Structural Organization of the Bone Mineral

A. A. Doktorov, Yu. I. Denisov-Nikol'skii, and B. A. Zhilkin

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Structural organization of the bone mineral phase formed by intra- and interfibrillar lamellar crystals is studied by scanning electron microscopy and cryofractography. Rod-shaped crystals are found only in collagen fibrils of the bone lacuna walls. Intra- and interfibrillar crystals form numerous contacts, ensuring the integrity of the bone mineral phase.

Key Words: *bone; collagen fibrils; bone mineral crystals*

Mechanical and physicochemical properties of mineralized tissues strongly depend on the interactions between the organic matrix and embedded mineral crystals [1,8]. Some aspects of these interactions still remain unclear. For example, what determines the shape and size of these crystals as well as their localization and orientation in the collagen framework, which represents the basis of an organic matrix of most mineralized tissues [8]. Mineral crystals fill the interfibrillar spaces [3,5-8]. Mineralized collagen fibrils (CF) are considered to be the main building blocks of mineralized tissues in vertebrates [12].

Calcifying tendon from a domestic turkey is a convenient model of biomineralization [3,6,7,9,10]. It has been shown that intrafibrillar crystals are located both in the holes and overlaps of CF with their C axes paralleling each other. The adjacent crystals may fuse and form larger mineral particles. The shape and spatial organization of interfibrillar crystals in a calcifying tendon have not been described in detail.

Little is known about the shape and spatial organization of mineral crystals in the bone matrix. It has been hypothesized [11] that in a lamellar bone the crystals are coplanar and arranged in parallel layers. In some lamellae, these layers are parallel to the lamellar surface, while in others they are arranged at an angle.

The aim of the present study was to investigate the spatial organization of intra- and interfibrillar mineral crystals in the matrix of a human lamellar bone.

Research Center of Biological Structures, Moscow

MATERIALS AND METHODS

Fragments of compact substance of human femur and tibia (from 19-44-year-old individuals) were studied. Bone and articular pathologies were excluded. Bone fragments for scanning electron microscopy (SEM) were split off perpendicularly to its long axis. Specimens (1-2 mm thick) were cut out from the bone below the fracture, organic components were removed by incubation in sodium hypochlorite and hydrazine solutions [1,2], routinely processed, and examined under an SEM-515 scanning electron microscope (Philips). The absence of organic matter was confirmed as described previously [12]. Hydrazine- and hypochlorite-treated specimens were frozen-fractured in a BAF-400D installation (Balzers) and shaded with platinum and carbon. The replicas were examined under an EM-420 transmission electron microscope (Philips).

Fig. 1. Organization of bone mineral crystals on a natural surface of bone lacuna. *a*) randomly oriented mineralized collagen fibrils (CF) and bundles forming the surface of a bone lacuna. The openings of osseous canaliculi are indicated with arrows. *Zone of lacuna wall coated with mineralized ground substance. SEM. ×8250; *b*) CF-associated chains of bone mineral crystals oriented at a small angle to its axis. Cryofractography. Transmission electron microscopy (TEM). ×90,000; *c*) transverse fracture of a mineralized CF with superficial rod-shaped crystals (arrows) and deeper lamellar crystals (double arrows). *Lamellar crystals surrounding the CF. Cryofractography. TEM. ×240,000; *d*) tile-shaped crystal organization in mineralized ground substance on bone lacuna surface. Cryofractography. TEM. ×145,000.

