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# ELECTRON MICROSCOPIC STUDYING OF RAT BONE CELLS UNDER MICROGRAVITY CONDITIONS

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**Abstract**

In the experiments on rats (SLS-2) with the use of electron microscopy there was made the study of the osteoclasts population and the peculiarities of resorptive processes in a spongy bone of the epiphyses and the iliac crest.

The results of investigation permit to suppose that the processes of resorption of bone tissue become more intensive in zones of adaptive remodeling and destruction of the spongy bone under microgravity that is performed by several ways. One of mechanisms is the increasing of the functional activity of osteoclasts (appearance of "giant" osteoclasts). As a result it is the local demineralization and subsequent destruction of superficial areas of the bone matrix. The other mechanism is activation of osteocytic osteolysis was also investigated.

**Keywords:** osteoclasts, osteocytes, bone remodeling, femoral bones, microgravity, electron microscopia.



## 1. Introduction

The most typical changes for the bone under the space flight conditions and a long-term hypokinesia are the following: the decreasing of bone mass, the demineralization and reducing of mechanical strength. It can lead to osteopenia and osteoporosis development. Also it increases the risk of fractures of supporting bones. Osteopenies that are caused by the microgravity are partially connected with the increasing of a reduction of trabecular bones [1–7]. Cytological mechanisms of gravity-dependent reactions in the bone tissue remain not clear in many respects [6, 7].

Under conditions of functional load deficit there occurs lowering in the mineral content of the skeleton that deteriorates the mechanical characteristics of bones particularly of those that carry the supporting load. This leads to a reduction of their hardness and appears as a risky factor of the bone-muscle system damages. In the space experiments the reduction of bone mass especially of a spongy bone was established [6, 7].

However the mechanisms of bone tissue remodeling following the lowering of supporting load still remain little-studied. It is topical to elucidate how the processes of bone resorption and destruction increase under conditions of microgravity and prolonged hypokinesia and which their mechanisms are. So in the histomorphological study of rat bones exposed on SLS-1 [8, 9], was shown a statistically verified increase of the bone superficies covered with osteoclasts, as well as an increase in their number as compared to a synchronous control. According to the other data [9, 10], the osteoclasts number in a spongy bone of vertebrae and tibial bones of rats, as well as their resorptive activity do not change under conditions of supporting load lowering (Cosmos 2044) [9–11].

The aim of our investigations is to study the peculiarities of resorptive processes in zones of adaptive remodeling in spongy bone under the influence of microgravity with the use of electron microscopy.

## 2. Material and methods

In mission SLS-2 an electron microscopic study was made of biological speciments of spongy bone from the epiphyseal zone of femoral bone of mature white rats exposed to microgravity (F) during 14 days and control animals (synchronous control (Sc) and vivarium control (v)).

An experiment on rats in the conditions of the real microgravity was conducted within the framework of international cooperation consonant with the Russian and American scientists onboard the American Space Station of SLS-2. Animals (mature white rats of the Wistar line (males), the middle-weight of which made 250 gr.) were parted on a few groups. There were 5 animals in every group.

The first group is flight (F). Rats of this group were in space during 14 days onboard SLS-2 in the special containers. The second group is synchronous control (Sc). The third group is vivarium control (V). Rats from the group of synchronous control were in the conditions analogical to the ones in space they were in the same containers as rats in a space station, at the same temperature, humidity and other, got a ration analogical to the ration of the space group. Rats from the group of vivarium control were in a vivarium.

After the end of space flight selection of biomaterial was taken simultaneously from the rats of flight group, synchronous to vivarium controls. Before selection of speciments of long bones the rats were killed by the chloroform. The proximal metaphyses of femoral bones of rats served as material.

Biosamples were fixed in a 2 % glutaraldehyde solution with an addition of 1,5 % paraformaldehyde on a phosphate buffer. pH 7,2, during 24 hrs. Afterwards the samples were washed out in a 70 % ethanol, postfixed in a 1 % $OsO_4$ solution, dehydrated in ethanol and embedded in araldite. Ultrathin sections were stained according to Reynolds and examined in TESLA-BS-500 electron microscope (Czechia).

**3. Results**

The electron-microscopic studies show that the population of osteoclasts may contain various forms. We discerned among them young, functionally active, functionally non-active and resting cellular forms. Young osteoclasts contain 2 to 3 nuclei on sections. Functionally active cells have 5 and more nuclei, the well-developed cytoplasm. The cytoplasm of functionally active osteoclasts is not homogenous, concerning the content and distribution of organelles, and we conditionally divided it into two zones: a zone of concentration of organelles responsible for biosynthesis and energy supply (1) and a zone of structures providing for specific functions (2).

But it is prevailed by functionally active osteoclasts which are characterized by the presence of 3 to 5 nuclei in a section, a well-developed cytoplasm and the “brush border”. However we could discern among them the “giant” multinuclear cells. Such “hyper-osteoclasts” are almost not found in a spongy bone of control animals. Concerning the content of organelles (nuclei, mitochondria, polysomes, etc) the “giant” osteoclasts have a structure typical of functionally active osteoclasts. Such osteoclasts are characterized by an intensive development of the fibrillar zone and the “brush border” which occupy a significant area. The “brush border” penetrating the mineralized matrix destroys it to fragments which subsequently split by endo- and phagocytosis.

