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KØBENHAVN
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Anatomical Introduction.

The hard part of a tooth is composed of three distinct substances viz. the dental substance proper, dentine, the enamel, and the cement. The dentine constitutes by far the largest portion; the enamel is found in a comparatively thin layer partly covering the dentine; and the cement covers the surface of the root in a thin layer. In the case of the canines of cats we found the weight of the enamel ash to be 11,2 % of that of the dentine ash, the weight of the enamel before ashing being equivalent to about 9,7 % of that of the dentine.

The dentine is penetrated throughout by fine tubes (dentinal tubes) starting from that side of the dentine which faces the pulpa cavity; they have an initial diameter of 2 to 8 μ and do not much diminish in size at first as they approach the surface; the distance between adjacent tubules is about two or three times their width. From the tubules numerous immeasurably fine branches are given off and penetrate the hard intertubular substance. Near the periphery of the dentine, the tubules, which by division and subdivision have become very fine, terminate imperceptibly in free ends. It is reported that tubules have been observed passing into the enamel in the teeth of marsupial animals, and to a less marked degree in human teeth. In this case they pass, not into the enamel prisms, but into the inter-prismatic substance. The enamel is made up of microscopic columns, very hard and dense, arranged close side by side, and fixed at one extremity on to the subjacent surface of the dentine. The enamel columns have the form of six-sided prisms. Their diameter is about 0.005 mm. They are united by a small amount of substance which appears to be similar to the intercellular substance of an epithelium. The small amount (about 1 %)[1] of organic matter in the enamel is probably found to a large extent in the above mentioned connective substance. In marsupials and some rodents there are regular canaliculi in the interprismatic substance.

The central cavity of a tooth is occupied by a soft and very vascular dental pulp, containing cells, blood-vessels, nerves, and fine connective-tissue fibres. The cells are partly disseminated in the matrix and partly form a stratum at the surface of the pulp. These superficial cells, the odontoblasts, send out elongations into the tubules in the dentine. It is through the intermediary of the pulp that constituents of the blood get into the hard tissues of the teeth.

Chemical composition of the teeth.

a) Dentine.

On analysing a great number of dry human dentine samples Bowes and Murray[2] found a loss in weight of the fresh tissue on ignition amounting to 29—29.7 %. The losses on ignition found in some of our experiments can be seen in table 1, in which we have also included for the sake of comparison the values found for the tibia and jaw.

[1] J. H. Bowes and M. M. Murray, Biochem. J. **29**, 12, 2721, 1935.
[2] J. H. Bowes and M. M. Murray, Biochem. J. **30**, 977, 1936.

Table 1.

Albino rat 200 g.

Organ		Loss on ignition in % of fresh weight
Incisors	Proximal end	33.6
	Distal end	25.0
	Average	26.4
Molars		27.0
Tibia	Head	79.1
	Average	63.2

Cat 4 kg.

Incisors	32.0
Canine	35.0
Molar	38.0
Jaw	50.4
Tibia epiphysis	66.8
Tibia diaphysis	36.7

The average values found for the chief constituents of the dentine by BOWES and MURRAY are seen in table 2.

Table 2.

Analyses of dentine of human teeth (% in dry dentine).

	Slight hypoplasia	Severe hypoplasia
Ash	71.09	70.28
Ca	27.79	26.96
P	13.81	13.5
CO_2	3.18	3.10
Mg	0.835	0.728
Cl	—	0.023

BOWES and MURRAY give the following average figures for the composition of the enamel:

Table 3.

Analyses of enamel of human teeth.

	Slight hypoplasia	Severe hypoplasia
Ash	95.38	94.67
Ca	37.07	35.81
P	17.22	17.72

	Slight hypoplasia	Severe hypoplasia
CO_2	1.952	2.434
Mg	0.464	0.477
Cl	0.3	0.19
Fe[1]	0.25	—

As is seen from the above figures phosphorus is the second most abundant mineral constituent of the teeth, its share in the dentine amounting to 13.5—13.8 % and in the enamel to 17.2—17.7 % while in the dentine ash 18.2—18.4 % in that of the enamel 18.4—19.4 % were found. In the ash of the incisors of rats an even higher phosphorus content of 20 % was found. Bone ash contains an only slightly lower amount of phosphorus than tooth ash, the values found varying between 17.9 and 18.5 %.

In distinction to the chief constituents of the teeth the minor constituents vary within wide limits. The composition of the mineral constituents of the teeth corresponds approximately to a mixed crystal of the minerals hydroxide-apatite and carbonate apatite, the former predominating strongly. As in apatite minerals the OH^- ions of the tooth apatite can be replaced to a certain extent by F^- ions for example. The degree of replacement of OH^- by F^-, will depend primarily on the fluorine content of the blood during the development period of the tooth and also on that which circulates in the fully calcified tooth. The fluorine content of the blood will depend on the fluorine content of the food and water taken up. It is thus easy to explain why the fluorine content of the teeth varies within wide limits (see table 4). The high fluorine content of the teeth

[1] G. Montelius, J. F. McIntosh and Y. C. Ma J. dent. Res. **13**, 73, 1933. The amount of copper present in teeth is 10^{-4}—10^{-5} gm. per gm. E. Tiede and H. Chomse, Ber. d. dt. chem. Ges. **67**, 1988, 1934.

of human beings living at Colorado Springs is due to the high fluorine content of the water which amounts to up to 2 mgm. per liter. The high fluorine content of the teeth of some North African sheep is to be explained by the high fluorine content, above 0.02 %, of the soil on which they graze. On such soil plants of high fluorine content grow, are eaten by the sheep, and lead to an abnormally high fluorine content of the blood plasma, which in turn leads to an abnormally high replacement of OH^- by F^- in the teeth.

