RENEWAL OF PHOSPHATE IN BONE MINERALS

I. RENEWAL RATE OF PHOSPHATE IN RELATION TO THE SOLUBILITY OF THE BONE MINERALS

by

ROLF ZETTERSTRÖM

with the technical assistance of MARGARETA LJUNGGREN

Department of Metabolic Research, Wenner-Gren Institute, Stockholm (Sweden)

Almost immediately after the introduction of radioactive phosphate into the circulation, some tracer phosphate can be found in the calcified tissues[1]. The apatite structure in epiphyseal bones has a higher uptake of radioactive phosphate than in diaphyseal bones[2–4]. In both structures, however, the uptake of labeled phosphate takes place much too rapidly to account for new calcification or rebuilding of the skeleton. As an explanation for this fact the hypothesis was put forward[5–7] that the exchange between bone phosphate and phosphate in surrounding fluids was due to a replacement process between the phosphate ions situated on the surfaces of the apatite crystals and those of the plasma, the atoms situated inside the crystals being prevented from doing so. The differences in uptake of labeled phosphate between different structures of bone and between bone and enamel were interpreted as due to differences in crystal size[5,7,8] and to differences in the contact between bone minerals and circulating fluid[5,7]. A rapid ion exchange on the crystal surfaces and a much slower penetration into the inner parts of the crystals could also explain the observation of MANLY, HODGE AND MANLY[9] that bones have a dual composition; one part rapidly attaining an equilibrium with orthophosphate in plasma, the other reaching equilibrium very slowly.

The inorganic part of bone cannot be considered as to be a distinct chemical compound of constant chemical composition[10]. Alterations and substitutions take place in the lime throughout the development of newly deposited bone[10,11]. Furthermore the relative content of carbonate is quite variable and seems to depend on definite factors such as age, disease and composition of the diet[12–16]. Since the uptake of labeled phosphate in the skeleton cannot be accounted for by surface reactions[17] only it seems reasonable that different fractions of bone minerals, having different composition, also differ in renewal rate.

This report gives evidence that it is possible to extract different fractions of bone minerals with different renewal rate with the aid of salt solutions.

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EXPERIMENTAL

As experimental animals male white rats or rabbits have been used. In experiments on rats, the animals were injected intraperitoneally with 0.2–0.3 millicuries (mc) of radioactive phosphate (^{32}P) as Na_2HPO_4, containing negligible amounts of carrier phosphate. In experiments on rabbits, the animals were given an intraveneous injection of 2 mc of ^{32}P. The animals were sacrificed by decapitation at intervals ranging from 1.5 to 45 hours after the injection. Blood was collected from the carotid arteries and immediately deproteinized with the same volume of ice-cold 15% (wt/vol) trichloroacetic acid (TCA). The femurs and tibiae were removed and dissected free of flesh and connective tissue; the diaphyseal and epiphyseal bone were separated and roughly freed of metaphyseal tissue. The diaphyseal bone was freed of marrow as much as possible. The bone specimens were then frozen with the aid of solid carbon dioxide and were pulverized in a chilled mortar in the frozen state.

The bone powder obtained, about 150–300 mg, was extracted, at −10° C, with 3 ml of saturated ammonium sulphate of pH 7.5 with the aid of steel homogenizer*. The extraction time was about 10 min. The samples were then filtered through Büchner funnel (4 cm) and the filters were washed three times with 3 ml of saturated ammonium sulphate. Following this first extraction the residue together with the filter paper was reextracted with 4 ml of 15% TCA, stirring with a glass rod driven by compressed air. The solution was filtered off and the residue washed twice with 2 ml of 15% TCA. Extracts and washings from the two fractions were combined and made up to a definite volume, suitable for analytical purposes.

