COMPOSITIONAL, INFRARED, AND X-RAY ANALYSIS OF FOSSIL BONE

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ABSTRACT

Fossil bone exhibits an increase in F over modern mammalian bone (3% *vs* 0.18%) but CO_2 (3.7% *vs* 4.4%) and H_2O (5.36% *vs* 6%) are essentially the same. Heating of fossil bone, modern bone and carbonate-bearing apatites to 1250°C produces no separate CaO phase detectable by X-ray diffraction, but Na is subsequently leached preferentially. Infrared spectrograms provide no evidence of physically discrete CO_2-bearing phases in carbonate apatites and fossil bones. The $(CO_3)^{2-}$ ν_3 vibration in the 7μ region shows a bidentate or tridentate split, even in apatite from Renfrew, containing only 0.75 percent CO_2, suggesting that two $(CO_3)^{2-}$ sites exist, one of which is asymmetrical. The model of McConnell (1960) for carbonate apatites fits these data most closely.

INTRODUCTION

More than twenty varieties of apatite and fossil bone have been analyzed in an attempt to gain a better understanding of (1) the carbonate bearing species, and (2) the relationship between apatite and ossified tissue. X-ray, infrared, and chemical analyses have been made on samples heated to various temperatures in an effort to correlate chemical and structural changes.

The francolite end member of the apatite group most closely approximates fossil bone with its high fluorine and carbonate content. But these francolites, with high F, CO_2, and H_2O^+, are more difficult to fit into the structure model proposed by McConnell (1960) than are the dahllites, with less than 1 percent F and generally lower H_2O^+ content.

EXPERIMENTAL METHODS

Samples of fossil bone, various apatites, and modern bone were examined initially by X-ray diffraction. Optical studies were ineffective due to the small crystallite size in most material investigated. Ground samples were leached in 0.1 N HNO_3 to remove any calcite present as cement or encrustation. Leaching produced no chemical or structural changes other than removal of calcite. Samples were then analyzed at room temperature, and after heating to 1250°C, by X-ray, infrared, and chemical methods. Many samples were analyzed after heating at close temperature intervals up to 1400°C.

All X-ray analyses were performed with a Norelco geiger-counter diffractometer using $CuK\alpha$ radiation and a 1° 2θ/minute traverse speed.

After considerable experimentation the following analytical procedure was adopted. No determination of H_2O^- is included in the analyses because initial studies showed that it was not part of the unit cell. Carbonate was determined gravimetrically as $BaCO_3$ after the gas evolved during solution of the apatite in 1:1 HNO_3 was bubbled through a train of

three vessels containing 0.1 M. $Ba(OH)_2$. Duplicate or triplicate analyses were made since any error had a cumulative effect in the determination of H_2O^+. Fossil bone samples were surprisingly free from organic material, but, when present, organic carbon did not come off as CO_2 during solution in 1:1 HNO_3 at temperatures below 85°C. Calcium was determined in triplicate gravimetric analyses after precipitation as the oxalate at pH 4, and subsequent decomposition at 500 ± 25°C for two hours to assure removal of any water of crystallization. Magnesium, potassium, and sodium were determined by flame photometry on a Beckman model DU spectrophotometer. Background corrections were not needed but standard solutions of the ions had to be run before each analysis to ensure standard aspiration rate. Due to the depressing effect of PO_4 on Mg flame emission, spiked samples were run for various concentrations of phosphate and magnesium. Phosphate was determined colorimetrically by the molybdicvanadic acid method of Boltz (1958); the inhibiting effect of fluoride ion on the color is not significant in the ranges of concentration encountered if a 30 minute reaction time is allowed. Fluorine was determined colorimetrically after distillation by the method of Willard and Winter. Zirconium was used as the complexing ion and eriochrome syanine R was the chromophore (Kolthoff, *et al.*, pp. 238–242, 1961). When present, chlorine was determined by potentiometric titration with $AgNO_3$ plus 50 percent acetone at 5°C. Due to the unusual stability of oxygen-hydrogen groups, H_2O^+ was determined gravimetrically after heating to 1250°C. Minus 200 mesh material was heated for four or more hours until cessation of weight loss in an oven controlled at 1250 ± 10°C by a Bristol controller with a Pt-Pt, Rh thermocouple. Volatilization of fluorine was negligible at 1250° but complete analyses of most samples were made to confirm the weight loss as only CO_2, organic matter when present, and H_2O^+.

