and expression of enzyme-encoding genes related to glutathione in hematopoietic cancers and solid tumors / S. Zmorzynski, G. Uwiderska-Kobacz, D. Koczkodaj [et.al] // BioMed Research International. – 2015. – P.1-6. DOI: http://dx.doi.org/10.1155/2015/853573

17. The concentration of thiobarbituric acid reactive substances (TBARS) and paraoxonase activity in blood of patients with osteoarthrosis after endoprosthesis implantation / D. M. Olszewska-Słonina, D. Mątewski, R. Czajkowski [et. al] // Medical Science Monitor. – 2011. – №17(9). – P.498–504. DOI: 10.12659/MSM.881936.

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ASSESSMENT OF CHANGE OF ERYTHROCYTES BY METHOD OF RASTER ELECTRONIC MICROSCOPY AT THE PERSONS WHO DIED OF FATAL HYPOTHERMIA

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ABSTRACT

Red blood cells (RBC) morphology of deceased from various causes (injury, hypothermia) was examined in this study by using scanning electron microscopy. The obtained data shows that the appearance of certain forms of cadaveric erythrocytes depends on the causes of death. Therefore, when death is caused by stabbing and gunshots RBC take acanthocyte forms and in cases of fatal hypothermia RBC take echinocyte forms. The results of an experiment carried out in vitro, at small negative temperatures,show the appearance of acanthocytes in observed blood samples. Based on theobtained data and on the ability of echinocyte to return to normal form, it can be concluded that the probability of restoring the vital activity of the frozen organismsare possible.

Keywords: red blood cells, erythrocytes,hypothermia, scanningelectron microscopy.

Introduction. Frostbite and hypothermiaare the most severe types of cold trauma, often leading to a high level of disability and death of the affected [1]. Till present time, the questions of death from freezing in conditions of extremely low temperatures (below -40°C) have not been fully examined in the world. In real conditions, people who died from hypothermia (by external signs) without carrying out resuscitation measures are delivered to the morgue. The key-point is that during the first 2 days when the victims are in a state of cold anabiosis (a very rare pulse, low blood pressure) it is may be possible to restore the vital activity of the organism(there are cases of preservation of the vital functions of the heart and other organs in frozen animals).The results of our work suggest a theoretical probability of the restoration of frozen tissues, organs, and also the revitalization of the frozen organisms.

At present time, in the literature available to the public, there are no data on the SEM study of the RBCs of people who diedfrom the cold temperatures.

Revealing the features of the morphology of the blood cells of the deceased from hypothermia may possibly supplement the idea of the cellular-molecular mechanisms of the process of general cooling of the organism and confirm the possibility of restoring the vital activity of the organism.

The purpose of this article is toexamine

the morphological changes in RBCs from cadaveric material (cause of death: hypothermia) using a scanning electron microscopy.

Materials and methods of research. The study was carried out using a high-resolution scanning electron microscope (SEM) JSM-7800F («Japanese Electron Optics Laboratory» - «JEOL», Japan) in UNTL «Graphene nanotechnology» M.K. Ammosov NEFU. The subjects of the study were blood smears from cadaveric material of the deceased from various causes of death, including hypothermiaprepared by the Bureau of Forensic Medical Examination of the Ministry of Health of the Republic of Sakha (Yakutia).

The device has a Schottky thermo-field emission cathode and a superhybrid objective lens that significantly reduces chromatic and spherical aberration and significantly increases the resolution, especially at low accelerating voltages. The SEM used in the experiment (enlargement range of 25 - 1 000 000) allows us to to examine the object at an accelerating voltage of 0.1-30 kV. The device is equipped with 4 types of detectors: the upper electron detector, the upper secondary electron detector, the backward reflected electron detector and the lower secondary electron detector. In this study a lower secondary electron detector was used. This mode allows reducing the effect of electrostatic charge on the surface of the sample, which significantly improves the image quality of the erythrocyte surface.

Used in the study of blood smears, the SEM JSM-7800F is equipped with the «Gentle Beam» system, which allows to reduce the speed of electrons falling on the sample and accelerate the electrons emitted by them, which significantly improves image quality at low accelerating voltages.

The «Gentle Beam» system allows for the study of erythrocytes in blood smears without the deposition of conductive coatings and without damaging the objects under investigation for a time sufficient to make the necessary photographs [2].

Investigations of blood smears were carried out at accelerating voltages of 1 and 2 kV with a voltage applied to the investigated object of 8-10 V.

