# The Form of the Protein-Mineral Union in Bone Matrix

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The main object of this paper is to present a new theory of the way the protein, collagen, and the mineral, calcium phosphate unite to form bone matrix.

The form of the protein-mineral union in bone has puzzled a number of investigators (1, 2, 3, 6, 12, 15). Between 1949 and 1953, five annual Macy conferences on Metabolic Interrelations (17) discussed all of the known relations in the nature of bone; however, these conferences failed to achieve a resolution of this particular matter.

A theory that is currently accepted by many osteologists was mentioned there. Briefly expressed, that theory relates the protein and mineral in bone matrix by way of a third material, a ground or cement substance, playing the role of a *medium* uniting the two framework components (17a). A second aim of this paper is to suggest that this glutinous matrix theory is untenable.

Before considering the inadequacy of this theory and the proposal of a new explanation of the protein-mineral relationship, however, let us review some relevant, known facts about bone matrix and some of its extracellular components.

A preferred orientation of calcium phosphate crystallites together with collagen polypeptide chains in bone matrix, the chains aligned with a definite bone axis, is an established fact. This fact has been *repeatedly* observed and *independently* confirmed by several different optical techniques (2, 6, 7, 15, 16). This pattern of orientation is, of course, not visible on an anatomic or even on an histologic level; but it is clearly visible on a submicroscopic level. The calcium phosphate crystallites are hexagonal; and although colloidal, these crystallites are seen with the c-axes of their unit cells oriented parallel with the direction of the main axes of the collagen chains. In a long bone, the preferred direction of the main collagen chains and of the c-axes of the unit cells of the crystallites is that of the longest axis of the bone.

One way of observing directly this disposition of the crystallites in relation to that of the chains is by only partially decalcifying a long bone specimen and then examining it with X-ray diffraction (Figure 1) (9, 11). A significantly similar *oriented* crystallization of calcium phosphate may be induced *in vitro* into the organic matrix of completely decalcified bone specimens (Figure 2) (9, 11). Recently, it also has been shown that oriented crystallization of sodium carbonate monohydrate, sodium sesquicarbonate, and other inorganic salts may be introduced *in vitro* into *nonosseous* matrix of rat tail tendon (13).

A merit of the previously defined glutinous matrix theory is that it appears to fit much of the optical data on bone matrix. This result should not be surprising, since the theory was cut out and designed over a century ago especially to fit available microscopic data (1, 5). The theory becomes less obviously tenable when it is applied to measured data on the mechanical strength of bones. The torsional, shear, longitudinal, compressive and tensile strengths of an ordinary human femur are approximately all of the order of 10,000 p. s. i. (5a, 14). No chain is stronger than its weakest link. The speculation about the ground substance in the human femur matrix functioning as a cement substance therefore requires that this substance possess an intrinsic strength of the order of 10,000 p. s. i. Assume all of the extracellular, amorphous ground substance of the tissue in the case of a femur to be nothing but such a cement or glue. Then the protein fibers and mineral crystallites imbedded in this glutinous material

would form a three-component structure. The conception of the femur matrix as such a three-component structure is both insufficient and unnecessary because (1) there is no evidence that the amorphous (5b) ground substance possesses inherent strength to withstand stresses of the order of 10,000 p. s. i. and (2) a two-component structure of protein fiber and mineral crystallite appears ample without any cement to build a femur structure capable of withstanding the measured stresses.

Regard bone matrix material then as essentially a two-component structure of protein and mineral. Two contrary conceptions of their union which might be thought of and actually have been proposed are (a) that the protein and mineral are chemically combined (3, 6, 10) and (b) that these two components are physically mixed (8). The author presents now a third hypothesis (c) implying that the union of protein and mineral in bone is neither a physical mixture nor a chemical compound in the usual sense of these terms. According to this view, while the protein and mineral together spontaneously form a single entity, their union is due to a spatial fitness like that of a bolt in a series of lock nuts.