By this method, there is no possible means of obtaining absolute and quantitative separation of two distinct fractions. With saturated ammonium sulphate a part of the rest fraction also seems to be dissolved. The solubility of the first fraction is, however, much greater than that of the remaining one. For this reason the predominant part of the mineral salts dissolved in ammonium sulphate belongs to the most soluble material, when limited amounts of the extraction solution are used. However, if the amount of bone powder extracted is too small in relation to the extractant, a greater part of the rest fraction will be dissolved in the first extraction solution**.

In some experiments the bone powders were extracted with acetate buffers of varying ionic strength prior to extraction with saturated ammonium sulphate. These extractions were performed exactly in the same way as the extraction with ammonium sulphate.

Ashed bone samples, freed of the organic matrix, were obtained by consecutive 24 hours' extraction with ethanol and ether in a Soxhlet apparatus followed by boiling in 3% potassium hydroxide in ethyleneglycol[18]. In some cases fat was removed by extraction of the powder with chloroform overnight. Ignition was carried out by heating the powder in an oven at about 700° C until the weight remained constant.

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* Later experiments have shown that a better extraction can be obtained when bone powder is extracted together with quartz sand by using a glass rod driven by compressed air.

** As a reference it can be mentioned that about 100 μg P can be dissolved from $Ca_3(PO_4)_2$ per ml of saturated ammonium sulphate, at −10° C. Under the same conditions it has been possible to extract up to 1 mg P per ml ammonium sulphate from bone powder.

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ANALYTICAL METHODS

From the blood extract the bulk of TCA was removed by three shakings with ether; ether was then removed by aeration. The specific activity (*i.e.*, number of impulses/μg P) of the orthophosphate was determined by a previously described method[19], based upon the principle of MARTIN AND DOTY[20]. The measurements of radioactivity were carried out in a Scaler 64 Electronic Counter (A/S Brüel a. Kjaer, Copenhagen, Denmark), yielding $3 \cdot 10^8$ counts per min per mc ^{32}P.

Phosphate in the bone extracts was determined by the method of FISKE AND SUBARROW[21], the concentration of ammonium sulphate in the diluted extracts being too low to interfere with the color development. Radioactivity was determined as follows: an aliquot, usually 0.5 ml, was pipetted into an aluminium dish and dried under an infrared lamp, activity then being measured under the counter*.

Calcium was determined in the Beckman flame spectrophotometer at wavelength 4227 A. Citrate was determined by the pentabromacetone method[22].

RESULTS

Table I shows the specific activity of the ammonium sulphate fraction and the remaining fraction in epiphyseal and diaphyseal bone in relation to the specific activity of orthophosphate in the blood** (relative specific activity). Young rats were sacrificed at different time intervals after injection of ^{32}P. The table clearly demonstrates that in short period experiments the fraction which could be extracted with saturated ammonium sulphate had a much higher uptake of labeled phosphate than the remaining part of the bone minerals. In each of the two fractions the uptake of ^{32}P was greater in epiphyseal than in diaphyseal bone. In long period experiments the relative specific activities of both fractions showed a tendency to approach the same value.

TABLE I

RELATIVE SPECIFIC ACTIVITY OF PHOSPHATE IN THE AMMONIUM SULPHATE FRACTION AND IN THE REMAINING FRACTION OF EPIPHYSEAL AND DISPHYSEAL BONE MINERALS AT DIFFERENT TIME INTERVALS AFTER THE INJECTION OF ^{32}P

Specific activity of orthophosphate in blood = 100. Young rats, about 4 weeks old, were used for the experiments.

Hours after injection of P^{32}	Relative specific activity			
	Epiphyseal bone		Diaphyseal bone	
	$(NH_4)_2SO_4$ fraction	Remaining fraction	$(NH_4)_2SO_4$ fraction	Remaining fraction
2	26	5.8	14	1.6
10	23	13	17	3.7
29	46	22	24	9.0
45	55	44	40	22

The very high uptake and rapid turnover of bone phosphate in the experiments referred to in Table I was due to the fact that young animals were used; the renewal rate of phosphate in the skeleton being more rapid in actively growing bones than in

* Control experiments showed that the amount of esterified radioactive phosphate was negligible, thus being without influence upon the determinations of specific activity.

