One possible pathway for the pathogenic action of the agents of infectious diseases of the intestine may thus be the toxic action of their products on the neurohumoral regulatory systems, caused by the penetration of bacterial toxins into the blood, as a result of which the cardiovascular system and the gastrointestinal tract, with their complex functional interrelations, are involved in the pathogenetic process; this leads to the development of an infectious disease and determines its course and outcome.

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RHYTHM OF STRUCTURAL AND FUNCTIONAL CHANGES IN HEPATOCYTES AFTER EXPOSURE TO PESTICIDES

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The biological transformation of Dursban was investigated during perfusion of the livers of rats exposed for 4, 8, and 15 days to poisoning by CCl_4 and Milbex (an inhibitor and inducer, respectively, of microsomal enzymes). The ultrastructure of the hepatocytes was studied in rats poisoned with these substances. Three stages of structural and functional changes were identified. After the eighth day, the pathways of transformation of Dursban were reorganized: dealkylation processes, leading to the formation of less toxic metabolites, were intensified.

KEY WORDS: *perfusion of the liver; microsomal oxidases of mixed function; pesticides; Dursban — biological transformation.*

The body can adapt itself to some extent to the action of harmful factors that differ in strength. Adaptive structural and functional changes reflects both the intensity and the rhythm of action of the pathogenic factor [9-11]. Sarkisov et al. [8] distinguished four types of structural and functional responses aimed at securing homeostasis. The first three responses they regard as nonspecific, while the fourth is to some extent specific for it is aimed "against" a particular toxic factor. According to the same [8] and other workers [1, 7], and also the present writers' observations [4], during daily exposure to poisons the structural and functional changes in the hepatocytes are more clearly expressed during the

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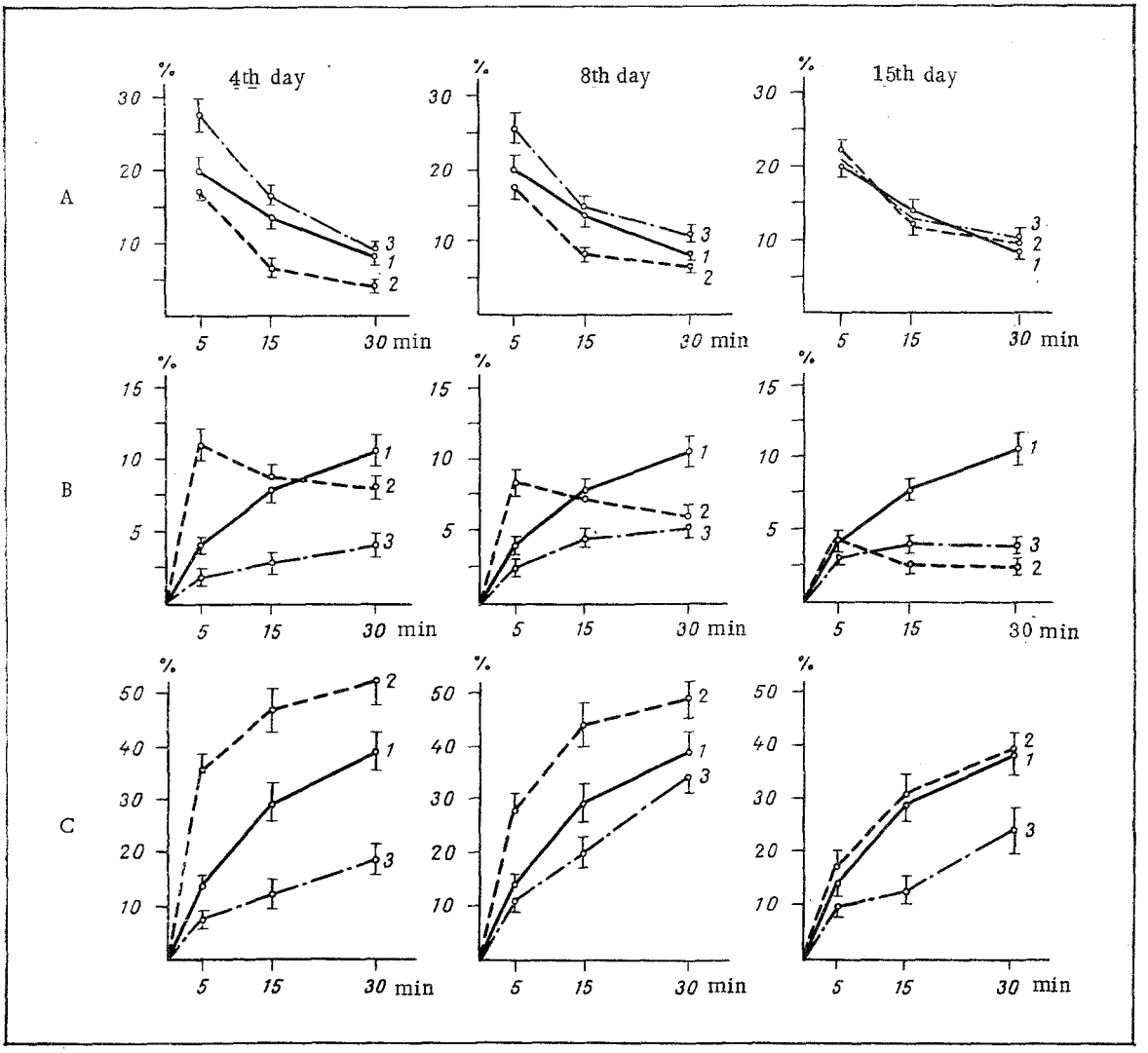

