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Influence of Lead Acetate on the Histological, Ultrastructural and Histochemical Picture of the Livers of Albino Rats

Wpływ octanu ołowiu na obraz morfologiczny, ultrastrukturalny i histochemiczny wątroby szczura białego

The amount of lead pollution in the environment is increasing proportionally to the development of industry. Lead is capable of damaging the organism in many ways due to its high affinity to various tissues, different enzymes and serum proteins and its tendency to cumulate (1, 14). Acute lead poisoning occurs in people who have had intense, but short-term contact with organic lead compounds (tetraethylolead) used as an antidetonant in motor fuels, or plumbous orthoplumbate (red lead), which is an important component in anticorrosive paints (4, 6). Chronic poisoning by lead and lead salts, which used to occur in printers and workers in battery factories, is now a threat to the whole human population because of the hundreds of thousands of tons of tetraethylolead used as a fuel additive. This substance pollutes not only the atmosphere, but also the soil and the water and because of this, the food (10).

The toxic effects of lead on the central and peripheral nervous system and the hematopoetic system is well known (9, 12). Less clear, however, are the toxic effects of this metal on the liver. As of now, there are still different views on the existence of negative effects of lead on the livers of people and animals exposed to this metal. Some researchers found no changes in the liver in cases of long-term contact with lead (3).

The goal of our work was to examine the influence of lead on the histological and ultrastructural pictures as well as on some histochemical reactions in the liver. Only a few morphological works are presented on this subject.

MATERIALS AND METHODS

For this examination we used 75 male rats of the Wistar race, which were kept under stable environmental conditions for the duration of the experiment. The rats' mass was between 180 and 240 grams. The animals were divided into three main groups, including two experimental groups of 30 rats each, one control group of 15 rats and 7 subgroups, each with different times of duration of the

experiment. The rats in the experimental groups received Merck brand lead acetate in a saline solution intraperitoneally twice a week at the same time of the day.

The experimental group I received lead acetate in a dose of mg/kg body mass in 1 ml of saline:
subgroup "a" — duration of the experiment — 1 month;
subgroup "b" — duration of the experiment — 2 months;
subgroup "b" — duration of the experiment — 3 months.

The experimental group II received lead acetate in a dose of 30 mg/kg body mass in 1 ml of saline:
subgroup "a" — duration of the experiment — 1 month;
subgroup "b" — duration of the experiment — 2 months.

The control group received 1 ml of saline in the same manner and time as the prior groups.

Liver samples taken from dead rats after 1, 2 or 3 months of lead acetate administration were examined histopathologically, histochemically and ultrastructurally.

For histological investigations specimens of liver tissue were fixed in 10% formalin and embedded in paraffin. Paraffin specimens were stained by the following methods: 1) staining with hematoxylin and eosin; 2) histochemical staining of lipids by the Sudan IV method; 3) histochemical staining of glycogen according to Kiszeli; 4) silver reticulin Gomori staining; 5) staining of collagen fibres by the van Gieson method.

For histochemical studies liver specimens were fixed in baker fluid and then sliced with a freeze microtome. The activity of the following enzymes in the liver was evaluated histochemically: 1) acid phosphatase according to Gomori; 2) alkaline phosphatase according to Gomori; 3) adenosinetriphosphatase (ATP-ase) according to Wachstein and Maisel.

For ultrastructural investigation liver specimens were fixed in 4% glutaraldehyde buffered with cacodylate and postfixed in 1% OsO_4, then dehydrated in graduating contcentrations of ethanol and embedded in Epon 812. Semithin sections were stained with 1% methyl blue and 1% Azur II in 1% borax solution. Ultrathin sections were stained with uranyl acetate and lead citrate. Semithin and ultrathin sections were cut using a Tesla BS 412 ultramicrotome. Ultrathin sections were then examined using a Tesla BS 500 electron microscope.

