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Zakład Histologii i Embriologii. Instytut Biologiczno-Morfologiczny. Akademia Medyczna w Lublinie
Kierownik: prof. dr hab. n. med. Józef Staszyc

Irena KRÓLIKOWSKA-PRASAŁ,
Teresa ZAKRZEWSKA-GRZYCKA

Histochemical Research on the Liver Tissue in Acute and Chronic Lead Stearate Poisoning

Badania histochemiczne tkanki wątrobowej w ostrych i przewlekłych zatruciach stearynianem ołowiu

Гистохимические исследования печёночной оболочки при острых и хронических отравлениях стеаринианом свинца

One of the substances which can produce acute and chronic poisoning is lead stearate — $(C_{17}H_{35}COO)_2Pb$. It was introduced into industry as a thermic stabilizor in the production of plastic material and as an addition to bearing greases (7, 11).

On the basis of available information concerning lead stearate poisoning (6, 7), the changes which occur in the liver are still discussed and that is why we have decided to trace the histochemical reactions in the liver of animals which have undergone acute and chronic poisoning by this substance. The investigations were also carried out with the aim to find out if, and to what degree, the changes are reversible.

MATERIAL AND METHOD

The reasearch was carried out on 50 male white rats of our own breed, body weight being about 250 g. All the animals, experimental and control were kept in identical conditions and fed on a standard diet throughout the experimental period. The experiment was carried out in two series A and B. Each series was divided, dependent on the lead stearate doses, into 4 experimental groups and 2 control group (K_1 and K_2). The experimental rats in series A and B were given

lead stearate dissolved in oil, at a permanent volume of 1 ml, by a stomach tube 4 times a week for 5 weeks. In the control group K_1 the animals received oil and in K_2 nothing except the normal diet. The rats in series A were decapitated in 24 hours and in series B 5 a week after receiving the last dose of lead stearate. The division into groups is given in Table 1.

Table 1. The arrangement of experimental groups in the A and B series

Groups	Series A mg/kg	Series B mg/kg
I	25	25
II	15	15
III	10	10
IV	5	5

The material for examination was collected from the right liver lobe, on which histochemical reactions were made, for succinic dehydrogenase activity according to Nachlas et al and for lactic dehydrogenase according to Pearse, using in both cases Nitro BT electrons as an acceptor. The incubation time was 45 min. at a temp. 37°C. The phosphatase activity was marked according to Gomorie's method. The control tests for the examined enzymes were made in incubation liquid which did not contain the substrate. Apart from the histoenzymatic reaction, glycogen was revealed by using the PAS reaction according to McManus, nucleic acid according to Brachet's method, and neutral lipids by Lill's method, using oil red. Hematoxylin and eosin staining was carried out on control slides.

RESULTS

The sections of the livers of experimental animals which were stained with hematoxylin and eosin did not show any significant differences in comparison to the pictures in the control group. Attention was drawn to a somewhat decreased size of the nucleoli, weaker eosinophilia and exsanguination in the I groups receiving the highest dose of lead stearate in series A and B.

Succinic dehydrogenase — the enzymatic marker of mitochondria indicated a decreased activity in comparison with that in the control group (Figs. 1 and 2) in its reactions in the liver of the experimental animals in series A. The smallest enzymatic reaction product occurred in group I of series A. Whereas in the liver of the animals in series B a decrease of the succinic dehydrogenase activity was smaller than that in the respective groups of series A. Group IV B showed identical histochemical reactions as those in the control group.

On the liver slides of the experimental animals a decrease of the enzymatic reaction of lactic dehydrogenase activity was observed in groups I and II of series A (Figs. 3 and 4) and only in group I of series B.

The microscopic pictures of the remaining groups were similar to those observed in the control groups.

An increase in the acid phosphatase activity and an increase in the number and size of phagocytic cells (Figs. 5 and 6) was found in the liver of the animals of all the groups of series A. The greatest visible changes were observed in groups I and II. In the histochemical reactions of series B an increase in the acid phosphatase activity was observed only in groups I and II, in the other no changes were observed.

The reaction to glycogen in the hepatocytes of the animals in series A in groups I—IV was distinct and increased. The largest amount of glycogen was observed in the hepatocytes of group I which received the largest dose of lead stearate. In this group the difference in the reaction intensity between the central and peripheral part of the lobule faded. Nearly all the cells were filled with glycogen granules to the same degree (Figs. 7 and 8). A decreased reaction to glycogen in the preparation of series B were only observed in groups I and II. However, when compared with the appropriate group in series A the amount of glycogen was somewhat smaller.

