Probable Phase Composition of the Mineral in Bone

F. C. M. Driessens

Institute of Dental Materials Science, Subfaculty of Dentistry, University of Nijmegen, P. O. Box 9101, 6500 HB Nijmegen, The Netherlands

Z. Naturforsch. **35 c,** 357–362 (1980); received January 15, 1980

Bone, Mineral, Phase Composition, Citrate

Formulas proposed for the mineral of bone were reviewed. Literature data were collected where Ca, P, Na, Mg and CO_3 are determined in the same samples. These data were analyzed for their conformity to the above mentioned formulas. According to this analysis Mg is contained in a phase having the Ca/P of magnesium whitlockite within the limits of error. Na is contained in a carbonated calcium phosphate phase which in analogy with synthetic systems must have the apatite structure. The Ca/P ratio of the remaining "rest phase" is 2. This is based on the composition of 101 bone mineral samples taken from fishes, reptiles, amphibians, birds and mammals. The CO_3 content of the bone samples agrees with the formula

$$Ca_8\,(PO_4)_4\,(CO_3)\,(OH)_2 \cdot x\,H_2O$$

for the "rest phase" within the limits of experimental error. Such a compound has, however, not been found in synthetic systems. Human bone contains about 15% magnesium whitlockite, 25% of the Na and CO_3 containing apatite and the rest is the carbonated calcium phosphate with Ca/P = 2. It is presumed that this compound has a structure similar to that of octo calcium phosphate and that most of the citrate ions which always occur in bone mineral samples are incorporated in that phase.

Introduction

Early X-ray diffraction studies revealed the apatite structure of the mineral in bone [1, 2]. In one study [2] the mineral was claimed to be hydroxyapatite with the formula

$$Ca_{10}\,(PO_4)_6\,(OH)_2 \qquad (1)$$
$$Ca/P = 1.667.$$

In another study [1] it was thought to consist of carbonated apatite of the formula

$$Ca_{10}\,(PO_4)_6\,CO_3 \qquad (2)$$
$$Ca/P = 1.667.$$

Klement and Trömel [3] expressed as their view that carbonate was present as calciumcarbonate and that constituents like sodium, magnesium and chloride should be considered as unimportant impurities.

In more recent studies on synthetic systems it has been established that the CO_3^{2-} ion can replace both OH^- ions and PO_4^{3-} ions in the apatite structure [4, 5]. Bonel [6] proposed the formula

$$Ca_9\,V(Ca)\,[(PO_4)_4\,(CO_3OH)_2]\,V(OH)_2 \qquad (3)$$
$$Ca/P = 2.25$$

for carbonated sodium-free apatites prepared by precipitation. Here V(Ca) and V(OH) are vacancies in the Ca and OH sublattice respectively. However, according to Montel [7] these apatites conform to the formula

$$Ca_8\,V(Ca)_2\,[(PO_4)_4\,(CO_3)_2]\,V(OH)_2 \qquad (4)$$
$$Ca/P = 2.$$

For Na^+ and CO_3^{2-} containing apatites prepared by precipitation from aqueous solutions a whole range of compositions can be obtained which are more or less between [8] hydroxyapatite (1) and

$$Ca_7\,Na_2V(Ca)\,[(PO_4)_3\,(CO_3)_3]\,(OH)\;V(OH)$$
$$Ca/P = 2.333. \qquad (5)$$

However, it is extremely difficult to establish whether precipitated apatites are single phase. Therefore, Schaeken and Driessens (1979) have carried out high-temperature preparations of Na^+ and CO_3^{2-} containing single-phase apatites. They found the existence of solid solutions between carbonated apatite (2) and

$$Ca_{8.5}\,Na_{1.5}[(PO_4)_{4.5}\,(CO_3)_{1.5}]\,CO_3 \qquad (6)$$
$$Ca/P = 1.889.$$

It is a well known fact that the mineral of bone contains variable amounts of CO_3^{2-}, Na^+ and Mg^{2+}

Reprint requests to Prof. F. C. M. Driessens.

