

## Conference Paper

# Toxic Effect of Lead on the Red Marrow Cells of Birds

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**ORCID:**Alexander I. Vishnyakov: <http://orcid.org/0000-0002-7246-9321>**Abstract**

The purpose of this study was to determine the main patterns of the toxic effect of lead on the red marrow cells of birds in the first three days after exposure. The electro-microscopic study revealed that the toxic effect of lead nitrate on bone marrow cells begins even at a slight MAL excess after 1 day of intoxication. Hematopoietic and stromal marrow cells mainly show the signs of dystrophic changes, which increase as the term after exposure to lead nitrate increases. In the cytoplasm of many cells, heterogeneous lipid inclusions are formed, which are likely the lipid products of cell membrane destruction. In lead intoxication of chickens, the structure of the cell nuclei in the bone marrow is changed, and the morphological characteristics reveal a decrease in the transcription of ribosomal RNA genes and the preparation of many cells for apoptosis.

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**Keywords:** lead toxic effect, birds, marrow cells.

## 1. Introduction

Lead is one of the most commonly known toxic microelements. Biological and medical significance of lead is determined by its high toxicity, ability to penetrate into the body and accumulate there producing the polytropic effect [1]. Lead toxicosis primarily affects hematopoietic organs (anemia), nervous system (encephalopathy and neuropathy), senses, kidneys (nephropathy) and cardiovascular system. The overfrequency of asthenospermia, hypospermia and teratospermia was noted, which is associated with the direct toxic effects of lead on android glands. Excess lead in the body was also shown to reduce vital elements (Ca, Fe, Zn and Se) in organs and tissues [2, 3, 6].

Like other heavy metals, lead is included into various cell enzymes, and as a result, these enzymes can no longer perform their intended functions in the body [4, 5].

The chronic administration of low doses of lead to rats showed an increase in the response of lymphocyte transformation to phytohemagglutinin and concanavalin

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starting from week 5 and a subsequent decrease by week 14. The response of B lymphocytes to lipopolysaccharide activation was also increased and maintained throughout the observation period, while the functional activity of macrophages was significantly reduced [7]. Lead causes suppression of antibody response (Ig M and Ig G), reduces antibody-dependent and mitogen-induced cell cytotoxicity and suppresses macrophage functions, reduces NK activity for all types of tumor cell cultures [8].

The purpose of the study was to determine the main patterns of lead toxic effect on red bone marrow cells in the first three days after exposure.

## 2. Materials and Methods

Broiler chickens of *Smena-7* crossing took part in the experiment. Together with the basic feed the chickens of the experimental groups received 1.5 MAL of lead as a nitrate salt, which corresponds to 4.5 mg of lead per 1 kg of feed.

The material for electro-microscopic study was collected after one and three days. For ultrathin study, the bone marrow was fixed in 2.5% glutaraldehyde solution on a phosphate buffer followed by an hour-long postfixation with 1% OsO<sub>4</sub> solution on the same buffer. After treatment with saturated uranyl acetate solution on 70% ethanol, the material was dewatered in ascendant alcohols and placed into an epon. Ultrathin sections were studied on the transmission electron microscope JEM-100 CX II (JEOL, Japan).

## 3. Results and Discussion

As early as 1 day after the lead nitrate got into the body of chickens with feed at a dose of 1.5 MAL, the signs of dystrophic changes were detected in bone marrow cells. The most pronounced disorders were detected in macrophages, which are known to perform many functions, including protective functions, and, as a result, are the first to undergo intoxication. Their cytoplasm was non-uniform, with many different electron densities, large and small inclusions, and sometimes containing numerous light vacuoles.

In varying degrees of expression, the monocytoïd cell of the bone marrow also showed the signs of dystrophic changes. Mature monocytes with darker cytoplasm and a nucleus, along the periphery of which a dense heterochromatin band was located, were also detected.

The granulocytic cells had a variety of changes. Most promyelocytes retained their structure. The body of cells is more likely to be oval-shaped, with a large oval or nephroid

nucleus with a small amount of peripheral heterochromatin, sometimes one or two nuclei.