Table 4.

Fluorine content of teeth ash.

		%
Man[1]	teeth	0.03
Marine animals	teeth	0.69 —0.74
Rats[2]	teeth	0.006—0.03
Man[3] New York	dentine	0.065
Man New York	enamel	0
Man[3] Colorado Springs	dentine	0.112
Man Colorado Springs	enamel	0.065
Man[4]	dentine	0.030
Man[4]	enamel	0.005
Calves[4]	dentine	0.022
Calves[4]	enamel	0.0057
Sharks[4]	teeth	0.89
Sheep, young from neighbourhood of Norwegian aluminium factory where fluorides are utilised[4]	incisors	0.45—0.49
Sheep[5] North Africa	teeth	0.04
Sheep[5] North Africa, attacked by fluorine disease	teeth	0.32—0.46

The much higher fluorine content of animals living in sea water is also due to the comparatively high fluorine con-

[1] R. Klement, Naturw. **21,** 662, 1933.

[2] G. R. Sharpless and E. V. McCollum, J. Nutrit. **6,** 163, 1933. J. H. Bowes and M. M. Murray, Biochem. J. l. c.

[3] H. Boissevain and W. F. Drea, J. Dent. Res. **13,** 495, 1933.

[4] K. Roholm, Fluorine Intoxication, Copenhagen 1937 p. 260.

[5] M. Gand, A. Chavnot and M. Langlais, Bull. Inst. Hyg. Maroc. Nos. I—II, 1934, comp. also Roholm loc. cit. p. 42.

tent of the latter. In the same way that F^- replaces OH^- in the apatite lattice, magnesium for example replaces calcium. Human dentine ash has a magnesium content[1] of 1.18—1.39 % whereas human enamel ash has only 0.42 %. While the calcification of the tooth tissue is presumably the result of specific cell activity, it is quite possible that later on a replacement of calcium by for example magnesium takes place, governed chiefly by solubility (chemical affinity) conditions, and it is quite conceivable that in the course of time more and more calcium is replaced by magnesium if the magnesium: calcium ratio is in favour of such an exchange. The enamel being characterised by a decidedly poorer lymph circulation than the dentine, the much lower magnesium content of the former can easily be accounted for by a reference to the above considerations. The 0.42 % magnesium found in the human enamel possibly got into the latter wholly or to a large extent during the formation of the enamel tissue. The presence of as much as 4 % of magnesium in elephant dentine is possibly due to a high magnesium content of its food or to a high magnesium retention in its blood. It is also of interest to remark that the magnesium content of the teeth found in prehistoric skeletons is only one third of that found in teeth of recent generations, furthermore that carious teeth[2] show a greatly increased magnesium content. Besides the elements discussed above, spectroscopic investigation[3] revealed the presence of traces of Na, Ag, Sr, Ba, Cr, Sn, Zn, Mn, Ti, Ni, V, Al, Si, B and Cu in dental tissue.

[1] M. M. Murray, Biochem. J. **30**, 1568, 1936.

[2] T. Francia, Ann. Clin. Odoniat. 8, 685, 1931; M. M. Murray and J. H. Bowes, Brit. Dent. J. **61**, 473, 1936.

[3] E. Lowater and M. M. Murray, Biochem. J. **31**, 837, 1937.

That the concentration of the minor constituents of the teeth does not fluctuate between still wider limits is due to the narrow limits within which the concentration of most elements in the blood plasma is restricted. This is caused partly by a prevention of the resorption of excessive quantities of the elements, conspiciously shown in the case of calcium, and partly by prompt removal chiefly through the kidneys of excessive amounts of the mineral constituents present in the plasma. But even in spite of this levelling mechanism of the blood plasma some of the mineral constitutents are deposited to a noxious extent in the tooth tissue, as is seen above in the case of fluorine, and it is quite possible that even an excessive replacement of for example the calcium by magnesium, sodium, or potassium might lower the resistance of teeth to disease.

While the conclusions given above are based partly on hypothetical assumptions, in the case of lead, which also replaces calcium in the crystal lattice, the accumulation in the teeth with time can clearly be shown. While small children have only negligible amounts of lead in their teeth, the lead content increases with age[1], the increase being markedly greater in the case of carnivorous than herbivorous animals, presumably on account of a greater lead intake in their normal nourishment. In the case of lead poisoning the lead content of teeth is greatly increased. All these observations support the hypothesis, that even in fully formed teeth an exchange of mineral constituents is regularly taking place. To test this hypothesis we have studied the exchange of phosphorus by means of labelled phosphorus atoms.

[1] F. Pfrieme, Arch. f. Hyg. **111**, 232, 1934.

Phosphorus exchange in teeth.

We investigated the movement of the phosphorus atoms both in the teeth of fully grown and growing animals by using labelled phosphorus atoms as an indicator. By adding radioactive phosphorus, prepared from sulphur by the action of neutrons, to food administered to animals at a known date, it is possible to distinguish the phosphorus atoms which were present in the food sample and which have been retained and deposited in the organism, from those already present in the body and the teeth at the start of the experiment. We can thus follow the movement of the phosphorus atoms taken in for example a glass of milk and investigate if and to what extent these particular atoms get into the teeth and how they are distributed there.

