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DERMAL TOXICITY OF PARAQUAT

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Abstract: Dermal toxicity of paraquat in rats was studied, as well as its influence on internal organs, phagocytic and bactericidal activity of the neutrophile system, and the behaviour of the rats. The studies were conducted on 30 female rats of Wistar strain. The animals were divided into three groups, of which two groups were experimentally exposed to dermal absorption of paraquat (1/2 LD_{50} or 1/10 LD_{50}), and one group was exposed as a control to dermal absorption of the solvent. The animals were exposed to dermal absorption for 4 hours daily for a period of 4 weeks. After 28 days, the rats were anaesthetized and blood was drawn from the heart to evaluate the activity of the neutrophilic system while the internal organs were excised for histological and ultrastructural studies. Histological and ultrastructural changes were observed in the lung, manifested by widened interalveolar septa filled with erythrocytes, lymphocytes, plasmatic cells, or alveolar macrophages. In the interalveolar septa bunches of collagen fibres were noted, while in the lumen of alveoli exudate and erythrocytes were observed. Histological and ultrastructural changes were also noted in the heart. They manifested themselves by focal hypertrophy of the interstitial tissue and by the increase of collagen fibres in bunches between cardiomyocytes. Less severe pathological changes were observed in kidney and liver. In the brain histological changes occurred in the neutrocytes. The bactericidal activity of the neutrophilic system increased in both experimental groups. Stimulation of phagocytosis was noted only in animals exposed to 1/10 LD_{50} - the lower dose of paraquat. The cognitive activity increased in both experimental groups after 2-weeks exposure to dermally applied paraquat, but returned to normal 2 weeks later.

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Key words: Dermal toxicity, paraquat, histopathology, ultrastructure, neutrophile activity, behaviour.

INTRODUCTION

Paraquat (dichloride 1, 1-dimethyl-4, 4-bipyridylium) was introduced commercially by Plant Protection Ltd. in 1958, and is now one of the most widely used herbicidal chemicals in agriculture throughout the world. Formulations containing the dichloride are sold under the trade names: “Gramoxone”, “Dextrone”, and “Weedol”, which also contain diquat. Mixtures of paraquat with various residual herbicides are marketed as “Dexuron”, “Tota-Col”, “Gramuron”, “Para Col”, “Pathclear” and “Gramonol”.

The acute oral LD_{50} for rats is 150 mg/kg, for mice 104 mg/kg, for sheep 70 mg/kg, for dogs 25-50 mg/kg, and for hens 262 mg/kg. The acute dermal LD_{50} for rabbits is approximately 236 mg/kg, for male rats 80 mg/kg, and for female rats 90 mg/kg [14].

According to the acute oral toxicity, expressed in milligrams per kilogram of animal body weight, paraquat belongs to the second class of toxicity.

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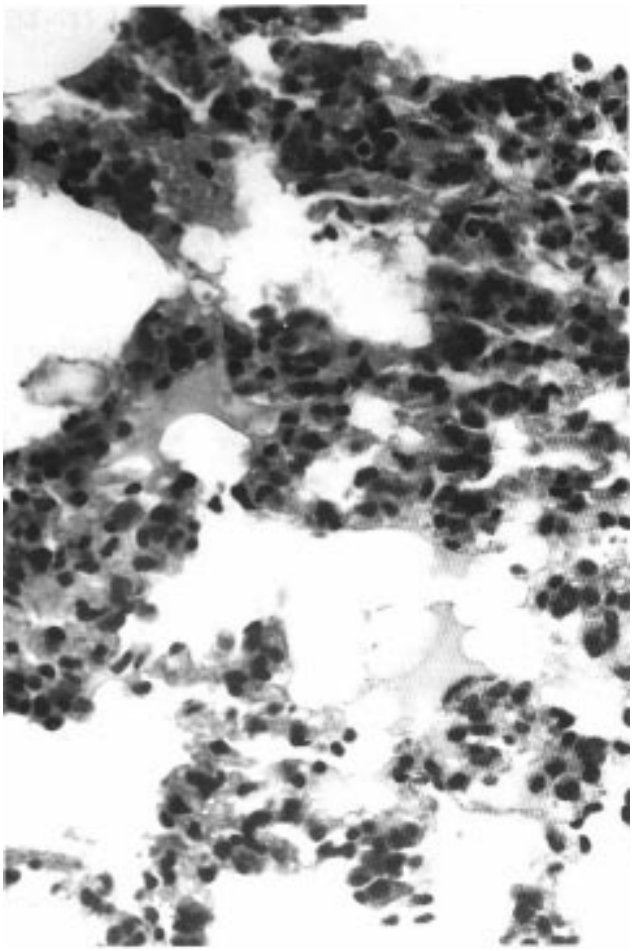

Figure 1. Rat exposed to 1/2 LD_{50} of paraquat. Exudate is present in alveoli. H-E, × 100.

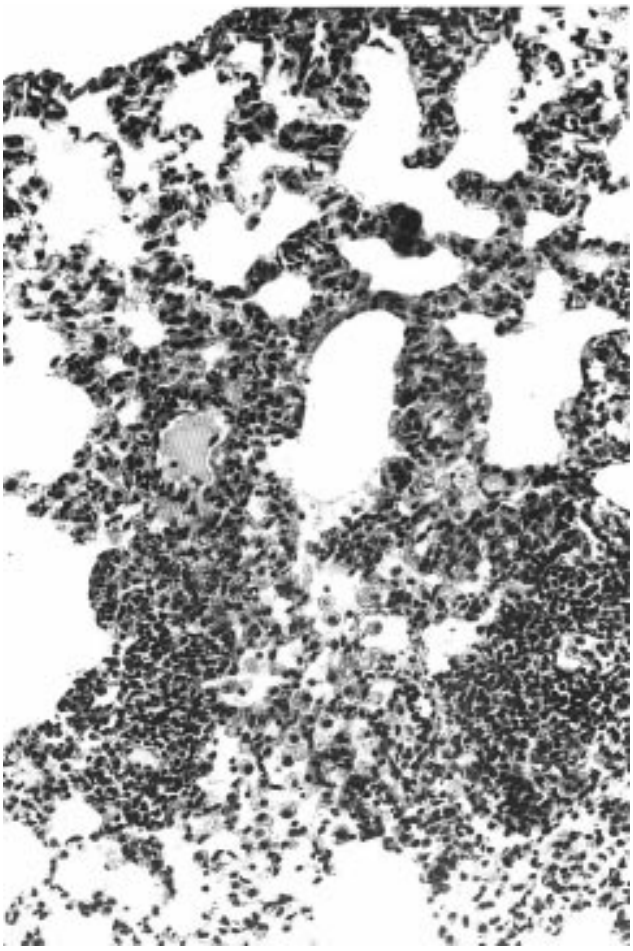

Figure 2. Rat exposed to 1/2 LD_{50} of paraquat. Wider interalveolar septa because of infiltration with lymphocytes and alveolar macrophages. H-E, × 50.

