



## Effect of Pb exposure on the cells and matrix of the intervertebral disc of rats

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Degenerative spine diseases are common throughout the world. There are many factors that lead to these diseases. One of these exogenous factors may be Pb exposure. Most of the fundamental investigations about the negative effects of the Pb exposure have investigated the impact of high doses of Pb, while Pb exposure in low concentrations has been insufficiently studied. The nutrition of the intervertebral disc is provided by the vessels that grow into the vertebral bodies and the paravertebral tissue. This suggests the possibility that Pb penetrates from the bone to the intervertebral disc together with nutrients. This article reports an experimental study which was performed in rats aged 1.5 months age ( $n = 40$ ). Rats in the experimental group received lead acetate solution (230 mg of Pb per 1 liter of distilled water) as drinking water and the animals in the control group received distilled water for 10 weeks. In the isolated intervertebral discs, the content of Pb was determined by X-ray fluorescence spectrometry. The effect of lead on the structural organization of the intervertebral disc was investigated by light microscopy. The ultrastructural features of cells and the matrix intervertebral disc were studied by transmission electron microscopy. In the young animals after Pb exposure, a significant increase was found in the content of Pb in the intervertebral disc tissue, of 4.6 times compared to the control group. The histological analysis showed degenerative changes in the annulus fibrosus (AF) of the intervertebral disc. In the outer region cracks and defibration of the lamellae were observed. Large isogenic groups were detected in the inner region of the intervertebral disc. A decrease in the height by 12.2% in the outer parts and by 10% in the central part of the intervertebral disc was recorded. Also a reduction in the width of the lamellae by 8% was shown. Cell density of the annulus fibrosus decreased by 12% and in the gelatinous nucleus pulposus (NP) by 24.2%. Electron microscopic analysis of the inner region of the AF of the intervertebral disc showed chondrocytes with electron-dense mitochondria, vacuolization of the cytoplasm, fragmented nuclei and destructive cavities. The matrix of the lamellae of the AF had a diverse electron density, so areas with the high electron density of various sizes were observed. Collagen fibrils of the AF had blurred outlines, sometimes the transverse striation disappeared or it was irregular, which can indicate the matrix mineralization and possibly the accumulation of Pb in these areas. In the NP, the most notochordal cells revealed the formation of myelin figures. The effect of Pb at low concentration results in its accumulation in the tissues of the intervertebral disc of rats and causes degenerative changes in the intervertebral disc structure, leading to the reduction in the height of the intervertebral disc, decrease in the width of the lamellae and in cell density, thereby violating both the structures of matrix and cells. Future investigations of the collagen content of intervertebral disc after Pb exposure are needed.

**Keywords:** degenerative disc; histology; transmission electron microscopy; lead

### Introduction

An important research problem of our time is the reduction of the incidence of various diseases in the population. Degenerative diseases are a significant cohort of diseases which are associated with the influence of various factors, both the environment and the characteristics of the organism itself. The degeneration of the intervertebral disc is considered an unsolved problem of modern medicine, due to the huge number of factors causing it, as well as the peculiarity of the mechanisms of cartilage tissue regeneration. This pathology leads to back pain, both episodic and permanent, in many individuals (Smith et al., 2011; Clarençon et al., 2015; Izzo et al., 2015). In this regard, studies are being conducted, which consider both the different treatment options for this group of diseases and the factors that cause them. Given the probability of the influence of environmental factors on the occurrence of degeneration of the intervertebral disc, it can be assumed that one of them may be the toxic effect of Pb.

The high content of Pb in the earth's crust, as well as its frequent use in various industries, has led to its presence in the

life of every person, as well as its more or less pronounced effect on the body. Every year there is a decrease in the amount of Pb in the environment, and in the industrial enterprises. However, as a result of clinical studies, it has been established, that even in a sufficiently low concentration in the blood Pb causes various disorders such as the decrease of the intellectual capacity in children (Reuben et al., 2017), hypertension (Almeida Lopes et al., 2017), coronary heart disease (Ding et al., 2016), kidney dysfunction (Buser et al., 2016), dental caries (Wiener et al., 2015), and psychiatric disorders (Modabbernia et al., 2016, 2017). Thus, the problem of studying the influence of the low concentrations of Pb on the human body seems to be significant.

