INTRODUCTION

Mammoths, extinct mammals, have always attracted the attention of scientists around the world. In recent years the mammoth body parts discovery and excavation allowed us to think about the possibility of reconstruction of the body of this fossil animal. The discovery of mammoth carcass in 2013 on the Maly Lyakhov island (the Laptev Sea) became a historical event. According to paleontologists, the age of this find is 43 thousand years. The main features of the Malolyakhovsky mammoth are preservation of the soft tissues and a detection of the dark-brown liquid substance, which was found along the bottom of the mammoth's trunk.

THE AIM

Firstly, to reveal and study preservation of cell elements of biomaterial received at excavation of 2013. Secondly, to find out and study preservation of cell elements taken from a blood vessel of a front extremity, during preparation of the Malolyakhovsky mammoth trunk which was carried out in March, 2014.

MATERIALS AND METHODS

The research study was done on the basis of educational and scientific clinical diagnostic laboratory of Clinic of MI NEFU, Yakutsk, Russia. The material of our research study: dark brown liquid taken out during the time of excavation of Malolyahovsky Mammoth (2013), biomaterials taken out during the dissection of the mammoth carcass (March 2014).

To study the morphological properties of the biomaterials we used the microscopic examination of swabs by the classical Romanovsky-Gieims [2] staining method for formed elements and we used binocular microscope «Olympus CX31» (10F eye lens FN20i wide field of view, zooming in 40-1000).
in which the acidophilic elements are stained in various shades of red, and basophil elements - from purple to blue.

Hematological analysis of biomaterial was performed in an automated analyzer Advia 2120 (Siemens Healthcare Diagnostics Inc., 2010), which allowed us to detect and differentiate accurately the cellular elements in a wide linearity and range (0.2 - 400 x 10^9 / L). In addition to these, we used two-dimensional laser diffusion of light technique, which allowed us to identify each cell and determine its individual properties. To study hemoglobin we used technique of non-cyanide definition [4].

RESULTS AND DISCUSSION

During the research study of similar to blood fluid swabs, the main background was gray-pink, the background is a homogeneous mass of destroyed nuclear-free cellular elements, which is similar to the hemolysis in its morphology. Against this background, we found individual cellular elements having morphological picture similar to the description of formed blood cells of large mammals (neutrophils, lymphocytes, and monocytes).

NEUTROPHILS

The discovered cellular elements had intense color and morphologically were similar to polymorphonuclear leukocyte (neutrophils). Being studied the cells had clear contours, diameter sizes ranged from 5 to 7 micrometers. The cell plasmolemma had more distinct color and thickness. Basophilic cytoplasm was filled with dust-like grains and preserved nucleus. Nuclear inclusions were deep purple color, consisted of 4-6 segments. Some parts of the nucleus were connected by thin constrictions, the nuclear chromatin had inhomogeneous large - lumpy structure (Fig. 1).

The microscopic study of the concentration taken from a blood vessel of the forelimb during the mammoth carcass thawing in 2014 showed us cells identical to the initial biomaterial similar to neutrophils (Fig. 2).

The swab background had a completely different staining - smoky-blue, there were numerous clusters of destroyed cellular elements, preserved cells were rare in the field of vision. Simple structures were present (Fig. 3).

Hematologic study showed us that total number of leukocytes was 1.6 x 10^9/L. The research study of the peroxidase channel shows that the number of leukocytes - 1.4 x 10^9/ L. The analyzer differentiated absolute neutrophil number (0.2 x 10^9/ L) and the relative neutrophil number was 11.9%. (Table 1).

In the microscopic study of the concentration taken from a blood vessel of the forelimb during the mammoth carcass thawing in 2014 we found identical cells, like the cells of a dark brown liquid (Fig. 5).

Hematologic study of the vessel concentrations tested on the analyzer, confirmed the presence of the lymphocytes in the composition, the absolute and relative number of which was 0.8 x 10^9 / L and 54.6%, respectively (Table 1).