Due to the extensive world-wide use of paraquat, cases of paraquat poisoning frequently occur. This was demonstrated at the Second European Symposium on Paraquat Poisoning in 1986, attended by delegates from over 20 countries, where it was reported that in Japan 1,300 persons die each year from paraquat poisoning [9].

The toxic effect of paraquat on plants is connected with the production of paraquat free radicals which, after the reoxidation with oxygen molecules, cause disorders in photosynthesis.

In warm-blooded animals reoxydation leads to the production of superoxide anion 0_{-2} and to the oxydation of unsaturated lipids to superoxide forms, thereby producing free radicals. These free radicals react with multiunsaturated lipids of the cell membranes, thus beginning lipid peroxidation. The biological effect of free radicals is wide damage to cellular membranes, and disturbance of their functional infragibility.

In cases of fatal paraquat poisonings in humans the most severe changes are observed in the lungs, appearing as very intensive hyperaemia and prompt total airlessness; in the interalveolar septa and in the lumen of alveoli, cells with a large number of silver-absorptive fibres occur [29]. Lung oedema and destruction of the lung epithelium were also observed [28]. This is probably due to the active transportation of paraquat to the lung where its concentration is 10 times higher than in plasma [20].

Paraquat causes a distinct reduction of the lecithin fraction to 75%, which leads to collapse of the alveoli in the lung [13]. Simultaneously, there occur severe disturbances of the metabolic activity of superoxide dismutase in the microsomes of the lung. These disturbances, however, are not observed in liver or kidney, in which takes place the detoxication of superoxide radicals stimulated by paraquat [16].

Apart from the characteristic lung symptoms in the cases of fatal paraquat poisonings, there may also exist necrosis of the adrenal cortex or necrosis of the heart muscle [15, 17]. Povoa *et al.* [17] found cardiac involvement due to paraquat in 40% of patients. The clinical picture of this involvement had a wide spectrum, ranging from minimal changes in ECG to acute and extensive myocardial necrosis.

The paraquat poisonings most often described in Germany were burns, gastrointestinal disturbances and lung oedema, while only rarely were reported diseases of kidney, liver and heart [6].

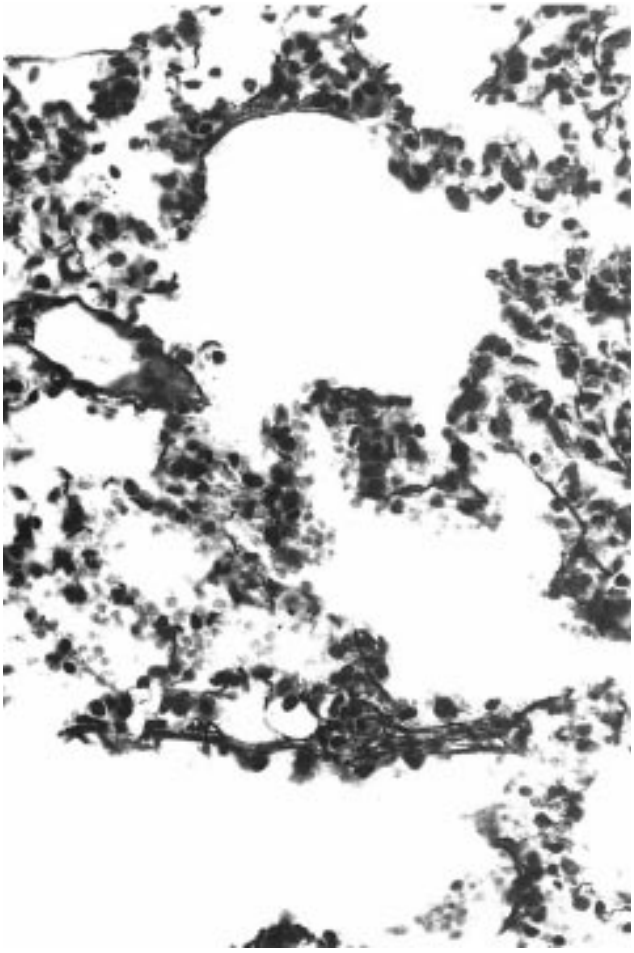

Figure 3. Rat exposed to 1/2 LD_{50} of paraquat. A slight increase in the number of fibres in the alveolar septa. H-E, × 100.

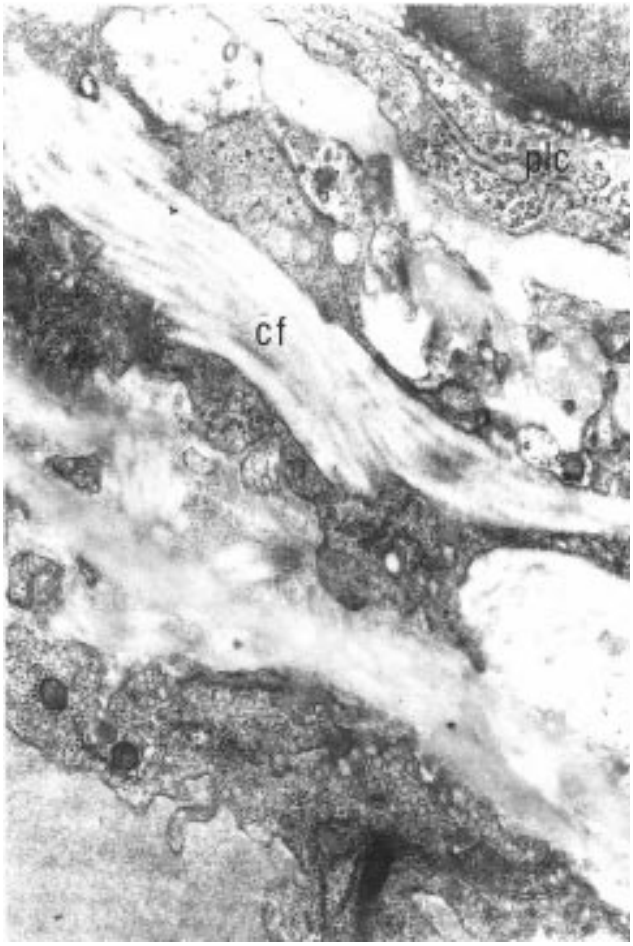

Figure 4. Rat exposed to 1/2 LD_{50} of paraquat. Interalveolar septa contain thick bunches of collagen fibres (cf) and infiltrative plasmatic cells (plc). EM, × 25000.

Grant *et al.* [7] described eight patients who died of brain damage caused by paraquat poisoning, with general oedema, small haemorrhages, astrocytic glial proliferation and meningeal inflammation.