An experimental study of volunteer blood cells in vitro was also conducted. Blood, placed in a glass tube, was gradually cooled, and then warmed. During the cooling and heating, 6 blood smears were made alternately: immediately after venous blood sampling at a temperature of + 36.6 ° C; then at temperatures of + 8.0 ° C; + 2.0 ° C; - 1.7 ° C; + 12.5 ° C and + 31.0 ° C. Blood sampling from deceased persons from hypothermia was made from the brain region where the temperature was maximum (from +6 ° C to 20 ° C) compared to other tissues and organs. The minimum temperature in the soft tissues of the upper and lower extremities was -40 ° C, in the lungs +12 ° C, in the liver +10 ° C.

Results and discussion. There is a problem of differentiation of the forms of acanthocytes and echonocytes, therefore in this work for the evaluation of erythrocyte species the results of other studies [3] were used, where the characteristic parameters of differentiation of different forms of erythrocytes and their association with diseases based on images obtained with the scanning electron microscope SAM). Figure 1 shows the characteristic forms of erythrocytes - acanthocytes (A), echinocytes (E) and schizocytes (S).

Echinocytes have small identical protuberances distributed evenly, while acanthocytes are protuberances of various sizes and are unevenly distributed over the surface of the cell.

During the study of blood smears, REM images of erythrocytes were obtained for various causes of death. In Fig.2.images of red blood cells of a young man whose blood smear was made the day after his death are presented. Death occurred within 30 minutes as a result of a stab wound.

In the field of vision with an increase in 1000 times, the prevalence of dysmorphic erythrocytes is observed in comparison with normal ones. It should be noted that the dysmorphic erythrocytes are close in shape to the acanthocytes. Unchanging cells form small «coin pillars», and the modified cells are tightly connected to each other by processes. In this image, fragments of hemolyzed erythrocytes are not observed.

In Fig. 3 shows the erythrocytes of a man who died of a gunshot wound whose blood smear was made on the second day after the onset of death.

In the case of a gunshot wound, the changed erythrocytes are similar in form to acanthocytes. The same dysmorphic erythrocytes are also observed in Fig. 1 in the case of a stab wound wound. In contrast to Fig. 2 there are signs of hemolysis of erythrocytes, i.e. a small number of fragments of erythrocytes are seen, almost all cells form conglomerates. Unlike the first case, the amount of unchanged red blood cells is significantly less, which may be due to a temporary factor: in the second case, a smear was made on the second day after the onset of death, while in the case of a stab wound cut, a day later.

In Fig. 4 depicts images of red blood cells of a 25-year-old man who died from hypothermia. The blood was taken a day later.

In this figure, all the red blood cells are changed and most likely they can be attributed to echinocytes, and they are evenly distributed in one layer. In contrast to the previous images in this figure, it is observed that all red blood cells are interconnected very tightly and form small groups. The processes of erythrocytes in the frozen have a more acute form than those of the deceased from wounds, and also more evenly distributed over the entire surface of the erythrocytes.

