vented determination of the decrease in the erythropoietin titer in the intact rats. However, the depression of synthesis of high-polymer RNA observed during hyperoxia in both the "endocrine" and the intact kidney indicates that a short period of hyperoxia is sufficient to change the chain of biosynthetic reactions at the genome level leading to a reduction in formation of the hormone or of its precursor [7]. Fractionation of RNA in the kidneys, carried out in the writers' previous experiments [3], showed that hyperoxia for 4 h caused the maximal decrease in synthesis of DNA-like RNA [3]. The connection between processes of RNA synthesis in the kidney and its endocrine function, confirmed also by the "endocrine kidney" model, is evidence that DNA-dependent RNA synthesis is one of the mechanisms included in erythropoietin biogenesis.

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BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN LUNG AND LIVER TISSUES OF ALBINO RATS WITH EXPERIMENTAL PARAQUAT POISONING

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KEY WORDS: herbicide paraquat; superoxide dismutase; free radicals; histomorphology of the lungs and liver.

Metabolic conversions of the herbicide paraquat (1,1-dimethyl-4,4-bipyridyl dichloride) are carried out with the participation of a microsomal NADPH-dependent system of oxidases of mixed function [3]. It has been suggested [4, 5] that during conversion of paraquat in vivo superoxide radicals responsible for the specific toxic action of the compound are formed.

The object of this investigation was to study the effect of paraquat on the ability to form free radicals in the tissues of the lungs and liver and on superoxide dismutase (SOD) activity, an enzyme with whose participation superoxide radicals are detoxicated, and to study pathomorphological changes in these organs, developing under the experimental conditions used.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. The animals were poisoned by peroral administration of paraquat in three doses, each of 25 mg/kg, or in repeated doses of 12.7 and 6.35 mg/kg daily for 30 days, equivalent to 0.2, 0.1, and 0.05 LD_{50}. The animals were decapitated 1, 5, and 30 days

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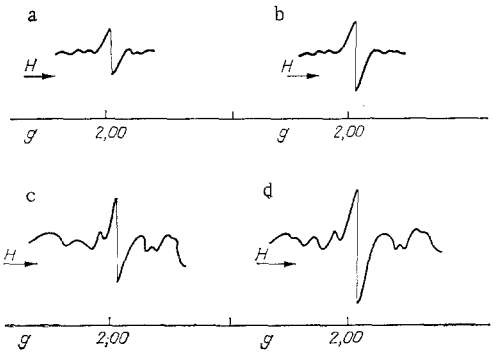

Fig. 1. EPR spectra of rat tissues under the influence of paraquat: a) lungs of control rat; b) lungs of rat after three doses of the poison (0.2 LD_{50}, 5th day); c) liver of control rat; d) liver of rat after administration of three doses of paraquat (0.2 LD_{50}, 1st day).

TABLE 1. Changes in SOD Activity in Rat Lung and Liver Tissues after Administration of Paraquat (M ± m)

Experimental conditions	Time of investigation, days	SOD activity, units of activity/g protein	
		lungs	liver
Control	—	0,72±0,07 (5)	1,57±0,03 (6)
Administration of three doses of paraquat each of 25 mg/kg	1	0,46±0,07* (5)	1,64±0,12 (5)
	5	0,51±0,1* (5)	1,95±0,07* (6)
Repeated administration (for 30 days) of paraquat in a daily dose of:			
12.7 mg/kg	30	0,36±0,04* (5)	1,05±0,08* (6)
6.35 mg/kg	30	0,71±0,08 (5)	1,25±0,08* (5)

Legend. *) $P < 0.05$. Number of animals in parentheses.

after the beginning of the experiments. The lung and liver tissues were perfused with 1.15% KCl and homogenates were prepared in which the ratio of the weight of tissue to volume of solution was 1:3 for the lung and 1:4 for the liver. The isolation medium was 1.15% KCl. The postmitochondrial fraction was obtained by centrifugation of the homogenates for 20 min at 15,000g. SOD activity was determined spectrophotometrically at 480 nm by measuring the rate of formation of adrenochrome from adrenalin [8]. The unit of activity of SOD was taken to be the quantity of enzyme used to inhibit autooxidation of adrenalin by 50%. Free radicals were determined by EPR-spectroscopy. Samples of lung and liver tissues frozen to 77°K were investigated on the "Varian" Mark E-109 EPR spectrometer. The intensity of the signal with a g-factor of 2.00 was studied by comparing the amplitude of the signal from the experimental samples with those from the controls.

