MORPHOLOGY OF THE MINERAL PHASE OF BONE

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In the study of the size and shape of crystals composing the ground substance of mineralized tissues, data of roentgenographic analysis and transmission electron microscopy of nondecalcified material are usually used. The existing data show [1, 4, 12] that mature lamellar bone is characterized by the presence of many mineral particles in the form of tiny needles and rods, arranged chiefly along the course of collagen bundles and fibers. They may reach 50 nm in length but their width does not exceed 3-4 nm. Meanwhile, investigations [5, 9, 10] have shown the presence of lamellar crystals as well as needles in the matrix of bones. They have been described as lamellar structures, sometimes hexagonal but varied in shape, measuring 32-36 nm. They are arranged in the spaces between collagen fibrils. Some workers [8] consider that all crystal structures of the mineral phase of bone are lamellar in shape, and that the discovery of needle-shaped crystals is an indication of deformation of the lammelae during preparation of ultrathin sections [13]. Recently published work [14] indicates the existence of definite interconnections between structures of the bone mineral in the composition of the matrix of mature lamellar bone. As a result it has been suggested [11] that mutually intersecting networks of collagen fibers and mineral particles may be present in formed bone. Investigations [2, 6, 7] have shown that treatment of specimens of bone with solutions of sodium hypochlorite can completely remove the organic components of bone substance and can make the surface of the mineralization front accessible for study. Under these circumstances the structure and chemical composition of the bone mineral are unchanged.

The aim of this investigation was to study the spatial organization of particles of the mineral phase of lamellar bone in preparations obtained after removal of the organic part of the bony matrix.

EXPERIMENTAL METHOD

Specimens of compact substance from the human femoral diaphysis (age range 19-44 years) were studied. Cases of diseases of the bones and joints were excluded. The organic components of the bone were removed with grade A sodium hypochlorite (State standard 11086-76) by the method in [7]. Specimens of the mineral phase, after washing in distilled water and dehydration in alcohols, were embedded in methacrylate. Unstained semithin sections were studied in polarized light on the "Nu" instrument. Shear preparations of the mineral phase were obtained and their surface sprayed with copper and studied by scanning electron microscopy (SEM) on a "Philips SEM 515" instrument. Ultrathin sections were studied by transmission electron microscopy (TEM). Freezing and shearing of specimens of the mineral phase also were carried out on a "BaF-400D" instrument (Balzers), and the shear surface was sprayed with platinum/carbon; the resulting replicas were studied in an EM-420 electron microscope (Philips). To evaluate the strength properties of the mineral phase of the bone, the specimens, after removal of organic matter, were tested on an "Instron 6022" apparatus by the method developed previously [3]. The strength of the specimens was determined by testing their compression. The index of microhardness was determined on the PMT-3 instrument. The mechanical characteristics of the mineral phase were studied under two conditions: immediately after removal of organic matter and after drying of the specimens to constant weight at 105°C. At all stages of investigation similar native specimens of bone and specimens decalcified in EDTA served as the control.

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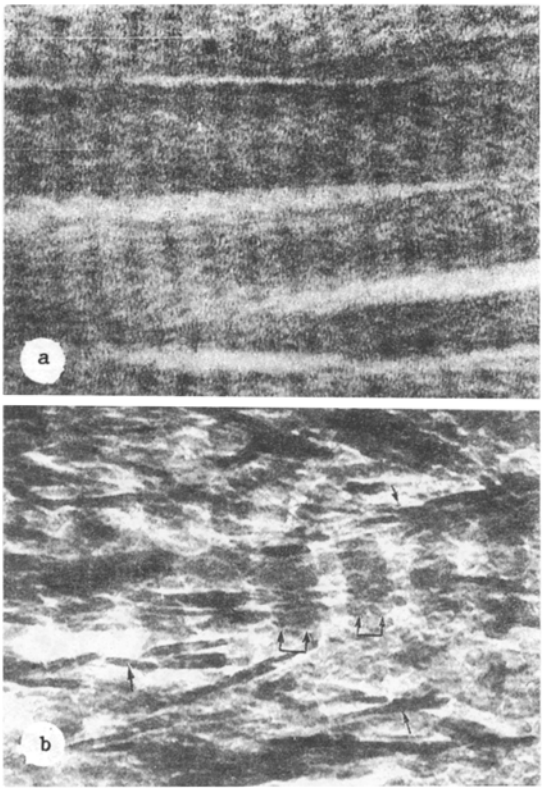

Fig. 1. Principal structural components of bone matrix according to TEM data. a) Collagen fibrils, decalcified preparation, b) crystals of bone mineral in interfibrillary spaces (one arrow) and along course of single fibrils (two arrows); undecalcified preparation. 95,000×.

