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Cytological mechanisms of gravity-dependent changes in a bone tissue

The study was made of main changes in the osteoblasts and osteocytes ultrastructure in bone tissue cells of monkeys (Macaca mulatta), flown for two weeks aboard the biosatellite BION-11. By the use of methods of electron microscopy, cytochemistry and morphometry we researched biopsy material of an iliac bone. It was established by ultrastructural indexes that under microgravity conditions in the osteoblasts population little active forms (4th morphofunctional type) prevail. Also we discovered osteoblasts of a fibroblastic type and local zones of fibrosis. In the osteocytes population we found not only typical cells but also octeocytes with developed RER. Processes of osteocytic osteolysis increased.

INTRODUCTION

Medical and biological investigations aboard space satellites and stations, have shown, that bone tissue is one of the most important targets of microgravity influence on human and animal organisms. Growth and osteoplastic processes in bones are reduced and mineral filling, especially, Ca containing, and mechanical strength of bone tissue decrease. It is noted general decreasing of bone mineral mass and the tendency to redistribution of mineral substances to high districts of skeleton [3, 10].

The loss of bone substance and osteopenia make worse the mechanical properties of bones, carrying the supporting load lead to reducing of their strength and is the risk factor of bone-joint system breaches. It was established in experiments on monkeys that osteoplastic processes in skeleton bones, especially in zones with metabolically active spongiose decreases. The bone structure undergoes destruction. The spongiose in long bones reduces [3].

Structural and metabolical reconcructions in the bone tissue cells, which are still unclear, underlie these changes. The slowing of osteoplastic processes under microgravity conditions is still connected with a decrease of functional active osteoblast number, and the development of resorptive reconstructions — with osteoclast quantity increasing [3, 4]. However, the question about correlation of these processes and mechanisms of bone substance loss still remains unclear. In evaluation of cytological mechanisms of microgravity effects developing in the bone skeleton, we don't take into account morpho-functional heterogeneity of osteoblast, osteoclast and osteocyte population [11].

The purpose of our investigation is to study cytological mechanisms of gravity-dependent changes in a bone skeleton under space flight conditions and also the clearing-up of a role of gravity in bone tissue formation.

The research task was to study ultrastructural changes in bone tissue cells of monkeys (Macaca mulatta), flown for two weeks aboard the biosatellite BION-11.

MATERIAL AND METHODS

Material for research (by the way of biopsia with the use of isofluorethane anesthesia) taken from a crest of an iliac bone, was fixed in 2 % glutaraldehyde on the phosphate buffer with paraformol addition (1.5 %), postfixed in 1 % osmic acid on the phosphate buffer, dehydrated in alcohols and embedded in araldite. The activity of acid phosphomonoesterase was demonstrated with the using of natrium beta -glicerophosphate according to the Gomori method. The ultrathin sections were studied in the electron microscope «Tesla BS-500». Biosamples of monkey bones from the flight experiment, as well as ground and synchronous controls were studied. The morphometric analysis of on electron microphotograph were per-

formed by point counts with the use of Student criteria.

RESULTS AND DISCUSSION

Osteoblasts. We can judge about the intensity and peculiarities of osteogenesis in weightlessness only on the basis of the ultrastructural study of the morpho-functional cell alterations in the osteogenic zones. It is known, that the collagen and protein components of the proteoglycans are synthesized in the rough endoplasmic reticulum (RER) polysomes. The synthesis of the alkaline phosphatase occurs there too. Golgy complex (GC) participates in the sulphated glycosaminoglycans synthesis. It plays a leading role in the accumulation and transport of the proteins and polysaccharide substances, alkaline phosphatase, Ca and P compounds. The accumulation P and Ca ions took place on the RER membranes. The degree of the development and state of these organelles are important indices of differentiation and specific functional activity in the bone cells.

