Sebaceous Glands of the Woolly Mammoth, Mammothus primigenius Blum.: Histological Evidence

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GENERAL BIOLOGY

Sebaceous Glands of the Woolly Mammoth, *Mammothus primigenius* Blum.: Histological Evidence[1]

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The study of the remains of fossil animals and the comparison of the morphology of their organs with modern species is very important for understanding the evolutional relations in nature. No unambiguous answer has been found to the question if mammoths had skin (e.g., sebaceous) glands [1, 2, 4, 5, 11]. Theoretically, the living conditions of the woolly mammoth, *Mammothus primigenius* Blum., at high latitudes did not exclude substantial evolutional differences from elephants. It is known that elephants have no sebaceous glands, and the only facial gland of elephants has been described as a modified sweat gland actively working during the reproduction period [5]. Here, we present a documentary proof of the presence of sebaceous glands in the woolly mammoth, *Mammothus primigenius* Blum.

The remains of mammoths found to date that are suitable for examination are not numerous. K. Möbius was the first to describe in detail the mammoth hair and report some data on the histological structure of the skin. Skin glands were not found [3]. The overdrying of specimens could prevent Möbius from examining the skin in full detail. A detailed histological examination of the skin and muscles of the Berezovka and Lyakh mammoths did not allow finding skin glands either [8, 13]. Later studies also did not detect skin glands in mammoth skin [4, 5, 9, 14]. At the same time, the study of the skin of the Indigirka fossil bison showed that sebaceous glands were well preserved in permafrost, while sweat glands were present only as individual vacuoles [12].

The statement of Magadan scientists about finding sebaceous glands in the skin of a mammoth foot found at the Enmyveem in 1986 [15] brought a discord. Unfortunately, the scientific description of this event has not been published. Note that the same group of authors that examined the Magadan young mammoth in detail did not find skin (sebaceous) glands during a detailed examination of the hair and skin of the young mammoth [14].

An expedition to the region of the Muksunuokha River in Sakha Republic (Yakutia) with the coordinates 71°34′56.9″ N, 141°37′37.6″ E was organized in the framework of the ISTC project "The Study of Macro- and Microorganisms Isolated from Permafrost" in August 2002 to find mammoth remains in frozen ground. As a result of excavations, the foots of one fore and one hind legs of the mammoth were found in the frozen ground as a frozen monolith. A piece of skin with underlying tissues (6 cm thick) was taken for examination. Skin layers were clearly determined in the section. A very dense dark brown, almost black layer, 3–5 mm in thickness, was the closest to the surface. It corresponded to skin epidermis. A brown 15-mm layer that was looser and had white veins (derma properly) lied below. A light yellowish brown layer lied still deeper; it was soapy to the touch and stained well. Pieces of skin and underlying tissues of the extremities in a frozen state (–4°C) were placed in 4% buffered formaldehyde cooled to about 0°C and stored on the permafrost surface. The preparations were periodically washed in fresh portions of fixer solution for the first three days.

Further treatment of specimens was performed in a laboratory using standard histological techniques for preparation and staining [7, 10]. However, the peculiarities of tissues that had lied frozen in permafrost for thousands of years required some modifications. For instance, the tissue poorly absorbed paraffin, which made it difficult to obtain high-quality sections in a mixture of xylol and paraffin and paraffins (for 48 h). Analysis of the material showed that this long-term exposure to the thermal factor did not influence the preservation of the structures: no notable loss of tissue took place. Histoplast (melting temperature, 52–56°C), which proved to be good for filling solid tissues, was used as a filling medium. We managed to prepare 5- to 7-μm sections of satisfactory quality from the blocks obtained. The preparations were stained with hematox-

[1] This article was submitted by the authors in English.

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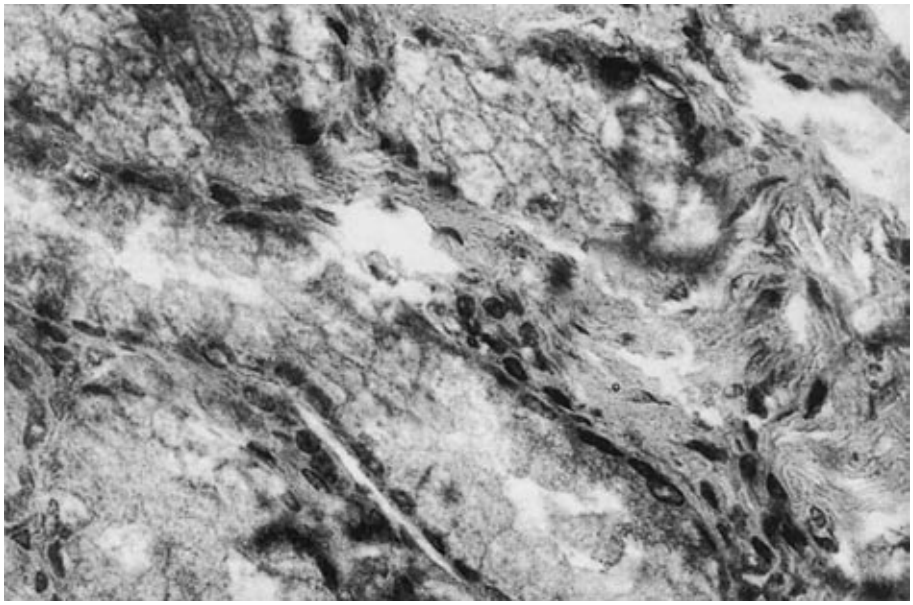

Fig. 1. Sebaceous glands. Light microscopy. Hematoxylin–eosin staining. Magnification, ×400.