EXPERIMENTAL RESULTS

The study of the control material revealed all the basic structural features of mature lamellar bone. In the decalcified sections bundles of collagen fibers in the composition of individual bony lamellae could be distinguished clearly (Fig. 1a). They had the characteristic cross-striation, and their diameter usually did not exceed 100 nm. In undecalcified preparations, numerous mineral particles were detected in the composition of the matrix of the bone, and lay between bundles of collagen structures. In ultrathin sections they had the appearance of elongated rods or needles, with a maximal length of 40-50 nm. Large rods usually were located in the intervals between bundles of fibrils. Along the course of a single fibril small mineral particles of elongated shape could be seen (Fig. 1b). The strength index, obtained during compression testing of the native bone specimens, was 158 ± 2.3 MPa, and the microhardness was 372 ± 3.4 MPa. For demineralized bone specimens, the compression strength was 11 ± 1.3 MPa and the microhardness was close to zero.

The study of bone specimens after removal of the organic components of the matrix showed that in their configuration they do not differ from the analogous native preparations. They were bright white in color. In semithin unstained sections groups of mineral lamellae of different configuration, arranged in consecutive groups, with a maximal size of 20-25 μm and minimal of 5 μm, were seen (Fig. 2a). On comparison with the corresponding control sections of bone, chains of these lamellae were found to correspond to the position of collagen bundles. By light microscopy it is impossible to assess the mutual arrangement of these mineral lamellae or the design of their contacts.

During analysis of the material by SEM, the extent of individual mineral lamellae and their configuration could be clearly determined (Fig. 2b). As a rule these are rectangular structures with uneven edges. Their greatest length could reach 36 μm, although quite often similar lamellae, not more than 10 μm in length, could be seen. In most cases the edges of the lamellae were in close contact with one another and narrow spaces or irregularly shaped openings could be seen between individual lamellae. No structures resembling rods or needles in their configuration could be found.