MONOCYTES

Per field of vision we also found concentrations of larger cells with the diameter of 13-14 micrometers, they had precise contours and irregular shapes. Plasmolemma was thin, cytoplasm - basophilic (smoky-blue) and there were numerous dust-like granules. The nucleus had large, polymorphic form, it was non-segmented. Chromatin was loose and irregular. (Fig. 6).

In the microscopic study of the concentration taken from a forelimb blood vessel during the mammoth carcass thawing in 2014 we found the same cells like monocytes. Swab background was smoky-blue, it had numerous ghosts of destroyed cellular elements (Fig. 7).

Hematological study of the blood vessel concentrations we conducted in analyzer confirmed the presence of monocytes in the fluid composition, the absolute and relative amount of which was (0.2 x 10^9/ L) and 15.5%, respectively (Table 1).

The obtained results of microscopic examination of the initial dark-brown liquid, allowed us to conclude that the liquid cell comprises elements in their morphology similar to mammoth blood elements: monocytes, neutrophils, lymphocytes containing nuclear inclusions. We made a comparative analysis of cells found in the dark brown liquid swab obtained during the excavations of the Mammoth, the swab was taken from the bottom of the trunk and a swab of biological material taken from a blood vessel of the forelimb, during the dissection of the carcass, conducted in March 2014. The analysis showed us the same morphological structure of cell elements in both swabs.

Thus, the microscopic examination of swabs made from dark brown liquid vessel concentrations and the front limbs having the same morphological structure of cellular elements, allow us to conclude that the blood of the fossil animal indeed exists in the composition of dark-brown liquid.

PHAGOCYTOSIS

The study of swabs, to our surprise, showed us some cellular elements in the various stages of phagocytosis. Neutrophils and monocytes (macrophages, in tissues) are referred to one of the types of professional phagocytes mammals. As is known, one of the initial steps of the phagocytosis is chemotaxis wherein the directed migration of leukocytes [6] take place. On the surface of the object there were the molecule receptors on the surface of phagocytes, so the next step is adhesion. We registered adhesion and activation stages of membrane in the swab.
Table 1. Malolyahovsky mammoth neutrophil, lymphocytes, monocytes hematological indices obtained during the thawing in 2014.

<table>
<thead>
<tr>
<th>Defined parameters</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>WBC – White blood cell count (x10E3 cells / micro-L)</td>
<td>1.6 x 10^9 /l</td>
</tr>
<tr>
<td>WBCP – White blood cell count from the peroxidase method (x10E3 cells / micro-L)</td>
<td>1.4 x 10^9 /l</td>
</tr>
<tr>
<td>NEUT /n – Absolute count of neutrophils (x10E3 / micro-L)</td>
<td>0.2 x 10^9 /n</td>
</tr>
<tr>
<td>NEUT % – Percent of neutrophils</td>
<td>11.9 %</td>
</tr>
<tr>
<td>LYMPH # – Absolute count of lymphocytes (x10E3 / micro-L)</td>
<td>0.8 x 10^9 /n</td>
</tr>
<tr>
<td>LYMPH % – Percent of lymphocytes</td>
<td>54.6 %</td>
</tr>
<tr>
<td>MONO # – Absolute count of monocytes (x10E3 / micro-L)</td>
<td>0.2 x 10^9 /n</td>
</tr>
<tr>
<td>MONO % – Percent of monocytes</td>
<td>15.5 %</td>
</tr>
</tbody>
</table>

Fig. 1. Malolyahovsky mammoth fluid obtained during the excavations of 2013 (zooming in 10 × 1000).

Fig. 2. The concentration of the Malolyahovsky mammoth blood vessel obtained during the mammoth carcass thawing in 2014. (Zooming in 10 × 1000).

Fig. 3. The concentration of the Malolyahovsky mammoth blood vessel obtained during the mammoth carcass thawing in 2014. (Zooming in 10 × 1000).

Fig. 4. Malolyahovsky mammoth fluid obtained during the excavations in 2013. (Zooming in 10 × 1000).

Fig. 5. The concentration of the Malolyahovsky mammoth blood vessel obtained during the mammoth carcass thawing in 2014. (Zooming in 10 × 1000).