It is known that the maximum absorption of Pb is observed in children and pregnant women (up to 50% with food), and is involved in the process of growth in the case of children, and the formation of the embryo skeleton in pregnant women (Warm, 2013). Thus, the bodies of children are very susceptible to the effects of Pb due to its active accumulation in the body. In the case of the increasing rates of the bone resorption which occurs after

menopause or in the various diseases associated with the remodeling process, Pb embedded in the structure of bone tissue is released into the blood and its negative effect on the body can increase (Theppeang et al., 2008; Brito et al., 2014). As a consequence, the recent decrease of the level of Pb in the environment and, correspondingly, in the blood, does not reduce its stable content in the bone tissue and, therefore, it continues to exert a stable long-term negative effect on the body (Hu et al., 2007).

In connection with the established decrease of Pb in the environment, special attention is paid to the study of the effect of this element on the organism, especially considering that earlier studies aimed to study the effect of Pb at higher doses, which was due to the higher level of Pb in the human environment at the time of those studies. Consequently, the influence of Pb in the concentration accepted as normal (10 µg/dl, in the blood) has not been sufficiently studied. Moreover researchers face the question that there may not be a safe level of Pb (Grandjean, 2010).

The vertebrae is a kind of depot for the accumulation of Pb and due to the long half-life term (25 years) of Pb, bones are also a source of it in the blood during remodeling even with no direct impact on the environment. It is known that the nutrition of the intervertebral disc is provided by a few blood vessels permeating the vertebral bodies and paravertebral tissue. This suggests the possibility that Pb penetrates from the bone to the intervertebral disc together with nutrients. Pb has an affinity to many elements contained in the human organism and affects the formation and metabolism of bone tissue (Pemmer et al., 2013). The main component of the bone is hydroxyapatite, the main constituent elements of which are calcium and phosphorus. It is known that Pb can replace calcium in the structure of hydroxyapatite whereby it violates the biomechanical properties of the bone matrix. In the study of samples of degenerative intervertebral discs, obtained after dissection, it was found that Pb level increases with age (Nowakowski et al., 2015). Furthermore, there is evidence that microscopic calcification occurs in the intervertebral discs in the norm, namely in the NP (Rutges et al., 2010). This indicates the possibility of the accumulation of Pb in the nucleus pulposus (NP) of the intervertebral disc by replacing calcium in the structure, as in the bone tissue.

In most clinical and experimental studies, the effect of Pb on bone tissue was studied at an early stage during the growth of the skeleton. However, some researchers suggest that Pb can affect the cartilaginous tissue (Brodziak-Dopierala et al., 2011; Meirer et al., 2011; Holz et al., 2012; Roschger et al., 2013; Tomaszewska et al., 2016). Currently, in such studies a relationship between the level of Pb in the blood in women and the development of osteoarthritis has been found, which suggests that Pb can be involved in the pathogenesis of this disease (Jordan et al., 2007; Nelson et al., 2011).

The aim of the study was to reveal the characteristic features of the influence of Pb on the structural organization of cells and the matrix of the intervertebral disc of laboratory rats.

## Materials and methods

The experimental research was conducted on white laboratory rats. The animals were housed in groups of 5 individuals in a vivarium cage. The rats had access to the normal balanced diet for rodents with a 12 hour day/night schedule. The experimental investigation was carried out with the approval of the Committee of Bioethics of Sytenko Institute of Spine and Joint Pathology, Academy of Medical Science, Kharkiv, Ukraine (protocols No 127 of 24 February 2014; No 159 from 19 December 2016) and conducted in accordance with European legislation and the law of Ukraine "On protection of animals from cruelty".

Rats aged 1.5 months (n = 40) were separated randomly into two groups of twenty animals each (the experimental group and the control group). The rats in the experimental group received Pb acetate solution (230 mg of Pb per 1 liter of distilled water) as drinking water and in the control group the animals received distilled water for 10 weeks. The choice of the concentration is due

to researches of other authors, where it was found to be the closest environmentally equipment to the level of human exposure (Carmouche et al., 2005). The animals were removed from the experiment by decapitation under ether anesthesia at the age of 4 months.

The fragments of lumbar spine LI-LIV were harvested for further analysis.

Samples of intervertebral discs were removed, washed with phosphate buffered saline, fixed in 96° alcohol and dried at 60 °C overnight. After that, X-ray fluorescence spectrometry was conducted on the samples intervertebral discs in an energy dispersive spectrometer "Sprut"-K (Ukrrenthen, Ukraine) with SDD detector X-123 (Amptek, USA) to determine the Pb content in the samples of the intervertebral disc.

