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THE CALCIUM EXCHANGE REACTION OF BONE *IN VITRO*. EFFECT OF PARATHYROID EXTRACT

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Abstract—By combining isotope techniques with a method of separation of bone into fractions of various specific gravity, the percentage of exchangeable calcium found in bone samples derived from animals treated with parathyroid extract was shown to be increased as compared to normal controls. This is interpreted as having resulted from an alteration of the ultrastructural relationships which exist between the organic and the mineral phases of bone, prior to resorption of high density bone. Pyrophosphate determinations confirmed the fact that new bone formation did not increase in these acute experiments.

IN RECENT studies it has been possible to describe the mechanisms which determine the *in vitro* uptake of radioactive calcium by bone.[1] By comparing results obtained in aqueous and alcoholic solutions, isoionic exchange was differentiated from various remodelling phenomena such as intracrystalline exchange due to thermal vibration[2] and recrystallization.[2, 3] It was also shown that there exist in bone ashed under anhydrous conditions reactional groups that can fix radio-calcium and are very labile in aqueous solutions. The presence of these groups taken together with the fact that the percentage of exchangeable calcium is reduced from 8 per cent in wet-ashed bone to less than 1 per cent in whole unashed bone, has led to the suggestion that mechanical and/or chemical relationships exist between the organic and inorganic phases of bone.[1]

Utilizing these techniques, we now report the effect of parathyroid extract (PTE) on bone tissue. The results indicate that in bone removed from the PTE-treated animals treatment has altered the relationships that exist between the organic and inorganic phases prior to the resorption of high density bone.

MATERIALS AND METHODS

1 *Animals*

All experiments were done with female Sprague–Dawley rats. Their weight averaged 149 g (s.d.†: 3 g) and they were between 52 and 55 days old. One group of animals was fed Purina Chow (1·61% Ca; 0·91% P) and tap water *ad libitum*. Another group was fed a calcium-free diet (Nutritional Biochemicals) containing 0% Ca, 0·15% P, 0·25% Mg and redistilled water.

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† s.d., standard deviation.

2. *Treatment*

The animals received parathyroid extract (Lilly) by intraperitoneal injection, 25 USP units twice daily for periods of from 1 to 4 days, and were killed 16 hr after the last injection. The extract was tested on animals identical with those used in the experiments by comparing the urinary calcium output during 24 hr following an injection of 25 units of PTE with that of the same animals in the preceding 24 hr. Calciuria was increased by 334 per cent in four treated compared with 4 per cent in four control animals.

3. *Sample preparation*

The animals were killed by exposure to ether; the long bones were dissected out and carefully cleaned of adhering soft tissue. Each bone was then divided into upper epiphysis, lower epiphysis and diaphysis, and an intermediate fraction called metaphysis. The epiphyseal and diaphyseal fractions were identified by proper anatomical reference marks for each bone; the fraction called metaphysis was an intermediate fraction between ends and shafts. No attempt was made to separate cartilage from bone. The bones were defatted in a mixture of alcohol–ether and the various fractions from bones of one or two animals were pooled (see tables for details). The bones were ground in an Abich mortar and passed through calibrated sieves. In the exchange experiments, the size of the particles used was between 250 and 500 μ, though particles size has been shown not to be critical.[1] The sample prepared in the above-described fashion corresponds to what has been called "os total 1" in a previous publication.[1]

4. *Wet-ashing*

An aliquot of the whole bone powder was ashed by a method adapted from Gabriel[3] by boiling in redistilled glycerol that contained 6% KOH. In contrast with Gabriel's method, the powder was not washed with water, but successively with ethanol, formamide and ethanol to minimize remodeling.[4]

5. *Separation*

Diaphyseal bone powders were separated into fractions of various specific gravities by method of centrifugation previously described.[5]

6. *Analytical methods*

Calcium of the liquid phases was measured by precipitation as the oxalate and titration with perchloratoceric acid;[6] calcium of the solid phases was determined by EDTA titration with Eriochrome Blue as indicator.[7] Calcium determinations were done in triplicate and the s.d. was held to 2 per cent.