** It would have been more correct to calculate this value in relation to orthophosphate in plasma, since plasma orthophosphate is the precursor of bone phosphate. However, the error introduced is negligible as blood corpuscles have an extremely low content of orthophosphate[23].

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adult bones[17]. As is shown in Table II, the rate of renewal of both fractions declined with increasing age of the animal in epiphyseal as well as in diaphyseal bone.

TABLE II

RELATIVE SPECIFIC ACTIVITY OF PHOSPHATE IN THE AMMONIUM SULPHATE FRACTION AND IN THE REMAINING FRACTION OF EPIPHYSEAL AND DIAPHYSEAL BONE MINERALS IN RATS OF DIFFERENT AGE

Specific activity of orthophosphate in blood = 100. The animals were injected with ^{32}P 15 hours before sacrificing

Weight of the animal g	Relative specific activity			
	Epiphyseal bone		Diaphyseal bone	
	$(NH_4)_2SO_4$ fraction	Remaining fraction	$(NH_4)_2SO_4$ fraction	Remaining fraction
51	31	8.5	27	5.7
77	24	5.7	15	3.3
133	16	2.4	13	2.0
160	16	2.5	10	1.5

According to MANLY, HODGE AND MANLY[9] the "labile" part of bone minerals, *i.e.*, the part which rapidly comes to equilibrium with plasma orthophosphate, can account for $^1/_5$ of the total content of phosphate in the skeleton. The value is, however, somewhat higher in epiphyseal than in diaphyseal bone. Table III shows the amount of bone phosphate in the ammonium sulphate fraction relative to that in the remaining fraction, in experimental animals of different ages. A greater part of the bone minerals could be extracted with saturated ammonium sulphate from epiphyseal bones, than from diaphyseal bones. The relative amount of phosphate extractable with ammonium sulphate decreased with increasing age of the animal.

TABLE III

RELATIVE AMOUNT OF THE AMMONIUM SULPHATE FRACTION IN EPIPHYSEAL AND DIAPHYSEAL BONE IN RATS OF DIFFERENT AGE

Weight of the animal g	Epiphyseal bone Percentage	Diaphyseal bone Percentage
51	52	44
77	46	40
133	37	29
160	28	30

The composition or structure of mineral salts extractable with saturated ammonium sulphate can either be different from that of the remaining portion or may be the same. Part of a homogeneous inorganic substance could be dissolved in the saturated salt solution until a certain ionic product was reached. In order to obtain information concerning these problems the following experiments have been performed. Varying amounts of a carefully mixed bone powder from the tibia and femur epiphyses of an adult rabbit, injected with ^{32}P, have been extracted with the same volume of saturated ammonium sulphate. After filtering the residue was re-extracted with the same volume

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of ammonium sulphate instead of being washed. After re-extraction the residue was washed three times with the extractant; the washing fluids were then combined with the second extract. Table IVa shows that the specific activity was the same when different amounts of bone powder were extracted; the specific activity also being the same both in the first and second ammonium sulphate extract. The relative amount of phosphate extracted was also the same in all cases*. As is shown in Table IVb, the pH of the extractant can vary between 6.5 and 8.5 without significant influence on the amount of phosphate extracted.

TABLE IVa

SPECIFIC ACTIVITY OF PHOSPHATE IN THE AMMONIUM SULPHATE FRACTION AND IN THE REMAINING FRACTION WHEN EXTRACTING DIFFERENT AMOUNTS OF BONE POWDER

A rabbit, weighing about 2 kg, was injected with 2 mc of ^{32}P 1.5 hours before sacrificing. Epiphyseal bone powder was used. For experimental details cf. METHODS