RESULTS

The histological picture of the livere of rats from the control group was normal. In all of the experimental groups, histological examination revealed inflammatory infiltration of mononuclear cells in the intermediate and marginal zones of the lobules. Small granulomas consisting of macrophages, lymphocytes and small number of plasmocytes were also seen in the intermediate zone of the lobules (Figs. 1 and 2). Uneven staining of hepatocyte cytoplasms varying in intensity was observed in all of the groups. The nuclei of hepatocytes with an eosinophilic cytoplasm often had condensed chromatin and were shrunken (pycnotic). The hepatocytes with the condensed cytoplasms and shrunken nuclei were not surrounded by inflammatory cells, which is typical of coagulative necrosis, therefore on this basis one can assume that the picture observed corresponds rather to apoptosis than to necrosis of these hepatocytes. The presence of numerous round deposits in the hepatocyte nuclei, as seen in semithin sections, attracted attention. These inclusions either occurred singularly, or there were several in the nucleus and they stained with varying intensity. The intensity of all of the above changes increased

proportionally to the length of time of lead acetate administration and the dose administered.

In all of the examined histological specimens the architecture of the liver lobules was preserved and the quantity of collagen fibres, evaluated by the van Gieson staining, was similar to that of the control group. Also, the Sudan IV reaction showed no lipids present in any of the examined livers. In our histochemical examinations we found a marked increased reaction of acidic phosphatase as well as a slight increase in the activity of alkaline phosphatase and adenosinetriphosphatase.

The ultrastructural picture of the hepatocytes in the control group were in the norm. In the group I (lead acetate administered in a dose of 15 mg/1 kg body mass) after 1, 2 and 3 months of lead administration, similar ultrastructural changes were found, with the most intense changes appearing in the subgroup "c", which received lead acetate for 3 months. The noticed changes had a focal character and were of low intensity. The most common change was the increase of primary and secondary lysosomes and peroxisomes not only around the bilary pole, but in other zones of the hepatocyte as well. In all of the subgroups, especially subgroups "b" and "c" (2 and 3 months of lead acetate administration) hepatocytes with a significantly condensed (shrunken) cytoplasm were sometimes seen. Intense swelling of the mitochondria and slight widening of the canals of the smooth endoplasmic reticulum were traits that appeared consistently in these hepatocytes (Fig. 3). Ultrastructural features of liver regeneration such as irregular shapes of the nuclei, open pores of the nucleus' envelope and concentrations of polyrybosomes, were also found (Fig. 4).

In all experimental subgroups, atypical deposits were seen loosely scattered about the hepatocyte hyaloplasm. They exhibited significant electron density and a homogeneous fine-granular or fine fibrillar structure (Fig. 3). The size of mentioned deposits varied from small to large and they occurred in the hepatocytes that showed no signs of significant damage. In addition to this, numerous autophagic vacuoles were noticeable in the livers with the mentioned deposits. They contained glicogen, myelin figures, disintegrated fragments of hepatocyte cytoplasm, and sometimes homogeneous, electron dense fine-granular or fine fibrillar deposits similar to those found in the hepatocyte hyaloplasm and described above (Fig. 5). Similar deposits were observed inside the phagocytic vacuoles or loosely scattered about the hyaloplasm of the Kupffer cells. In the livers of rats from the subgroups "b" and "c" deposits located in the hepatocyte nuclei were rarely observed.

In the experimental group II (lead acetate administered in a dose of 30 mg/1 kg body mass) in both subgroups, the changes observed had a focal character and were definitely more intense than in the experimental group I in the subgroup "b" (2 months of lead acetate administration) however, the changes were more intense than in the subgroup "a" (1 month of lead acetate administration). As for

deviations from the norm, an increased number of primary and secondary lysosomes and peroxisomes were seen in different fields of the hepatocyte's cytoplasm (Fig. 6). Proliferation of the smooth endoplasmic reticulum of the hepatocytes was a frequently occurring phenomenon. Its numerous vesicles filled up large areas of the hepatocytes. Slight or significant widening of the canals of the smooth and rough endoplasmic reticulum and signs of degranulation of the rough endoplasmic reticulum membranes were found more frequently than in the hepatocytes of the experimental group I (Fig. 6). The mitochondria were usually normal and signs of slight swelling that were found were the exception. In the experimental group II, especially in the subgroup "b", shrunken hepatocytes with condensed cytoplasms were often observed. Electron dense, homogeneous or fine-granular deposits lying loosely in the hepatocyte hyaloplasm were observed far more seldom than in the experimental group I. Frequently, however, they made up the contents of larger or smaller autophagic vacuoles in the hepatocytes (Fig. 7) or in the Kupffer cells. Numerous phagocytic vacuoles were often observed in the Kupffer cells and apart from above mentioned deposits they also contained disintegrated constituents of the cytoplasm and even the nucleus.