After removal, fragments of the lumbar spine were fixed in 10% neutral formalin overnight and decalcified in 10% solution of formic acid for two weeks. Fragments of the spine after decalcification were washed in running water and were dehydrated in a series ethanol solutions with increasing concentrations and chloroform and were embedded in paraffin (An and Martin, 2003). Histological sections (5 µm) were made in the slide microtome "Reichert" and were stained with Weigert's iron hematoxylin and eosin. The analysis of the histological sections was performed using a light microscope Olympus BX-63 (Japan). Measurements were carried out on photos which were made from 3 slices in 4 fields of view under  $\times 10$ ,  $\times 20$ ,  $\times 40$  objectives and using ImageJ software and cellSens (Olympus BX-63). The height of the intervertebral disc in the outer regions (µm) and central region (µm), the width of lamellae (µm) of collagen fibers of AF were measured. Also, the density of the fibrochondrocytes in the AF and notochordal cells in the NP was evaluated in mm<sup>2</sup>.

The isolated fragments of the intervertebral disc were cut into pieces the size  $\approx 1 \text{ mm}^3$  and processed by the standard method of electron microscopy in accordance with the recommendations of B. Weekly. The collected samples were prefixed in 5% Karnovsky fixator, after which we performed additional fixation in 1% osmium tetroxide solution (OsO<sub>4</sub>) and dehydration in ethanol solutions of increasing concentration (from 50° to 96°) and acetone. Thereafter specimens were embedded in a mixture of epon and araldite epoxy resins.

Semi-thin (1–2 mm) and ultra-thin sections (0.05–0.09 µm) were made using glass knives on the ultramicrotome UMPT-3M (Sumy, Ukraine). The differential staining of semi-thin sections were performed in 1% solution of methylene blue solution and 1% solution of basic fuchsin by the method of Aparicio (1969). Ultra-thin sections were contrasted in Pb citrate and uranyl acetate by the method of Reynolds (1963). Ultrastructural analysis was provided by using transmission electron microscope EMR-100BR (Sumy, Ukraine).

The mean value (x) and the standard error (SE) were determined for each of the measured parameters in the study. The comparison of mean values for identification of the impact of certain factors in case of the normal distribution and equality of group variances of two independent samples were performed using t-Student test. In the statistical analysis we calculated the achieved significance level (P) and the critical level of significance in this study was accepted as 0.05.

## Results

X-ray fluorescent spectrometry. In young animals, after Pb exposure, we found a significant increase in the content of Pb in the intervertebral disc tissue which was 4.6 times (P = 0.02) that of the control group (Table 1).

**Table 1**

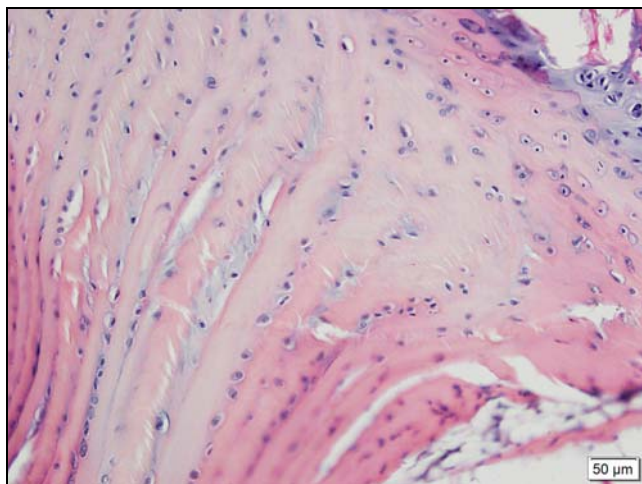
Mass fraction of Pb in the intervertebral discs of rats (%)

Groups	Mass fraction of Pb	P*
Experimental group	$(0,37 \pm 0,093) \cdot 10^{-2}$	0,02
Control group	$(0,08 \pm 0,031) \cdot 10^{-2}$	

Note: \* – t-Student test.

During the histological analysis, the signs of degenerative changes in the annulus fibrosus (AF) of rats after Pb exposure were shown. The defibrillation of collagen fibers in the lamellae of AF and gaps between bundles of collagen fibers in the longitudinal and transverse directions were observed in the animals' intervertebral discs (Fig. 1). Especially large cracks were located in areas bordering the NP. Partial necrosis of the tissue areas was noted. chondrocytes with pyknotic nuclei were located in the regions with the disturbance structure of collagen fibers of lamellae of AF (Fig. 1).

In addition, among the fibrochondrocytes there were typical cartilaginous cells – chondrocytes in capsules. It indicates a violation of the specific cell phenotype of the intervertebral disc in the outer region of AF. The chondrocytes, located in the inner region of AF, had the typical structure of the normal intervertebral disc in the control and experimental groups of rats.