Our investigation demonstrated that the normal osteogenic cell population is not homogenous. Osteoblasts are distinguished by the shape, ultrastructure, biosynthetic activity and topographic relation with the mineralization zone. We distinguish four morpho-functional types (or stages) among them. In the zones of active osteogenesis there are young osteoblasts (1st type) with narrow RER channels and well-developed GC, the mature functional active osteoblasts with enlarged RER channels and cisterns (2nd type). Osteoblasts with hyperthrophic RER are revealed too (3rd type). They serve for the secret accumulation. In the zones of the osteoplastic process fading osteoblasts turned into non-active state (4th type). These cells do not participate in the specific biosynthesis and have narrow RER channels, a great number of the autofagolysosomes (ATL). They lie parallel to the calcified matrix.

Using radionuclides (^{3}H-glycin, ^{35}S-sulphate, ^{45}Ca) we demonstrate, that the presence of osteoblasts of the different functional states is conditioned by some asynchrony in the bone matrix synthesis in the different cells. It was established, that proteoglycans and alcaline phosphatase biosynthesis, calcium compound accumulation and secretion are predominates in the 1st type osteoblasts. The synthesis of collagen proteins predominants in the 2nd type osteoblasts. The 3rd type osteoblasts secrete proteins and glycosaminoglycans. These cells are characteristic for the intensive osteogenic zones.

In the bone of animals from the flight group the osteoblasts population is more uniform. It does not consist of the osteoblasts of different functional states, as it is characteristic for the normal osteogenesis in the ground and synchronous control. Intensive osteogenesis takes place only in some areas of the bone trabecules.

Osteoblasts likes 4th- type ones are predominant in the population (See Fig. 1). They have oval or elongated forms and lie parallel to the mineralization zone, near the trabecule surface in 1-2 layers or separately. They have low nuclei-to-cytoplasm ratio and well developed RER with the narrow (0.1—0.2 mkm) channels. The state of the endoplasmic reticulum suggested about relatively low level of the bone matrix biosynthesis. Mitochondria have the dense matrix and sometimes cristalline inclusions, which demonstrate disturbance of the calcium metabolism. The nuclear chromatin is arranged on the perimeter and in the small aggregations.

The cytoplasm borders, attached to the bone matrix, have sharp countures. The narrow or slightly expanded RER channels lie compactly. In the GC the vesicles and vacuoles are predominant. There are autophagolysosomes with the RER membrane fragments. This ultrastructure picture testifies to a low biosynthetic activity in comparison with the 2nd and 3rd type osteoblasts. In the norm the collagen proteins of 4th type osteoblasts are transferred from the RER polysomes to the GC where they become bound with glycosaminoglicans and are transported to the intercellular space by vesicles. In the osteoblasts of all types the alcaline phosphatase, phosphates and calcium compounds are excreted by exocytosis, as a result of separation of the vesicles from the cytolemma, and destruction of the cell surface areas. The vesicles are registred as the mineralization centres in the mineralization zone. In the flight group the mineralization zones are narrower than in the control. Functionally active 2-type osteoblasts are rare (I per 5-10 of the 4-type osteoblasts). Osteoblasts of the 3rd type with hypertrophic endoplasmic reticulum are single cells. Table 1 presents data about the changes of the specific volume of cell organelles in osteoblasts of the animals from flight and control groups.

In the flight group the morphological pattern of the osteogenic cells shows a lower level of the growth and synthetic processes in comparison with the control. This effect is conditioned by the disturbance of the optimal balance of the bone matrix compounds biosynthesis by osteoblasts. A relatively small number of the 2 and 3 type osteoblasts in the osteogenic cells population among the predominant 4 type osteoblasts reflects a decrease of the collagen synthesis and secretion intensity in comparison with the control. It