Hypothesis (c) affirms that calcification of bone matrix occurs in such a way that calcium phosphate crystallites grow about collagenous chains which serve as the nuclei for the crystallization process. Thereby the chains become trapped in tunneled spaces that occur within portions of the oriented crystallites along the direction of the unit cell-c-axes. Specifically, the nature of the orientation of the crystallites appears to the author to be such that a linear array of unit cells of a linear sequence of crystallites is strung like a row of beads on the individual, helical, polypeptide chain. This single collagen chain is a useful abstraction even though it does not exist by itself. Actually it is connected with adjacent chains by the so-called side and backbone chains to form a loose three dimensional web of fibrous collagen. It is the existence of bridges between adjacent chains that aparently produces the further order in the arrangement of the crystallites about the fibers, both of the kind noticed by Cagliotti (6, 13) and the further kind noticed by Robinson (15, 16).

There are those who will say of this hypothesis (c) that it is a wild speculation, because there are no sub-microscopic tunnels in the bone mineral crystallites; and even if there were any tunnels, they could not possibly be of the right size to accommodate the collagen polypeptide chain. The critics may be right; but before dismissing the hypothesis, let us briefly examine the evidence for it.

First, evidence for the existence of voids in the mineral substance of bone matrix can be deduced from certain considerations of Hendricks (17b). If he is right, the conclusion that voids are present in bone mineral follows from the dropping of the average of the measured refractive indices of this mineral below that of the corresponding indices of the apatites, a family of minerals to which bone mineral belongs. Dallemagne (8) reports the mean index of refraction of bone mineral to be 1.590 and assigns the value of 1.645 for a typical mineral of the apatite group. Hendricks specifies a value of 1.640-1.650 for hydroxyapatite and 1.630 or 1.620 for carbonate apatite. The conclusion that voids occur in the bone mineral does not establish whether the voids occur *within* the crystallites or *between* them in some way. It does indicate, however, the rashness of those who deny the possibility of voids or tunnels in the mineral substance of bone.

Next, it is reasonable to infer from available X-ray data something more about the voids in this mineral, whether they are *inside* the crystallites or *between* them. The voids are probably submicroscopic in size and occupied by submicroscopic units of collagen. For the mutual orientation of protein and mineral, which X-ray shows, relates to submicroscopic periods in the structures of these materials.

Let us suppose that submicroscopic voids do in fact occur *inside* the crystallites. Now when on the basis of this supposition we seek some way in which the protein could fill submicroscopic voids in the crystallites, an interesting possibility comes to notice.

Figure 3 shows schematically several faces (dotted lines) of some unit cells of a bone mineral crystallite in relation to an array of hexagons (solid lines), the corners of which represent the locations of calcium atoms in the crystal structure. These faces of the unit cells have sides equal to the axial length, a=9.4A, and lie transverse to the axial length, c=6.88A. The observed orientation of the mineral crystallites in bone means that the c-axes of most unit cells not only of one crystallite but also of neighboring crystallites are parallel to each other.

The contents of the calcium phosphate unit cell in general includes more than calcium at the corners of the hexagons in Figure 3. In the case of the complete apatite structure (4b), the unit cell is postulated to have room enough for other calciums, some phosphorus, many oxygens, and other atoms, which together fill in the available cell volume rather completely; but every unit cell of a bone mineral crystallite cannot and must not be supposed necessarily to possess this complete apatite structure. A bone mineral crystallite resembles crystallographically an apatite crystal without being identical with it in every or any other respect. One essential difference is in the environments in which their crystallization begins. Originally, polypeptide chains of collagen probably provide the nuclei around which the bone mineral crystallites begin their orderly growth by dislocations. The author maintains that two effects naturally emerge from the growth of the crystallites about their polypeptide chain nuclei. Both effects are confined to the submicroscopic regions where the nuclei and crystallites meet.

