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HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES OF RATS EXPOSED TO CARBARYL

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Abstract: The aim of the study was to assess the general toxic effects of dermally applied carbaryl, based on histological and ultrastructural examinations of internal organs and to relate these effects to earlier own studies where ^{14}C carbaryl was used for determining the pesticide penetration. The pesticide was applied in doses of 1/5 and 1/10 LD_{50}, administered to the tail skin of male Wistar rats 4 hours daily, for 4 weeks except Saturdays and Sundays. After the experiment, the animals were anaesthetized and the following organs were taken for histological study: brain, lung, heart, liver, kidney, skin from the site of exposure and skin from a place at least 2 cm distant from the exposure site. Lung, liver, kidney, heart and skin were used for ultrastructural studies. Dermal application of carbaryl resulted only in slight histological changes in skin, liver, brain and lung. Even in brain and liver, where large amounts of ^{14}C carbaryl, compared to other organs (lung, kidney, heart), where the intensity of histologic changes was earlier stated to below. Ultrastructural changes were observed in skin, liver, lung, heart and kidney.

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Key words: Dermal toxicity, carbaryl, histopathology, ultrastructure.

INTRODUCTION

Carbaryl is a carbamate insecticide of low toxicity, for both humans and other vertebrates. In our previous studies, after oral application of this preparation to rats in doses of 1/10, 1/50 and 1/100 LD_{50} for a period of 3 months, no histopathological changes were observed in internal organs [10]. However, it was confirmed that carbaryl possesses embryotoxic properties for rats [18] and chick embryos [15, 16]. Its toxic effect was also noted in a chick embryo fibroblast tissue culture [17].

Application of ^{14}C carbaryl enables the observation of its kinetics and metabolism in organs and tissues. Studies *in vitro* with the use of ^{14}C carbaryl show that the preparation was relatively easily absorbed through the skin [3]. The rate of absorption depended on the dose of the preparation and type of solvent [1]. These parameters may change the toxicity of the pesticide.

This study was preceded by an experiment with the use of ^{14}C carbaryl dermally applied to rats. Various amounts of this pesticide accumulated temporarily in the skin and internal organs. During a 4 h dermal application of ^{14}C carbaryl, 0.15 $\mu g/cm^2$ of the pesticide penetrated into the skin at the exposure site. The preparation penetrated into the skin not only in a vertical direction reaching blood vessels and organs, but also horizontally to the adjacent areas of skin. The intensity of penetration depended on the type of exposure. It was observed that 6 h after single, double or triple exposures the amounts of carbaryl in the area surrounding the exposure zone were circa 5%, 30%

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and 80% of the absorbed amount, respectively. At 20 h after exposure, the amount of carbaryl was similar at the site of exposure and in adjacent areas, approximating 4% of the absorbed dose [20].

OBJECTIVE

The aim of this study was an evaluation of subacute toxicity of dermally applied carbaryl, based on histological and ultrastructural analysis of internal organs and skin, as well as an attempt to determine the relationship between the progression of histological and ultrastructural changes and the amount of ^{14}C carbaryl detected in these organs [20].

MATERIALS AND METHODS

Carbaryl (1-Naphtyl-N-methyl-carbamate) produced by the Institute of Organic Industrial Chemistry in Warsaw as a standard with 99.0% purity, was used for the experiment. For dermal application, it was suspended in an emulsion of gum arabic, olive oil and water in proportion 1 : 2 : 1.5, and applied to the tail skin in two doses: 1/5 and 1/10 LD_{50}, i.e. 22 mg/cm^2 and 11 mg/cm^2 respectively.

The study was conducted on male Wistar rats aged 3 months with no macroscopically detected changes of the tail skin. The animals were fed with standard feed LSM [9] and provided with water *ad libitum*. The initial body mass of the rats was 220 g ± 20 g.

The experiment was conducted on 3 groups of rats: 2 experimental and 1 control group, 10 animals in each. Experimental animals received for 4 weeks (except Saturdays and Sundays) an oil emulsion of carbaryl (22 mg/cm^2 and 11 mg/cm^2), applied to the skin of the tail according to Massmann's method in own modification [19]. The time of exposure was 4 hours daily. The animals of the control group were dermally exposed to the emulsion vehicle (as above) at the same time and under the same conditions. During the entire experiment, the body mass of all rats was determined once a week.

For histological and ultrastructural studies, heart, liver, kidney, lung, brain, skin from the site of exposure, and skin from a place at least 2 cm distant from the exposure site were taken. The organs for histological examinations were fixed in 10% neutral buffered formalin, embedded in paraffin and stained with H+E. The brain for histological examinations was perfused with a solution of methanol, formalin and glacial acetic acid, embedded in paraffin and stained by the Nissel method [22]. For ultrastructural studies, organs were fixed in 4% glutaraldehyde buffered to a pH of 7.2–7.4 with 0.1 M sodium cacodylate, and post-fixed with 1% solution of OsO_4 in water. Dehydration was carried out by ethyl alcohol in graded concentrations up to absolute. The material was embedded in Poly/Bed® 812 medium (Polysciences, Inc., Warrington, PA, USA), Ultrathin sections were observed and photographs taken using a Tesla BS 500 electron microscope.

Table 1. Degenerative changes in internal organs of Wistar rats after dermally applied carbaryl.

Examined organs	Examined groups[a]		
	1/10 LD_{50} N = 10 %	1/5 LD_{50} N = 10 %	Control group N = 10 %
Liver	-	50	-
Kidneys	-	10	-
Lungs	50	60	10
Heart	-	40	-
Brain	50	80	-

[a]In each group, percent of animals with changes is shown.