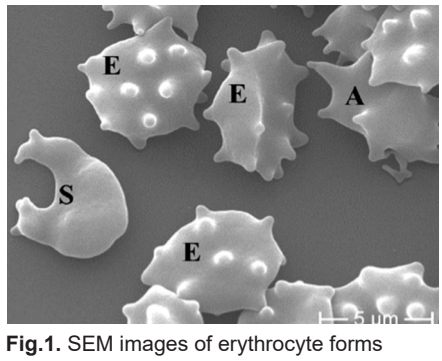

Fig.1. SEM images of erythrocyte forms

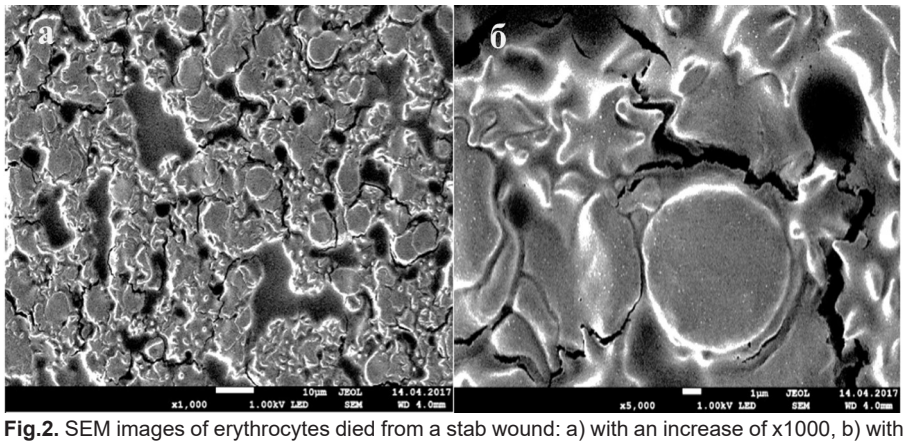

Fig.2. SEM images of erythrocytes died from a stab wound: a) with an increase of x1000, b) with an increase of x5000

In addition to studies of blood cells from cadaveric material, REM studies of blood cells of a young male volunteer were performed.

In Fig. 5-6 presents SEM images of erythrocytes of blood smears of a living healthy person on a slide, obtained at various temperature effects on a blood sample.

The blood in the test tube was cooled gradually and as the cooling was carried out applying blood smears to the slide. Obviously, with positive blood temperature values (from + 36.6 ° C to + 2.0 ° C), no significant changes in the morphology of erythrocytes are observed (Fig. 5). However, at a negative temperature (-1.7 ° C), dermorphic erythrocytes appear in a small amount in a form close to the acanthocytes (Figure 6), as well as a small number of schizocytes. In addition, the formation of coins and conglomerates of erythrocytes is observed. Then gradually the blood was heated. The REM images of erythrocytes obtained with the heating of blood do not differ from their images obtained after exposure to negative temperature.

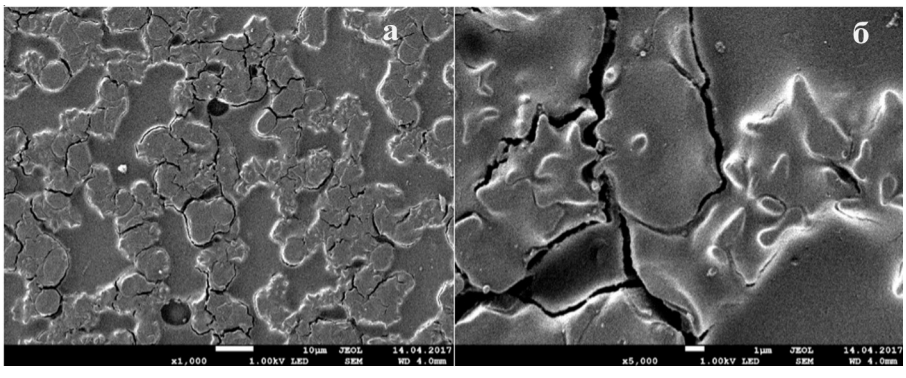

Fig.3. SEM images of erythrocytes died of a gunshot wound: a) with an increase of x1000, b) with an increase of x5000

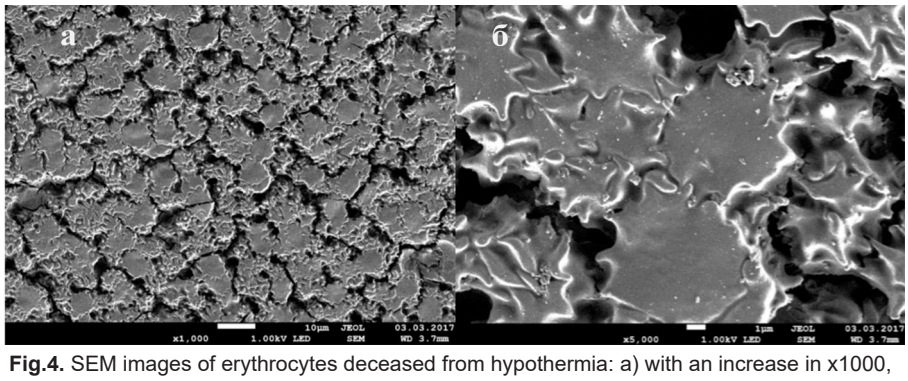

Fig.4. SEM images of erythrocytes deceased from hypothermia: a) with an increase in x1000, b) with an increase of x5000

