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COMPARISON OF HISTOLOGICAL AND ULTRASTRUCTURAL CHANGES IN MICE ORGANS AFTER SUPPLEMENTATION WITH INORGANIC AND ORGANIC SELENIUM

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Abstract: Two organic compounds of selenium, 4-o-totyl-selenosemicarbazide p-chloro-benzoic acid (chain compound) produced at the Chemistry Department of the University Medical School in Lublin, and one inorganic compound of sodium IV selenite (Na_2SeO_3) were used. The preparations were used per os in doses of 1 mg/kg body weight and 0.5 mg/kg body weight. The studies were conducted on female Swiss mice, covering seven groups of animals, i.e. 6 experimental and 1 control. Histopathologic changes were observed in liver, kidney, lung and heart. Ultrastructural changes were observed in liver and kidney. Our studies indicate a dose-dependent effect of selenium on histopathologic and ultrastructural changes. It is possible therefore, that the extent of excess of selenium exerts a greater influence on a cell than the form of supplemented selenium.

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INTRODUCTION

Selenium in combination with proteins and amino acids plays an important role in maintaining homeostasis in an organism [3]. As a main component of glutathione peroxidase, it performs the function of an antioxidant in a number of metabolic and immunologic processes [4, 5, 7]. Selenium shows protective properties in neoplastic and cardiovascular diseases [7], and provides for the normal functioning of the spleen and liver [2,12]. Various cell types, organs and tissues were investigated with regard to selenoprotein content and selenium antioxidant enzyme activity in blood and muscles [7], thyroid gland [1], reproductive cells and male gonads, liver, kidney, brain, serum, and urine [5].

In body disorders caused by selenium deficiency, supplementation with this bioelement is applied. It seems that due to a high Se toxicity an organic form of the compound is more advantageous for an organism [5]; however, in the case of selected types of cancer the application of inorganic selenium produces better results [7]. Organic Se compounds (selenosemicarbazide) are the form more easily assimilated, compared to inorganic sodium selenite, which is confirmed by an elevated content of Se in the internal organs of mice after organic and inorganic supplementation with selenium [9, 10]. In respect of the very narrow interval between the doses which are toxic or safe for the organism *vs.* considering not only the dose volume but also the form of Se compound, it seemed justified to analyze the potential

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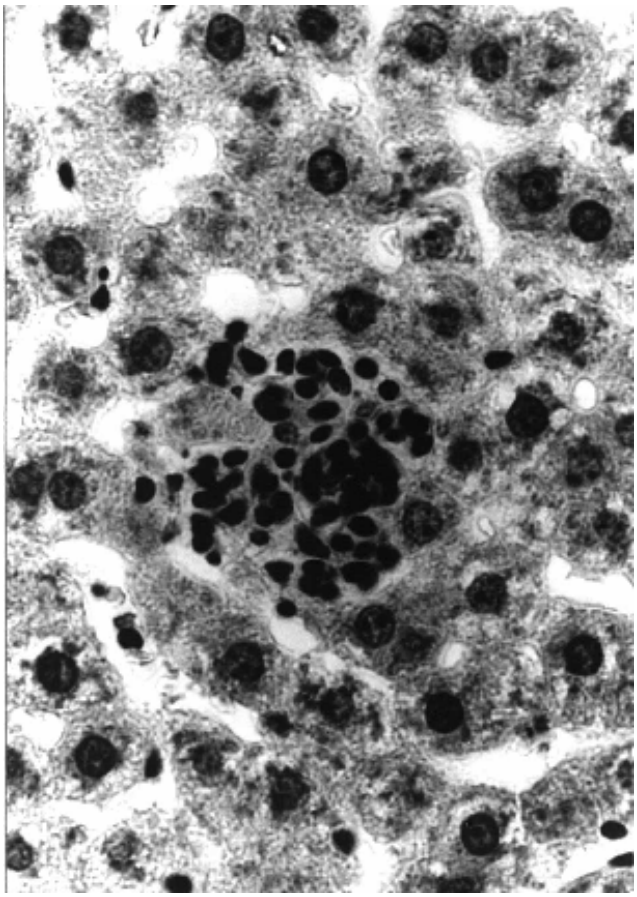

Figure 1. Liver of mice. Supplementation of selenium sodium (0.5 mg/kg). Fine inflammatory infiltration. H+E, × 160.