Fig. 1. Dynamics of biological transformation of Dursban and of depression of cholinesterase activity during perfusion of rat liver. A) Concentration of Dursban in perfusion fluid; B) concentration of P—O analog of Dursban; C) degree of depression of cholinesterase activity. 1) Control; 2) administration of Milbex; 3) administration of CCl_4.

first 1-15 days. In the investigations cited above, only disturbances of liver function [1, 4, 7] or changes in the subcellular structures and synthetic activity of the hepatocytes [8] were evaluated.

The object of the present investigation was to discover changes in the subcellular structures and associated functions of the hepatocytes by the use of electron microscopy and perfusion of the isolated liver.

EXPERIMENTAL METHOD

Sexually mature noninbred male rats were used. The experimental model of induction of the hydroxylating enzyme system of the endoplasmic reticulum of the liver, known as oxidases of mixed function (OMF), was produced by daily oral administration of the organochlorine pesticide Milbex in a dose of 30 mg/kg (0.025 LD_{50}). Depression of OMF activity was produced by daily oral administration of the classical industrial poison — carbon tetrachloride (CCl_4), in a dose of 200 mg/kg (0.025 LD_{50}). This dose was given as an emulsion in sunflower oil.

The livers were perfused by means of an apparatus designed by the writers [5]. The liver donors were rats exposed for 4, 8, and 15 days to the action of Milbex and CCl_4, and also control rats. The technique of the operation and perfusion of the liver was described previously [6].

The organophosphorus pesticide Dursban was used as the model substance with which to study biological transformation processes. A solution of the chemically pure preparation was added to the perfusion fluid in a dose of 10 μg/ml before the liver was connected to the artificial circulation. Dursban and its main metabolites were determined by gas and thin-layer chromatography. Cholinesterase activity in the perfusion fluid was determined by Hestrin's method [15]. The material for investigation (perfusion fluid) was taken before and 5, 15, and 30 min after connecting the organ to the artificial circulation.

An electron-microscopic investigation of the ultrastructure of the hepatocytes was carried out on 17 rats: 3 control rats and 14 experimental animals, tested in groups of 2 or 3 on the 4th, 8th, and 15th days after the beginning of poisoning with Milbex and CCl_4.

EXPERIMENTAL RESULTS

The kinetics of Dursban poisoning (as reflected in the decrease in its concentration in the perfusion fluid and accumulation of its P—O analog) and the dynamics of depression of cholinesterase activity of the perfusion fluid are illustrated in Fig. 1. Curves were drawn through points corresponding to the arithmetic mean of the results of four perfusions. For clarity the results are represented as percentages of the initial level. The dynamics of the decrease in the Dursban concentrations during perfusion of the liver of rats exposed for 4, 8, and 15 days to the action of Milbex and CCl_4 is shown in the top 3 graphs (A). The middle three graphs (B) illustrate the dynamics of accumulation of the P—O analog of Dursban, and the bottom three graphs (C) show the increase in the degree of inhibition of cholinesterase activity in the course of perfusion.

The rapid depression of cholinesterase activity even at the beginning of perfusion can only be explained by the high velocity of the reaction of oxidative desulfuration which takes place with the participation of the liver OMF. The product of this reaction, the P—O analog of Dursban, is 4 times more toxic than Dursban itself; and unlike the latter, it possesses high anticholinesterase activity [2].