Zone 1 includes nuclei, mitochondria, polysomes, structures of endoplasmic reticulum and Golgy complex. In zone 2 in which the cells are directed towards the mineralized matrix the content of organelles in the cytoplasm is lower. This zone contains a thin-fibrillar component - actin (in the light microscope these regions look light and are, therefore, called a “light zone”) and a “brush border”. The resting osteoclasts are multinuclear cells, located on the bone surface, have a compact cytoplasm deprived of the “brush border” **(Fig. 1)**.

Zone 2 regarding its dimensions (in sections) occupies an area 1–2 times much than zone 2 in a typical active osteoclast **(Fig. 2)**, has a “light” fibrillary zone and a great number of vacuoles, many of which include fragments of mineralized matrix.

Such osteoclasts are characterized by an intensive development of the fibrillar zone and the “brush border” that occupy a significant area **(Fig. 3)**. The “brush border” penetrating the mineralized matrix destroys it to fragments, which subsequently split by endo- and phagocytosis **(Fig. 4)**.

The analysis of electron micrographs shows that the formation of the fiblillar layer is preceded by the development of “brush border”. Its areas directed towards a cytoplasm contain polysomes, canals of rough endoplasmic reticulum, mitochondria. This is probably associated with the participation of these organelles in biosynthesis of contractile proteins forming microfilaments of the fibrillar layer. The movement of “brush border” villi, their active penetration into the mineralized matrix, formation and translocation of endocytose vacuoles in the cytoplasm are performed by the activity of contractile structures of the fibrillar layer and the “brush border”. It is supposed that the appearance and functioning of these “giant” osteoclasts is conditioned by the necessity of development of quick adaptive resorption of the mineralized matrix in the most “vulnerable zones” of bone after removing gravity load in space.