The dentine contains 14 % and the enamel 17.5 % of phosphorus in the form of phosphate (PO_4). It is the movement of these phosphate radicles which we actually investigate. For the sake of brevity we shall often use the word phosphorus in discussing the behaviour of the phosphate[1] radicle. We may recall that the phosphorus taken with food, amounting in the case of an adult to somewhat more than 1 gm. per day, is to a large extent (in most cases up to about 80 %) absorbed from the gut and gets into the blood stream. Adult human blood contains 44—50 mgm. % of phosphorus of which only 2—5 mgm. % are present as inorganic P. Very different views have been put forward on the formation of the bone and tooth tissue, but they all consider the blood plasma as saturated or nearly saturated with calcium phosphate and the precipit-

[1] The expression radioactive phosphate is ambiguous, since in such a radicle either the phosphorus or the oxygen atoms, possibly even both, may be radioactive.

ation of the latter from the plasma as being of paramount importance for the ossification process. The solubility of calcium phosphate in the plasma is very strongly affected by the presence of proteins, carbonate and bicarbonate ions, and possibly also other constituents. It is also dependent on the acidity of the blood, slight changes in which may be sufficient to produce precipitation. It seems very probable that it is not simple calcium phosphate but a complex salt of the apatite type, a solid solution of hydroxide apatite and carbonate apatite, that precipitates.

In addition to the inorganic phosphate, blood contains a phosphoric ester at a comparatively high concentration which is mainly found in the corpuscles; as it cannot yield phosphate ions by dissociation, this ester does not affect the saturation of the blood with respect to calcium phosphate. However, as ROBISON[1] discovered, the cartilage and osteid contain an enzyme, phosphatase, which hydrolyses this ester, thus setting free inorganic phosphate, whereby the concentration of the phosphate ions increases and a supersaturation occurs, followed by a precipitation of the calcium phosphate in the matrix of the tissue. With the discovery of the bone phosphatase a second agency (in addition to the acidity change) of great importance was found, regulating the calcium phosphate precipitation leading to ossification. ROBISON found that the enzyme had the greatest activity in ossifying cartilage, bones, and teeth of very young animals, the activity per unit weight of tissue decreasing with age. Although the plasma contains on an average only 0.5 mgm. of phosphorus present as phosphoric ester per 100 ccs. this is completely hydro-

[1] Comp. R. ROBISON, The significance of phosphorus esters in metabolism. New York 1912.

lysable by the bone phosphatase and thus supplies phosphate ion amounting to about $^1/_6$ of the inorganic phosphorus present in the plasma, an amount amply sufficient to bring about a supersaturation and a subsequent precipitation of calcium phosphate, or more correctly of the apatite-like bone substance, from the already nearly or fully saturated plasma. The conclusions arrived at in this paper are independent of the special mechanism assumed for the ossification process.

Distribution of labelled phosphorus in the incisors of rats.

The rapidly growing incisors of rats are very suitable for studying the distribution of phosphorus. According to

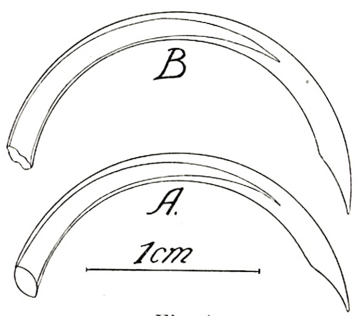

Fig. 1.

Fridericia and Gudjonsson[1] the average extrusive incisor growth per week is 2.7 mm. in the case of adults and 3.4 mm. for young rats. As seen in Fig. 1 A the cross section of the pulpa is very large at the proximal end and gets narrower toward the distal end, the last millimetres

[1] L. S. Fridericia and S. V. Gudjonsson, Kgl. Danske Vid. Selsk. Biol. Medd. XIII, 12, 1936; comp. also W. G. Downs jun., Proc. Soc. exp. Biol. and Med. 28, 813, 1931, who finds an average growh per week of 2.78 mm. for 6 months old animals.

of the teeth being free of pulpa. The problem we have to investigate is how the distribution of newly formed calcium phosphate in the incisor takes place. Two extreme cases must be envisaged:

a) the labelled phosphate is deposited in close proximity to the pulp from which it is derived, while the tissue formed at an earlier date is pushed along in the direction of growth;

b) the labelled phosphate is equally distributed throughout the incisor.

Cutting incisors transversally into pieces and analysing these separately revealed the fact that the largest part of the labelled phosphate is found in those regions of the incisor where the pulpa is strongly developed, but that some of the labelled phosphate is found all through the incisoral tissue (Tables 5 and 6).

Table 5.

Distribution of labelled phosphorus, contained in the normal diet, found in the incisor after 2 days. Weight of the rat 210 gm. + denotes upper ÷ lower teeth.

Part of the incisor	Weight of ash in mgm.	% of labelled P taken found	% of the labelled P per mgm. ash
Proximal I + ...	38.2	0.42	0.011
Proximal II + ...	40.8	0.47	0.012
Proximal I ÷	29.2	0.37	0.013
Proximal II ÷	27.2	0.38	0.014
Middle	92.6	0.125	0.00135
Distal I + ...	115.2	0.072	0.00063
Distal II ÷	36.4	0.008	0.00022

Percentage of labelled P found in the total incisors = 1.85. Average per 1 mgm. ash = 0.005. Biggest ratio between proximal and distal end = 60.

Table 6.