0341-0382/80/0500-0357 $ 01.00/0

ions [10]. Perhaps the most widely accepted hypothesis to explain this fact has been hitherto the formation of an apatitic solid solution containing these ions in variable amounts [11]. However, it has been proven [12] that Mg^{2+} ions are not incorporated in the apatite lattice. Another calcium phosphate, *i. e.* whitlockite, is formed preferentially in the presence of Mg^{2+} ions [13]. There are strong indications that this phase is often so finely disperse that it appears as amorphous to X-ray diffraction, although the ionic product of whitlockite is constant over such amorphous precipitates [14]. Therefore, it is not surprising that the presence of whitlockite has not been detected in bone mineral by X-ray diffraction. However, a more appropriate technique like electron diffraction elucidated the presence of whitlockite in the magnesium rich peritubular regions of dentine [15]. The composition of magnesium whitlockite is given by the formula

$$Ca_9\,Mg(HPO_4)\,(PO_4)_6 \qquad (7)$$
$$Ca/P = 1.286.$$

An alternative explanation put forward to explain the variable composition of the mineral in different samples of bone is the occurrence of a "paracrystalline apatitic texture" [16, 17] or, more precisely, that of a sandwich structure between apatite and octocalciumphosphate [18, 19]. The latter has in its pure form the formula

$$Ca_8\,(PO_4)_4\,(HPO_4)_2 \cdot 5\,H_2O \qquad (8)$$
$$Ca/P = 1.333.$$

Hayek [20] has been able to prepare carbonate containing octo calium phosphates being solid solutions of (8) and

$$Ca_8\,(PO_4)_4\,(CO_3)_2 \cdot 5\,H_2O \qquad (9)$$
$$Ca/P = 2$$

which apart from the water content shows a strong similarity with (4). Precipitated compounds like (8) and (9) are hardly distinguishable from precipitated apatites by X-ray diffraction [19].

In view of these facts it seems possible that the mineral in bone consists of more than one phase. The Mg content [10] points to the occurrence of whitlockite (7) whereas the Na^+ and CO_3^{2-} contents which show a significant correlation [21] indicate the occurrence of a Na^+ and CO_3^{2-} containing apatite, of which we will prefer here formula (6). The purpose of this paper is to find evidence for the nature of the phases possibly occurring in the mineral of bone.

The Mg containing phase

By differential dissolution in ammonium citrate solutions Gabriel [10] has proven that Mg is contained in the most fast dissolving phase in bone mineral. In order to estimate whether this phase has a high or a low Ca/P molar ratio the correlation was calculated between the Mg content and the overall Ca/P ratio of both bone mineral and dentine mineral. The results are given in Table I together with the sources of reference for the experimental data. The overall expression for the relation between Mg content on ash basis and Ca/P molar ratio becomes

$$\%Mg = 9.41 - 5.11\,(Ca/P). \qquad (10)$$

If we introduce the Mg content of whitlockite (7) in this relation, we obtain $Ca/P = 1.39 \pm 0.12$. This equals the value for whitlockite within the limits of error. In the following sections we will assume that the Mg content reflects the content of the whitlockite phase in bone mineral.

Eanes, Termine and Posner [31] have indicated that part of the bone mineral is amorphous to X-ray diffraction. The ratio between the amorphous and the crystalline part in the bone mineral of rats decreases with age [32]. In synthetic systems the amorphous calcium phosphate is stabilized by Mg^{2+} ions [33], but its solubility product constant is that of whitlockite [14] as mentioned before. In human bone

Table I. Correlation between Mg content on ash basis and Ca/P molar ratio of the mineral in bone and dentine

Tissue	Reference	Number of samples	Coefficient of correlation
Bone	Marek *et al.*, 1935 [22]	2	−0.63
	Marek *et al.*, 1934 [23]	10	
	Morgulis, 1931 [24]	16	
	Kick *et al.*, 1933 [25]	8	
Dentine	Murray, 1936 [26]	6	−0.78
	McClure *et al.*, 1960 [27]	12	
	Burnett *et al.*, 1957 [28]	4	
	Logan, 1935 [29]	4	
	Gabriel, 1894 [10]	1	
	Klement, 1938 [30]	1	
Both	Total	64	−0.82

the Ca/P ratio is low initially whereas the Mg content is high [34, 35]. Within 15 weeks it rises to the normal value. This combined evidence suggests that the low Ca/P ratio right after the initial mineralization is due to a high whitlockite content of the bone mineral and not to a high octo calcium phosphate content as proposed earlier [19].