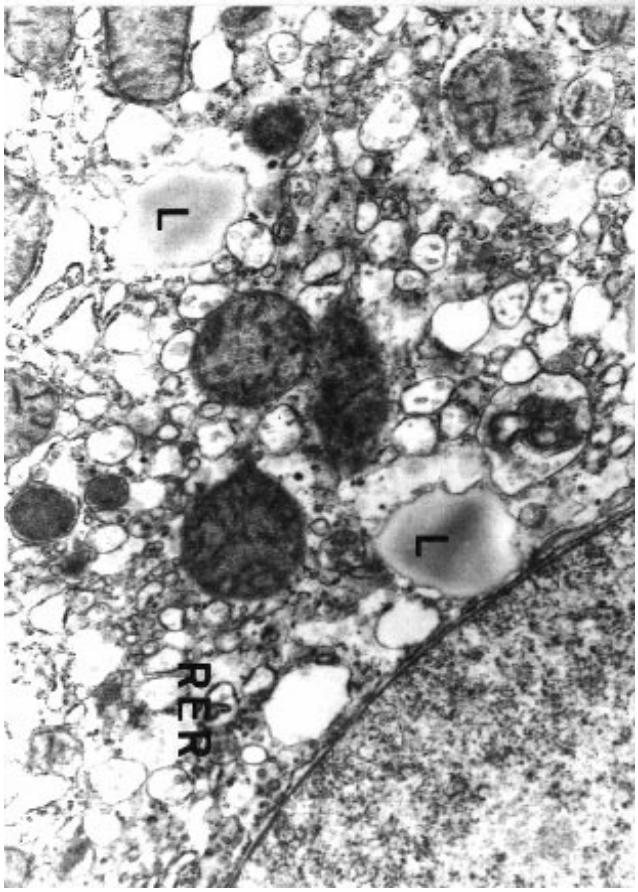

Figure 2. Liver of mice. Supplementation of selenium sodium (0.5 mg/kg). Dilation of cisternae of the rough endoplasmic reticulum (RER) and lipid droplets (L). EM, × 15000.

histopathologic and ultrastructural changes in selected internal organs in mice after the supplementation with selenosemicarbazide and selenium selenite.

MATERIALS AND METHODS

Two organic compounds of selenium, 4-o-totyl-seleno-semicarbazide p-chlorobenzoic acid (chain compound) and 3-(p-chlorbenzoilamino-)-2(o-tolylimino)-4-phenyl-4-selenazoline (cyclic compound) produced at the Chemistry Deparment of the University Medical School in Lublin [10], and one inorganic compound of sodium IV selenite (Na_2SeO_3) were used. All experimental compounds were suspended in the form of an emulsion consisting of arabic gum, oil and water in the proportion 1 : 2 : 1.5. The preparations for the application per os were used in doses of 1 mg/kg body weight and 0.5 mg/kg body weight.

The studies were conducted on female Swiss mice. The animals were fed with standard fodder LSM [8] and watered *ad libitum*. At the beginning of the experiment, the body mass of mice ranged from 21–24 g.

The study covered 7 groups of animals, 6 experimental and 1 control. Each group consisted of 10 mice. The experimental groups were administered the preparations for 10 days. The animals of the control group were administered per os the same emulsion during this time and in conditions corresponding to the experimental group. The following organs were taken in order to evaluate histologic changes: liver, heart, kidneys, lungs and spleen. The liver and kidneys were taken in order to evaluate ultrastructural changes.

The material for the histologic study was fixed in neutralized formalin diluted with water in the proportion 1 : 9. Preparations were stained with H+E. Material for the study by electron microscopy was fixed in 5% glutaraldehyde solution in 0.1 M. cacodylate buffer (pH 7.2–7.4) for a period of 5 hours, and for 1 hour in 1% solution of OsO_4 in the same buffer. The material was dehydrated in ethyl alcohol and embedded in Poly Bed. Ultrathin specimens were observed and photographed using a BS 500 Tesla electron microscope.

