Additional materials to the lecture of A.Tikhonov, B. Buigues "Permafrost of Siberia and Alaska — the freezer of the genetic material of the Ice Age"

It should be noted that the interest taken in preservation of rare and disappearing species is constantly growing. Striking advance in cloning of animals gave a new impulse to the development of this problem. It means that mankind is at the stage of development when it's expedient to try to reconstruct extinct macroorganisms such as mammoths and their contemporaries - wool rhinoceros, bison, horse, cave-lion and other representatives of mammoth fauna. Great progress has been made by the scientists of USA, Germany, France, England, Canada, Denmark and Russia. The success of the researches first of all depends on degree of safety of the tissues of carcass remains in the frozen ground. Arctic Siberia is known to be one of the main deliverers of the well-preserved materials of fossil animals. Its entrails bound by the permafrost show us wonderful natural storage of the preserved remains of the Pleistocene animal carcasses until nowadays.

Mammoth, as the entire mammoth fauna, had reached its florescence in the late Pleistocene, in the period from 100,000 to 10,000 years ago. By that time in the Siberian Arctic the sharply-continental climate and the cold tundra-steppe landscapes with rich grass vegetation had been formed. Judging by the radio-carbon analysis of famous mammoth carcasses and of the other their contemporaries we can divide two periods of their most frequent death - the Karginskyi interglacial epoch (50,000-25,000 years ago) and the end of Sartan glacial epoch - the beginning of the Holocene climatic optimum (13,000 – 9,000 years ago). At those time periods the consequence of strengthening thermokarst (melting) processes and river activity had created the wildest different natural traps for the big mammals. There are numerous publications about taphonomic conditions of ancient animals' burial places by N.K. Vereshchagin (1979, 1995), P.A. Lazarev (1980) and by other investigators. There had been different swamps, mud and solifluctian streams, deep ravines and clefts in the frozen ground, etc. The extinction of mammoth, woolly rhinoceros and some other representatives of the mammoth fauna have been connected with the beginning of the Holocene climatic optimum, that has caused the changes of natural environment - melting of permafrost, swamping of northern territories, etc. However, some species of the mammoth fauna (reindeer, muskox, snow sheep), almost all the predators have adopted to the new ecological environmental conditions and have been preserved until nowadays.

The mammoth, a large herd animal from the elephant family lived in the northern hemisphere during the Pleistocene. Africa is considered to be the place of its origin. In the north of Siberia the evolution of mammoth took place under the conditions of cold spell of climate with short epochs of interglacial rises in temperatures. 2 cm-thick skin and 1 m-long hair with thick and soft underwooll as well as relatively small ears have been the result of the mammoth adaptation to the cold climate. Some big individual had the height of 4 m and the weight up to 6 tones. The primitive hunters had considered the mammoth and its contemporaries as a source of life. They used their meat as a food, created a dwelling for themselves from their bones and skins, made clothes, implements of work and hunting.

Every finding of the mammoth carcass remain was a sensation and of great interest for the scientists. In the authors' opinion, one the most interesting finding is the skeleton remains of the Shandrin mammoth with internal organs preserved inside the ribs in a form of a frozen monolith. The burial place of this mammoth was discovered in 1971 on the bank of the Shandrin River at the mouth of the Indigirka by the inhabitant of that region D. Kuz'min. Specialists of 15 Russian research institutes were invited for complex study of the internal organs of the Shandrin mammoth. Mummified internal organs presented an integral monolith weighing more than 300 kg. The mammoth's absolute age is 41,000 years. The morphologic investigations using histologic methods revealed the presence of abdominal walls of the diaphragm, pancreas, liver, spleen and kidneys, small and large intestines. Biochemical analyses detected a considerable amount of protein among the large intestines contents. Bots of the intestinal beast-fly were collected on the monolith surface. They were classified under a new species differing from the contemporary one.