Many cases of Parkinson's disease were described among greenhouse workers who used insecticides (parathion), fungicides (zineb, ziram) and herbicides (paraquat) [24]. In some regions of Canada a significant correlation has been noted between using paraquat and developing Parkinson's disease. This suggests a direct effect of paraquat on the central nervous system, and demonstrates that this herbicide may enter into the brain by passing through the blood-brain barrier [4].

Pesticides may also influence the immunological system. According to Sterzl [25], this influence may be immunotoxic, immunosuppressive or immunostimulating, depending on the dose of substance applied. After oral application of pesticides to experimental animals, a distinct decrease in the immunological mechanisms was observed [12].

There have been no reports concerning the immunotoxic influence of dermally-applied paraquat, although it is generally known that dermal penetration of pesticides is most dangerous during occupational exposure.

OBJECTIVE

The aim of this study was to evaluate the following:

- Dermal toxicity of paraquat in rats based on histological and ultrastructural examinations.
- Immunotoxicity of the dermal penetration of paraquat based on the functioning of the neutrophilic system.
- Neurotoxicity of paraquat in rats based on their behaviour, especially that of cognitive functions.

MATERIALS AND METHODS

Paraquat. Active substance of paraquat (dichloride -1, 1-dimethyl-4, 4-bipyridylium), Sigma Chemic-D-8024, was used. Paraquat, in a solution containing water and alcohol (4 : 1), was applied dermally in two doses: 1/2 LD_{50} and 1/10 LD_{50}.

Animals. The study was conducted on female rats of Wistar strain, aged 3 months, in good condition, and without macroscopic skin changes. The animals were fed with standard grain-based granulated fodder LSM [10] and watered *ad libitum*. The body weight of the rats at the beginning of the study ranged from 220 g to 250 g.

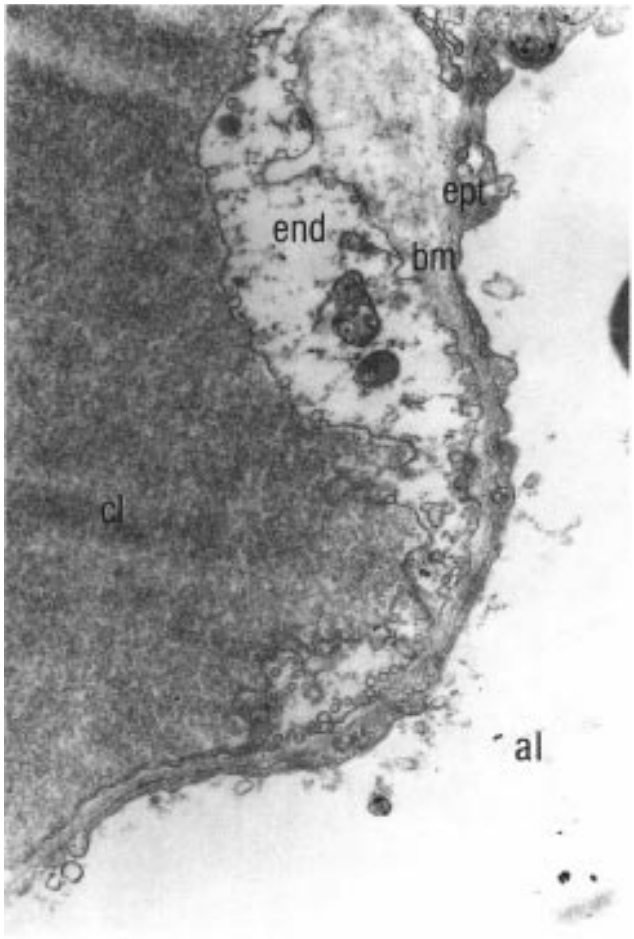

Figure 5. Rat exposed to 1/2 LD_{50} of paraquat. Respiratory barrier. Endothelium of the pulmonary capillary (end) is swollen and contains increased number of pinocytic vesicles; al - alveolus, bm - alveolocapillary membrane, cl - capillary lumen, ept - respiratory epithelium. EM, × 25000.

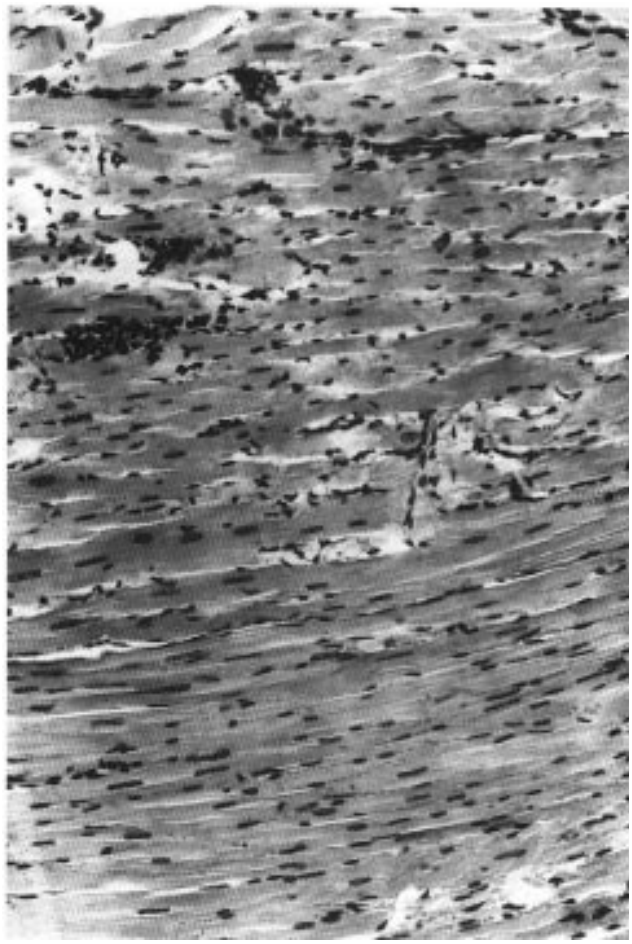

Figure 6. Rat exposed to 1/2 LD_{50} of paraquat. Between the fibres of heart muscle occur small focuses of mononuclear cells. H-E, × 50.

Dermal application of paraquat. The animals were divided into three groups, 10 rats in each. Two experimental groups received daily 1/2 LD_{50} or 1/10 LD_{50} of paraquat dermally for a period of 4 weeks, excepting Saturdays and Sundays.

For the dermal application of paraquat a band of absorptive fabric FPP-15 was used to which the pesticide was added and then isolated with aluminium foil. Daily time-exposure was 4 hours.

Animals of the control group were simultaneously, and under the same conditions, exposed to dermal absorption of the solvent alone.

During the study the body weight of all animals was tested twice a week.