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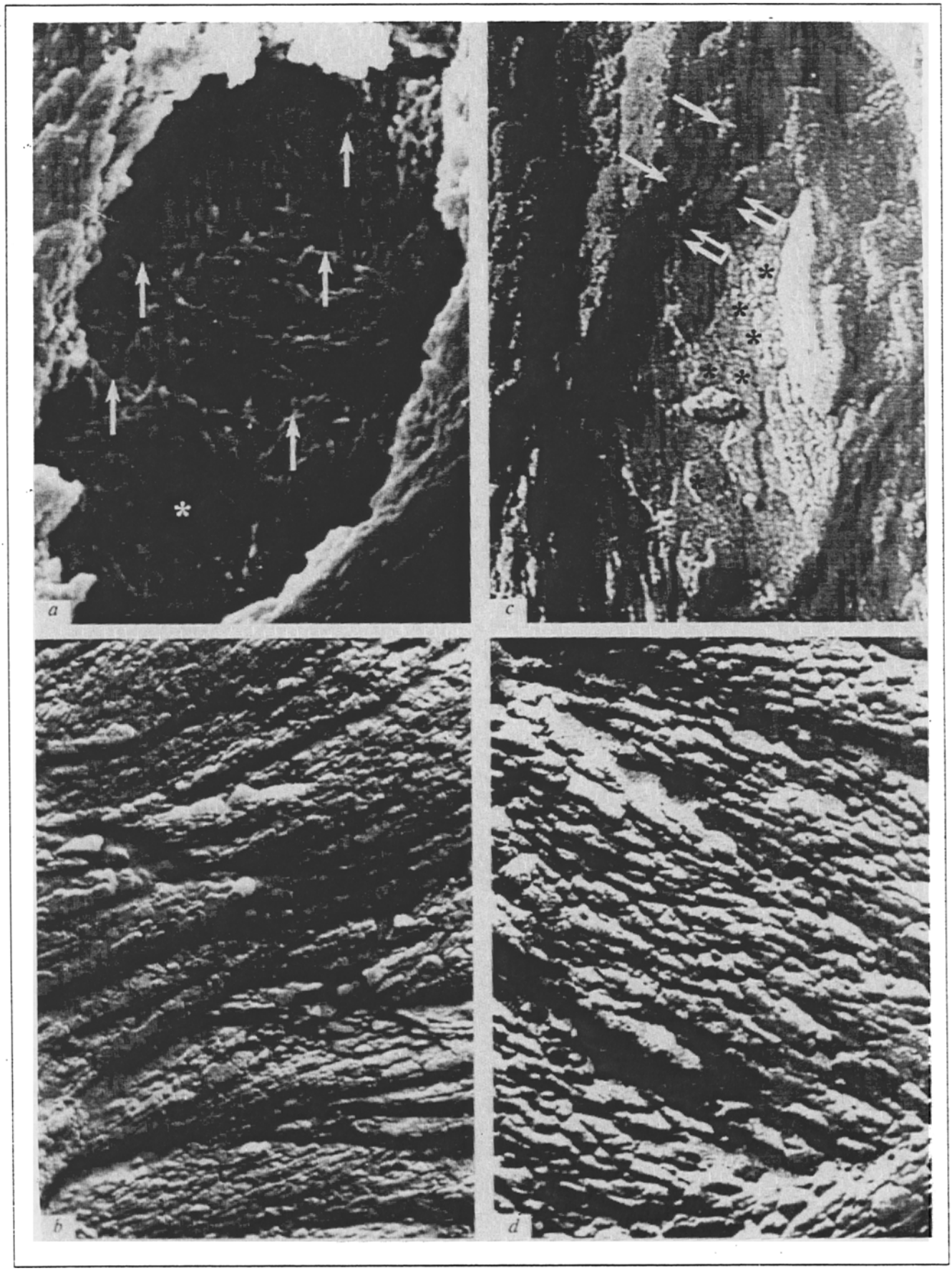
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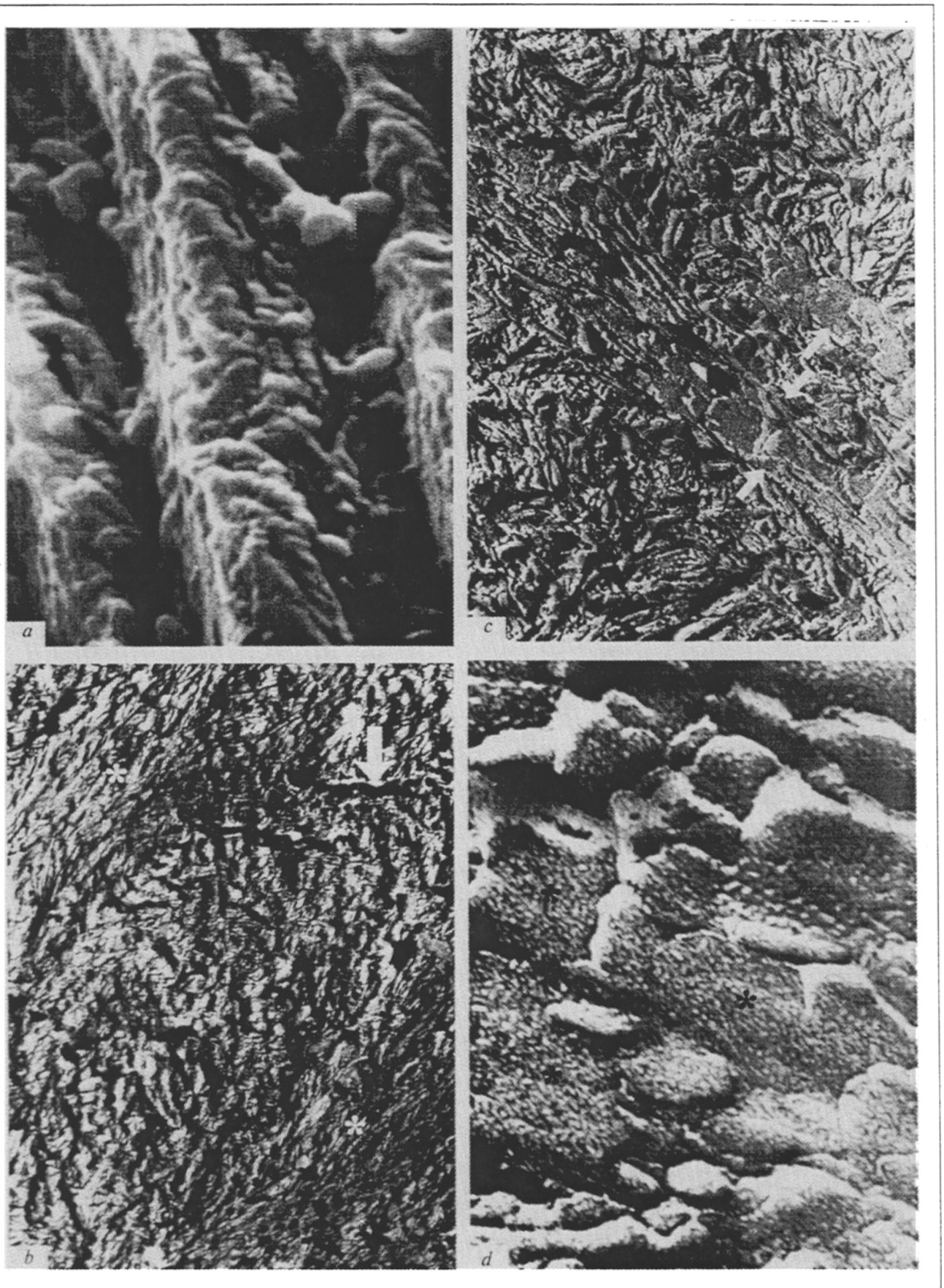
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RESULTS