In the hepatocytes of series A a decreased RNA reaction in the cytoplasm was observed and the number of nucleoli also decreased, being often single and small in size. The highest number of hepatocytes with the above changes were observed in the animals of group I which were given the highest dose of lead stearate (Figs. 9 and 10) the least in group IV. The changes in the animals in series B, group I were similar to those in group A-I, whereas in group B-II the cytoplasm pyronine absorption was greater than that in A-II. The nucleoli were larger and the cells with 2 nucleoli were frequently found. The remaining groups in series B showed no changes.

In the groups of series A a distinct increase of lipids was observed on the periphery as well as in the central parts of the liver lobules. Groups I—IV of this series differed in the size of lipid droplets. The largest amount of hepatocytes with big lipid droplets was found in group I (Figs. 11 and 12) and in group IV which obtained the lowest dose of lead stearate the cells contained small lipid droplets.

In the livers of animals in series B a large amount and large balls of lipid were ascertained only in group I and exclusively on the outer limits of the lobule near the triad. Whereas in group II—IV the steatosis concerned the whole lobule, but was weaker in comparison with the parallel group in series A. It is worth noting that the reactions to lipids in the experimental groups were compared with the both control groups K_1 and K_2 as in group K_1 which received oil, an unsignificant increase in the reaction to lipids was noticed.

DISCUSSION AND CONCLUSIONS

The lead salts of organic acids can accumulate in the tissue in a way similar to that of inorganic lead salts (9, 11). Guerdjikoff et al. (3), assumed that the toxicity of lead compounds in the body may depend on their ionization. Although lead stearate as a hydrophobe compound dissolves in lipids and undergoes ionization, its harmful influence on the body has been shown to be small.

Lead stearate effects the behaviour of the liver enzymatic system which is especially sensitive (2, 4). Therefore we have thorougly analyzed, apart from the general morphological structure of the liver tissue, the changes in the activity of succinic dehydrogenase, lactic dehydrogenase, acid phosphatase and, additionally, that of glycogen, lipids and RNA.

In the liver of the animals in series A the reaction to succinic dehydrogenase showed a decrease in the enzymatic activity and was the smaller the larger was the dose of lead stearate which was given to the experimental animals. Whereas in series B a decrease in the succinic dehydrogenase activity was lower than in the respective groups of series A. Group IV-B did not show any histochemical reaction at all. This may lead to an assumption, that changes produced by lead stearate gradually retreat during 5 weeks when the animals cease to be given the compound.

Lead stearate caused only a decrease in the lactic dehydrogenase activity in the liver of the animals in groups I and II, whereas the activity in groups III and IV of series A unchanged. The liver of animals in series B a change in the lactic dehydrogenase activity was only observed in group I. This indicates a lower sensitivity of lactic dehydrogenase to the action of lead stearate than succinic dehydrogenase and a greater ability to retreat the changes caused by it.

The observed by us, as well as by Valade and Coste (10), distinct rise in the acid phosphatase activity and lysosome increase in the liver of animals receiving lead stearate and increase in the phagocyte cells in the liver cavities, may be an expression of an infectious condition of the liver or it may be evidence of degeneration processes in the hepatocytes. Sassi et al. (8) also indicated that lead stearate causes, in persons working in its production, damage to the liver tissue, similar symptoms are also given by inorganic lead compounds. Whereas Głuszcz and Mylak (1) did not find any liver damage in 4 clinical cases of lead stearate poisoning.

The carried out by us histochemical reactions to glycogen indicate an increase in its quantity in the liver of animals in series A which is proportional to the size of the lead stearate dose. Whereas distinct reaction differences in series B were not shown. The observed changes in the

glycogen level in the hepatocytes of animals poisoned by lead stearate indicate that disturbances in the glycogen accumulation occur shortly after the administration of the preparation and depend on the amount of the dose, whereas in the later period the change retreat.

The decrease in the reaction to RNA in the groups of series A also depends on the dose of lead stearate, the amount of RNA decreases alongside the increase in the size of the dose. Similar changes which were also observed in series B may be evidence of a decrease in the protein synthesis process in the cell, which leads to a decrease in the organism resistance (5).

The lipids content in the liver tissue of animals in series A was distinctly higher and its distribution within the periphery of the lobules differed from the physiological conditions. The steatosis was extensive and embraced the whole of the liver lobule. Similar observations were made by Valade and Coste (10) who experimented on dogs and rabbits. Such steatosis is equivalent to degeneration changes and leads to the necrosis of the liver.