In the experimental group II, especially in the subgroup "b", electron dense deposits were often found in the hepatocytic nuclei. Each nucleus contained one or more of these deposits, and besides this, polymorphism was visible, suggesting that the deposits went through different stages of formation (Fig. 7). Small, newly forming deposits looked like entwined threads and filaments (Fig. 8) or appeared in smaller or larger concentrations of electron dense granules. Large, fully formed deposits consisted of an electron dense homogeneous or fine-granular core surrounded by a belt of gentle randomly dispersed fibrils (Fig. 9). We noted a coexistence of deposits in both the nucleus and the cytoplasm of one hepatocyte: the cytoplasms of hepatocytes containing deposits in the nucleus usually did not show any signs of damage. The nuclei containing the deposits, however, had an irregular shape, condensed chromatine with increased electron density, enlarged nucleoli and open pores of the nuclear envelope (Fig. 8 and 9).

DISCUSSION

In the histological pictures observed in the experimental groups I and II we discovered focal pathological changes difficult for interpretation. The most constant change was an inflammatory infiltration consisting of mononuclear inflammatory cells. Similarly, other investigators (11, 16) observed inflammatory infiltration in the liver after exposition to lead. The damage of the hepatocytes was slight and was manifested in a variable staining of the cytoplasm. Most frequently this damage was observed in peripheral and intermedial zone of hepatic lobule. In all investigated livers we observed single hepatocytes with condensed cytoplasm and shrunken nuclei. These hepatocytes were not surroun-