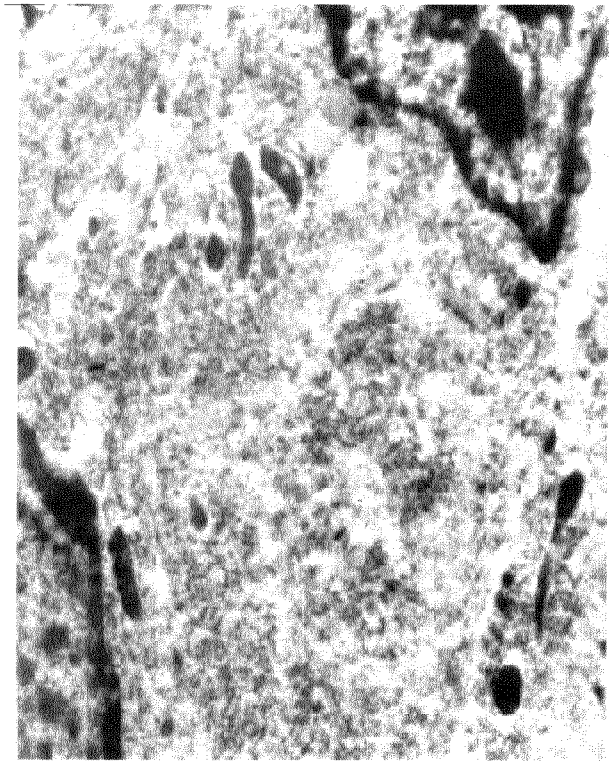

Fig. 1. The fragment of osteoblast. Flight. N — nucleus; M — mineral matrix; RER — rough endoplasmatic reticulum; GC — Golgy Complex. Electron microphotography X3000

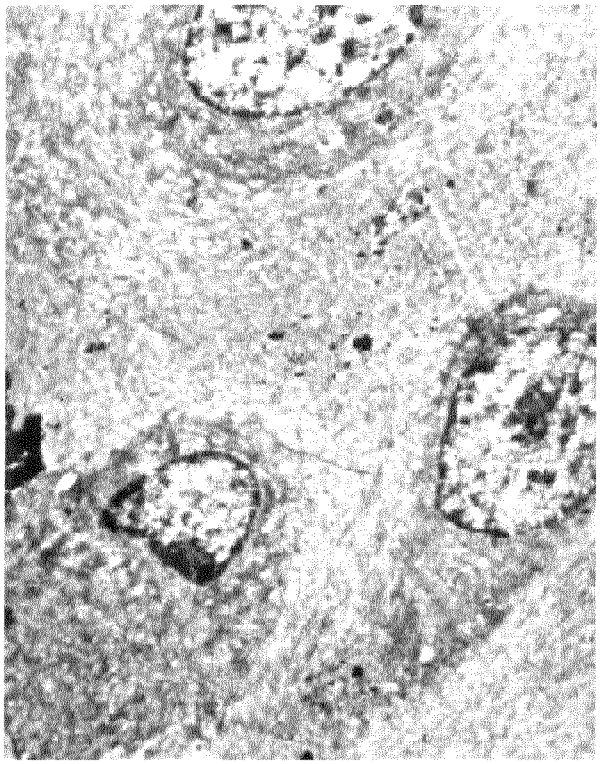

Fig. 2. The osteoblast of fibroblastic type. Flight. See Fig. 1 for designations. Electron microphotography X3500

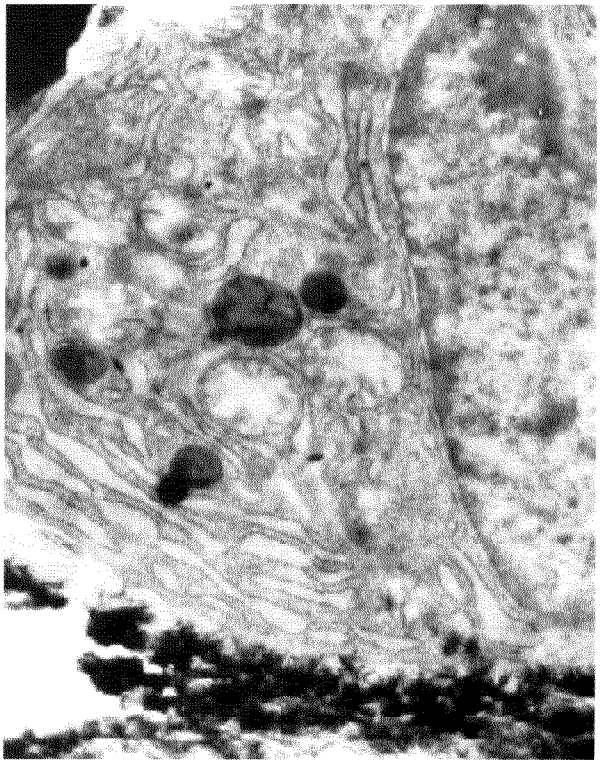

Fig. 3. The fragment of osteocyte with developed RER. Flight. See Fig. 1 for designations. Electron microphotography X15500