RESULTS

The body mass of all animals, both in the experimental and control groups, increased about 70 g during studies lasting 28 days.

Histologic studies of the liver showed the presence of slight infiltrations of mononuclear cells between hepatocytes. Degenerative changes were sporadically observed in individual hepatocytes in 50% of the animals after dermal application of carbaryl in the dose 22 mg/cm^2 (Tab. 1). The submicroscopic structure of hepatocytes showed a decrease in amounts of the rough endoplasmic reticulum, as well as an increase in amounts of the smooth endoplasmic reticulum in the cytoplasm (Fig. 1).

In the widening interalveolar septa of the lung, macrophages were observed after both doses of carbaryl (Tab. 1) (Fig. 2). In the apical parts of the organ compensatory emphysema was noted. Ultrastructural studies of the lung showed swelling of the endothelium of capillary vessels (Fig. 3).

In 40% of animals, after administration the higher dose of carbaryl (Tab. 1), inflammatory infiltrations were observed in the heart, between the cardiac fibres (Fig. 4). The submicroscopic structure of cardiomyocytes showed swollen mitochondria, as well as swelling of the endothelium of capillary vessels (Fig. 5).

Histopathologic studies did not show changes in kidney; ultrastructural analysis, however, detected an increase in the number of autophagous vacuoles in the cells of proximal tubuli (Fig. 6).

Changes in the brain were manifested after both doses of carbaryl in 50–80% of animals (Tab. 1). A focal concentration of neurocytes cytoplasm in the hypothalamus nucleus, CA 3 hippocampus layer (Fig. 7), and in the area of the pyriformis cortex cerebri were observed. Degenerative changes were also noted in the granular layer and in Purkinje cells of the cerebellum (Fig. 8).

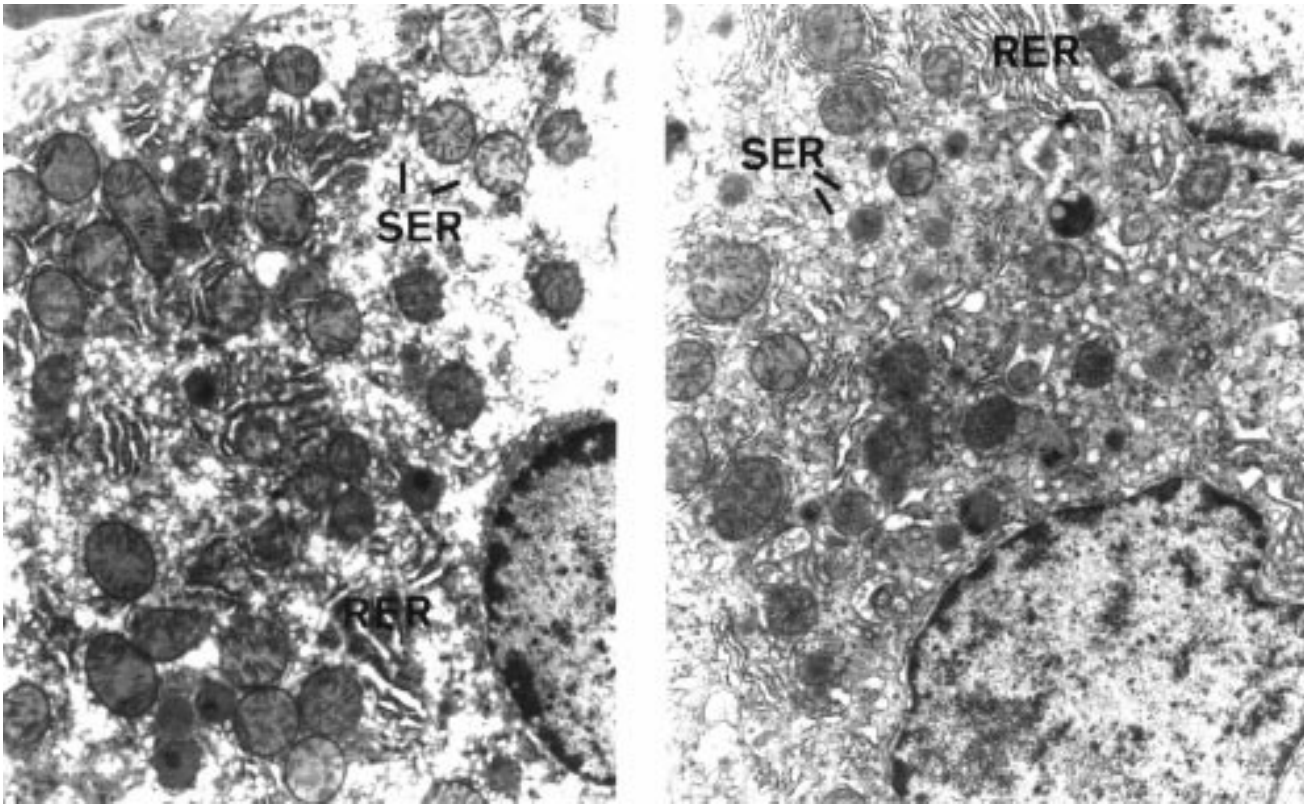

Figure 1. a: Decrease in amounts of the rough endoplasmic reticulum (RER), and increase in amounts of the smooth endoplasmic reticulum (SER) in hepatocytes (1/5 LD_{50}). EM, × 8 000. b: Control group, no changes. EM, × 8 000.

