Ultrastructural Changes in Albino Mouse Liver Caused by Isogenous F1$^+$ and F1$^-$ Strains of *Yersinia pestis* and their Correction with Ceftriaxone

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Fatty dystrophy and partial cytoplasmic necrosis predominate during the preagonal period in parenchymatous organs of mice infected with *Yersinia pestis* 231 F1$^+$. Endothelial damage, fibrin precipitation, and microcirculatory disorders occur in sinusoidal capillaries. More pronounced changes in the liver develop during the preagonal period in mice infected with *Y. pestis* 231 F1$^-$. Treatment with ceftriaxone leads to 100% survival in both groups and substantial restoration of liver structure.

Key Words: *experimental plague; liver; ceftriaxone; ultrastructure*

Strains of the causative agent of plague with reduced or lost ability to produce fraction 1 capsule antigen (F1$^+$ and F1$^-$, respectively) have been isolated from naturally occurring foci during the decline of an epizootic. The effectiveness of some antibacterial preparations drops in experimental plague induced by F1$^-$ strains [3,4]. However, ceftriaxone (CT), a third-generation cephalosporin, is highly effective in mice infected with the F1$^+$ and F1$^-$ strains, although the ED_{50} still tends to rise [2].

Our objective was to study the ultrastructure of albino mouse liver during the preagonal period and after CT therapy of plague caused by the F1$^+$ and F1$^-$ strains of *Yersinia pestis*.

MATERIALS AND METHODS

Isogenous 231 F1$^+$ and 231 F1$^-$ strains with the same LD_{50} for albino mice — 4-10 microorganisms according to the turbidity standard developed at the L. A. Tarasevich State Institute of Standardization and Control of Medical and Biological Preparations — were used. Mice were infected by subcutaneous injection of 24-h agar cultures (28°C) of *Y. pestis* in a dose of 10,000 microorganisms (about 1000 LD_{50}). Group 1 mice (n=25) were infected with the F1$^+$ strain and group 2 (n=25) with the F1$^-$ strain. Three animals from each group were killed by ether overdose on days 1, 2, 3, 4, and 5 after infection, and bacteriological study of internal organs and tissues was performed. The phenotype of isolated microorganisms was controlled with the antibody neutralization test. Untreated animals died on day 3 (F1$^+$) or 5 (F1$^-$) on average. Group 3 (n=20, 231 F1$^+$) and group 4 (n=20, 231 F1$^-$) mice were treated with CT (Roche). The preparation was injected intramuscularly once a day in a dose of 50 mg/kg during a 7-day period. The treatment was started 24 h after infection. The animals were killed after 30 days, and their internal organs and tissues were studied bacteriologically. For electron microscopy liver specimens from three mice in each group were collected in groups 1 and 2 during the preagonal period and in groups 3 and 4 on day 30 after infection. The material was fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, washed with the same buffer, postfixed with 1% OsO_4, dehydrated, and embedded in Epon. Semithin sections were stained with Toluidine Blue. Ultrathin sections were