Infrared analyses were made on all samples, with special concentration on the active carbonate ion. The best spectra were obtained with pellets pressed from 1 mg of -200 mesh sample in 250 mg of optical grade KBr at 20,000 psi for 5–10 minutes. The spectra were very good in the region 6-15 microns, but the water region from 2–6 microns was complicated by interference from water adsorbed on the KBr. Spectra were confirmed by the hexachlorobutadiene mull method.

Attempts to study oxygen-hydrogen groups with nuclear magnetic resonance, and with fluoralube mulls in the 2–6 micron region, were not successful. Fluoralube oil from the 3M Company gives a highly desirable flat spectrum from 2–7.5 microns, but disconcertingly shifted all resonance bands to longer wavelengths. The high porosity of the bone samples will not allow significant density measurements. Thus no comparison of theoretical and experimental densities can be made.

Results of X-Ray Analyses

Fossil bone has the advantage of providing large quantities for X-ray, chemical, and thermal analyses, and also of essentially being "cleaned" of all organic matter during fossilization. It does not produce extreme line broadening nor contain complex organic material to interfere with interpretation of chemical analyses. Heated fossil bone samples produce more distinct diffraction patterns, and retain an apatite structure to at least 1400°C (Table 1). Only one heated sample of dahllite inverted to whitlockite below 1400°C.

Intensity differences were compared between carbonate and noncarbonate varieties. Intensity of the (121) and (112) reflections decreases and (300) increases with increasing carbonate content in the manner

TABLE 1. X-Ray Diffraction Data[a]

hkl	1. Fossil bone unheated d(Å)	I/I₀	2. Fossil bone heated to 1250°C d(Å)	I/I₀	3. Fossil bone heated to 1400°C d(Å)	I/I₀	4. Francolite (Analysis 2, Table 2) d(Å)	I/I₀	5. Dahllite (Analysis 1, Table 2) d(Å)	I/I₀
100					8.13	36	8.04	18	8.13	18
110					4.692	20	4.668	2	4.692	4
200			4.046	9	4.055	30	4.037	16	4.064	10
111			3.871	7	3.872	22	3.863	2		
002	3.437	17	3.444	45	3.437	65	3.433	20	3.430	16
102			3.173	15	3.163	28	3.162	5		
210	3.040	9	3.066	21	3.058	43	3.054	34	3.076	27
211			2.799	100	2.799	100	2.790	55	2.811	80
112	2.778	37	2.773	50	2.772	75	2.769	15	2.774	27
300	2.690	21	2.707	70	2.700	100	2.692	100	2.717	100
202	2.617	11	2.623	30	2.621	51	2.619	8	2.627	11
301			2.520	6	2.520	15	2.506	3	2.526	4
003	2.290	7	2.290	7	2.290	16	2.284	2	2.293	5
310	2.240	11	2.252	26	2.249	49	2.240	45	2.261	33
311	2.128	9			2.136	15	2.134	4	2.149	6
400					2.018	13	2.021	4		
222	1.934	10			1.936	51	1.931	12	1.941	15
132					1.884	28	1.887	7	1.889	7
230	1.843	100			1.837	53	1.855	7	1.841	12
213							1.834	10		
231					1.797	92	1.790	11	1.805	16
410					1.769	31	1.783	25	1.781	12
303					1.747	27	1.754	7	1.757	7
004					1.722	24	1.720	6	1.720	4

$a = 9.357$ Å	$a = 9.375$ Å	$a = 9.357$ Å	$a = 9.346$ Å	$a = 9.419$ Å
$c = 6.888$ Å	$c = 6.875$ Å	$c = 6.888$ Å	$c = 6.887$ Å	$c = 6.886$ Å

1. Bone, *Equus* scotii, (locality unknown).
2. Bone, *Parahippus*, (locality unknown).
3. Bone, *Equus* scotii, (locality unknown).
4. Francolite, Staffel, Germany. See Analysis 2, Table 2.
5. Dahllite, Allendorf, Saxony. See Analysis 1, Table 2.
[a] Cu radiation, Ni filter.

noted by McConnell (1952). This might be produced by preferred orientation in the fibrous carbonate apatites, as has been pointed out by Posner (1961) and Carlstrom (1955). However, differences were linear with CO_2 content and more critical study might confirm their significance.