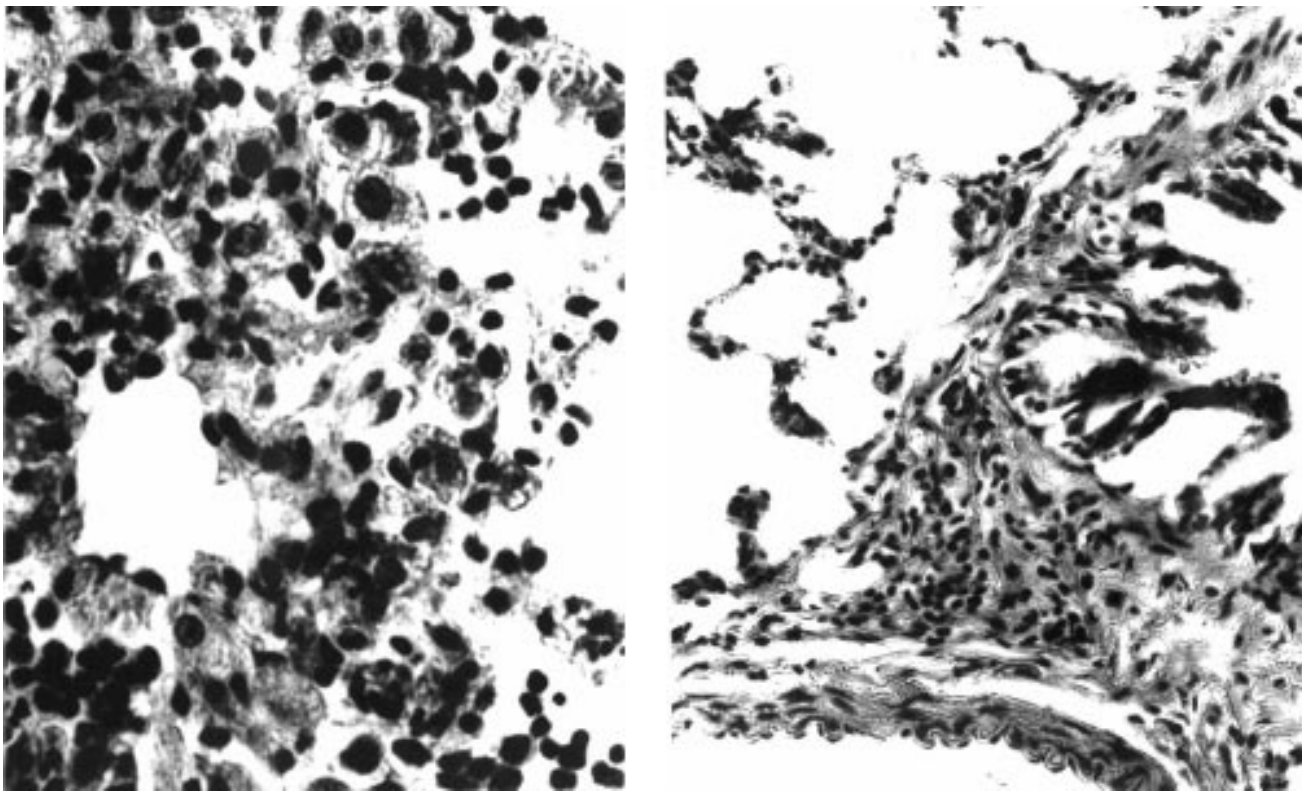

Figure 2. a: Macrophages in the widening interalveolar septa of the lung (1/10 LD_{50}). H+E, × 160. b: Control group, no changes. H+E, × 160.