The influence of lead stearate on the liver tissue depends on the duration of its administration, size of dose, the break after the last administration and also on the way it was administered. On comparing the dose given by us per os with the dose given by means of inhaling by other authors (1) the latter would be lower in comparison to the changes observed in our experiment. This may be significant to professional diseases where the poison enters through the respiration system.

On the basis of the obtained results one can make the following conclusions:

1. Lead stearate given to rats by stomach tube causes liver damage. This damage is manifested by extensive steatosis of the liver tissue, a decrease in the activity of succinic and lactic dehydrogenase and lower RNA reactions and an increase in the activity of acid phosphatase and in the reaction to glycogen.
2. The degree of damage depends on the size of the given dose.
3. Changes caused by lead stearate indicate a certain tendency to disappear after a five week break in the drug administration.

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EXPLANATION TO FIGURES

Fig. 1. Reaction to succinic dehydrogenase activity in the liver of control animals. Method of Nachlas et al. Magn. ca 200×.

Fig. 2. A liver of an experimental rats, group I series A. Reaction to succinic dehydrogenase activity. Method of Nachlas et al. Magn. ca 200×.

Fig. 3. Reaction to lactic dehydrogenase activity in the liver of control rats. Magn. ca 200×.

Fig. 4. Reaction to lactic dehydrogenase activity in the liver of experimental rat, group I series A. Magn. ca 200×.

Fig. 5. Reaction to acid phosphatase activity in control groups. Magn. ca 200×.

Fig. 6. A liver of an experimental rat, group I series A. Reaction to acid phosphatase activity. Magn. ca 200×.

Fig. 7. Reaction to glicogen in the liver of control group. Magn. ca 200×.

Fig. 8. Reaction to glicogen in the liver of experimental rat, group I series A. Magn. ca 200×.

Fig. 9. Reaction to RNA in the liver of control group. Magn. ca 400×.

Fig. 10. RNA in the liver of experimental group I, series A. Magn. 200×.

Fig. 11. Reaction to fats in the liver of control animals. Magn. 200×.

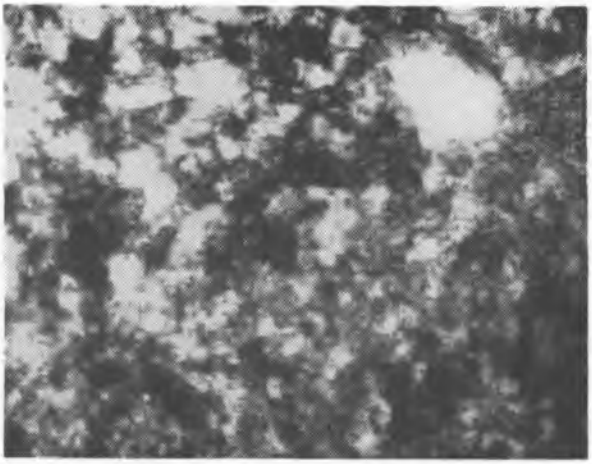
Fig. 12. Reaction to fats in group I, series A. Magn. ca 200×.

STRESZCZĘNIE

Badania histochemiczne przeprowadzono na skrawkach wątroby szczurów białych, którym podawano dożołądkowo stearynian ołowiu w 4 różnych dawkach przez okres 5 tygodni. Wyniki badań wskazują, że wielkość zmian w odczynach histochemicznych zależy od wielkości dawki stearynianu ołowiu. Dawka 5 mg/kg ciężaru ciała daje najmniejsze zmiany w przeprowadzonych reakcjach i są one odwracalne po 5-tygodniowej przerwie od zakończenia podawania tego związku.

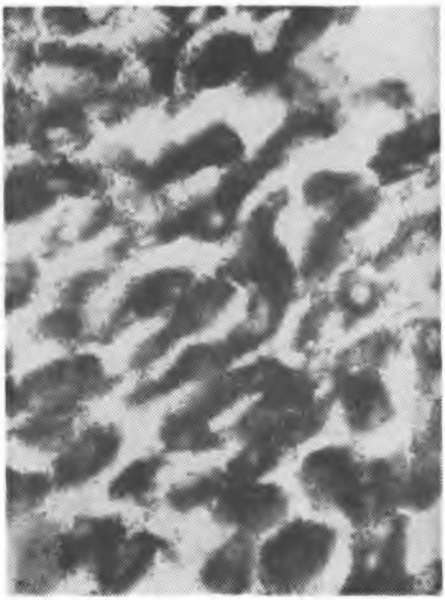
РЕЗЮМЕ

Гистохимические исследования проведены на печени белых крыс, которым давали дожелудочно стеариниан свинца в 4 дозах в течение 5 недель. Результаты исследований указывают на то, что величина изменений в гистохимических реакциях зависит от дозы стеариниана свинца. Доза 5 мг/кг вызывает наименьшие изменения в проведенных реакциях и они оборотные после пятинедельного перерыва от последней дозы этого химического соединения.