RESULTS

Sodium selenite supplementation in a lower dose (0.5 mg/kg body weight) caused microfocal infiltrations in the liver of only 40% of the animals (Fig. 1). In the ultrastructure of hepatocytes, widened ergastoplasm's canals and single lipid drops were observed, while mitochondria showed shortened crista (Fig. 2). A higher dose (1 mg/kg body weight) resulted in infiltrations of mononulear cells which were observed in all animals, and the inflammation was

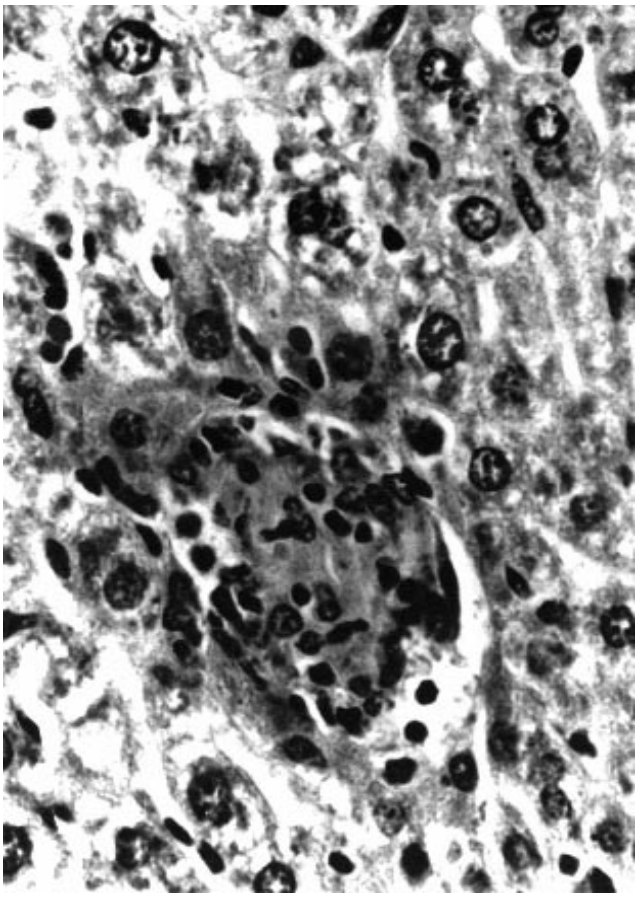

Figure 3. Liver of mice. Supplementation of selenium sodium (1mg/kg). Degenerative changes of hepatocytes with infiltration of mononuclear cells. H+E, × 160.

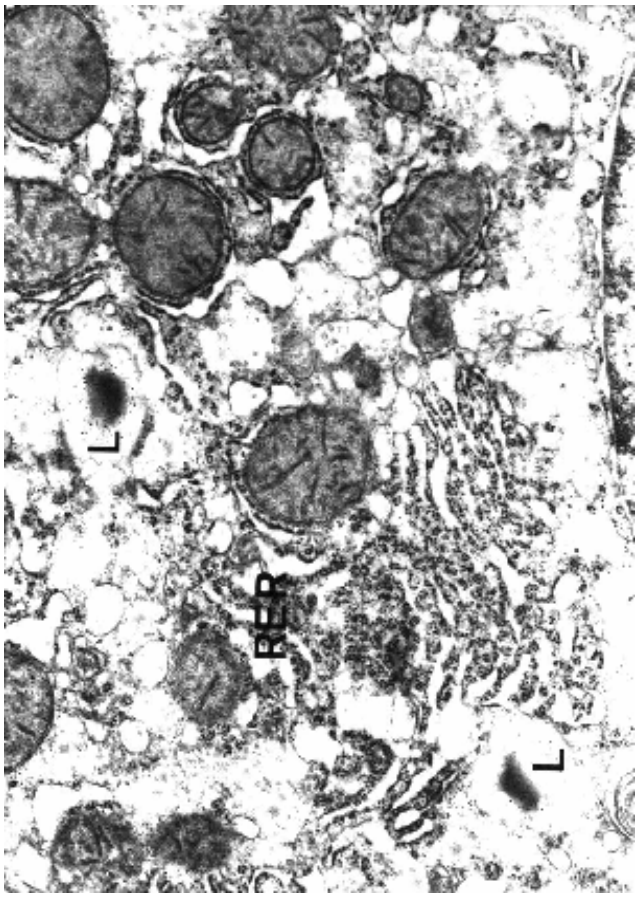

Figure 4. Liver of mice. Supplementation of selenium sodium (1mg/kg). Dilated ergastoplasm's canals (RER) and lipid-like vacuoles (L). EM, × 15000.