No evidence of diffraction maxima attributable to calcite was found in any samples, other than that clearly introduced during fossilization (and usually visible in thin section), and removed easily by leaching with 0.1 N HNO_3. Carlstrom (1955) stated he found CaO lines in heated samples and presented them as evidence of an adsorbed calcite phase in bone and apatite crystals. In this study, using the same method, no CaO lines or other evidence was found for an adsorbed phase of any sort.

RESULTS OF CHEMICAL ANALYSES

Over fifty complete and partial chemical analyses were performed on natural and heated samples, a few of which are given in Table 2. Analyses 1, 2 and 3 (Table 2) fall in the range of previously analyzed dahllites and francolites. Analysis 4 (Table 2) is a representative of the many fossil bones that contained large amounts of CO_2, H_2O^+, and F; six others are averaged in analysis 5.

Finely ground samples of staffelite, dahllite, extracted bone, fossil

TABLE 2. CHEMICAL ANALYSES OF CARBONATE APATITES

	1	2	3	4	5	6
CaO	48.87	49.96	48.87	51.42	51.58	53.71
MgO	1.27	00.95	2.12	1.35	1.16	0.10
Na_2O	2.09	1.12	1.68	1.17	0.80	0.20
K_2O	0.57	0.23	Tr.	0.38	NP	—
P_2O_5	33.42	35.03	37.17	40.33	39.39	38.71
CO_2	3.74	3.89	4.44	2.70	3.51	3.01
F	3.00	2.69	0.18	3.89	0.44	0.10
Cl	Tr.	Tr.	NP	Tr.	Tr.	0.61
H_2O^+	6.02	5.36	6.03	0.63	1.48	3.42
Wt. loss (1250°C)	(9.76)	(9.78)	(10.47)	(3.33)	(4.99)	
Insol.	2.01	1.97	Tr.	Tr.	Tr.	0.27
	100.99	101.20	100.49	101.87	98.36	100.13
less O = F	1.26	1.13	0.08	1.64	0.19	
Total	99.73	100.07	100.41	100.23	98.17	

1. Fossil bone, Parahippus, locality unknown.
2. Average of 6 fossil bones.
3. Modern bone, extracted with ethylene diamene.
4. Francolite, Staffel, Germany.
5. Dahllite, Allendorf, Saxony.
6. Parahippus enamel (Brophy and Hatch, 1962).

bone, and fluorapatite were heated at 1250° for four hours, treated with boiling water for several minutes, the solution decanted, and analyzed. Sodium showed a marked decrease from 1.22 to 0.54 percent (staffelite), 0.80 to 0.20 percent (dahllite), 1.68 to 0.71 percent (extracted bone), 1.71 to 0.21 percent (fossil bone), and 1.33 to 0.05 percent (fluorapatite); other cations (Ca, Mg, K) remain constant. At first it was thought that heating had destroyed bonding in an adsorbed sodium phase, converting it to soluble Na_2O. However, the largest loss (96 percent) was from a large crystal of fluorapatite, which probably could not adsorb Na^+. Thus the sodium loss must represent selective removal, as has also been noted for the alkali feldspars. Probably $(H_3O)^+$ exchanged for Na^+.