Ryc. 1

Ryc. 2



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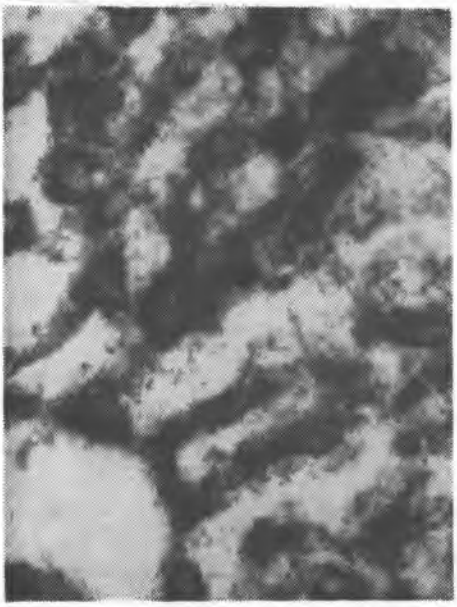

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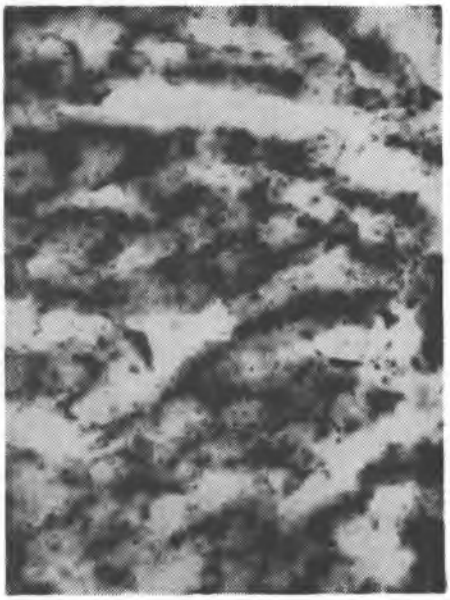

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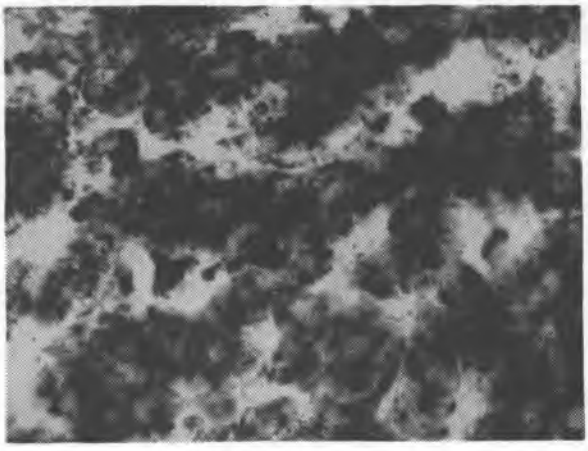

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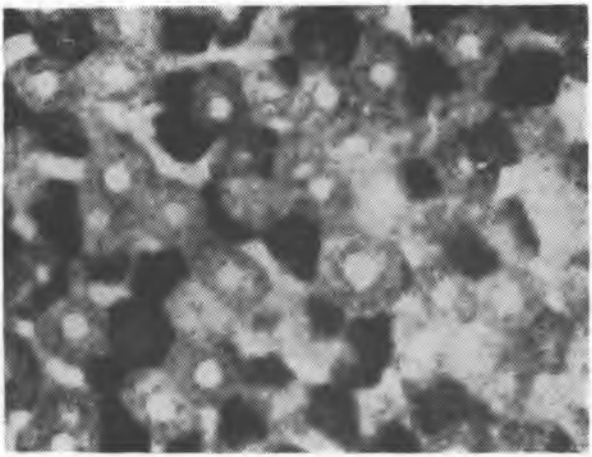