Parallel with the above, pieces of lung and liver were fixed in 10% neutral formalin for subsequent histomorphological study. Dewaxed sections of the organs were stained with hematoxylin and eosin and also impregnated with silver nitrate by Gomori's method to detect argyrophilic fibers.

EXPERIMENTAL RESULTS

Injection of paraquat was shown to lead to an increase in the concentration of free radicals. Triple administration of paraquat caused an increase in the intensity of the EPR-signal with a g-factor of 2.00 in the lungs on the 5th day by 2.2 times compared with the control. In the liver, the signal was increased by 1.2 times after only 24 h. Changes of this intensity also were found after 5 days (Fig. 1).

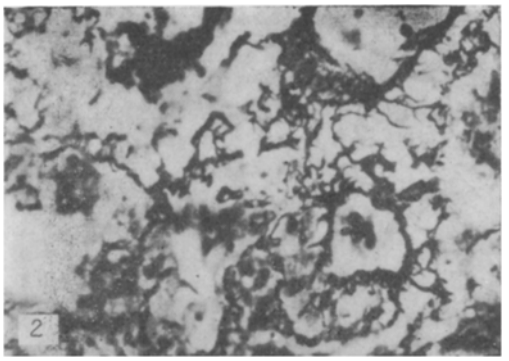

Fig. 2. Administration of three doses of paraquat (0.5 LD_{50}, 5th day). Lung. Coarsening and disturbance of structure of argyrophilic network. Impregnation with silver nitrate by Gomori's method, 90×.

In the liver and lungs of the control animals, SOD activity was discovered; its level in the liver was 2.1 times higher than in the lungs, in agreement with data in the literature [10].

Acute paraquat poisoning lowered the SOD activity in the lungs relative to the control by 37% after 1 day and by 31% after 5 days; in the liver it was increased by 24% but not until 5 days after injection of the compound (Table 1).

After repeated injections of paraquat in a dose of 0.1 LD_{50} SOD activity in the lungs was inhibited by 50% and in the liver by 34%. Exposure to 0.05 LD_{50} did not affect SOD activity in the lungs, but in the liver it remained depressed by 34%.

The changes discovered in SOD activity in the lungs and liver after injection of paraquat were to a definite degree determined by the physiological functions of their tissues, the level of enzyme activity, and the distribution of the compound in the body and its metabolism. Investigations using paraquat-^{14}C [12, 13] have shown that it is concentrated maximally in the lungs.

Administration of three doses of paraquat in a dilution of 0.2 LD_{50} led to the development of distinct histomorphological changes in the organs studied. In the lungs focal hemorrhages were observed, together with considerable perivascular and peribronchial edema, with infiltration of the tissue by histiocytes, lymphoid and plasma cells, and eosinophils; foci of plasmarrhagia were present. The interalveolar septa were thickened and rich in cells, among which eosinophils also were seen. The tissue was poorly aerated, areas of atelectasis and vicarious emphysema were visible, and complexes of large pale cells, with oval, weakly basophilic nuclei, were conspicuous. The few alveoli which still remained intact were filled with a vacuolar serous or blood-stained fibrinous effusion, with desquamation of the cells of the alveolar epithelium. Coarsening and destruction of the argyrophilic fibers of the alveolar septa were observed (Fig. 2). In the liver, cells in the lobules were haphazardly arranged, and anuclear hepatocytes were found singly and in groups. There was a marked increase in the number of binuclear cells of the parenchyma and of cells with polyploid nuclei.