Distribution of labelled phosphorus, administered in the normal diet, found in the incisor after 7 days. Weight of the rat 240 gms.

Part of the incisor	Weight of ash in mgm.	% of labelled P taken found	% of the labelled P per mgm. ash
Proximal I + ...	25.0	0.28	0.011
Proximal II + ...	23.6	0.31	0.013
Proximal I ÷	21.8	0.29	0 013
Proximal II ÷	31.6	0.32	0.010
Middle + ...	81.6	0.204	0.0032
Middle ÷	68.0	0.206	0.0031
Distal + and ÷	29.3	0.020	0.00074

Percentage of labelled P found in the total incisors = 1.69. Average percentage per 1 mgm. ash = 0.006. Biggest ratio between proximal and distal part = 18.

In the experiments now to be described the distal part of the incisor was removed by operation one day before labelling the phosphorus present in the blood. In these experiments the radioactive P was not added to the food but given in the form of subcutaneous injections. 2 days, 5 days and 8 days after the administration of the labelled phosphorus the end part of the freshly grown incisor was again removed by operation and its radioactivity ascertained. The distal parts removed were all outside the range of the pulp. The figures obtained are seen in Table 7 and those from a similar experiment in Table 8.

Table 7.

Days after intake of labelled P	Weight of the tissue in mgm.	% of the labelled P found in 1 mgm. fresh tissue
2	42.8	0.00089
5	16.4	0.00030
8	20.4	0.00066
13 (rat killed)	26.6	0.00090

Percentage of the labelled P found in 1 mgm. of average incisor tissue = 0.0076. The removed distal ends contained 8 to 25 times less labelled P than the average tissue.

Table 8.

Days after intake of labelled P	Weight of the tissue in mgm.	% of the labelled P found in 1 mgm. fresh tissue
5	14.4	0.00040
8	11.0	0.00044
13 (rat killed)	21.3	0.00062

Percentage of the labelled P found in 1 mgm. of average incisor tissue = 0.0062. The removed distal ends contained 10 to 16 times less labelled P than the average tissue.

Though the figures in the tables above clearly show that the deposition of labelled phosphorus is not restricted to the regions in the vicinity of the pulp, but that the labelled phosphorus is to be found even in the most remote part of the incisors, we attempted to obtain incisors with an appreciably larger pulp-free part. As is well known, rats, being rodents, grind their teeth and thus continually remove parts of the pulp-free end of the growing incisors. By eliminating the upper incisors the animal was prevented from gnawing and incisors were thus obtained in which the distal pulp-free end had a length of 10.5 mm. as shown in fig. 1 B. The result of this experiment is seen in Table 9 and the diagram fig. 2.

Table 9.

Distribution in the incisor after 3 days of labelled phosphorus, injected subcutaneously.

Weight of the rat about 200 gms.

Part of the incisor (comp. Fig. 2)	Weight of the tissue in mgm.	Weight of the ash in mgm.	% labelled P found per mgm. tissue	% labelled P found per mgm. ash
I (Proximal end) . .	13.1	9.2	0.0103	0.0151
II	14.5	11.0	0.0079	0.0116
III	24.0	17.1	0.0026	0.0040
IV	15.3	11.0	0.00021	0.00030
V (Distal end)	12.0	9.0	0.000033	0.000044

The content of labelled P varies between 0.01 % at the proximal end and 0.000033 % at the pulp-free distal end, thus diminishing by a factor of 1/300. On comparing the activity of the ash obtained by igniting the incisor the figures work out to be 0.015 % and 0.000044 % respectively, corresponding to a factor of 1/340. The average content of labelled P in 1 mgm. tissue was found to be 0.0041 %, in 1 mgm. ash 0.0059 %. Fig. 2 shows both the location of

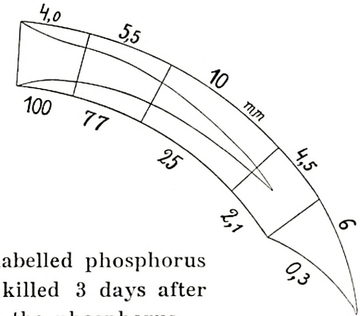

Fig. 2. Distribution of labelled phosphorus in the incisor of a rat killed 3 days after the administration of the phosphorus.

The figures below give the relative amounts of labelled phosphorus present in 1 mgm. of fresh tissue in the section in question. The figures above give the length of the section in mm.

the pulp and the distribution of the labelled phosphorus. It is seen clearly that the bulk of the labelled phosphorus atoms are to be found in the vicinity of the pulp but an amount which is far from being negligible reaches even the remotest part of the incisor. In Fig. 2 we have inserted the relative abundance figures of the labelled phosphorus present in the different parts of the incisor. A part of the first sector amounting to 1.4 mm. grew during the time which elapsed between the injection of the labelled P and the killing of the animal; the other parts were present before. Out of 204 parts of labelled phosphorus only 100 were found in the first sector and consequently not more than 30 in the part actually grown, the remaining 174 or more being at least partly located in the parts present before injecting the phosphorus.

In seeking an explanation of the presence of labelled phosphorus at a very considerable distance from the pulp we must remember that even the most remote incisal part of the tooth contains organic constituents. The constituents of the blood plasma penetrate through the latter and exchange of phosphate radicles and possibly also some ossification occurs in situ, though only to a modest extent on account of the poor circulation in comparison with that in the vicinity of the pulp. For part V (fig. 2) we found a loss of weight on ignition amounting to 25 % of the weight of the tissue dried in a vacuum dessicator; part I lost 29.8 %; and the average loss on ignition was found to be 27.4 %. That bones rich in organic constituents, i. e. such in which a comparatively effective circulation takes place, take up more radioactive phosphorus than the diaphysic bones poor in organic constituents had already been found previously in our investigations and is also to be seen in an example discussed on page 23.