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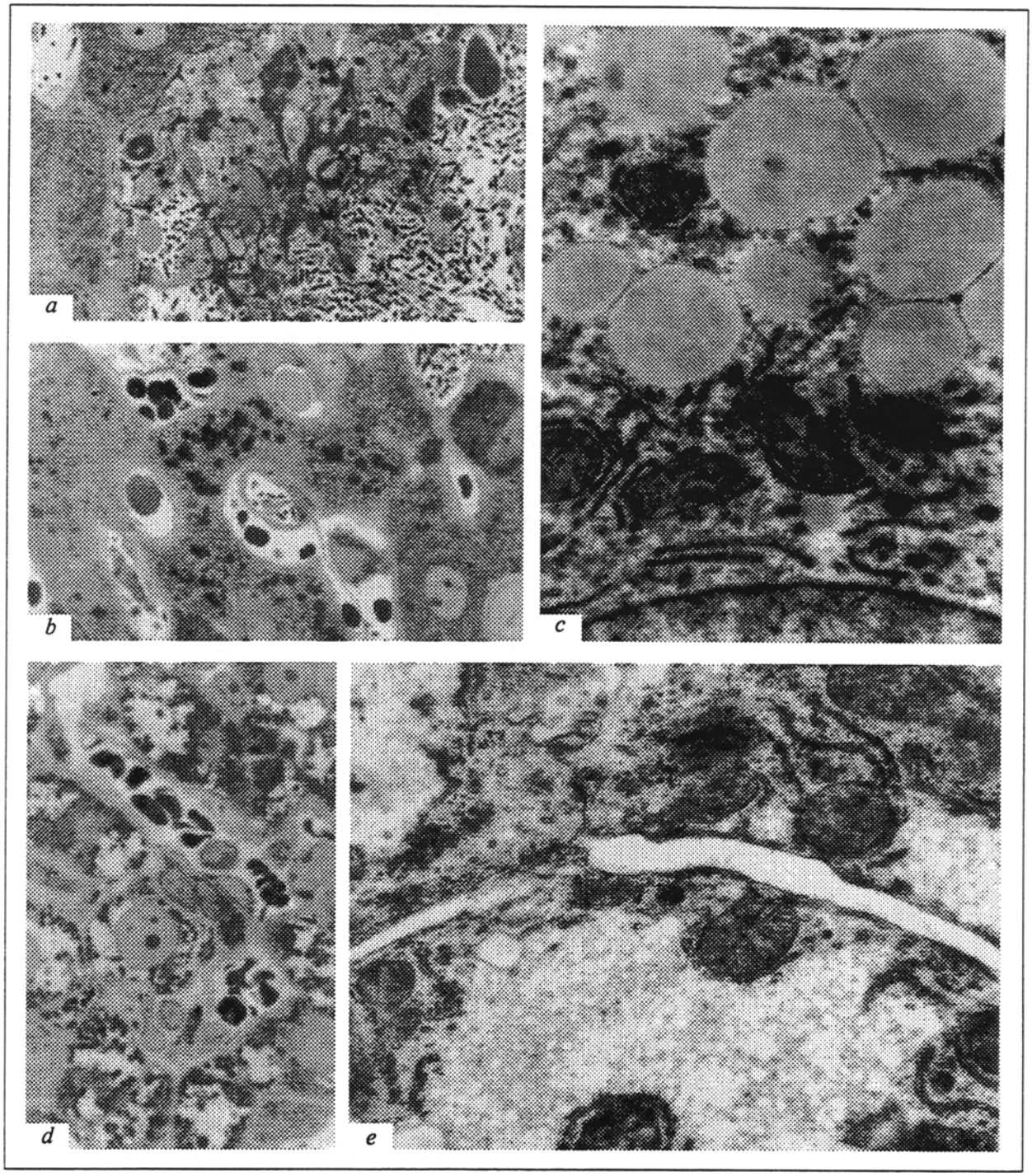

Fig. 1. Structural changes occurring during the preagonal period in the liver of albino mice infected with *Y. pestis* 231 F1$^+$ (*a*–*c*) and the isogenous strain 231 F1$^-$ (*d*, *e*).

a) numerous microorganisms in sinusoidal capillaries and the space of Disse (semithin section, Toluidine Blue staining, × 1000;

b) numerous lipid inclusions and vacuolization of hepatocyte cytoplasm (semithin section, Toluidine Blue staining, × 1000);

c) cytoplasm of parenchymatous cell with fragments of granular cytoplasmic reticulum, a few mitochondria, and lipid inclusions, × 8000.

d) colliquative necrosis of some hepatocytes (semithin section. Toluidine Blue staining, × 1000);

e) widening of intercellular spaces, absence of glycogen, partial necrosis of hepatocytes at the biliary pole, × 10,000.

cut in an LKB 8800 microtome, contrasted with uranyl acetate and lead citrate, and viewed in a JEM-100B electron microscope.

RESULTS

Despite the same virulence of the *Y. pestis* strains, infection caused by 231 F1⁻ lasted longer. In mice infected with yersinia 231 F1⁺ generalization of the process was observed by the 48th hour and in mice infected by yersinia 231 F1⁻ by the 72nd hour after infection. Group 1 mice survived for 3.5 days and group 2 mice for 5.4 days. Microorganisms isolated from the animals retained their original phenotype (F1⁺ or F1⁻).

