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STRUCTURAL-MORPHOLOGICAL CHANGES OF BONE TISSUE IN PATIENTS WITH CHRONIC RECURRENT HEMATOGENOUS OSTEOMYELITIS

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***ABSTRACT**--To study the morphological features of the structure of bone tissue in patients with chronic recurrent hematogenous osteomyelitis.The studied material of scanning and transmission electron microscopy were bone fragments of 40 patients obtained during surgery from various pathologically altered sections of the affected tubular bones.Morphological studies revealed microscopic foci of necrosis, leukocyte infiltrates and microabscesses both in the main focus and in other parts of the bone marrow canal.Microabscesses detected by microscopy represent the morphological basis of chronic hematogenous osteomyelitis, can be located at a considerable distance from the main necrotic purulent focus and, under appropriate conditions, cause a relapse of the disease. Morphological studies have shown that surgical debridement of the lesion with ultrasonic cavitation of the lesion and the medullary canal contributes to a significant reduction in the degree of microbial contamination, followed by a decrease in pathological changes in bone tissue.*

***Key words**-- chronic osteomyelitis, morphology, bone tissue.*

LIST OF ABBREVIATIONS

CT - computed tomography
MRI - magnetic resonance imaging
TEM-transmission electron microscopy
SEM scanning electron microscopy
CGO - chronic hematogenous osteomyelitis
HRGO - chronic recurrent hematogenous osteomyelitis

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I. INTRODUCTION

Chronic hematogenous osteomyelitis (CGO), characterized by a long recurrent course, continues to be one of the most severe and frequently occurring purulent-inflammatory diseases in children. According to various authors, CGO makes up from 12.5 to 47% in the structure of purulent-inflammatory pathology of childhood [4, 6]. Despite the existing methods of complex treatment, the frequency of repeated operations (12-31%) and relapses of the disease (22-78.4%), leading to disability of patients, remains high [1, 2, 10]. The success of treatment of chronic osteomyelitis, according to many authors, depends on the radicalization of sanitation of the purulent necrotic focus, in particular, according to some authors, one of the reasons for unsatisfactory outcomes of surgical treatment of chronic osteomyelitis is "inadequate surgical technique" [3, 9]. Despite the availability of modern diagnostic methods, such as CT, MRI, etc., some authors believe that histological examination remains the gold standard in the diagnosis and treatment of CVD [2, 5].

Pathomorphological changes in hematogenous osteomyelitis, especially in the acute and chronic stages, have now been studied quite well [7, 8]. However, the requirements for the diagnosis and treatment of the often relapsing process and exacerbations of the chronic stage dictate the need for a new understanding of this issue.

II. MATERIAL AND METHODS

Morphological studies were carried out in the pathological anatomy laboratory of the Republican Specialized Scientific and Practical Medical Surgery Center named after Academician V. Vakhidov under the guidance of Professor I. Baybekov.

The studied material was bone fragments obtained during surgery from various pathologically altered sections of the affected tubular bones in 40 patients with chronic recurrent hematogenous osteomyelitis (HRGO).

The material for light-optical research was fixed in 10% formalin solution according to Lilly. Bone fragments were decalcified in Trilon-B solution for 10-15 days. Paraffin sections were stained with hematoxylin and eosin.

For transmission electron microscopy (TEM), decalcified samples after fixing with glutaraldehyde on a phosphate buffer were fixed with a 1% solution of osmium tetroxide in a similar buffer. After dehydration in alcohol - acetone, it was poured into epon - araldite. Ultrathin sections obtained on an Ultracut Reichert-Iung ultratome were contrasted in an Ultrastainer LKB. Viewing and photographing preparations was carried out in TEM.

For scanning electron microscopy (SEM), the preparations after the fixation described above were subjected to dehydration in alcohol-acetone, then dried by the critical point method in an HCP-2 apparatus and sprayed with gold in an IB-2 apparatus. Semi-thin epoxy sections stained with methylene blue - fuchsin were also investigated by the light-optical method.