The Na containing phase

As mentioned before, there is a strong positive correlation between the Na content and the CO_3 content of bone mineral [21]. This means that Na occurs in a carbonated phase. However, the correlation between Na content and the Ca/P molar ratio is very low. Therefore, the Ca/P ratio of the Na containing phase must be rather moderate compared to that of the other phases. For this reason formula (6) is preferred here over formula (5).

Table II. Ca/P molar ratio of the "rest phase" in bone mineral

Reference	Ca/P	Remarks
Gabriel, 1894 [10]	1.957	human
	1.999	bovine
	2.015	goose
Klement, 1936 [36]	1.789	bovine
	2.038	bovine
	1.946	bovine
	2.001	bovine
	1.992	bovine
	1.961	bovine
	1.837	bovine
	1.963	bovine
	1.918	bovine
	1.928	human
	1.902	human
	1.951	human
	1.910	human
	1.965	human
	1.978	human
Klement, 1938 [30]	1.929	human
	1.932	bovine
	2.087	sea-horse
	2.350	kormoran
	2.023	gull
	2.126	lumme
	2.122	auk
	2.131	duck
	1.790	turtle
	1.783	tuna
	1.836	saw-fish
Armstrong, 1965 [37]	1.790	bovine
Vatassery *et al.*, 1970 [38]	2.110	human
Average	1.970 ± 0.022	

Table III. Theoretical and experimental CO_3 content of bone mineral

Experimental (e)	Theoretical (4)	Theoretical (11)	Difference (e) – (4)	Difference (e) – (11)
7.99	11.67	7.51	– 3.68	0.48
6.90	10.75	7.14	– 3.85	– 0.24
5.60	9.91	6.74	– 4.31	– 1.14
5.93	11.21	6.18	– 5.24	– 0.20
5.20	9.82	5.91	– 4.62	– 0.71
4.76	10.11	6.23	– 5.35	– 1.47
5.06	10.18	6.02	– 5.12	– 0.96
4.99	10.18	6.00	– 5.19	– 1.01
5.30	10.36	6.14	– 5.06	– 0.84
5.17	11.08	6.35	– 5.91	– 1.18
5.23	10.43	6.26	– 5.20	– 1.03
4.98	10.55	6.23	– 5.57	– 1.25
5.40	11.15	6.60	– 5.75	– 1.20
5.49	11.31	6.59	– 5.82	– 1.10
6.51	12.02	7.25	– 5.51	– 0.74
5.36	11.28	6.47	– 5.92	– 1.11
5.53	10.93	6.38	– 5.40	– 0.85
6.11	12.09	7.26	– 5.98	– 1.15
5.69	11.42	6.67	– 5.73	– 0.98
5.15	10.37	6.12	– 5.22	– 0.97
5.59	11.06	6.77	– 5.47	– 1.18
5.33	9.31	5.70	– 3.98	– 0.97
4.95	11.14	6.68	– 6.19	– 1.73
4.38	10.44	6.22	– 6.06	– 1.84
5.04	11.12	6.84	– 6.08	– 1.80
4.40	10.70	6.24	– 6.30	– 1.84
6.13	10.14	5.92	– 4.01	0.21
4.68	10.62	6.33	– 5.94	– 1.65
3.22	9.46	5.40	– 6.24	– 2.18
2.83	8.05	5.62	– 5.22	– 2.79
5.91	10.12	6.25	– 4.21	– 0.34
	Average		-5.29 ± 0.77	-1.07 ± 0.68

The "rest phase"

The main components of bone mineral are Ca, P, CO_3, Na, Mg and water in one or another form [10]. As water and carbondioxyde can evaporate from the mineral, the analytical determinations of the contents of Ca, P, Na and Mg are most confident. If formula (6) and (7) are accepted for the Na and the Mg containing phases respectively and if it is assumed that there is only one other phase present in bone mineral, then it is possible to calculate the Ca/P molar ratio of that phase for those literature data where the Ca, P, Na and Mg content of bone mineral are given. These data are summarized in Table II as far as they are obtained on bone mineral isolated by glycol-KOH or glycerol-KOH extraction. This bone mineral is free of the mineral constituents which are dissolved in the aqueous gel of bone

under *in vivo* conditions and thus do not belong to the solid phases of bone mineral.

