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Kierownik: prof. dr hab. n. med. Daniel Chibowski

Jadwiga SIEROCIŃSKA-SAWA, Franciszek WOŹNIAK

Morphological and Histochemical Research into the Influence of the Continuous Administration of Lead Acetate on the Microstructure of the Male Gonad in the White Rat

Badania morfologiczne i histochemiczne wpływu stałego podawania octanu ołowiu na mikrostrukturę gonady męskiej szczura białego

Chemical contaminations of the environment by heavy metals are commonly regarded as the most dangerous form of such contaminations. Among those contaminants lead is ranked uppermost in terms of toxicity. It is generally referred to as "the element of death". A long-term cumulative exposure to even small doses of toxic lead compounds is a threat to human and animal organisms and may have negative genetic consequences. Lead is capable of impairing many elements of the systems, which results from its great affinity to tissues, various enzymes and serological systems. It may also accumulate.

The toxic influence of lead on gonads, especially on the male gonad, is little known since the latter is rarely subjected to a detailed histopathological examination. Nevertheless, as it is generally assumed, the male gonad is a very sensitive indicator of toxicity of administered chemical compounds.

The purpose of the present study was to show whether the continuous action of lead compounds may effect some histopathological and histochemical changes in the male gonad of the white rat and, if so, to trace and define those changes. The study also aimed at proving the influence of a selected lead compound on the spermatogenesis and spermiogenesis in the rat. It also sought to prove the compound's influence on the interstitial cells of Leydig as well as on the biological activity of the male rats which comes into play here.

MATERIALS AND METHODS

75 white male rats of the Wistar breed were used for study. They were genetically homogeneous, weighed 200 g on the average, lived in constant environmental conditions during the experiment, and were fed by a standard LSM food and water *ad libitum*. They were clinically healthy.

The animals were divided into three groups (I—III). Two groups were experimental (I and II) and numbered 30 animals each. One control group (III) numbered 15 animals. The rats from the experimental groups received intraperitoneally lead acetate dissolved in 1 ml of physiological salt twice a week in the dosage of 15 and 30 mg/kg respectively of the body mass. The control group rats

received 1 ml of physiological salt. Time of administration of lead acetate and psysiological salt to the animals of groups I—III was a) 1 month, b) 2 months, c) 3 months.

On completion of the lead acetate administration the rats were killed after 1, 2 or 3 months. During the same period, the rats from the control group were killed in tandem. The testes along with the epididymis taken from the killed rats were first assessed on the macroscopic scale (their appearance, size, weight, consistency). Then they were fixed in 10% neutral formalin. The preparations made of paraffin segments were then dyed using the following methods:

The histological methods: 1) hematoxylin — eosin stain; 2) safranines stain; 3) silver impregnation technique for argentaffin fibres according to Gomori; 4) azan according to Heidenhain; 5) resorcin — fuchsin according to Weigert; 6) periodic acid — Schiff reaction (PAS).

The histochemical methods: 1) lipid dyeing according to Lillie; 2) reaction to acid phosphatase according to Gomori; 3) reaction to alkalic phosphatase according to Gomori; 4) reaction to adenosine triphosphatase according to Wachstein; 5) reaction to 5-nucleotidase according to Wachstein and Meisel; 6) reaction to non-specific esterase according to Barnet and Seligman.

Numerical data on testes mass obtained in this manner were subjected to statistical analysis. The analysis of the toxic factor (lead acetate) was carried out on the masses of both testes in the control group (III) and in both experimental groups (I and II).

THE HISTOLOGICAL FINDINGS

No significant changes have been reported in the animals from the control groups during the experiment. In group IIc (which received lead acetate in the dose of 30 mg/kg of the body mass) no rat lived longer than three months of the experiment's duration. The most apparent changes reported in the animals which received lead acetate were a significant reduction of the body mass and testes' mass, proportional to the dose and time of administration.

In all rats from the experimental group some clear-cut histopathological changes have been reported, which were intensified during the experiment. In the group which received lead acetate in doses of 15 mg/kg of the body mass there was apparent necrosis and exfoliation of the immature germ cells into the lumen of the seminiferous tubules and edema was seen in the intertubular spaces (Fig. 1). In group Ic there was observed a massive exfoliation of the degenerated and necrotically changed spermatocytes at various stages of development into the lumen of the seminiferous tubules along with the formation of the cellular embolus (Fig. 2). Degenerative and necrotic changes in the seminiferous epithelium and quite numerous divisive figures in the germ cells were also highly prominent.

