte organelles can be detected. For example, in biliary cirrhosis of the liver, antibodies to membranes of the Golgi complex and to mitochondria can be found in the patients. Autoantibodies to mitochondria of the hepatocytes also are found in biliary cirrhosis of the liver [15]. Finally, comparatively recently it was found that antibodies to cytochrome P-450 appear in the serum of patients with autoimmune hepatitis [1]. The authors cited consider that autoantibodies are formed against those forms of cytochrome which are involved in the metabolic activation of chemical agents. At the same time, none of the investigators attempted to study the mechanisms lying at the basis of these pathological processes.

The role of LPO in the etiology of liver pathology is unknown. Only the details of these processes and the role of various LPO products in the disturbance of the morphological and functional state of the hepatocytes have been discussed [12]. Activation of LPO and oxidation of membranes evidently constitutes the basis for development of necrotic changes in the cells. Destruction of the membranes and consequent loss of proteins, including enzymes, lead to their escape from the cell into fibrin surrounding the hepatocytes, which can evidently adsorb cytosol proteins on itself. The ability of enzymes to escape from the cell was demonstrated a long time ago, and the diagnosis of many diseases of the liver, heart, and kidneys may actually be made on its basis [7-9]. Our investigation has expanded modern views on the possible staggering of development of autoimmune diseases, starting from the first stages of cell damage, for we showed that enzymes leaving the cells may bind with extracellular components of the connective tissue.

It was found in [2] that one stage of repair of damage and of collagen formation is absorption of fibrin by macrophages. Since the latter are antigen-presenting cells for lymphocytes, it can be postulated that unusual complexes of adsorbed enzymes become unique haptens or adjuvants, triggering the autoimmune process. Evidence in support of this view in our model situation is given by infiltration of the liver by macrophages, lymphocytes, and plasma cells (i.e., morphological evidence of clinical autoimmune liver damage). The developing immune response may in turn cause damage to new cells and may become long-lasting because of the firm bond with cytochrome P-450 and fibrin, whereas P-450 itself possesses relatively weak immunogenicity. Similar processes of autoimmunization may probably be characteristic of other membrane-bound proteins also.

The data described above, as a whole, may serve as important proof of the hypothesis of a connection between activation of LPO, necrotic changes, and autoimmune diseases.

LITERATURE CITED

1. P. M. Beane, D. Mansuy, L. Kiffel, and C. Amar, Liver Cell and Drug, Paris and London (1987), pp. 175-180.
2. S. M. Bosib, Kidney Int., **33**, 966 (1988).
3. R. Brands, M.D. Snider, Y. Hino, et al., J. Cell Biol., **101**, 1724 (1985).
4. R. F. Burk, R. A. Lawrence, and J. M. Lane, J. Clin. Invest., **65**, 1024 (1980).
5. G. Frens, Nature, **241**, 20 (1973).
6. W. Graninger and J. S. Smolen, Acta Med. Aust., **15**, 150 (1988).
7. D. J. Hearse and S. M. Humphrey, J. Molec. Cell Cardiol., **7**, 463 (1975).
8. G. Horpacsy, G. Farkas, T. Barankay, et al., Enzyme, **19**, 102 (1975).
9. M. Koseki, W. Nishida, T. Kamiike, and K. Nakao, Biomed. Res., **9**, 47 (1988).
10. M. Hanno, F. de Matteis, and L.J. King, Biochem. Pharmacol., **37**, 81 (1988).
11. M. Plebani, V. Chiaramo, A. Floreani, and R. Bertileani, Clin. Chim. Acta, **181**, 201 (1989).
12. G. Poli, E. Albano, and V.U. Dianzani, Chem. Phys. Lipids, **45**, 117 (1987).
13. E. Rubin, Iatrogen. Hepat. Injury, **11**, 312 (1980).
14. R. J. Smith and S. C. Spease, Inflammation, **10**, 132 (1986).
15. H. Sudoyo and S. Marzuki, Biochem. Biophys. Res. Commun., 158, 220 (1989).