III. RESULTS AND DISCUSSION

A study of the pathomorphology of bone tissue destruction sites in HRHC showed that the overall architectonics of the bone was preserved in the cortical form of the lesion. In particular, in the bone itself around the Haversian canals, concentrically located bone plates, as well as relatively well-preserved interstitial plates, are

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clearly defined. However, there is an expansion of bone lacunae with osteoblast remnants (Fig. 1). In addition, quite numerous osteocytes with relatively good preservation of nuclei, as well as Haversian and Volkman channels, are distinctly revealed on the sections. On the outer surface of the bone, the periosteum is not determined. There are homogeneous basophilic masses representing bone detritus (Fig. 2). In addition to the widened gaps of the Haversian canals, slit-like formations are formed in the bone tissue, which can merge with each other. This, apparently, is the structural basis for the formation of sequesters, which can have different sizes (Fig. 3).

A characteristic morphological picture of the emerging sequesters is the lack of expression of the bone platelets, however, wrinkled hyperchromic osteocytes, sometimes only their shadows, are determined. The basophilic masses of detritus are located on the outer surface of the newly formed sequesters, which have not yet completely separated from the main bone (Fig. 4). The formed osteomyelitic sequestra are characterized by a complete violation of the architectonics of bone tissue. The latter is replaced by a rather homogeneous eosinophilic mass with poorly distinguishable osteocyte residues.

At the same time, areas are preserved where, against the background of complete melting of the bone plates and complete loss of the characteristic architectonics of the bone tissue, numerous osteocytes are determined. As the osteonecrotic process progresses, the zone expands where there are no osteocytes, characteristic bone plates are not determined, and the bone tissue itself is represented by rather coarse fibers. These fibers are often concentrically arranged around cavities of regular round shape of various sizes, which on yellow semi-thin epono-araldite sections stained with methylene blue-fuchsin have a yellow color characteristic of lipids.

The absence of osteoblasts in the gaps, the relative preservation of the structure of neighboring osteocytes indicates that the latter are more resistant to inflammatory and destructive changes in bone tissue that occur with hematogenous osteomyelitis.

It is known that the inner and outer bone plates that form the bone marrow cavity and the outer part of the tubular bones do not form concentric structures around haversian canals. With osteomyelitis in these areas of the bone, coarse fiber structures are determined with overlays of a different nature on the surface (Fig. 5). Probably, these overlays are formed from fibrin films and clusters of microorganisms that form a kind of biofilm.

Another morphological feature of HRHC is the presence of microabscesses with inclusions of detritus on the surface of large caverns of tubular bones and in the lumen of the enlarged Haversian tubules. The contents of the microabscess are represented by altered cells and accumulations of microorganisms (Fig. 6).

Surgical debridement of the lesion with ultrasonic cavitation of the lesion and the medullary canal causes a significant decrease in the degree of microbial contamination, which subsequently helps to reduce pathological changes in bone tissue. So, after rehabilitation, the lumens of the cavities of the Haversian tubules become much smaller, the cracks in the bone tissue narrow significantly and their length is reduced. Numerous osteocytes are determined among bone plates, many of which have a normal structure (Fig. 7). Moreover, between the fiber components of bone tissue, binuclear osteocytes are detected (Fig. 8).

More significant changes in the ultrastructure of bone tissue during cortical necrosis on the basis of HRHC are also detected by transmission electron microscopy. Thus, organelles are not determined in the cytoplasm of osteocytes, the nuclei are predominantly heterochromatin densely condensed throughout the nucleoplasm. In the lumen of the Haversian tubules, the processes of osteocytes clearly contour. The osmiophil plate is well defined (Fig. 9).

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With a deeper lesion of bone tissue in the lumen of the lacunae, the remains of osteocytes are determined. The fiber components of bone tissue are presented as amorphous material of moderate electron density (Fig. 10).

A characteristic feature of hematogenous osteomyelitis is the detection by TEM of numerous microorganisms in the damaged areas of the tubular bones. These microorganisms are both sticks and cocci of various sizes. With a more severe form of damage to the bones of the cocci (apparently, staphylococci) form characteristic colonies. The surrounding bone tissue undergoes complete destruction (Fig. 11).

In bone biopsies obtained after surgical debridement of a purulent lesion with ultrasonic cavitation, a noticeably smaller number of microorganisms and destructive manifestations of bone tissue are noted. In sanitized bone tissue, neutrophilic polymorphonuclear leukocytes and phagocytic microorganisms - microphages are much more often detected. In some preparations, microorganisms were determined only in the cytoplasm of microphages, undergoing lysis here. However, the ultrastructure of the microphage is also violated.