Calcium-45 was determined with the aid of an automatic gas-flow counter (C 115, Nuclear Chicago) with a background of less than 3 counts per minute (counts/min). The constancy of the counting efficiency was checked with a ^{14}C source (2508 counts/min; s.d., 31 counts/min). Samples of the liquid phases were prepared for counting by evaporating 0·1 ml of the alcoholic solution in nickel-plated planchets. Samples of the solid phases were prepared by precipitating the calcium as calcium oxalate after dissolution with HCl.[8] Adequate standards were used for both types of planchets. The precision of the radioactivity determinations was kept within 4 per cent s.d.

Phosphorus was determined as the orthophosphate on the Technicon Autoanalyzer by a method derived from Fiske and Subbarow.[9] Pyrophosphate groups in bone samples heated to 325°C for 16 hr were separated from the orthophosphate groups by column chromatography, using Amberlite IRA-400; they were hydrolyzed to orthophosphate with $HClO_4$ and measured as such.[10]

The exchange reaction

When a sample of bone powder is suspended in a solution containing either PO_4 or Ca ions labeled with ^{32}P or ^{45}Ca, radioactivity is transferred from the liquid to the solid phase, even though no detectable transfer of mass occurs. This observation, which requires a carefully defined set of conditions,[1, 11] indicates that ions have been transferred from the liquid to the solid phase and that an equal number of the same ions has been transferred from the solid to the liquid phase. This phenomenon has been called isoionic exchange.[12, 13]

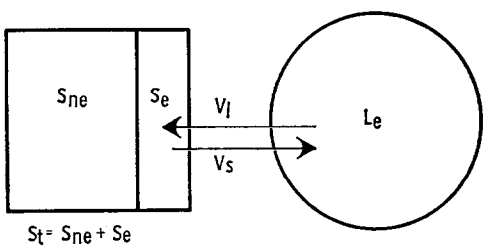

FIG. 1. Model describing the calcium exchange reaction.
The mass of calcium of the solid phase (*St*) is made up of an exchangeable fraction (*Se*) and a non-exchangeable fraction (*Sne*). The liquid phase contains a mass of calcium, *Le*. Calcium is transferred from the liquid phase to the exchangeable fraction of the solid at a rate v_l, whereas calcium is transferred from the exchangeable fraction of the solid to the liquid at a rate v_s. By definition, v_l is equal to v_s.

Figure 1 shows the simplest model that describes isoionic exchange. The percentage of exchangeable calcium is defined by,

$$\alpha(\%) = \frac{Se}{St} \times 100 \tag{1}$$

where *Se* is the mass of exchangeable calcium in the solid phase, *St* is the total mass of calcium in the solid phase, and α is the percentage of exchangeable calcium.

α in equation (1) is obtained experimentally from,

$$\alpha(\%) = \frac{\left(\frac{^{45}Ca}{Ca}\right)_{sol}}{\left(\frac{^{45}Ca}{Ca}\right)_{liq}} \times 100 \tag{2}$$

i.e. the ratio of the specific activities of the solid and the liquid phases at equilibrium.[14]

Two important conditions must be met for the model in Fig. 1 to describe isoionic exchange adequately:

(a) the percentage of exchangeable calcium defined by equation (1) must be independent of the mass of calcium present in the liquid and the solid; and

(b) it must be constant between the time of equilibrium and $t \rightarrow \infty$ (Fig. 2)

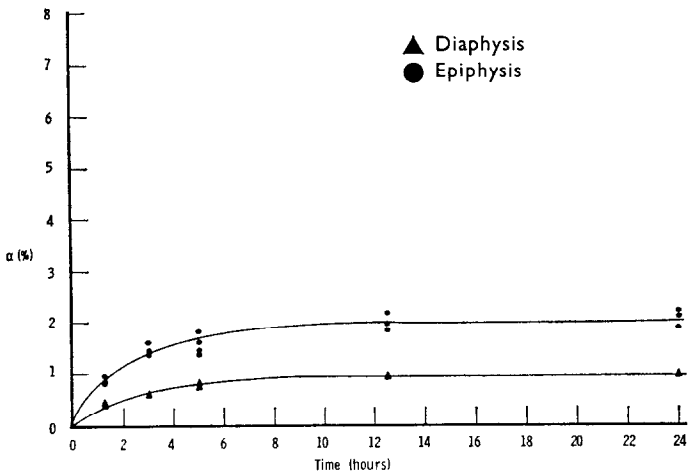

FIG. 2. Equilibrium of the calcium exchange reaction in various samples of bone.

