TABLE I

INDUCTION OF CRYSTAL FORMATION BY COLLAGEN

Determination of the point of precipitation was accomplished by techniques published earlier (Fig. 1, ref. 5). Conditions were $\mu = 0.16$; $t = 37°$; pH 7.4; time of equilibration, 3 days.

	No. expts.	Point of precipitation[a] Mean	Standard deviation	Standard deviation of mean
Control solutions	7	50	4.1	1.6
Collagen-seeded	11	20	4.7	1.4

[a] Expressed as Ca × P_i in (mg. %)².

Presumably, this phenomenon results from localized acid production by the living bone cells themselves.[8,9]

In the absence of preformed solid phase, it is the K_{sp} of $CaHPO_4 \cdot 2H_2O$ which seems to govern the stability of the aqueous calcium:phosphate system.[5] Spontaneous precipitation never has been described in solutions having products, Ca × P_i, less than about 35 (mg. %)[5] although varying degrees of metastability have been observed (depending upon conditions) with solutions having products as high as 50. These general findings are summarized in Fig. 1.

Until now the idea that collagen can function as a crystal nucleator *in vivo* has suffered from a serious quantitative defect. Crystal induction by collagen has been demonstrated in several laboratories.[5,10,11] The process has been shown to be exquisitely specific[4,10] and morphologically the early phases of crystal induction are very similar both *in vivo* and *in vitro*.[10,12] Nonetheless, until the present, these examples of crystal induction have all involved solutions in the region of metastability ($>K_{sp}$ $CaHPO_4 \cdot 2H_2O$) involving products of Ca × P_i from 35 to 50 (mg.%)².[3,5,11,13] Yet to be functional in the living animal, collagen must be shown to induce crystal formation at physiological products (*ca.* 20), a region of undersaturation with respect to solid formation.

Quite recently,[14] crystal induction has been demonstrated with demineralized, fresh dentin at products, Ca × P_i, of 20, well below the critical K_{sp} $CaHPO_4 \cdot 2H_2O$. This represented the first occasion, to our knowledge, of crystal nucleation by a non-living system under physiologically meaningful conditions. The tissue residue employed, however, undoubtedly contained substances other than collagen. Hence, the case for collagen as the nucleator, though greatly strengthened in a quantitative sense, was still not proven. In experiments just completed, a collagen[15] prepared by the method of Einbinder and Schubert[16] also has proved able to cause crystal formation in solutions of physiological concentrations of calcium and phosphate. These data are summarized in Table I.

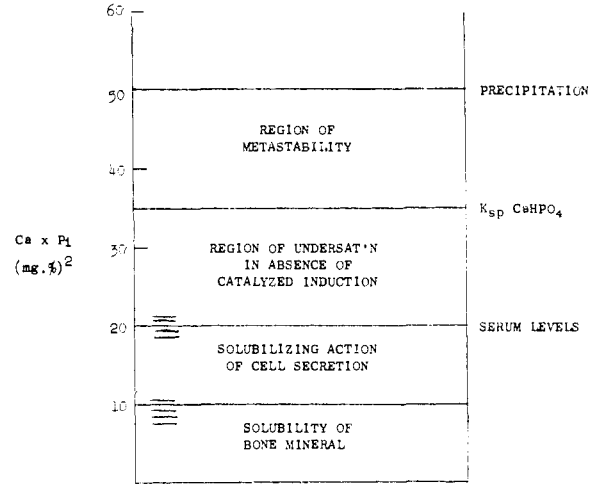

Fig. 1.—A summary of present information concerning dissolution and formation of bone mineral given in terms of the serum product, Ca × P_i, in (mg. %)².

The case for collagen now seems complete. It *can* specifically cause crystal formation under physiological conditions. *Whether* it does this *in vivo* and *how* it accomplishes this remain questions yet to be answered by further research.

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PROTON RESONANCE SPECTRUM AND STRUCTURE OF THE AZULINIUM ION

Sir:

The structure of the azulinium ion is of considerable interest in connection with the electron charge distribution in azulene itself. Protonation of azulene in acid solutions may be expected to occur at the 1 and 3 positions, which, according to molecular orbital calculations,[1] have the greatest excess π-electron charge density. It is also known that the protons at the 1 and 3 positions of azulene are readily exchanged in D_2SO_4 solutions.[2] We have now been able to confirm the structure of the azulinium ion on the basis of a high-resolution proton resonance spectrum. A previous attempt[3] to determine the structure from the proton resonance in concentrated sulfuric acid was unsuccessful due to inadequate resolution.

Figure 1a shows the proton resonance spectrum of a 7 mole % solution of pure azulene in CCl_4. A scale in c./s., referred to the resonance signal of CH_2Cl_2 (dotted line), is shown at the bottom of the figure. The assignment and analysis of the azulene proton spectrum has been described previously.[4] Shown on the same scale in Fig. 1b is the proton resonance spectrum of a 7 mole %