After administration of paraquat in a dilution of 0.1 LD_{50} for 30 days more considerable changes were observed in the lungs. Besides thickening of the alveolar septa, infiltration of the tissue with histiocytes, lymphocytes, and plasma cells, poorly aerated regions were very constantly seen, together with extensive airless zones rich in polymorphic cells, including large cells of fibroblast type with pale, oval nuclei; numerous mitotic figures also were seen (Fig. 3a). Proliferation of the epithelium of the alveolar passages, bronchioles, and small and medium-sized bronchi could be seen, with partial filling of their lumen with newly formed cells. The proliferative changes in many parts of the lung were adenomatous in character (Fig. 3b). Collections of eosinophils were frequently observed, mainly perivascular and peribronchial in situation (Fig. 3c).

The lumen of the residual alveoli was filled with homogeneous protein contents, weakly or strongly eosinophilic, partly vacuolated, with a delicate network of fibrin, and with desquamated alveolar epithelial cells and erythrocytes. Extensive regions of vicarious emphysema could be seen.

Polymorphism of the hepatocyte nuclei was observed in the liver, many parenchymal cells with polyploid nuclei and binuclear cells were observed, and occasionally trinuclear cells and mitotic figures were visible.

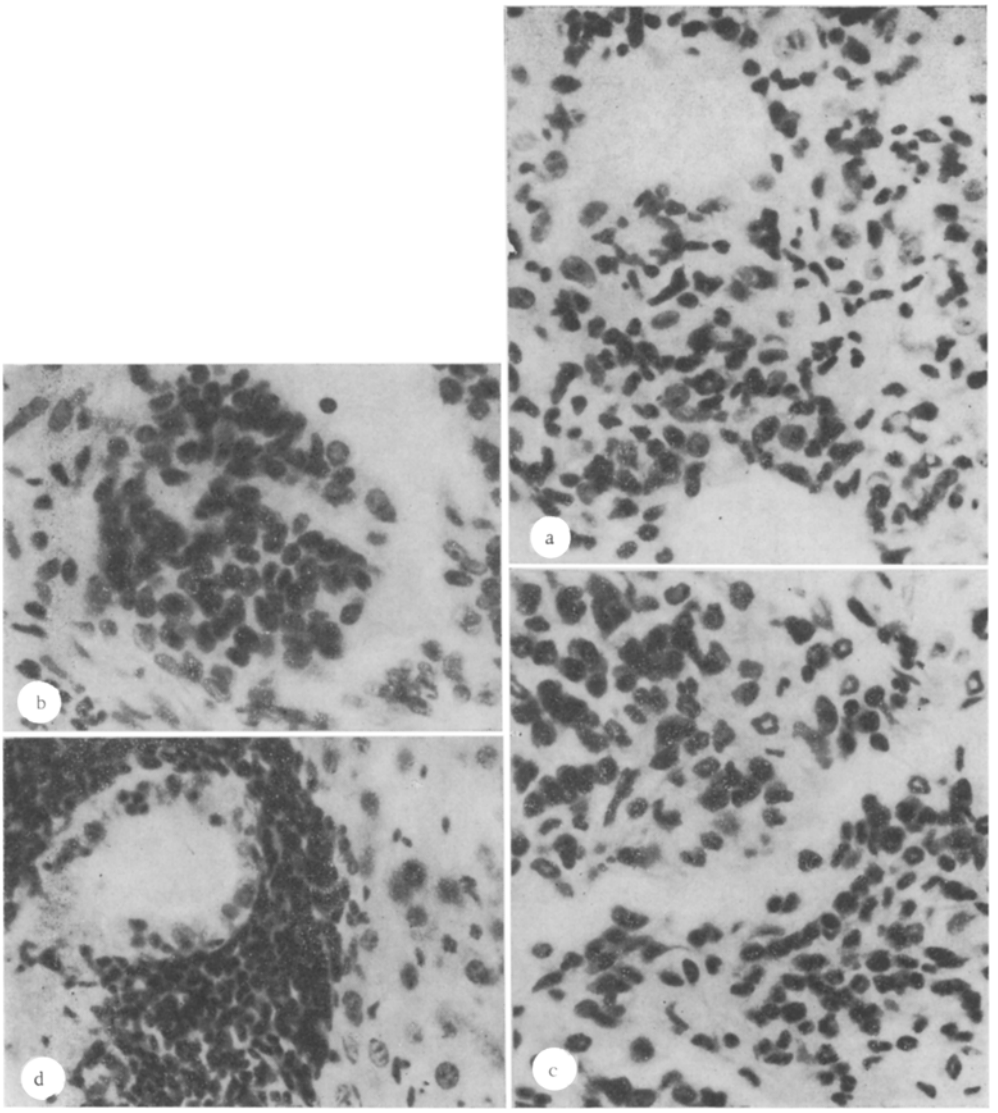

Fig. 3. Daily injection of 0.1 LD_{50} paraquat for 30 days: a) lung. Intensive proliferation of alveolar epithelium and local connective tissue cells. Mitoses; b) lung. Region of adenomatous proliferation of bronchiolar epithelium; c) lung. Extensive accumulation of eosinophils; d) liver. Focal infiltration consisting of histiocytes, and of lymphoid and plasma cells. Hematoxylin-eosin. Magnification: a-c) 200×, d) 140×.

The epithelium of the small and medium-sized bile ducts showed moderate proliferation in some areas. Focal collections of histiocytes and of lymphoid and plasma cells could be seen in the interlobular connective tissue (Fig. 3d).

In the 30-day experiments in which the animals were given 0.05 LD_{50} of paraquat, the changes discovered in the organs were analogous or similar. Proliferation of the cells in the lungs of some animals was less intensive.

To judge from the morphological evidence obtained, proliferative changes in the lungs are of great importance in paraquat poisoning. Closely similar changes have been described in cases of paraquat poisoning in man [7, 9, 11]. In one investigation [9] a fibroblastic response, leading to the development of pulmonary insufficiency, was regarded as the cause of death of a child poisoned with paraquat. Some workers claim [6] that proliferative changes in the lungs can continue even after the entry of paraquat into the body has ceased. The widespread reaction of the epithelium and fibrosis and repair processes in the lungs (the latter were observed also by other workers [1, 2]) undoubtedly are responsible for the sharp decline in the functional capacity of the organ, which may be incompatible with life.