In all rats from the second group which received lead acetate in doses of 30 mg/kg of the body mass clear-cut histopathological changes have been reported ,which intensified in the course of the experiment. In many visible sites narrowing in the diameter of the seminiferous tubules was apparent. There were extensive degenerative, necrotic and atrophic changes of the seminiferous epithelium with a total lack of clarity in its structure. The seminiferous tubules often contained only the immature, and to a considerable extent degenerated and

exfoliated cells of the seminiferous epithelium. Some tubules contained only the cells of Sertoli.

During the experiment the changes became more and more intense. There were apparent atrophic areas of the seminiferous tubules lined with Sertoli's cells alone (Fig. 3). Numerous syncytial gigantic multinucleate cells were formed from the exfoliated degenerated spermatocytes. Edema was present in the intertubular spaces. Annular fibrination and amelification of the seminiferous tubules' own membranes and also fibrination of the intertubular space was highly marked. In the fibrinated connective tissue "annuli" of the tubules there was a marked multiplication of the number of elastic fibres. Some atrophic seminiferous tubules were present with the concretes of dystrophic calcification. The ducts of the epididymis were often totally empty.

THE HISTOCHEMICAL FINDINGS

In the routine histopathological studies carried out during the experiment Leydig's cells did not manifest greater changes, except in those sites in which cells underwent hyperplasia.

In those foci there is an increased accumulation of lipids and a decrease in the number of androgenic seeds in cells, with a concomitant multiplication of the number of elastic and argentaffin fibres in those sites where the cells were. The impairment of the seminiferous epithelium of the contorted tubules is often accompanied by the growth of Leydig's cells.

The histopathological changes in the seminiferous epithelium of the rat's testis and in the interstitial gland of Leydig's cells were accompanied by or preceded by serious histoenzymatic changes in the cells of those elements of the testis.

The changes also affected the seminiferous tubules in which no essential histopathological changes have been reported or were minimal. They also affected the Leydig's cells'interstitial gland. The changes were: an increase in the enzymatic activity of acid phosphatase (ACP-ase) and non-specific esterase (N—E). The activity of alkalic phosphatase (ALP-ase) and 5-nucleotidase (5-N-ase) did not, however, change.

Out of the phosphatases under study the change in the activity of the reaction was manifested by acid phosphatase (ACP-ase) and adenosine triphosphatase (ATP-ase). The strongest activity of acid phosphatase in physiological conditions may be reported in Leydig's cells, and markedly weaker in the immature seminiferous cells, lying close to the seminiferous tubules' own membrane. The behaviour of acid phosphatase is a sensitive indicator of the impairment of spermiogenesis.

The increase in the enzymatic reaction on the acid phosphatase in Sertoli's cells is most probably connected with phagocytosis in the impaired dead cells of the

seminiferous epithelium, and to an increase in the lysosomal activity of Sertoli's cells during that phagocytosis. Leydig's cells also manifested an increased reaction to acid phosphatase. It is certainly associated with the removal of ageing and impaired cells by lead cells. Yet it is probably also related to the decrease in the activity of some enzymes which take part in the steroidogenic action of Leydig's interstitial gland.

The research proved that under the influence of lead the enzymatic activity of non-specific esterase (Fig. 4) increases in Leydig's and Sertoli's cells. The increase in the activity became more and more intense in each of the groups.

We have reported the increase in the activity of adenosine triphosphatase in the seminiferous tubules'own membranes, in the wall of blood vessels and in the immature seminiferous cells. The enzymatic activity of ATP-ase increased in proportion to the dose and time of lead acetate administration. The latter did not have impact on the enzymatic activity of 5-nucleotidase. An increased furring up of lipids in the epithelium of the seminiferous tubules and cells of the interstitial gland was also observed.

DISCUSSION

The histopathological changes that we have reported appeared in all experimental groups as the focal, rarely diffused, inhibition of spermatogenesis and spermiogenesis. They also appeared most often as focal impairment of the seminiferous epithelium and histopathological changes in Leydig's cells' interstitial gland. The focality of those changes is most probably related to the cyclical manner of the changes in the seminiferous epithelium of the contorted tubules, and consequently, in various sensitivities to deleterious factors.

Degenerative changes in the seminiferous epithelium, ranging from the hydropic degeneration, and steatosis of the seminiferous cells to their necrosis inclusively, caused exfoliation of the immature, degenerating cells into the lumen of the tubules. Sometimes one observed creation of gigantic cells which were visible in the lumen of the seminiferous tubules, and also the degenerated seminiferous cells and semen in the epididymis ducts. The gigantic cells were found almost in every case of impairment of the seminiferous epithelium due to various impairing factors (5, 11—13, 16, 17). The gigantic cells originate from the young, degenerating germ cells, most often spermatocytes and spermatids. Only in few seminiferous tubules, in which the impairment of the epithelium was most prominent, there was reported almost total absence of the seminiferous epithelium with the preservation of unaffected Sertoli's cells which, as it is well-known, are most resistant to the action of deleterious factors.

