## Evidence for Epitaxy in the Formation of Collagen and Apatite

A NUMBER of structural relationships between the organic and inorganic components of bone are known from X-ray diffraction and electron microscopy studies. The interrelationships have led to a theory of mineralization involving the process of epitaxy, that is, the orientated overgrowth of bone mineral on certain well crystallized areas of the organic matrix. This differs in principle from theories of mineralization which assign no active role to the organic matrix in the mineralization process. Epitaxy clearly requires some structural symmetry, but considerable mismatch seems to be permissible in the process of crystal seeding. Results reported here indicate a structural symmetry in collagen and apatite in the presence of copper ion and support the concept of epitaxial growth; areas of identical crystal order on apatite and the organic substrate have been demonstrated as would be expected if epitaxy were a relevant mechanism.

In the technique used, the essentially diamagnetic substances to be studied are labelled with an inorganic paramagnetic ion. The ion is detected by electron paramagnetic resonance (EPR), and from an analysis of the shape of the signal the crystal field symmetry, if any, at the ion site is inferred. The cupric ion, Cu(II), was chosen as the labelling ion because (*a*) it has a known affinity for the substrates studied[1], (*b*) its hyperfine lines are sensitive to the local crystal fields[2], and (*c*) there have been previous studies involving Cu(II) bound to a variety of substrates

which provide a basis for comparison with the present results[3-7].

Clinically normal human tendon was obtained during surgery and stored in an air-dried state until studied. Bovine tendon and animal skin gelatine were purchased. Human bone mineral was extracted by refluxing whole cortical bone pieces approximately $2 \times 3 \times 5$ mm in ethylenediamine for 72 h, then washing for 24 h in distilled deionized water at room temperature. Bone collagen was prepared by treating pieces of cortical bone with either 1·0 M formic or 1·0 M HCl for 72 h.

Bone mineral was exposed intact (that is, as the same crystalline aggregate as the original sample of refluxed bone) to solutions containing known concentrations of Cu(II). The ratio of the weight of solid to volume of solution was about 500 mg/l., and the standard exposure time for all materials was 24 h. The samples were rinsed, air-dried and reduced to powder. Human and bovine tendons and bone collagen were minced with scissors to a length of about 1 mm. After treatment with Cu(II) at a solid to solution ratio of 200 mg/l., they were rinsed and dried in the same way as the mineral. Cu-gelatine films were prepared by evaporating to dryness 5 per cent gelatine solutions made 0·068–0·83 mM with respect to Cu(II).

The native materials not treated with Cu(II) solutions exhibited either no resonances[1] or weak lines at $g = 2{\cdot}0$ which did not interfere with the present analysis[8-10].

After immersion in solutions containing Cu(II), bone mineral exhibited a well defined Cu(II) EPR spectrum (Fig. 1*a*). For solutions in the range 0·034 mM to 0·350 mM with respect to Cu(II) the spectrum is sharp and distinct and the overall line shape remains constant. As the molarity is raised above 0·35 mM the low field lines are less resolved and the main absorption peak becomes featureless. These results do not depend on the anion. Equimolar solutions of the sulphate, nitrate and chloride of Cu(II) yield identical spectra. Cupric ions absorbed on collagenous structures from solutions in the range 0·008 to 0·080 mM yield an EPR spectrum similar to that of copper labelled bone mineral (Fig. 1*b*). The set of four constants of the spin Hamiltonian which characterize each resonance are, within experimental error, identical for the two materials; thus the results indicate that there are sites of the same symmetry on each material. To give some idea of the spread in the spin Hamiltonian constants describing absorbed Cu(II), some representative values from the literature are given in Table 1. The virtual identity of the constants for bone mineral and collagen as compared with the spread in values shown in Table 1 is what is meant by "sites of the same symmetry" on collagen and bone mineral.

We find no difference between the various collagen structures and gelatine with respect to the short range order about artificially added copper sites (Table 1). This seems to indicate that the secondary protein structure is not an important factor in determining the site crystal field symmetry.

Table 1. REPRESENTATIVE VALUES OF THE CONSTANTS IN THE HAMILTONIAN WHICH DESCRIBE THE SPECTRUM OF THE ABSORBED CUPRIC ION

| | $g_{\parallel}$ | $g_{\perp}$ | $A^*$ | $B^*$ | Ref. |
|---|---|---|---|---|---|
| Laccase | 2·048 | 2·197 | 90 | — | 6 |
| Ceruloplasmin | 2·056 | 2·209 | 80 | — | 6 |
| Glass | 2·06 | 2·32 | 157 | 23 | 3 |
| Collagen, apatite, gelatine | 2·07 | 2·27 | 170 | < 20 | — |
| DNA, RNA | 2·08 | 2·35 | 153 | < 30 | 7 |
| 'Dowex-50' | 2·099 | 2·40 | 128 | — | 5 |

* ($\times 10^{-4}$ cm$^{-1}$).

This work was supported in part by a grant from the National Institutes of Health, US Public Health Service, and by the Veterans Administration Research Service.

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Received September 15, 1969; revised January 22, 1970.

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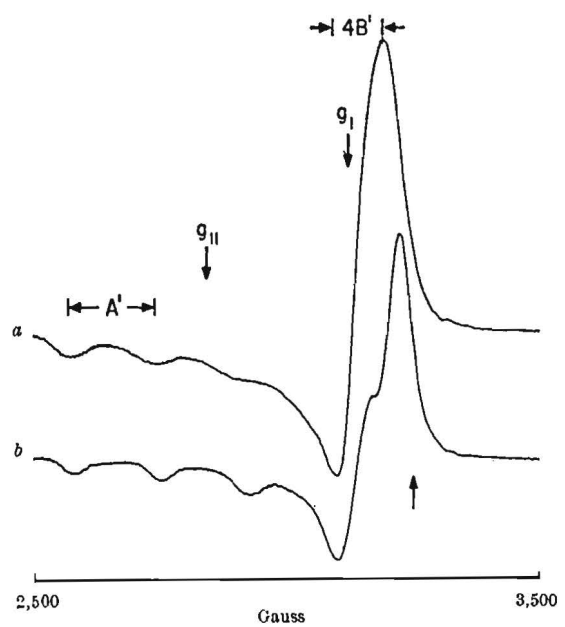


Fig. 1. EPR spectrum from (*a*) bone mineral, (*b*) tendon collagen after treatment with cupric ion. The arrow denotes the position of $g = 2{\cdot}00$.