These conditions are not met when the exchange reaction of calcium in bone is measured in aqueous solutions, even under well-controlled conditions of equilibration of the solid and the liquid phases.[11, 13] Even under such experimental conditions, there appears to occur a transfer between *Se* and *Sne* via the solution, because of recrystallization or other aging phenomena. When ethanol rather than water was used to dissolve $^{45}CaCl_2$, the distribution of radioactivity between *Le* and *Se* was found to attain an equilibrium. Under these conditions, the only mechanisms of transfer between *Se* and *Sne* appeared to be intracrystalline exchange by thermal vibration which at room temperature is too slow to be apparent.[1, 2] Figure 2 shows the establishment of an equilibrium in the distribution of radioactivity for various samples of bone powders from normal animals.

It must be emphasized that the model just described does not account for the variation with time of the percentage of exchangeable calcium before equilibrium is reached. To analyze this phenomenon, it is necessary to derive and solve systems of differential equations describing suitable multicompartmental models capable of generating the experimental curve.[14]

The percentages of exchangeable calcium reported in this article are defined by the dilution model of Fig. 1 and equation (1). They were derived from the specific activities of the liquid and the solid phases measured at equilibrium (24 hr) as described by equation (2). Details of the experimental conditions have been described and studied previously.[1, 11]

RESULTS

Table 1 shows that there was no significant difference between the average calcium content of the various fractions of bone from the control (A–D) as compared to the treated animals (E–J). Table 2 compares the experimental values and the derived percentages of exchangeable calcium in whole diaphyseal bone samples of four groups of two control animals (A–D) with those of six groups of two animals (E–J) treated

TABLE 1. CALCIUM CONTENT (% DRY WEIGHT) IN VARIOUS FRACTIONS OF BONE IN CONTROL AND PTE TREATED ANIMALS

Group	No. animals	Diaphysis (mg %)	Upper epiphysis (mg %)	Lower epiphysis (mg %)	Metaphysis (mg %)
Control (A–D)	8	24·08 (s.d.: 0·8)	19·15 (s.d.: 0·4)	20·24 (s.d.: 1·1)	21·61 (s.d.: 1·3)
Treated (E–J)	12	23·22 (s.d.: 1·2)	19·89 (s.d.: 0·9)	19·58 (s.d.: 0·8)	21·01 (s.d.: 0·5)

respectively with 50, 100, 200 units PTE. Table 3 is a comparison of the percentages of exchangeable calcium in the epiphyseal and the metaphyseal fractions of the same animals, and Table 4 indicates the percentages of exchangeable calcium in a series of wet-ashed samples derived from the same groups of animals. The radioactivity values of Table 2 were standardized to a total of 10,000 counts/min to facilitate comparison; this was justified by the fact that the total radioactivity recovered in each experiment (counts/min in the liquid + counts/min in the solid at equilibrium) was found to be constant within 3·10 per cent s.d.

The results indicate that treatment with parathyroid extract did not measurably affect the exchangeable calcium in the various wet-ashed samples (Table 4) but that it increased significantly ($P < 0{\cdot}01$) the percentage of exchangeable calcium in whole diaphyseal bone samples (Table 2). There is no clear-cut dose–effect relationship, but the calcium concentration of the liquid phase at which equilibrium between the liquid and the solid was attained was higher for the samples from animals treated with 200 units PTE. This might be due to a slightly higher solubility of the mineral phase of these samples. The difference found in the diaphyseal samples was not observed in the epiphyseal or metaphyseal samples (Table 3). This may be the result of a greater variability of these samples in both control and treated animals reflected by the higher standard deviation of the mean. It may also indicate that the changes observed in the diaphysis did not occur as such in the epiphysis. Epiphyseal and metaphyseal samples showed a higher percentage of exchangeable calcium than the corresponding diaphyseal samples.

The relatively high calcium intake (1·6% Ca in the diet) did not prevent the effect of PTE in this experiment, but we found later that the level of calcium in the diet was important and that a much lower calcium intake was required to insure experimental success. In the series reported in Tables 2–4, the animals were kept on the regular stock diet (1·6% Ca) for less than 1 week, including the 1 to 4 days of the experimental period.