Widened and plethoric capillaries containing great numbers of *Y. pestis* were seen on semithin sections prepared from the livers of F1⁺-infected mice collected within 3 days after infection (preagonal period). The microorganisms were also found in the space of Disse. In some preparations, fibrin precipitates were seen (Fig. 1, *a*). Characteristic changes in hepatocytes were lipid inclusions, often combined with smaller vacuoles, lending the cells a peculiar honeycomb structure (Fig. 1, *b*). The first signs of irreversible changes in the mitochondria and nuclei were observed.

Hepatocytes contained lipid inclusions of various size, which occupied large areas of the cytoplasm (Fig. 1, *c*). There were fragments of granular cytoplasmic reticulum and mitochondria with a few cristae and an electron-dense matrix. Margination of chromatin with its aggregation at the inner layer of the nuclear membrane was seen in almost all preparations. In advanced cases, the karyoplasm became transparent due to karyolysis, and glycogen was practically absent from the cytoplasm of parenchymatous cells located in the zone of partial necrosis. The intercellular spaces were widened considerably, and the hepatocytes lost contact with each other. Fragments of microvilli and cellular detritus were seen in biliary capillaries at the biliary pole. The microvilli oriented towards the space of Disse were indistinct and partially reduced. Irreversible damage to endothelial cells facilitated their desquamation, leading to the formation of deendothelialized areas through which *Y. pestis* and blood cells passed (blood cells can also leave the vessels by diapedesis).

On day 5, more uniform changes were revealed by light microscopy in livers of rats infected with the 231 F1⁻ strain. These changes included circulatory disorders (plethora of sinusoidal capillaries) and colliquative necrosis (Fig. 1, *d*). Lipid inclusions were quite often seen in hepatocytes; however, their accumulation in the cytoplasm was less pronounced compared with that occurring in group 1.

A wide range of changes (from reversible shifts to necrosis) was revealed by electron microscopy. The cytoplasm became more transparent, and the number of organelles dropped considerably. In some hepatocytes such changes occurred only at the biliary or vascular pole. Glycogen was absent from almost all preparations; it was not even found in the areas with preserved organelles (Fig. 1, *e*). The intercellular spaces were widened along the whole stretch between adjacent parenchymatous cells (Fig. 1, *e*). The endothelium was often edematous with local damage to the luminal plasmalemma. Destroyed polymorphonuclear leukocytes and activated Kupffer cells were seen.

Treatment with CT, which was begun simultaneously in both groups, led to 100% survival of mice. There were no macroscopic changes in their internal organs on day 30 after infection with both strains. However, distinct differences between the structural changes induced by these strains were revealed under the light microscope. There were no partial necrosis zones in hepatocytes of mice infected with the F1⁺ strain, and capillary plethora was moderate. There were no microorganisms in blood vessels or in the spaces of Disse (Fig. 2, *a*). Electron microscopy showed that most liver cells had restored their structure. The increased number of binuclear parenchymatous cells with recovered glycogen can be regarded as an adaptive reaction. The number of lipid inclusions was markedly decreased (Fig. 2, *b*). Some hepatocytes with preserved organelles remained separated by widened intercellular spaces.

Plasma soaking of perisinusoidal spaces, plethora of some capillaries, and necrotic zones in liver cells were seen on semithin sections prepared from the livers of mice infected with *Y. pestis* 232 F1⁻ and treated with CT (Fig. 2, *c*). However, ultrastructural study revealed both damaged and undamaged functional units including mitochondria, nuclei, and cisternae of granular cytoplasmic reticulum (Fig. 2, *d*). There were fewer lipid inclusions located predominantly at the cell periphery.

Our results show that some changes (microcirculatory disorders and intravascular coagulation) are similar to those induced in the liver by lipopolysaccharide of Gram-negative microorganisms [6-8]. The replenishment of the glycogen stores and the appearance of binuclear hepatocytes, which are more active than mononuclears, are manifestations of intracellular regeneration and promote the restoration of tissue homeostasis [1,5].