In experimental paraquat poisoning a definite parallel is thus observed between the biochemical and morphological changes in the tissues. It can be tentatively suggested that depression of SOD activity and the increase in the concentration of free radicals found in this condition are among the causes of development of proliferative processes manifested as a combination of characteristic pathological and morphological changes.

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EFFECT OF KUPFFER CELL BLOCKADE ON THE DEVELOPMENT OF ACUTE TOXIC HEPATITIS

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KEY WORDS: carbon tetrachloride; hepatitis; Kupffer cells.

In previous investigations we showed that recovery of the mass of the liver after partial resection [3, 4] and injury and repair of the liver structure after administration of a single dose of a hepatotropic poison [2] depend on the functional state of the hepatic stroma and, in particular, of the Kupffer cells. Preliminary stimulation of the system of mononuclear phagocytes by the bacterial polysaccharide prodigiosan [1] was found to promote the more rapid regeneration of hepatocytes after partial resection of the liver, and the resistance of the hepatocytes to CCl_4 was increased. Conversely, preliminary loading of the Kupffer cells with colloidal iron carbonyl, with a strictly determined granule size, retarded repair of the liver after partial hepatectomy by inhibiting the rate of DNA synthesis in the hepatocytes and their mitotic division.

The object of this investigation was to study the course of acute toxic hepatitis in rats after blockade of the Kupffer cells.

EXPERIMENTAL METHOD

Wistar rats weighing 200-250 g were used. The animals in the experiment received an intravenous injection of 1 ml of 10% colloidal iron carbonyl-phosphate, grade R-100F, with a particle size of 0.8-1.5 μ, suspended in 5% starch in 0.85% NaCl solution. The control rats received 1 ml of starch solution. The animals of the experimental and control groups were given a subcutaneous injection of 0.2 ml of a 40% solution of CCl_4 in vegetable oil per 100 g body weight 2 h after the intravenous injection. Rats of the experimental and control groups were decapitated in batches of 4-6 at a time, 16, 24, 48, and 72 h later. The liver was fixed in Carnoy's mixture and embedded in paraffin wax. Sections were stained with hematoxylin-eosin and examined under the light microscope.

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