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Research Article

Erythrocytes and Their Transformations in the Organism of Cows

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ABSTRACT

A study was conducted to study the respiratory function of blood and the morphology of erythrocytes in the organism of black-mottled cows in the conditions of the t echnogenic province formed in the zone of distribution of emissions of JSC "Uchalinsky GOK" ("Uchalinsk ore mining and processing enterprise"). In blood samples of 3 (group I), 4-5 (group II) and 5-6 year old (group III), the number of erythrocytes, hemoglobin, mean cell hemoglobin, morphology of erythrocytes were determined. The results of the research show that in the blood of cows in conditions of the natural and territorial complex subjected to technogenic pressure of JSC "Uchalinsky GOK", the number of red blood cells in the blood stream increases with age by 40.65% as the hemoglobin decreases by 1.41 -11,06% due to the change in the size of red blood cells and the average content of hemoglobin in a separate red blood cell. The state of the respiratory function of the blood is associated with the tendency of the erythrocytes to transform their form. In 3-year-old cows, the modified cellular forms are contained in 72.73% of blood smears, 4-5-year-olds at 90.91% and in 5-6-year-olds - 100%. In blood smears, in which erythrocytes have abnormalities in morphology, the number of normal discocyte cells, respectively, is 27.61±1.74; 12.94±0.65 and 2.50±0.32%. The age of animals influences the morphological changes in red blood cells. In the blood smears of 3-year-old cows, they were mainly represented by megalocytes (6.31±0.68%), macrocytes (26.88±1.09%) and anulocytes (4.13±0.78), most of which were connected to strand coins. Microcytes (35.14±1.48%), anulocytes (19.17±3.59%) and acanthocytes (16.53±0.61%) prevailed in 4-5-year-old animals. Similar picture was observed in the smears of 5-6 year old cows: microcytes (12.30±1.21%), anulocytes (13.87±0.75%), and acanthocytes (62.68±0.86%).

Key words: Blood, Respiratory function, Transformation of erythrocyte form, Cows, Technogenic province

INTRODUCTION

A study was conducted to study the respiratory function of blood and the morphology of erythrocytes in the organism of black-mottled cows in the conditions of the technogenic province formed in the zone of distribution of emissions of JSC "Uchalinsky GOK" ("Uchalinsk ore mining and processing enterprise"). In blood samples of 3 (Group I), 4-5 (Group II) and 5-6 year olds (Group III), the number of erythrocytes, hemoglobin, mean cell hemoglobin, morphology of erythrocytes were determined. The results of the research show that in the blood of cows in conditions of the natural and territorial complex subjected to technogenic pressure of JSC "Uchalinsky GOK", the number of red blood cells in the blood stream increases with age by 40.65% as the hemoglobin decreases by 1.41-11,06% due to the change in the size of red blood cells and the average content of hemoglobin in a separate red blood cell. The state of the respiratory function of the blood is associated with the tendency of the erythrocytes to transform their form. In 3-year-old cows, the modified cellular forms are contained in 72.73% of blood smears, 4-5-year-olds at 90.91% and in 5-6-year-olds - 100%. In blood smears, in which erythrocytes have abnormalities in morphology, the number of normal discocyte cells, respectively, is 27.61±1.74; 12.94±0.65 and 2.50±0.32%. The age of animals influences the morphological changes in red blood cells. In the blood smears of 3-year-old cows, they were mainly represented by megalocytes (6.31±0.68%), macrocytes (26.88±1.09%) and anulocytes (4.13±0.78), most of which were connected to strand coins. Microcytes

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(35.14±1.48%), anulocytes (19.17±3.59%) and acanthocytes (16.53±0.61%) prevailed in 4-5-year-old animals. Similar picture was observed in the smears of 5-6 year old cows: microcytes (12.30±1.21%), anulocytes (13.87±0.75%), and acanthocytes (62.68±0.86%).

The problem of anthropogenic pollution of the environment is one of the priorities in the world. Especially unfavorable ecological situation develops in the industrial regions, as the activities of enterprises form the background conditions of the environment, which exert direct and indirect influence on the processes of vital activity in humans and animals (Mactaggart *et al.*, 2017; Kakimov *et al.*, 2017). Chelyabinsk region is not an exception. It belongs to one of the most developed industrial regions of Russia, in which mining, metallurgical, energy, chemical and other enterprises are located.

