Calcif Tissue Int (1987) 41:137–144

Calcified Tissue International

Age-Related Changes in Mineral of Rat and Bovine Cortical Bone

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Summary. The mineral of cortical bones has been studied in newborn, growing, and adult rats and in the calf and cow, using X-ray diffraction and infrared spectroscopy during the thermal decomposition of bones and by microassay of carbonate. The mineral of all the bone samples, regardless of species or age, was found to be a calcium-deficient apatite containing both CO_3^{2-} and HPO_4^{2-} ions in the crystal lattice. The crystal size, Ca/P molar ratio, and CO_3^{2-} ion content of cortical bone all increased with increasing age in both the rat and the bovine. The Ca/P ratio varied from 1.51 in newborn rats to 1.69 in adults but remained that of Ca-deficient apatite even though its value was close to that of stoichiometric hydroxyapatite (1.67). Both the carbonate ion and the hydrogenophosphate ion contents varied from one animal species to another and with age within a given species. Maturation was correlated with an increase in carbonate ion content, which replaced the HPO_4^{2-} ions. In contrast, the calcium ion number per unit formula did not vary during maturation. Cortical bone mineral, in both species, regardless of age, can therefore be represented by the following formula: $Ca_{8.3}(PO_4)_{4.3}(CO_3)_x(HPO_4)_y(OH)_{0.3}$; y decreased and x increased with increasing age, (x + y) being constant, equal to 1.7.

Key words: Cortical bone mineral — Aging — Apatite — CO_3^{2-} ions — HPO_4^{2-} ions.

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Many studies have been published on the mineral component of bone. It has been shown [1–4] that the Ca/P molar ratio increases with age, rising from 1.43 in young animals to 1.70 in adults. This change led some authors [2, 4] to conclude that bone mineral approaches the ideal hydroxyapatite (HA) stoichiometry (Ca/P = 1.67) at maturity. The low Ca/P ratio found in young animals has been explained by assuming that the initial phase in calcified tissues is not HA but a precursor having a low Ca/P ratio. This precursor would develop by hydrolysis, with the Ca/P ratio increasing towards that of HA. Many hypotheses have been proposed as to the nature of this precursor. It has been suggested that amorphous calcium phosphate (ACP) is the initial phase formed in the early stages of mineralization [5, 6]. Octacalcium phosphate, $Ca_8H_2(PO_4)_6$, $5H_2O$ has also been considered to be the precursor of apatite in bone and teeth [7, 8], while brushite has been suggested for the same role in embryonic bone [9]. However, recent studies [10] from the same laboratory indicate that the crystalline brushite previously identified in poorly mineralized embryonic bone [9] was an artifact resulting from the fractionation process, and that there are no significant amounts of ACP in embryonic bone [4]. Thus, the nature of the initial mineral phase has not yet been clearly defined and remains the topic of some discussion.

Although it may not be necessary to postulate nonapatitic phases to account for the low Ca/P ratio in embryonic bone and during the initial stages of mineralization, the existence of nonapatitic phases in bone mineral during the postnatal period cannot be excluded. In earlier studies on the bone mineral of growing rabbits and rats [11, 12] we found only one mineral phase. This was an apatite which was deficient in Ca ions and had Ca/P ratios whose values depended upon the HPO_4^{2-} and CO_3^{2-} ion

contents. Other published reports have examined the age-related changes in the Ca/P molar ratio [1–4], crystal size [13–15], and the CO_2 ion content. Nevertheless, the relationship between these characteristics and the chemical and structural nature of bone mineral during growth and maturation remains poorly understood.

We have attempted to resolve some of these ambiguities in the present study by using physicochemical methods to analyze the structural and chemical composition of cortical bone from two species, the rat and bovine. Bone samples were taken at different stages of postnatal growth, during the growing period, and at maturity.

Materials and Methods

Preparation of Cortical Bone Samples

Long bones, including tibias, femurs, and humeri, were removed from 40 newborn rats, 20 growing male rats (30 days old), 15 mature male rats (1 year old), 2 calves (4 months old), and one cow (7 years old), and were prepared as previously described [16]. Briefly, the bones were removed immediately after sacrifice and carefully stripped of all surrounding fibrous tissue. They were then dried in a vacuum dessicator at room temperature and sawed into 150–200 μm slices. The diaphysis cortical bone was removed by microdissection and ground for 20 min in a percussion mill (model 6700 Freezer Mill. Spex Industries) operating in liquid nitrogen. The mill was operated intermittently to avoid heating. The bone was reduced to 1–10 μm particles (average 5 μm, determined microscopically).