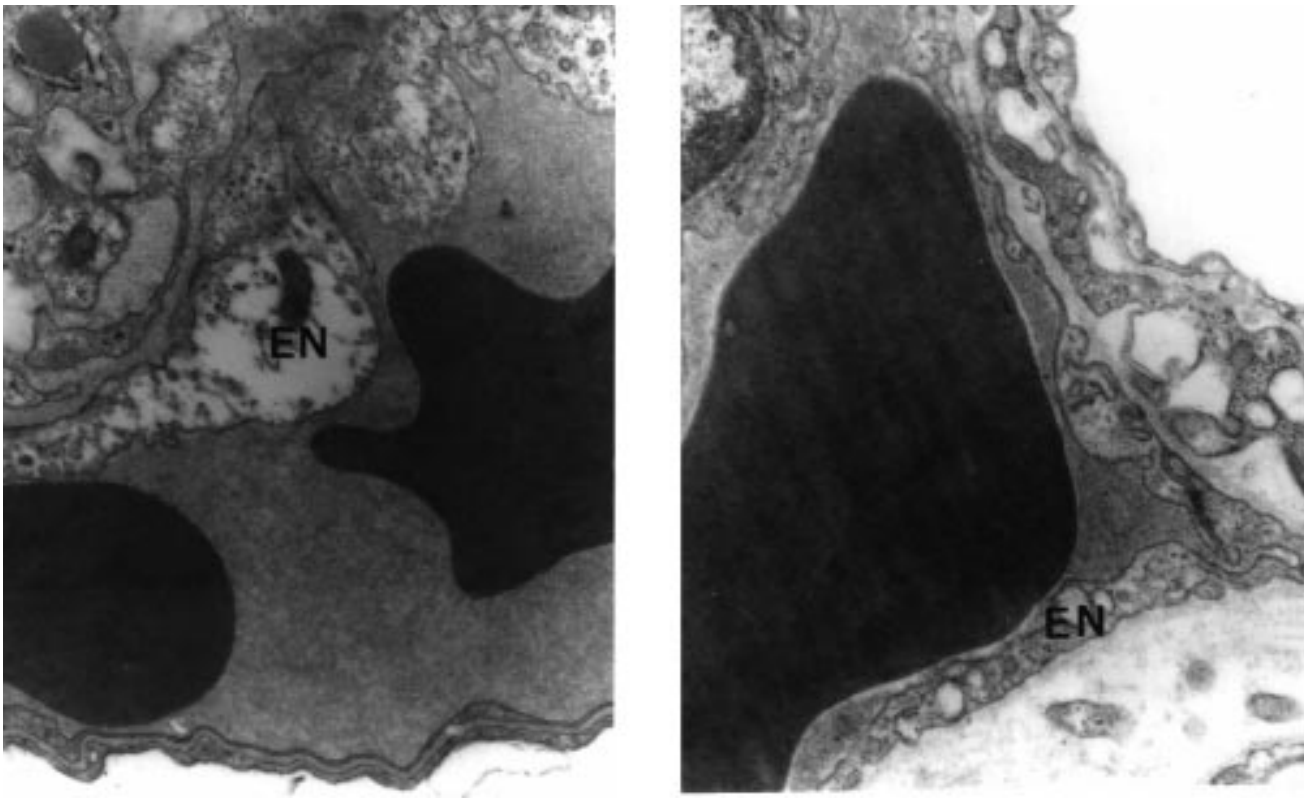

Figure 3. a: Swelling of the endothelium (EN) of capillary vessels in the lung (1/5 LD_{50}). EM, × 16 000. b: Control group, no changes. EM, × 16 000.

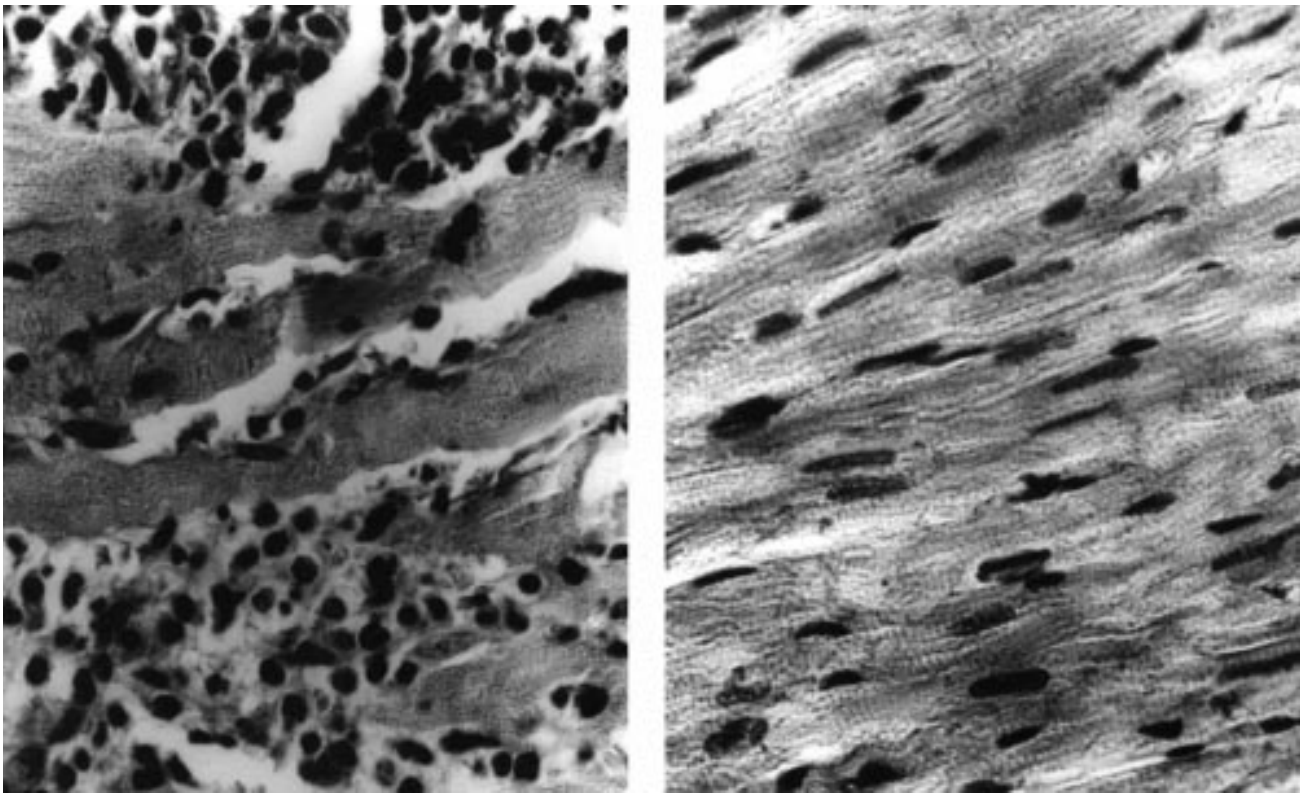

Figure 4. a: Inflammatory infiltration between the cardiac fibres in the heart (1/5 LD_{50}). H+E, × 160. b: Control group, no changes. H+E, × 160.