In the descriptions of the ultrastructural study of testes under electron transmission and scanning microscope an increase may be noted in the number and in the swelling of lysosomes in Sertoli's cells (1, 9).

Saxena et al. (11, 12), Chowdhury et al. (2, 3) and Veit et al. (15) described the changes which they observed, including the disappearance of testes, inhibition of spermatogenesis tearing off the single seminiferous cells from the baseline membrane, and degeneration of the seminiferous cells. There was also a narrowing of the lumen of seminiferous tubules and a slight edema in the intertubular space. In the foci where Leydig's cells underwent proliferation there was an enhanced aggregation of lipids and a decrease in the number of androgenic seeds in the cells, with a concomitant multiplication of the number of elastic and argentaffin fibres in the sites where those cells were. The impairment of the seminiferous epithelium was often accompanied by the proliferation of Leydig's cells, which was observed in rats after experimental administration of cadmium, pesticides, insecticides and with protein deficiencies (6, 7, 11, 16, 18—20).

The histopathological changes in the seminiferous epithelium and in Leydig's cells which were described were accompanied by or preceded by serious histoenzymatic changes in the cells of those elements of the testis. The changes were the following: an enhanced enzymatic activity of ACP-ase, ATP-ase and N—E. The activity of ALP-ase and 5-N-ase, however, did not change. The reaction of ACP-ase is a sensitive indicator of the spermiogenesis impairment (4). What proves the latter is a heterogeneous enzymatic activity in particular seminiferous tubules probably linked with the periods of spermatogenic cycle (4, 18). Non-specific esterases are a group of enzymes highly resistant to the activity of impairing factors. The enhanced activity of N—E increased proportionally with the dose and time of lead acetate administration.

Miętkiewski and Fichna (8), who gave the rats Endoxan, and Cieciura (4), who irradiated their bodies with X-rays, also reported an increase of N—E activity in the interstitial tissues of the testis and in the seminiferous tubules' own membrane. The enzymatic activity of ALP-ase in the study did not undergo any change in the groups. Chowdhury et al. (3) in his research proved, however, a decrease in ALP-ase activity in the tissue of the rats' testes after the administration of large doses of lead.

We proved that the activity of ATP-ase in the seminiferous tubules' own membrane, in the wall of blood vessels, and in the immature seminiferous cells increased in proportion to the dose and period of lead acetate administration. Saxena et al. (10, 11) proved the inhibition of ATP-ase activity, of succinate dehydrogenase and of glucose-6-phosphatase, which is most probably connected with the pathological changes and decrease in the number of sperms in the testes of those rats which received lead. The absence of changes of 5-N-ase activity goes without saying if in the tissues under study it is not concomitant with ALP-ase.

The above disturbances in enzyme activity in steroidogenesis in the testis (without having a direct part in it) are intermediate morphological indices of a decrease in testosterone synthesis in the testis. This is reflected in a decrease

in sexual activity in male white rats. Undoubtedly, a long-term lead acetate administration has a cytotoxic impact on the male gonad of the rat. On the basis of our research we can state, in line with Stow and Goyer (14), that in the case of toxic reaction to lead the irregularities of the reproduction of sperm are proportional to the continuous exposure to lead.

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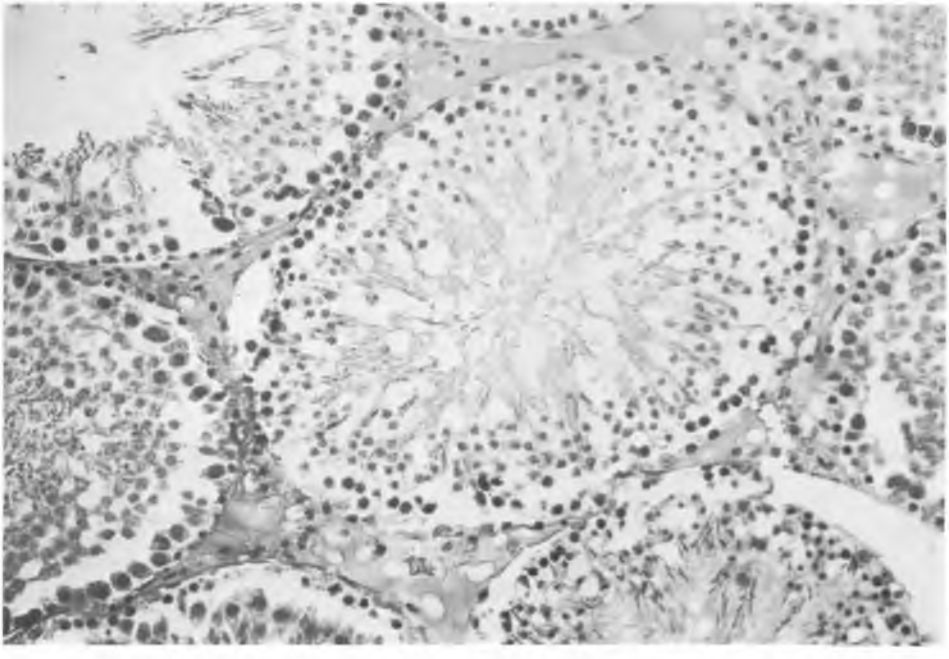