Analytical Methods

The thermogravimetric experiments were carried out with a Setaram MTB 10-8 microanalyser at $360h^{-1}$ under a dynamic air flow. Powder X-ray diffraction (XRD) patterns were photographically recorded on a Seeman-Bohling Camera (CGR diameter 114 mm). The Cokα radiation (λCo $K\bar{\alpha}$ = 1,7902 Å) was selected using a quartz curved crystal monochromator. The fine homogeneous sample was glued onto Mylar foil to form thin pellets. Typical exposure times were between 3 and 4 hours.

Bone crystal size was followed by half width (width at the half maximum intensity β) measurement of 002 apatite reflexion β_{002}. The half-widths of the diffraction lines were obtained by scanning the photographs with a double-beam recording microdensitometer (M K III C Joyce Loebl and Co, Ltd). These measurements do not take the lattice strain effect into consideration; however, they give an estimate of the average size of the long axis (C-axis). Measured widths were corrected for instrumental broadening by subtracting the square of β_{002} for a fully crystalline HA from the square of the sample value and taking the square root of the difference. L values were calculated from these measurements using the SHERRER equation [17]:

$$L = \frac{k\lambda}{\beta\frac{1}{2}\cos\theta}$$

where λ is the X-ray wavelength and θ the diffraction angle. $\beta\frac{1}{2}$ is the peak width at half height and K is a constant approximately equal to 1, but varying slightly with crystal shape. A value of 0.94 was chosen for the elongated crystallites of bones. Precision was estimated to be about ± 3% of L from duplicate measurements on the same batch of bone.

Ca/P molar ratios were determined by analysis of the X-ray diffraction (XRD) pattern of powdered bone tissues heated to 900°C, as previously described [16]. At this temperature the bone mineral was recovered as a mixture of two well-crystallized phases, HA and β tricalcium phosphate (TP). These could be considered to be approximately stoichiometric. The Ca/P molar ratio was therefore related to the proportions of HA and TP in mixture at 900°C. Other cations, such as Mg^{2+}, could be neglected [16]. As the proportions of HA and TP are a function of the relative intensity of the specific HA and TP diffraction lines, the Ca/P ratio could be obtained by measuring the amounts of both components in the mixture. The coefficient of variation was 0.3%.

The infrared spectra (IR) of all specimens were determined on a Perkin Elmer 457 instrument operating in the absorbence mode. The samples were mixed with potassium bromide (1 mg sample/300 mg KBr) and pressed into thin discs.

The carbonate ions content of samples was determined by a technique based on gas phase chromatography of the traces of carbon dioxide formed when calcified tissue is treated with acid [18]. The chromatographic determination could be carried out on a few milligrams of sample with reproducible results: the coefficient of variation was 2%.

Calculation of the HPO_4^{2-} and OH^- Ion Contents

The well-known structure of synthetic apatites provides a reference for the study of the structure of bone apatites as they have many features in common: they all contain Ca^{2+}, PO_4^{3-}, HPO_4^{3-}, CO_3^{2-} and OH^- ions. In 1963, Kühl and Nebergall [19], confirmed in 1982 by Meyer and Fowler [20], proposed that calcium-deficient apatites which contain HPO_4^{2-} ions could correspond to the following formula:

$$Ca_{10-y-y'}(PO_4)_{6-y}(HPO_4)_y(OH)_{2-y-2y'} \quad \text{with } 0 \leq y \leq 2, \; y' \leq 1 - y/2$$

However, for calcium-deficient apatite which contains CO_3^{2-}, Kull and Nebergall found that there was a partial saturation of defects by addition of Ca^{2+} and $2OH^-$, and proposed the following general formula, which has been confirmed by Bonel et al. [21] and Labarthe et al. [22] for the carbonated deficient apatite:

$$Ca_{10-x+x'}(PO_4)_{6-x}(CO_3)_x(OH)_{2-x+2x'} \quad 0 < x < 2, \; x' \leq x/2$$

When the ions CO_3^{2+} and HPO_4^{2-} are both present in the apatite structure, we assume that the opposite effect of the terms x′ and y′ made it possible to consider as negligible the Ca^{2+} $2OH^-$ vacancy compensation.