After 28 days the rats were anaesthetized and blood from the heart was drawn to evaluate the effect on the neutrophilic system, and organs were excised for histological and ultrastructural studies.

Study of neutrophile activity. The phagocytosis assay with Bacto-Latex (Difco, USA) was used to evaluate the phagocytic functioning of neutrophils. The microbicidal functioning of neutrophils was examined by the nitroblue-tetrazolium (NBT) test according to Park's method [19].

In both tests 100 cells were analysed. The cells which contained three or more granules of latex were considered positive in the phagocytosis test, while in the NBT test the cells containing dark deposits of formazane (reduced NBT) were considered positive. The percentages of positive cells were designated as "scores" in both tests used.

Histological studies. For histological studies the following organs were excised: brain, lung, heart, liver, spleen, kidney, thymus and lymph nodes. Perfusion of brains was carried out using a solution of methanol, formalin and glacial acetic acid; brain slides were stained with cresyl violet. The remaining organs were fixed in formalin diluted 1 : 9 with water, and stained with hematoxylin and eosine (H + E). For staining the elastic fibres, resorcin-fuchsine was used according to Weigert [30].

Ultrastructural studies. For ultrastructural studies, the following organs were excised: lung, heart, liver and kidney. The samples for the electrone microscopic study

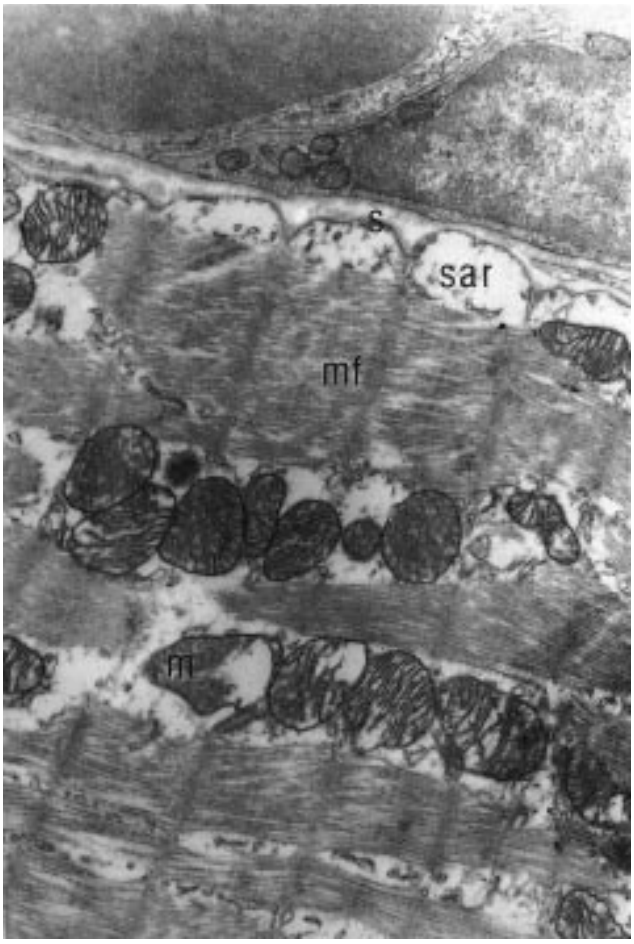

Figure 7. Rat exposed to 1/10 LD_{50} of paraquat. Cardiomyocyte: Focal clarifications are observed in the sarcoplasm (sar). Some of the mitochondria (m) are swollen; mf - myofibrils, s - sarcolemma. EM, × 15000.

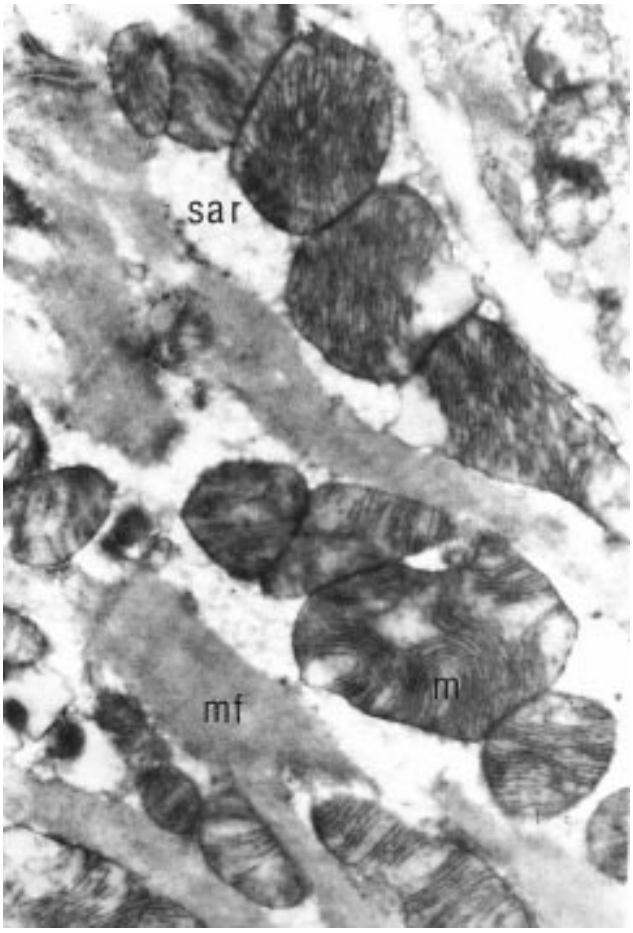

Figure 8. Rat exposed to 1/2 LD_{50} of paraquat. Cardiomyocyte: In sarcoplasm (sar) structures resembling new, immature fibres are visible; m - mitochondrion, mf - myofibrils. EM, × 20000.

were fixed in 5% glutaraldehyde buffered to pH 7.2-7.4 with 0.1 M sodium cacodylate for 5 hours. They were then washed in 0.1 M cacodylate buffer and postfixed in 0.1% OsO_4 in Michaelis buffer for 1 hour, dehydrated in graded ethanol solutions and embedded in Epon 812. Ultrathin sections were first stained with uranyl acetate, then poststained with lead citrate. They were viewed using Tesla B-S-613 electron microscope.

Evaluation of neurotoxicity by study of behaviour. The behaviour was studied three times: before exposure, two weeks after dermal application, and at the end of the study, i.e. after four weeks of dermal exposure to paraquat.

To evaluate the neurotoxicity of paraquat the "open field" method was applied [21]. Rats were placed on a square white plate (100 cm × 100 cm), divided into 25 identical fields. The area of fields was lighted equally. Four blocks were put on the middle of the plate in a square, each of them 20 cm far from the edge. The rats were placed in the middle of the plate and observed for 5 minutes. During that time their behaviour was noted, i.e. crossing the grid lines on the plate, washing themselves (toiletes), climbing on the blocks (raisings), interest in blocks, defecation.