**Fig. 1.** The outer region of AF of the intervertebral disc of a rat: defibrillation of bundles of collagen fibers in the lamellae; pyknotic nuclei in chondrocytes near the cracks in lamellae; Pb exposure; hematoxylin and eosin

Measuring of the height of the intervertebral disc of rats allowed us to establish a decrease of this index by 12.2% in outer departments and by 10% in the central region after Pb exposure (Table 2). Also a reduction in the width of the lamellae of collagen fibers in the annulus fibrosus by 8% was shown compared to the control group (Table 3). The density of fibrochondrocytes in the lamellae of the experimental animals was lower by 12% than equivalent indices in the control animals (Table 3).

The lamellar structure of the inner region of AF of the rats was less pronounced. Among thin lamellae of collagen fibers cracks were observed and near them some chondrocytes had piknotic nuclei (Fig. 1).

**Table 2**

Height of the intervertebral disc of rats ( $\bar{x} \pm SE$ ,  $n = 40$ )

Regions	Control group	Experimental group	P*
Outer region, $\mu\text{m}$	$898,8 \pm 13,8$	$789,4 \pm 14,4$	0,01
Central region, $\mu\text{m}$	$720,8 \pm 15,0$	$648,7 \pm 17,8$	0,01

Note: \* – t-Student test.

**Table 3**

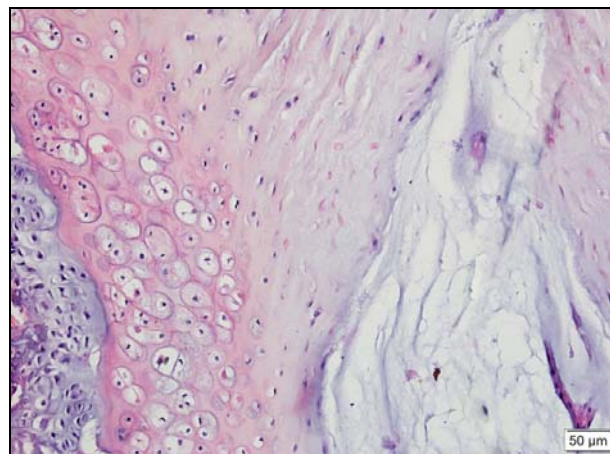
Morphometrical parameters of the intervertebral disc of rats ( $\bar{x} \pm SE$ ,  $n = 40$ )

Parameters	Control group	Experimental group	P*
Width of lamellae of AF, $\mu\text{m}$	$36,3 \pm 0,2$	$33,4 \pm 0,2$	0,01
Density of fibrochondrocytes of AF, $\text{mm}^{-2}$	$1897,8 \pm 35,7$	$1669,4 \pm 28,2$	0,01
Density of notochordal cells in the NP, $\text{mm}^{-2}$	$4693,5 \pm 87,5$	$3557,0 \pm 45,8$	0,01

Note: \* – t-Student test.

Destruction of intercellular substance in the areas with cracks was less pronounced. Alongside these areas single large groups of

isogenic cells were observed. Also the overgrowth of cartilage on the border of the annulus fibrosus and NP was observed (Fig. 2).

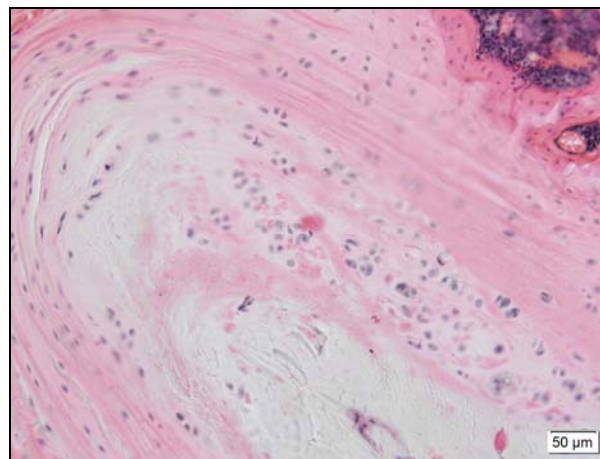


**Fig. 2.** The inner region of AF of the intervertebral disc of a rat: defibrillation of matrix; Pb exposure; hematoxylin and eosin

In this area of the intervertebral disc chondrocytes formed large isogenic groups with 5–8 cells, each of which was surrounded by a matrix typical for hyaline cartilage matrix.

The matrix had slight eosinophilic staining, the fibrous structure did not have clear contours. Cells in these isogenic groups were with different characteristics. Some of them had pyknotic nuclei or vacuolization of cytoplasm. Also cells with large hypochromic nuclei surrounded by a narrow rim of cytoplasm were observed (Fig. 3).

