Apatite crystallites in bone

The characteristics of the wide-angle X-ray reflections from the inorganic component of compact adult bone suggest that this component is present in the form of very small crystallites which are markedly elongated in the direction of the crystallographic *c*-axis. Calculations from line-width measurements give the order of size of the crystallites as up to a few hundred Angstroms and a more specific estimate of the length of the long axis from considerations of *c*-axis reflections alone gives a value of about 200 A[1]. Experiments with synthetic apatites show that crystallites of these dimensions produce an intense low-angle X-ray scatter.

Studies of the continuous low-angle scatter from intact bone have indicated the presence in the system of strongly scattering units measuring approximately 200 A × 40 to 70 A[2,3]. Such units persist, and scatter even more strongly, after the removal of the organic components from the bone. When the specimen is heated to high temperatures, the size of these scattering units appears to increase, and it is also observed that the definition of the wide-angle reflections is markedly improved, thus indicating a probable increase in the size of the apatite crystallites. In embryonic bone[4] it has been observed that the initial apatite wide-angle diffraction pattern does not show any preferred orientation (*i.e.* crystallites not well-oriented), but the collagen diffraction pattern is highly oriented. The diffuse low-angle scatter from these specimens shows no orientation.

Thus on the grounds of X-ray diffraction data alone one can conclude that the wide-angle reflections and at least a part of the low-angle scatter probably arise from apatite crystallites. As the low-angle scatter can be interpreted in terms of a single set of particles of uniform shape and size and similar orientation, we suggest that the scatter arises mainly from the apatite crystallites, and that scatter from other particles must either be relatively weak or be of a form similar to that from the apatite. The study of the low-angle scatter from collagen[5] suggests that this too contains scattering units of a similar form and probably relatively weak. We consider this work to support our earlier contention that there is some close relationship between the organisation of the apatite crystallites and the structure of collagen[6].

It is to be hoped that the complete picture will eventually be revealed by the electron microscope, but the results so far obtained have been inconclusive.

The recent comments by CAGLIOTI *et al.*[7] on our conclusions regarding the low-angle scatter from bone emphasise the difficulties encountered in correlating the information obtained from various types of study but contribute little towards resolving these difficulties. The idea that the low-angle scatter comes mainly from holes filled with ossein ignores the independent evidence for the presence of strongly scattering apatite crystallites.

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Pyridine nucleotide-dependent reduction of the α-keto acid analogue of lysine to L-pipecolic acid*

Conversion of lysine to pipecolic acid has been demonstrated by tracer studies in plants[1,2,3] and in the intact rat[4]. That the α-keto acid analogue of lysine may be an intermediate in this conversion is consistent with (a) the observation that the α-amino group rather than the ε-amino group of

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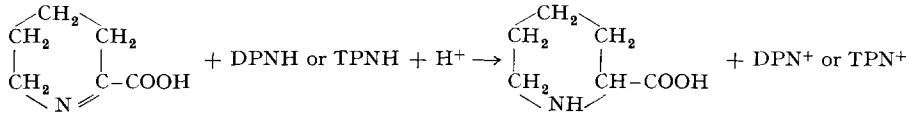
Abbreviations: DPN, diphosphopyridine nucleotide; TPN, triphosphopyridine nucleotide.

lysine is lost during conversion of lysine to pipecolic acid in the rat[4], and (b) the finding that the α-keto acid analogue of lysine, recently prepared in this laboratory[5], exists in solution in equilibrium between open-chain (α-keto-ε-aminocaproic acid) and cyclic (Δ^1-piperidine-2-carboxylic acid) forms. It has also been found that lysine is oxidized to its α-keto acid analogue by L-amino acid oxidase[5], and there is isotopic evidence for the conversion of the product of such oxidation to lysine and to pipecolic acid *in vivo* in *Neurospora*[6].

We now report the optically-specific pyridine nucleotide-dependent reduction of the α-keto acid analogue of lysine to L-pipecolic acid catalyzed by an enzyme present in rat and rabbit liver. This enzyme system, which has been purified approximately 25-fold from rat liver by means of differential centrifugation, ammonium sulfate fractionation, and treatment with calcium phosphate gel, catalyzes the following reaction:



The reaction has been followed by quantitative determination of the pipecolic acid formed[7] and by observation of the decrease in the characteristic absorption band of reduced coenzyme at 340 mμ (Fig. 1). The reaction goes to greater than 90% of completion, and proceeds most rapidly (in 0.1 *M* potassium phosphate buffers, pH 6.0–8.5, 26°), at pH 6.0. The enzymically-formed pipecolic acid was isolated as the hydrochloride at the end of the reaction, and was identified by co-chromatography with a synthetic sample of pipecolic acid. The enzymically-formed product was not oxidized by purified kidney D-amino acid oxidase under conditions whereby DL-pipecolic acid was oxidized, indicating that the product is L-pipecolic acid. TPNH prepared by reduction of TPN with *iso*citric dehydrogenase is also active in the pipecolic acid-forming system. The enzyme preparation (with DPNH or TPNH) also catalyzes reduction of the next lower homologue of Δ^1-piperidine-2-carboxylic acid (*i.e.*, Δ^1-pyrroline-2-carboxylic acid) and of Δ^1-pyrroline-5-carboxylic acid to proline. Reduction of Δ^1-pyrroline-5-carboxylic acid to proline in the presence of TPNH or DPNH by *Neurospora* extracts[8] and in the presence of DPNH by liver preparations[9] has been reported. The question as to whether the same enzyme catalyzes reduction of the piperidine and to the two isomeric pyrroline compounds must await further purification and study of the enzyme.

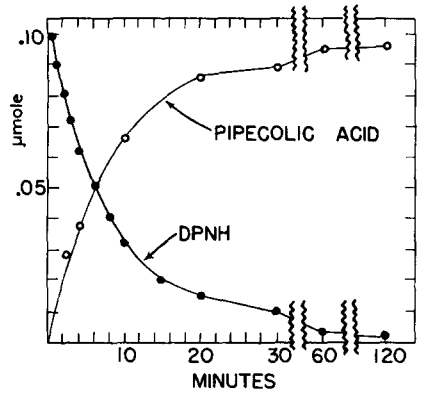


Fig. 1. Reduction of Δ^1-piperidine-2-carboxylic acid to L-pipecolic acid. Reaction mixtures consisted initially of Δ^1-piperidine-2-carboxylic acid (0.1 μmole), DPNH (0.1 μmole), rat liver enzyme preparation (200 γ) in a final volume of 0.05 ml of 0.08 *M* potassium phosphate buffer (pH 6.0); 26°. The reaction mixtures were analysed for pipecolic acid[1], or, after dilution with 1 ml of water, for DPNH (absorption at 340 mμ).

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