Peculiarities of the bone-marrow hemopoiesis, peripheral blood, and the elemental status of the red bone marrow of poultry resulted from copper introduction

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Abstract. Metals in an electrically neutral form produced with the method of vacuum condensation from metals are finely dispersed powders. The role of chemical elements in an organism is versatile and multifunctional. However, it is necessary to pay attention to the reaction of an organism on the introduction of chemical elements with different biological roles, which is predetermined by the physiological role of organs and body systems. These include the red bone marrow, which primarily responds to endogenous and exogenous factors by its functional significance. In order to study the influence of copper nanopowders on hematopoiesis of birds, we selected 150 10-day-old broiler chickens which formed 5 groups by the method of analogs (n = 30). All said above allows us to conclude that copper nanoparticles ambiguously affect the bone-marrow hemopoiesis of poultry; increasing the dose and changing the type of introduction activate bone marrow hematopoietic function, in particular, granulocyto-, megakaryocyto-, and erythropoiesis. Different dosages and ways of introduction of copper nanoparticles, in our opinion, ambiguously affect the bone-marrow hemopoiesis of poultry when using copper in doses of 1.7 mg/kg of feed, which is 50% of the daily needs of the organism. E A Sizova et al. proved a stimulating effect of copper nanoparticles in the same dosage on liver cells of chicks.

1. Introduction
Metals in an electrically neutral form produced with the method of vacuum condensation from metals are finely dispersed powders [1]. Professor E V Burlakova reported that, under introduction of finely dispersed powders into an animal organism, the powders can perform the role of a depot which the element enters the body from for a long time [2].

The advantage of a substance in the electrically neutral form over the ion one is associated with higher permeability through the cytoplasmic cell membrane of the first, which is achieved by higher solubility in lipoprotein matrices. In a cell, under the influence of the environment, the valence of elements changes, they take charge and expose their biological activity [3,4].

The advantage of finely dispersed metal powders over salts when applied as a source of microelements consists of the following. Firstly, they are low-toxic. Secondly, metals entered into the
body in the form of powder become a long-standing source of elements, which provides their prolonged action. Thirdly, studying the biological effects of metal powders found higher efficiency of their impact on various biological systems of the body in comparison to salts [5,6].

Finely dispersed metal powders introduced into the body have the significant advantage of particle proportionality (50-100 nm) with sizes of capillaries, and procure their transmission and distribution all over the organs, lymph and blood flow. Due to electroneutrality, metal particles can easily penetrate into tissues and affect vital processes [7]. In addition, particles of metals in various organs and tissues are gradually transformed into a structure with a changed nature of exchange interaction between atoms of iron [8].

Thus, the role of chemical elements in an organism is versatile and multifunctional. However, you should pay attention to the reaction of the organism on the introduction of chemical elements with different biological roles, which is predetermined by the physiological role of organs and body systems. These include the red bone marrow, which primarily responds to endogenous and exogenous factors by its functional significance [9,10].

2. Materials and methods
In order to study the influence of copper nanopowders on hematoxixis of birds, we selected 150 10-day-old broiler chickens which formed 5 groups by the method of analogs (n=30) (table 1).

| Object | Group         | Stage of experiment |
|--------|---------------|---------------------|
|        |               | preparatory registry|
|        |               | age, days           |
|        |               | 10-20               |
|        |               | 21-50               |
| Broiler chickens of cross “Smena-7” | control         | MR1                 |
|        | I experimental| MR2                 |
|        | II experimental| MR3                |
|        | III experimental| MR4                |
|        | IV experimental| MR5                |

MR – the main ration in the preparatory stage; MR1 – the main ration in the experimental (registry) stage; MR3 – the main ration + copper NPs 2.0 mg/kg of weight i.m.; MR5 - the main ration + copper NPs 0.2 mg/kg of weight i.m.

Copper nanoparticles of type Cu10x represent spherical particles sized 103.0 ± 2.0 nm with 6 nm thick oxide layer obtained at Institute for Energy Problems of Chemical Physics of the Russian Academy of Sciences (Moscow). The method of X-ray phase analysis helped to determine their composition: crystal copper 96%, copper oxide 4%.

Statistical processing of received data was carried out using generally accepted methods in such software as Excel from “Office XP” and “Statistica 6.0", including the definition of the arithmetic mean value (x), standard error of the mean (Sx). To identify statistically significant differences, we applied the Student’s criterion, for similarity (difference) between the means of all groups – the Kruskal-Wallis test. Also, we used the discriminant analysis to classify individuals on the effects of various factors [2].

3. Results and discussion
Analyzing the myelogram of birds after the various ways of copper NP introduction into the body and the different dosages, we found that, by the end of the experiment, the total number of bone marrow cells in all groups was lower than the initial values: in the second group – 12.54% lower (p < 0.05), in third – 26.32% lower (p < 0.001), for the fourth – 14.75% lower (p < 0.05), with exception for the first experimental group, where this index was 45.51% higher (p < 0.001) (Figure 1).

Considering discrete hematopoietic lineage, we revealed that a similar trend was observed in cells of granulation and erythroid groups (Figure 2). So, the second group had 4.57% and 18.14% (p < 0.05)
lower values; the third group was lower by 12.71% and 32.95% (p < 0.01); and the fourth group had 3.49% and 26.24% (p < 0.01) lower values compared to the control while the first one had 44.13% and 51.56% (p < 0.001) higher values.

**Figure 1.** Changes in the total number of bone marrow cells of birds under effects of Cu NPs.

Concentration of lymphoid cells changed as follows: in the first and third groups, their numbers were 16.29 and 37.88% (p < 0.01) lower than those of control values, for the fourth group, it was 45.11% (p < 0.001) higher, and for the second group, it was on the same level as those of animals that had not been given copper nanoparticles (Figure 3).