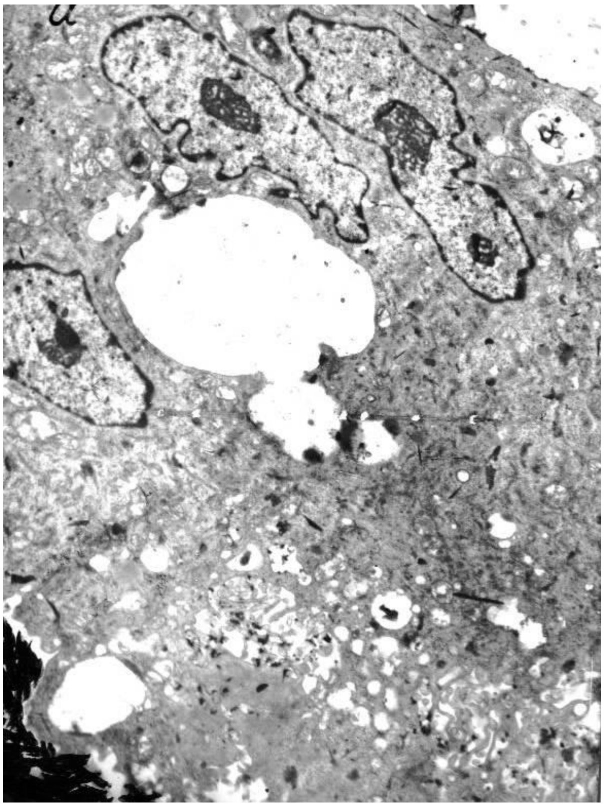

**Fig. 1.** The fragment of the “giant” osteoclast cytoplasm with nuclei (Flight).
Electron micrograph, ×18000

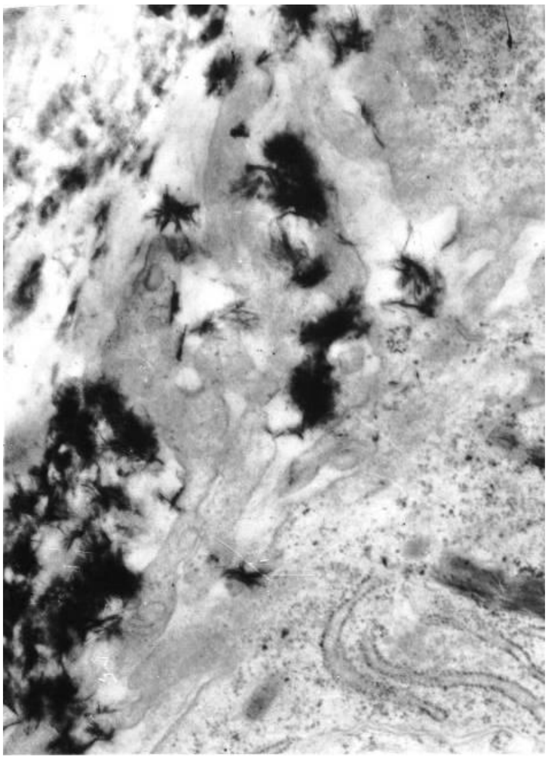

**Fig. 2.** The fragment of zone 2 (the “brush border” in the “giant” osteoclast (Flight).
Electron micrograph, ×8000

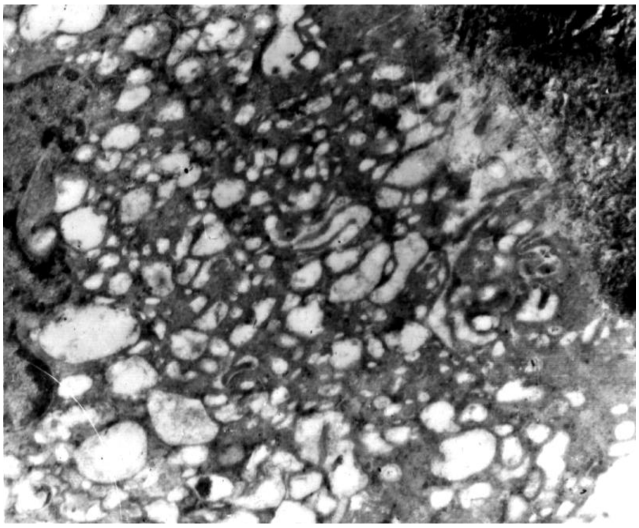

**Fig. 3.** The fragment of zone 2 (the developed "brush border" in the "giant" osteoclast (Flight). Electron micrograph, ×1800

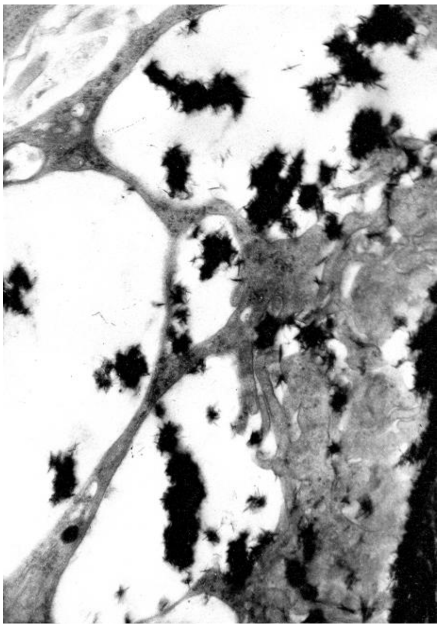

**Fig. 4.** The fragment of well-developed "brush-border" with a mineral matrix undergoing resorption. Microgravity. Electron micrographs, ×10000

Such "giant" osteoclasts are not found in the metaphyses of the rat control groups, the majority of osteoclasts are represented by typical forms.

Some resorption zones are characterized by the appearance of a "fringe" along the margins of the mineralized matrix. The fringe originates from the non-mineralized collagen fibrils or from those that have lost its crystals. Thus there is evidence of dissolution of superficial layers of the mineral component and exposure of the organic matrix in bone. Demineralized regions may be of a significant size, contain a fibrillar or a fine-granular component. Moreover the superficial regions of mineralized bone can disintegrate to conglomerates that subsequently undergo destruction.

As far as the bone matrix resorption proceeds the osteoclasts with the peripheral part of their cytoplasm penetrate osteocytic lacunae and take part in lysis of the mineral substance surrounding the osteocyte. In some cases it is possible to observe also pictures of mineralized matrix destruction in the periosteocytic lacunae due to osteolytic activity of osteocytes without the osteo-

clasts involvement. Resorption is performed by lysosomal enzymes secreted by osteocytes by the way of exocytosis.

**4. Discussion**

The function of osteocytes in osteolysis is already established [12, 13]. Osteocytic osteolysis as well as osteoclastic one is considered as a physiological hormonal-dependant mechanism of resorption. During osteolysis some part of osteocytes is destroyed. Then the osteoclasts penetrate to the empty lacunae utilizing the surrounding mineral matrix.

The role of osteocytes has been defined as mechanosensitive bone cells and that of the lacuno-canalicular network – as the way for mechanosensitivity transduction [13]. The loss of osteocytes may be a signal for an increase of the osteoclasts' number and enhancement of their activity. Bronkers A. L. (2001) [14] has described a positive relationship between osteocytes apoptosis and osteoclasts attack in bone.

The above-described peculiarities of the resorptive processes in a spongy bone under microgravity as well as our earlier data [15–21] about the changes in the population of osteoclasts are, in our opinion, within the limits of adaptive reactions of the osteogenesis system to the microgravity action and other flight-concomitant factors.

**5. Conclusions**

In the experiments on rats (SLS-2) with the use of electron microscopy there was made the study of osteoclasts population and the peculiarities of resorptive processes in a spongy bone of the epiphyses and the iliac crest.

The results of investigation permit to suppose that the processes of resorption of bone tissue become more intensive in zones of adaptive remodeling and destruction of the spongy bone under microgravity that is performed by several ways: increase of the functional activity of osteoclasts (appearance of "giant" osteoclasts) and also activation of osteocytic osteolysis as well as a result of local demineralization and subsequent destruction of superficial areas of the bone matrix.

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