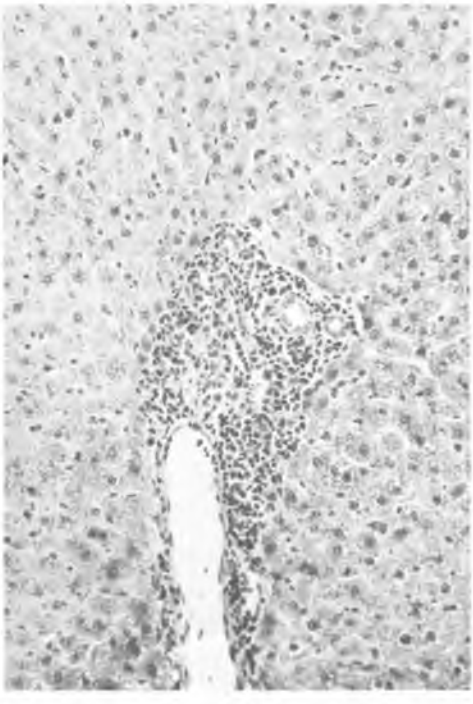

Fig. 1

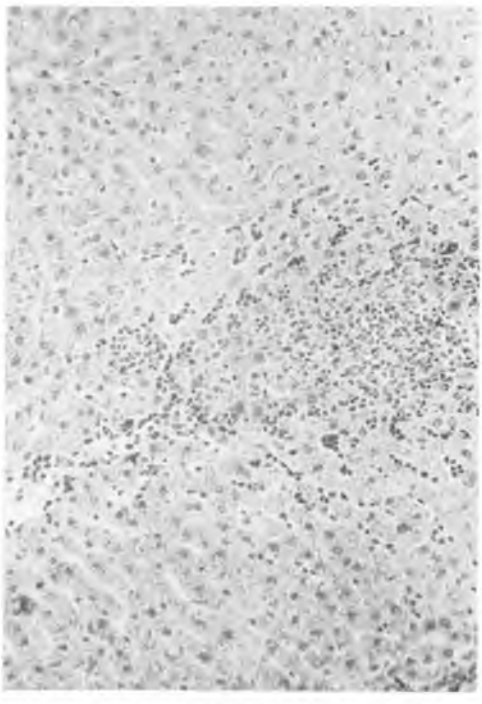

Fig. 2

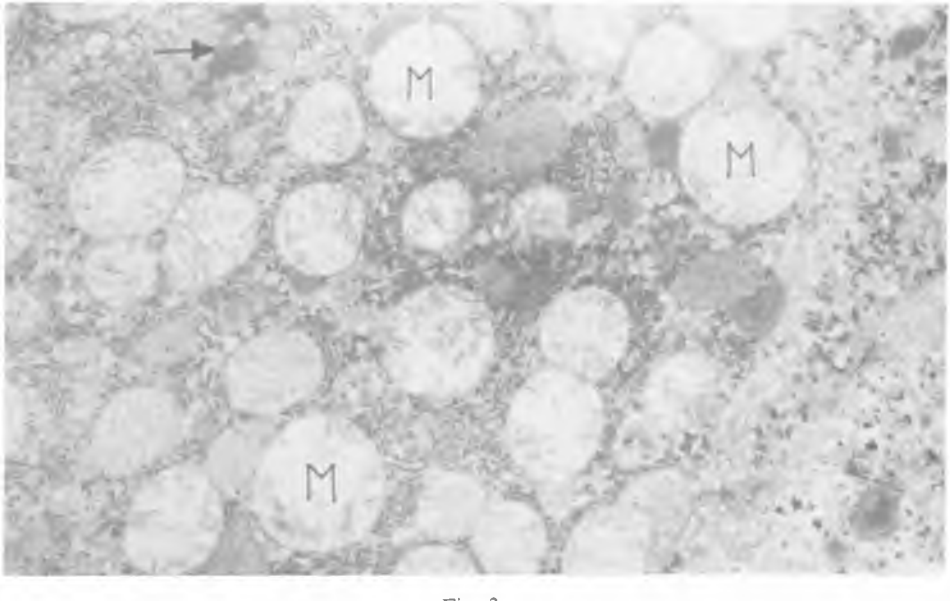

Fig. 3

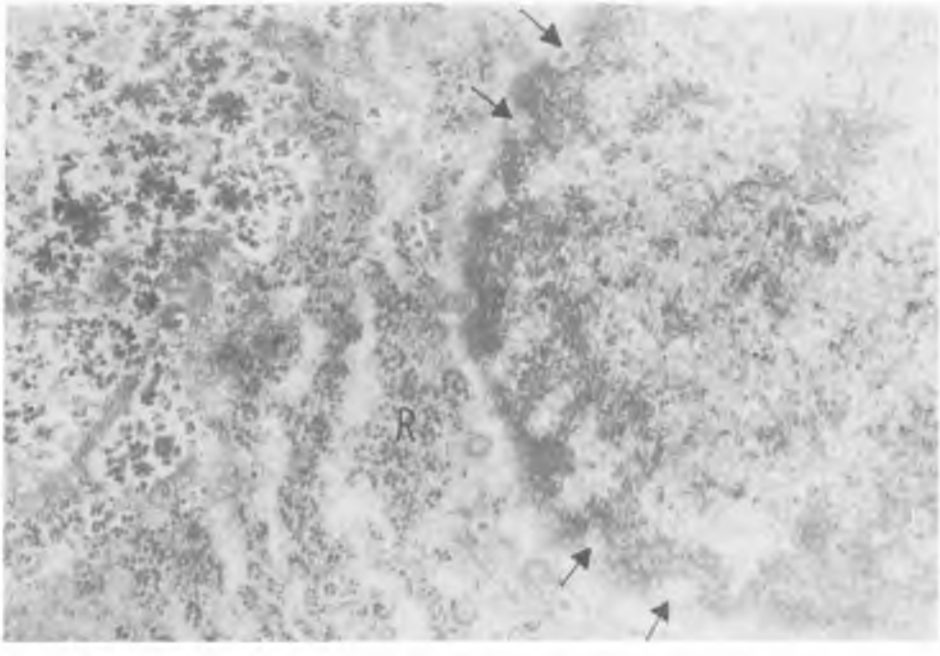

Fig. 4

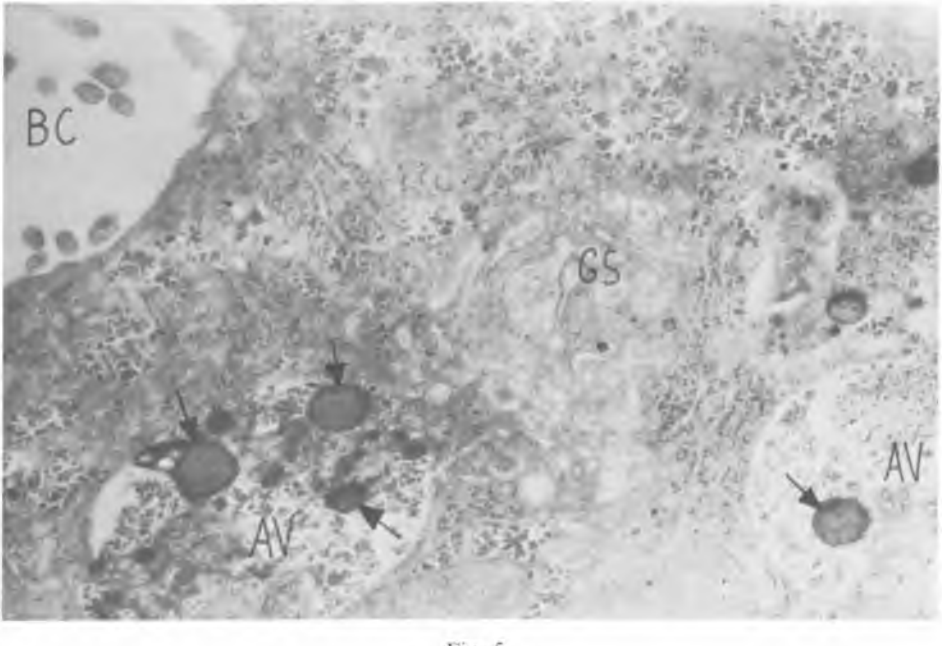

Fig. 5

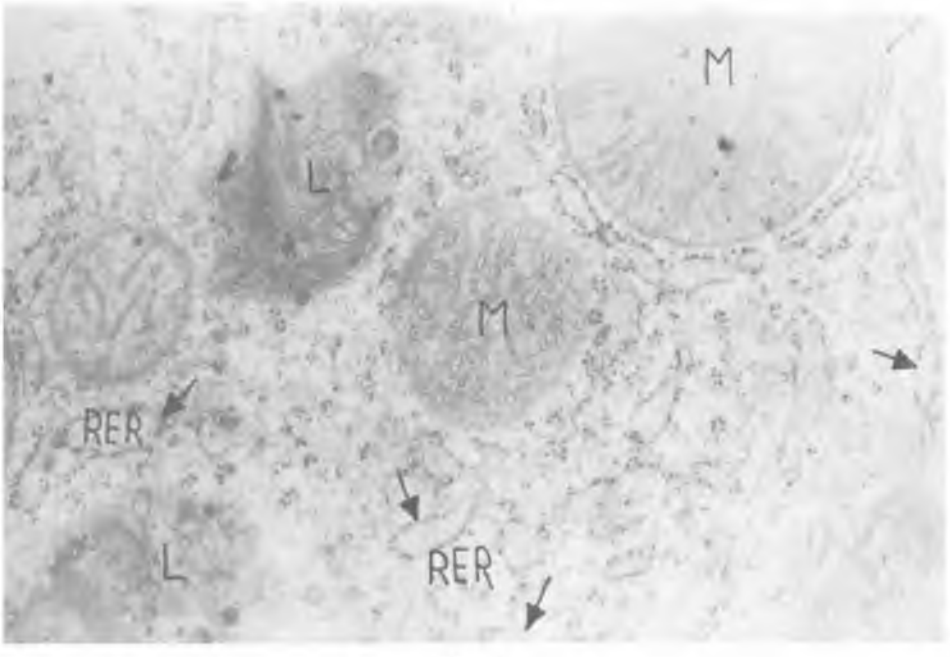