**Figure 2.** Changes in number of cells of granulation and erythroid hematopoietic lineages under effects of Cu NPs.
Figure 3. Changes in concentration of lymphoid, monocyte-derived, and megakaryocytic cells in the red bone marrow under Cu NPs.

In the first and the fourth group, we observed an increase of monocyte-derived cells by 23.09% (p < 0.01) and 4.84%, respectively, while the second and the third groups showed a decline of 2.76% and 16.94% (p < 0.05).

The number of cells of the megakaryocytic haematopoietic lineage in all groups was higher than that before the start of the experiment by: 4.37 times (p < 0.001); 8.74%; 11.52% (p < 0.05), and 6.88% respectively; on the contrary, the content of plasma cells was lower than the initial values by: 78.14% (p < 0.001); 21.93% (p < 0.01); 6.73, and 3.89 times (p < 0.001), respectively. The content of histiocytic cells showed the following: a decline of 5.68% for the second group, of 0.13% for the third, 36.19% (p < 0.001) for the fourth, and 1.74 times higher values (p < 0.001) in the first group.

The absolute content of the reticuloendothelial cells and cells in various stages of mitosis in the experimental groups was higher than the control values: by 45.72% and 28.58% (p < 0.001) for the first group; by 9.29% and 11.52% for the second; by 11.52% and 4.96% for Group III; by 17.57% and 71.01% (p < 0.001) for Group IV, respectively.

We revealed the following changes in the peripheral blood: the hemoglobin content by the end of the experiment was significantly higher than the initial values: by 18.63% for the first group (p < 0.01); 28.61% higher in the third group (p < 0.001); and 15.76% higher for the fourth (p < 0.01), except the animals of the second group (3.23% lower). The concentration of erythrocytes in all groups was higher than that of the background: 24.56% higher (p < 0.001), by 3.37%, by 26.18% (p < 0.001), and by 14.85% (p < 0.01), respectively; the leukocyte concentration in the first group was 39.63% higher (p < 0.001), it remained at the level of the initial values in the other groups. The erythrocyte sedimentation rate in all groups increased by 2.4, 4.0, 2.01, and 1.86 times (p < 0.001), respectively.

We noted the following changes in the leucogram: the number of eosinophils in all groups decreased, single basophils appeared in samples of the first and second groups, band eosinophils in the fourth group were 2.01 times lower (p < 0.001), the other groups maintained the same level of them; segmented pseudoeosinophils were higher by: 22.78% (p < 0.001), 8.97%, 25.61% (p < 0.001), and 19, 74% (p < 0.01), respectively.

The content of lymphocytes and monocytes was lower than that before the start of the experiment: in the first group – by 33.33% and 44.20% (p < 0.001), in the second – by 6.84% and 2.17 times (p <
Analyzing changes of biochemical indicators, we found a 17.57–46.86% (p < 0.01) increase of total protein by the end of the experiment in all groups, at that, albumin decreased by 18.28–48.10% (p < 0.01) and globulins increased by 1.76–2.95 times (p < 0.001). The content of residual nitrogen in the first and second groups was not significantly higher than the initial values when in the fourth it was 18.72% higher (p < 0.01), and in the third group, we recorded a non-significant reduction.

According to our experiments, the concentration of urea in the chicks before the experiment was 1.62 ± 0.508 mmol/l. By the end of the research, the index declined in the first and third groups by 95.20% (p < 0.001) and 65.31% (p < 0.001), and it rose in the second and the fourth by 41.30% (p < 0.001) and 21.74% (p < 0.01), respectively.

The concentration of creatinine in the fourth group increased during the experiment by 3.01%, while in other groups it declined: by 54.82% (p < 0.001) for the first group, by 27.58% for the second (p < 0.01), and by 38.56% for the third (p < 0.001). In all groups cholesterol increased by 22.12% in the first group (p < 0.01), by 12.29% in the second (p < 0.05), by 9.19% in the third and by 27.08% for the fourth (p < 0.01). A similar trend was revealed for the content of glucose: in the first group, there was recorded a 3.36 times (p < 0.001) increase, in the second – 1.61 times (p < 0.001), 3.15 times for the third group (p < 0.001), and a 27.36% increase in the fourth (p < 0.01).

Analyzing the changes of the elemental content in the red bone marrow, we pointed out both similar and different trends, it depended on the dose of copper nanoparticles and the introduction means.

So, we found that introduction into organism of copper in the form of nanopowder both with feed and intramuscularly reliably caused an increase of the content of such elements as arsenic, copper, silicon and a decrease of calcium, potassium, magnesium, phosphorus, boron, cobalt, iodine, lithium, sodium, zinc, tin and strontium in the marrowy aspirate. Moreover, compared to the first group (p < 0.01), increasing doses of nanopowders caused a reliable rise in the arsenic and tin concentrations and a decline of iodine and strontium.

The other elements behaved in the same way. The contents of sodium, chromium, selenium, vanadium, aluminum in the first and fourth groups were higher than the initial values (p < 0.001), and lower in the second and the third groups (p < 0.01).

4. Conclusion
The above mentioned aspects make it possible to conclude that copper nanoparticles ambiguously affect the bone-marrow hemopoiesis of poultry; increasing the dose and changing the type of introduction activate bone marrow hematopoietic function, in particular, granulocyto-, megakaryocyto-, and erythropoiesis.

Different dosages and ways of introduction of copper nanoparticles, in our opinion, ambiguously affect the bone-marrow hemopoiesis of poultry when using copper in doses of 1.7 mg/kg of feed, which is 50% of the daily needs of the organism. E.A. Sizova et al. proved a stimulating effect of copper nanoparticles in the same dosage on liver cells of chicks [11].

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