On the average the Ca/P molar ratio of the rest phase equals that of the apatite of formula (4) and of the octocalcium phosphate of formula (9). The CO_3 content found by the different authors is mentioned in Table III. It is compared with the theoretical CO_3 content of the basis of formula (4). (For formula (9) it was not calculated as it would be nearly the same.) Comparison of the experimental with the calculated values reveals that neither one of the formulas (4) or (9) applies. However, the agreement with the theoretical values derived from the formula

$$Ca_8 (PO_4)_4 (CO_3) (OH)_2 \cdot x\, H_2O \qquad (11)$$

is acceptable within the limits of error.

This conclusion is entirely new. A compound of formula (11) has not been found in synthetic samples yet nor has it been mentioned as a possible constituent of bone mineral.

Discussion

With respect to the data in the tables it must be noted that the errors in the four determinations of Ca, P, Na and Mg propagate in the theoretical values of Ca/P and the CO_3 content of the rest phase. In conjunction with the facts that the average value for the Ca/P ratio is very close to one of the expected stoichiometries of formulas (1) through (9) and that the average theoretical CO_3 content equals that of a simple formula like (11) within the limits of error, it is presumed that the variations in these calculated values hardly reflect biological variations but are rather due to the above mentioned errors in the analytical procedures. This conclusion is justified if it can be assumed that the relative errors in the determinations of Na and Mg in all studies have been about 10% as was indicated by Vatassery *et al.* [38] in their study.

Several literature data on the Ca, P, Na and Mg content of bone mineral have been obtained on samples prepared by ashing of whole bone. In this procedure the aqueous gel contributes especially to the apparent Na and Mg content. For the data of bone samples of 16 vertebrates found by Biltz and Pellegrino [39] there is a fair correlation between the initial water content of the bone and the apparent Ca/P ratio calculated for the rest phase on the basis of the ash composition (see Fig. 1). Extrapolation to 0% H_2O results in a Ca/P ratio of 2.05 ± 0.06. Further Pellegrino and Biltz [40] have analyzed 54 human bone samples both for water and for mineral constituents in the total ash. The calculated average Ca/P ratio for the rest phase is 1.92 ± 0.02. The water content was always very close to 8%. Using Fig. 1 for estimating the necessary correction results in a value of 1.98 ± 0.04 for the Ca/P ratio of the rest phase in these 54 samples.

The conclusion that the average value of the Ca/P ratio of the rest phase in bone mineral equals 2, is thus based on at least 101 bone samples. It is noteworthy that this group includes several types of vertebrates like fishes, reptiles, amphibians, birds and mammals. It is also remarkable that the same Ca/P ratio was found for the "rest phase" in the mineral in dentine but not in enamel [41] and that the CO_3 content of the rest phase in dentine was also in agreement with formula [11]. In enamel the "rest phase" could be identified as nearly pure hydroxyapatite and it appeared to contain most of the F^- and Cl^- ions of the enamel mineral [41]. The compound of formula (11) does not or hardly occur in enamel, but in human dentine and bone mineral it amounts to about 50%. Similarly, the citrate content

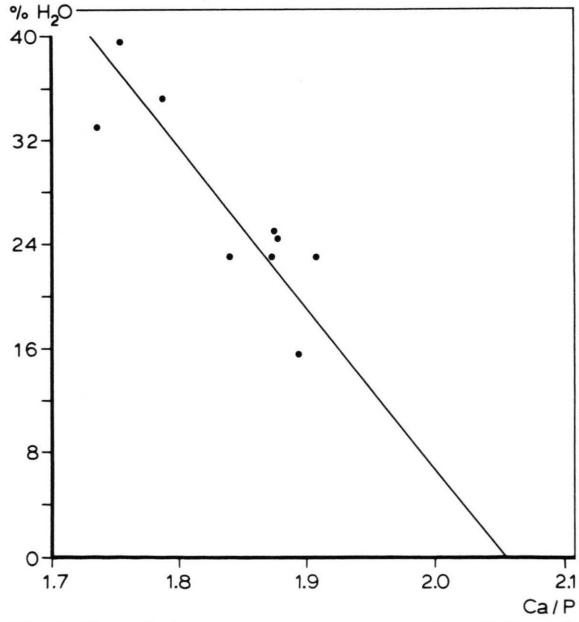

Fig. 1. Plot of the water content versus the Ca/P ratio calculated for the "rest phase" in different samples of bone mineral as determined by Biltz and Pellegrino [39].