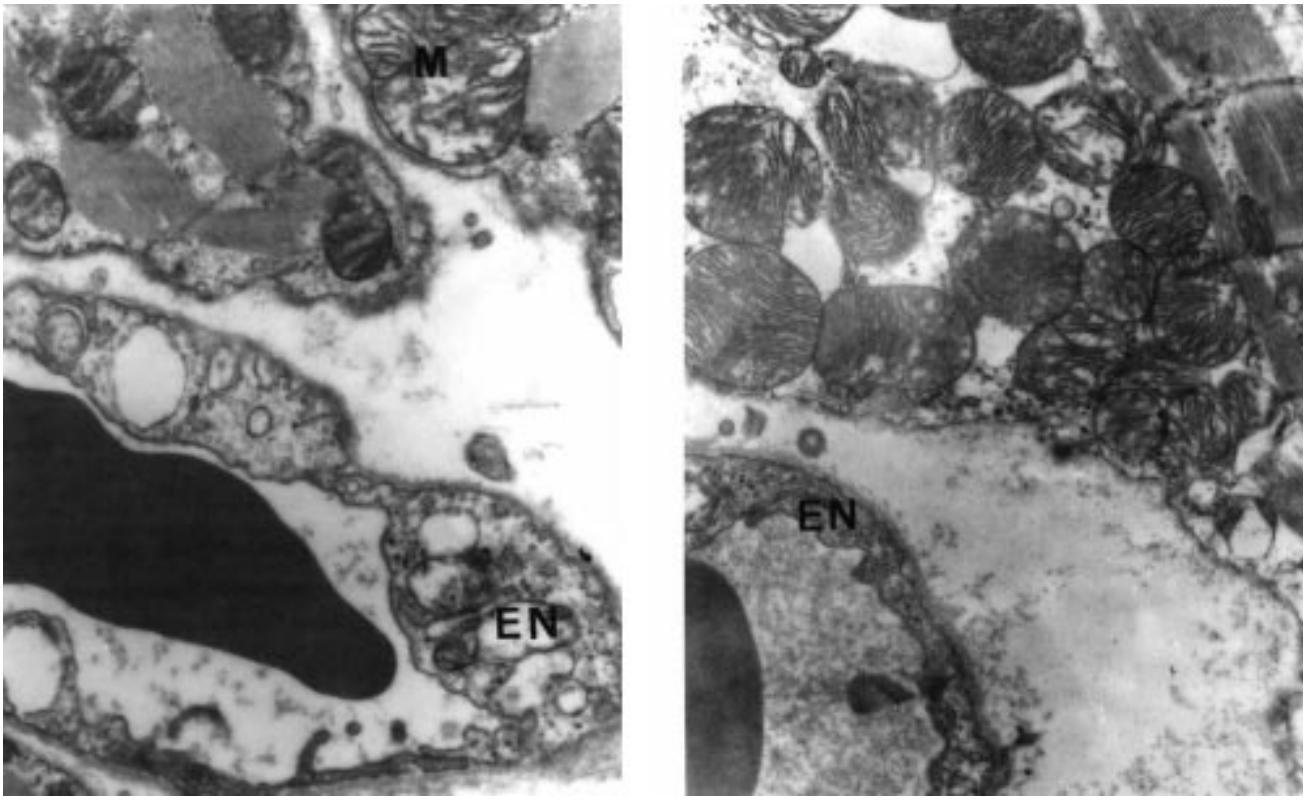

Figure 5. a: Swollen mitochondria (M), and swelling of the endothelium (EN) of capillary vessels in cardiomyocytes (1/5 LD_{50}). EM, × 16 000. b: Control group, no changes. EM, × 16 000.

