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# MORPHOLOGICAL CHANGES OF PROSTATE GLAND DUE TO HEAVY EXPOSURE TO HEAVY METALS SALTS

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### Abstract

*The problem of the growing number of diseases of the prostate gland is not only of medical but also social importance, since this group of diseases increasingly embraces the male population of both elderly and reproductive age. High level of pathology of the prostate gland in men, ever increasing incidence of prostate cancer cause heightened interest in the search for possible etiologic factors of prostate diseases. Special significance is attributed to the effect of adverse environmental factors due to its negative impact on biological processes in organs and tissues of an organism and their ability to induce various pathological processes. Numerous studies have shown the dependence of prostate pathology on various exogenous factors. However, there is very little information on the effects of salts of heavy metals on the morphogenesis of the prostate gland.*

*The objective of this paper was to investigate the morphological features of the prostate gland tissue parameters under heavy exposure to the salts of heavy metals in an experiment.*

*The experiment was carried out on 60 outbred male rats aged 6 months. Animals were divided into two groups: I – control and II – animals treated with distilled water with a combination of heavy metal salts.*

*As a result of the experiment it was established that in a simulated microelementosis there is a change of the balance of stromal-parenchymal component and increase of lineal-weight indices of prostate gland; expressed atrophic, dystrophic and degenerative processes, and reduced secretion and functional activity of the prostate are defined.*

*Thus, the results of the experiment demonstrate that exposure to a variety of heavy metal salts induces structural changes at all levels of the prostate morphology along with a change of its microelement composition.*

**Keywords:** *prostate gland, salts of heavy metals, microelementosis, morphology.*

## Introduction

The problem of the growing number of diseases of the prostate gland is not only of medical but also social importance, since this group of diseases increasingly embraces the male population of both elderly and reproductive age [*Alexander F, Boyle P, 1994; Guess H, 2001*]. High level of pathology of the prostate in men, ever increasing incidence of prostate cancer cause increased interest in the search for possible etiologic factors of prostate diseases.

One of the most significant causes of untimely death of men is prostate cancer. This disease is the second largest cause of death from malignancy in men [*Jemal A et al., 2004*]. In Ukraine, as in most countries, there is a trend to increased morbidity and mortality from prostate cancer. In 2012 there were first detected 7512 cases of prostate cancer in Ukraine. The largest group included patients with 3 or 4 stages of the disease [*State Statistics Service of Ukraine, 2012*].

Numerous studies have shown the dependence of prostate pathology on various exogenous factors [*Hiatt R et al., 1994; Bostwick D et al., 2004; Johansson M et al., 2012; Leitzmann M, Rohrmann S, 2012*]. However, there is very little information on the effects of heavy metal salts on the morphogenesis of the prostate gland.

The objective of this study was to investigate the morphological parameters of the prostate tissue under the influence of increased intake of the salts of heavy metals in the experiment.

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## Materials and Methods

The study was performed on 60 outbred rats – males aged 6 months. Animals were divided into two groups: I – control, II – animals treated with



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distilled water with a combination of heavy metal salts (zinc ($ZnSO_4 \cdot 7H_2O$) – 5 *mg/l*, copper (CuSO4 · $5H_2O$) – 1 *mg/l*, ferrum ($FeSO_4$) – 10 *mg/l*, manganese ($MnSO_4 \cdot 5H_2O$) – 0.1 *mg/l*, lead (Pb ($NO_3$) 2) - 0.1 *mg/l*, chrome ($K_2Cr_2O_7$) – 0.1 *mg/l*). Duration of the experiment was 60 days. During the experiment, laboratory animals were kept in accordance with regulations adopted by the European Convention for the Protection of vertebrate animals used for experiments and scientific problems (Strasbourg, 1986), the Principles of the Helsinki Declaration adopted by the General Assembly of the World Medical Association (1964-2000), "General ethical rules of experiments on animals" approved by the National Congress on Bioethics (Kyiv, 2001).

