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Polymetallic Industrial Dust Affects Mice Spermatogenic Cells and Chromosomes

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Polymetallic industrial dust as a waste product in lead-zink factory near Assenovgrad (Bulgaria) contains Cd, Pb, Cu and Zn which accumulate in liver, kidney and spleen but its effect on spermatogenesis is unknown. ICR mice were given a diet enriched of heavy metals Cd, Pb, Cu and Zn for 15, 40, 60 and 90 days. The ultrastructural alterations in male germ cells and chromosome reactivity were studied. Spermatogonia and spermatocytes were found the most sensitive cells. The disturbances were seen predominantly in the cytoplasm and nuclei of the spermatocytes. Chromosome aberrations of chromatide and isochromatide type, Robertsonian translocations and changes in chromosome spiralization were significantly increased in all experimental groups. There was no direct correlation between duration of the treatment and the percentage of aberrant mitoses. The presence of metaphases with altered coiling and "C-banding" effect related with changes in chromatin spiralization were confirmed by ultrastructural observation on spermatogenic cells.

Key words: heavy metals, spermatogenesis, chromosomes.

Introduction

The drastic increase in number of toxic chemicals in environment results irreversible adverse effects in animals and human beings. Reproductive system is very sensitive to different environmental factors and could be used as a useful tool to solve many ecological problems. Mammalian testis is a unique organ producing both sperm and androgens serving to maintain reproductive function [5]. Due to complexity and long duration of the spermatogenic process, the testes of mammals are highly sensitive to damage produced by environmental expose to chemicals [6]. The process of spermatogenesis is under the control of hormones and local growth factors acting through paracrine and/or autocrine mechanisms. Testicular germ cells are situated in their own environment – Sertoli cell cytoplasm. In this respect germ cells are environmentally well preserved cells and Sertoli cells act as a mediators in transmission of different molecules. The polymetallic industrial dust as a waste product in lead-zinc factory near Assenovgrad (Bulgaria) is very rich in heavy metals Cd, Cu, Pb and Zn. In our previous article we have published the bioaccumulation of the above heavy metals in liver, kidney, spleen

and bones in mice [7] and the effect on body weight, blood, oxygen consumption, mitotic index etc. [8]. In the present study we focus on the in vivo effect of Cd, Cu, Pb and Zn on subcellular structure of spermatogenic cells and chromosome reactivity in mice under ecologo-toxicological experiment.

Material and Methods

Male 10 weeks old ICR mice were given a diet enriched of heavy metals (mg/kg) as follows:

	n	Cadmium	Lead	Copper	Zinc
Experimental	5	64.1± 10.5	784± 244	20.9 ±7.3	1945±429
Control	5	3.5 ±1.9	61.8± 20.9	1.3 ±1.1	90.9± 25.9

Polymetallic industrial dust from electrofilters of the lead-zinc rafinery near Assenovgrad (Bulgaria) was mixed mechanically at 1 % ratio from conventional animal food. Samples were taken on the 15, 40, 60 and 90 day of treatment. Two hours before sacrification mice were given C-methyl 3H-thymidine (NEW Products, Boston, MA)(spec act. 20 mCi/mmol) in a dose of 2 μCi/g of body weight. Pieces of testes were fixed in Serra's fixative or in 2.5% glutaraldehyde and proceeded for routine histological, autoradiographic and ultrastuctural study. Chromosome preparations "slides" were obtained from colchicine blocked bone marrow cells (2–4 g/kg b.w.) according to a routine protocol [4]. Air dried slides were stained with 5% Giemsa solution and observed under light microscope.

Results and Discussion

The testicular tissue in control mice displayed normal appearance and well distinguished stages of seminiferous epithelium. Histological assessment did not show significant differences between the testes of control and experimental animals. On the autoradio-

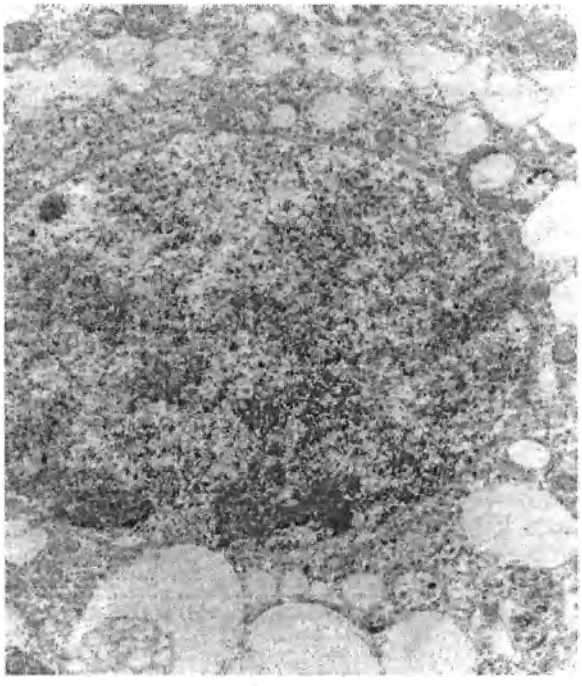

Fig. 1. Mouse testis on the 15 day of the treatment with polymetallic industrial dust. Spermatocyte with well preserved nucleus and highly vacuolized cytolpasm (× 7000)

