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Little and Radford's demonstration that their data and conclusions (*1*), showing the existence of areas of relatively high levels of $^{210}$Po in human lung tissue, do not disagree (despite appearances to the contrary) with those of Hill (*2*) and Rajewsky and Stahlhofen (*3*) is significant. One should note, however, that the discrepancies are still not fully resolved either in the light of existing data or regarding some theoretical considerations.

First, the autoradiographic measurements by Hill (*2*) on specimens of vacuum-dried epithelium, from bronchial bifurcations taken from smokers, showed an upper limit of $\alpha$-activity of 0.01 pc/cm$^2$, equivalent to a concentration of less than 2 pc/g. This upper limit is thus quite a little lower than the average value of 4.5 pc per gram of wet tissue determined from similar specimens by Little *et al.* (*1*). Second, this latter specific activity of epithelium is higher than that of dried smoke, 2 pc/g, as shown by Kelley (*4*) and Hill (*2*) and confirmed by us; it could be explained by preferential retention of the nuclide in the tissue over other components of the smoke, but this point is not established.

Finally, the origin of the high levels of $^{210}$Po (up to 7 pc/g) (*1*), especially in nonsmokers, is of interest since the location at the bifurcations indicates that it is acquired by precipitation of that nuclide itself, or of its parent $^{210}$Pb, from inhaled air. Thus these tissues, if they weigh 0.1 g and the concentration is 4 pc/g, might contain a total of 0.4 pc, a quantity representing the total weekly intake of $^{210}$Po or 1 day's intake of $^{210}$Pb. Considering that only a small fraction of the activity, say 5 percent, precipitates in these areas, the rate of build-up of $^{210}$Po would approach the rate of its physical half-life, 138 days. Clearance processes operating on the deposited materials would make this build-up still more unlikely. On the other hand, build-up of $^{210}$Pb presents these difficulties to a lesser extent, not only because of the higher concentrations in the atmosphere (0.02 pc of $^{210}$Pb per cubic meter compared to about 0.002 pc of $^{210}$Po), but also because of the much longer half-life of the parent, 21 years. Lead, however, may be more mobile in soft tissues.

It appears from these speculations that more evidence is necessary because of the high concentrations of $^{210}$Po with the consequently high radiation level. Detailed and careful analyses, similar to those of Little *et al.* (*1*), and autoradiographic studies of the distribution of $^{210}$Pb in the lung could perhaps establish the existence of high localized concentrations of $^{210}$Po. Such studies might also provide insight into the origin and metabolism of $^{210}$Pb and $^{210}$Po.

RICHARD B. HOLTZMAN
*Radiological Physics Division, Argonne National Laboratory, 9700 South Cass Avenue, Argonne, Illinois*

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## Infrared Absorption of Carbonate Apatite

Termine and Posner (*1*) have concluded that the inorganic component of rat bone is composed of a two-phase mixture of crystalline and amorphous (noncrystalline) calcium phosphate. This they have done through consideration of a "splitting fraction measurement for the 600 cm$^{-1}$ phosphate ion antisymmetric bending frequency in the calcium phosphates." They state that they "found a straight line correlation between the degree of this infrared splitting and percentage crystallinity (weight fraction of crystalline apatite) in synthetic samples containing these two phases." They refer to these synthetic substances as hydroxyapatite even though the substance of bone is not hydroxyapatite (*2*) and state that carbonate apatites were excluded from their investigation. Their basis for excluding the carbonate apatites is related to the size of individual crystallites in rat bone. There is no fundamental theory that will permit one to deduce the chemical composition of a crystalline phase from a knowledge of the size of the crystals, as Termine and Posner have done.

Elsewhere Posner *et al.* (*3*) have attempted to determine the "degree of crystallinity" of bone mineral by measuring line broadening of x-ray diffraction maxima, but incipient line splitting cannot, in general, be distinguished from line broadening (*4*).

The interpretation of their infrared absorption data is also erroneous. Coles (*5*) has pointed out: "The synthetic carbonate apatites have infrared spectra very different from those of other apatites. Distinguishing features are the strong carbonate band at about 1450 cm$^{-1}$ and lack of resolution of the main $PO_4^{-3}$ band into a doublet." By "main $PO_4^{-3}$ band," Coles apparently means the pronounced absorption at about 1050 cm$^{-1}$. A similar lack of resolution has been observed in the absorption band at about 600 cm$^{-1}$, and was described by Zapanta-LeGeros *et al.* (*6*): "This band [635 cm$^{-1}$] is only very weakly present or even absent in the spectra of synthetic carbonate apatite, staffelite (carbonate-F-apatite), and biological apatite. This absence is believed due to the presence of the carbonate ion."

It is this reduction of resolution (absence of splitting) that Termine and Posner (*1*) used as a criterion to indicate the presence of an amorphous phase in rat bone, although the same reduction of resolution occurs for a holocrystalline, mineralogical carbonate apatite (*6*). Furthermore, although bone has been examined by both electron microscopy and electron diffraction by several investigators, no one has presented direct evidence of the existence of an amorphous inorganic phase, either phosphate or carbonate.

Thus, Termine and Posner have wrongly assumed that rat bone is a simple hydroxyapatite (instead of a carbonate hydroxyapatite) and have used a measurement that is spurious when carbonate apatite is present. They have furnished no evidence that an amorphous phase is present in bone.