Weight of the sample mg	Specific activity			Percentage of total phosphate extracted in the combined $(NH_4)_2SO_4$ extracts
	First $(NH_4)_2SO_4$ extraction	Second $(NH_4)_2SO_4$ extraction	Remaining fraction	
177	248	255	45	32
310	283	310	48	23
441	287	254	35	27

TABLE IVb

INFLUENCE OF DIFFERENT pH OF THE AMMONIUM SULPHATE SOLUTION UPON THE AMOUNT OF PHOSPHATE EXTRACTED AND UPON THE SPECIFIC ACTIVITY OF THE TWO FRACTIONS

The experimental conditions were the same as in IVa

pH of the extractant	Specific activity			Percentage of total phosphate extracted in the combined $(NH_4)_2SO_4$ extracts
	First $(NH_4)_2SO_4$ extract	Second $(NH_4)_2SO_4$ extract	Remaining fraction	
6.5	230	214	46	29
7.5	258	246	44	29
8.5	285	292	48	24

Table V shows that the fraction extracted with saturated ammonium sulphate was by no means homogeneous as regards the renewal rate of phosphate. Bone powders from a rabbit injected with ^{32}P were extracted with acetate buffers of varying ionic strength, and of the pH 5.0 and 5.5, prior to the extraction with ammonium sulphate. More phosphate was extracted by solutions of higher ionic strengths and at the lower pH. When increasing amounts of phosphate were extracted the relative specific activity of this first fraction decreased. The Table thus demonstrates that the most soluble fractions of bone salts also have the most rapid rate of phosphate renewal. The specific activity of the fraction dissolved in the buffer solution with the lowest dissolving capacity was

* When bones from young animals are used, however, the second $(NH_4)_2SO_4$ fraction has a lower specific activity than the first fraction. Thus the more distinct difference between the two fractions appears in the adult state.

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almost as high as that of blood orthophosphate. This fraction was, of course, mixed with blood orthophosphate. But this contamination, however, could not seriously interfere with the results, as the content of orthophosphate in blood was extremely low in comparison to that of bone. The bone salts extractable with the acetate buffers must belong to the fraction which was extractable with saturated ammonium sulphate, since in all cases the remaining fraction was of the same relative magnitude and had the same relative specific activity.

TABLE V

RELATIVE SPECIFIC ACTIVITY OF PHOSPHATE IN DIFFERENT FRACTIONS OF BONE MINERALS, SEPARATED BY THE USE OF ACETATE BUFFERS

A rabbit, weighing about 2 kg, was injected with 2 mc of ^{32}P 1.5 hours before sacrificing. In each experiment the same amount of epiphyseal bone powder was used. The bone powder was first extracted with acetate buffer, then with saturated ammonium sulphate and finally with TCA

Extractant	Percentage phosphate extracted			Relative specific activity		
	Acetate fraction	$(NH_4)_2SO_4$ fraction	Remaining fraction	Acetate fraction	$(NH_4)_2SO_4$ fraction	Remaining fraction
M acetate buffer; pH 5.0	27	22	51	41	8.8	2.2
M acetate buffer; pH 5.5	19	31	50	60	10	2.4
0.1 *M* acetate buffer; pH 5.5	4.7	53	42	79	22	2.3
0.01 *M* acetate buffer; pH 5.5	2.1	51	47	88	23	2.6

Table VI demonstrates some experiments performed in order to obtain information concerning the nature of the different labeled portions of bone minerals. Prior to the extraction of the minerals salts, powdered bone from a rabbit, injected with ^{32}P, had been treated in different ways.