Fig. 6

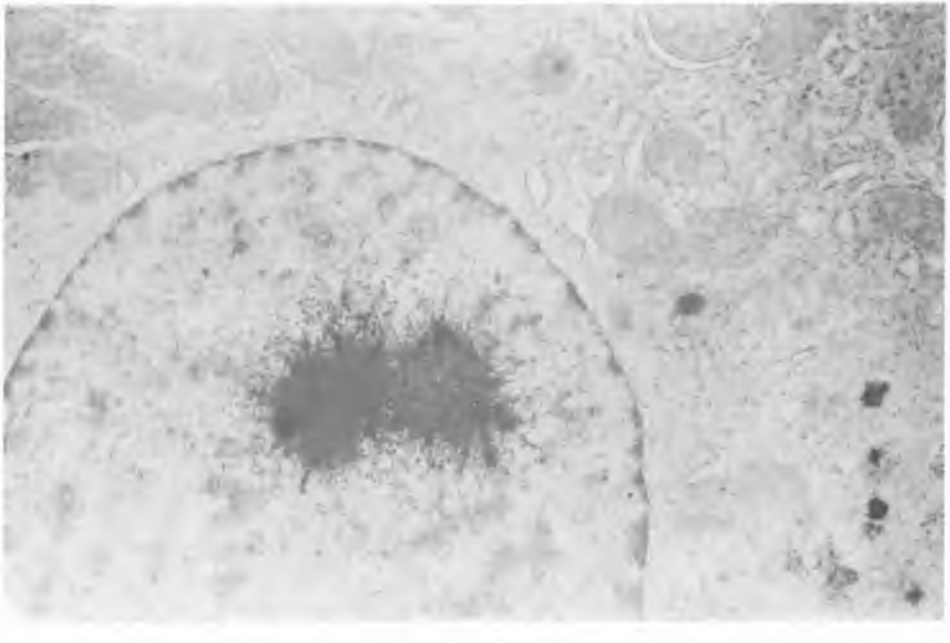

Fig. 7

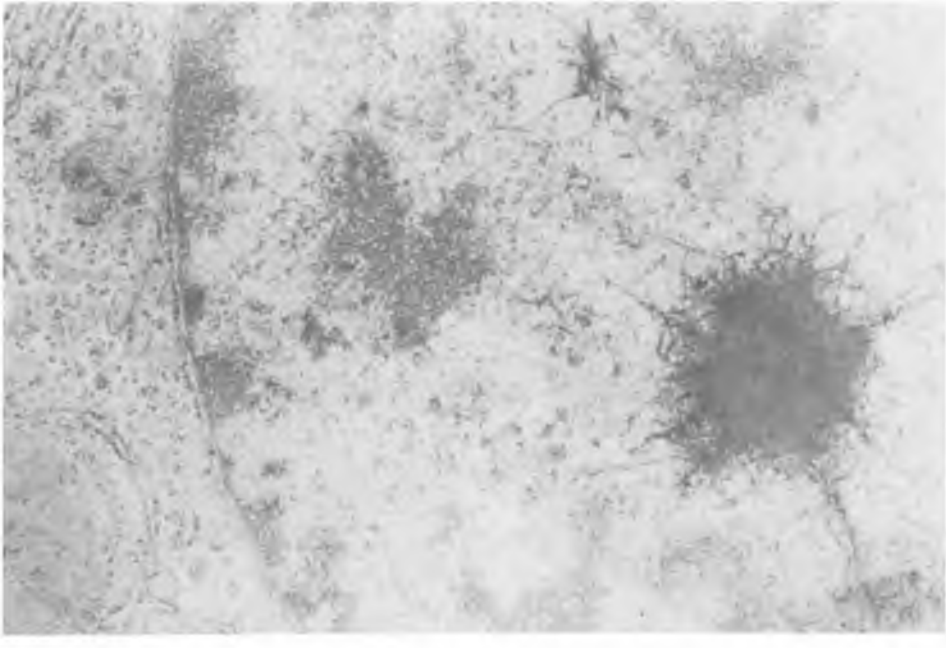

Fig. 8

Fig. 9

ded by inflammatory cells and on this basis we assume that the pictures seen through light and electron microscopy correspond to apoptosis rather than to coagulative necrosis of the hepatocytes. Similarly, other authors suggested that apoptosis is a constant phenomenon in the course of lead poisoning (8). Contrary to the opinion of other investigators (11, 16) we did not discover necrosis and fatty degeneration.