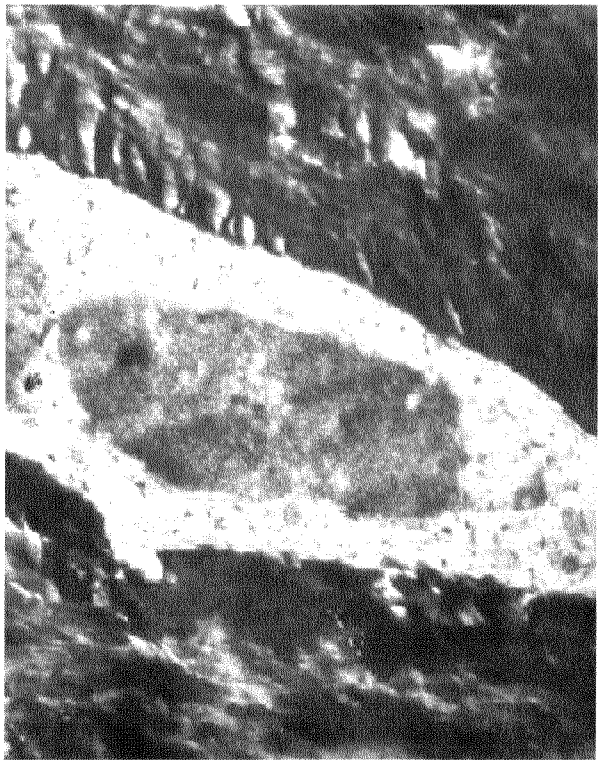

Fig. 4. Destroyed osteocyte with the picnotic nucleus. Flight. See Fig. 1 for designations. Electron microphotography X5800

seams that there are no asynchrony of the specific biosynthesis, a part of it is disconnection of the collagen proteins synthesis and glycosaminoglycans synthesis, which is characteristic for the intensive osteogenic zones.

We obtained the similar data during our investigation a microgravity influence on osteogenesis, which was conducted on the rat metaphyses and epipyses from biosattelite «Cosmos-2044 and SLS-2. The study of the biochemical peculiarities of the osteoporosis development under the microgravity action is demonstrated alterations in the collagen and glycosaminoglycans metabolism: deceleration of the synthesis and intensification of catabolism. The comparison of these alterations with the bone structure damages (such as reduction of the Ca and P content and increase of their excretion with the biological fluids) permits to suggest the interrelation between the disturbance of the glycosaminoglycans synthesis and the mineralization state [10].

In some bone zones around the osteoblasts the large areas with collagen fibrills are revealed in biosamples from the flight group This is evidence of the destruction of the osteogenic function in the cells and exhibits a tendency to cells conversion into the fibroblastic ones (Fig. 2). Table 2 shows data about the changes of the specific volume of cell organelles in the typical for flight osteoblasts and osteoblasts of the fibrotic type of the animals from flight and control groups.

We can suppose, that disappearance of the gravitation overload induces osteoblasts to synthesize the collagen proteins that are characteristic for the fibroblastic phenotype. In some studies the possibility of the new forms of the collagen synthesis in the bone tissue and of the specific gene expression inhibition (e.g. the osteocalcin-producing gene) is postulated under microgravity condition [9]. It leads to the disturbances of the osteoid mineralization processes and to the appearance of fibrosis zones. These phenomena may be considered as a pattern of the phenotypic diversity that reflects the adaptive processes.

Osteocytes. In the flight group, the osteocytes from animal bone samples generally preserve a typical structure similar to that of the control. Osteocytes of superficial zones of bone trabeculae are mostly oval and have short processes. The chromatin in nuclei is concentrated as granular accumulations in whole nuclei and at their periphery. However, there are differences compared to the control: some osteocytes differ in RER and GC development.