Despite the more pronounced disorders in the liver of mice infected with the *Y. pestis* 231 F1⁻ strain,

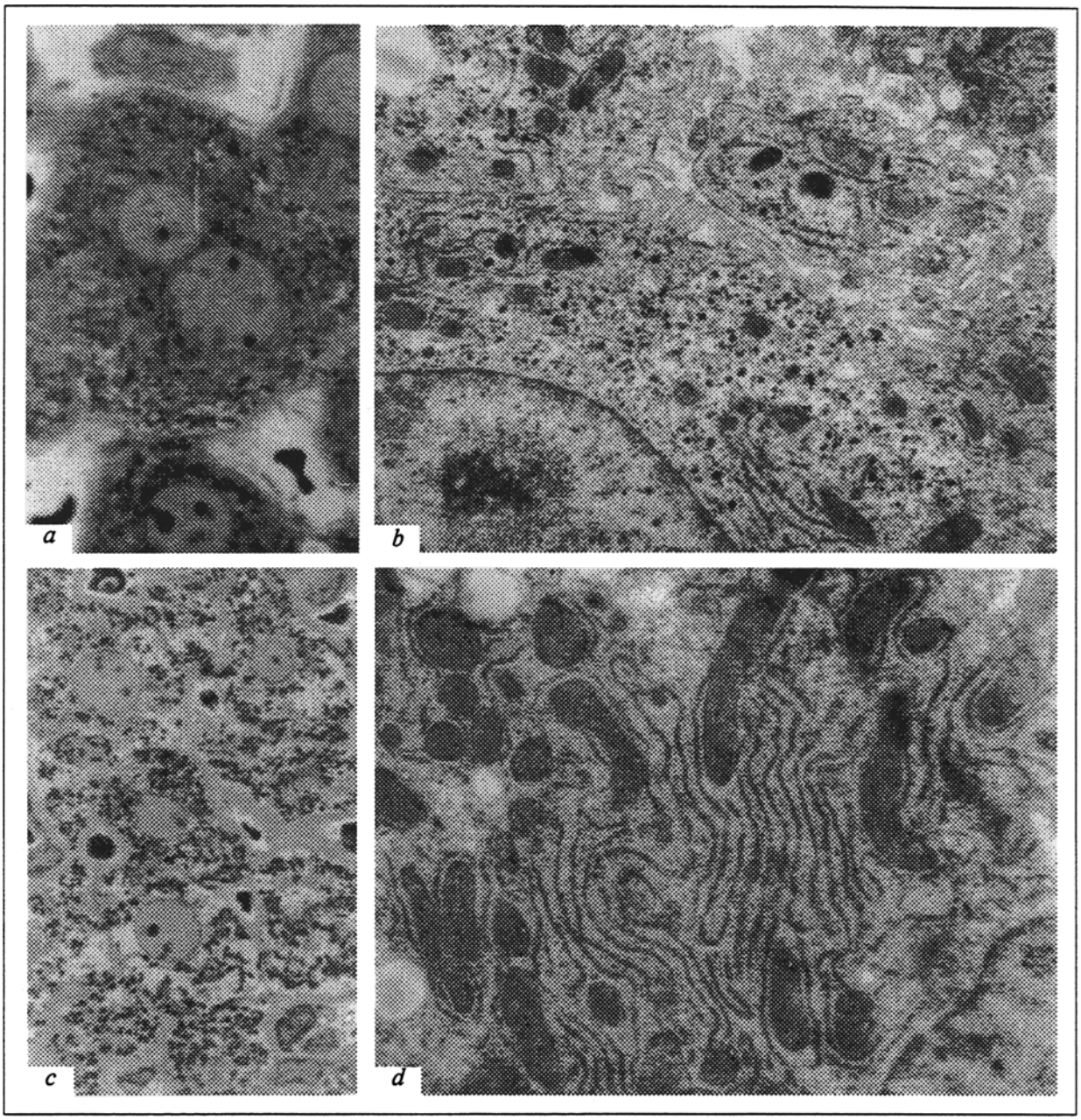

Fig. 2. Effect of CT on structural changes in the liver of albino mice infected with *Y. pestis* 231 $F1^+$ (*a*, *b*) and the isogenous strain 231 $F1^-$ (*c*, *d*). *a*) restored structure of hepatocytes, no changes in sinusoidal capillaries (semithin section, Toluidine Blue staining, × 1000); *b*) restored ultrastructure of parenchymatous cell, × 6000; *c*) necrotic zones in some hepatocytes, no changes in sinusoidal capillaries (semithin section, Toluidine Blue staining, × 1000); *d*) preserved organelles and the absence of glycogen, × 5000.

the development of a complex of compensatory-adaptive changes caused by etiotropic therapy indicates that CT is a promising drug for the treatment of plague caused by the $F1^+$ and $F1^-$ forms of *Y. pestis*.

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