Statistical analysis. For the evaluation of differences between the groups, Student's t-test was used.

RESULTS

Body weight. In animals exposed to dermal penetration of 1/2 LD_{50} paraquat no increase of body weight was observed during the whole month of study. At the beginning of the study the body weight of the rats ranged from 220–250 g, and it remained the same after four weeks, ranging then from 210–250 g. Similar results were obtained in animals exposed dermally to 1/10 LD_{50} of paraquat.

Histological and ultrastructural changes. In rats exposed to dermal absorption of 1/10 LD_{50} of paraquat histological changes in the lung were manifested as wider interalveolar septa filled mainly with lymphocytes and alveolar macrophages, together with slight peribronchial and perivascular infiltrations.

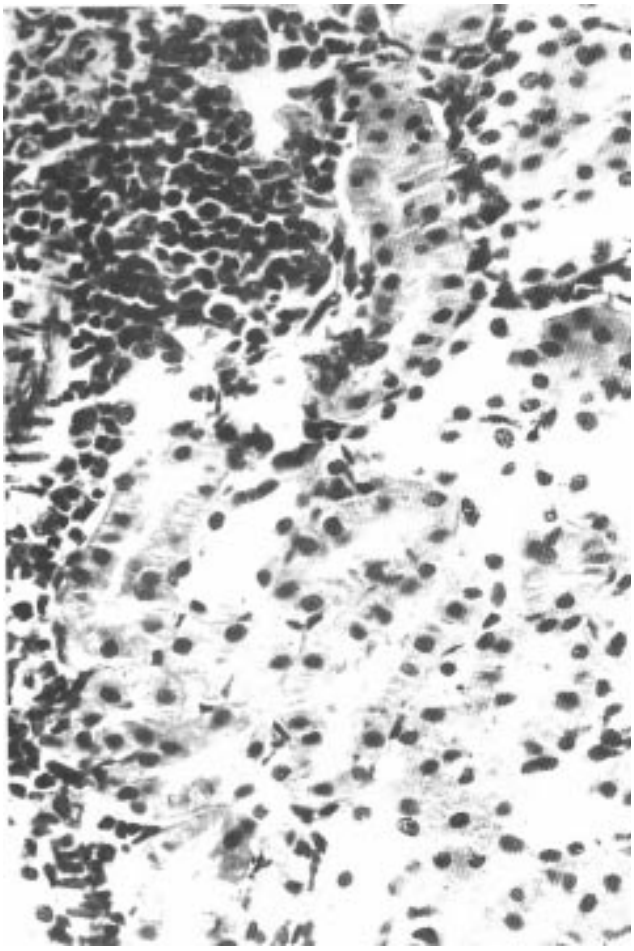

Figure 9. Rat exposed to 1/2 LD_{50} of paraquat. Lymphatic infiltrations in the kidney. H-E, × 100.

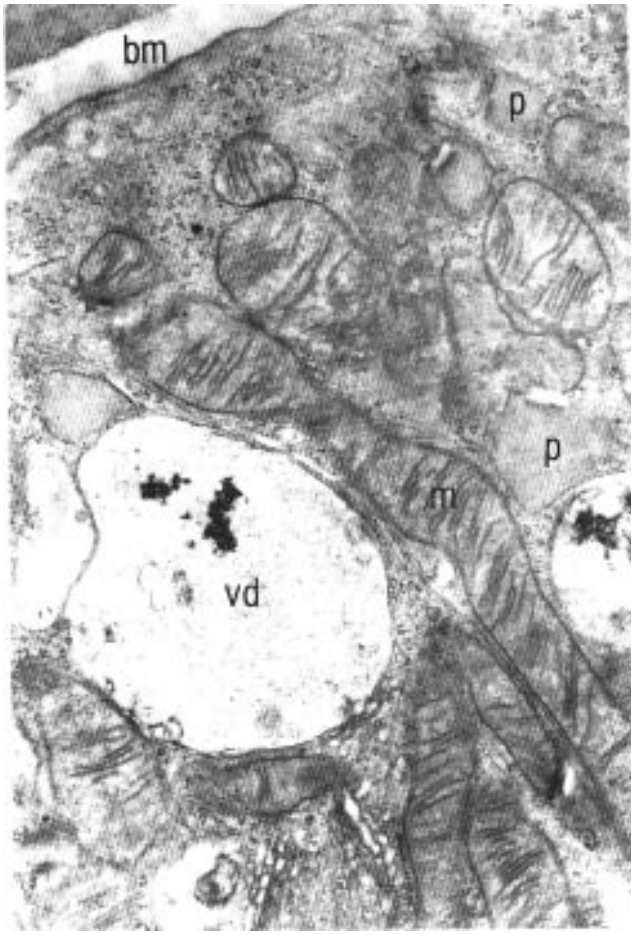

Figure 10. Rat exposed to 1/10 LD_{50} of paraquat. Renal proximal tubule: Digestive vacuole (vd), irregulary in size and shape peroxisomes (p), and mitochondria (m) are noted; bm – basement membrane. EM, × 25000.

In the ultrastructural studies, slight changes of the respiratory barrier were observed. Thus, the cells of the vascular endothelium and the pneumocytes type I showed features of oedema. In the pneumocytes type II wider channels of the endoplasmic reticulum occurred. The internal structure of granules in those cells was similar to that in rats of the control group.

In the animals exposed to dermal absorption of the higher dose of paraquat (1/2 LD_{50}) exudate and erythrocytes in the alveoli were observed (Fig. 1). The wider interalveolar septa were filled with erythrocytes, lymphocytes or alveolar macrophages. These infiltrations were more extensive than in animals exposed to the lower dose of paraquat (Fig. 2). A slight increase in the number of fibres in alveoli or in interalveolar septa was noted only in the animals exposed to the higher dose of paraquat (Fig. 3).

The above-described results were confirmed by the ultrastructural studies. In the interalveolar septa relatively thick bunches of collagen fibres were noted (Fig. 4), as were infiltrations consisting of neutrophils, eosinophils, lymphocytes and plasmatic cells. Changes in the endothelium were different, while some of the cells became thinner, others swelled. The number of pinocytic vesicles near the vascular wall increased (Fig. 5). The cytoplasm of pneumocytes type I was greatly dilated. Continuity of the respiratory barrier remained intact. In the cytoplasm of pneumocytes type II the channels of endoplasmic reticulum were wider when compared with animals exposed to the lower dose of paraquat. Changes were also observed in the lamellar granules. They contained a substance of slight electron density, which did not form osmophilic structures typical for lamellar granules. Those granules were most often larger than normal.

Myocardial changes were seen only in the rats exposed to the higher dose of paraquat. Focal hypertrophy of the interstitial tissue and homogenous basophilous cytoplasm in muscle fibres were noted. Between the fibres of heart muscle there were small focuses of infiltrations containing mononuclear cells (Fig. 6). Nearby vessels excised, the amount of fibres increased.