By analogy with synthetic apatites, the mineral of cortical bone containing HPO_4^{2-} and CO_3^{2-} ions corresponds to the following general formula:

$$Ca_{10-x-y}(PO_4)_{6-x-y}(HPO_4)_y(CO_3)_x(OH)_{2-x-y}$$

As the Ca/P molar ratio and the CO_3^{2-} content are known, it is possible to calculate the value of the parameters x and y, and thus the values of HPO_4^{2-} and OH^- ions.

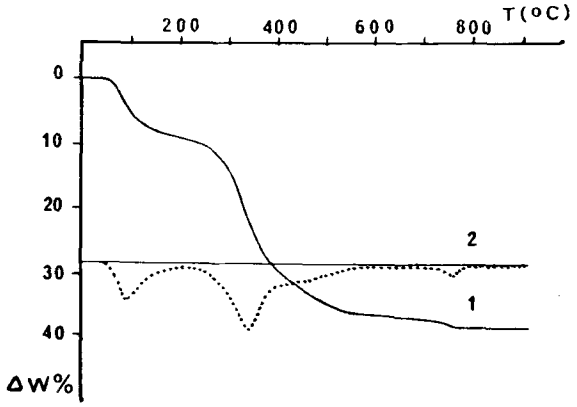

Fig. 1. Calf cortical bone thermogram. (1) ΔW/dt, (2) differential of curve 1 d(ΔW)/dt.

Results

The thermogram of calf cortical bone showed that cortical bone lost weight in three steps when heated to 900°C (Fig. 1). The weight losses and the ash contents at 900°C for each bone cortical sample are given in Table 1. Ash content increased with age from birth to adult in the rat.

The X-ray diffraction patterns of all nonheated samples studied were very diffuse, even in cortical bone of the 7-year-old cow (Fig. 2). The 002 line and the envelope of the 211-113-300 lines indicated that the mineral of cortical bone had the structure of a poorly crystallized apatite regardless of the age of the animals. No other mineral phase was detectable.

The apparent crystal size increased with the age for the two species studied (Table 1).

When rat cortical bone samples were heated to 900°C, the mineral was found to be transformed into two crystalline phases, as shown by the X-ray diffraction patterns. These two crystalline phases were clearly identified as HA and β-TP by X-ray diffraction. The Ca/P molar ratios, calculated from the proportion of these HA and TP at 900°C, indicate an increase in this ratio with age in rats (Table 2). Similarly, the cortical bone samples from the calf decomposed into HA and TP at 900°C. But in the cow, the cortical bone mineral was decomposed into HA and CaO at 900°C. These decomposition products were always typical of apatites, but of apatites in which the Ca/P ratio is greater than 1.67 [22]. The strongest line of the CaO diagram (d = 2.41; I = 100) was very weak compared to the intensity of the line d = 2.72 of the apatite (I = 75), indicating that the quantity of CaO formed at 900°C was very small. By comparison with synthetic mixtures of HA and CaO, this value was about 2% by weight, corresponding to a Ca/P molar ratio of about 1.69 (Table 2).

The IR spectra of unheated calf cortical bone (Fig. 3) and of all species studied at different ages appear to be identical to that of synthetic B-type carbonated apatite, in which carbonate ions are substituted for phosphate ions. The presence of CO_3^{2-} ions is demonstrated by the absorption bands at 875, 1410, and 1460 cm^{-1} (Fig. 3). These carbonate bands disappeared completely on heating to 750°C, indicating that the carbonate ions were then totally eliminated. Moreover, the IR absorption band at 875 cm^{-1} is characteristic not only of CO_3^{2-} but also of HPO_4^{2-} ions. Table 2 indicates the CO_3^{2-} content of all unheated samples and the carbonate content of bone ashes obtained from the sample weight loss during ignition at 900°C. Carbonate content appeared to increase with age.

As the Ca/P molar ratios and the CO_3^{2-} ion content are known, the number of HPO_4^{3-} ions (Y) and CO_3^{2-} ions (X) per unit formula were also calculated. The results are shown in Table 2. Like the carbonate ion contents, the number of CO_3^{2-} per unit formula increased with age. In contrast, the number of HPO_4^{2-} ions per unit formula decreased with age.

From the general formula proposed for the mineral of cortical bone

$$Ca_{10-x-y}\,(PO_4)_{6-x-y}\,(CO_3)_x\,(HPO_4)_y\,(OH)_{2-x-y}$$

the formula for each cortical bone studied at various ages had been calculated and is reported in Table 3.