accompanied by granulocytes and focal hepatocyte degeneration in some animals (Fig. 3). In the submicroscopic structure of hepatocytes, a higher dose of selenite caused a considerably greater widening of canals of the ergastoplasm and an increase in the amount of smooth endoplasmatic reticulum. In addition, specific lipid-like vacuoles were observed, which contained osmophilic material inside (Fig. 4).

In the kidney, after a lower dose of sodium selenite no changes were noted; however, a higher dose caused parenchymatous degeneration of renal tubules in 60% of animals.

Ultrastructural studies of the cells of renal proximal tubules after the application of both doses of solium selenite showed only swollen mitochondria.

In the lungs in single animals, peribronchial infiltrations were observed as well as hyperemia and blood extravasations into the bronchiole, irrespective of dose. In the heart, no changes were noted.

Organic selenium (chain compound) supplementation in a lower dose caused inflammatory infiltrations in the liver of all animals. After the application of a higher dose, apart from infiltrations, numerous foci of necrosis were observed. Organic selenium (cyclic compound) supplementation in a lower dose resulted in the occurrence of inflammatory foci in 60% of mice. A higher dose caused parenchymatous degeneration of hepatic cells and inflammatory infiltrations with the presence of granulocytes in 80% of animals. Ultrastructural studies showed changes in hepatocytes which were not connected with the form of the supplemented organic compound of selenium, but with its dose. After the supplementation of a lower dose, vasiculation endoplasmatic reticulum was observed in the cytoplasm of hepatocytes. Fine lipid drops were noted between the alveoli of the endoplasmatic reticulum. Mitochondria generally had short crista, some of which were swollen and had vacuoles (Fig. 5). The application of a higher dose resulted in the segregation of organelles in the cytoplasm. Mitochondria showed polymorphism, with some having paracrystalline inclusions. Peroxisomes were of various sizes and occurred in slightly increased quantities (Fig. 6).

In the kidney, after the supplementation of both forms of organic selenium in a lower dose, no changes were observed in histopathologic studies. A higher dose of, the cyclic compound of Se did not cause any changes. However, after the application of a higher dose of the chain compound of selenium, infiltrations in the renal cortex were noted in 40% of mice, as well as parenchymatous dimness of the renal tubules in 20% of animals. In the heart, only a higher dose of chain organic selenium resulted in the occurrence of inflammatory foci in 20% of mice. Changes in the ultrastructure of proximal