Ultrasonic cavitation, contributing to an effective reduction in the number of microorganisms in the affected bone, at the same time does not have a significant negative effect on the ultrastructure of the treated bone tissue. In the lacunae, the normal structure of osteocytes is quite distinct, the fiber components of the bone also retain their characteristic ultrastructure (Fig. 12).

The presence in the osteocytes of profiles of the granular endoplasmic reticulum, the Golgi complex, and mitochondria indicate that they retain the ability to heterosynthesis.

Conducted studies of bone surface cleavages with HRG using scanning electron microscopy revealed significant changes in the microrelief. These changes were in violation of the rhythm of the microrelief, the appearance of uneven ridges and depressions on the surface, the presence of detritus and rounded spherical structures of various sizes, representing both red blood cells and cocci (Fig. 13).

In cortical osteonecrosis caused by the inflammatory process with hematogenous osteomyelitis, fibers of various sizes, red blood cells, and individual cocci are clearly differentiated on the surface of the structures (Fig. 14).

SEM of the intraosseous abscesses shows a complete loss of the characteristic three-dimensional architectonics of the bone. Extensive necrosis with lysed fragments of bone marrow tissue, an abundance of purulent exudate, detrital masses, fibrin and proliferation of connective tissue elements in the affected fragments is noted. Disorders of intraosseous circulation leads to an increase in intraosseous pressure and the expansion of ischemic zones and the emergence of new microscopic foci of necrosis, leukocyte infiltrates and microabscesses at a distance from the main lesion. It is these aberrant microabscesses detected by microscopy that represent the morphological basis of the recurrent course of CGO. Confirmation of the aforesaid can be considered the fact that microabscesses can be located at a considerable distance from the main necrotic purulent focus and, under appropriate conditions, cause a relapse of the disease. At the same time, in the areas of bone adjacent to the microabscess, the fiber base with individual migrated cells is preserved (Fig. 15).

After sanitation of the purulent lesion in the studied SEM biopsy specimens obtained from the surface of the treated part of the bone, better preservation of bone tissue is revealed than we observed in the biopsy specimens obtained from the destruction zone: interwoven fibers and lacunae with partially preserved osteocytes are clearly visualized (Fig. 16).

Conclusion A study of the pathomorphology of bone tissue destruction sites in HRHC showed that the revealed pathomorphological phenomenon of the presence of aberrant, distal from the main focus, intraosseous abscesses requires an expansion of the scope of the operation to sanitize all foci of purulent-inflammatory destruction.

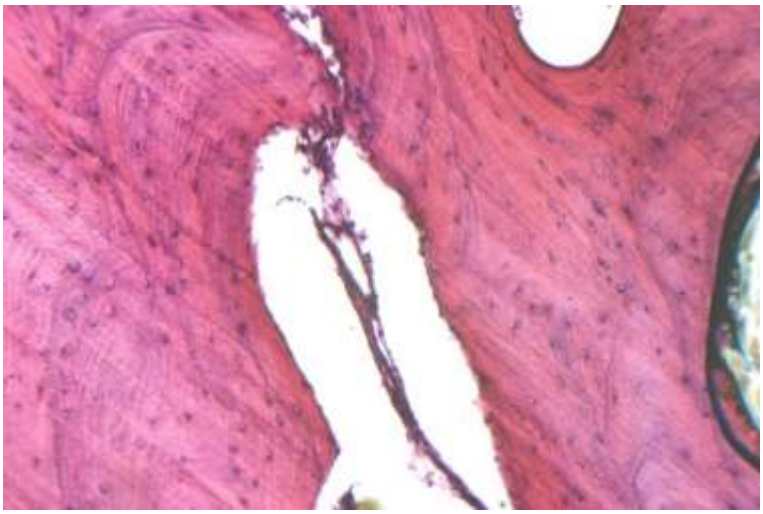

Figure 1: Expanded bone lacunae, preservation of the architectonics of bone plates.