Induction of OMF by the organochlorine pesticide Milbex on the fourth day after the beginning of its administration led to a statistically significant increase in the rate of fall of the Dursban concentration in the perfusion fluid (Fig. 1A). Parallel with this effect, a threefold increase in the concentration of the P—O analog and a 3 times more marked depression of cholinesterase activity were observed (at the fifth minute). Accumulation of the less toxic dealkylated product of the P—O analog in the course of perfusion shows that, parallel with the process of oxidative desulfuration, the product of which was more toxic than the original preparation (toxification), processes of detoxication also took place. The results also confirm Doterman's hypothesis [3] of the importance of this pathway in the biological transformation of thiophosphates.

At the same time (fourth day of exposure to Milbex and during investigation of the ultrastructure of the hepatocytes) signs of degeneration and hyperplasia of the endoplasmic reticulum were observed. In some areas there was fragmentation of the granular endoplasmic reticulus. In the peripheral cells it surrounded the mitochondria and was dilated here and there. It could be seen to communicate with the outer membrane of the nuclei.

The statistically significant slowing of the decrease in the Dursban concentration in the course of perfusion of the liver of the rats receiving CCl_4 for 4 days (Fig. 1A), revealed by these experiments, confirms the observations of those workers [12-14] who found that this poison inhibits OMF activity. At all times of perfusion there was also a significant decrease in the concentration of the P—O analog of Dursban (Fig. 1B) and in the degree of inhibition of cholinesterase activity (Fig. 1C). The dealkylated derivative of the P—O analog of Dursban could not be detected. Investigations of the ultrastructure of the livers of the rats exposed for 4 days to CCl_4 showed that besides areas of micronecrosis of the hepatocytes, there were also cells with well-marked degenerative changes.

The biological transformation of Dursban in the liver of the rats poisoned for 8 days with Milbex was rather slower than at the previous time (fourth day). Differences from the control were significant at the 15th minute after the beginning of perfusion (Fig. 1A). The dynamics of accumulation of the P—O analog (Fig. 1B) and of the increase in the degree of depression of cholinesterase activity (Fig. 1C) was similar to that obtained after 4 doses of the poison, but the differences from the control were less marked.

The ultrastructure of the hepatocytes at this time (eighth day of exposure to Milbex) was characterized by a marked increase in the quantity of the smooth endoplasmic reticulum. Among areas of evenly dilated cisterns of the latter structure there were small areas of granular endoplasmic reticulum and also free ribosomes and polysomes.

After exposure to CCl_4 for the same time (8 days) the processes of the toxicokinetics (Fig. 1A, B) and toxicodynamics (Fig. 1C) differed less from the control than after the fourth dose of the poison. The features of adaptation to repeated administration of the poisons were accompanied by various structural changes in the hepatocytes. They were heterogeneous and "mosaic" in character. Side by side with areas of necrosis and degeneration, which were similar to those described above (the fourth day of CCl_4 poisoning), hypertrophy of the nuclei of the hepatocytes and foci of proliferation of the smooth and granular endoplasmic reticulum were observed.

Analysis of the results of the study of the toxicokinetics (Fig. 1A, B) and toxicodynamics of Dursban during perfusion of the liver of rats exposed for 15 days to the action of Milbex and CCl_4 indicates features of functional equilibrium. This conclusion is based, first, on the identical course of the curves of the decrease in Dursban concentration in the perfusion fluid (Fig. 1A). By contrast with the control, the biological transformation of Dursban took place not through the accumulation of its P—O analog, but through the intensification of its dealkylation. By this stage of exposure to Milbex and CCl_4, the normal rate of biological transformation of Dursban was thus maintained through the reorganization of its metabolic pathways. The features of functional equilibrium discovered at this stage (15th day) rested on a material basis, namely the mosaic pattern of changes in the cell organoids.

During poisoning with CCl_4, three stages of structural and functional changes are thus distinguishable: 1) degeneration of the structures with a decrease in functional activity (4th day), 2) reparative regeneration with some restoration of the functions of the hepatocytes after their initial injury (8th day), and 3) structural and functional equilibrium (15th day).

In the case of Milbex poisoning, the boundaries between the first 2 stages are ill defined. After the eighth day, an adaptive reorganization of the pathways of biological transformation took place.

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