of enamel mineral is very low, but in dentine and bone it is about 1% [42]. Although part of the citrate might be adsorbed onto the surface of the crystals like the amount occurring in tooth enamel, the larger part is certainly incorporated in the mineral of bone as was shown by the high correlation between citrate content and mineral content of bone samples [21]. Obviously, the citrate ion is incorporated in the phase with formula (11).

As mentioned in the introduction, the compounds proposed in formula (4) and (9) have the apatite and the octo calcium phosphate structure respectively. The compound of formula (11) most probably has one of these structures. The above mentioned evidences that citrate is incorporated in this compound obtains a special meaning in this respect. Within the structure of octo calcium phosphate (9) and also that of a defective apatite (4) two neighbouring HPO_4^{2-} or CO_3^{2-} ions occupy a space of nearly cylindrical form having a length of 9.5 Å and a diameter of 5.5 Å. These dimensions belong also to the citrate ion, whereas its electric charge is only slightly less.

The probability that two CO_3^{2-} ions in a defective apatite structure like that of formula (4) are neighbours is very small for electrostatic reasons. However, in the structure of an octo calcium phosphate like that of formula (9) they should be neighbours automatically as they would replace the HPO_4^{2-} ions rather than the PO_4^{3-} ions [19]. Further, there has not been reported a special interaction between apatites and citrate ions, but it is known that citrate ions stabilize the octo calcium phosphate structure [43]. For these reasons the octo calcium phosphate structure is preferred over that of apatite for the compound of formula (11). Octo calcium phosphate can form a sandwich structure with hydroxy apatite [44]. Similarly, most probably the compound of formula (11) will form a sandwich structure with the Na and CO_3 containing apatite in bone mineral and this might explain the paracrystalline nature of this mineral [17].

Pellegrino and Biltz [45] pointed out the high coefficient of correlation (0.95) between the Ca/P ratio and the CO_3 content of bone mineral. This is in complete agreement with the present analysis of literature data according to which the two carbonated phases have both a high Ca/P ratio and the non-carbonated phase has a low Ca/P ratio. In the authors view, however, there is no reason to assume the presence of a separate $CaCO_3$ phase [45], although part of the carbonate can be extracted from bone mineral with NH_4Cl solutions [39] and although the loss of CO_2 by heating of bone mineral shows some resemble to that of $CaCO_3$ [46]. As pointed out by Termine and Lundy [47] part of the carbonate in bone mineral may be very loosely bound so that by chemical analysis after isolation of bone mineral a composition like that of formula (11) is found for the "rest phase" although the composition *in vivo* may approach that of formula (9).