1. Phosphate groups and calciums not at the corners of hexagons are missing from some of the unit cells of some of the crystallites wherever collagen chains are close by.

2. The remaining calciums in these exceptional unit cells of bone crystallites form continuous hexagonal channels, in each of which is just enough room for one but only one polypeptide chain of collagen.

It is the size of such a channel cross section which indicates that at least one chain but no more than one can be accommodated within each channel. Referring again to Figure 3, the greatest distance between any two corners of the hexagon is 10.8A. This distance represents a length between the centers of two calciums. Now since a calcium ion has a diameter of about 1A, the maximum effective dimension of the void would be about 9.8A, which is the same order as the length of the side chains of collagen=10.4A (4a, c; 5d). The dimension perpendicular to this dimension in the plane of the cross section is 9.4A, which is twice the backbone thickness of the primary chain=4.65A (4a, 5d).

Thus, the unit cells of both crystallites containing channels and of adjacent crystallites having their full complement of oxygen, phosphorus, calcium, and other atoms provide continuity for the channels in which the collagen chains are contained; thus in the light of the evidence as the author sees it, the crystalline protein and crystalline mineral fit together in a bone matrix like a hand in a glove. Amorphous material, both inorganic and organic (including protein and polysaccharide), also belongs in the matrix; but these amorphous components exist relatively independently of each other and of the framework matrix materials.

A compound of fibrous protein and crystalline mineral in bone matrix by a spatial juncture and by a snug filling of pores in the mineral by the protein would not be a unique compound. Within the last twenty years, a new, large class of such compounds has been recognized to exist (18).

Material combinations of this class must be distinguished from the more familiar stoichiometric chemical compounds and the Werner-Pfeiffer coordinate complexes of chemistry, because spatial relations instead of valence bonds govern combinations for this class of compounds.

Cagliotti's conception (6) closely resembles the author's conception morphologically in spite of his assumption that the protein and mineral form a chemical compound. He correctly ascribes bone texture to the formation of a semicombined lattice by collagen chains and calcium phosphate crystallites. Bone matrix material simulates a chemical compound in the sense that the physical properties of this material are quite distinguishable from the properties of either of its main two framework constituents separated from each other. Nevertheless, it is evident that this matrix material does not qualify as a chemical compound; since when forming the matrix, the collagen and calcium phosphate constituents do *not* lose their chemical identities and are *not* stoichiometrically combined.

After the above paper was prepared, the author learned about two recent papers by Cagliotti *et al.* (19,20) which appear to provide further evidence in favor of hypothesis (c). According to their interpretation of the low-angle scatter of x-rays from bone tissue, this scattering pattern is evidence for the existence of holes in the mineral substance of bone matrix. The holes represent the space occupied by the micelles of the organic substance of the bone matrix. These investigators also offer additional, collateral evidence for their conception that the low-angle X-ray scattering pattern of bone tissue is produced by voids in the mineral material instead of by the needle shape of the mineral particles, as proposed by others (21,22).

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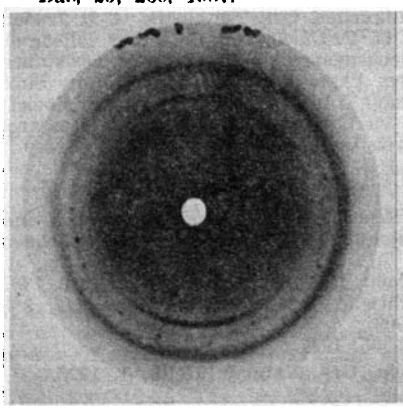