TABLE 2. PERCENTAGES OF EXCHANGEABLE CALCIUM IN WHOLE DIAPHYSEAL BONE

Sample	Treatment	No. animals	Liquid phase			Solid phase			α (%)
			Calcium (mg)	^{45}Ca (counts/min)	Specific activity (counts/min per mg $\times 10^{-3}$)	Calcium (mg)	^{45}Ca (counts/min)	Specific activity (counts/min per mg $\times 10^{-3}$)	
A	None	2	3·425	9347	2·729	23·0	653	0·0284	1·04
B	None	2	3·400	9289	2·729	24·3	711	0·0293	1·07
C	None	2	3·250	9450	2·908	24·6	550	0·0226	0·77
D	None	2	3·375	9345	2·768	24·4	655	0·0268	0·97
Mean		8							0·96 (s.d.: 0·14)
E	50 U PTE	2	3·400	9119	2·682	23·7	881	0·0371	1·39
F	50 U PTE	2	3·225	9075	2·814	23·5	925	0·0394	1·40
G	100 U PTE	2	3·200	9213	2·879	23·2	787	0·0339	1·19
H	100 U PTE	2	3·225	9178	2·846	22·6	822	0·0364	1·28
I	200 U PTE	2	3·775	8928	2·365	23·4	1072	0·0458	1·84
J	200 U PTE	2	3·650	8974	2·459	23·4	1026	0·0438	1·78
Mean		12							1·48 (s.d.: 0·26)

At time 0, the liquid phase contained 3·750 mg of calcium and 78,450 counts/min as $^{45}CaCl_2$ dissolved in 25 ml of ethanol.
The solid phase was made up of 100 mg of the selected sample.
The total radioactivity has been standardized to 10,000 counts/min for each experiment (see text).

Table 5 gives the data and the percentages of exchangeable calcium derived from whole diaphyseal bone samples of twelve control animals (nos. 1–12) as compared to those from twelve animals treated with 150 units PTE (nos. 13–24). This dose was selected to exclude the solubility effect mentioned above. These animals were maintained on a calcium-free diet for 1 week prior to the experimental period. As previously,

TABLE 3. PERCENTAGES OF EXCHANGEABLE CALCIUM IN WHOLE EPIPHYSEAL AND METAPHYSEAL BONE

Sample	Treatment	No. animals	Percentages exchangeable calcium (α%)		
			Upper epiphysis	Lower epiphysis	Metaphysis
A	None	2	2·53	2·10	2·12
B	None	2	2·33	2·75	2·20
C	None	2	2·67	2·32	2·13
D	None	2	3·26	2·64	2·16
Mean		8	2·70 (s.d.: 0·45)	2·45 (s.d.: 0·32)	2·15 (s.d.: 0·04)
E	50 U PTE	2	2·79	2·96	2·22
F	50 U PTE	2	2·22	1·84	1·92
G	100 U PTE	2	1·97	2·89	1·89
H	100 U PTE	2	2·14	2·68	2·09
I	200 U PTE	2	2·93	2·54	2·15
J	200 U PTE	2	3·00	2·85	2·15
Mean		12	2·51 (s.d.: 0·50)	2·63 (s.d.: 0·54)	2·08 (s.d.: 0·12)

Same conditions as in Table 2.

TABLE 4. PERCENTAGES OF EXCHANGEABLE CALCIUM IN VARIOUS SAMPLES OF WET-ASHED BONE

Pools	Treatment	Percentages of exchangeable calcium (α%)				
		Diaphysis	Upper epiphysis	Lower epiphysis	Mean	s.d.
A–D	None	9·03	8·06	9·04	8·71	0·47
E–F	50 U PTE	9·54	10·97	10·50	10·34	0·85
G–H	100 U PTE	8·46	8·92	8·29	8·55	0·37
I–J	200 U PTE	7·89	8·57	8·69	8·38	0·47

Same conditions as in Table 2.

the total radioactivity recovered in each experiment was found constant within 2·59 per cent s.d.; this justified standardizing the radioactivity values of Table 5 to 10,000 counts/min per experiment. As in Table 2, the samples of the treated animals showed a higher percentage of exchangeable calcium than their normal controls. The mass of calcium in the liquid phase at equilibrium did not differ in the two groups, nor did the calcium content of the bone samples. There was no difference in the exchangeable