Ultrastructural changes in the heart were observed already in rats exposed to the lower dose of paraquat. In the sarcoplasm of cardiomyocytes focal clarifications were observed which were most distinct in the peripheral

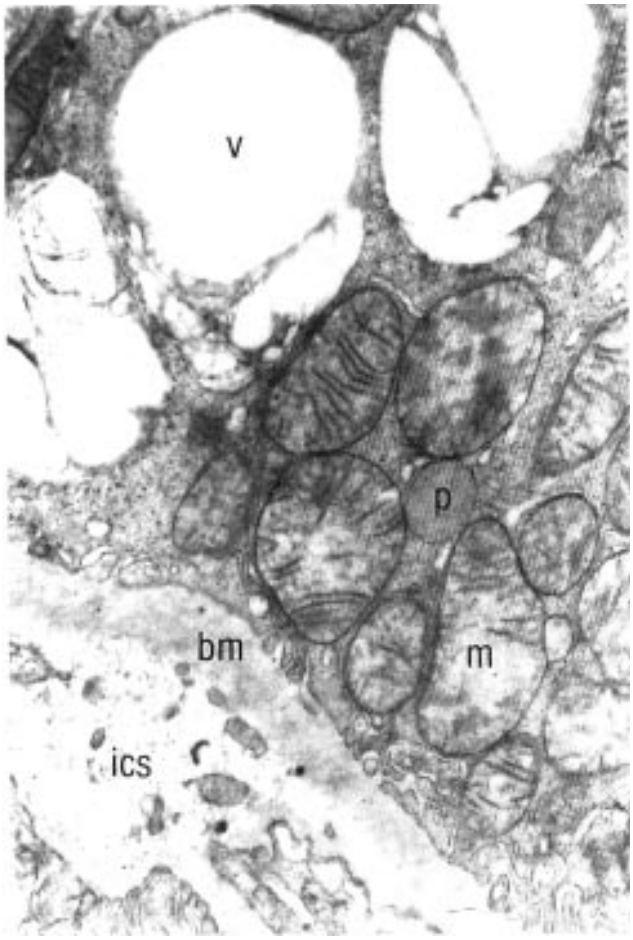

Figure 11. Rat exposed to 1/2 LD_{50} of paraquat. Renal proximal tubule: Electron bright vacuoles (v) are observed in cytoplasm of the cell. The intertubular space (ics) is wide and contains the interstitial elements; bm - basement membrane, m - mitochondria, p - peroxisome. EM, × 25000.

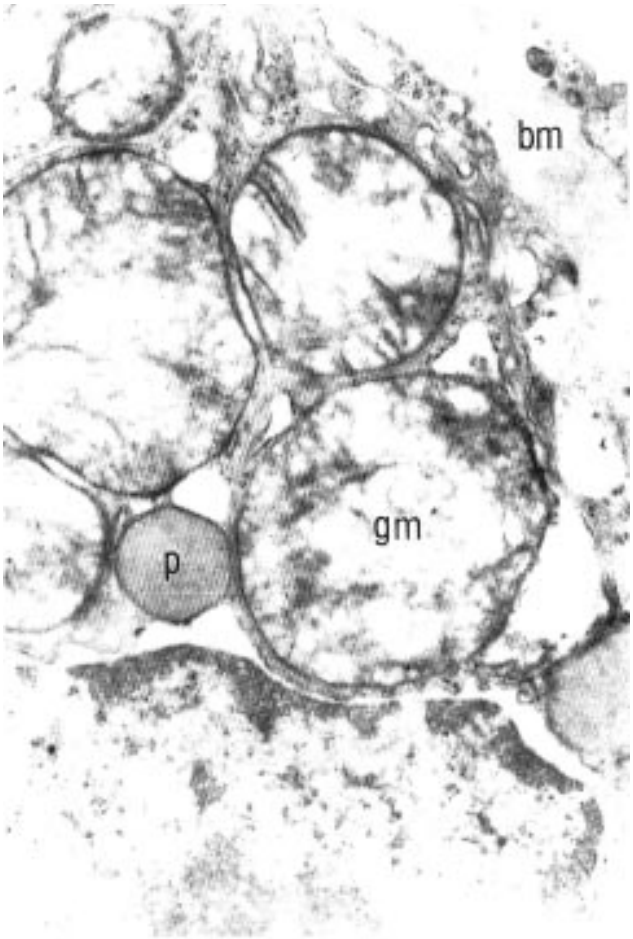

Figure 12. Rat exposed to 1/2 LD_{50} of paraquat. Renal proximal tubule: Giant mitochondria (gm) are seen in the cell; bm - basement membrane, p - peroxisome. EM, × 25000.

part below the sarcolemma. Sarcoplasmatic reticulum and mitochondria were swollen (Fig. 7).

More extensive changes were observed after exposure to the higher dose of paraquat. The endothelium of vasa between the muscle fibres was extensively swollen. Between the cardiomyocytes a great amount of collagen fibres occurred in bunches. Many changes were observed inside the cells. Under the sarcolemma accumulation of electron light material was noted. Structures resembling new, immature fibres were sometimes observed in this material. Similar material also cumulated in the sarcoplasm between the elastic fibres, probably due to partial loss of myofibrils and the disappearance of stripped pattern. Mitochondria were also changed. Many of them were very large in size, with numerous crista. Focal clarification in the electron dark matrix was occasionally observed (Fig. 8).

In most animals of both experimental groups, slight foci of lymphatic infiltrations in the cortex and medulla of kidney were noted (Fig. 9). The ultrastructural studies showed injuries to the proximal tubuli of the kidney, related to mitochondria, peroxisomes, and congestive vacuoli. Some swollen mitochondria showed a tendency to produce large, electron bright vacuoli. Others were large, with a very irregular shape. The number of peroxisomes slightly increased and their shape changed from round to irregular. Near to the organella the vacuoli of smooth endoplasmic reticulum were cumulated. The amount of digestive vacuoli in cytoplasm increased, and their size and internal structure were different (Fig. 10).

After exposure to higher dose of paraquat, wider spaces between the tubuli contortus in the kidney were found (Fig. 11), which were filled with interstitial elements. The changes in the mitochondria were different: some of them were slightly swollen and their loss of crista and brightness of the matrix were distinct. Other “giant” mitochondria were many times larger than those described above. Their matrix was bright and the number of crista was not extensive. The number of peroxisomes remained normal (Fig. 12), however, a smaller amount of endoplasmic reticulum was observed, which usually accompanies those organella. A large number of electron light vacuoli containing some electron dense material were observed in cytoplasm of many cells.