For example, in the mining regions of the Bashkir Trans-Urals and the Chelyabinsk region, the prior source of pollution is the enterprise of “Uchalinsky GOK”, production waste and especially industrial effluents of which are the main reason for the formation of technogenic provinces in the territories located far beyond the deposits (Uchalinskoye, Uzelginskoye, Talganskoye, Molodezhnoe) and an ore mining and processing enterprise. At the same time, the main pollutants are heavy metals, entry into the environment of which, on the one hand, is superimposed on the specific geochemical background of mining areas, and on the other hand presents a potential threat to human and animal health through inclusion in food chains and disturbances in natural balance (Rybyanova and Derkho, 2017).

The organism of animals in technogenic provinces is forced to adapt to the conditions of existence due to the launch of adaptive reactions and mechanisms of natural self-regulation, as a result of the impact of a complex of anthropogenic factors (Derkho and Sotskiy, 2008). At the same time, the adaptation mechanisms that have evolved in the body are insolvent and unable to maintain the state of homeostasis, which initiates the appearance of diverse morphological and functional disorders in physiological systems (Baimova, 2009; Khaziakhmetov *et al.*, 2018).

The main indicator reflecting adaptive rearrangements in the animal organism is blood, which circulates through the blood vessels, touches the cells of all organs and tissues and, through this, reflects any changes in their state (Gromyko, 2005; Sotskiy and Derkho, 2009). In addition, blood is the most common and available biomaterial for laboratory research, as it is technically easy to obtain by simple manipulations.

The toxicity of heavy metals has been proven. Metals getting into the body of animals and humans, form complexes with cellular compounds through interaction with sulfur-, oxygen- and nitrogen-containing functional groups. The resulting complexes inhibit the activity of enzyme systems or modify the structure of "critical" protein molecules, which ultimately leads to dysfunction of the cells of organs and tissues or their death (Pages *et.al.*, 2008; Derkho and Sotskiy, 2008; Hernández-García *et.al.*, 2014; Muratbayev *et.al.*, 2018). The degree of manifestation of toxic effects is determined by the level of intake of metals into the body, the chemical state (organic, inorganic), the type of action (acute, chronic), age of animals, sex, etc. One of the little studied aspects of the manifestation of the toxicity of heavy metals in animals with chronic intoxications is the morphological features of red blood cells, which adapt to their action by transforming the shape of cells.

Objective: The study of the respiratory function of blood and morphological features of erythrocytes in the organism of black-mottled cows in the conditions of the technogenic province formed in the zone of distribution of emissions of JSC “Uchalinsky GOK”.

MATERIALS AND METHODS

The experimental part of the work was completed in 2016-2018 on the basis of LLC "Preduralie" Verkhneuralsky district of the Chelyabinsk region (Novoahunovo, Russia). The Verkhneuralsky region is agrarian, but it has its own geochemical features due to the presence of copper-zinc pyrite deposits in its territory that are part of ore deposit of JSC “Uchalinsky GOK”, as well as the flow of rivers into which mine and colliery water is carried off. In the components of the cows diet and drinking water, the content of zinc, copper, cadmium and lead exceeded MRL and MPC by 3-5 times.

The research was approved by the Commission on Bioethics of the Federal State Agrarian University of Higher Education "South Ural State Agrarian University" (Russia).

The object of the study was black-mottled cows before drying off (280-300 days of lactation). To perform experimental work on the principle of approximate analogues, taking into account the age, physiological state (lactation and pregnancy period), 3 experimental groups were formed (n = 11). The first group included cows after the first calving at the age of 3 years, the second after the second calving at the age of 4-5 years and the third after the third calving at the age of 5-6 years. The daily yield of the cows under investigation in the control milking results ranged from 5 to 6 kg. The feeding ration was balanced by the main nutrient and biologically active substances, including brome hay (6 kg), concentrates (crushed barley) - 6 kg, haylage - 25 kg.