The fragmentation of the syncytium structure was found in rats of the experimental group. In addition, clusters of notochordal cells were observed (Fig. 3). Among them was a significant amount of cells with pyknotic nuclei, which indicated degenerative changes. There was a decrease in the density of notochordal cells by 24.2% after Pb exposure compared with the control group (Table 3).

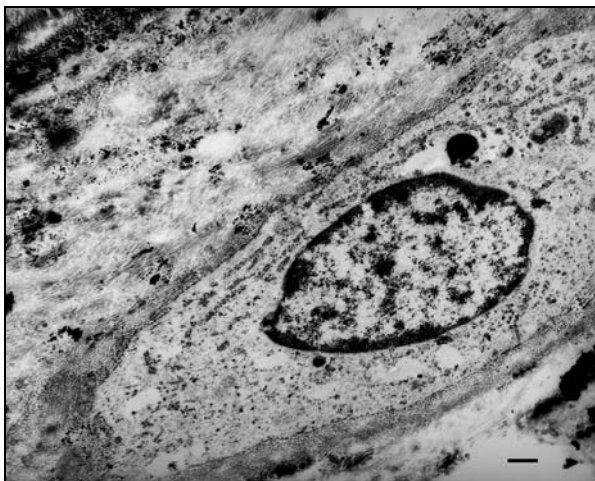


**Fig. 3.** The inner region of AF of the intervertebral disc of a rat: great isogenic groups of chondrocytes; the fragmentation on syncytium in NP; Pb exposure; hematoxylin and eosin

Thus, decrease in the height of the outer regions of the intervertebral disc occurs due to violation of the structure of the lamellae in the annulus fibrosus. We hypothesize that changes in the central region of the intervertebral disc were manifested mainly by decrease in the density of notochordal cells. These cells synthesize of glycosaminoglycans, which are structural components of the NP and indirectly ensure the preservation of the volume of intervertebral disc (Erwin and Hood 2014).

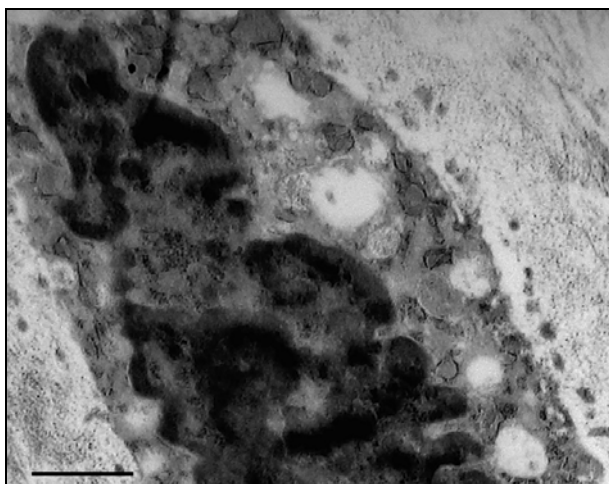
Single fibrochondrocytes in the outer region of the annulus fibrosus of the intervertebral disc in the experimental group were identified. Most cells had a characteristic phenotype. The vacuoles and individual electron-dense granules were revealed in the cytoplasm of some cells (Fig. 4).





**Fig. 4.** The outer region of AF: chondrocyte with single profiles of rough endoplasmic reticulum and vacuolization of cytoplasm; Pb exposure; contrasted by Reynolds; electron micrograph; scale bar: 1  $\mu$ m

In addition, cells containing electron-dense mitochondria along with vacuolization of cytoplasm and individual profiles of rough endoplasmic reticulum were located in the isogenic groups of chondrocytes. Some cells in isogenic groups had fragmented nuclei, indicating apoptosis. In these cells against a background of preservation of the plasma membrane individual profiles of rough endoplasmic reticulum among large vacuoles were found (Fig. 5).



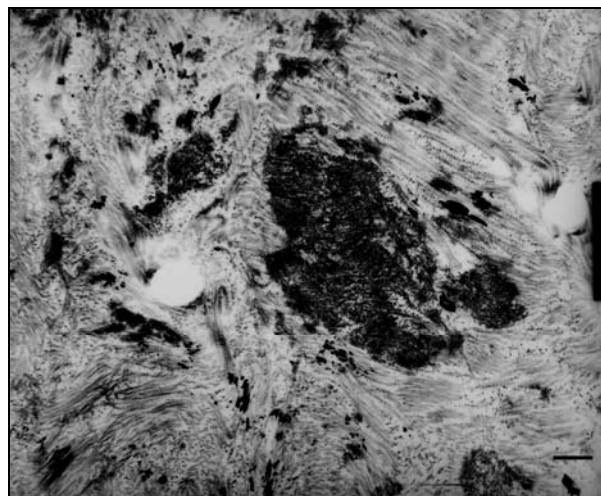
**Fig. 5.** The outer region of AF: fragment of chondrocyte with large vacuoles in cytoplasm and electron-dense nucleus; Pb exposure; contrasted by Reynolds; electron micrograph; scale bar: 1  $\mu$ m

Matrix of lamellae of AF had a diverse electron density. Areas with high electron density of various sizes were observed (Fig. 6).