[1] H. H. Roseberry, A. B. Hastings, and J. K. Morse, J. Biol. Chem. **90,** 395–407 (1931).
[2] R. Klement and G. Trömel, Z. Physiol. Chem. **213,** 263–269 (1932).
[3] R. Klement and G. Trömel, Klin. Wochenschr. **12,** 292–294 (1933).
[4] R. Z. Legeros, O. R. Trautz, E. Klein, and J. P. Legeros, Experientia **24,** 5–7 (1969).
[5] G. Bonel and G. Montel, Compt. Rend. Ser. C. **263,** 1010–1013 (1966).
[6] G. Bonel, Ann. Chim. **7,** 127–144 (1972).
[7] G. Montel, Biol. Cell. **28,** 179–186 (1977).
[8] C. Vignoles, G. Bonel, and G. Montel, Compt. Rend. Ser. C. **280,** 361–364 (1975).
[9] H. G. Schaeken and F. C. M. Driessens, unpublished results.
[10] S. Gabriel, Z. Physiol. Chem. **18,** 257–303 (1894).
[11] A. S. Posner and S. R. Stephenson, J. Dent. Res. **46,** 257–264 (1953).
[12] W. F. Neuman and B. J. Mulryan, Calc. Tiss. Res. **7,** 133–138 (1971).
[13] E. Hayek and H. Newesely, Monatsh. Chem. **89,** 88–95 (1958).
[14] J. L. Meyer and E. D. Eanes, Calcif. Tiss. Res. **25,** 59–68 (1978).
[15] J. Vahl, H. Höhling, and R. M. Frank, Arch. Oral Biol. **9,** 315–320 (1964).
[16] H. Newesely, Experientia **19,** 620–621 (1963).
[17] E. J. Wheeler and D. Lewis, Calcif. Tiss. Res. **24,** 243–248 (1977).
[18] H. Newesely, Dtsch. Zahnärztl. Z. **24,** 484–485 (1969).
[19] W. E. Brown, Clin. Orthoped. **44,** 205–220 (1966).
[20] E. Hayek, Klin. Wochenschr. **45,** 857–863 (1967).
[21] F. C. M. Driessens, J. W. E. van Dijk, and J. M. P. M. Borggreven, Calcif. Tiss. Res. **26,** 127–137 (1978).
[22] J. Marek, O. Wellmann, and L. Urbanyl. Z. Physiol. Chem. **234,** 165–175 (1935).
[23] J. Marek, O. Wellmann, and L. Urbanyl, Z. Physiol. Chem. **226,** 3–17 (1934).
[24] S. Morgulis, J. Biol. Chem. **93,** 455–466 (1931).
[25] C. H. Kick, R. M. Bethke, and B. H. Edgington, J. Agr. Research **46,** 1023–1037 (1933).
[26] M. M. Murray, Biochem. J. **30,** 1567–1571 (1936).
[27] F. J. McClure and H. G. McCann, Arch. Oral Biol. **2,** 151–161 (1960).

[28] G. W. Burnett and J. A. Zenewitz, J. Dent. Res. **36,** 684–689 (1957).
[29] M. A. Logan, J. Biol. Chem. **110,** 375–389 (1935).
[30] R. Klement, Naturwissenschaften **26,** 145–152 (1938).
[31] E. D. Eanes, J. D. Termine, and A. S. Posner, Clin. Orthop. **53,** 223–235 (1967).
[32] J. D. Termine and A. S. Posner, Calc. Tiss. Res. **1,** 8–23 (1967).
[33] E. D. Eanes and A. S. Posner, Calc. Tiss. Res. **2,** 38–48 (1968).
[34] J. W. T. Dickerson, Biochem. J. **82,** 56–61 (1962).
[35] W. W. Swanson and L. V. Iob, Am. J. Diseases Children **54,** 1025–1029 (1937).
[36] R. Klement, Ber. Dtsch. Chem. Ges. **69,** 2232–2238 (1936).
[37] W. D. Armstrong, Clin. Orth. Rel. Res. **38,** 179–190 (1965).
[38] G. T. Vatassery, W. D. Armstrong, and L. Singer, Calcif. Tissue Res. **5,** 183–188 (1970).
[39] R. M. Biltz and E. D. Pellegrino, J. Bone Joint Surg. **51 A,** 456–466 (1969).
[40] E. D. Pellegrino and R. M. Biltz, Medicine **44,** 397–418 (1965).
[41] F. C. M. Driessens, to be published.
[42] I. Zipkin, Art and Science of Dental Caries Research (R. S. Harris, ed.), p. 29–41, Academic Press, New York 1968.
[43] J. L. Meyer, personal communication.
[44] W. E. Brown, L. W. Schroeder, and J. S. Ferris, J. Phys. Chem. **83,** 1385–1388 (1979).
[45] E. D. Pellegrino and R. M. Biltz, Nature **219,** 1261–1262 (1968).
[46] M. J. Dallemagne and C. Fabry, Bone Structure and Metabolism (G. E. W. Wolstenhome and C. M. O'Connor, eds.), p. 14–35, J. and L. Churchill, London 1956.
[47] J. D. Termine and D. R. Lundy, Calc. Tiss. Res. **13,** 73–82 (1973).