TABLE VI

SPECIFIC ACTIVITY OF PHOSPHATE IN THE AMMONIUM SULPHATE FRACTION AND IN THE REMAINING FRACTION AND THE RELATIVE AMOUNT OF PHOSPHATE EXTRACTABLE WITH AMMONIUM SULPHATE AFTER DIFFERENT TREATMENTS OF THE BONE POWDER

Freeze-dried epiphyseal bone powder from a rabbit, injected with ^{32}P 1.5 hours before sacrificing, was used. For experimental details cf. METHODS

Experiment No.	Treatment of the sample	Specific activity			Percentage of total phosphate extractable with $(NH_4)_2SO_4$
		Whole sample	$(NH_4)_2SO_4$ fraction	Remaining fraction	
1	Original	73	165	23	35
2	Defatted	62	150	18	32
3	Stored in watery solution for 4 hours	64	140	23	35
4	Ashed in ethylene-glycol for 2 hours	55	75	41	37
5	Ignited at 700° C	67	—	67	0

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Experiment 1 represents a sample which was extracted directly.

As can be seen from the next experiment, the lipid content of bone was without influence upon the extractability of the two fractions.

In the third experiment the sample was stored in destilled water at 20° C for 4 hours before the extractions. The specific activity of the powder became somewhat lower after this treatment, since a small amount of highly labeled phosphate passed into solution. However, no exchange occurred between the two fractions of bone minerals, since the specific activity of the remaining fraction was the same as found originally.

In the fourth experiment, the sample, after previous extraction of lipid materials, was ashed with 3% potassium hydroxide in boiling ethyleneglycol. Some of the highly labeled phosphate was dissolved in the ethyleneglycol solution; for this reason the specific activity of the entire bone powder was lowered. Furthermore the specific activity of the ammonium sulphate fraction was lower whereas that of the remaining fraction was higher than before. MANLY AND LEVY[24] have observed, under similar experimental conditions, that the calcified tissues adsorb phosphate from an ethyleneglycol solution. So it seems as if the bone powder contains bone crystals with different degrees of labeling. Highly labeled phosphate dissolved by the ethyleneglycol solution from crystals with rapid phosphate renewal can secondarily be adsorbed on bone crystals that are originally lowly labeled and not soluble in saturated ammonium sulphate. In that way the degree of labeling of the remaining fraction is increased. Thus, the result seems to give support to the view that the different fractions of bone phosphate cannot be present in the same crystals. If the highest uptake of labeled phosphate was due to an ion exchange of like magnitude on the surfaces of all bone crystals, such a phenomenon would not occur. The same results were also obtained with rat bones. The original specific activity of the two fractions of bone powder from a rat, injected with ^{32}P 1.5 hours before sacrification, were 246 and 35, respectively. After boiling in ethyleneglycol for 6 hours the specific activities of these same fractions were 137 and 96.

The last experiment in the same Table shows that, after ignition at 700° C, no phosphate can be extracted from the bone powder with saturated ammonium sulphate. This observation seems to give evidence that the nature of the inorganic constituent of bone is considerably changed by ignition.

Table VII shows that fresh bone *in vitro* also takes up the greatest amount of radioactive phosphate in the fraction soluble in ammonium sulphate.

TABLE VII

In vitro UPTAKE OF RADIOACTIVE PHOSPHATE IN THE AMMONIUM SULPHATE FRACTION AND IN THE REMAINING FRACTION OF BONE MINERALS

100 mg defatted fresh epiphyseal bone powder was incubated in 10 ml of a radioactive 0.001 *M* phosphate buffer of pH 7.3 for 3 hours, under constant stirring. After the incubation the powder was carefully washed with distilled water

Fraction	Specific activity
First $(NH_4)_2SO_4$	40
Second $(NH_4)_2SO_4$	39
Remaining	6.3

As yet there have been no extensive studies concerning the nature of bone minerals

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extracted in the two fractions. Investigations on rat bones have shown that the molar Ca: P ratio was about the same in the ammonium sulphate fraction as in the remaining fraction. DICKENS[25] has found that the hard substance of bone has a high content of citrate and has suggested that this citrate may readily be available as an endogeneous source of supply. About 75% of the citrate in bone has been found to be extractable with saturated ammonium sulphate; the presence of citrate in a part of the bone salt complex must increase the solubility of this fraction (*cf.* KUYPER[26]).