The material of the studies was blood, which was obtained by vacuum method from the stitched vein in the morning, before feeding. Blood smears were made immediately after taking blood, stained using the method of Romanovsky-Giemsa. Evaluation of the morphological features of erythrocytes was carried out with the help of an immersion objective, and their number was determined in Goryaev's chamber. To determine the amount of hemoglobin, ready-made sets of “Klini Test-Gem Ts” reagents (St. Petersburg) were used. The mean hemoglobin content in erythrocyte (MCH, Pg) was calculated by the formula:

$$MCH = \frac{\text{RBC}}{Hb},$$

Where *RBC* is the number of red blood cells, 10^{12} / l,
Hb is the hemoglobin concentration, g/l.

The object of the study was cows of black and motley breed before the launch (280-300 days of lactation). To perform experimental work on the principle of

approximate analogues, taking into account the age, physiological state (lactation and pregnancy period), 3 experimental groups were formed (n = 11). The first group included cows after the first calving at the age of 3 years, the second after the second calving, having the age of 4-5 years and the third after the third calving at the age of 5-6 years. The daily yield of the cows under investigation in the control milking results ranged from 5 to 6 kg. The feeding ration was balanced by the main nutrients and biologically active substances, including hay bonsai (6 kg), concentrates (crushed barley) - 6 kg, haylage - 25 kg.

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Statistical processing of the data was carried out on a PC with the help of Microsoft Excel 2003 table processor using the 'Biometrics" application programme. The reliability of the differences between groups of traits was assessed using Student's t-test.

RESULTS

When assessing the age variability of parameters reflecting the oxygen transport function of the blood, we found the following (Table 1):

1. The number of erythrocytes in the peripheral blood of the cows of group I was 4.28±0.27 10^{12} / l, which was less than the lower limit of the norm by 14.40%. With age, there was an increase in the number of cells in the bloodstream. The maximum was noted in animals aged 5-6 years (6.02±0.16 1012 / l). The age-related increase of the indicator was 40.65% (P≤0.001). At the same time, the concentration of hemoglobin, on the contrary, decreased. If in group I the parameter value corresponded to the norm limits, in II and III it was less than the lower value of the interval by 1.41-11.06%.

2. The average hemoglobin content in erythrocyte (MCH), characterizing the cell size, decreased 1.63 times with the cows' age (P≤0.001). Comparison of this value with the norm limits showed that erythrocytes-macrocytes circulated in the blood of 3-year-old animals, and erythrocytes-microcytes circulated in 4-5 and 5-6-year-old animals, as a result of changes in the intensity of hemoglobin synthesis and cell life time (Muravyev *et al.*, 2013).

The age of cows and the number of calving were associated with the number of deviations in the morphology of red blood cells in blood smears (Figure 1). So, in group I the altered cellular forms were contained in 72.73% of blood smears. One type of deformation of cells was presented only in 9.09% of smears, two types in 27.27% and three types in 36.37%.

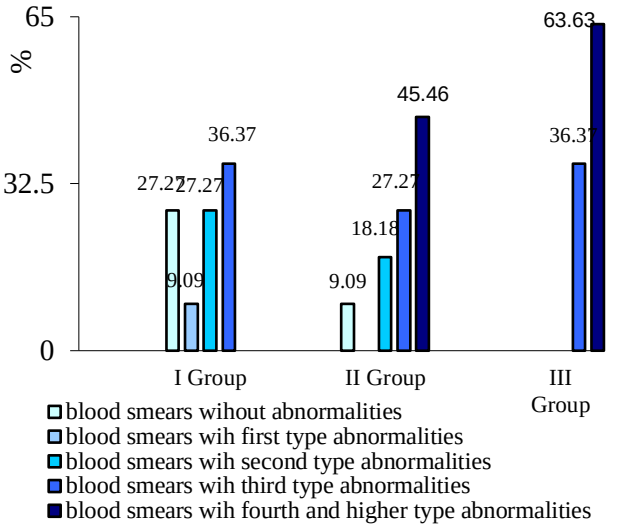

Fig. 1: Occurrence of blood smears with abnormalities in the morphology of erythrocytes.