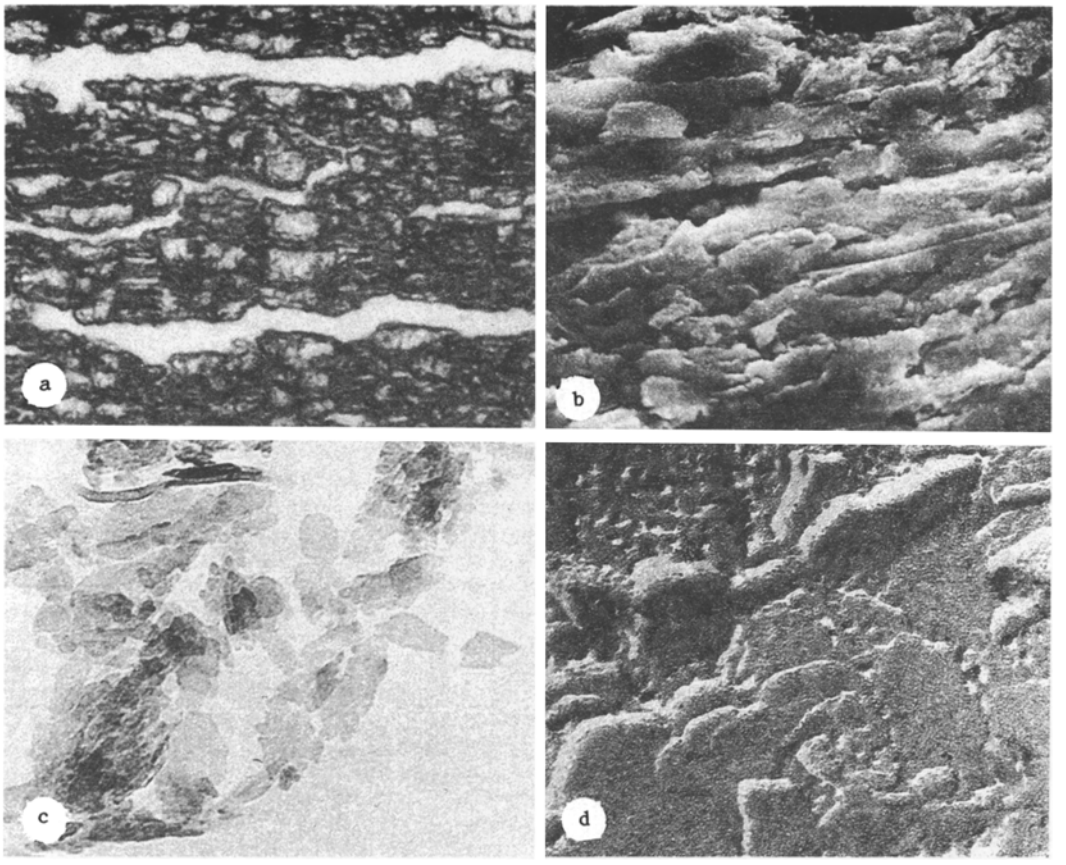

Fig. 2. Structure of mineral phase after removal of organic components of bone. a) Mineral particles located along course of collagen bundles. Unstained section, 360×; b) the same as in *a*, SEM, 900×; c) lamellar structures of bone mineral. TEM. 50,000×; d) Mineral lamellae on shear surface of bone after removal of organic matter. Replica, TEM, 180,000×.

Electron-microscopic investigation of ultrathin sections of the mineral phase of the bone revealed thin translucent mineral lamellae of complex configuration, not exceeding 100 nm in average size. They were arranged chaotically, without any definite correlation with each other (Fig. 2c). The impression was created that they were splinters from large mineral formations. In some cases groups of lamellae, lying side by side, and with their edges in contact, could be detected; outgrowths of one lamellae, moreover, corresponded to depressions of the edge of the other. Typical prismatic or needle-shaped crystals of bone mineral could not be detected.

The study of replicas taken from the shear surface of the mineral phase of bone revealed a distinct lamellar structure of the bone mineral (Fig. 2d). Lamellar structures of the mineral phase were arranged in layers, in close contact with one another. The lamellae had uneven contours, and in places where they were in contact, small spaces and pinholes could be seen. The greatest size of the lamellae was 200 nm, although structures were seen whose long axis did not exceed 50 nm. The lamellar structure of the bone mineral was clearly visible in regions in which the shear surface passed through structures of osteons, and also on mineralized surfaces bounding the bony canals and walls of the bony lacunae. No mineral particles of needle shape were found.

The results of the mechanical tests on specimens of the mineral phase of bone showed that the strength index for compression of these samples was 10.7 ± 0.55 MPa, and their microhardness was 55 ± 2.6 MPa. Preliminary drying of samples of the mineral phase at a temperature of 105°C led to a considerable increase in the values of these parameters, which exceeded the initial level by 7 and 6 times respectively.

Thus this investigation provides evidence for the view that the mineral phase of mature human bone consists mainly of mineral structures in the form of lamellae. These particles are arranged in the matrix of the bone in close contact with one another and not chaotically. As a result a group of lamellae occupying the spaces of the interfibrillary processes in the bone matrix is formed. They can in general be identified also at the light-microscopic level, but during preparation of bone sections,

only small fragments of them could be observed. Interaction between mineral lamellae most probably take place at the level of the hydration layers of crystallites of the bone mineral. It can be tentatively suggested that the forces of this interaction are large enough for the mineral phase, separated from organic matter, to preserve the configuration of the bone specimen and also sufficiently high mechanical strength.

LITERATURE CITED

1. V. N. Bogatov, Usp. Sov. Biol., **85**, No. 1, 71 (1978).
2. A. A. Doktorov and Yu. N. Denisov-Nikol'skii, Arkh. Anat., **82**, No. 5, 61 (1982).
3. I. V. Matveichuk and Yu. I. Denisov-Nikol'skii, Current Problems in Biomechanics [in Russian], No. 4, Riga (1987), pp. 175-183.
4. E. P. Podrushnyak and A. I. Novokhatskii, Ortoped. Travmatol., No. 8, 15 (1983).
5. D. Bocciarelli, Calcif. Tiss. Res., **5**, 261 (1970).
6. A. Boyde and M. Hobdell, Z. Zellforsch., **99**, No. 1, 98 (1969).
7. A. Boyde and S. Jones, Principles and Techniques of Scanning Electron Microscopy, M. Hayat (ed.), Vol. 2, New York (1974), pp. 123-139.
8. S. Chatterji, J. Wall, and J. Jeffery, Calcif. Tiss. Intern., **33**, 567 (1981).
9. E. Iohansen and H. Parks, J. Biophys. Biochem. Cytol., **7**, No. 4, 743 (1960).
10. S. Jackson, A. Cartwright, and D. Lewis, Calcif. Tiss. Res., **25**, 217 (1978).
11. Z. Ralis and I. Turner, Microscop. Acta, **84**, No. 4, 385 (1981).
12. R. Robinson, J. Bone Jt. Surg., **34A**, No. 2, 389 (1952).
13. K. Towe and G. Hamilton, J. Ultrastruct. Res., **22**, 274 (1968).
14. I. Turner, Mech. Prop. Biol. Mater. Symp., Cambridge (1980), pp. 457-458.