Prostate tissue was fixed in a solution of 10% neutral formalin, embedded in paraffin; 5 microns thick sections were made on a rotary microtome. Histological preparations stained with hematoxylin and eosin, solution of picric acid and fuchsin by Van Gieson, histochemical staining by Gomori and PAS-reaction were performed. To determine the ratio of the volume fraction of the components of the prostate tissue, stereometric grid of 100 points was used for histological examination with magnification 200x. Chemical analysis involved immersion of paraffin sections of the prostate tissue in xylene three times, each for 10 minutes. After the procedure the sections were washed with distilled water for 5 minutes, placed in hematoxylin and in 96% ethyl alcohol for 10 minutes. Lastly, paraffin sections were thoroughly washed with distilled water for 4-5 minutes. Paraffin sections of the prostate tissue were placed on pre-polished carbon plate in order to perform the most qualitative chemical analysis. Chemical analysis of the prostate tissue was performed with scanning electron microscopy and X-ray spectral microanalysis electron microscope Remme 100 V IN Electron (Ukraine). Electron microscopy involved microelement analysis, performed on scanning electron microscope SEM-100E (Ukraine). These products were studied and photographed with a digital system of image output SEO Scan Lab 2.0 (Ukraine). The resulting material was documented in the form of digital photos. The results of morphometric measurements were processed by standard statistical methods.

## RESULTS AND DISCUSSION

Glandular structures of the prostate of rats in the control group (Fig. 1) are shown as high prismatic or cubical epithelium, with their area being greater by numerous folds, protruding into the lumen of acinus. Acinus lumens are filled with secretion. Blood-vascular system around glandular elements is characterized by a presence of dense capillary net with sinuous capillaries. Stroma is formed with bundles of smooth muscle fibers, separated from each other by layers of loose connective tissue.

In macro-anatomical study of the prostate of rats treated with a combination of the salts of heavy metals an increase was found in lineal-weight indices compared with the intact rats (Table 1).

*TABLE 1.*

Morphometric parameters of the prostate gland

| Test-results | Study groups | |
|---|---|---|
| | Control | Experiment |
| Absolute volume ($mm^3$) | | |
| Glandular lumen | 177.91±2.81 | 204.19±4.64* |
| Epithelium | 193.44±2.08 | 180.57±1.88* |
| Muscular stroma | 61.14±0.35 | 64.76±0.77* |
| Connective tissue stroma | 81.42±0.85 | 89.75±0.97* |
| Volume ratio (%) | | |
| Glandular lumen | 34.62 | 37.87 |
| Epithelium | 37.64 | 33.48 |
| Muscular stroma | 11.90 | 12.01 |
| Connective tissue stroma | 15.84 | 16.64 |
| Prostate weight (mg) | 471.74±6.01 | 522.99±8.41* |

***Note***: * – $p<0.05$ .

As Table 1 shows, under the influence of the salts of heavy metals the relative volume of stroma is increased (mainly due to a connective tissue component) as well as the lumen of the prostate glands compared with the control group, which indicates a change in morphofunctional activity of the prostate and the negative impact of the combinations of salts of heavy metals under study.

Appearance of pronounced dystrophic and atrophic changes is indicative for prostate parenchyma of mature rats treated with a combination of salts of heavy metals (Fig. 2). The lumens of most of the acinus contain no secretion, which indicates low morphofunctional activity of the prostate glands. The area of secretory lumens in comparison with

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*FIGURE 1. Prostate gland of mature rat in the control group. Secretory areas are lined by prismatic epithelium with centrally located nuclei (1); acinar secretion is stained with eosin (2).*

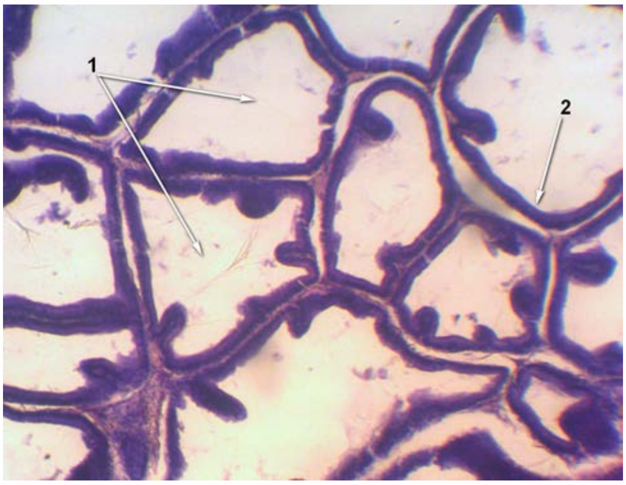


*FIGURE 2. Prostate gland of rat in experimental group. Acinus lumens contains no secretion (1), epithelium is flattened, atrophied (2). Staining with hematoxylin and eosin. Magnification: 300 x*