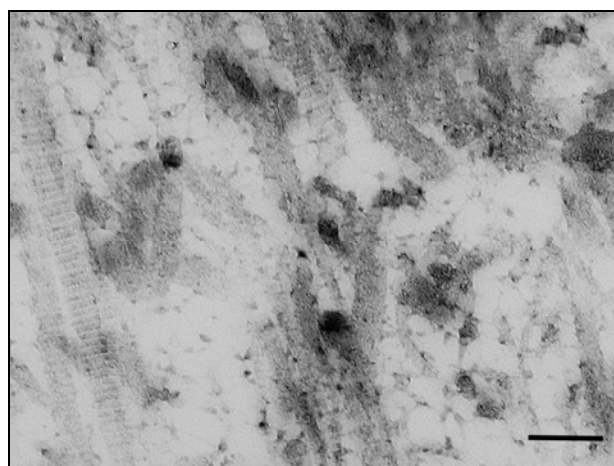
Collagen fibrils of lamellae had blurred outlines, sometimes the transverse striation disappeared or it was irregular (Fig. 7). Also the electron-dense circular structures, consisting of densely arranged collagen fibrils, were located in these areas. Most chondrocytes had large round nuclei, sometimes with nucleoli and single profiles of rough endoplasmic reticulum in the cytoplasm in the inner region of AF of the experimental animals.

Also chondrocytes with two nuclei were observed and they corresponded to each other as the key to the lock. It can be evidence of a recent division by the amitosis of these cells. The cytoplasm of these chondrocytes was less differentiated compared to other chondrocytes, it can suggest of the immaturity of these cells. Chondrocytes were distributed in groups of 2–4 isogenic cells sometimes 5. Some chondrocytes had lysosomes in the cytoplasm, possibly with enzymes destroying the degenerative matrix. Also, some cells with exocytosis phenomenon were found. In the cytoplasm of most cells of the inner region of AF, the varying size

of destructive cavities and its location or the formation of very large vacuoles were located. Similar changes are observed in chondrocytes in the articular cartilage in osteoarthritis (Pascarelli et al., 2015). As was observed, some of these cells had an electron-dense outline around the cell membrane, which is possibly due to a violation of the forming of the matrix structure or the cell capsule formation and the death of mitochondria.



**Fig. 6.** The outer region of AF: irregular density of collagen fibers in matrix: the area of possible calcification or Pb accumulation in the matrix; Pb exposure; contrasted by Reynolds; electron micrograph; scale bar: 1  $\mu$ m



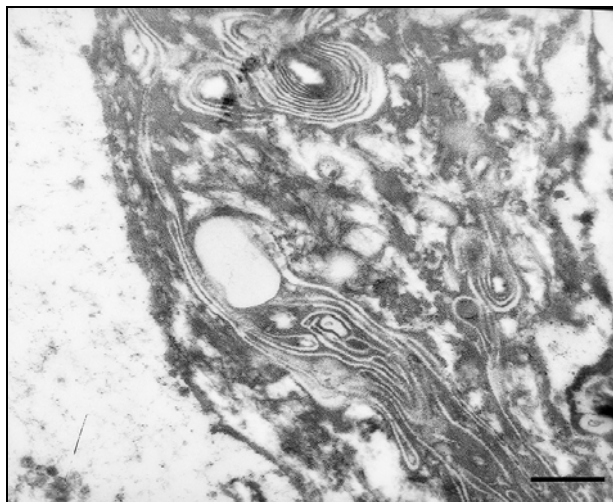
**Fig. 7.** The outer region of AF: the collagen fibrils with different range of destructive changes in the structure and electron-dense structures in the matrix; Pb exposure; contrasted by Reynolds; electron micrograph; scale bar: 0,5  $\mu$ m

Notochordal cells with heterochromatic nuclei were found in the NP of rats both in the experimental group and in the control group. In the cytoplasm of some notochordal cells of rats in the experimental group large vacuoles were observed, probably filled with the products of biosynthesis. Additionally, some cells had a hyperchromic electron-dense nucleus, indicating degenerative changes in the cells. Also, myelin figures were revealed in most notochordal cells (Fig. 8).