RESULTS OF INFRARED ANALYSIS

Infrared analysis of carbonate apatites has provided significant chemical and structural data. No natural or synthetic single crystals of carbonate apatites are available at this time for X-ray structure analysis, so information obtained from infrared studies, concerning the $(CO_3)^{2-}$ and $(PO_4)^{3-}$ groups, is critical. The most important region is that of the ν_3 vibration for $(CO_3)^{2-}$ near 7μ, (Figs. 1 and 2). Splitting of this vibration into two or three bands is unusual and important. Adler and Kerr (1963) analyze this behavior for the $(CO_3)^{2-}$ group: splitting of the ν_3 vibration can signify either lower molecular symmetry or the presence of two types of high symmetry $(CO_3)^{2-}$ groups. Tridentate splitting requires at least two different $(CO_3)^{2-}$ sites one of which can be a symmetrical group; or one band may be produced by a weakly bonded adsorbed phase. Posner and Duyckaerts (1954) concluded that the splitting was produced by two adsorbed phases, magnesite and calcite. The unusual magnesite and calcite spectra they present are little justification for this conclusion. Heated dahllite and fossil bone (Fig. 2) showed greater change in the 7.02μ band than the 6.9μ band. If these bands represent adsorbed calcite and magnesite respectively, heating should produce the opposite effect due to the lower thermal stability of $MgCO_3$.

Bidentate or tridentate splitting of the $(CO_3)^{2-}$ ν_3 vibration is observed in all carbonate-bearing apatites. Large crystals of skarn-type fluorapatite such as at Renfrew, Ontario, containing less than 0.75 percent CO_2, still have split bands at 7 microns. Carbonate spectra do not always produce the maximum number of bands allowable by the asymmetry of the structure. For example, the ν_3 vibration for aragonite should be bidentate but rarely is (Adler and Kerr, 1963). Infrared spectra for unheated and heated bone and dahllite (Fig. 2) show the marked decrease in absorption of the 7.02 band in heated samples. The bands at longer wavelength must represent a less tightly bonded group to satisfy the require-
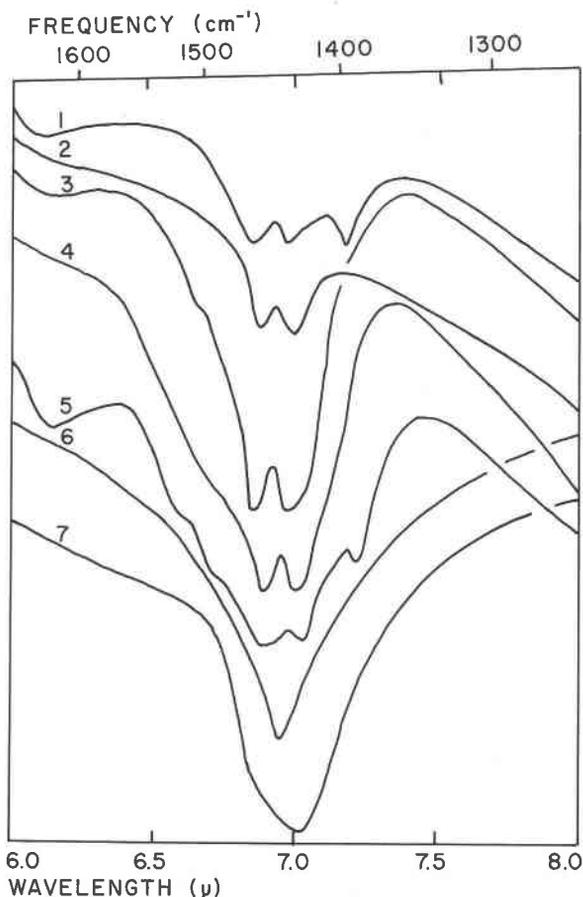
FIG. 1. Infrared spectra of apatites, calcite and magnesite.

1. Fluorapatite, Guide Copper Mine, Transvaal, U. S. Africa.
2. Fluorapatite, 0.58% CO_2, Renfrew Co., Ontario, Canada.
3. Dahllite, 3.51% CO_2, Allendorf, Saxony.
4. Francolite, 2.70% CO_2, Staffel, Germany.
5. Parahippus, 3.74% CO_2, locality unknown.
6. Magnesite, Chewelah, Washington.
7. Calcite, Butte, Montana.

ments of a simple harmonic oscillator, and this group would be driven from the structure before one more tightly bonded.