Table 1: Blood indicators

Indicator	Red blood cell, 10^{12}/l	Hemoglobin, g/l	MCH, Pg
Group I	4,28±0,27	102,53±0,65	23,95±2,14
Group II	4,97±0,17	80,05±1,43***	16,11±1,00**
Group III	6,02±0,16***	88,73±0,91***	14,73±0,49***
Norm	5,0-7,5	90-120	16,5-18,5

Note: *** - P≤0,001 in comparison with the level of group I.

Table 2: Abnormal erythrocytes frequency (%) in blood smears (n=11), x±Sx

Name	I group	II group	III group
Normal erythrocytes	27.61±1.74	12.94±0.65	2.50±0.32
Abnormal in morphology erythrocytes including:	72.39±1.74	87.06±0.65	97.50±0.32
- megalocyte	6.31±0.68	-	-
- macrocyte	26.88±1.09	0.76±0.17	-
- anulocytes	4.13±0.78	19.17±3.59	13.87±0.75
- rouleaux	10.05±1.07	7.03±0.58	-
- ovalocyte	5.45±1.27	3.23±0.81	-
- microcyte	-	35.14±1.48	12.30±1.21
- acanthocyte	14.01±0.48	16.53±0.61	62.68±0.86
- echinocyte	5.00±0.34	-	-
- stomatocyte	-	5.20±0.41	-
- spherocyte	-	-	4.00±0.63
- teardrop cells	-	-	4.65±0.32
- drepanocyte	0.56±0.11		

With the increase in the age of the animals, the number of calving and, as a consequence, the duration of contact with the factors of the technogenic habitat, the number of blood smears increased in which erythrocytes with a modified form were found. Thus, in the group II, their number was 90.91%, and in the group III it was 100%, that is, under hypoxic conditions, the tendency of cells to reversible and irreversible transformations of the form increased, which according to the data of (Muravyev *et al.*, 2013) determines their gas transport function, as well as the time of their life in the bloodstream, as they become less elastic and easily destroyed, both inside the vessels and as a result of intracellular hemolysis (Vorobyev, 2017). To similar data in their studies came (Ranjan *et al.*, 2014). They noted that the functional

integrity of the membrane skeleton is also necessary for the transformation of the shape of red cells. In this case, the effect of chemical exogenous substances increases the tendency of erythrocytes to transformations.

Therefore, in blood smears of cows of experimental groups II and III 4 types of abnormalities in the morphology of erythrocytes were recorded. So, in the group II, their number was 45.46% and in III - 63.63% (Figure 1).

Consequently, the existence of cows in the technogenic environment that is formed in the zone of distribution of emissions of JSC "Uchalinsky GOK" is accompanied by the transformation of the form of erythrocytes.

Analysis of the frequency of occurrence of transformed erythrocyte forms in blood smears, in which deviations in cell morphology were revealed, showed that the number of normocytes (discocytes) in the bloodstream of animals does not correspond to the norm limits and decreases with age. If in group I the number of biconcave erythrocytes in blood smears was 27.61±1.74%, then in III – only 2.50±0.32% (Table 2).

Types of "reversibly and irreversibly changed" erythrocytes (Lipunova and Skorkina, 2007) and their number also depended on the age of cows and, accordingly, the duration of their existence in the conditions of the technogenic province. Thus, in the group I of animals (Table 2, Figure 2) cells circulating in the bloodstream differed in both reversible and irreversible transformations. Reversibly transformed cells were mainly represented by echinocytes, the appearance of which indicated a change in the ion composition of the medium surrounding the erythrocyte (Lipunova and Skorkina, 2007). In a pool of irreversibly transformed cells, those that retained the discoid form predominated. They were represented by megalocytes (6.31±0.68%), macrocytes (26.88±1.09%) and anulocytes (4.13±0.78%).

Most of these cells, as a result of changes in the surface charge, were connected into strand coins. In addition, elliptocytes (6.01±1.27%), that is, erythrocytes of the oval form, and acanthocytes (14.01±0.48%) were detected in blood smears.

