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Calcification by *Escherichia coli*

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Escherichia coli K-12, grown in a synthetic medium containing metastable calcium phosphate, formed intracellular biological apatite crystals.

The purpose of this study was to examine *Escherichia coli* K-12 for calcification. Most work on microbiological calcification, recently reviewed by Ennever and Takazoe (1), has been with *Bacterionema matruchotii*. The organism forms intracellular calcium phosphate deposits indistinguishable from biological apatite, the major mineral of vertebrate calcifications. If a standard-strain *E. coli* were to calcify, it should prove more useful than *B. matruchotii* for an in depth study of the phenomenon since the latter organism is relatively poorly characterized.

E. coli K-12 (University of Texas, Houston, #6299) was grown at 37 C in 250-ml volumes of a chemically defined liquid medium known to provide for calcification by *B. matruchotii* (2). The medium is a metastable calcium phosphate solution ($Ca \times P_i = 48$ $mg^2\%$) combined with the essentials for *B. matruchotii* growth (3) in 0.15 M *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.35). During incubations the pH of the medium ranged between 7.22 and 7.38, thus providing for continued metastability of the calcium phosphate. At 18 h, 24 h, and thereafter at 24-h intervals, a culture was harvested by centrifugation at 34,000 $\times$ *g* for 5 min at 5 C and water washed. Most of each yield was air dried at 45 C and low-temperature ashed (no. 40 Radio-Frequency Asher, Coleman Instruments, Maywood, Ill.) for 2 to 3 h. The residue was analyzed by X-ray diffraction. The remainder of each yield was processed for and examined by electron microscopy. The X-ray diffraction and electron microscopy instrumentation and techniques were the same as those used previously (2) with *B. matruchotii*.

The earliest calcification detected was in 3-day cultures. Ashed residues gave X-ray diffraction patterns for apatite (Fig. 1A). A pattern obtained with powdered human dentin (Fig.

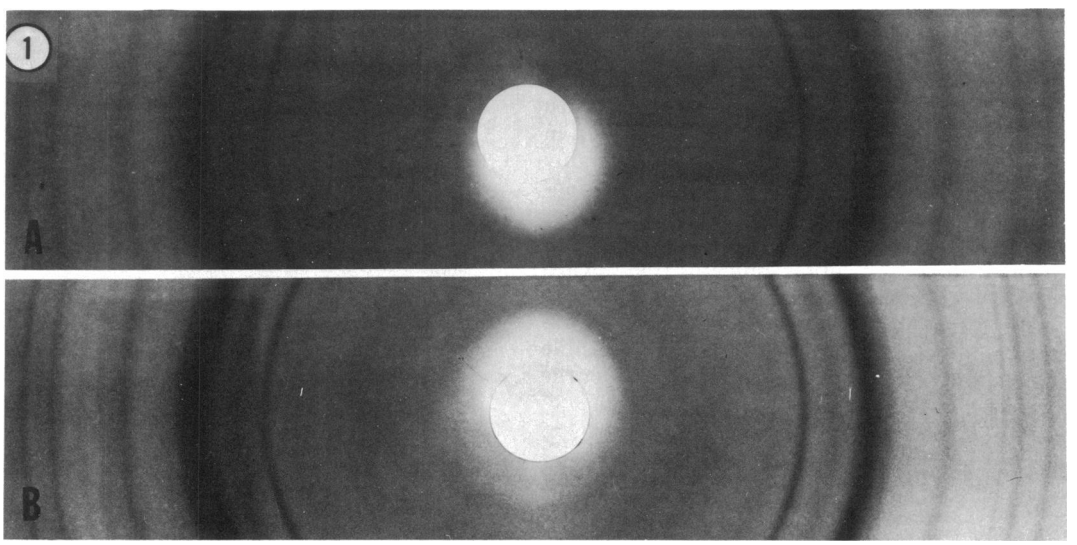

FIG. 1. *X-ray diffraction patterns. (A) Ashed residue of calcified E. coli K-12; (B) powdered human dentin.*