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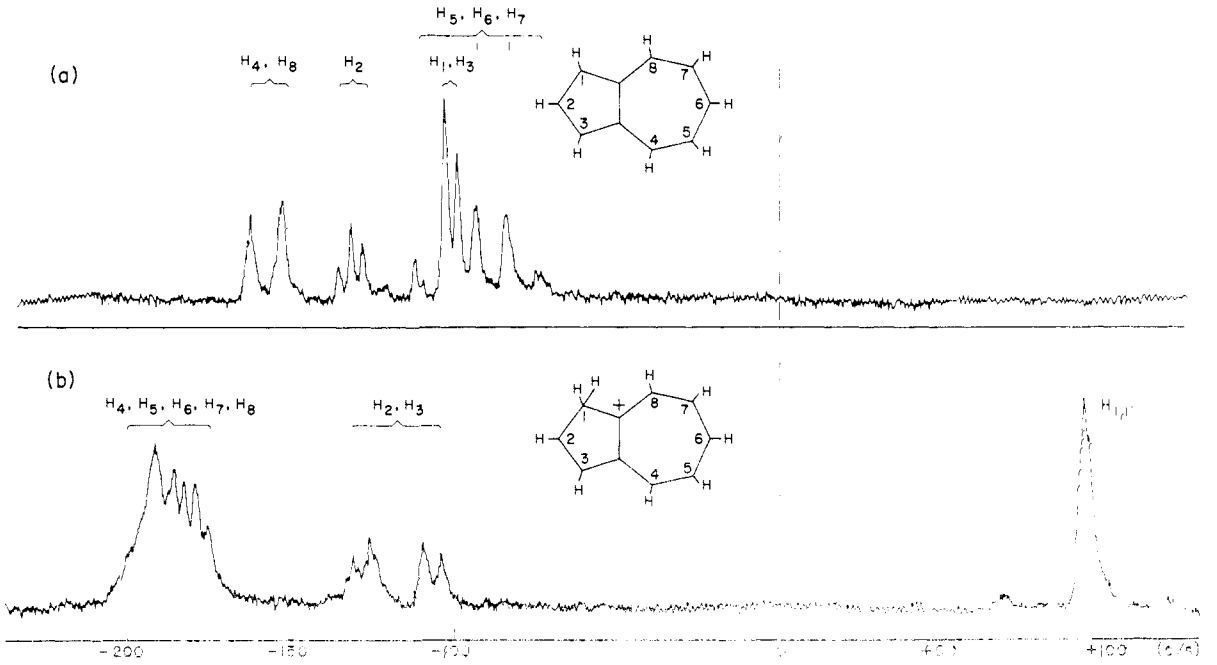

Fig. 1.—Proton magnetic resonance spectrum at 60 Mc./s. of (a) 7 mole % solution of azulene in CCl_4, and (b) 7 mole % solution of azulene in CF_3COOH. External reference signal (dotted line) is CH_2Cl_2.

solution of azulene in trifluoroacetic acid. It is evident that under these conditions the azulene is converted completely to the protonated ion. The signal at high field, +93 c./s., is characteristic of methylene protons which are assigned to the 1 (or 3) position. The quadruplet centered at −117 c./s., which is equal in intensity to that of the methylene proton signal, is a typical AB spectrum for two non-equivalent protons and arises from protons 2 and 3 on the 5-membered ring. A partially resolved spin-coupling ($J\sim1$ c./s.) between these protons and the two methylene protons is evident. The chemical shifts of protons 2 and 3 are only slightly altered from their values in pure azulene. The corresponding changes in the signals of the protons in the 7-membered ring are more pronounced; in the ion these protons tend to be more nearly equivalent and show a marked decrease in screening. These changes are consistent with the conclusion that the electron deficiency in the ion resides almost exclusively on the 7-membered ring, where it tends to be shared with all the carbon atoms in the ring. In the formula in Fig. 1b, as suggested by Heilbronner and Simonetta,[5] a positive charge is indicated as localized on the "branch" carbon atom, but may in fact be more delocalized in the 7-membered ring and tend to approach the configuration of the tropylium ion.

From the width of the proton resonance lines it can be concluded that the mean "life-time" of the protonated azulene ion is at least of the order of one second and probably greater.

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(5) E. Heilbronner and M. Simonetta, *Helv. Chim. Acta*, **35**, 1049 (1952).

COMPETITIVE INHIBITION OF ENZYMATIC REACTIONS BY OXAMYCIN

Sir:

Inhibition of *S. aureus* by oxamycin ((D)cycloserine, (D)4-amino-3-isoxazolidone), which results in accumulation of the uridine nucleotide, UDP-GNAc-lactyl-(L)ala-(D)glu-(L)lys,[1] is competitively reversed by (D)alanine.[2] The antibiotic is a structural analog of this natural substrate. (D)-alanine is formed and incorporated into a uridine nucleotide through the reactions[3]

$$\text{Eq. 1} \quad \text{(L)ala} \xrightleftharpoons[\text{pyridoxal phosphate}]{} \text{(D)ala}$$

$$\text{Eq. 2} \quad \text{(D)ala} \xrightarrow[Mn^{++}]{\text{ATP}} \text{(D)ala-(D)ala}$$

$$\text{Eq. 3} \quad \text{UDP-GNAc-lactyl-(L)ala-(D)glu-(L)lys} + \text{(D)ala-(D)ala} \xrightarrow[Mn^{++}]{\text{ATP}} \text{UDP-GNAc-lactyl-(L)ala-(D)glu-(L)lys-(D)ala-(D)ala}$$

The purpose of this communication is to report that oxamycin is a competitive inhibitor of the enzymes catalyzing reactions 1 and 2.

Reaction 1 is catalyzed by alanine racemase.[4] Oxamycin is a competitive inhibitor of the reaction in both directions (Fig. 1). The Michaelis constants (K_m) for (D)alanine ($6.1 \times 10^{-3}M$) and for (L)alanine ($6.5 \times 10^{-3}M$) are nearly identical. Similarly, K_i for oxamycin measured in the direction of (D)alanine formation ($0.6 \times 10^{-4}M$) is

(1) Abbreviations: UDP, uridine diphosphate; GNAc-lactyl, an ether of acetylglucosamine and lactic acid (acetylmuramic acid).

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