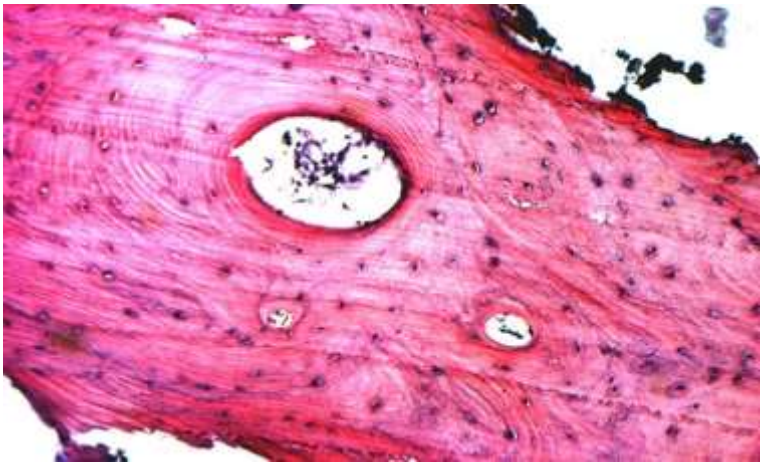

Figure 2: Lack of periosteum and overlay on the surface of the bone.

Figure 3: The formation of cracks in bone tissue, the expansion of lacunae.

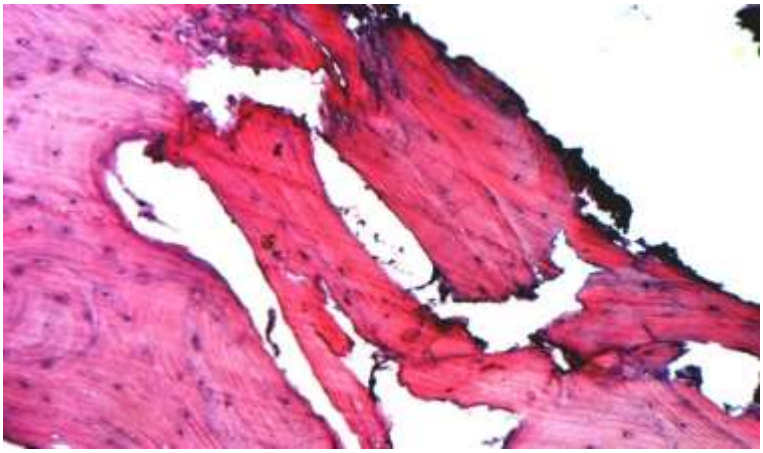

Figure 4: The formation of sequesters of necrotic bone with detritic masses on the surface.

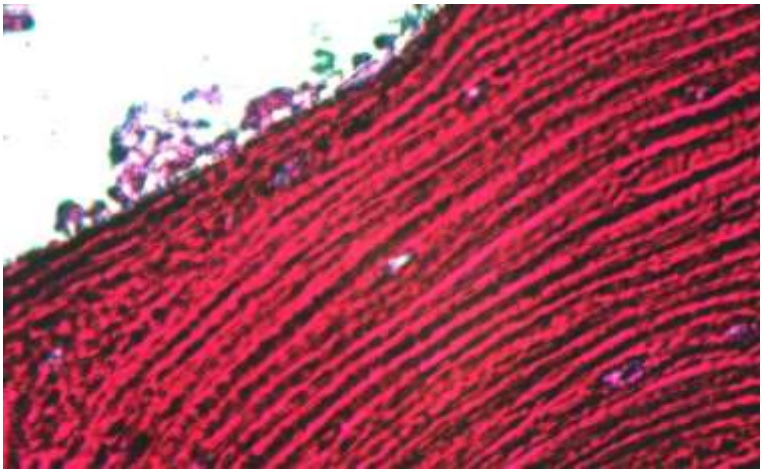

Figure 5: Coarse fibers of the bone surface with overlays**.**

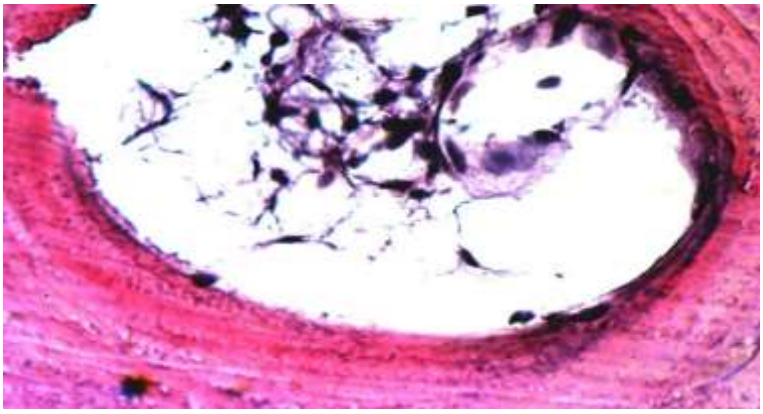

Figure 6: Microabscess with detritus in the lumen of the expanded gap.