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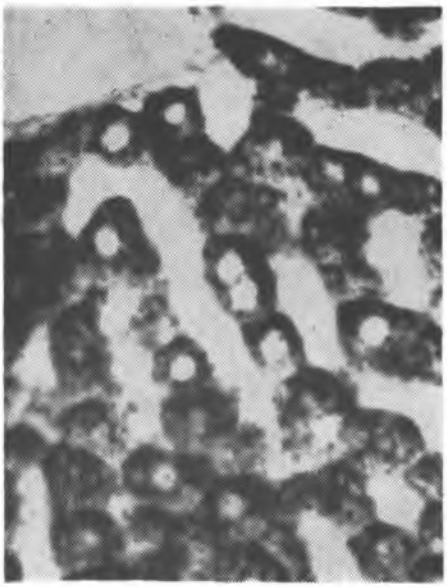

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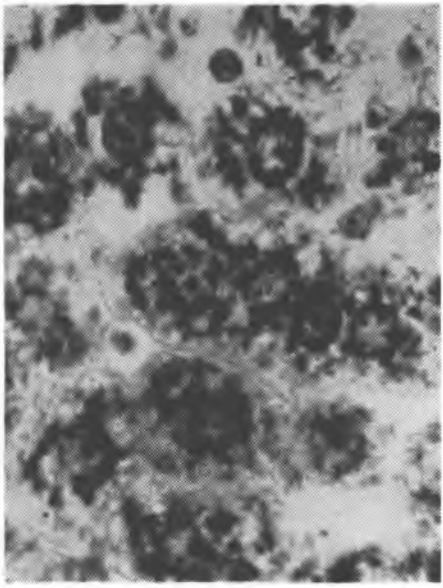

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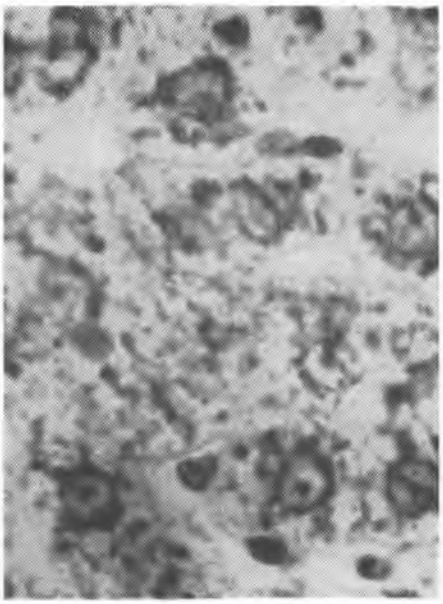

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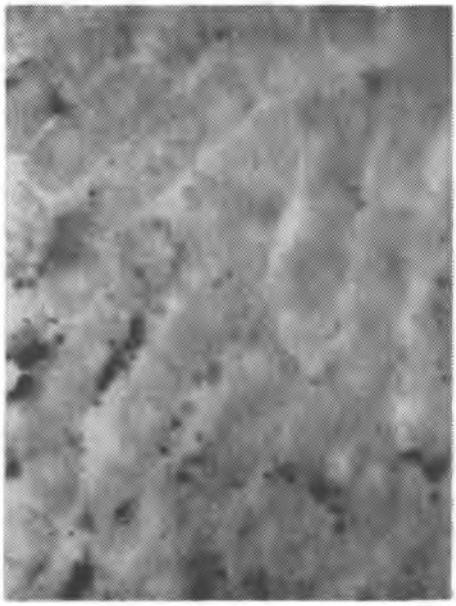
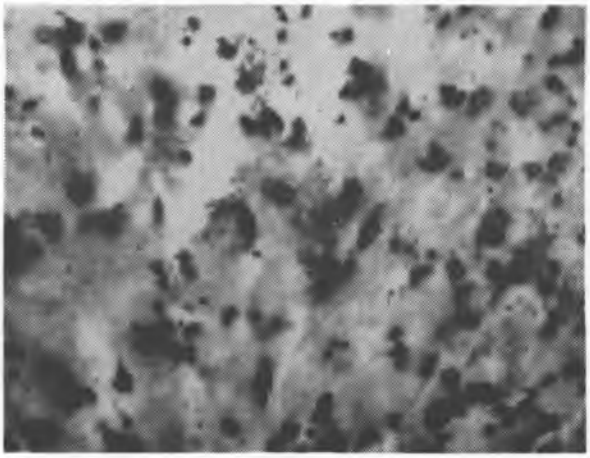


Ryc. 11



Ryc. 12