Histochemical investigations showed an increase in the intensity of reaction of acidic phosphatase and a small increase in the intensity of reactions of alkaline phosphatase and adenosinetriphosphatase, which is in accordance with observations of other authors (13). The described changes most probably are included in the widely understood parameters of liver adaptation to the action of destructive factors.

Analysing the ultrastructural pictures of our material and data taken from literature we assume that the deposits in the hyaloplasm and hepatocyte nuclei described above may be lead deposits. The deposits presented in this paper are identical to the ones described by Goyer in the cells of the anterier horns of the spinal cord and the ones described by Stiller (15) and Cramer (5) in the nuclei of epithelial cells of renal tubules. We assume that these deposits are formed in the hepatocyte nucleus because this is where their different phases of development can be observed. In the nuclei of the hepatocytes which cumulated lead deposits, other changes, such as nodular clumping of chromatine and the enlargement of the nucleoli were observed. From a morphological point of view the presented ultrastructural picture is seen in embryonic, regenerating, displastic and neoplasmatic cells. The occurrence of benign and malignant neoplasms in animals which were administered lead compounds was early reported (2).

The above changes were not accompanied by ultrastructural signs of intracellular regeneration. This may reflect a disturbance of protein biosynthesis in the hepatocyte, including structural proteins, which can intensify the process of apoptosis.

Further studies on the effect of long term exposure to large doses of lead are imperative, for it seems that the progressive cumulation of this metal in the organism may cause uncontrolled cell proliferation.

Conclusions

1. The degree of damage to the liver after the administration of lead acetate depends upon the dose, whereas the intensity of changes in the liver depends upon the length of time during which it was administered.

2. The increase in the activity of acidic phosphatase, alkaline phosphatase and adenosinetriphosphatase is within the widely understood parameters of liver adeptation to damaging factors.

3. After administering lead acetate in a dose of 15 mg/ 1kg body mass, deposits, probably composed of lead, cumulate in the hepatocyte hyaloplasm (sporadically in the nuclei) and are quickly eliminated by way of autophagocytosis and exocytosis.

4. After administering lead acetate in a dose of 30 mg/1 kg body mass many lead deposits cumulate in the hepatocyte nuclei (few in the hyaloplasm) causing simultaneously, the enlargement of the nucleoli and the condensation of chromatine into nucleus, which may be a sign of activity of the nucleus.

REFERENCES

1. Barry P. S.: A comparison of concentrations of lead in human tissues. Brit J. Ind. Med. **32,** 119, 1975.
2. Boyland E. et al.: The induction of renal tumours by feeding lead acetate to rats. Brit. J. Cancer **16,** 283, 1962.
3. Carmignani M. et al.: Cardiovascular actions of lead in rats as related to the level of chronic exposure. Arch. Toxicol. Suppl. **12,** 326, 1988.
4. Committee on Lead in the Human Environment: Lead in the Human Environment. National Academy of Sciences. Washington D.C. 1980.
5. Cramer K. et al.: Renal ultrastructure, renal function and parameters of lead toxicity in workers with different period of lead exposure. Brit. J. Ind. Med. **31,** 113, 1974.
6. Cullen M. R. et al.: Adult inorganic lead intoxication: Presentation of 31 new cases and a review of recent advances in the literature. Medicine **62,** 221, 1982.
7. Harman A. W. et al.: Induction of microsomal drug metabolism in man and in the rat by exposure to petroleum. Brit. J. Ind. Med. **38,** 91,1981.
8. Groniowski J.: *Apoptosis* — ubywanie komórek. Pat. Pol. **38,** 1, 1987.
9. Holtzman D. et al.: The pathogenesis of lead encephalopathy. Effects of lead carbonate feedings on morphology, lead content, and mitochondrial respiration in brains of immature and adult rats. Virch. Arch. Pathol. Anat. Hist. **387,** 147, 1980.
10. Mahaffey K. R.: Nutritional factors in lead poisoning. Nutr. Rev. **39,** 353, 1981.
11. Markiewicz K. et al.: Ostre zatrucie czterotlenkiem ołowiu (minią). Pol. Arch. Med. Wewn. **72,** 115, 1984.
12. Martin A. H., Ognivie D.: Behavioral malfunctions and histopathology of mouse central nervous system after pre-or postnatal exposure to lead. Anat. Res. **3** (194), 749, 1979.
13. Przybyłowski J.: Zmiany histopatologiczne i histochemiczne w wątrobie i płucach oraz zachowanie się niektórych parametrów biochemicznych w surowicy krwi w przewlekłym doświadczalnym zatruwaniu benzyną i etyliną. Pat. Pol. **30** (3), 387, 1979.
14. Rabinowicz M. B. et al.: Kinetic analysis of lead metabolism in healthy humans. J. Clinic. Invest. **58,** 260, 1976.
15. Stiller D., Friedrich H. J.: Ultrastructural and ultrahistochemical investigations of lead-induced intranuclear inclusion bodies in rat kidney. Exp. Path. **24,** 133, 1983.
16. Szarek J. et al.: Obraz patomorfologiczny narządów wewnętrznych szczurów intoksykowanych octanem ołowiu w dawce frakcjonowanej i wybranymi insektycydami fosforoorganicznymi. XI Zjazd Naukowy PTP. 1989, 109.