TABLE 5. PERCENTAGES OF EXCHANGEABLE CALCIUM IN WHOLE DIAPHYSEAL BONE

Rats 1–12: Ca-free diet; no treatment.
Rats 13–14: Ca-free diet; 150 U PTE

Animal	Liquid phase			Solid phase			
	Calcium (mg)	^{45}Ca (counts/min)	Specific activity (counts/min per mg $\times 10^{-3}$)	Calcium (mg)	^{45}Ca (counts/min)	Specific activity (counts/min per mg $\times 10^{-3}$)	α (%)
1	3·410	9373	2·748	24·08	627	0·0260	0·95
2	3·450	9390	2·722	23·84	610	0·0256	0·94
3	3·420	9390	2·746	23·52	610	0·0259	0·94
4	3·360	9388	2·794	23·92	612	0·0255	0·91
5	3·410	9363	2·746	24·00	637	0·0265	0·97
6	3·410	9380	2·751	24·56	620	0·0252	0·92
7	3·420	9426	2·756	24·08	574	0·0238	0·86
8	3·430	9397	2·756	24·08	603	0·0250	0·91
9	3·420	9288	2·716	24·24	712	0·0294	1·08
10	3·410	9381	2·735	24·40	619	0·0254	0·93
11	3·420	9428	2·757	24·24	572	0·0236	0·86
12	3·420	9348	2·733	23·20	652	0·0281	1·03
Mean	3·415			24·01			0·94 (s.d.: 0·02)
13	3·400	9347	2·749	23·76	653	0·0275	1·00
14	3·390	9253	2·729	25·28	747	0·0295	1·08
15	3·420	9281	2·714	24·00	719	0·0300	1·11
16	3·430	9262	2·700	23·36	738	0·0316	1·17
17	3·430	9190	2·679	24·96	810	0·0325	1·21
18	3·430	9251	2·697	23·60	749	0·0317	1·18
19	3·410	9159	2·686	24·00	841	0·0350	1·30
20	3·410	9183	2·693	24·64	817	0·0332	1·23
21	3·410	9230	2·707	24·33	770	0·0317	1·17
22	3·300	9050	2·742	24·84	950	0·0382	1·39
23	3·360	9234	2·748	24·56	766	0·0312	1·14
24	3·390	9125	2·692	24·56	875	0·0356	1·32
Mean	3·398			24·32			1·19 (s.d.: 0·03)

At time 0, the liquid phase contained 3·750 mg of calcium and 208,258 counts/min as $^{45}CaCl_2$ dissolved in 25 ml of ethanol. The solid phase was made up of 100 mg of the selected sample.

The total radioactivity has been standardized to 10,000 counts/min for each experiment (see text).

calcium in wet-ashed samples of the control or treated animals of this group, though the percentage of exchangeable calcium was higher (control: 13·06 per cent, s.d.: 0·83; treated: 13·06 per cent, s.d.: 1·32) than in the previous groups; this was probably related to the fact that the animals were fed a calcium-free diet.

DISCUSSION

Percentage of exchangeable calcium

As in previous work with adult cow bone,[1] we found in these experiments with rats that the percentage of exchangeable calcium is reduced from 8–9 per cent in wet-ashed bone to 1 per cent in whole diaphyseal bone. We also found an exchange value of 2·5 per cent for whole epiphyseal bone. These values represent the ratio $Se/St \times 100$ as defined by the model shown in Fig. 1.

St is the total mass of calcium present in the solid phase, but what *Se* represents is less obvious. We have previously postulated[1] that the exchangeable calcium of bone is the calcium present at the surface of the microcrystals. One can correlate the value of the percentage of exchangeable calcium observed in our experimental conditions with data on the shape and the size of the microcrystals obtained by X-ray diffraction and electron microscopy. If the needle-like crystals seen on electron micrographs are assimilated to cylinders, one can write,

$$\alpha'(\%) = \frac{2\pi Rh + 2\pi R^2}{\pi R^2 h} \times 100$$

where $\alpha'(\%)$ = the ratio of the surface of a cylinder to its volume,
R = the radius of the base,
h = the length.