Phosphorus exchange in growing rats.

It is tempting to explain the parallelism between the abundance of organic substance present in the tissue investigated and the percentage of labelled phosphorus present by assuming that the latter is chiefly present in the organic substance and not in the calcium phosphate of the bone or teeth. This possibility must however be discarded because blood weighing as much as an incisor contains after the lapse of few days less than 0.01 % of the labelled phosphorus taken, while that found in the incisors exceeds 1 %. In view of the importance of this point we tested the effect of the removal of the pulp on the exchange data. The experiment was carried out on a young rat which increased in weight from 87 to 110 gm. in the course of the 5 days which elapsed between the subcutaneous administration of the labelled phosphorus and the killing of the animal. Before the analysis the pulp was removed from

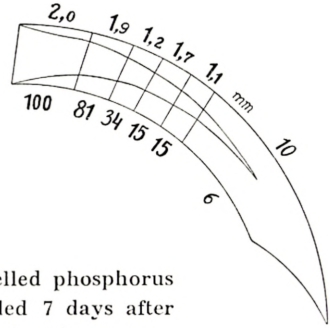

Fig. 3. Distribution of labelled phosphorus in the incisor of a rat killed 7 days after administration of the phosphorus.

The figures below give the relative amounts of labelled phosphorus present in 1 mgm. of fresh tissue in the section in question. The figures above give the length of the section in mm. 2.0 should read 2.8.

the two upper incisors and the activity of these incisors compared with that of the two lower incisors containing the pulp: we also measured the activity of the extracted pulp. The results are seen in Table 10.

Table 10.

	Fresh weight	Ash weight	% of the labelled P found per mgm. ash	Loss of weight on ignition
Lower Incisors .. + pulp	58.2	37.7	0.0192	35.2 %
Upper Incisors .. — pulp	33.2	22.9	0.0185	31.0 %
Tibia	800.1	123.2	0.0234	84.6 %

The activity of the extracted pulp was very weak and only amounted to about 3 % of that of the upper incisors. Should the exchange of phosphorus in the calcium phosphate of the teeth be very small, it is however quite possible that the amount of labelled phosphorus present in the pulp would no longer be negligible (comp. p. 26).

While in the experiments described above emphasis was laid on the investigation of the remote incisal end, in the following experiment we cut the proximal end of the incisor into small pieces and compared their activity with that of the distal end. The results are seen in Table 11, I denoting the united parts nearest to the jaw of all four incisors (comp. Fig. 3).

Table 11.

Distribution of labelled phosphorus, injected subcutaneously, in the incisor after 7 days. Weight of the rat about 200 gm.

Part of the incisor (comp. Fig. 3)	Length in mm.	Weight of the fresh tissue in mgm.	% of the labelled P found per mgm. tissue
I	2.8	7.3	0.0156
II	1.9	16.8	0.0127
III	1.2	21.3	0.0054
IV	1.7	25.1	0.0025
V	1.1	28.2	0.0025
VI	10.0	163.7	0.00096

Average % of labelled P per mgm. tissue = 0.0028, per mgm. ash 0.0038.

The investigation of the labelled P content of the head (A), the central (B) and lower part (C) of the tibia gave the following figures.

While in the proximal end of the incisor the phosphorus exchange is much greater than in any part of the tibia, the exchange of the average phosphorus atoms in

Table 12.

	Weight of the fresh tissue in mgm.	Weight of ash in mgm.	% of the labelled P found per mgm. ash
A	1131.1	144.5	0.0080
B	312.4	130.9	0.0026
C	243.8	63.5	0.0027

Average % of labelled P per mgm. tissue = 0.00098; per mgm. ash 0.0049.

the tibia is about 29 % greater than that of the average P atoms of the incisor; this is due to the fact that contrary to the tibia a large part of the incisor exchanges phosphorus atoms in the course of 7 days only to a very small extent. The figures in Table 12 cannot be compared directly with those obtained from fully grown animals for the following reason: the growing animal being much smaller the percentage of labelled P obtained for the same weight of the organ becomes larger; furthermore growth much facilitates the uptake of phosphorus. We can, however, compare the ratio of the labelled P content of the incisor and of other organs; the value of this ratio for the tibia, for example, is found to be not appreciably different in the cases discussed above. As to the labelled phosphorus content of the blood, this amounted after the animal was killed to only 0.04 % per gram of blood; assuming a total amount of 10 ccs. of blood all but 0.4 % of the labelled phosphorus administered left the blood of the animal in the course of 5 days.

The exchange of labelled phosphorus in molars.

In contrast to the incisors, molars of adult rats do not grow, so the labelled phosphorus found in the latter is due solely to exchange processes; the blood stream circulating through the molar carries labelled phosphate ions which enter into exchange processes with the calcium phosphate of the molar tissue. Such exchange processes also take place in the incisors simultaneously with the formation of new ossification products. In the molars of adult animals, however, we encounter chiefly the former process; but though growth can be excluded we cannot discard the possibility of dissolution of tooth tissue at one place and a corresponding precipitation of calcium phosphate at another along the boundary between the circulating fluid and the tooth tissue. Small fluctuations in the acidity or parathormone concentration of the blood are sufficient to cause such a process. The molars of the rat decribed on p. 15 showed a content of labelled phosphorus amounting to 0.0013 % per mgm. of tissue and 0.0018 % per mgm. of ash, which is less than in the average incisor. The loss on ignition was found to be 26.9 %. We thought furthermore that it would be of interest to compare the labelled P content of the incisors, molars and skeleton, choosing the tibia as representative of the latter. The figures obtained are seen in Table 13. The

Table 13.