DISCUSSION

NEUMAN AND MULRYAN[17] have reported that fresh bone taken from actively growing areas has a higher uptake *in vitro* of radioactive phosphate than bone from the non-growing shaft. However, the magnitude of the exchangeable fraction was markedly reduced, while that of the compact shaft was increased as a result of ashing. As an explanation it was suggested that fresh bone, especially in newly-formed structures, unlike ash, undergoes a process of recrystallization at the same time as there is an ion exchange on the surfaces of other crystals.

There are many reasons for believing that bone minerals are of a much more complex type than the simple hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, though the fundamental structure of the bone salt is that of an apatite[27]. There seems to be good evidence that the carbonate in bone is part of the apatite[27, 28, 29], and that also magnesium[30] and alkali metals[14, 27] are parts of the main apatite crystals.

In agreement with other investigations (cf. BRANDENBERGER AND SCHINZ[31]) it has, been shown[32] that different structures of bone and Haversian systems, of varying age, and with different content of mineral salts, all show the same X-ray diffraction patterns, under normal and pathological conditions. The crystalline orientation as well as the crystal lattice thus is the same in all kinds of bone and in all parts of the same bone. The reported results[32] also indicated that there can be relatively large variations in the composition of the mineral salts without being detectable by X-ray diffraction techniques, which findings also are in agreement with other investigations[33, 34]. So the fact that the diffraction patterns are the same in all parts and all structures of bone does not refute the suggestions of SHEAR AND KRAMER[35] and of LOGAN[11], that substitutions can take place long after the minerals have been deposited.

The data reported in the present communication indicate that bone minerals are composed of an unknown number of fractions having different solubilities in acetate buffers and in saturated inert ammonium sulphate. The most soluble parts have the most rapid rate of phosphate renewal. Since no detectable amounts of organic phosphorus compounds were present in any of the extracts, the activity in all fractions must derive from the apatite crystals.

When bone is ashed in ethyleneglycol, an exchange of phosphate takes place between the different fractions of bone minerals (*cf.* Table VI). It seems to be thermodynamically impossible that phosphate in the centre of the crystals could exchange in a finite time without complete dissolution of the crystals themselves. Therefore it is suggested that there are very wide variations in the labeling of different bone crystals following the administration of radioactive phosphate. Furthermore, the highly labeled portion cannot originally be situated on the surface of the crystals only.

Shortly after the administration of the labeled phosphate the most soluble fraction

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had almost the same specific activity as the orthophosphate in the blood (*cf.* Table V), thus being almost in equilibrium with the serum phosphate. So, the salts in this fraction seem to be recrystallized at an extremely rapid rate. In contradiction to what occurs in the whole sample, in *in vitro* experiments with ashed bone powder[8], it has not been possible to demonstrate the attainment of a "quasi-equilibrium"[8] in any one of the various fractions. Therefore, as the fixation of radioactive phosphate does not follow the FREUNDLICH isotherm[24], it may be suggested that the uptake of labeled phosphate is always due to a rebuilding of the bone crystals. The mineral metabolism of bone thus must be a solution and reprecipitation of calcium phosphates. However, *in vivo* calcified tissues do not exchange calcium and phosphate in the same rate[36], phosphate has a much higher renewal rate than calcium. This fact seems to indicate that bone crystals might, at least to a certain extent, be mixed crystals (*cf.*[33]), and that these crystals are only partly dissolved and reprecipitated at a rapid rate. Parts containing a higher content of calcium are renewed more slowly than other parts.