Lymphoblasts in the form of large mononuclear cells, up to 25  $\mu\text{m}$  in diameter, having a large rounded or oval nucleus with numerous grains of heterochromatin around the periphery, sometimes with 1-2 nuclei, were found in lymphopoiesis cells. The cytoplasm contained several rounded mitochondria, several short GER cisterns, and a large amount of RNA as clusters of free ribosomes and polyribosomes. Sometimes lymphocytes had a broad cytoplasm almost at complete preservation of the nucleus, with pronounced dystrophic changes in the form of destruction of intracellular organelles, enlargement and expansion of cytoplasm areas.

Rarely seen young progenitor cells (blast cells) of chicken marrow in this experimental group were rounded or somewhat oval-shaped, with a large rounded or oval nucleus. With extensive cytoplasm containing small light bubbles, vesicles, a small number of rounded mitochondria and short GER cisterns, many free ribosomes and polyribosomes, sometimes single small Golgi complexes. Despite the preservation of most intracellular organelles, the changes in the form of "melting" chromatin nuclei and weakening carioplasm density were often detected within the nucleus. Perinuclear spaces ranging from weak to strong expanded in some blast cells in varying degrees of expression [2].

One day after intoxication of chickens with lead nitrate in the dose of 1.5 MAL, the dystrophic changes were subjected not only to hematopoietic, but also to stromal bone marrow cells. In their cytoplasm, the number of organelles decreased, single swollen mitochondria, various vesicles and bubbles were determined. In nuclei, chromatin was also partially destroyed, although the shape of the nuclei did not change.

Three days after lead intoxication of chickens, already pronounced destructive changes were detected in most hematopoietic and stromal bone marrow cells, mainly in the cytoplasm. Organelles were completely destroyed or evacuated, and heterogeneous lipid inclusions were often found in the cytoplasm instead of them.

Young forms of bone marrow cells were difficult to differentiate by structure in terms of their belonging to a certain series, they also had signs of dystrophic changes expressed in varying degrees. This was the case of light cells in the cytoplasm of which organelles were destroyed, as a result of which it was evacuated, and a pronounced swelling of perinuclear space was determined. The cells with darker cytoplasm and a nucleus, in which heterochromatin was destroyed, were also detected. In some such cells, large lipid droplets were found instead of organelles.

The cytoplasm of monocytoïd cells was dark, sometimes showing short GER channels, rounded dark mitochondria, small rounded lysosomes, many bubbles, vesicles,

rarely Golgi plate complex. The cells with a large number of heterogeneous phagosomes in the cytoplasm were found, large of which resembled lipid inclusions.

Lymphocytes, with the preservation of the rounded large nucleus, had a broad cytoplasm with pronounced dystrophic changes in the form of destruction of intracellular organelles, enlargement and expansion of cytoplasm areas.

After three days of lead intoxication of chickens in the study of bone marrow agents in most cells, regardless of the cell series, the same changes in the structure of the nucleus were noticed.

First, there were signs of decreased transcription of ribosomal RNA genes – nuclear chromatin condensed tightly around the periphery of cariolemma or in carioplasm, and the nuclei turned pale. Second, in many cells the nuclear chromatin formed quite wide dense areas, and it seemed that the cell was preparing for apoptosis. Cells with a picnotic dense dark nucleus were determined in some areas.

## 4. Conclusion

Thus, the results of the study revealed that the toxic effect of lead nitrate on bone marrow cells begins even at a slight MAL excess after 1 day of intoxication. Hematopoietic and stromal marrow cells mainly show the signs of dystrophic changes, which increase as the term after exposure to lead nitrate increases. In the cytoplasm of many cells, heterogeneous lipid inclusions are formed, and in our opinion, they represent lipid products of cell membrane destruction. In lead intoxication of chickens, the structure of cell nuclei in the bone marrow is changed, the morphological characteristics reveal a decrease in the transcription of ribosomal RNA genes and the preparation of many cells for apoptosis.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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