Microcyts (35.14±1.48%) and anulocytes (19.17±3.59%), as well as cells with primary abnormalities in the functions of the lipid component of the membrane - acanthocytes, prevailed in group II. A similar picture was observed in smears of animals of group III.

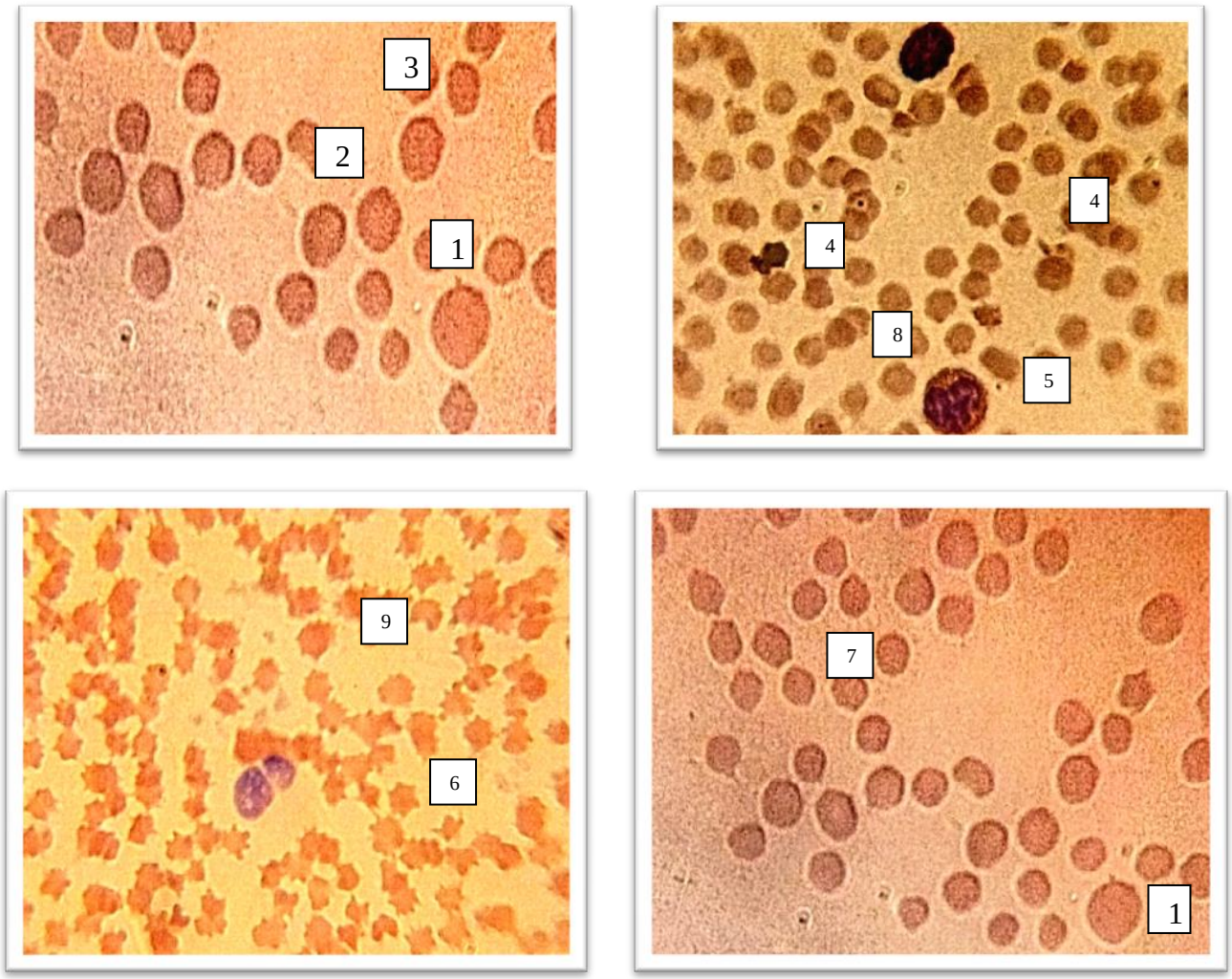

Fig. 2: Erythrocyte shapes in cow's blood smearsв: 1 - megalocyte, 2 – macrocyte, 3 - elliptocyte, 4 – «rouleaux»; 5 – microcyte; 6 acanthocyte; 7) normocyte; 8) anulocytes; 9) drepanocyte.