## Discussion

Degenerative diseases of the spine are often found among the populations of the different countries of the world. However, there is still a wide range of unidentified factors which cause this pathology. In particular, the influence of Pb on the structure of the intervertebral disc has not been determined. Rats are one of the generally accepted models for the reproduction and studying of degenerative intervertebral disc diseases, due to their availability,

low cost, and structural features (Norcross et al., 2003, Gebhard et al., 2011). Nevertheless, the distinctive feature of the structure of the intervertebral disc of rats is the absence of differentiation of the notochordal cells into chondrocytes. As a result, these cells remain in the gelatinous nucleus throughout the lifetime of the rats, whereas in humans they almost entirely disappear at the age of 4–10 years due to bipedalism. According to a recent study, the preservation of notochordal cells in the gelatinous core of rats does not prevent their use as a model of the degeneration of the intervertebral disc due to the fact that the chondrocyte-like cells, which are the result of the differentiation of the notochordal cells, due to axial load on the upright, appear in the jelly nucleus of a person with ageing. Accordingly, the pathological or physiological stages of ageing or the degeneration of the notochordal cells are displayed. The content of collagen, glycosaminoglycans and water in the annulus fibrosus of rats corresponds to that of humans (Showalter et al., 2012).



**Fig. 8.** Myelin figures in the cytoplasm of notochordal cell; Pb exposure; contrasted by Reynolds; electron micrograph; NP; scale bar: 1 μm

In the intervertebral discs of young rats after exposure of Pb we found a decrease the height of the intervertebral disc and the width of lamellae, along with the decrease in the cell density as in the annulus fibrosus as in the NP. If decrease in the height of the intervertebral disc was observed to be almost the same in the outer region as in the central region, the cell density is reduced more in the NP. The presence of signs of the degeneration in notochordal cells of the NP after Pb exposure, such as the chromatin condensation and the formation of myelin figures in the cytoplasm which are associated with the cell membrane and the destruction of its structure as a result of certain negative factors (Fig. 5) is a sign of cell death (Majno et al., 2004). Probably it indicates the possibility of the penetration of this element with nutrients into the NP and the inhibition of the biosynthetic functions of the notochordal cells by disturbance of their structure as result of Pb which leads to their death. The presence of the electron-dense mitochondria in the chondrocytes of the experimental groups of rats probably indicates their death due to the accumulation of Pb. It is known that the accumulation in the mitochondria of cationic metals, including Pb, is mainly due to the presence of calcium in the transport channels (Meyer et al., 2013). One can also mention the possibility of the accumulation of Pb in the endoplasmic reticulum through its close relationship with the functioning mitochondria, it can also support the accumulation of Pb in the mitochondria.

In chondrocytes of the outer region of the annulus fibrosus after Pb exposure vacuolisation of the cytoplasm and the condensation of chromatin in the nucleus were found, indicating the disruption of cells, namely their biosynthetic functions, and this is consistent with our finding that Pb exposure causes an increase in the content of collagen fibers of collagen type III, which is uncharacteristic for the structure matrix of the intervertebral disc (Maltseva, 2016).

In separate experimental studies in vivo and in vitro, conducted to identify the mechanisms of action of Pb on the articular cartilage, the authors also discovered changes in the biosynthetic characteristics of chondrocytes, as well as their phenotype. Zuscik et al. (2006) found that the action of Pb on the articular chondrocytes contributes to the disruption of the basic characteristics of these cells, in connection with their hypertrophy, which subsequently causes the degradation of the articular matrix and its mineralization. An in vitro study on chondrocytes of articular cartilage of chickens also confirmed that the action of Pb causes chondrocyte hypertrophy, leading to inhibition of the synthesis of proteoglycans and type II collagen, which are the main permanent components of the cartilage matrix (Holz et al., 2012). However, the mechanisms of the appearance of the established changes under the action of Pb in the cartilaginous tissue remain undisclosed. Probably, a similar process occurs in the intervertebral disc, causing the inhibition of biosynthesis in the chondrocytes of matrix components.