The ν_4 vibration (at about 14μ) is inactive in all leached samples, and is additional evidence that no calcite phase is present in carbonate apatites. In mechanical mixtures of calcite and apatite (Fig. 3) the presence of the
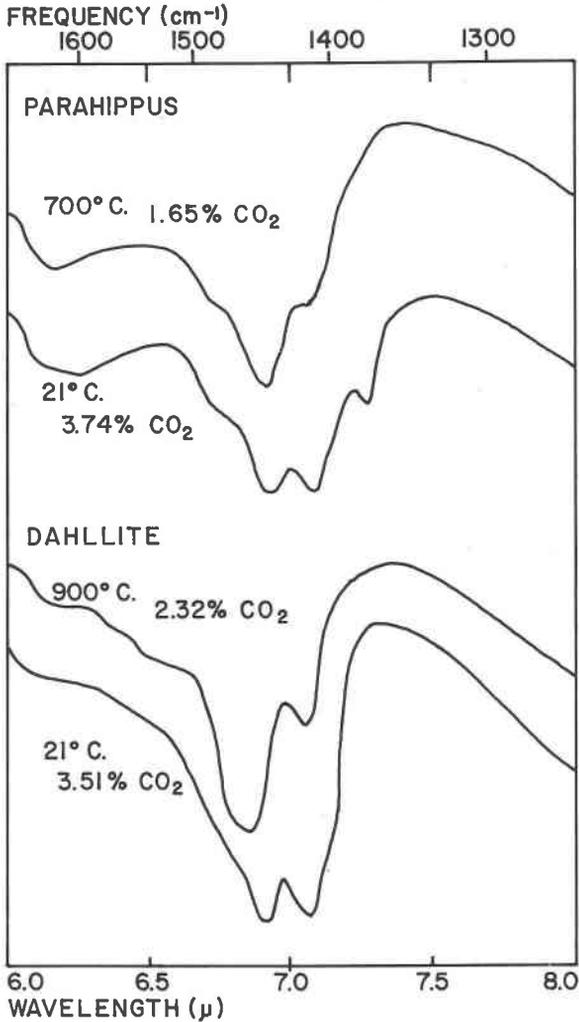
Fig. 2. Effect of heating on the split CO_3^{2-} vibration. The upper two curves are from *Parahippus* (see analysis 1, Table 2). The lower two curves are for dahllite (see analysis 5, Table 2). In both cases the band at longer wavelength changes first for the heated samples.

ν_4 band is readily noted. The vibration was still detectable in mixtures containing as little as 0.5% $CaCO_3$.

McConnell (1960) proposes that $(CO_3)^{2-}$ substitute (1) in some Ca ion positions so that the $(CO_3)^{2-}$ groups lie parallel to (00.1) and (2) for $(PO_4)^{3-}$ groups and lie perpendicular to (00.1) distributed about an axis

of essentially threefold symmetry. The observed splitting of the infrared spectra in the 7μ region lends support to this model.

Structural Calculations

Errors in chemical or structural analysis are compounded during calculation of a chemical analysis into proper unit-cell positions, especially in the carbonate apatites where many substitutions are possible, and density and structure are indefinite. The major question is, whether the CO_2 should be assigned totally to the structure or partly to absorption on the crystallite surface. This study has formed no evidence for an adsorbed calcite or $(CO_3)^{2-}$ phase. Infrared data requires carbonate substitution by
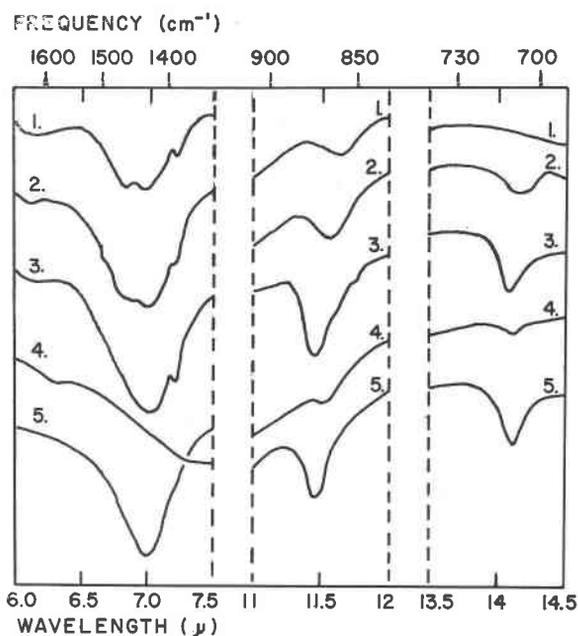

Fig. 3. Effect of calcite on the infrared spectra of fossil bone and apatite.