From Table 3 it was ascertained that Ca^{2+} ions per unit formula were constant, mean 8.32 ± 0.04, regardless of species or age. The number of PO_4^{3-} ions per unit formula were also constant: 4.32 ± 0.04. The number of OH^- ions per unit formula were constant: 0.32 ± 0.04.

Discussion

The mineral of the cortical bone of newborn, growing, and adult rats, calves, and cows was found to be poorly crystallized and characterized by an apatitic structure deficient in Ca ions and containing both CO_3^{2-} and HPO_4^{2-} ions. The crystal size, Ca/P molar ratio, and CO_3^{2-} ion contents of cortical bone increased with increasing age in both species. These results are consistent with previous reports [1–4] and [13–15].

The X-ray diffraction patterns of unheated rat and bovine cortical bone have the characteristics of

Table 1. Weight loss during thermal decomposition; ash content at 900°C and crystal size of rat and bovine cortical bone

Animals	Weight loss			Ash % at 900°C	Crystal size Å
	20–200°C	200–600°C	600–900°C		
Rat					
at birth	11.9 ± 1.1	37.0 ± 1.1	1.4 ± 0.5	50 ± 2	97 ± 3
30 days old	12.1 ± 0.8	26.3 ± 1.1	1.3 ± 0.4	60 ± 1	133 ± 4
1 year old	10.3 ± 0.5	23.0 ± 0.8	2.0 ± 0.4	65 ± 1	157 ± 4
Bovine					
calf 2 months	9.8 ± 1.9	26.0 ± 0.5	2.3 ± 0.4	62 ± 2	91 ± 3
7 years	9.1 ± 1.2	26.4 ± 0.8	2.3 ± 0.4	62 ± 2	206 ± 6

The results are expressed as mean ± SEM. Number of determination of each samples: 4 for rat at birth and 6 for all others

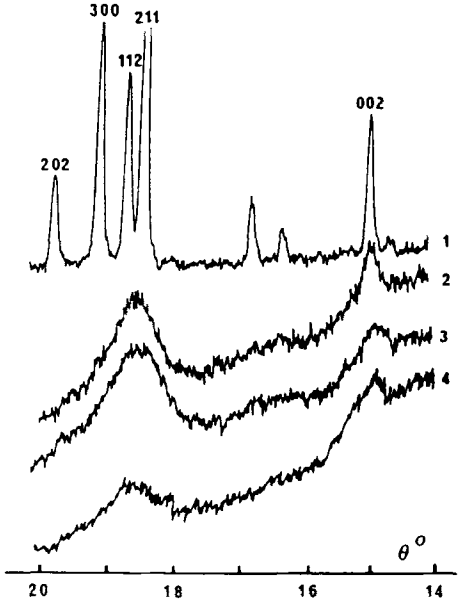

Fig. 2. Photometric tracings of X-ray powder diffraction patterns of synthetic apatite and various cortical bones. (1) Hydroxyapatite, (2) cow 7 years old, (3) calf 2 months old, (4) rat at birth. Powder diffraction patterns were photographically recorded on a Seeman-Bohlin Camera. The patterns of the diffraction lines were obtained by scanning the photographs with a double beam recording microdensitometer.

a poorly crystalline apatite. IR analysis also indicated that the unheated mineral corresponded to a B-type carbonated apatite with the CO_3^{2-} ions located at the PO_4 sites. No other mineral phase has been detected. However, the possibility of another, amorphous mineral phase, which would not be detected by the methods used, X-ray diffraction and thermogravimetry, could not be neglected. The concept of a single mineral phase has been debated for a number of years and there has been considerable interest in the possibility of amorphous calcium phosphate (ACP) as a bone component. Termine and Posner [5, 6] have suggested that as much as half the mineral in bone may be in the form of ACP. However, Posner and Betts [23] concluded that "fully developed bone mineral cannot contain over 10% of a phase which is closely analogous to synthetic ACP," and attributed earlier high apparent ACP contents to problems in the preparation of crystalline standards. As long ago as 1973, Steve-Bocciarelli et al. [24] showed, in an electron diffraction study of selected areas of periosteal bone, that there were no differences in the intensities obtained from zones almost completely covered with low density, supposedly amorphous, material and those of adjacent zones rich in normal crystalline forms. More recent support for the presence of a single bone mineral phase has been provided by Grynpas et al. [4] by their determination of the radial distribution of atoms in the mineral phase of calcified tissue. The results of the present study on cortical bones at different ages are in full agreement with these findings, demonstrating the presence of only one mineral phase in cortical bone. Moreover, the simultaneous phenomena observed during the thermal behavior of all the cortical bone samples studied, crystallization and loss of CO_3^{2-} ions between 500 and 900°C, indicate that both phases which were found at 900°C were formed from only a single original mineral phase by breakdown of the poorly crystallized initial carbonated apatite during ignition. The presence of a single mineral phase was also clearly shown in a previous study on periosteal bone of growing rabbits by electron diffraction [12].