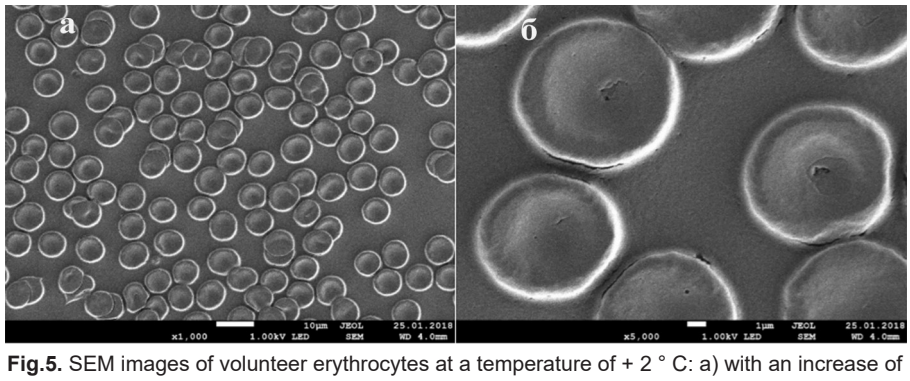

Fig.5. SEM images of volunteer erythrocytes at a temperature of + 2 ° C: a) with an increase of x1000, b) with an increase of x5000

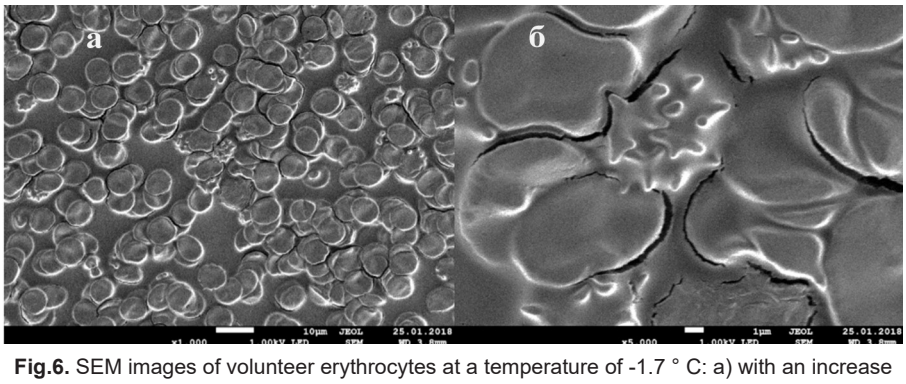

Fig.6. SEM images of volunteer erythrocytes at a temperature of -1.7 ° C: a) with an increase of x1000, b) with an increase of x5000

The results obtained by us show that the appearance of certain forms of erythrocytes of cadaveric material depends on the causes of death. So, when dying from a stab-cut and gunshot wounds, erythrocytes take acanthocyte forms, and when undercooling, they take echinocyte forms.

In the results of the experiment conducted in vitro, at small negative temperatures in SEM images, the appearance of acanthocytes is observed as in cases of death from wounds. In the deceased from hypothermia and other causes, blood samples were taken at approximately the same small positive temperatures. It should be noted that in the case of in vitro red blood cells did not change their forms up to a temperature value of + 2.0 ° C. This suggests that the appearance of acanthocytes in the blood of the deceased from hypothermia is possible only with prolonged stagnation of the corpse in a medium with a negative temperature, i.e. Provided that the temperatures of the internal vital organs decrease to negative values.

According to the results of this study, the comparison of dysmorphic erythrocytes during supercooling and cooling of blood samples in vitro shows that the mechanisms of the changes in the forms of red blood cells in these cases are different.

It is known that the acanthocyte forms of erythrocytes are determined by the defect of the structural membrane [4, 5], whereas the echinocyte forms can be induced and canceled by pH, osmolality, biochemical and even electrical changes [6-9]. Irreversibility of changes in the forms of acanthocytes is confirmed in experiments in vitro: acanthocytes, which appeared as a result of a drop in the temperature of the blood sample under study to negative values, do not disappear with and without an increase in the temperature of the blood in the field of view of SEM images. Unlike acanthocytes, erythrocytes of the echinocyte form, which are observed in persons who died from hypothermia, can

be restored.

Conclusion. Thus, the results of the SEM study obtained by us indicate that the forms of erythrocytes in persons who died from various causes differ, namely, during overcooling - erythrocytes take echinocytic form, and when wounded - acanthocytic. This fact gives us the opportunity to assume that the process of dying during hypothermia has its own characteristics. It is necessary to further in-depth study of this pathology at the molecular-cellular level to find solutions to restore the body's vital activity in the first days after death from hypothermia.