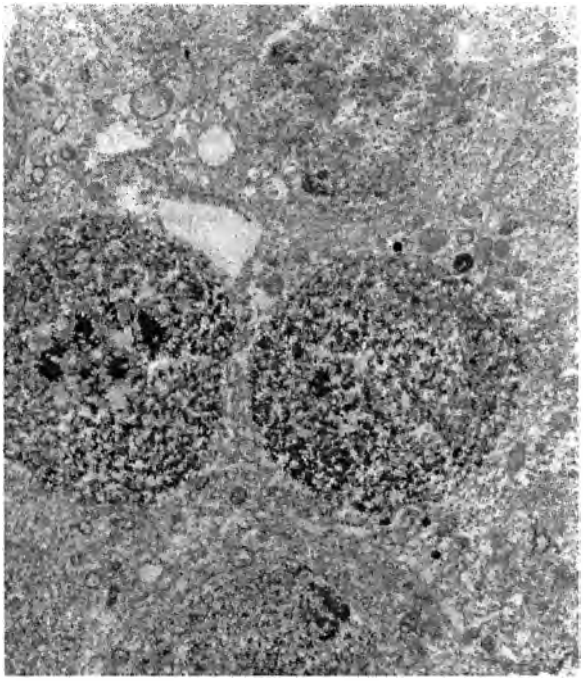

Fig. 2. Mouse testis on the 40 day of the treatment. Two degenerative spermatocytes are visible (× 3000)

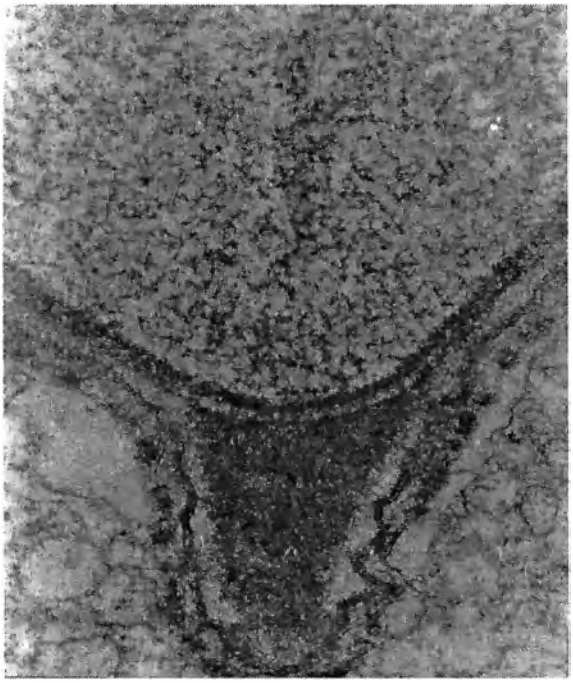

Fig. 3. Mouse testis on the 60 day of the treatment. Spermatid with undulated acrosomal membrane is visible (× 30 000)

graphs the percentage of labelled spermatogonia in control mice was 42±3 without significant difference between control and experimental groups with exception of day 40 where 20±2% of spermatogonia were labeled.

At the ultrastructural level in the experimental groups different alterations were visible. On day 15 of the experiment the cytoplasm of some spermatogonia and spermatocytes was highly vacuolized (Fig. 1) but the nucleus was relatively well preserved. On day 40 the nuclear membrane of some spermatocytes was strongly affected and the chromatine was condensed in amorphous heterochromatine accumulations (Fig. 2). The tripartite structure of synaptonemal complexes (SC) was well preserved even in completely degenerated pachytene spermatocytes. The higher resistance of SC was observed after in vivo X-ray irradiation due to a high degree of chromatin condensation and the strength of DNA-protein complexes [3]. On day 60 rarely some spermatids have shown abnormal acrosome with deep foldings of the acrosome membrane (Fig. 3). Fully degenerated spermatocytes were visible at day 90 of the experiment (Fig. 4).

In addition to damaged spermatogenic cells, majority of spermatogonia, spermatocytes, sprmatids and spermatozoa have shown normal ultrastructure. Sertoli and Leydig cells showed normal appearance as well. The cytoplasm of some Sertoli cells was rich in lysosomes as an expression of increased phagocytic activity. The higher bioaccumulation of heavy metals in liver, kidney and bones on the 40, 60 and 90 day of the experiment [7] coincides with the above described ultrastructural alterations of spermatogenic cells. Obviously the excess exposure of the organism to heavy metals disturbs the whole homeostasis including reproductive system. More studies are required to follow the effect of interactions between several heavy metals like Cd/Zn, Cd/Cu, Pb/Cu on the process of spermatogenesis.