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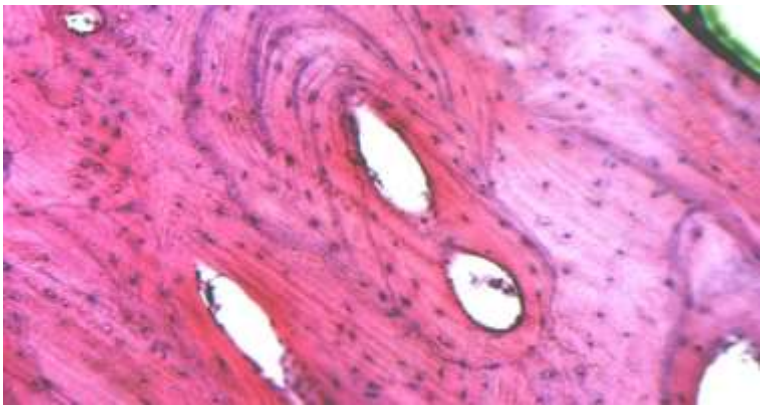


Figure 7: The best preservation of the architectonics of the bone, the small size of the lumen of the lacuna after rehabilitation and ultrasonic cavitation.

Figure 8: Binuclear osteocytes after ultrasonic cavitation**.**

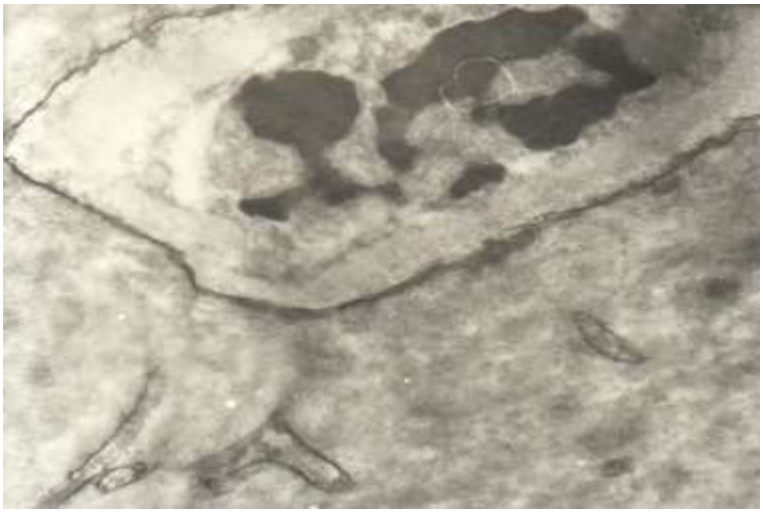


Figure 9: The relative safety of the osteocyte ultrastructure**.**

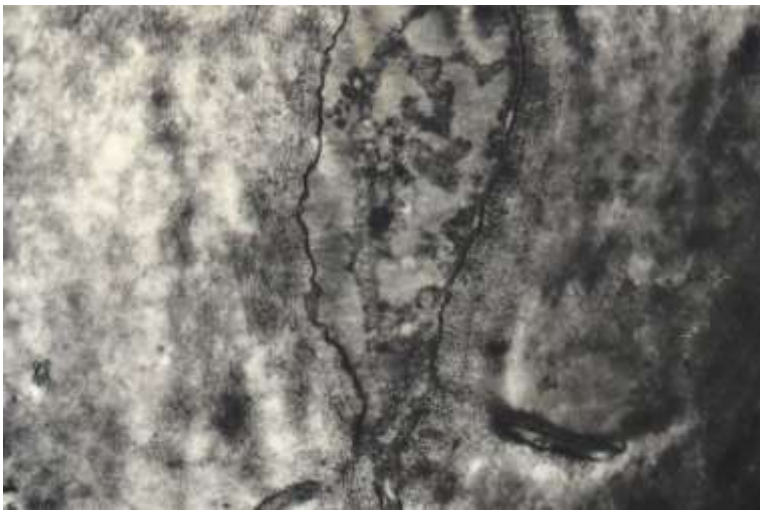


Figure 10: The destroyed osteocyte in the gap.