Organ	Labelled P found in 1 mgm. fresh tissue as a percentage of the amount given	Labelled P found in 1 mgm. ash as a percentage of the amount given	Loss in weight on ignition
Incisor	0.0033	0.0044	26.4 %
Molar	0.0013	0.0018	26.9 %
Tibia	0.0024	0.0064	63.2 %

fresh tissue of the incisors contains more labelled P than equal weights of either the molars or the tibia, but comparing the ashes the tibia has a content three times as large as the incisors and eight times as large as the molars.

A comparison of the labelled P content of the ash is in general preferable to that of the fresh tissue, the former comparison giving information about the percentage of phosphorus atoms replaced by labelled ones. The total P content of the ash of the incisors varies between 19.6 and 20.0 %, and that of the molars and the tibia is only slightly smaller, about 18 %. A closer analysis of the tibia revealed the parallelism already mentioned between the content of organic tissue and labelled phosphorus; that is shown in Table 14. The rat was killed 3 days after the administration of the labelled P.

Table 14.

Part of the tibia, see fig. 4	Weight of the fresh tissue in mgm.	% of the labelled P found in 1 mgm. tissue	% of the labelled P found in 1 mgm. ash	Loss on ignition
a_1	215.4	0.0028	0.0134	79.1
b_1	64.4	0.0033	0.0064	48.3
c	78.8	0.0019	0.0033	42.0
b_2	33.1	0.0015	0.0028	47.5
a_2	81.5	0.0014	0.0034	58.7

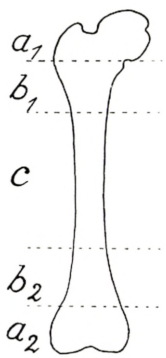

Fig. 4.

As seen from the figures of Tables 13 and 14 the head of the tibia has exchanged a part of its phosphorus content eight times as large as that exchanged by the molars.

Furthermore we compared the labelled P content of the incisors, the molars, and the tibia in the case of a rat weighing 220 gms. killed 1 hour after the labelled phosphorus had been administered by subcutaneous injection. The results are seen in Table 15.

Table 15.

Organ	Labelled P found in 1 mgm. fresh tissue as a percentage of the amount given	Labelled P found in 1 mgm. ash as a percentage of the amount given	Loss in weight on ignition
Incisor	0.00046	0.00062	26.0
Molar	0.00025	0.00034	27.4
Jaw	0.00125	0.0020	36.3
Tibia head	0.0024	0.0077	68.7
Tibia residue	0.00068	0.0014	52.7

The incisor was cut in 5 pieces the labelled P content of which is seen below, I denoting the proximal end.

Table 16.

	Weight of the fresh tissue in mgm.	Labelled P found in 1 mgm. fresh tissue as a percentage of the amount given
I	6.1	0.0062
II	14.4	0.0027
III	236.2	0.00040
IV	33.0	0
V	19.6	0

One cc. of plasma contained 0.5 % of the activity injected; assuming a plasma content of 10 cc., only 5 % of the labelled phosphorus injected was present in the blood after the lapse of 1 hour. Only 1 hour after administering the labelled phosphorus the tibia phosphorus was found to be 1000 times less active than the blood phosphorus, while for the molars the corresponding ratio was found to be 5000 and for the incisors (inclusive of growth) 2700. We also determined the activity of the acid soluble phosphorus extracted from the muscles of the rat and found 1 mgm. to contain 0.042 % of the labelled P given. From this figure and those found for the activity of the tooth and tibia phosphorus to be seen in Table 16 it follows that a comparatively fast phosphorus exchange is taking place in the muscle compared with that ascertained in the bones and the teeth.

Exchange of phosphorus in the teeth of cats.

For the teeth of young cats[1] killed a few hours after the subcutaneous injection of the labelled phosphorus the results seen in Table 17 and 18 were obtained.

Table 17.

Cat weighing 2 kg. killed after $3^1/_2$ hours.

Tooth	Weight of ash in mgm.	% of injected labelled P present in the tooth	% of the labelled P per mgm. ash of the tooth
Upper molar.....	123	0.016	0.00013
Lower molar	110	0.014	0.00013
Upper canine	108	0.044	0.00041
Lower canine	91	0.040	0.00044

Table 18.

Cat weighing 2.5 kgm., killed after $1^1/_2$ hours.

Tooth	Weight of ash mgm.	% of injected labelled P present in the tooth	% of the labelled P per mgm. ash of the tooth
10 Incisors....	94.8	0.0032	0.000034
Canine	148	0.019	0.00013
Jaw	126.3	—	0.00037

We also investigated fully grown cats. A cat weighing about 4.5 kgm. and killed three days after administration of the labelled P gave the figures seen in Table 19. In this experiment the labelled P injected was not of negligible weight but amounted to 15 mgm. (corresponding to about 75 mgm.