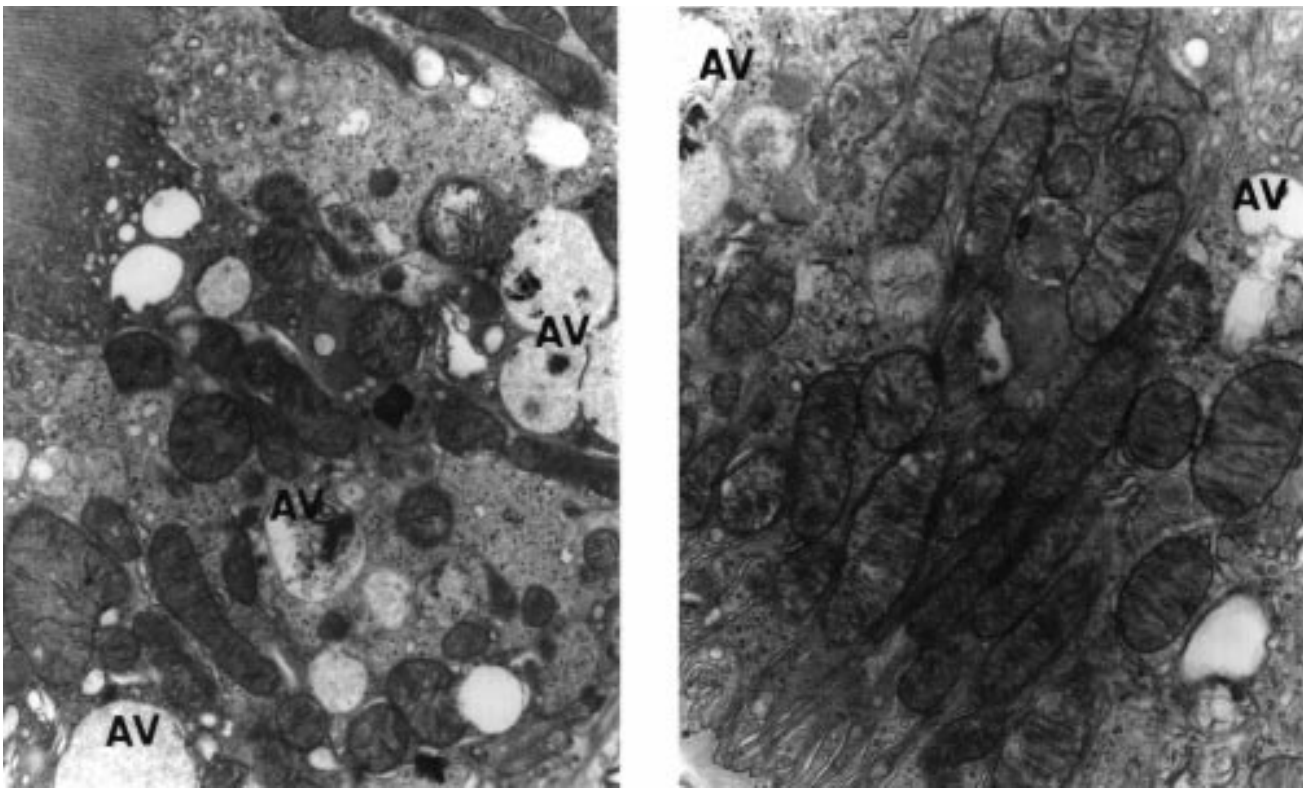

Figure 6. a: Increase in number of autophagous vacuoles (AV) in the cells of proximal tubuli in kidney (1/5 LD_{50}). EM, × 8 000. b: Control group, no changes. EM, × 16 000.

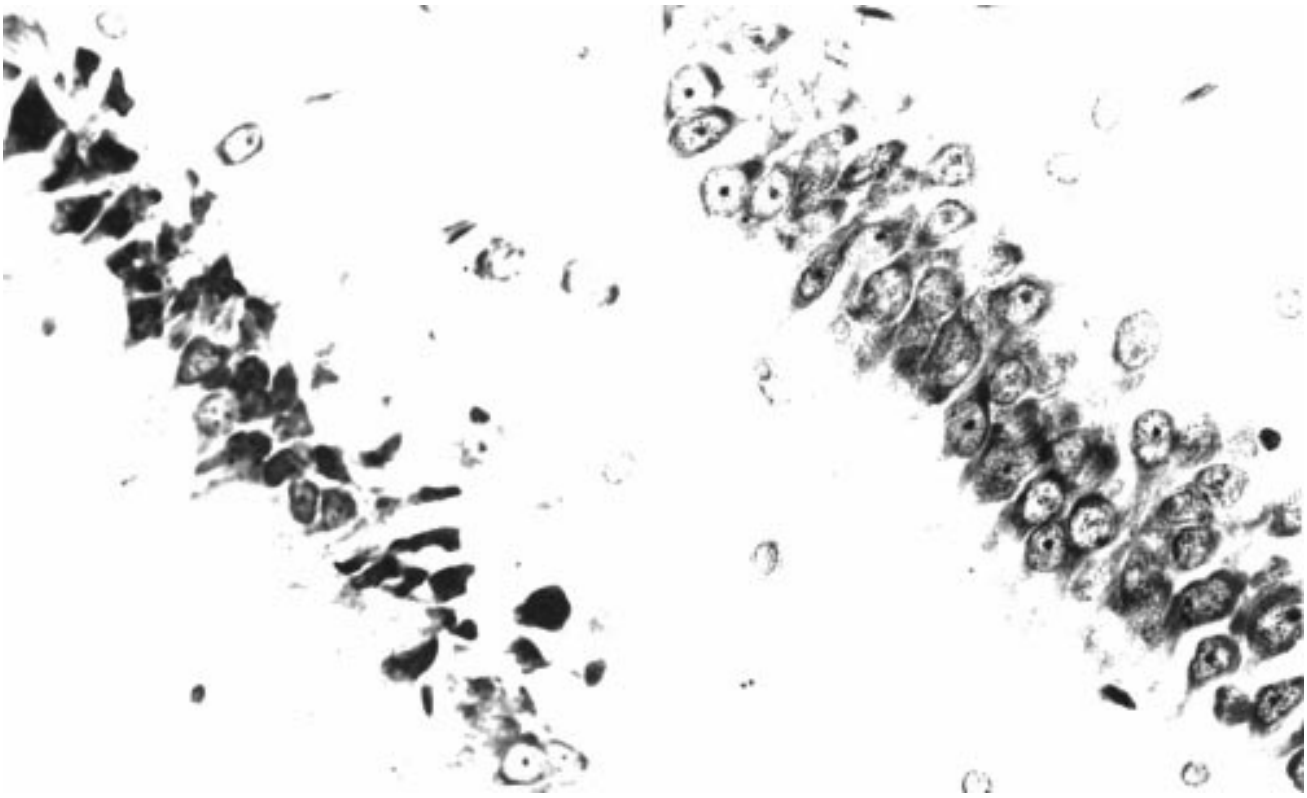

Figure 7. a: A focal concentration of neurocytes cytoplasm in the CA 3 hippocampus layer in the brain (1/10 LD_{50}). Stained according to the Nissel method, × 160. b: Control group, no changes. Stained according to the Nissel method, × 160.