The presence of CO_3^{2-} ions in the apatitic mineral phase of cortical bone was clearly indicated by the IR spectra, which also showed that these CO_3^{2-} ions were located in the B-sites of the apatite structure. These results are in agreement with a number of published findings [25–28]. The presence of CO_3^{2-} ions could be related to the poor crystal-

Table 2. Ca/P molar ratio; CO_3^{2-} ion contents in unheated and ashed cortical bones

Animals	Ca/P (at 900°C)	% CO_3^{2-} (unheated)	% CO_3^{2-} (at 900°C)	x[a]	y[b]
Rat					
at birth	1.51 ± 0.003	1.8 ± 0.3	3.6 ± 0.3	0.51 ± 0.04	1.20 ± 0.03
30 days	1.60 ± 0.003	3.2 ± 0.1	5.3 ± 0.2	0.80 ± 0.03	0.88 ± 0.02
1 year	1.65 ± 0.002	4.7 ± 0.2	7.4 ± 0.2	0.99 ± 0.03	0.74 ± 0.02
Bovine					
calf 2 months	1.65 ± 0.004	4.1 ± 0.2	6.6 ± 0.2	0.92 ± 0.03	0.70 ± 0.02
7 years	1.69 ± 0.010	4.7 ± 0.1	7.6 ± 0.2	1.06 ± 0.03	0.59 ± 0.02

[a] x = number of CO_3^{2-} ions and [b]y = number of HPO_4^{2-} ions per unit formula

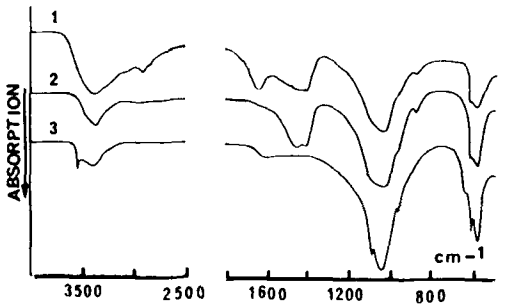

Fig. 3. Infrared absorption spectra of calf cortical bone. (1) Unheated sample, (2) heated at 500°C for 2 hours, (3) heated at 900°C for 2 hours.

Table 3. Formulae of cortical bone mineral

Animals	Bone mineral formulae
Rat	
at birth	$Ca_{8.29}(PO_4)_{4.29}(HPO_4)_{1.20}(CO_3)_{0.51}(OH)_{0.29}$
30 days	$Ca_{8.32}(PO_4)_{4.32}(HPO_4)_{0.88}(CO_3)_{0.80}(OH)_{0.32}$
1 year	$Ca_{8.27}(PO_4)_{4.27}(HPO_4)_{0.74}(CO_3)_{0.99}(OH)_{0.27}$
Bovine	
calf 2 months	$Ca_{8.38}(PO_4)_{4.38}(HPO_4)_{0.70}(CO_3)_{0.92}(OH)_{0.38}$
7 years	$Ca_{8.35}(PO_4)_{4.35}(HPO_4)_{0.59}(CO_3)_{1.06}(OH)_{0.35}$