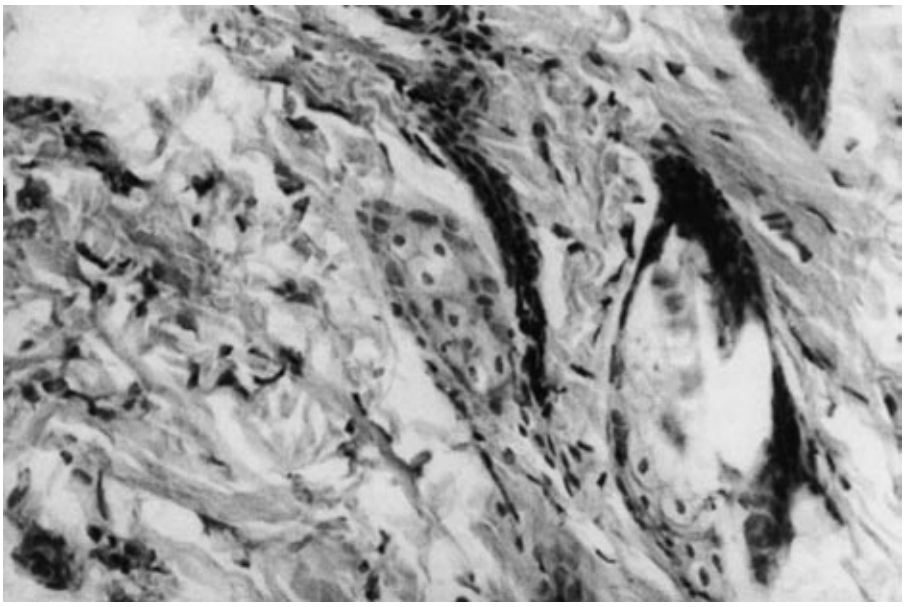

Fig. 2. A sebaceous gland and a hair follicle. Light microscopy. Hematoxylin–eosin staining. Magnification, ×400.

ylin and eosin [7, 10]. The sections were examined under a "Jenamed" microscope (Germany) at magnifications from ×100 to ×1000.

The histological examination showed that all skin structures underwent different postmortem changes but, on the whole, preserved their structure and spatial organization and acquired the staining typical of a normal skin. All skin layers were clearly identified, the fibers of loose unorganized connective tissue stained well; they corresponded to the papillary layer of the derma, and the walls of blood vessels were seen. Deeper layers were formed by solid connective tissue organized in a form of a rough net of thick bundles of collagen fibers. Hair follicles and hair, sebaceous and sweat glands, and small blood vessels were located in this layer. A considerable deformation of tissues was observed; it was most noticeable in the hypodermal cellular tissue and the muscular layer where large cavities resulting from injury with big ice crystals were localized. The arteries and veins of the hypodermal layer of muscular type had thick walls, in which the inner, the middle and the outer membranes were clearly distin-

guishable. The layer of endothelial cells was usually destroyed. There were no signs that the tissues were lifetime injured while the animal was alive. No signs of bacterial decomposition were observed either, although individual bacteria resembling *Escherichia coli* in shape and size were found in these tissues. The absence of a large number of bacteria is surprising, as a large portion of tissues are in the state of adipocere, and its formation requires the presence of bacteria [6]. Structures that could be undoubtedly called glands were found in some skin pieces. The ducts of sweat glands and sebaceous glands were observed in the sections (Figs. 1, 2). Sebaceous glands had a structure typical of ordinary alveolar glands of holocrine type. A layer of flat cells with prolonged nuclei is located at the periphery. Large round cells are above them, and big round nuclei are observed in the fundal part of the gland. Sebaceous glands are surrounded by loosely arranged collagen fibers.

In general, the study of paraffin sections of the skin of fossil mammoth has demonstrated that the cells stain well, and their tinctorial properties do not differ from modern cells. However, the staining is unstable, the sections fade soon and do not stain again.

Thus, our study is a documentary confirmation of the presence of sebaceous glands in the hairy mammoth. Sebaceous glands are a sign of cold adaptation. The presence of sebaceous glands in mammoths is a convincing argument in the discussion of the question if mammoths really lived in cold climatic zones.

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