[1] The heads of the cats were kindly given to us by Professor Lundsgaard; they were obtained in the course of an investigation on the distribution of labelled phosphorus carried out by him and one of the present writers. In the first mentioned case 1 mgm. plasma P was found to contain after $3^1/_2$ hours 1.6 % of the activity injected, i. e. about 2300 times as much as that present in 1 mg. of the upper molar P.

sodium phosphate). The labelled phosphorus used in this experiment was kindly presented to us by Prof. LAWRENCE and was prepared by the action of high speed deuterium ions on phosphorus and accordingly contained a comparatively large amount of normal phosphorus. The injection of 15 mgm. P into a cat contained in its blood only about 10 mgm. inorganic P leads to an accelerated excretion and the figures are thus not entirely comparable with those of the last described experiment, which was furthermore carried out on a growing cat.

Table 19.

Cat weighing 4.5 kgm., killed after 3 days.

	Weight of ash in mgm.	% of injected labelled P present in the teeth	% of the labelled P per 100 mgm. ash of the tooth
Molars	690.5	0.0080	0.0012
Upper canines..	768.4	0.0076	0.0010
Lower canines..	635.2	0.0068	0.0013

The corresponding enamels weighed 29.3, 34.3 and 55 mgm. The canine enamel was found to contain less than 1/80 of the labelled P content of the corresponding dentine.

In another experiment a strong preparation was administered in three portions, 5 days, 2 days and 1 day before killing the animal, each portion containing 40 mgm. P. The results are seen in Table 20.

Table 20.

Cat weighing 4 kgm., killed after 5 days.

	Weight of teeth in mgm.	Weight of ash in mgm.	% of injected labelled P present in the teeth	% of the labelled P per 100 mgm. ash of the tooth
Molar	323.3	186.0	0.0027	0.0015
Canine	422.2	274.3	0.0038	0.0014
8 Incisors.	172.5	117.5	0.0021	0.0018

The enamel obtained is discussed on page 28. In investigating the incisors of rats we found the activity to be due almost exclusively to the phosphate of the mineral constituents, the pulp being only slightly active. In the earlier experiments conditions were however very different from those obtaining in the above mentioned case. The uptake of labelled P in the teeth of a cat is much smaller than in the incisors of a rat and correspondingly the ratio of labelled P in the plasma to labelled P in the teeth is much larger in the case of the cat. Now a high blood activity will lead to a comparatively high pulp activity and we must expect a greater share of the pulp[1] in the total activity of the tooth in the case of cat teeth. To test this point we removed the pulp of some of the canine teeth and compared the activity of the dissected and the total canine. We found an activity ratio of 3 : 4, showing that a quarter of the activity of the canines of a fully grown cat is due to the pulp.

A comparison of the figures of Tables 17 and 18 with those of 19 and 20 shows that the uptake of labelled P in young animals is greater than in fully grown ones and also that while in the former case the canines take up 3 to 4 times as much labelled P (per mgm. ash) as the molars, in the latter case no such difference is found. As has already been mentioned above the figures for the two sets of experiments are not entirely comparable, but no objection can be raised against a comparison of the ratio of the canine and molar uptake, which differs very markedly in the case of growing rats from the ratio for fully

[1] Human tooth pulp was found by H. C. Hodge (Proc. Soc. Exp. Biol. Med. **35**, 53, 1936) to contain 0.70 % phospholipins besides other phosphorus compounds.

grown animals. The following is a possible explanation of this difference: the labelled P uptake in the teeth of young rats is due partly to a growth of the teeth and not to an exchange process; since in the cat the canines grow faster than the molars the uptake is greater in the former case. One would be inclined to object to this explanation on the the short duration of the experiment, the growth in ground of the course of few hours being considered entirely negligible. This objection is however unwarranted. The molars of the growing cat weighed 116 mgm. and those of the fully grown animal 691 mgm. It does not take longer than a few years for the growing cat to become fully grown so the yearly growth of a molar will be above 100 mgm. Let us now calculate the amount of tooth ash formed on the assumption that the labelled phosphorus found in the tooth is due to growth. A molar of the growing cat took up 0.016 % labelled P during 3.5 hours. The labelled P which we injected into growing cats had in most cases a negligible weight originally, but very soon after the injection it mixed with the inorganic phosphate of the plasma (corresponding to about 5 mgm. P) and from that moment we must consider the labelled P as having a weight of about 5 mgm. 0.016 % of the labelled P will therefore correspond to 0.0008 mgm. P. The next step is that a large part of the labelled phosphorus leaves the plasma and is replaced by other phosphorus atoms coming from different bodily organs and also from the blood corpuscles. The result is that 0.016 % of the activity given no longer represents 0.0008 mgm. P but a greater weight, our scale of indication becoming less and less sensitive. From the experiences of Prof. LUNDSGAARD and one of us on the exchange of phosphorus present in the plasma we can estimate roughly that the amount of P which corresponds after the lapse of 3.5 hours to 0.016 % of activity is about 0.008 mgm. in the case discussed. To transform from phosphorus weight to ash weight we have to multiply by six. The weight of the tooth thus increases by 0.04 mgm. in 3.5 hours and about 100 mgm. in a year. The order of magnitude of the growth observed and that calculated on the assumption that the uptake of labelled P is due to growth and to exchange is thus the same.

A very simple but instructive calculation can be carried out in the case of a fully grown cat into which as much as 120 mgm. labelled P was injected. We can calculate how many milligrams of these 120 mgm. are to be found after the lapse of 5 days in a single tooth. Making use of the figures quoted in table 20 we find that a canine takes up 0.005 mgm. and a molar 0.003 mgm.

