ground substance show an orientation which corresponds to the period of collagen fibres.

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Résumé

Les auteurs ont étudié au microscope électronique, au moyen de la technique de la pseudo-empreinte, la structure de la substance fondamentale calcifiée d'os à structure lamellaire (fémur de bovidé). Ils ont pu ainsi démontrer que cette substance est formée de globules de nature organique, enclavés dans un fin réseau inorganique. Le diamètre des globules, ainsi que celui des mailles du réseau inorganique, est de l'ordre de 200–250 Å. Les globules organiques sont alignées parallèlement aux fibrilles collagènes.

The Exchange of Bone Calcium with Ca^{45} [1]

Introductory Background. (1) It is well established that KOH-glycol ashed bone will exchange calcium ions when suspended in a calcium solution tagged with Ca^{45} [2]. When equilibrium is obtained 14–15% of the bone calcium is found to enter into the exchange reaction[3].

(2) Calcium phosphates with Ca/P weight-ratios ranging from 1·72 to 2·26 have been described as a series of defect pseudo-apatites with more or less calcium occupying lattice positions[4]. It is possible to fill these missing positions by suspending the material in lime solution[5]. These pseudo-apatites differ chemically and physically from true hydroxyapatite despite the fact that their X-ray diffraction patterns are similar. The compound with the greatest Ca/P (2·26) is similar to the principal inorganic component of bone.

If we prepare this calcium phosphate (Ca/P = 2·26) by suspending a defect pseudo-apatite in lime solution, the various types of calcium ions in the resulting solid are:

(a) the original 9 Ca ions stoichiometrically bound to the phosphates groups,

(b) the additional 1½ Ca ions included in some manner in the pseudo-apatite of Ca/P = 2·26,

(c) moreover, especially in the case of bone salts, some physically adsorbed Ca ions.

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(3) Hydrochloric acid can preferentially dissolve the Ca ions bound to the carbonate in bone[1]. In the first stages of the acid action, the Ca/P ratio of the liquid phase is very high, decreasing slowly as the fundamental phosphate (Ca/P = 1·94) goes into solution. The action of HCl on bone is just the reverse of adding Ca from lime solution to a defect calcium phosphate as above. Unfortunately, this acid action is not quite selective and it is not possible to dissolve the calcium ions which fill the defects without destroying the fundamental tricalcium phosphate to some extent.

Experimental procedure. Combining the techniques of radioactive exchange and HCl attack, we were able to obtain information regarding the nature of exchangeable calcium in bone mineral. The starting material for our experiment was KOH-glycol ashed bone which was exposed for a month to $Ca^{45}Cl_2$ solution and then filtered and dried to constant weight at 105°C. The specific activity of this stock bone ash was $9{\cdot}48 \times 10^3$. Fractions of this material (500 mg) were suspended for 10 min in 25 ml of varying HCl solutions. The amount of HCl ranged from 0·2 mE to 6·25 mE. The amount of HCl sufficient fully to dissolve 500 mg of bone ash was 6·85 mE. After filtration and drying at 105°C, each sample was weighed; the calcium, phosphorus and specific activity were determined for both the liquid and residual solid phase. See Table for a tabulation of the results.

Results and discussion. The weight loss of the samples in HCl solution increased with the increase of HCl employed. As the HCl concentration increased, the specific activity of the liquid, very high for the small HCl quantities, decreased. However, the specific activity was always higher in the liquid than in the corresponding residual solid phase. For each experiment, the liquid phase always contained more calcium than required for 9 Ca per 6 P (Ca/P = 1·94). This excess represents the additionnal Ca ion content of bone structure. On the other hand, the Ca/P weight-ratio in the solid phase decreased slowly until it reached a value of 1·98.

Instead of regarding the ratio (counts/m)/total Ca, which decreases in both phases, we can calculate the ratio: (counts/m)/excess Ca. This turns out to be a constant value ($61{\cdot}9 \pm 5{\cdot}2$) independent of the extent of HCl attack on the solid.

We feel that the Ca^{45} exchanged by bone mineral is exclusively the excess 1½ moles of calcium of the saturated pseudo-apatite. These calcium ions are not only on the surface, but are contained in the structure of the solid, for they are still present after severe HCl attack. This is supported by a recent experiment in this laboratory which studied the uptake of Ca^{45} by a defect pseudo-apatite of Ca/P = 1·94. The material was dried at 105°C and exposed to a solution containing *low* calcium concentrations. Only pseudo-apatites with a Ca/P greater than 1·94, such as bone, can exchange in the presence of small Ca concentrations.