Recently molecular investigations of specimens of bones and soft tissues of mammoths and other animals excavated from frozen ground in the north of Siberia have been carried out. A number of specialist performed microbiologic investigations in order to reveal philogenetic relations between the mammoth and African and Indian elephants. The German researchers G. Hauf, F/K. Zimmerman, J. Joger and others (1994, 2000) supposed that the African elephant could be the closest relative of the mammoth after they had carried out amplification of the mitochondrial cytochrome d from the mammoth's ossein (bone). However, they do not consider this result as the final one and are going to continue research in this field. The Japanese scientists T. Ozava and S. Hayasi as well as the Russian cytologist V. Mikhelson (1995) succeeded in amplifying and sequencing the mammoth's mitochondrial cytochrome b and concluded that the mammoth and the Indian elephant were the sister groups. Proceeding from the comparative analysis of the exterior morphologic characteristics (the trunk structure, the ear form and the body size), the authors also consider the mammoth and the Indian elephant to be the closest genetic chains in the evolution of elephants. Other researchers are seeking to obtain well-conservable DNA from the cells of the mammoth soft tissues and to revive a mammoth by cloning. Experimental molecular works have not given positive results yet.

Ever-frost produces a destroying effect on the remains of the extinct animals. It deforms and saps them. Satisfactory conservation of soft tissues is possible only under ideal burial conditions after the animal's spontaneous death as described above. The French scientists H. Bocheren, A. Mariotti and others studied the excess of stable carbon and nitrogen isotopes in the ossein collagen and soft tissues of the mammoth, the wool rhinoceros and other animals found in frozen sediments of Siberia. It has been found out that the isotope concentrations in herbivorous and carnivorous species of extinct Pleistocene and modern animals of

Siberia are similar. Mammoths' specimens contained lower amounts of carbon and larger amounts of nitrogen than those of other herbivorous animals. This fact was difficult to explain. The ossein collagen was found to have preserved very well over many thousand years in the carcasses of extinct animals buried in frozen sediments of Siberia and Alaska. The Japanese scientists K. Goto and Sh. Okutsu (Agricultural University of Kagosima) and A. Iritani (University of Kinki) believe that insemination of a female elephant with the sperm of a male mammoth taken from the frozen carcass could result in obtaining a hybrid animal, which could be made maximally closer to the mammoth by selective work. The Japanese researchers say that sperm is very stable and must have preserved well under permafrost conditions.

A special attention should be paid to the study of microorganisms. Though people recollect them mostly in case of a disease or when food goes bad. They can be found in volcano craters or frozen in polar ice. But the activity of the whole biosphere is directly dependent on microorganisms' vital functions [Zavarzin, 1984]. The small chains of DNA of retro-virus was found in the marrow which was taken from the Holocene mammoth bone samples from the Wrangel Island (Greenwood et al., 2001).

The reason for poor knowledge of the microorganisms' role is that microbial world is beyond the capabilities of human eyes. They became known only 300 years ago when the first primitive microscope appeared. The molecular ecologists' studies of biodiversity have shown that only 5 to 10 % of all the microorganisms' species were isolated and studied. That means that we are still to learn a lot about the huge variety of microbial species. It is reasonable to ask ourselves: where are those 1 1mln. 430, 000 non-discovered fungi species? May be they should be searched for in the unique natural habitats like Kamchatka and Baikal Lake in Russia, or Yellowstone Park in the US? Or may be they are deep in the earth bowels? The microbial studies fulfilled by the US and Russian scientists under the IPP Thrust I Project and the Russian Program "Baikal-Drilling" have shown that life exists in samples of age estimated as ten million years and more, and the diversity of life is not significantly decreasing with the growing depth [Repin, Andreeva, et al, 2000], but depends on specific local conditions [Fliermans, Balkwill, 1989]. Based on the results of traditional identification tests the following bacteria species have been found to survive in the depth: *Bacillus, Pseudomonas* и *Actinomycetes*. Besides these, the representatives of the following genus (17 all in all) have been identified: *Acinetobacter, Arthrobacter, Chryseobacterium, Clavibacter, Curtobacterium, Methylobacterium, Micrococcus, Nocardia, Paenibacillus, Rhodococcus, Salmonella, Sphingobacterium, Sphingomonas, Staphylococcus, Stenotrophomonas* [Torok, Repin, 2001]. Additional comparative studies of DNA will show how much these representatives differ from their modern species. It was considered previously that Antarctic is the area without microbials and low temperature prevent bacteria from intensive reproduction. But recent studies managed to find specific microorganisms (psychrophils) adjusted to low temperature [Brock, Rose, 1969]. Moreover, permafrost zones of Siberia and Alaska are probably the biggest and the least investigated regions.