DISCUSSION

Adaptation of animals to constantly changing environmental conditions occurs with the help of ready adaptive mechanisms, among which an important role belongs to antihypoxic reactions aimed at preserving the body cells with the necessary amount of oxygen. An important role in these processes is played by erythrocytes, the main function of which in the animals is the transport of oxygen due to the presence of a hemoglobin protein in their composition (Moroz *et al.*, 2012). The blood system is one of the first to be exposed to heavy metals, which is reflected both in the number of erythrocytes and their morphological characteristics as a result of changes in the physical and chemical properties of cell membranes.

Analysis of the age-related variability in the concentration of erythrocytes, hemoglobin, and the average hemoglobin content in the erythrocyte allows us to state that the respiratory function of the blood and, as a consequence, the supply of oxygen to the cells of the body's tissues decreased, with an increase of the age of the cows and the number of calving, which was the result of adaptation of animals to the conditions of the technogenic habitat . At the same time, the increase in the number of red blood cells in the blood was the result of the homeostatic response of the body to a decrease in the amount of hemoglobin in the cells. According to the data (Sharma *et al.*, 2014) under hypoxic conditions, red blood cells are able to act as a sensor, which initiates the subsequent emission of regulatory molecules that activate the processes of erythropoiesis.

The result of our studies is consistent with the data of other authors. Thus, it was noted in (Bersényi *et al.*, 2003; Pages *et al.*, 2008; Hernández-García *et al.*, 2014) that the change in the gas transport properties of blood during metallotoxicosis is due to hemolysis of erythrocytes due to the direct action of toxicants. Similar conclusions were reached (Vorobyev, 2017), studying the effect of copper-zinc pyrite ore on the organism of laboratory rats. According to research (Sotskiy and Derkho, 2009; Rybyanova and Derkho, 2017) in the conditions of man-made provinces in the human body and agricultural animals signs of hypoxia develop. However, some authors stated that excessive intake of heavy metals in the body initiates a simultaneous decrease in erythrocytes and hemoglobin (Sotskiy and Derkho, 2009), others noted a decrease in the number of erythrocytes against a background of increased hemoglobin (Derkho *et al.*, 2013) or an increase in erythrocytes against the background of hemoglobin decrease (Gromyko, 2005).

The gas transport properties of blood are determined not so much by the quantity of erythrocytes as by their ability to transport oxygen in the microcirculatory bed, since the size of the "mature" cell is much larger than the diameter of the small capillaries. Therefore, the basis of efficient oxygen transport is the tendency of erythrocytes to deform. This indicator determines the lifetime of cells in the bloodstream, and the physical and chemical properties of the erythrocyte membrane, and size. Therefore, the evaluation of the morphology of erythrocytes plays an important role in the recognition of the mechanism of hypoxia, since the shape, size, diameter of the cells affect the concentration of hemoglobin in them, the resistance to transformation and fragmentation, which together determines the rate of their circulation in the circulatory system and the gas transport capacity.

The population of red blood cells is not uniform in shape and size. Normally the bulk consists of biconcave cells - discocytes, as well as aging forms of erythrocytes (echinocytes, stomatocytes and spherocytes) (Lipunova and Skorkina, 2007). Found reasons for deviations in the morphology of erythrocytes are changes in the membrane complex that initiate the transformation of its viscosity, elasticity and fluidity, and direct exposure to xenobiotics (Vorobyev, 2017). According to the data (Moroz *et al.*, 2012; Krylova and Derugina, 2011), the degree of morphological changes in the erythrocyte is proportional to the level of oxidative stress.