Mean Ca ± SEM = 8.32 ± 0.04; mean PO_4 ± SEM = 4.32 ± 0.04; mean OH ± SEM = 0.32 ± 0.04

linity of mineral in cortical bones. The latter may be due to the existence of defects in the crystal lattice caused by the presence of such ions as carbonate ions [29]. Moreover, the CO_3^{2-} ion content varies, increasing with age from 1.8% in the newborn rat to 4.7% in the adult. The increased carbonate ions content could be related to variations in crystal size; the latter was also found to increase with the age of the animal, in agreement with published results [14, 15]. This relationship seems to contradict the observations made on synthetic apatites which show that carbonate incorporation in the lattice reduces the size of crystallites [30–33]. However, the inconsistency is only apparent. The carbonate content of synthetic apatites varies between 2 and 20%, whereas the range is much smaller for cortical bones; that of newborn rat cortical bone is 3.6% and the carbonate content of one-year-old rat cortical bone is only 7.4%. The small variation in carbonate ion content parallels the relatively restricted increase in crystallite size. Moreover, whereas the crystallite size in cortical bone doubles from the young calf to the 7-year-old cow, the mineral content of the ash remains the same, suggesting a decrease in the number of crystallites in cortical bone during maturation and aging. This could indicate that, during growth and bone maturation, small crystals could be dissolved and replaced by larger crystals which are known to be thermodynamically more stable.

The decomposition products at 900°C were used to obtain the Ca/P ratios. These ratios were found to increase with age. They varied from 1.51–1.69 at birth to one year of age in the rat, and were significantly lower than that of synthetic B-type carbonated apatite (1.68–2.0). Such an increase in Ca/P values with age agrees with previous reports for human bone [3] and chick diaphysis [1, 4]

The thermal behavior during thermogravimetric studies also demonstrates the presence of HPO_4^{2-} ions in the cortical bones mineral. Their presence is suggested by the differences observed between the thermal decomposition products of the cortical bones heated to 900°C and those of synthetic B-type carbonated apatite. The synthetic carbonate apatite ($Ca/P > 1.67$) breaks down on heating to give a mixture of HA and CaO [21, 22] whereas cortical bones ($Ca/P < 1.67$) decomposed at 900°C to HA and TP. These differences can be attributed to the substitution of some PO_4^{3-} ions by HPO_4^{2-} ions in the unheated mineral apatitic phase of bone. Hydrogenophosphate ions were detected in the cortical bones of both species studied and at all the studied ages.

These results agree with those previously found on HPO_4^{2-} ions in the mineral of chicken diaphyses by Raman microprobe analysis [34] and nuclear magnetic resonance [35] and in the periosteal bone of rabbits by IR spectroscopy [12].

From the cortical bone mineral formulae established at different ages, as indicated in Table 3, most important new points emerge. First, the number of Ca ions does not change during bone maturation and aging in a given species. This number of Ca ions is the same from one species to another. The mean Ca value is 8.32 ± 0.04. This value is lower than that of stoichiometric HA (Ca: 10.0) and is similar to the value for octacalcium phosphate (Ca:8.0) described by Brown et al. [7] and Chickerur et al. [8]. Thus, the cortical bone mineral, whatever the age and the species, is always a Ca-deficient apatite.

The CO_3^{2-} and HPO_4^{2-} ions are located at the same crystallographic sites (XO_4) as the PO_4^{3-} ions in the apatitic structure. The ratio Ca/XO_4 defines the stoichiometry of the apatite. This calculated ratio (see Table 4) is identical for all the cortical bone studied. Its value, 1.39, corresponds to that of Ca ion-deficient apatite. The total calcium, phosphorus (expressed as PO_4^{3-}), and carbonate ion contents of bone mineral from very different animal species have been given in a number of publications. Some are reported by Biltz and Pellegrino [36]. These data have been used to calculate the Ca/XO_4 ratio for each result (see Table 5), assuming that XO_4 is equal to $PO_4 + CO_3$. The mean value obtained is 1.41, which corresponds well with the average value of the Ca/XO_4 ratio (1.39) obtained in this work for the rat and bovine cortical bone at different ages. The calcium, phosphorus, and carbonate ion contents of the whole bone mineral can be deduced using the formula for a Ca-deficient apatite.

In contrast to the calcium level, which remains constant during aging, the number of calculated hydrogenophosphate ions (HPO_4^{2-}) varies inversely as the animal's age: for the rat it decreases from 1.20 at birth to 0.88 in growing animals and reaches 0.74 in 1-year-old rats. These results are in agreement with those of Roufosse et al. [35], who found that the HPO_4^{2-} fraction was highest in the diaphyses of 17-day-old embryonic chicks and decreased progressively with increasing age until the chicks were 1 year old.