In some osteocytes, RER is well-developed, containing a net of channels and small cisterns that occupy a considerable part of the cytoplasm volume. The GC in these osteocytes is not well-developed and its structures exibit a compact localization. These osteocytes lie in large lacunae filled with collagen fibrils (Fig. 3). No mineralization places are observed. Such osteocytes can be destroyed during secretion of collagen proteins. This can be the result of slowing down of the transformation of osteoblasts into osteocytes in adaptive remodeling zones of bone under reduced functional loading due to flight conditions.

We can also see here the slowing down or cessation of mineralization processes of bone matrix formation. This happens probably because of the intensification of collagen protein biosynthesis of the in young osteocytes with a subsequent disturbance of the formation processes in osteocytic lacunae.

Such osteocytes are usually observed in bone tissue areas, where there is osteoblast reorganization (osteoblasts acquire a fibroblastic phenotype) and are surrounded by collagen fibrils without mineralization. We noticed this during osteoblast investigation in these biosamples. We assume that reduction of a gravity load causes some osteoblasts to synthese proteins, typical for fibroblasts. They can synthesize new collagen isoforms in bone tissue, and inhibit specific expression of genes in microgravity conditions. This may lead to a disturbance of mineralization processes and to the appearance of zones of fibrosis [12].

Such processes, we believe, also affect the formation of osteocytes in the bone-remodeling zone under microgravity. As a result, a fibroblastic tissue is formed instead of a mineralized bone tissue in zones of adaptive remodeling.

For mature osteocytes, which are in the depth-middle of bone trabeculas, the specific volume of structures of GC, lysosome-like bodies and autophagosomes typically increases compared to the control (Fig. 4). Table 3 demonstrates data about the changes of a specific volume of the cell organelles in the mature osteocytes of the animals from flight and control groups.

The GC vesicular component is especially well-developed. It is composed of primary lysosomes (d = 0.1—0.2 mkm), containing hydrolytic enzymes. Our view of this is presented below. First, the intensity autolytic processes increases in the cells, due to destruction of protein-synthesized organelles. Second, cells participate in the biosynthesis of glycosaminoglycans, which are secreted into the osteocytic lacunae space. This conclusion is confirmed by our the cytochemical and autoradiographical data: osteocytes incorporate ^{35}S-sulphate through out 30 min after its injection [11]. Third, the latter is observed: in octeocytic processes, related with an increased resorption of mineralized bone matrix (osteolysis).

Table 1. A specific volume of cells organelles in osteoblasts of the crest of iliac bone of monkeys (flight and controls)

Cell organelles	Ground-control, n = 5	Synchronous control, n = 3	Flight experiment, n = 2
RER	0.370 ± 0.018	0.367 ± 0.017	0.331 ± 0.011*
GC	0.230 ± 0.010	0.221 ± 0.008	0.172 ± 0.021*
AFL	0.007 ± 0.001	0.006 ± 0.002	0.026 ± 0.002*
Other structures	0.491 ± 0.030	0.403 ± 0.010	0.469 ± 0.002

* The distinctions are authentic as contrasted to control ($P < 0.05$)

Table 2. A specific volume of cells organelles in typical osteoblasts and osteoblasts of fibroblastic type of crest of iliac bone of monkeys (flight)

Cell organelles	Typical osteoblasts (4th type)	Osteoblasts of fibroblastic type
RER	0.331 ± 0.011	0.372 ± 0.019*
GC	0.172 ± 0.021	0.215 ± 0.008*
AFL	0.026 ± 0.002	0.012 ± 0.001*
Other structures	0.469 ± 0.002	0.597 ± 0.010

* The distinctions are authentic ($P < 0.05$)

Table 3. A specific volume of cells organelles in mature osteocytes of the crest of iliac bone of monkeys (flight and controls)

Cell organells	Ground-control, n = 5	Synchronic control, n = 3	Flight experiment, n = 2
GER	0.167 ± 0.013	0.159 ± 0.010	0.143 ± 0.011*
GC	0.034 ± 0.006	0.036 ± 0.005	0.052 ± 0.004*
AFL	0.018 ± 0.002	0.019 ± 0.003	0.032 ± 0.003*
Other structures	0.779 ± 0.003	0.783 ± 0.004	0.772 ± 0.004

* — the distinctions are authentic as contrasted to control ($P < 0.05$)