Table 6 gives a series of values for α' at various h/R ratios. It can be seen that the values

TABLE 6. SURFACE TO VOLUME RATIOS

h	R	h/R	$\alpha'(\%)$
10	25	0·4	28·00
25	25	1·0	16·00
50	25	2·0	12·00
75	25	3·0	10·70
100	25	4·0	10·00
125	25	5·0	9·60
150	25	6·0	9·34
175	25	7·0	9·14
200	25	8·0	9·00
250	25	10·0	8·81
375	25	15·0	8·53
500	25	20·0	8·40
1250	25	50·0	8·16
2500	25	100·0	8·08
3750	25	150·0	8·06
5000	25	200·0	8·04
12 500	25	500·0	8·02
25 000	25	1000·0	8·00

The solid is a cylinder; h is the length and R the radius of the base.

of α' are close to the values of α, as determined by the exchange reaction, at h/R ratios that correspond to the dimensions of the microcrystals of bone ($R = 25$; $200 \leq h \leq 500$) derived from X-ray diffraction and electron microscopy measurements.[15, 16] It must be noticed that, whereas α, which is the ratio of two masses, has no dimensions, α' is the reciprocal of a length (L^{-1}). In extending the expression α' to the mass of calcium present at the surface or in the volume of the crystals, the assumption is made that the surface is a plane volume, with the third dimension equal to unity. The significance of such dimensional analysis has been discussed by Stahl.[17]

With the aid of similar assumptions, one can attempt to calculate how many calcium ions may be exposed at the surface of a crystal of similar dimensions made up of the suitable number of unit cells properly oriented; the ratio surface calcium/total calcium appears then to be very different (51–52 per cent).

It has been shown that the percentage of exchangeable calcium, determined under conditions comparable to ours, will only decrease when wet-ashed bone has been heated to 300–400°C, a temperature high enough to induce sharpening of the X-ray diffraction lines.[2] This too indicates that the exchangeable calcium in wet-ashed bone is a function of the size of the microcrystals surface as defined by X-ray diffraction.

If the exchangeable calcium in wet-ashed bone is the calcium present at the surface of the microcrystals, then the fact that whole bone samples reach an equilibrium value when α equals only 1 per cent implies the existence at the ultrastructural level of mechanical and/or chemical relationships between the crystals and the organic phase. Moreover, any change of that low exchange value unaccompanied by a change in the percentage of exchangeable calcium in the corresponding wet-ashed material implies that the change is the result of an alteration of these mechanical and/or chemical relationships.

Though there is adequate documentation to support the hypothesis that the percentage of exchangeable calcium is determined by the size of the surface of the microcrystals, additional quantitative information is needed. The best approach would be a kinetic study of the exchange reaction so as to compare in whole and wet-ashed bone the masses and rates of exchange of the compartments making up the exchangeable fraction under experimental conditions like ours, where the uptake of radioactivity can be analyzed in terms of isoionic exchange only.[11]

Effect of parathyroid extract

As shown in Tables 2–5, treatment with PTE increased significantly the percentage of exchangeable calcium in whole diaphyseal bone samples, without affecting the exchangeable calcium in the corresponding wet-ashed samples.*

By autoradiography, it has been shown[18] that the *in vitro* uptake of radioactivity by bone is heterogeneous. In order to take that heterogeneity into account in interpreting the observed increase of α in whole bone samples derived from animals treated with PTE, we separated samples of bone into fractions of varying specific gravities

* The effect of the level of calcium in the diet will be discussed in a future publication, but the fact that the percentage of exchangeable calcium found for the animals fed a calcium-free diet ($\alpha = 13$ per cent) is higher than for the animals fed a high-calcium diet ($\alpha = 8$ per cent) would indicate that the crystals are equivalent to a cylinder where h is only 50 when R is 25 (Table 6) and therefore that crystal growth is limited.

after *in vitro* exchange (Fig. 3). The sample of the normal animals was derived from pool C (Table 2) and the sample of the PTE treated animals was derived from pool I (Table 2). Two facts emerged from this experiment: (a) in treated animals, less calcium was found in the fraction with a density 2·2 than in the controls; this indicates that high density bone has been resorbed as a result of treatment; (b) the higher percentage