A correlation may be established between the variation of HPO_4^{2-} and CO_3^{2-} ions, so that

$$\text{number } HPO_4^{2-} = -1.07 \text{ number } CO_3^{2-} + 1.74$$

with a correlation coefficient equal to -0.98 ($P < 0.001$). Thus, in the course of bone aging, one carbonate ion replaces one hydrogenophosphate ion, while the total number of CO_3^{2-} and HPO_4^{2-} ions remains constant and equal to 1.7. We have previously shown a similar relationship between these ions in the growing rabbit, cat, and rat [12]. Consequently, it is possible to propose a general formula which represents the mineral of cortical bone phase and which is independent of the animal species:

$$Ca_{8.3}(PO_4)_{4.3}(CO_3)_x(HPO_4)_y(OH)_{0.3}$$
$$\text{with } x + y = 1.7$$

These results are consistent with those found by Pellegrino and Biltz [1] in developing chick bone. These authors also noted a correspondence between HPO_4^{2-} and CO_3^{2-} ions.

Thus, the orthophosphate ions (PO_4^{3-} + HPO_4^{2-}), whose number per unit formula has been obtained from the proposed formulae and placed in Table 4, also vary according to age. Their number decreases during bone maturation. These variations in orthophosphate ions are sufficient to explain the increase in Ca/P ratio with age. This increase is in no way linked to an increase in Ca ion per unit formula but to a decrease of orthophosphate ion number during aging. The orthophosphate ions are replaced by carbonate ions.

We conclude that the mineral phase of cortical bone in the rat remains a calcium-deficient apatite containing both CO_3^{2-} and HPO_4^{2-} ions throughout growth and aging. The Ca/P ratio, which varies from 1.51 at birth to 1.69 in the adult, remains that of Ca-deficient apatite even though its value is close to that of stoichiometric hydroxyapatite (1.67). Ca/P increases with age, but the change is not due to content of Ca, which remains con-

Table 4. Number of (PO_4^{3-} + HPO_4^{2-}) ions and Ca/XO_4 ratio calculated from the formulae given in Table 3

Animals	Number of (PO_4^{3-} + HPO_4^{2-})	Ca/XO_4	Ca/P
Rat			
at birth	5.49	1.38	1.51
30 days	5.20	1.39	1.60
12 months	5.01	1.38	1.65
Bovine			
calf 2 months	5.08	1.40	1.65
7 years old	4.94	1.39	1.69
Mean + SEM		1.39 ± 0.01	

General formula of apatite: $Ca_{10}(XO_4)_6\,Y_2$
$XO_4 = PO_4^{3-} + HPO_4^{2-} + CO_3^{2-}$
$Y = OH^-$

Table 5. Calculation of Ca/XO_4 ratio for the principal mineral constituents of whole bone

	Values taken from Table 1 of Biltz and Pellegrino [36]				Values calculated from this study
		mol/g			
Source	Species	Ca	PO_4	CO_3	$Ca/PO_4 + CO_3$
Levy 1894	Man	6.25	3.58	.88	1.40
Gassmann 1910	Man	6.12	3.56	.53	1.50
Howland et al. 1926	Man	6.25	3.74	.82	1.37
Klement 1928	Bovine	6.03	3.40	.67	1.48
Bogert and Hastings 1931	Bovine	6.63	3.86	.91	1.39
Follis 1952	Man	6.42	3.76	.76	1.42
Eastoe 1961	8 vertebrates	6.41	3.81	.84	1.38
Taylor and Moore 1956	Chicken	5.60	3.29	.60	1.44
Agna et al. 1958	Man	6.25	3.58	.85	1.41
Armstrong and Singer 1965	Bovine	6.68	4.02	.79	1.39
Zipkin 1970	Man	5.63	3.32	.80	1.37
Biltz and Pellegrino 1969	16 vertebrates	6.56	3.81	.86	1.40
Mean value		6.24	3.64	.76	1.41
SEM					± 0.04

stant. It is the phosphate ion content that decreases with maturation. Both the carbonate and hydrogenophosphate ion contents vary from one animal species to another, and with age within a given species. Maturation is accompanied by an increase in carbonate ions which replace HPO_4^{2-} ions, while the number of calcium ions per unit formula does not vary. The cortical bone mineral can therefore be represented by the following formula regardless of the animal species or age:

$$Ca_{8.3}(PO_4)_{4.3}(CO_3)_x(HPO_4)_y(OH)_{0.3}$$

$$X + Y = 1.7$$

Acknowledgments. We are indebted to Dr. O. Parkes for revising the English of the text, and wish to acknowledge the skillful secretarial assistance of M. Courat.

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Received July 11, 1986, and in revised form February 3, 1987.