Fig. 1

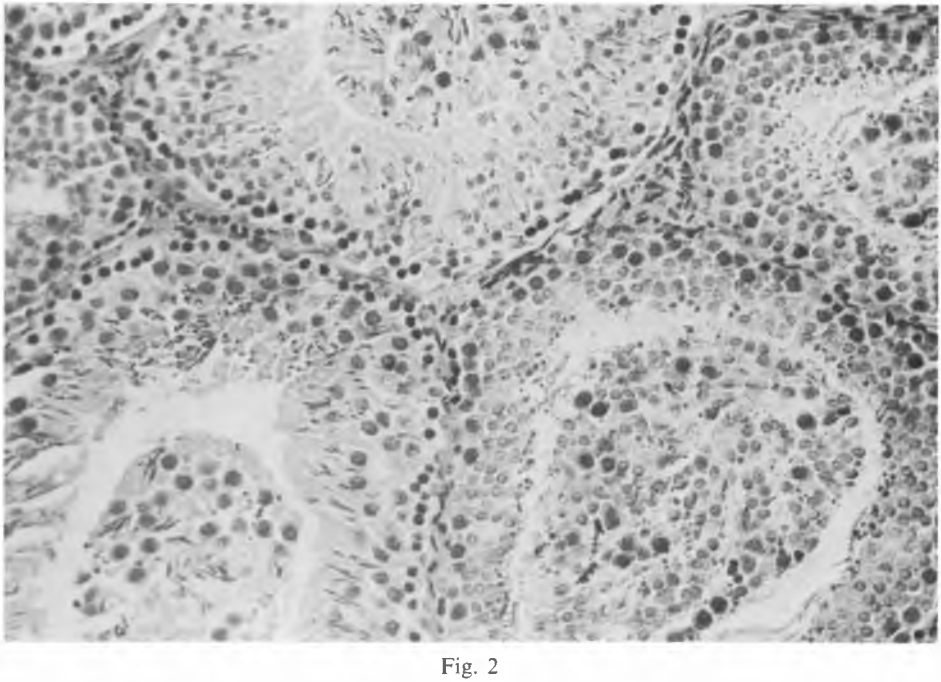

Fig. 2

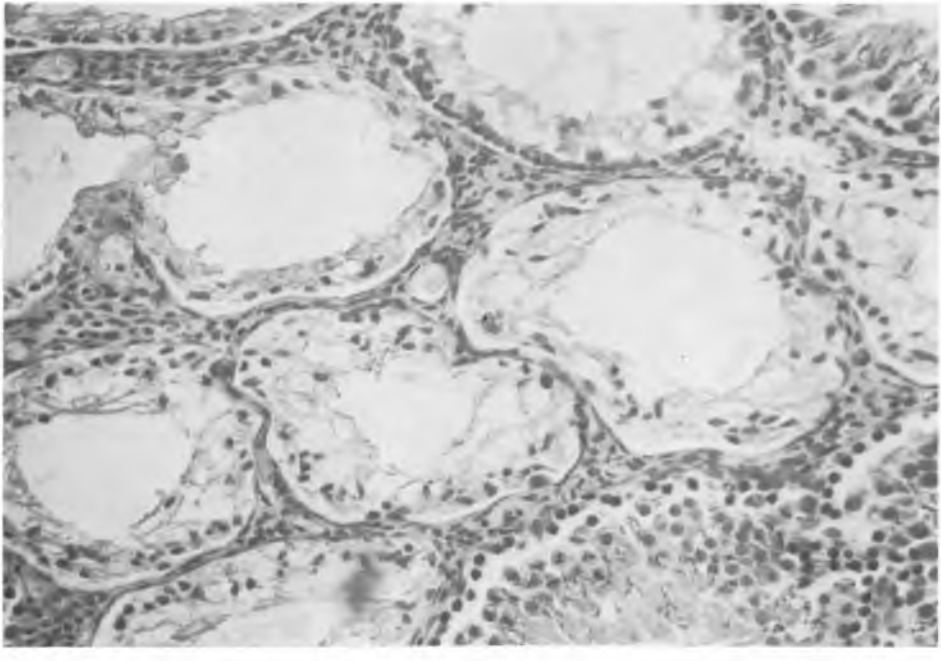

Fig. 3

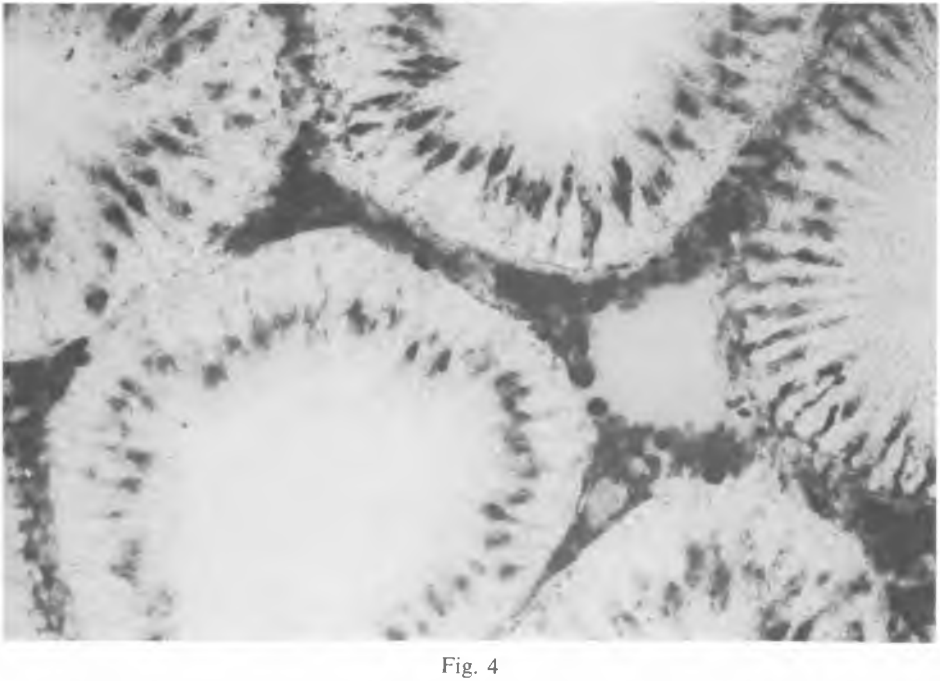

Fig. 4

EXPLANATION TO FIGURES

Fig. 1. Group I b. Necrosis and exfoliation of the immature germ cells into the lumen of the seminiferous tubules and edema of the intertubular spaces. Hematoxylin-eosin stain. Magn. 200 ×.

Fig. 2. Group Ic. Massive exfoliation of the degenerated and necrotically changed spermatocytes at various stages of development into the lumen of the seminiferous tubules along with the formation of the cellular embolus. Hematoxylin-eosin stain. Magn. 200 ×.

Fig. 3. Group IIb. Atrophic areas of the seminiferous tubules lined with Sertoli's cells alone. Hematoxylin-eosin stain. Magn. 200 ×.

Fig. 4. Group IIa. The increased activity of non-specific esterase in Leydig's and Sertoli's cells. Barnet and Seligman stain. Magn. 200 ×.

STRESZCZENIE

Badania przeprowadzono na 75 szczurach płci męskiej. Szczurom podawano octan ołowiu 2 razy w tygodniu dootrzewnowo w dawkach 15 i 30 mg/kg m.c. w okresie 1, 2 i 3 miesięcy. Pobrane od zabitych szczurów jądra oceniano makroskopowo i mikroskopowo, badano również aktywność enzymów: ACP-azy, ALP-azy, ATP-azy, N-E i 5-N-azy. Badaniem histochemicznym objęto także lipidy.

Stwierdzono wzrost aktywności ACP-azy, ATP-azy i N-E oraz wzmożone gromadzenie się lipidów w komórkach nabłonka nasiennego i komórkach Leydiga. Obserwowane zmiany histopatologiczne to zmiany zwyrodnieniowe i martwicze w nabłonku nasiennym. Towarzyszyło im zmniejszenie masy jąder będące wynikiem częściowego zaniku gonady.