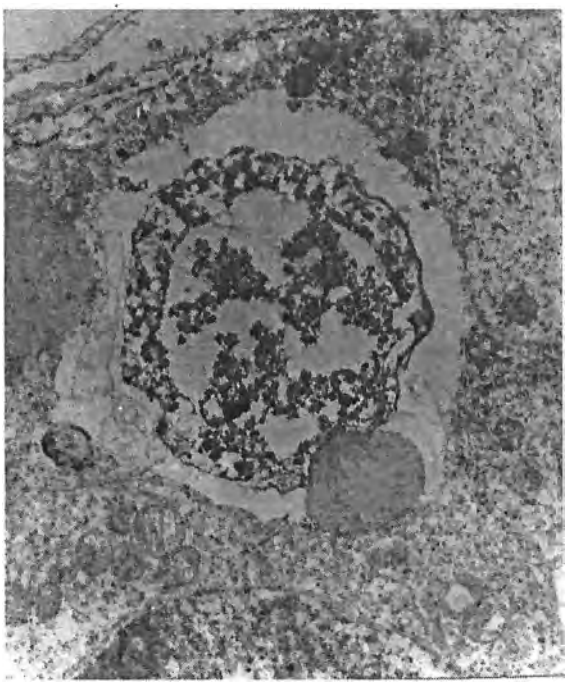

Fig. 4. Mouse testis on the 90 day of the treatment. Fully degenerated spermatocyte situated on the basal membrane (× 7000)

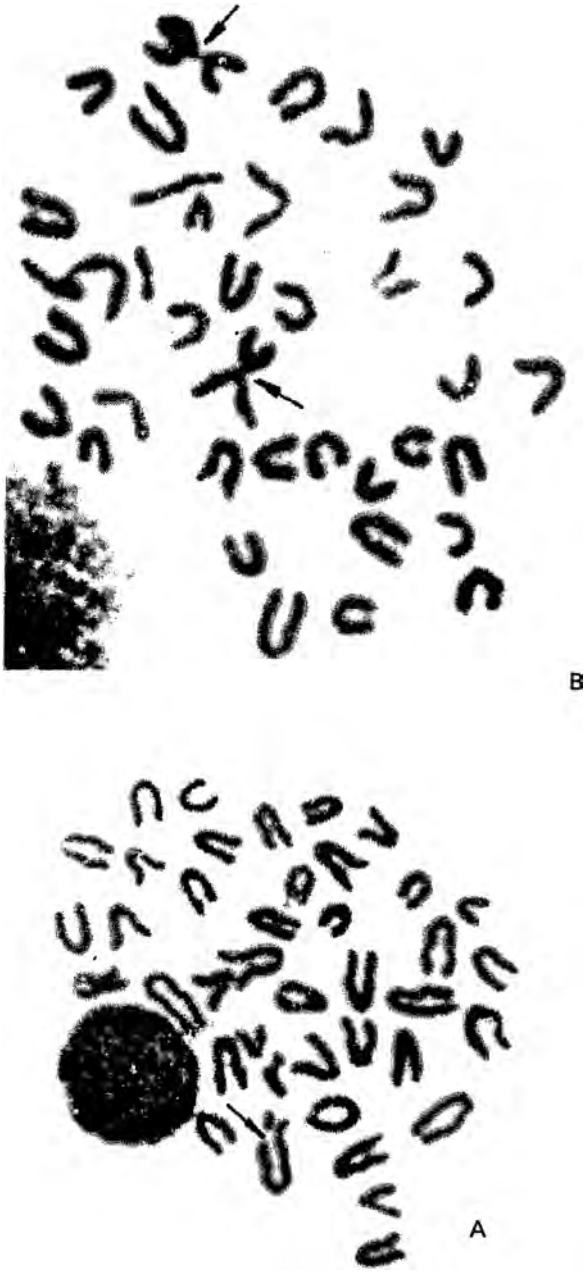

Fig. 5. Chromosome aberrations in bone marrow cells from mice treated with polymetallic industrial dust. A – Chromatide breaks (arrow); B – Centromeric fusions (arrows)

Analysis of the metaphase preparations including structural and numerical changes was carried out both on control and treated mice. Chromosome aberrations were of chromatide (breaks and fragments) or isochromatide type – the first prevailing (Fig. 5). The presence of ring chromosomes and Robertsonian translocations was also noticed. The analysis of characteristics of the chromosome aberrations suggests they are the results of clastogenic effect of Pb++ as a basic component of applied industrial dust [1, 2]. Metaphase analysis has shown a lack of direct correlation between duration of the treatment and the total percentage of aberrant metaphases. The constancy in harvested aberrations in rapidly proliferating bone marrow cells could be explained with an equilibrium between the process of induction of observed aberration by the mutagens and the elimination of the aberrant cells in the course of mitotic division.

More than 5% of analysed metaphases in the treated animals have shown changes in the spiralization of chromosomes and clear "c-banding" effects most probably related with changes in the chromatin spiralization. The highest percentage of metaphases with altered spiralization and "C-banding" effects in treated mice indicates an adverse influence of the heavy metals on chromatin structure, confirmed by ultrastructural observations on spermatogenic cells.

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