the control group is increased by 14.77%. Acinar epithelium is flattened, containing many epithelial-stromal outgrowths. Cells boundaries are indistinct. In some areas a periacinar reactive lymphoid infiltration, while destroying the basal plate, extended to the epithelial layer. In the lumen of the acinus there are modified epithelial cells with pyknomorphous nuclei. In separate acini epithelium metaplasia into transition zone, as well as its proliferation and the formation of papillary and cribriform structures.

The final parts of the excretory ducts are expanded, with symptoms of secretion stasis, the cells of desquamated epithelium and the cells of cluding neutrophilic and eosinophilic leukocytes (Fig. 3). There is a change of merocrine type of gland cells secretion into apocrine one, as evidenced by the increasing apoptosis index.

In some parts of the prostate gland of experimental animals there was found expressed diffusely-focal lymphocytic infiltration of the stroma with signs of fibrosis, proliferation of connective tissue components (Fig. 4). The thickness of connective tissue layers increases in the interstitium. There is an increasing number of fibrous stroma and degenerative structures.

Blood capillaries are expanded – venous congestion can be observed therein, edema of perivas-

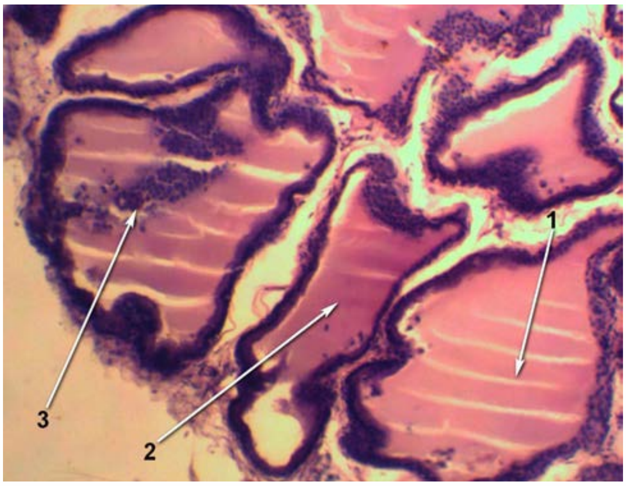


*FIGURE 3. Prostate gland of rat in experimental group. Acinum lumen is expanded (1); filled with intensely colored secretion (2); desquamated epithelium (3). Staining with hematoxylin and eosin. Magnification: 300 x*

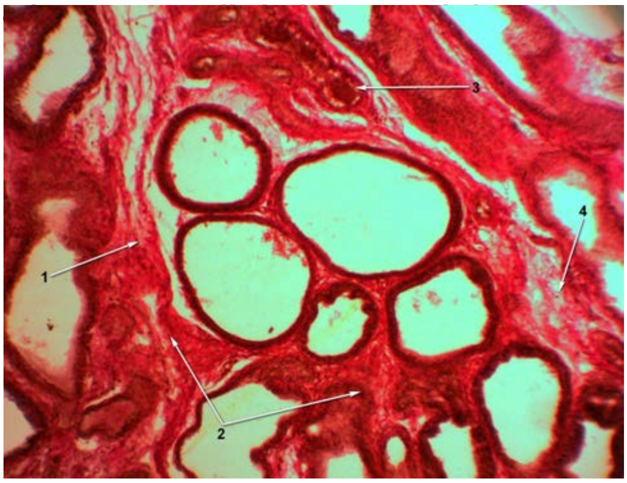


*FIGURE 4. Prostate gland of rat in experimental group. Lymphocytic infiltration of the stroma with signs of fibrosis (1); proliferation of connective tissue component (2); venous congestion (3); stromal edema (4). Van Gieson's stain. Magnification: 300 x.*

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changes in the endothelium are observed. There are pockets of impaired integrity of blood vessels.

For specification and verification of morphogenesis features of the prostate gland under the effect of salts of heavy metals the scanning electron microscopy was carried out.

In electronic scan of prostate tissue of intact animals (Fig. 5) glandular structures are presented by high prismatic epithelium, their area is increased by many folds that appear in the lumen of the acinum. Stroma of the gland is presented by uniformly distributed connective and muscular elements and blood vessels.