We have noted the division of chondrocytes in the inner region of AF of intervertebral discs along with the destructive changes in cells in the form of the cavities in the cytoplasm. It indicates the presence of a compensatory mechanism for repairing the structural homeostasis of the tissue. Increase of the cell proliferative activity through the formation of the large isogenic groups of chondrocytes, which was confirmed histologically, is a standard sign of the degenerative process in the intervertebral disc (Rutges et al., 2010; Majeed et al., 2016). According to Majeed et al. (2016) it is caused by a violation of biomechanical qualities of the intervertebral disc. The described changes in the inner region of the AF indicate cartilage matrix degeneration, namely changing its structure to the fibrous structure reminiscent of the structure of the hyaline cartilage. According to the opinion of Tsyvyan and Buruhin (1988) the transformation of the matrix of AF leads to local stresses in the areas of tissue heterogeneity, which in turn lead to further cracking of the matrix and enlargement of the zone of necrotic decay. Reorganization of the structure of the annulus fibrosus and its transformation into an excellent cartilaginous structure reduces the strength properties of the disc and its elasticity, which negates its main function – to bear load. Such a restructuring is supposedly an adaptation reaction in response to a violation of the organizational characteristics of the cartilage matrix, but it does not appear to be a complete replacement for the structure of the AF due to the inability of normal functioning.

Degenerative changes were observed in the form of cracks between and within the plates on the histological level and local destruction of collagen fibrils on electron-microscopic level in the matrix of the outer and the inner regions of the AF. The revealed features of the change in the structure of the matrix indicate the destruction of the intercellular substance of the cartilage of the AF. The formation of electron-dense areas in the structure of the matrix of AF can be evidence of the presence of calcification and is one of the signs of the degeneration of the tissue structure of the annulus fibrosus of the intervertebral disc (Fig. 6) (Rutges et al., 2010; Hristova et al., 2011). Also, it can confirm accumulation of Pb. The disarrangement of the structure of the matrix in the outer region possibly is caused by the changes of its support function in response to stress, which in turn causes the degenerative restructuring in the inner region of AF. According to Jin et al. (2014) the balance of catabolic and anabolic processes is disturbed in the degenerative disc, so negatively changing the synthesis and degrading the matrix components, which in turn leads to degenerative changes.

The accumulation of Pb and other elements in the intervertebral disc was studied in a clinical study where this process was associated with the mineralization of cartilage tissue of the intervertebral disc with ageing (Niedzwiedzki et al., 1997). In addition, the authors have identified the two types of mineralization of the intervertebral disc: visible and invisible. The visible mineralization is characterized by the presence of crystals of minerals during the microscopic examination of tissue of the intervertebral disc and invisible mineralization, considered by the authors as a primary

stage of the visible mineralization which could be determined only by chemical analysis of tissues. Our histological examination of rats' disc tissue after exposure to Pb did not reveal signs of the tissue mineralization, so we can assume that the established Pb content in the intervertebral disc is a sign of invisible mineralization, but may serve as the primary link in the degeneration of the disc.

Moreover the accumulation of Pb can occur not only in the cartilage matrix, but also in the cells, probably in chondrocytes. So by the electron-microscopic study of chondrocytes in the outer region of annulus fibrosus we have revealed electron-dense mitochondria, which can be a kind of cellular depot for the accumulation of heavy metals (Meyer et al., 2013) and residual bodies in cells, which perhaps can be a sign of the accumulation of Pb in chondrocytes. The formation of residual bodies in cells is the result of autophagy which occurs in normal and as the adaptive response to the impact of various factors on the intervertebral disc, but its expression increases in the chondrocytes with age in the case of the intervertebral disc degeneration (Jiang et al., 2014). According to the results of research Gruber et al. (2014), autophagy in chondrocytes of the AF is necessary for the elimination of dead mitochondria because this dysfunction occurs in disc degeneration. In the outer region of the AF matrix of the intervertebral disc we noted the formation of electron-dense sites with signs of calcification, which probably serves as the places of accumulation of Pb.

## Conclusion

The study has found accumulation of Pb in tissues of the intervertebral disc of young rats and an increase in its content by 4.6 times compared with the control. Histology has shown a reduction in the intervertebral disc height in the central region by 10%, and in the outer regions by 12.2%, caused by the cracks and defibration of lamellae in the AF and significant reduction of the cell density. The violations detected in the central part of the disc can be explained by the presence of degenerative changes in the notochordal cells. Pb exposure leads to similar degenerative changes in the chondrocytes of outer and inner regions of the AF such as the formation of the destructive cavities, the vacuolization of cytoplasm and the degradation of organelles. In the inner region of AF the formation of large isogenic groups of chondrocytes was marked, probably associated with the adaptive response to the degenerative changes in the intervertebral disc. In the matrix of AF of rats we noted the presence of the electron-dense areas, which could indicate a matrix mineralization and possible the accumulation of Pb in these areas.

Thus, Pb exposure leads to change in the phenotype of chondrocytes of the intervertebral disc and leads to the degenerative changes in the structure of the cartilage matrix in rats. Future investigations of the collagen content of the intervertebral disc after Pb exposure are needed.

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