Fig. 6. Malolyahovsky mammoth fluid obtained during the excavations in 2013. (Zooming in 10 × 1000).
One of the stages of phagocytosis is a phagosome formation stage. This stage per field of vision registered a single element, which had an irregular shape, and the boundaries of which were preserved, cytoplasm had inhomogeneous structure, nuclear inclusion were eccentrically located (Fig. 8).

Microscopic study of swabs often showed incomplete phagocytosis at the stage of interflowing and destruction (Fig. 9).

One of the final stages of phagocytosis is the release of degradation impurity. This stage was also registered in the swab of the original material. Against the background of hemolysis there were some ghosts of the destroyed cells and an isolated element in the process of release (Fig. 10).

Thus, it should be noted that in a liquid similar to blood taken during the excavations in 2013, some microscopic individual stages of phagocytosis were found out to our surprise.

RED BLOOD ELEMENTS RESEARCH STUDY
Microscopic examination of swabs of different liquid showed us aspect of hemolysis (Fig. 11). The background swab had blue-smoky staining, visible stroma of destroyed cellular elements called “gouts of red blood cells.”

In the context of hemolysis, the destruction of the erythrocyte membrane is accompanied by the release of hemoglobin into the blood plasma. This aspect of hemolysis let us suggest that there had been some quantity of hemoglobin, which was confirmed at the hematology study (Table 2).

Indices of Malolyahovsky mammoth hemoglobin (22 g/l) Malashevska mammoth when compared with the literature data G. Kleppel and R. Klepper significantly exceeded the content of hemoglobin in the blood of the living white elephants (13.72 g/l) [5].

Thus, we are the first to study and describe the fossil mammoth blood cells, which are related to leucocytes of hematherm: neutrophils, monocytes, lymphocytes.
The conducted research, in view of the lack of information in the literature about the probability of tissue preservation for such period, is certainly unique in the morphological reconstruction of the ancient animal.

CONCLUSIONS

The results of microscopic examination of dark brown liquid, allowed us to conclude that the liquid contains cell elements similar in their morphology to mammalian blood elements: monocytes, neutrophils, lymphocytes, containing nuclear inclusions.

Comparative analysis of cells found out in the swab of dark-brown liquid taken during the Malolyahovsky Mammoth excavation, and a swab of biological material taken from a blood vessel of the forelimb, showed the same morphological structure of cellular elements.

Thus, we are the first to study and describe the fossil mammoth blood cells, which are related to leucocytes of haematherm: neutrophils, monocytes, lymphocytes.

Study of primary biomaterial in hematological analyzer confirmed the concentration of these cells (lymphocytes, monocytes and granulocytes), as well as the presence of hemoglobin, the value of which was 22 g / l.

Thus, the initial results of hematological research studies of Malolyahovsky mammoth are unique materials and they require an integrative approach to the interpretation of the results.

REFERENCES

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Conflict of interest:
The Authors declare no conflict of interest.

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Table 2. Hemoglobin hematological parameters of Malolyahovsky mammoth obtained during thawing in 2014

<table>
<thead>
<tr>
<th>Defined parameters</th>
<th>Value</th>
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<tbody>
<tr>
<td>HGB –Hemoglobin ( g/dL)</td>
<td>22</td>
</tr>
<tr>
<td>RBC –Red blood cell count (x10E6 cells / micro-L)</td>
<td>0,03 x 10^n/n</td>
</tr>
<tr>
<td>HCT – Hematocrit (%)</td>
<td>0,2</td>
</tr>
<tr>
<td>MCV –Mean corpuscular volume ( fl)</td>
<td>67,3</td>
</tr>
<tr>
<td>MCH –Mean corpuscular hemoglobin (pg)</td>
<td>862,9</td>
</tr>
<tr>
<td>CH –Cellular hemoglobin content (pg)</td>
<td>15,8</td>
</tr>
<tr>
<td>MCHC –Mean corpuscular hemoglobin concentration (g/dL)</td>
<td>12831</td>
</tr>
<tr>
<td>CHCM - Corpuscular hemoglobin concentration mean (g/dl)</td>
<td>243 r/n</td>
</tr>
</tbody>
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