In both experimental groups changes in liver tissue were demonstrated by small lymphocytic infiltrations in

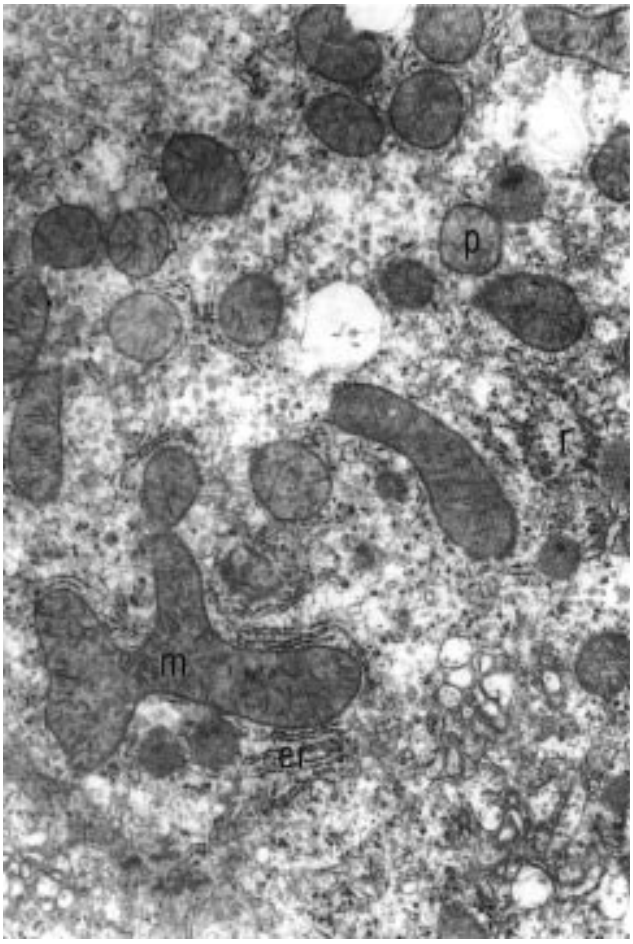

Figure 13. Rat exposed to 1/2 LD_{50} of paraquat. Hepatocyte: Pleomorphic mitochondrion (m) is seen in the cytoplasm. Endoplasmic reticulum (er) and peroxisomes (p) show normal structure; r - ribosomes. EM, × 15000.

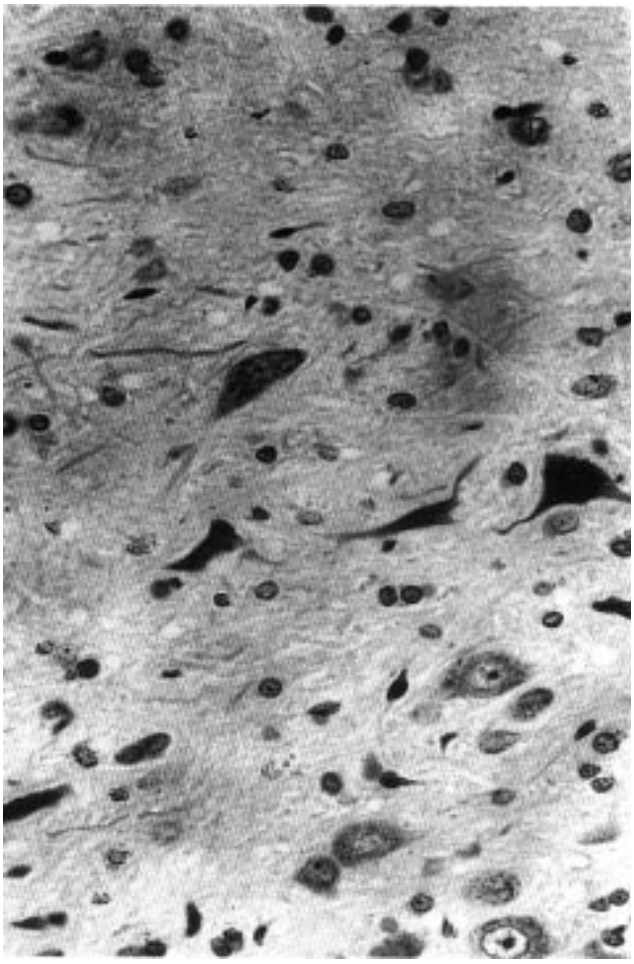

Figure 14. Rat exposed to 1/2 LD_{50} to paraquat. Accumulation of cytoplasm and cell nuclei in lateral nuclei of thalamus. H-E, × 100.

the areas of blood vessels and the accumulation of nuclei of hepatocytes in the subcapsular layer. After administration of a smaller dose of paraquat, no significant differences were noted in the ultrastructure of hepatocytes. Hypertrophy of smooth endoplasmic reticulum and a slight increase in the number of peroxisomes was observed only in some cells. Administration of 1/2 LD_{50} of paraquat resulted in widening of the channels of the smooth endoplasmic reticulum and an increase in the number of peroxisomes to a degree similar to the previous group. Moreover, mitochondria of increased size and very irregular shape, and occasionally, lipid inclusions occurred in hepatocytes (Fig. 13).

In the brains of rats dermally exposed to paraquat pathological changes were observed in neurocytes. In the first experimental group these changes were manifested by an accumulation of cytoplasm and cell nuclei, mainly of pyramidal cells of hippocampal gyrus (CA2), single cells of the granular layer of hippocampal gyrus and Purkinje cells in the ganglionic layer of the cerebellar cortex. In the group of rats which received a higher paraquat dose (1/2 LD_{50}) focal pyknosis was observed in neurocytes of all layers of the cerebral cortex, mainly in pyramidal cells. Pyknosis of single neurocytes was noted in the area of the fissura rhinalis, in the preoptical nucleus, in lateral nuclei of thalamus and cerebellum and in the layer of hippocampal pyramidal cells (CA3) (Fig. 14).

In spleen, no pathological changes were observed in both experimental groups, except for an increased number of giant cells.

In lymphatic nodes of some animals exposed to higher doses of paraquat the widening of subcapsular sinuses, blood extravasations, and the presence of macrophages filled with hemosiderin were noted. No pathological changes occurred in thymus except for blood extravasations.

Immunotoxicity. The bactericidal function of neutrophils expressed by the number and size of dark formazane deposits, which are produced as a result of the reduction of nitroblue-tetrazolium, varied in the experimental groups examined. NBT index, which reflects the number of neutrophils containing dark formazane deposits per 100 cells, was the lowest in the

Table 1. Results of the nitroblue-tetrazolium test and of the phagocytosis latex test in rats exposed to dermal absorption of paraquat.