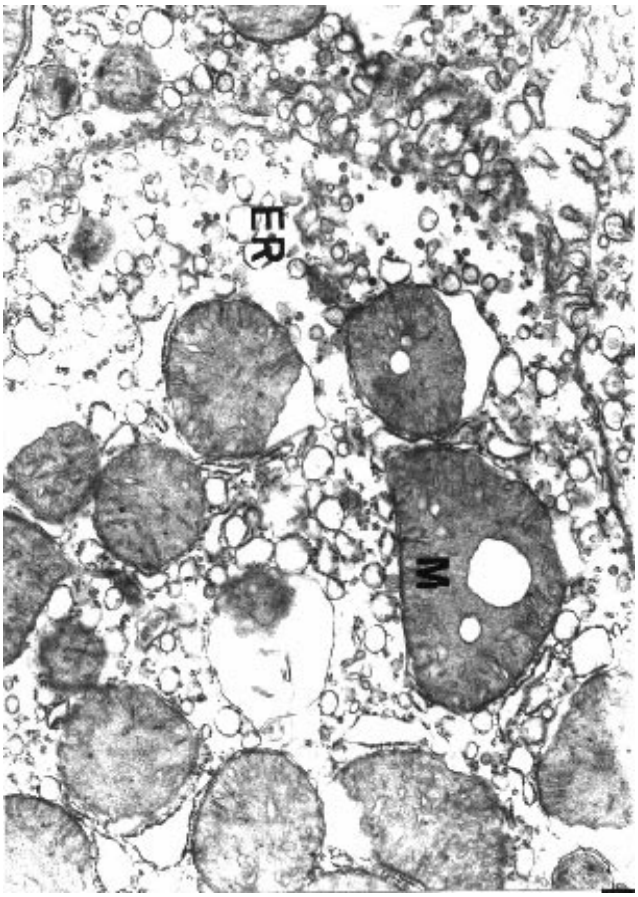

Figure 5. Liver of mice. Supplementation of organic selenium (0,5 mg/kg). Vesiculation endoplasmic reticulum (ER) and swollen mitochondria (M). EM, × 15000.

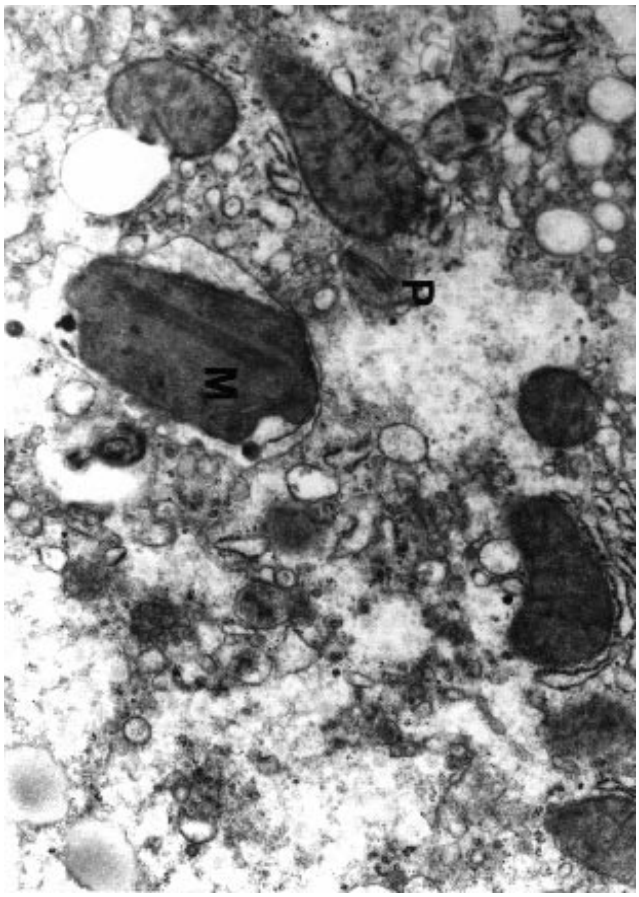

Figure 6. Liver of mice. Supplementation of organic selenium (1mg/kg). Mitochondria containing paracrystalline inclusions (M), peroxisome (P). EM, × 12000.

renal tubules after the application of a lower dose of both forms of organic selenium were comparable with changes observed after both doses of inorganic selenium. These changes covered swollen mitochondria and slight widening of the elements of the endoplasmatic reticulum. A higher dose of both forms of organic selenium caused more intensified changes, which covered not only swollen mitochondria, but also widening of Golgi structures and an increase in the quantity of electron dense bodies (Fig. 7).

In the lungs, in histopathologic studies, both forms of selenium in a lower dose did not lead to changes, while a higher dose caused thickening of interalveolar septa with the presence of alveolar macrophages and granulocytes in 20% of animals.

DISCUSSION

The anti-oxidation role of selenium in organisms results from the fact that it is built into the catalytic centre of glutatione peroxidase - the main enzyme which deactivates free radicals and protects against excessive lipid peroxidation [11]. Glutatione peroxidase in an active form occurs in cytosol and in the matrix of mitochondria, whereas it is in an inactive form in nuclei, liposomes and peroxysomes [5]. Its activity increases in the course of adding selenium to the diet.