Figure 1

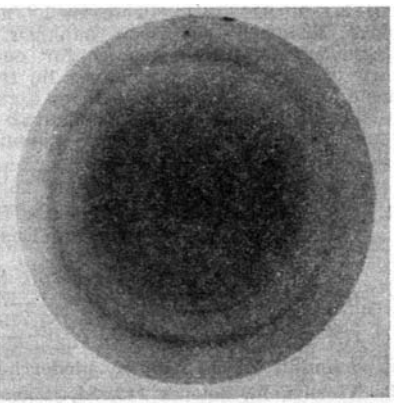

Figure 2

FIGURE 1 X-ray diffraction microcamera pattern of a partially decalcified chicken femur section. Superimposed on the typical collagen pattern is some of the diffraction pattern from the calcium phosphate crystallites, whose preferred orientation is partcularly inferred from arcing of the 002 diffractions. Long axis of bone parallel to vertical direction of Fig. 1 CuK$\alpha$. S-F distance 21.5 mm on original.

FIGURE 2 X-ray diffraction microcamera pattern of in vitro calcification of a fully decalcified chicken femur section. Saturated $CaCl_2$ solution was placed in lumen of bone completely decalcified in 5% $H_2NO_3$, the lumen sealed and the femur then placed for 30 days in saturated $Na_3PO_4$. Preferred orientation of freshly grown calcium phosphate crystallites in matrix is indicated by arcing of the 002 diffractions. Conditions same as in Fig. 1.

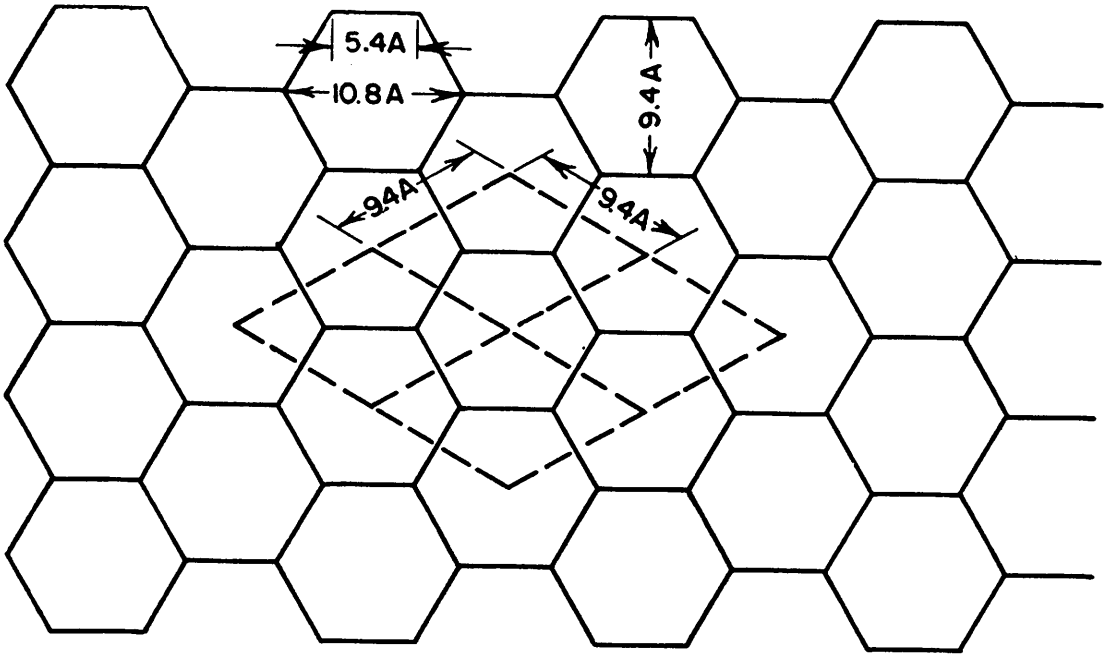


Fig. 3 DIAGRAM OF CROSS SECTION OF HEXAGON CHANNELS AND UNIT CELL FACES OF AN IDEAL BONE MINERAL CRYSTALLITE, REPRESENTING PERTINENT DIMENSIONS OF THE CROSS SECTION