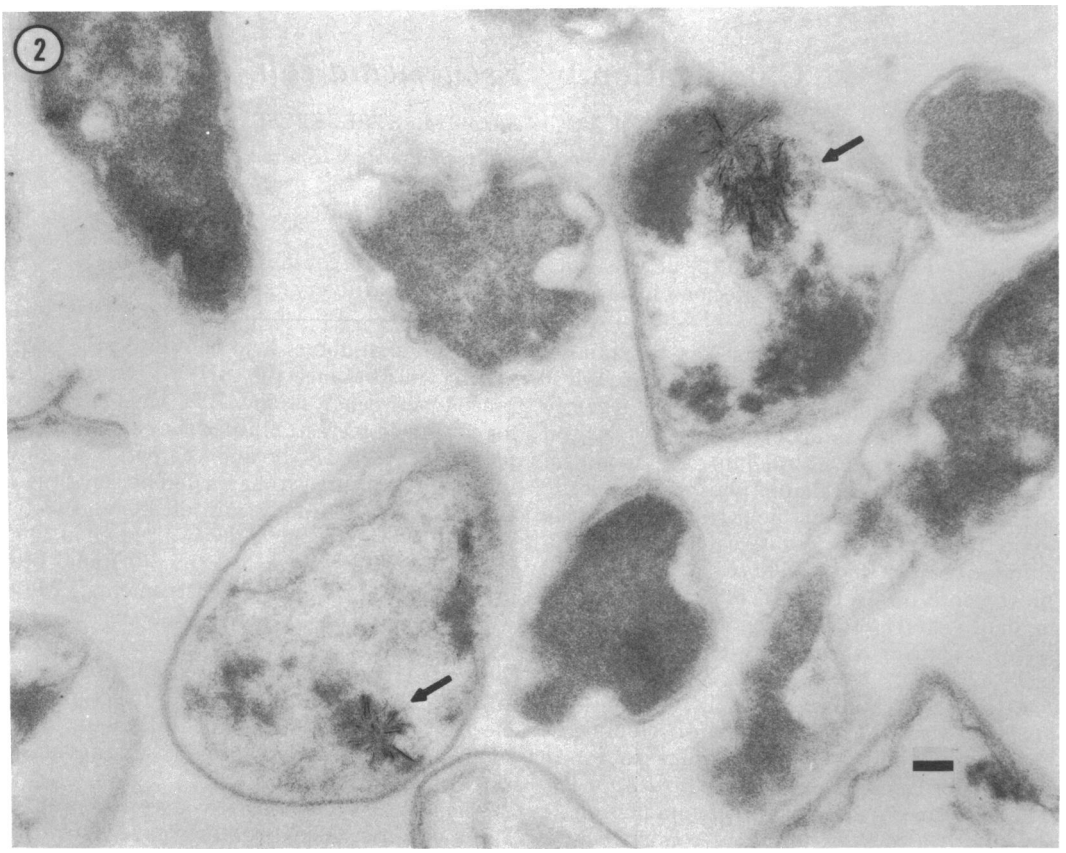

FIG. 2. *Biological apatite crystals (arrows) in E. coli K-12, unstained. Bar represents 0.1 μm.*

1B) is included in the illustration as an example of biological apatite. Based on maxima broadening, the crystals formed by the organism were somewhat smaller than those of dentin. The calcification was intracellular (Fig. 2) with typical needle-like apatite crystals arranged in clusters. Cells containing crystals invariably showed internal degeneration; therefore, orientation between calcification and subcellular structure was not possible. The 3-day period required for calcification indicates that calcification occurred subsequent to cell death.

A protein-containing component of the extractable phospholipid fraction is responsible for *B. matruchotii* calcification (4). Whether the same is true for *E. coli* remains to be established.

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