Therefore, the existence of cows in the conditions of the technogenic province was accompanied by appearance and a progressive increase of erythrocytes in the blood flow, the form of which differed from the disk form. According to the data (Björk and Backman, 1994), the main reason for these changes is the decrease in the antioxidant status of cells, as a result of their reaction to the action of the stimulus by changing their own metabolism (Makarova, 2013). At the same time (Hamada *et al.*, 1998), it is believed that poikilocytosis of erythrocytes in case of metallotoxicosis is the result of a change in the skeleton of cell membranes that initiates their intravascular hemolysis. According to the data of (Serebryakova Y.N., 2014), the main reason for the violation of cell morphology is the formation of stress erythropoiesis under hypoxia conditions, as a result of which erythroid progenitors differ in morphological features from those that are formed under physiological conditions. In combination, the above listed factors determine the appearance of morphological changes in erythrocytes during the adaptation of animals to the conditions of the technogenic habitat.

Consequently, hypoxia in the body of cows was the result of a change in the shape of red blood cells, which influenced their gas transport properties, as cells with anomalous form have a reduced capacity for deformation (Kumaravel and Singh, 1995).

The results of our studies are consistent with the data of (Valiullina *et al*, 2016). The authors also observed the appearance of pathological forms of erythrocytes (leptocytes, acanthocytes), as a result of changes in the structure of blood cells, their number and functions against the background of toxic effects of elements contained in copper-zinc pyrite ore. Similar conclusions were reached (Hamada *et al.*, 1998) with cadmium toxicosis.

Conclusion

1. The existence and exploitation of cows in the conditions of the natural and territorial complex, subject to technogenic pressure of JSC “Uchalinsky GOK”, is accompanied by the development of hypoxia in the organism of animals due to change in blood indicators reflecting the state of the oxygen transport function of the blood.

2. The number of erythrocytes in the bloodstream of animals increased by 40.65% with age, against a

background of a decrease in hemoglobin by 1.41-11.06% due to change in the size of red blood cells and the average hemoglobin content in a separate erythrocyte.

3. In the organism of cows, the age and length of life in the conditions of anthropogenic provinces determine the tendency of erythrocytes to transform the shape of cells. In 3-year-old cows, the modified cellular forms are contained in 72.73% of blood smears, 4-5-year-olds at 90.91%, and in 5-6-year-olds - 100%. The number of normal erythrocytes - discocytes, respectively, is 27.61±1.74; 12.94±0.65 and 2.50±0.32%.

4. The number of types of abnormalities in the morphology of red blood cells is determined by the age of the animals. In the blood smears of 3-year-old cows, they are mainly represented by megalocytes (6.31±0.68%), macrocytes (26.88±1.09%) and anulocytes (4.13±0.78), most which is connected to coins. Microbes (35.14±1.48%), anulocytes (19.17±3.59%) and acanthocytes (16.53±0.61%) prevail in 4-5-year-old animals. A similar pattern is observed in the smears of 5-6 year old cows: microcytomas (12.30±1.21%), anulocytes (13.87±0.75%) and acanthocytes (62.68±0.86%).

REFERENCES

Baimova SP, 2009. Heavy metals in the system "soil-plant-animal" in Bashkir Zauralye. PhD-thesis. Ufa, Institute of biology of Ufa Scientific Center.

Bersényi A, SG Fekete, Z Szöcs, E Berta, 2003. Effect of ingested heavy metals (Cd, Pb and Hg) on haematology and serum biochemistry in rabbits. Acta Veterinaria Hungarica, 51: 297-304.

Björk J, L Backman, 1994. Sodium selenite as modulator of red cell shape. Biochimica et Biophysica Acta, 1191(1): 141-146.

Derkho MA, PA Sotskiy, 2008. Effect of heavy metals accumulation in bull's body to the functional properties of liver. The Veterinarny Vrach, 1:16-19.

Derkho MA, YV Tkachenko and LF Maltcseva, 2013. Some haematological parameters of mice on cadmium intoxication treatment. Nauka, Special issue: 141-144.

Gromyko YV, 2005. Evaluation of the state of cow's body by biochemistry methods. North Caucasus Environmental Bulletin, 2: 80-94.

Hamada T, A Tanimoto, N Arima, Y Ide, T Sasaguri, S Shimajiri, Y Sasaguri, 1998. Altered membrane skeleton of red blood cells participates in cadmium-induced anemia. Biochemistry and Molecular Biology International, 45(4): 841-847.