According to SENDROY AND HASTINGS[37] the ion product $[Ca^{++}]\cdot[PO_4^{---}]$ is $10^{-26} - 10^{-27}$ for bone minerals, the same product in the blood plasma, calculated from its calcium and phosphate concentration, being 10^{-23}. The concept of solubility product for bone minerals seems, however, to be meaningless without reference to a known solid phase. Other difficulties in defining the solubility of the bone salt in the term of solubility product, is that equilibrium between the solution and the solid phase may not be attained even after 19 months at 25° C[38], and that the product of $[Ca^{++}]\cdot[PO_4^{---}]$ necessary to form the precipitate is greater than the product at which it will dissolve[11]. Precipitates of calcium phosphates formed in the physiological range of pH show the X-ray diffraction patterns of the final substance soon after their formation[32]. The solubility of the precipitate, however, decreases successively[26]. Furthermore, the structure of apatite permits large variations and substitutions, involving a considerable number of ions[39]. If different complex apatites exist in bone, it is very likely that each of them is "incongruently"[40] soluble and can establish equilibria of widely varying compositions.

In a subsequent paper[41] evidence will be presented that the greatest uptake of tracer phosphate takes place in the youngest osteons and in the periosteal and endosteal layers. When the osteons grow older the uptake of radioactive phosphate becomes less. The rapid dissolution and reprecipitation of certain parts of the inorganic salts, as indicated from the isotopic experiments, might be due to the fact that the solubility product constant of newly deposited inorganic material is the same as the ion product of ions entering the plasma. When the mineral deposits become older the solubility decreases.

The non-homogeneity of bone salts with respect to solubility has been impossible to establish in aqeous systems since tricalcium phosphate and hydroxyapatite do not exist as unique stoichometric compounds[42,40] in aqeous systems. For that reason the bone salts must be rearranged during the long periods necessary for establishing equilibria conditions. However, the use of concentrated inert salt solutions with the markedly increased solubility makes it possible to demonstrate the occurence of variations in solubility in aqueous systems.

As a conclusion the following hypothesis is put forward: Newly deposited apatite systems in the bone tissue exist in a solid solution, in ionic equilibrium with orthophosphate in plasma and bound to the bone tissue by the intermediation of organic

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substances. As the solid system becomes older some of the precipitated particles become larger, thus becoming less soluble than before. As a consequence, only a part of the particles become highly labeled, the rest taking up activity more slowly. When all particles have surpassed a critical size, the solubility becomes considerably decreased and the uptake of tracer phosphate is at a minimum. The overall rate for this reaction might be regulated by different electronic linkage to the organic constituents of bone tissue, *i.e.*, collagen. Changes in the collagen structure thus might be supposed to induce changes in the physical state of the inorganic part of bone. The intermediation of cells thus would be responsible for the initiation of the precipitation, for conditions controlling the renewal rate of the salts and for subsequent dissolution, *cf.* WILTON[43]. Furthermore, the solubility and renewal rate of bone minerals might be changed by substitutions in the apatite molecules.

SUMMARY

The inorganic part of bone tissue is composed of an unknown number of fractions with different solubility when acetate buffers and saturated inert ammonium sulphate are used as solvent systems. The most soluble fractions also have the most rapid rate of phosphate renewal: one fraction seems to be nearly in ionic equilibrium with orthophosphate in plasma. The significance of these observations has been discussed.

RÉSUMÉ

La partie inorganique du tissu osseux se compose d'un nombre inconnu de fractions ayant des solubilités différentes dans des tampons acétate et dans des solutions saturées de sulfate d'ammonium. Les fractions les plus solubles ont aussi les vitesses de "renouvellement" de phosphate les plus élevées: l'une des fractions semble presque être en équilibre ionique avec l'orthophosphate du plasma. Nous avons discuté la signification de ces observations.

ZUSAMMENFASSUNG

Der anorganische Teil des Knochengewebes besteht aus einer unbekannten Anzahl Fraktionen von verschiedener Löslichkeit in Acetatpuffern und gesättigtem Ammoniumsulfat. Die Fraktionen mit der besten Löslichkeit haben auch die grösste Phosphaterneuerungs-Geschwindigkeit: eine der Fraktionen scheint beinahe in Ionengleichgewicht mit dem Orthophosphat des Plasmas zu sein. Die Bedeutung dieser Beobachtungen wurde erörtert.

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