DUNCAN MCCONNELL
*Ohio State University, Columbus 43210*

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In our original report (*1*), we did not assign either structural or chemical identity to the poorly crystallized, apatitic fraction of bone mineral, since the exact nature of bone apatite is a matter of considerable debate (*2*). In

fact, our contention that the inorganic portion of skeletal tissue contains both amorphous and crystalline components incorporates the notion that bone apatite bears only a general or familial relation to the mineralogical apatites. In this regard, we referred to our synthetic standards as "crystalline apatites" and did not describe these substances as hydroxyapatites (*1*).

Crystal size came into our discussion in reference to the selection of synthetic crystalline standards for our infrared analysis of bone tissue and not with regard to defining chemical composition. Care must be taken to select a standard (100 percent crystalline) apatite which has the same crystal size and x-ray diffraction profile as the apatite portion of the bone under study, since measurement of the infrared (IR) splitting fraction depends not only on the amount of crystalline apatite present but also on the average crystal size of this phase (*1*, *3*). Thus, synthetic and mineralogical apatites of large crystal size, those containing carbonate as well as those without it, were not selected as crystalline standards for the analysis of bone tissue, which contains very small apatite crystals (*4*).

McConnell appears to be confused by the term "crystallinity index" as used earlier (*5*) and the term "percentage crystallinity" as employed in the report under discussion (*1*). Even though the earlier term has no bearing on the work in question, it would be useful to clarify this error. The first term is related to the size and/or imperfection of a crystal as determined by resolution of x-ray diffraction patterns, while the second refers to measurement of the percentage of a solid mass that exists in the crystalline, as opposed to the amorphous, state.

In general, McConnell is saying that carbonate-containing apatites exhibit a decreased resolution of the phosphate ion infrared absorption bands when compared with carbonate-free apatites. In particular, he states that the reduced phosphate band resolution we noted for bone tissue (*1*) is identical to that which he says occurs for 100-percent crystalline carbonate-apatites of either synthetic or mineralogical origin. For standardization purposes, we had prepared, by precipitation methods, finely divided crystalline apatites that were similar in crystal size and x-ray diffraction profile to bone apatite (*1*). These synthetic standards ranged in carbonate content from 1.5 to 6.0 percent, which is similar to the content in bone mineral (*6*). There were no differences in phosphate band resolution between any of these standards, regardless of their carbonate content. Furthermore, the phosphate band resolution of these samples could be reduced to levels found in bone tissue only by admixing these crystalline standards with amorphous calcium phosphate (*1*).

In the abstract of the thesis cited by McConnell, Coles states that she prepared her carbonate-apatites by phosphate ion replacement of carbonate ions in powdered calcite in alkaline media (*7*). According to the data of Ames (*8*), this method produces a carbonate-substituted apatite definitely different from bone apatite in crystal size and chemical composition. Our synthetic apatites, which gave x-ray diffraction profiles almost identical to the profile of bone apatite, were precipitated from aqueous solution at room temperature. This method, according to Ames, produces apatites which "contained adsorbed $CO_3^{-2}$ only" (*8*). It is probable, then, that Coles was examining samples that were far removed, both chemically and structurally, from our synthetic crystalline standards and from bone apatite.

McConnell also cites a paper that was given at the 1964 meeting of the International Association for Dental Research (*9*), which states that resolution of the 635-$cm^{-1}$ band in apatite IR spectra was affected by the presence of carbonate. This 635-$cm^{-1}$ band has been shown to arise from hydroxide ion librational, and not phosphate ion vibrational, motion (*10*). Indeed, a dependency, for this hydroxide ion band, on carbonate ion does not necessarily imply a similar dependency for the 605 $cm^{-1}$ and the 570 $cm^{-1}$ phosphate ion absorption doublet utilized in our IR analysis (*1*). Moreover, examination of our standards showed no dependency of this 635-$cm^{-1}$ band on carbonate concentration. This hydroxide ion absorption band is reduced in resolution when fluoride or chloride ions, or both, are present in the apatite structure (*10*). This could explain the reported decrease in resolution of this band in the holocrystalline, fluoride-containing, carbonate apatite, staffelite (*9*).

Our infrared absorption method for determination of percentage crystallinity (*1*), although empirical, yields results that are within ± 2 percent of values obtained by quantitative x-ray diffraction analysis (*11*). Furthermore, McConnell's statement that there is no evidence, obtained by electron microscopy, for the existence of an amorphous phase in bone mineral is in direct contradiction to the statement in our report that "These facts are consistent with evidence obtained by electron microscopy which indicates that a noncrystalline mineral phase appears prior to crystalline apatite during early stages of bone formation" (*1*, *12*). Molnar has shown that the mineralized areas of bone tissue that give a distinctively amorphous electron diffraction pattern have a different mineral morphology from areas containing apatite crystals (*12*).

Thus data obtained by infrared (*1*), x-ray diffraction (*11*), electron diffraction (*12*), and electron microscopy (*12*) point to the fact that bone mineral is a two-phased mixture of amorphous calcium phosphate and crystalline apatite.

JOHN D. TERMINE
AARON S. POSNER
*Department of Biochemistry,*
*Hospital for Special Surgery,*
*Cornell University Medical College,*
*New York 10021*

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15 December 1966