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EXPLANATION TO FIGURES

Fig. 1 and 2. Inflammatory infiltration consisting of mononuclear cells. H+E staining. Fig. 1 magn. 500 ×. Fig. 2 magn. 300 ×.

Fig. 3. Hepatocyte with a shrunken and condensed cytoplasm. Traits of mitochondrial swelling (M). A loosely lining, electron dense deposit is visible in the hyaloplasm (arrow). Magn. 800 ×.

Fig. 4. Perinuclear zone of the hepatocyte. Heterochromatine dominates in the nucleus and is marginally condensed. Also, open pores of the nuclear envelope are visible (arrow) along with concentrations of polirybosomes (R). Magn. 35,000 ×.

Fig. 5. Autophagic vacuoles (AV) on the biliary region of the hepatocyte contain glycogen, disintegrated fragments of cytoplasm, and regulary shaped, homogeneous, electron dense deposits (arrow). The lumen of the biliary canaliculi (BC) is significantly enlarged and the Golgo system (GS) is clearly active. Magn. 32,000 ×.

Fig. 6. Slightly widened canals of the rough endoplasmic reticulum (RER). Traits of focal degranulation of the rough membranes (arrow). In the cytoplasm secondary lysosomes (L), teleolysosomes and normal mitochondria (M) are present. Magn. 28,000 ×.

Fig. 7. Fully formed deposits in the hepatocyte nucleus are significantly electron dense with a homogeneous core and a margin made up of filaments and fine granules. Magn. 8,000 ×.

Fig. 8. A fully formed and newly forming deposit in the hepatocyte nucleus. The newly forming deposit resembles entwined thead, whereas the "developed" deposit consists of a dense homogeneous core surrounded by filaments. In the nucleus, large concentrations of heterochromatine are visible. Magn. 28,000 ×.

Fig. 9. Numerous fully formed "mature" deposits in a hepatocyte nucleus. Magn. 35,000 ×.

STRESZCZENIE

Na 75 szczurach badano działanie octanu ołowiu na wątrobę. Związek podawano zwierzętom dootrzewnowo w dawce 15 i 30 mg/kg m.c. w okresie 1, 2 i 3 miesięcy. Skrawki wątroby oceniano w mikroskopie świetlnym i elektronowym. U wszystkich zwierząt obserwowano w wątrobie występowanie z różnym nasileniem nacieków zapalnych złożonych z komórek jednojądrowych. W badaniach ultrastrukturalnych u zwierząt otrzymujących octan ołowiu w dawce 15 mg/kg stwierdzono obecność ołowiowych wtrętów głównie w cytoplazmie hepatocytów, natomiast po dawce 30 mg/kg wtręty ołowiowe występowały przeważnie w jądrze hepatocytów. W badaniach histoenzymatycznych stwierdzono w wątrobie niewielki wzrost aktywności fosfatazy kwaśnej, zasadowej i adenozynotrójfosfatazy.