The exchange reaction of the Ca in excess of 9 moles per unit cell in bone mineral is rapid, with ¾ of the equilibrium value being attained in 10 min and full equilibrium being attained in a few hours. Thus these excess Ca positions do not behave chemically in the same way as the original 9 Ca ions of the fundamental neutral phosphate.

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Table

Liquid Phase							
HCl mE/ 100 mg	% dissolved material	Ca mg	P mg	Ca/P	Excess Ca mg	c/m / total Ca	c/m / excess Ca
0·04	5·1	0·96	0·20	4·80	0·57	30·6	50·9
0·1	7·9	2·42	0·70	3·30	1·07	24·3	55·1
0·2	15·4	5·09	1·91	2·66	1·38	17·57	64·5
0·4	30·1	10·54	4·22	2·50	2·36	14·4	64·4
0·6	43·4	15·84	6·66	2·38	2·90	12·3	67·2
0·8	59·1	21·71	9·35	2·32	3·58	11·36	68·9
1·0	70·6	26·54	11·31	2·35	4·60	10·13	60·0
1·25	91·5	33·51	14·64	2·28	5·11	9·95	65·3
Solid Phase							
HCl mE/ 100 mg	% non dissolved material	Ca mg	P mg	Ca/P	Excess Ca mg	c/m / total Ca	c/m / excess Ca
0·04	94·9	35·13	15·74	2·22	4·60	7·83	61·4
0·1	92·1	33·67	15·24	2·21	4·10	8·19	65·1
0·2	84·6	31·00	14·03	2·21	3·78	8·13	65·1
0·4	69·9	25·55	11·72	2·18	2·81	7·35	63·0
0·6	56·6	20·25	9·28	2·18	2·25	6·98	61·0
0·8	40·9	14·38	6·59	2·18	1·60	6·29	55·6
1·0	29·4	9·55	4·63	2·06	0·57	6·08	—
1·25	8·5	2·58	1·30	1·98	0·06	5·04	—

All milligram values are calculated for 100 mg of bone mineral treated by HCl.

A certain amount of Ca in bone, above the 10½ mole value, is simply physically adsorbed (± 0·2 mg%). This exchanges very rapidly and reaches equilibrium with a Ca^{45} solution in a few minutes. Mineralized bone always achieves a higher specific activity than the same material leached with a small quantity of HCl (0·02 mEHCl/100 mg ash dissolves all the surface adsorbed Ca) when both are immersed in like solutions of Ca^{45}. Quantitatively this may be seen as follows: (a) after a long time: untreated bone mineral, specific activity = 9·13, leached bone 8·63; (b) after short exposure: untreated bone mineral 0·86 and 0·74, leached bone 0·63 and 0·59 respectively.

Conclusions. The exchangeable Ca of KOH-glycol ashed bone (dried at 105°C) is only the adsorbed calcium and the ions located at the tenth and the tenth and a half Ca positions in the pseudo-apatitic structure. These calcium ions correspond to about 15% of the total bone calcium which is in agreement with the percent exchange reported in the literature.

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Résumé

L'emploi combiné de l'isotope radioactif du calcium et de la dissolution progressive des sels osseux par HCl a permis de démontrer que le calcium échangeable de la substance minérale osseuse correspond au calcium adsorbé et à celui qu'elle renferme en excès par rapport au phosphate tricalcique fondamental.

Microradiographic Studies of Teeth

In 1933 the first interesting attempt was made to study teeth by means of the microradiographic technique[1]. On account of the coarse graining in the film used, the lack of sensitivity of the apparatus and the poor power of resolution by modern standards, the radiogram could only show the coarsest structures[2].

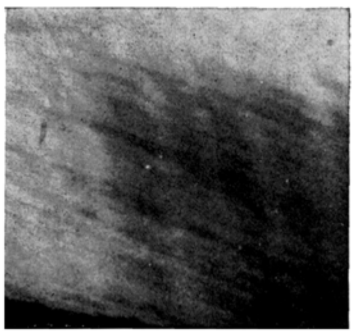

Fig. 1.–Enamel: Retzius' lines (horizontally) and the bands of Hunter-Schreger (vertically). 100 ×.

The pictures below are of ground sections (15–25 μ) of adult healthy teeth photographed by X-rays with wavelengths 2·4–4·0 Å. Calcium has the maximum absorption at 3·06 Å[3]. A Machlet type AEG 50 X-ray

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