References

1. Alekseev R.Z., Tomskij M.I., Gol'derova A.S. [et al.] Preduprezhdenie razvitiya nekroza pri otmorozheniyah s oledeneniem tkanej [Prevention of the development of necrosis during frostbite with icing of tissues] Mezhdunarodnyj zhurnal prikladnyh i fundamental'nyh issledovanij [International Journal of Applied and Fundamental Research.], 2015, V. 8-1. pp. 35-41.

2. Maksimov G.V., Mamaeva S.N., Antonov S.R. [et al.] Izmerenie morfologii ehritrocitov metodom ehlektronnoj mikroskopii dlya diagnostiki gematurii [Measurement of erythrocyte morphology by electron microscopy for the diagnosis of hematuria]. Metrologiya. Ezhekvartal'noe prilozhenie k Z. Izmeritel'naya tekhnika. [Metrology. Quarterlyannexto J. Measuringequipment], 2016, V.1, pp. 47-52.

3. Foglia A. The Acanthocyte-Echinocyte differential. Swiss Medical Weekly, 2010, V.140 (03-04). pp. 1-3; DOI:https://doi.org/10.4414/smw.2010.12963

4. Bosman G.J. Erythrocyte membrane abnormalities in neuroacanthocytosis. Evidence for a neuron-erythrocyte axis? / G.J Bosman., M.W. Horstink, W.J. De Grip // Neuroacanthocytosis Syndromes. Springer Verlag, 2005, pp.153–159.

5. Acanthocyteset hypercholesterolemia / J Perrin, A Georges, A Morali, [et al.] // Ann Biolo Clin., 2008, V.66 (5), P. 56972.

6. Reinhart W.H. Red Cell Rheology in Stomatocyte-Echinocyte Transformation: Roles of Cell Geometry and Cell Shape. / W.H. Reinhart, S.Chien // Blood., 1986, V.4. pp. 1110–1118.

7. Wong P.A. Basis of Echinocytosis and Stomatocytosis in the Disc-sphere Transformations of the Erythrocyte. J Theor Biol., 1999, V.196(3), pp. 343–361. DOI:10,1006 / jtbi.1998.0845

8.Mrowietz C, Hiebl B, Franke RP, et al. Reversibility of Echinocyte Formation after Contact of erythrocytes with various Radiographic Contrast Media. Clin. Hemorheol. Microcirc., 2008, V.39(1–4), pp. 281–286.

9. Schwarz S, Deuticke B, Haest CW. Passive Transmembrane Redistributions of Phospholipids as a Determinant of Erythrocyte Shape Change. Studieson Electroporated Cells .Mol. Membr. Biol., 1999, V.16(3), pp. 247–55.

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GAMMA BACKGROUND ENVIRONMENT DURING MAGNETIC STORMS AND THE CONTENTS OF SODIUM AND POTASSIUM IN ERYTHROCYTES IN PATIENTS WITH ARTERIAL HYPERTENSION DEPENDING ON THE TEMPERAMENT AND ANTIHYPERTENSIVE THERAPY

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ABSTRACT

Arterial hypertension is one of the major independent risk factors for complications of cardiovascular diseases, stroke, myocardial infarction, acute and chronic heart failure. In the structure of cardiovascular diseases arterial hypertension occupies the first position in disability and mortality of the working population. In recent years, this contributes to the growth of psycho-emotional stress, anxiety and depression. Periods of sharp changes heliometeofactors also combined with an increase in the proportion of individuals with complications of hypertension.

Thus, in the period of magnetic storms, there was an increase in gamma radiation power (within the regional norm), an increase in the degree of psycho-emotional stress and a change in the concentration of electrolytes in the blood of both healthy and persons with cardiovascular disease. The aim of the study was to establish the relationship between the dynamics of γ-background of the environment during magnetic storms and the content of potassium and sodium in red blood cells in patients with hypertension with different temperament and anxiety, taking options for antihypertensive therapy: targeted and not targeted (empirical) on the blockade of psychosomatic characteristics of patients, as well as to determine the most effective treatment option. The prevailing temperament – choleric, sanguine, phlegmatic and melancholy – was determined using the psychological test of John Eysenck and A. Belov, the presence and severity of depression – Je. Ahmetzhanov psychological tests. Potassium and sodium content in erythrocytes was determined by ion-selective method. The gamma background of the medium was measured using the dosimeter «Master».