The above-described method of processing bone specimens allows one to study both the surface of the fracture through the bone matrix and the mineralization fronts in natural bone surfaces such as the walls of lacunae and canaliculi. These surfaces are formed by mineralized collagen fibers of varying thickness and occasional chaotically oriented CF (Fig. 1, *a*). Sometimes, the walls of lacunae and canaliculi are evenly coated by mineralized ground substance, which is typical of inactive areas on the bone surface [4]. SEM resolution is not sufficient for visualization of the individual mineral crystals constituting the relief of the mineralization front in osseous lacunae and canaliculi. They are clearly seen on a platinum-carbon replica (Fig. 1, *b*), which enabled us to differentiate mineral CF-associated particles protruding into the bone lacunae and canaliculi from interfibrillar particles located in the surface layer of mineralized ground matter corresponding to inactive areas of the bone surface [4]. The CF-associated crystals form elongated spiral chains directed at a small angle to the fibrillar axis. The crystals of adjacent chains are usually shifted from one another and only occasionally form small orderly structures. Zones where the fracture plane is perpendicular to CF provide an insight into the spatial organization and cross-section of the intrafibrillar crystals (Fig. 1, *c*). The crystals are usually arranged as parallel layers tilted to the lacuna surface at an angle varying in consistency with the spiral arrangement of the crystal chains along the fibrillar axis. Both rod-shaped and lamellar particles are seen. The rod-shaped particles are usually located at the free surface of the fibril, whereas the lamellar particles reside in deep compartments. The adjacent crystals are densely packed and probably contact with each other.

Generally, interfibrillar mineral crystals have a lamellar shape and are arranged circularly along individual CF (Fig. 1, *c*). Alternatively, they are arranged in layers or dense clusters.

In the zones where a lacuna wall is covered with a layer of ground matter, all crystals are lamellar, densely packed, and aligned either in parallel to the bone surface or overlap each other (Fig. 1, *d*).