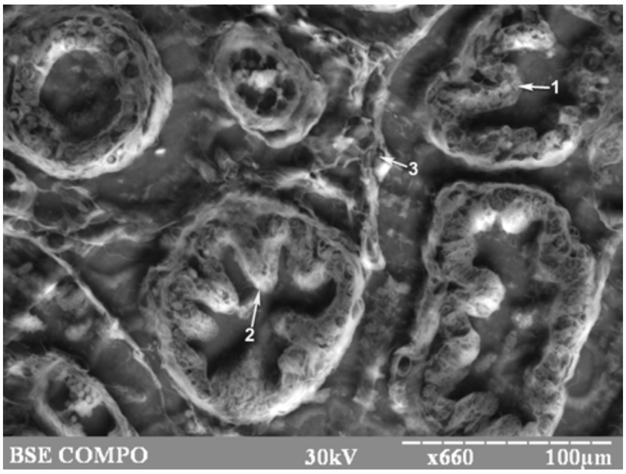


*FIGURE 5. Electronic scan of rat prostate in control group. 1 – high epithelium, 2 – folds of glandular epithelium, 3 – stromal component.*

In the prostate gland of the rat from experimental group (Fig. 6) the epithelium of secretory departments is flattened, their lumen is greatly expanded, no epithelial folds. Connective tissue between the acinum is presented by a dense network of randomly arranged collagen and elastic fibers and fascicles. Architectonics of prostate gland is defective.

After 60 days of the experiment, the following accumulation of microelements in the prostate tissue in comparison with the control was determined: ferrum – by 36.29% ($p \leq 0.01$), copper – by 59.94% ($p \leq 0.01$), chrome – by 235.59% ($p \leq 0.01$), manganese – by 89.40% ($p \leq 0.01$), lead – by 903.64%. Index of zinc decreased compared with control by 21.63% ($p \leq 0.01$). Clinical features of the change in the chemical composition of the prostate during the experiment are accounted for by synergistic and antagonistic interactions of these heavy metals with each other at different levels – absorption in the gastrointestinal tract, transport proteins, tissue and cellular levels [*Goyer R, 1997*].

## CONCLUSION

Under the effect of simulated microelementosis the changes of chemical composition of the prostate tissues were in progress, leading to the accumulation of ions Fe, Cu, Cr, Mn, Pb and decreased levels of zinc ions, which subsequently were reflected on the morphofunctional state of the body.

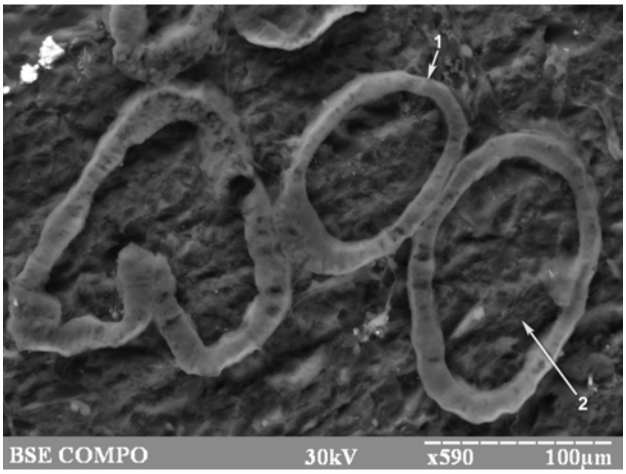


*FIGURE 6. Electronic scan of rat prostate from the experimental group. 1 - flattened epithelium, 2 - expanded lumen of gland.*

The negative impact of the salts of heavy metals causes a change in the ratio of stromal-parenchymatous component and increase of lineal-weight indices of the prostate gland, which indicates a change of morphofunctional activity.

The study had revealed the morphological changes in the prostate tissue of rats including dystrophic, atrophic and degenerative processes in the parenchyma and cellular infiltration with fibrosis formation in stromal structures. Simulated microelementosis causes a decrease in the secretory activity of the prostate epithelium of mature rats.

Thus, the results of the conducted study demonstrated that exposure to a variety of heavy metals salts causes structural changes at all levels of the prostate morphology along with a change of its microelement composition.

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