We also confirmed this from pictures of the destroyed mineralized matrix in periosteocyti lacunae. The bone matrix disintegrated into conglomerates. Resorption is facilitated by the lysosomal hydrolytic enzymes. We demonstrated of acid phosphatase in osteocytes and their lacunae with the help of electron cytochemistry. The lysosomal enzymes are secreted into lacunae by exocytosis. Sometimes we observe the osteoclastic resorption (fragments of the osteoclasts brush border in bone lacunae). Some of the osteocytes are destroyed in state of functional activity. Nuclei are subject to picnosis and the cytoplasm is destroyed (Fig. 4). Destroyed osteocytes also perform an osteolytic function, releasing lysosomal enzymes [7].

In flight conditions, the number of empty osteocytic lacunae and their volume increase in bone samples compared to the control. Therefore, the number of destroyed osteocytes increases. The quantity of empty lacunae located in a hystosection area increases from 5 to 7 % compared to control. Osteocytic and osteoclastic osteolysis can be considered as physiological hormone-dependant mechanisms of the regulation of mineral homeostasis [1]. This is why we suppose, that osteocytes can switch over from the osteolysis to the osteoplastic function (production of organic bone matrix) [11].

The removal of mechanical load is known to stimulate bone resorption, obviously due to citokynes production. In certain circumstances the conditions in the complex hierarchy of fluid- and ions regulation can initiate mobilization of Ca from bones. There exist morphological signs of activation of bone resorption, as one of the background of osteogenesis inhibition that has been shown mainly in experiments with animals, and in recent manned flights [8]. The metabolic hypothesis is also supported by the distinct specific topography of changes mostly observed in metabolically active trabecular bone structures. At present, wide recognition and experimental evidence receives the opinion according to which the reaction of bone tissue in space flight is to a considerable extent determined by local factors of bone metabolism [14]. In particular, there are data suggesting possible local inhibition ex vivo of the transformation of osteoprogenitors in preosteoblasts and differentiation of adult osteoblasts [13].

Data on changes in the bone cells activity ex vivo have been supported by results of the in vitro culture studies with a subsequent exposure to microgravity. Investigations of cultures of embryonic mice bone showed reduction of osteoid mineralization rate and glucose consumption by the culture and simultaneous increase in mineral resorption after space flight [15]. Syntheses of type-1 — collagen and alkaline phosphatase was also found to slow down in the culture of preosteoblast-like cells (line MN-7) under the influence of microgravity [2]. Culture studies of cartilaginous cells on the MIR station demonstrated shrinking of colonies, degradation of mechanical stability of the cultural structure and suppression of glucosaminoglicanes syntheses [5].

The microgravity effect is realized on a tissue level, with the involvement of local factors (peptides, as well TGF beta 2, IGF and others).

The role of gravity strain grasping structures in osteoblasts can play: mechanosensitive cationic channels; mechanochemical influences with the next remodeling of cytoskeletone as a gravity sensor [2].

In our researches we obtained data, that reflect some mechanisms, which lead to the local osteopenia (or osteoporosis) development in trabecular bone

structures of the lower part of the skeleton, increases a bone breaches risk under the space flight conditions.

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ЦИТОЛОГІЧНІ МЕХАНІЗМИ ГРАВІТАЦІЙНО-ЗАЛЕЖНИХ ЗМІН У КІСТКОВІЙ ТКАНИНІ

Н. В. Родіонова, В. С. Оганов

З використанням методів електронної мікроскопії, цитохімії та морфометрії проведено дослідження основних змін в остеобластах та остеоцитах клубової кістки у мавп (*Macaca mulatta*), що перебували в космосі на борту біосупутника БІОН-11 протягом двох тижнів. Біоматеріал було взято шляхом біопсії. За ультраструктурними показниками стану органел клітин було встановлено, що в умовах мікрогравітації в популяції остеобластів превалюють малоактивні форми. Також нами виявлені остеобласти фібробластичного типу та локальні зони фіброзу в губчастій кістці. В популяції остеоцитів нами знайдені типові клітини, а також остеоцити з розвиненим ГЕР. В кістці посилені процеси остеоцитарного остеолізису.