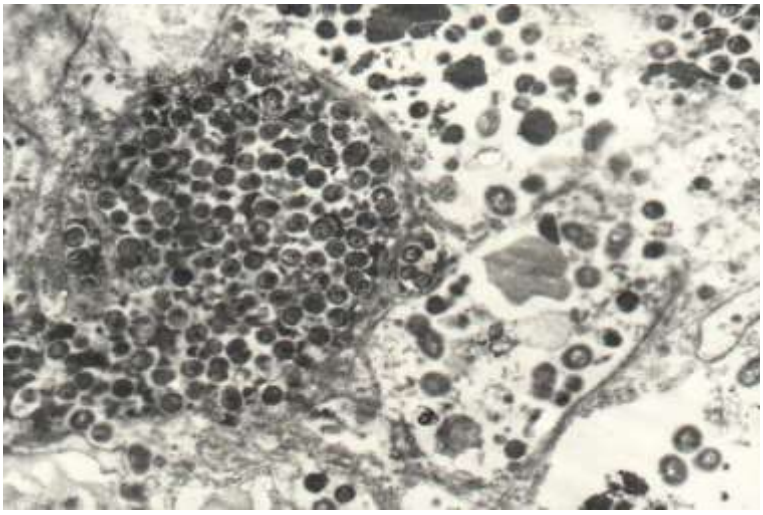
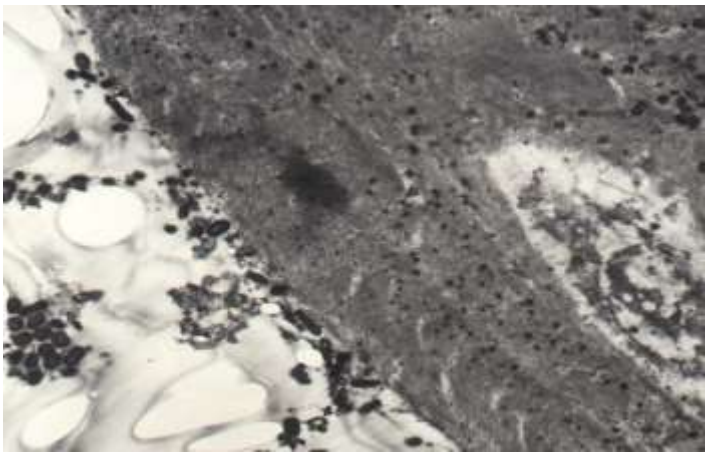


Figure 11: A colony of cocci in damaged bone tissue.



Figurer 12: The decrease in the number of microorganisms and the preserved ultrastructure of bone tissue after the rehabilitation of the pathological focus with ultrasonic cavitation.

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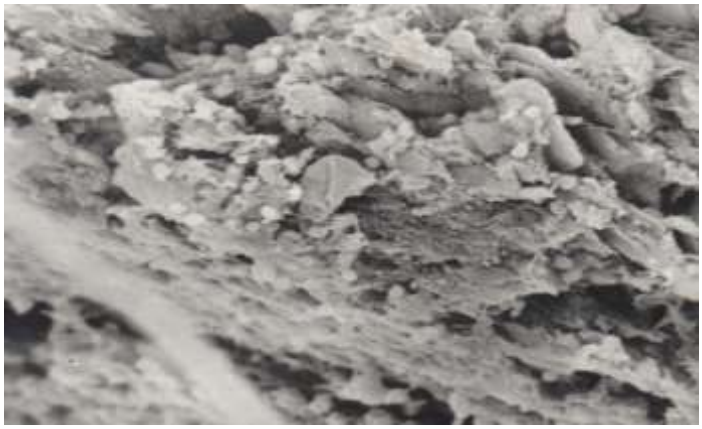

Figure 13: Violation of the rhythm of the microrelief, detritus and microorganisms on the surface of the bone.

Figure 14: Fibers and individual cells on the surface of the bone.

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