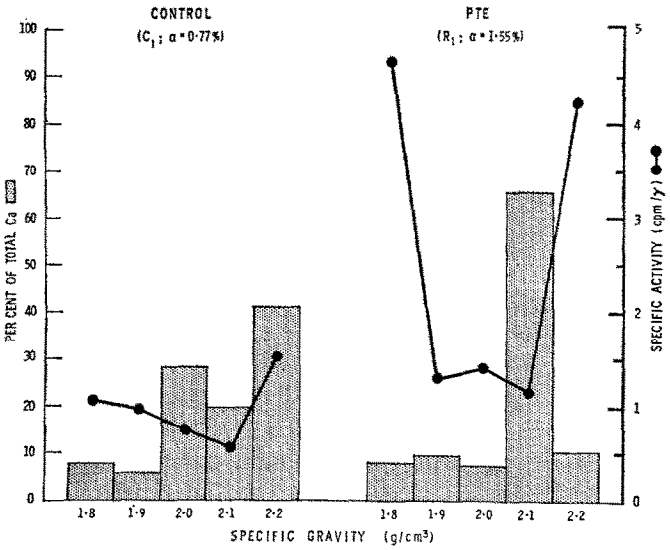

FIG. 3. Separation of bone samples derived from normal and PTE treated animals into fractions of varying specific gravities.

of exchangeable calcium resulting from PTE treatment was due to a systematic increase of the exchangeable calcium in all fractions, but particularly in the highest and lowest density fractions.

The pattern of specific activity distributed appeared similar in the the treated and in the control animals; the highest specific activities were found in both the low and high density bone, with lower values for the intermediate densities. A similar distribution has been found in adult cow bone.[5] It also appears from the separation that the higher percentage of exchangeable calcium in the treated animals was not due to a redistribution of the calcium into those fractions which normally have a high radioactivity uptake. In particular, the treatment did not alter the bone turnover rate so as to increase the formation of low density bone. This conclusion was strengthened by measuring the index of mineralization defined by P. François,[19] i.e. the ratio of calcium to pyrophosphate in bone heated to 325°C for 16 hr. Table 7 lists these ratios for two groups of twelve animals, one control and the other treated with 150 units PTE. As can be seen, no significant differences were observed.

Smeenk[20] has reported that the *in vitro* uptake of ^{32}P was higher in biopsy material obtained from patients suffering from chronic hyperparathyroidism than in similar material from normal controls. He found this higher uptake to be due to a larger than normal amount of low density bone as determined by microradiography. It appears

that in our acute experiments the resorption of high density bone induced by the treatment was not accompanied by the increased bone turnover found in chronic hyperparathyroidism.[20, 21]

TABLE 7

Group	No. animals	Ca (mg %)	PP* (mg %)	Ca/PP
Control	12	24·07 (s.d.: 0·14)	0·393 (s.d.: 0·081)	61·2
Treated	12	24·78 (s.d.: 0·50)	0·335 (s.d.: 0·135)	73·9

*PP = Pyrophosphate measured as phosphorus.

It thus appears that, prior to resorption of high density bone, parathyroid extract treatment induces an alteration of the ultrastructural relationships that exist between the mineral and the organic phases of bone. For us, this indicates that the activity of parathormone on bone not merely implies the solubilization of the mineral phase by an organic acid secreted by the osteoclasts,[22, 23] but also presupposes the existence of a mechanism whereby, without appreciably changing the dimensions of the crystals present or the specific gravity of the structure, the ultrastructural relationships between organic and mineral phases are altered. As was pointed out by Neuman,[24] it is difficult to conceive how any enzyme or other complex molecule can diffuse in compact bone; further work that could define the nature of the ultrastructural relationships that exist between collagen, mucopolysaccharides and the apatitic lattice of the crystals might provide an explanation as to why free diffusion is not normally possible and what kind of enzymes or metabolite should be looked for to explain the changes seen in our samples.

It is difficult to evaluate the significance of the observed phenomenon in terms of the regulation of calcium and bone metabolism. Nevertheless, it is interesting to point out that alterations in a matrix–mineral system might provide a mechanism for regulating calcium transfer between bone and the calcium pool of the body. The existence of such a mechanism must be postulated to explain that *in vivo* kinetic studies show much more calcium to be transferred unidirectionally from the pool to bone (v_{0+}, as defined in Refs. 25, 26) than can be accounted for by osteoblastic activity as measured by quantitative microscopic techniques.[27]

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