More than 85 % of seawater is known to have a temperature 5^oC or lower during the year, and more than 75 % is permafrost (have temperatures lower than 5^oC all the year round). This is a huge and poorly investigated natural reservoir where 95 % of the biomass are the microorganisms [Herbert, 1986].

XXI century brings for us outstanding finds of frozen carcasses of the Pleistocene beasts in Siberia. In 2000-2001 the remains of Jarkoff and Fishook Mammoths from Tainyr Peninsula were taken inside the huge blocks of permafrost and delivered to the ice-cave which now become the Museum of the permafrost in Hatanga settlement (Siberia). The samples which were taken from these remains show how are important to keep the remains in the frozen conditions because DNA preserved in it much better. At the same time the Yakutian expedition did the excavation on the coast of Arctic Ocean. As a result of the excavations, the foot of a front leg and the foot of a hind leg of a mammoth were taken out from permafrost as a frozen monolith and bring it unfrozen to the Museum of mammoth in Yakutsk. Our researches show that some cells from the skin looks like as undamaged in frozen conditions. Firstly sweat glands were found by the group of scientists (Repin, Tikhonov et al., 2002).

Undamaged state of cell structures is undoubtedly the most interesting question. Herz O.F. (1902) pointed to the contradiction between the macroscopic “undamaged state” of mammoth tissues and the microscopic picture of changes in their structure as far back as 1901 at the examination of the Berezovka mammoth. Zalensky V.V. (1905, 1909) revealed significant distortions in their microscopic structure and full absence of cells. Byalynitsky-Birulya (1904, 1909) supposed that the “prosperous” microscopic picture of tissues taken from permafrost did not reflect their true state. Korobko Yu. A. (1977) published a photo illustrating “nucleus-like” structures in smooth muscle cells of a fossil bison. Zhenevskaya R.P. (1977) gave histological description of sketetal muscles of a mammoth taken out of permafrost, pointing to fragmentation of muscular fibers and the absence of nuclei in them. Subbotin V.M. and Tum Yu.V. (1978) described the elements of paleopathology of soft tissues. The authors described the states of lungs and intestines of a mammoth taken out of permafrost. Interesting results were obtained at examining a young mammoth found near Magadan in 1977. Sokolov V.E., Sumina E.B. (1981) noted that fat cells of derma and subcutaneous fat cellular tissue preserved their both outer and inner structures. Mikhelson V.M. et al. (1981) published the results of light and electron fluorescent microscopy, where hepatic and kidney cells were described for the first time.

Studying tissue specimens of the same animal with a scanning electron microscope, Shoshani G. Et al. (1981) found blood cells: erythrocytes, leukocytes and thrombocytes.

After such results the next finds of the head and leg of Yukagir mammoth (2002), head of Oimyakon baby mammoth (2004), Yamal baby mammoth-Lyuba (2007), Kolyma rhino (2007) were preserved frozen. Some samples from these specimen gave for us the outstanding results in DNA study (wool of Yukagir mammoth i.e.)

At present, after successful experiments of cloning animals, there emerged the prospect of cloning of extinct animals (like mammoths) or disappearing animal species. In addition, the gene potential of the microorganisms has been studied very poorly too. Permafrost zones are very interesting from this point of view as nature-formed conditions intended for long-term storage.