Osseous lamellae with CF randomly oriented in the bone matrix were revealed by SEM of the fracture surface (Fig. 2, *a*). Crystals with different orientation corresponding to the CF orientation in the nearest lamellae were observed on platinum-carbon replicas (Fig. 2, *b*). In fractured CF, crystals form chains consisting of lamellar particles (Fig. 2, *c*). These particles contact by their edges and form larger lamellae consisting of several crystals (Fig. 2, *d*). The adjacent crystals and larger particles are often located in different planes so that the putative structure formed by parallel layers [11,12] cannot be observed. In neighboring fibrils of the same lamellae, only a tendency toward codirected orientation of crystal layers is noted. The fibril contours are smooth. This is usually observed in sites where interfibrillar spaces lack CF-surrounding particles. In these zones, either parallel layers or dense clusters of chaotically oriented crystals are seen. It should be noted that the crystals located in fibrils and interfibrillar spaces filled with the ground substance form contacts with each other. This ensures the integrity and ruggedizes the bone mineral phase [1] that constitutes bone lamellae and entire bone.

The present study shows that the rod-shaped mineral crystals occur only on the surface of mineralized CF protruding into bone lacunae and canaliculi, while the deeper layers contain only lamellar particles. This may reflect progressive mineralization of the surface of lacunae and canaliculi, since individual crystals may grow both in length and in width due to accumulation of calcium and phosphate ions [8].

Thus, the bone mineral phase consists primarily of lamellar crystals up to 45-55 nm long which are localized both in fibrils and in interfibrillar spaces. The intrafibrillar crystals form long spiral chains and are oriented at a small angle to the CF axis. The adjacent crystals can fuse and form large lamellae. Mineral deposits in interfibrillar spaces consist only of lamellar particles surrounding individual CF or being packed in parallel layers or dense clusters. Inter- and intrafibrillar crystals contact with each other, thus ensuring the integrity of the mineral phase of the entire bone.

Fig. 2. Organization of bone mineral crystals in lamellar bone matrix. *a*) various orientation of mineralized collagen fibrils (CF) in bone lamellae. SEM. ×12,000; *b*) mineral crystals composing lamellae of the lamellar bone. *Lamellae with codirected CF. A bundle of mineralized CF connecting the lamellae is indicated with an arrow. Cryofractography. TEM. ×45,000; *c*) alignment of bone mineral crystals in CF (between arrows). Cryofractography. TEM. ×90,000; *d*) lamellar mineral particles (asterisks) formed by edge-to-edge contact of individual crystals. Cryofractography. TEM. ×360,000.

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Choleragen-Induced Changes in the Ultrastructure of Epitheliocytes from the Large Intestine of Conventional and Gnotobiotic Minipigs

V. A. Shakhlamov, I. V. Isupov, and L. A. Nazarova

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The dynamics of changes in the absorbing epitheliocytes and goblet cells is studied in minipigs 1, 3, 6, 18, and 24 h after intragastral administration of cholera toxin. Light and dark cells are identified after the treatment. The dark cells are probably protected against the toxin by paracrine E_c-cells persisting throughout the entire observation period. Changes in the organelles of absorbing epitheliocytes appear 3 h after the treatment and persist for 24 h. During this period, the goblet cells actively secrete mucus.

Key Words: *choleragen; large intestine epitheliocyte; minipig; gnotobiotic and conventional animals*

In recent decades, minipigs have been widely used in laboratory research, since their internal organs are very similar to those of humans [2,10,11]. It should be noted that the intestinal microflora of minipigs of the Svetlogorsk population is identical or close to the symbiotic microflora of human intestine [3]. Therefore, the data obtained on minipigs have been extrapolated to humans [6]. However, the ultrastructure of epitheliocytes from the large intestine of gnotobiotic minipigs in the norm and during choleragenic intoxication has not been studied.

MATERIALS AND METHODS

This paper presents experimental data obtained on 15 gnotobiotic and 2 conventional minipigs (500-1500 g) after intragastral administration of cholera toxin (500-800 μg) in physiological saline. The large intestine and epitheliocytes were studied 1, 3, 6, 18, and 24 h after administration of the toxin. After a 24-hour period of food deprivation, the minipigs were given 1% sodium bicarbonate solution (pH 7.2) to neutralize gastric juice, after which cholera toxin was administered. Two control animals were given the same volume of physiological saline.

Specimens for light and electron microscopy were fixed with glutaraldehyde and postfixed with osmium tetroxide in a cacodylate buffer (pH 7.3). The specimens were passed through increased ethanol concentrations and embedded in Epon-Araldite. Both semithin and ultrathin sections were examined.

RESULTS

It has been generally recognized that gnotobiotic conditions affect all vital systems of the organism. The most pronounced ultrastructural changes occur

Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow; *Microb* Antiplague Research Institute, Saratov

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