Hernández-García A, D Romero, P Gómez-Ramírez, P María-Mojica, E Martínez-López, AJ García-Fernández, 2014. In vitro evaluation of cell death induced by cadmium, lead and their binary mixtures on erythrocytes of Common buzzard (Buteo buteo). Toxicology in Vitro, 28(2): 300-306.

Kakimov A, Y Zharykasov, Z Kakimova, Z Yessimbekov, G Mirasheva, M Baybalinova, I Smirnova, 2017. Specific activity of Cs-137 in milk of Semey region of East Kazakhstan area. Annual Research & Review in Biology, 12(5), ARRB-33391.

Khaziakhmetov F, A Khabirov, M Rebezov, A Basharov, I Ziangulov, E Okuskhanova, 2018. Influence of probiotics "Stimix Zoostim" on the microflora of faeces, hematological indicators and intensitivity of growth of calves of the dairy period. International Journal of Veterinary Science, 7 (4): 178-181.

Krylova VN, AV Derugina, 2011. Electrophoretic mobility of erythrocytes under stressors. Haematology and transfusiology, 56 (5): 18-21.

Kumaravel M, M Singh, 1995. Sequential analysis of aggregation process of erythrocytes of human, buffalo, cow, horse, goat and rabbit. Clinical Hemorheology and Microcirculation, 15: 291-304.

Lipunova YA, MY Skorkina, 2007. Blood physiology. Belgorod, BelGU Publishing House.

Mactaggart F, L McDermott, A Tynan, CA Gericke, 2017. Exploring the determinants of health and wellbeing in communities living in proximity to coal seam gas developments in regional Queensland. BMC Public Health, 18: 51.

Makarova NA, 2013. Principles of oxygen transfer functions of blood. Bulletin of South Ural State University. Series: Education, medicine, physical training, 13(1): 148-153.

Moroz VV, AM Golubev, AV Afanasyeva, 2012. Erythrocyte structure and function in normal and critical conditions. Critical care medicine, 1:52-60.

Muratbayev DM, ZK Tokayev, ON Akhmetzhanov, AS Ygieva, NN Mukhamadieva, 2018. Ovarian disorders treatment in dairy cows with infertility. Adv Anim Vet Sci, 6: 436-442.

Muravyev AV, VL Komlev, PV Milhailov, AA Akhapkina, AA Muravyev, 2013. Erythrocyte deformation: role in microcirculation. Yaroslavl Pedagogical Bulletin, 2 (3): 93-102.

Pages G, D Szekely, PW Kuchel, 2008. Erythrocyte-shape evolution recorded with fast-measurement NMR diffusion-diffraction. Journal of Magnetic Resonance Imaging, 28(6): 1409-1416.

Ranjan A, VK Dumka, ND Singh, 2014. Effect of flubendiamide, lead and their combined exposure on erythrocytic indices in water buffalo calves. Bulletin of Environmental Contamination and Toxicology, 92(4): 410-414.

Rybyanova ZS, MA Derkho, 2017. Erythrocyte morphology in calves' body in the condition of anthropogenic places. Agroind Russia, 24: 687-692.

Serebryakova EN, 2014. State of the erythrons in newborns with multiple-organ-failure syndrome. Doctor of Medicine's Thesis, Chelyabinsk.

Sharma B, S Singh, NJ Siddiqi, 2014. Biomedical implications of heavy metals induced imbalances in redox systems. BioMed Research International. http://dx.doi.org/10.1155/2014/640754.

Sotskiy PA, MA Derkho, 2009. Study the effect of heavy metals to haematological parameters of blood. The Veterinarny Vrach, 4:5-8.

Valiullina AR, LF Shaidullina, LM Saptarova, KR Ziyakaeva, 2016. Biochemical analysis of rats' blood in chronic intoxication by copper-zinc sulphide ore. Bulletin of Bashkir State Medical University, 4:113-118.

Vorobyev LV, 2017. Microscopy of native products. http://www.microscope.org.ua/wp-contenent upoads (accessed 10.12.2017).