1. *Parahippus.*
2. *Parahippus* before leaching and showing evidence of calcite presence by XRD.
3. *Parahippus* with a mechanical mixture of calcite (10% of total weight).
4. Fluorapatite (no CO_2 in analysis) from Llallagua, Bolivia, with 2% mechanical mixture of calcite.
5. Calcite, Butte, Montana.

Note that calcite with apatite (curves 2, 3, 4) does not produce the curve typical of carbonate apatites (curve 1). In all cases the presence of calcite is detectable in the 14μ region.

the same mechanism for all carbonate apatites regardless of CO_2 content or surface area. Although there is no evidence for an adsorbed phase, many analyses will not allow all components to be assigned to the unit cell by any of the presently held substitution models.

McConnell (1960) has presented the best model for the carbonate apatites. However, his example is not typical of fossilized bone and phosphorite, where fluorine is selectively substituted for $(OH)^-$ groups up to 5 percent by weight. Francolites with over 10 percent total F, CO_2 and H_2O^+ present structural problems not encountered in dahllite, and francolite with low H_2O content. Using the method of McConnell (1960), calculations (Table 3, nos. 1, 2, 3) for analyses of fossil bone (Table 2,

TABLE 3. RECALCULATION OF ANALYSES FROM TABLE II BASED ON A UNIT CELL WITH CATION CHARGES = 53

	1	2	3	4	5	6
Ca Position	9.91	9.26	8.94	9.57	9.41	9.64
(Ca, Mg, Na, K)	0.29 (C)	.74 (H$_3$)	1.06 (H$_3$)	.17 (H$_3$)	.68 (C)	.36 (H$_3$)
other (C, H$_3$)	10.20	10.00	10.00	9.74	10.09	10.00
P Position						
P	4.76	5.15	5.16	5.39	5.89	5.44
C	0.56	0.85	0.92	0.55	0.11	0.66
H$_4$	0.68	—	—	—	—	—
	6.00	6.00	6.08	5.94	6.00	6.00
F Position						
F	1.59	1.37	0.09	1.84	0.22	0.22
OH	0.41	0.63	1.91	0.16	1.59	1.78
	2.00	2.00	2.00	2.00	1.81	2.00
Distribution of H$_2$O$^+$						
a Total cation	6.77	5.76	6.14	0.66	1.59	3.79
b OH	0.41	0.63	1.91	0.16	1.59	1.78
c H$_3$	—	2.22	3.18	0.50	—	1.08
d H$_4$	3.13	—	—	—	—	—
$(a-(b+c+d))=XS$	3.64	2.91	1.05	0.00	0.00	0.93

nos. 1, 2, 3), cannot account for all the H_2O^+ in structural positions. There is good evidence that McConnell's model holds for the majority of carbonate apatites (Table 3, nos. 4, 5, 6) but apparently is not adequate for the extreme francolite end members. McConnell (pers. comm.) suggests that some of the excess H_2O may be present as water molecules in place of $(OH)^-$ in addition to $(H_4O_4)^{4-}$ substituting for $(PO_4)^{3-}$. All francolites (fossil bone and phosphorite most commonly) which cannot be

described adequately by McConnell's model are of minute particle size, (surface areas of over 150 m²/g), and produced extreme line broadening in diffraction patterns.

Conclusions

Fossil bone is a francolite end member. Infrared spectra indicates non-identical carbonate sites in both francolite and dahllite but the exact local symmetry cannot as yet be specified. The structure proposed by McConnell (1960) best explains the infrared spectra for C-O groups obtained in the 7μ region. There is no evidence to support the hypothesis that $(CO_3)^{2-}$ groups are present in apatite as a separate phase, such as $CaCO_3$. The failure of structural calculations on analyses of francolite (fossil bone) with high F, CO_2 and H_2O^+ content is evidence to the complexity of the problem.

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