Examined groups	Index of the nitroblue-tetrazolium test		Index of the phagocytosis latex test	
	$\bar{x}$	s	$\bar{x}$	s
Rats exposed to dermal absorption of 1/2 LD_{50} of paraquat (n = 10)	17**	10	54	11
Rats exposed to dermal absorption of 1/10 LD_{50} of paraquat (n = 10)	15**	7	41*	12
Control group (n = 10)	6	4	53	12

$\bar{x}$ - average; s - standard deviation; *–** significant difference compared to control group: * $p < 0.05$, ** $p < 0.01$.

control group (mean = 6). Dermally applied paraquat had a stimulatory effect on the function of neutrophils in both experimental groups, causing a clear increase in the NBT index compared to the control group (Tab. 1). Paraquat applied dermally in the amount of 1/2 LD_{50} caused an increase in the mean NBT index up to 17 ($p < 0.01$).

The results of the latex test in the control group and in the experimental group dermally exposed to the dose of 1/2 LD_{50} of paraquat, did not differ. The phagocytosis index proved to be significantly lower compared to the control group in rats exposed to a dermal absorption of 1/10 LD_{50} of paraquat (Tab. 1).

Neurotoxicity. In the experimental group examined after two weeks of dermal exposure to 1/2 LD_{50} of paraquat a smaller interest in blocks was observed, climbing on the blocks was less frequent, and, compared to the control group, defecations were also less frequent (Tab. 2). The differences were statistically significant. After the next two weeks, no significant differences were observed in the behaviour of animals of either group. The reason for this was probably the mobilization of adaptation mechanisms of the rats in the experimental group.

DISCUSSION

The fact that paraquat is more toxic for rats when applied dermally (80-90 mg/kg) than orally (110-115 mg/kg) probably reflects its poor absorption in the intestine, with decomposition quicker in the intestine than in the skin.

Dermal application of paraquat in a subacute experiment applied in this study induced the greatest microscopic and submicroscopic changes in lung tissue. In this respect, our results are consistent with numerous results reported by other authors who emphasize the special susceptibility of lung tissue to paraquat, irrespective of the route of absorption [3, 11, 13, 15, 16, 17, 20, 22, 26, 27, 28, 29, 31]. In paraquat poisonings an increase in the number of connective tissue fibres was noted based on biochemical and morphometric studies [23, 26]. This was confirmed by our histological and ultrastructural examinations. The accumulation of fibres was observed in alveolar septa of animals exposed to an absorption of 1/2 LD_{50} of paraquat. Apart from fibres, neutrophils, eosinophils, lymphocytes and plasmatic cells were also present.

The cytotoxic effect of paraquat, associated with the production of free radicals, was observed in the cells of vascular endothelium and types I and II pneumocytes [2]. Type I pneumocytes were swollen, while the endothelium cells became either thinner or swollen. The above-mentioned cells are the fundamental elements of the structure of respiratory barrier, we may therefore presume that abnormalities in their structure are closely associated with the functioning of this barrier. Moreover, the impairment of respiratory barrier may be associated with changes in granules of type II pneumocytes. Chen *et al.* [3] stress the affinity of paraquat to type II pneumocytes. Distinct changes in the picture of the granules within these cells may be the consequence of changes in the composition and amount of the surfactant which they produce, which is an important component of the respiratory barrier.

The subsequent organ in which significant histologic and ultrastructural changes due to paraquat dermal

Table 2. Results of the behaviour test in rats after exposure to 1/2 LD_{50} of paraquat.

Examined groups		Before exposure					After 14 days of exposure					After 28 days of exposure				
		A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Control group	$\bar{x}$	80.3	2.00	16.00	7.10	6.20	89.70	2.80	17.20	10.90	2.10	97.90	2.30	17.80	8.60	0
	SEM	7.52	0.63	2.78	0.85	1.96	13.20	0.80	2.10	2.03	0.64	17.70	0.54	3.26	1.87	0
Experimental group	$\bar{x}$	91.30	1.50	12.30	6.00	2.00	71.79	2.30	8.50	3.70	0.10	112.40	1.80	12.30	9.00	0
	SEM	16.08	0.22	2.59	1.31	0.20	14.28	0.30	1.95	1.92	0.10	14.65	0.33	2.00	2.19	0
Significant differences		NS	NS	NS	NS	≤0.01	NS	NS	≤0.01	≤0.005	≤0.01	NS	NS	NS	NS	NS

A - Crossed squares; B - Washing oneself; C - Climbing; D- Interest in blocks; E - Defecations; $\bar{x}$ - mean; SEM - standard error of the mean; NS - not significant.

absorption were noted, is the heart. Paraquat transported into the heart through blood vessels caused focal proliferal changes in the interstitial tissue, accompanied by infiltrations with mononuclear cells. Ultrastructural studies showed oedema of the endothelium of vessels and the increase in the number of connective tissue fibres between cardiomyocytes. In the sarcoplasma of cardiomyocytes and beyond sarcolemma, as well as between myofibrils, fibre-like material was observed. The result of accumulation of this material could be a partial loss in the number of myofibrils and the atrophy of their striation in certain sections. The stimulatory effect of paraquat on the creation of fibres has not been explained to date. In the studies of the mechanism of cytotoxicity of this preparation, however, special attention has been paid to its influence on the mitochondrial system of electron transportation [5, 31]. This was reflected in the morphological picture of mitochondria in the heart muscle examined, where these organellae were of large size and swollen, and had focal clarifications of the matrix.

Histological and ultrastructural changes observed in the liver and kidneys were slight, similar to studies by other authors [6, 8, 16]. The occurrence of only slight changes in these organs was probably due to a small concentration of paraquat in the blood reaching these organs in the case of dermal application. The histologic picture showed small infiltrations of lymphatic cells in both organs. Ultrastructural changes were reflected by the appearance of mitochondria, a slight increase in the number of peroxisomes and a widening of the channels of the endoplasmic reticulum. Changes in mitochondria were connected with disturbances in oxydo-reduction processes taking place in the organellae, whereas the increase in the number of peroxisomes may be associated with the cellular response to the toxic effect of free radicals induced by paraquat, as peroxisomes contain enzymes which inactivate these radicals.

In the remaining organs - brain, lymphatic nodes, and thymus - slight histological changes were observed. These changes were pyknosis of cerebral neurocytes and blood extravasations in the lymphatic nodes and the thymus. Paraquat administered to the brain caused pathological changes which covered large areas of the brain, and led to the necrosis of neurocytes [1, 2]. Slight pathological changes noted in the cerebral tissue in this study are associated with the dermal route of application of paraquat, at which only a small amount of the preparation reaches the brain.

CONCLUSIONS

1. The toxicity of dermally applied paraquat was manifested by histopathological changes in the lungs, heart muscle, kidneys, liver and brain. The most severe changes were observed in the lungs.
2. Microbicidal effect of neutrophils in rats was stimulated by paraquat.
3. The behaviour of rats exposed to paraquat absorption for 2 weeks was significantly disturbed, compared to the behaviour of animals from control group.

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