Selenium, in combination with vitamin E, which shows affinity to lipids, protects cellular membranes and intracellular organelles against oxidation by removing endogenic hydrogen peroxide and organic hyperoxides [5]. Selenium, both in excess and deficiency, can exert a pathogenic effect. An excess of this element, especially in the form of inorganic compounds and some of its metabolites, leads to changes similar to those observed in the case of deficiency, i.e. to the degeneration of free radicals, intensification of lipid peroxydation, and even to the inhibition of synthesis of proteins [6, 13].

The results of our study show that selenium supplemented to mice in excess in both the inorganic and organic forms, causes changes in the internal organs of the animals examined, especially in the liver and kidneys. Changes observed in the light microscope occurred mainly after the higher doses of supplemented compounds of organic and inorganic selenium. These changes covered the degeneration of hepatocytes after the supplementation of inorganic selenium, as well as degeneration and single foci of necrosis after organic selenium. In submicroscopic studies, great changes occurred, especially in the liver. In hepatocytes, vasiculation of endoplasmatic reticulum, changes in the appearance of mitochondria and peroxisomes, as well as the occurrence of numerous liposomes were observed. These are cellular organelles which, due to their protein-

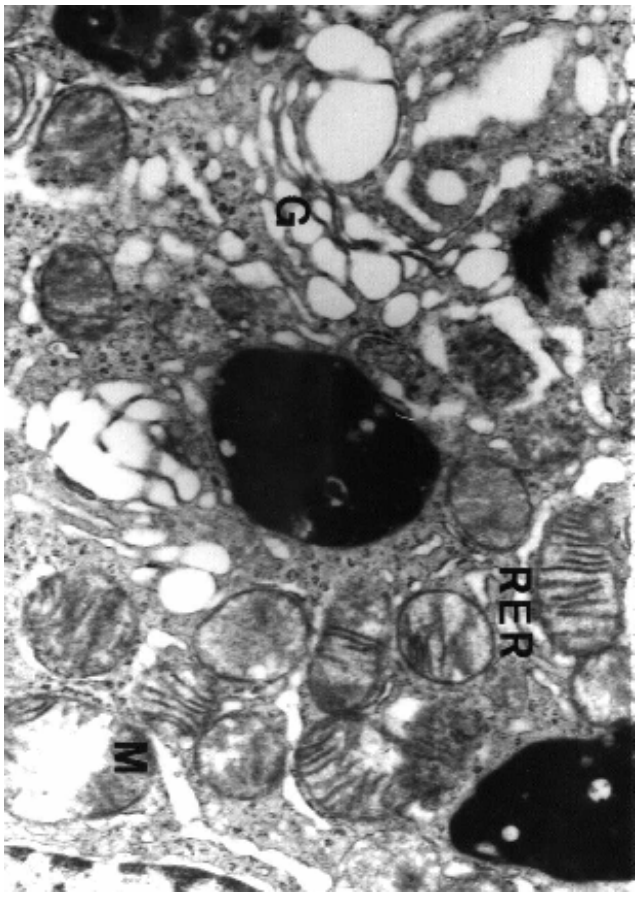

Figure 7. Kidney of mice. Supplementation of organic selenium (1mg/kg). Dilation of ergastoplasmic canals (RER) and of the Golgi structures (G), swollen mitochondria (M). EM, × 15000.

lipid membranes or the presence in them of glutatione peroxidase, may be a site of selenium 'activity'. It seems that both histopathologic and ultrastructural changes were more intensified in the case of supplementation of higher doses of selenium, the intensification of the changes observed being greater in the case of application of an organic form of selenium.

At first sight, our observations are not consistent with the data from literature, which show a greater toxicity of inorganic selenium, compared with selenium supplemented in the form of organic compounds [5]. However, it has been reported that better assimilation of Se from organic compounds would consequently cause a higher concentration of this element in the internal organs, compared to the supplementation of sodium selenite [10]. Our studies indicate a dose-dependent effect of selenium on histopathologic and ultrastructural changes. It is possible therefore, that the extent of excess of selenium exerts a greater influence on a cell than the form of supplemented selenium.

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