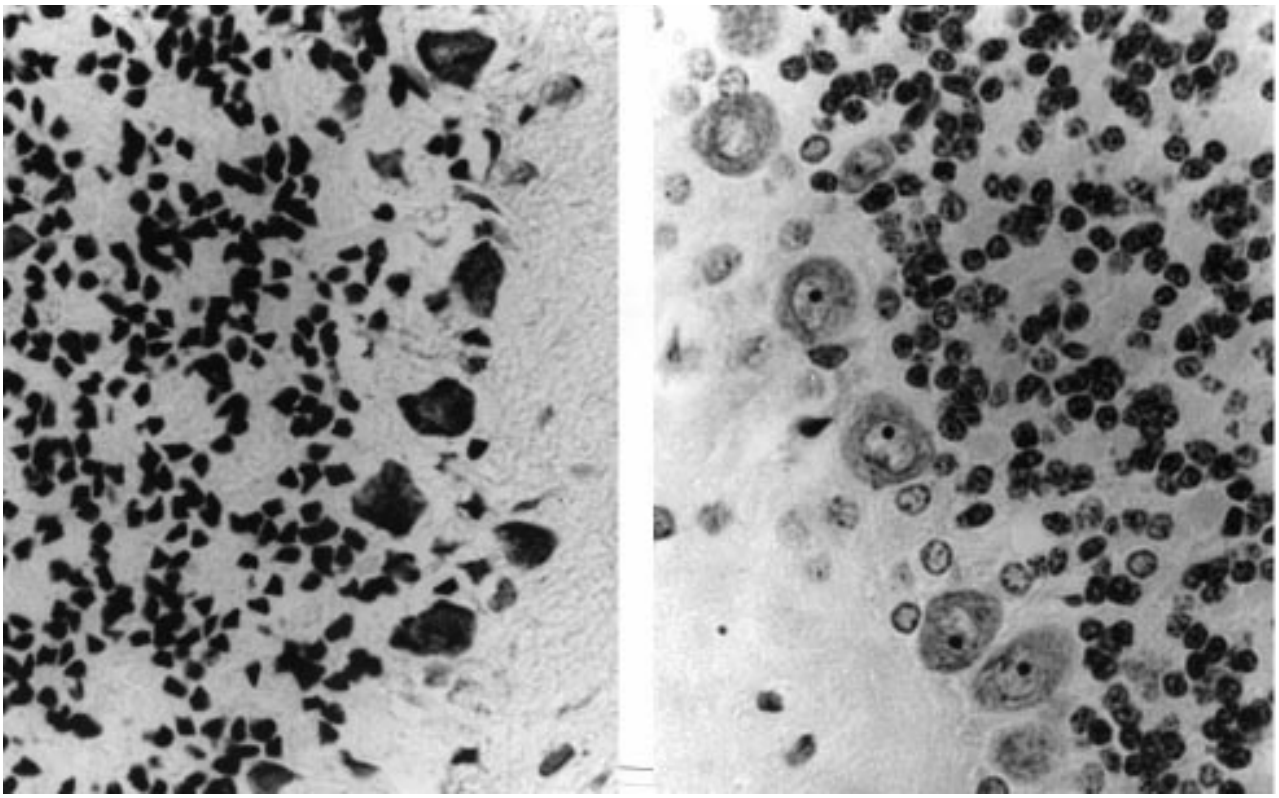

Figure 8. a: Degenerative changes in the granular layer and in Purkinje cells of the cerebellum (1/5 LD_{50}). Stained according to the Nissel method, × 160. b: Control group, no changes. Stained according to the Nissel method, × 160.

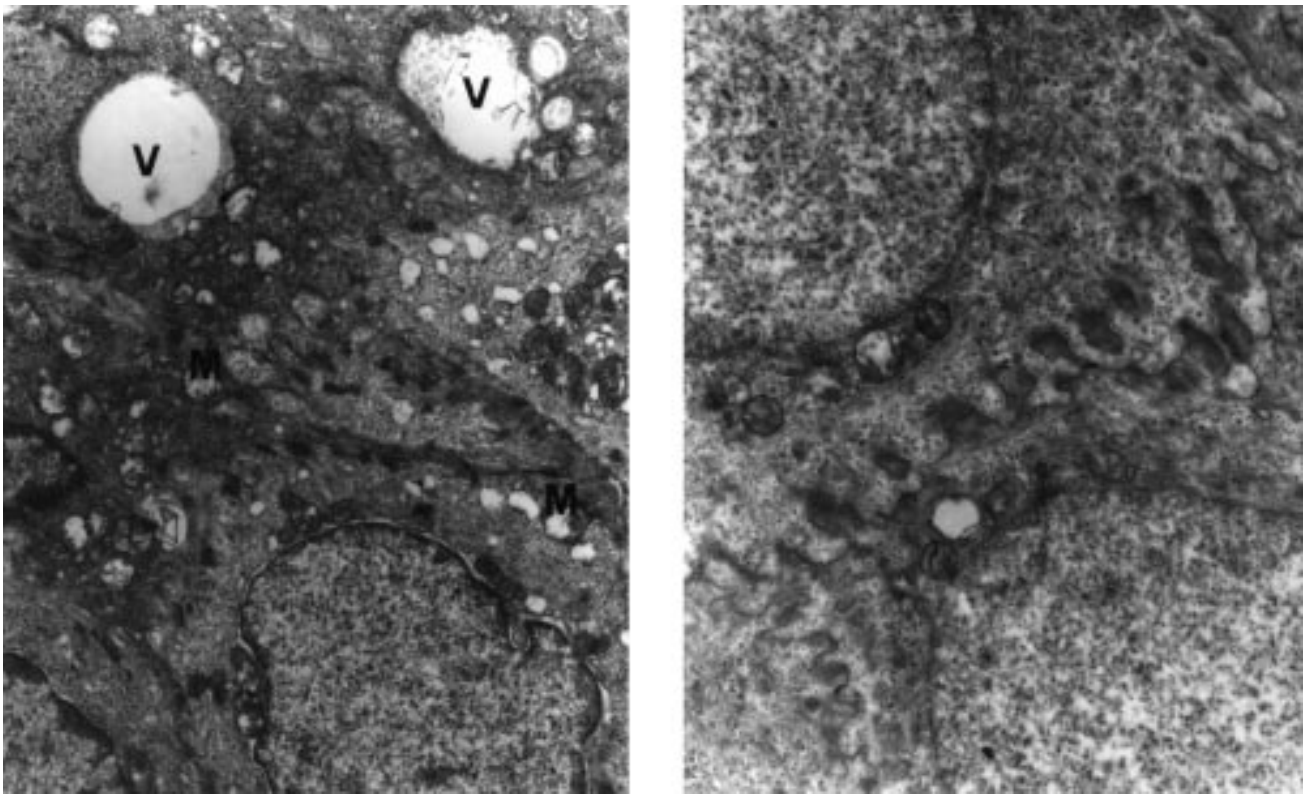

Figure 9. a: Large vacuoles (V), and swollen mitochondria (M) in the stratum spinosum of the skin (1/5 LD_{50}). EM, × 8 000. b: Control group, no changes. EM, × 16 000.

Histologic examination of the skin at the site of carbaryl application showed loosening of structure of all strata of the epidermis. Pyknotic nuclei surrounded by a transparent zone were observed in the stratum spinosum. A definite thickening of the stratum granulosum and stratum corneum was noted. Infiltration of mononuclear cells was present subepidermally, whereas an intensive hyperaemia was observed in the dermis. In the zone surrounding the application site oedema was clearly manifested in the stratum spinosum of the epidermis. Studies of the skin at the site of exposure, conducted by electron microscope, showed the presence of vacuoles in the stratum spinosum and stratum granulosum (Fig. 9), and swelling of mitochondria, especially in the stratum basale. Similar changes were noted in the skin surrounding the exposure zone.

DISCUSSION

Carbamates may be absorbed by the alimentary tract, respiratory system, skin and mucous membranes. Due to their high fat-soluble properties these compounds easily penetrate through cell membranes and are quickly distributed throughout the body. In addition, carbamates easily penetrate through the blood-brain barrier [2, 13].

The studies carried out by the use of marked ^{14}C carbaryl showed that this preparation applied to the skin of the back was absorbed by male rats at the rate of 0.18 µg/h/cm^2 [8]. Similar results were obtained in our percutaneous absorption study, where carbaryl applied to tail skin was absorbed by male rats at the rate of 0.15 µg/cm^2 during exposure lasting 4 h. Despite the elimination of the majority of ^{14}C carbaryl via urine and bile, residues of this pesticide were found in various organs, i.e. fatty tissue and cytosol of the liver [21], in the kidney, spleen and heart, and moreover in the eye and central nervous system in foetuses [5].

The persistence of blood radioactivity always correlates with an inhibition of cholinesterase activity in the plasm [7], this inhibition being higher the weaker the function of the liver, e.g. following hepatectomy [6]. Thus, carbaryl evidently acts through cholinesterase inhibition. However, is it the only mechanism by which this compound exerts its effect?

In the bodies of vertebrates, carbaryl is subject to hydrolysis and hydroxylation with the participation of specific enzymes, i.e. beta-glucuronidase and sulphatase, which results in the creation of metabolites identified as conjugated glucuronides and sulphates [4]. Mac Pherson *et al.* [12] observed that creation of the final products of carbaryl metabolism depended on the availability of metabolizing enzymes located in the liver.

A report concerning the effect of a mixture of carbaryl (50%), chlorfenvinphos (3%) and lindane (2%) described erythrorrhagia in brain, heart, lungs, liver and kidneys. Additionally, parenchymatous degeneration in the heart,

liver, kidneys, and small-celled infiltration and oedema in lungs were observed [14]. Lox [11] observed degenerative changes in the liver of rats after oral administration of carbaryl. However, a complete lack of pathological changes in the internal organs of rats after oral intoxication with carbaryl has also been described by other authors [10].

Our studies, conducted with the use of ^{14}C carbaryl applied dermally, show that within 6 h after exposure 3–4% of the pesticide which penetrated into the skin was found in the liver. In this organ, 20 h after dermal application, ^{14}C carbaryl was still detected at about 4% of the amount absorbed by the skin in the exposure zone. In the remaining organs (lungs, kidneys, and heart), the amount of ^{14}C carbaryl did not exceed 1% of the dermally absorbed dose [20].

It seems that the degree of progression of histologic and ultrastructural changes in the examined organs may be associated with the amount of carbaryl absorbed, but only to a limited extent. The intensity of histologic changes was not great, even in brain and liver where large amounts of carbaryl were present, compared to other organs, such as the lungs, kidney and heart.

In the brain, a concentration of neurocytes cytoplasm was noted in various areas of this organ, and in the cerebellum microfocal degenerative changes were observed. In the liver, histologic changes were manifested as microfocal inflammatory and sporadic degenerative changes. Similar changes were described by Lox [11]. On the ultrastructural level, the changed cytoplasmatic structures represent microsomal component of the liver, which according to Mac Pherson *et al.* [12] is responsible for carbaryl metabolism. Accordingly, the increase in amounts of the smooth endoplasmic reticulum and decrease of the rough endoplasmic reticulum suggest that these cytoplasmatic structures may participate in the metabolism of carbaryl. In the remaining organs, only ultrastructural changes were observed.

The products of biotransformation, as well as carbaryl in an unaltered form, are at first excreted by the kidneys, then by lungs and intestines [21]. Ultrastructural changes in the kidneys consisted only of an increase in the number of autophagous vacuoles observed in our studies. This may suggest an intensification of the processes of intracellular digestion.

Histopathological and ultrastructural studies of internal organs of rats after the dermal application of carbaryl in the doses of 1/5 LD_{50} showed slight histologic changes in the liver, brain, lungs heart and kidneys. In subacute intoxication with carbaryl used as a trade preparation Sewin containing 98% of carbaryl, administered orally, degenerative changes in the internal organs of rats were not observed [10]. The character of the observed ultrastructural changes suggests that the changed cytoplasmatic structures may participate in the metabolism of the examined pesticide. These results are evidence of low toxicity of carbaryl.

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