The behaviour of the enamel.

The difference in the mechanical properties of dentine and enamel is very pronounced. The hardness of anterior enamel is nearly half as great as that of hardened tool-steel, while dentine compares closely with brass[1]. The hardness is taken as the pressure in kilograms necessary to push a steel ball into the test piece.

The above mentioned difference is not due to a pronounced difference in the relative abundance of the mineral constituents of dentine and enamel, as discussed on p. 5, but to the following conditions. The amount of organic constituents + water found in dentine is about six times as large as the amount present in enamel, the calcification of the enamel tissue being thus carried through much more effectively than that of the dentine tissue. Bowes and Mur-

[1] H. C. Hodge, J. Dental Res. 15, 251 (1936).

RAY[1] found organic matter in human enamel to an extent of only 1 %. As there is more organic matter[2] in enamel near the junction with the underlying tissue, the dentine, than in the part equidistant from the dentine and the surface of the teeth, the outer part of enamel must contain even less than 1 % organic matter. The latter appears to be[3] a protein containing tyrosin and resembling reticulin.

Another outstanding difference between dentine and enamel seems to be the size and degree of orientation of the crystallites present in these. As to the orientation it has been stated[4] that enamel of high quality gives X-ray diagrams of a high degree of orientation, while enamel of poor quality does not. On igniting dentine an X-ray diagram characteristic of β-$Ca_3(PO_4)_2$ is often but not always observed[5]; this is never shown by ignited enamel. As it was found[6] that β-$Ca_3(PO_4)_2$ is formed when an excess of PO_4-ion is present, it was concluded that the dentine apatite often adsorbs an excess of phosphate ion which promotes the formation of β-$Ca_3(PO_4)_2$ on ignition. In the case of enamel forming larger crystallites, no excess of PO_4-ions being present, no β-$Ca_3(PO_4)_2$ formation was observed on ignition. While important information may be obtained by the study of X-ray diagrams the interpretation of the latter must be made with care.

Phosphorus exchange in the enamel.

In view of the connection found between the content of organic matter and phosphorus exchange in the teeth it

[1] J. H. BOWES and M. M. MURRAY, Biochem. J. 29, 721 (1935).
[2] C. F. BODECKER, J. Dental Res. 6, 2, 117 (1923).
[3] P. PINCUS, Nature 138, 970 (1936).
[4] J. THEWLIS, Naturw. 25, 42 (1937).
[5] W. F. BALE, M. L. LEFEVRE and H. C. HODGE, Naturw. 24, 976 (1936).
[6] G. TRÖMMEL and H. MÖLLER, Z. anorgan. Chem. 206, 227 (1932).

did not appear very promising to look for a pronounced exchange in the enamel. The enamel investigated by us was in some cases removed mechanically while in others we succeeded in separating the enamel of cat teeth after igniting the tooth very carefully. The enamel, having a different expansion coefficient from the dentine, splits off during the ignition process and can thus be removed. The method of separation used recently by various workers[1], in which the tooth is pulverized and placed in an organic liquid of suitable density when the heavier enamel settles to the bottom of the tube, is not suitable for our purpose. The reason is that some dentine often sticks on the pulverized enamel; assuming that the dentine is strongly active and the enamel not, we see that the presence of traces of dentine in the enamel might falsify the analysis.

We made several experiments with the enamel of cat teeth but in most cases with negative results, the exchange in equal weights of enamel being at least 20 times as small as that found in the molars of cats. In one case we got a positive effect, the canine of a fully grown cat five days after injecting the labelled phosphorus showing a radioactivity of 26 relative units (kicks per minute), one enamel sample showing 0.6, and another 0.7 kicks. The first mentioned enamel was separated by grinding it off from the dentine, while the second one was obtained by the same method from the uppermost enamel layer. The ash weight of the canine was 277.3 and that of the enamel samples 33.1 and 19.1 mgm. We are however reluctant to accept this positive result. On account of its smaller weight and greater distance from the underlying dentine, the outermost layer should be less active than the second enamel

[1] comp. P. J. Brekhus and W. O. Armstrong, J. Dental Res. 15, 23, 1935.

layer unless the labelled phosphorus present in the saliva (which latter contains[1] 13.4 mgm. per 100 ccs.) can interact with the outer layer of the enamel; this is not very probable, the uppermost layers excepted. We intend to follow up the problem of the phosphorus exchange in enamel using phosphorus preparations of greater activity.

Exchange of phosphorus in human teeth.

Other things being equal the exchange of phosphorus in teeth will be determined by the efficiency of lymph circulation in the tooth. Exchange experiments can thus be carried out to obtain information on the latter point. It does not look improbable that the growth of caries will be facilitated by a poor circulation; to decide this point we compared the phosphorus exchange in two teeth of the same individual (16 years old) removed simultaneously, one on account of caries, the other, a healthy one, to space the patients teeth better; about a two hundred thousandth part of the labelled phosphorus was found in each of the teeth investigated, a quantity sufficient to be measured but not large enough to permit the exact comparison necessary to decide the point discussed above. The weights of the whole fresh teeth were 800 and 540 mgm. and of the ash obtained on ignition 465 and 330 mgm. respectively; this corresponds to a loss on ignition of 58 and 61 % respectively. The time which elapsed between the injection of the radioactive phosphorus and the extraction of the teeth was 7 days. Through the very great kindness of Professor Lawrence we were able to continue these experiments using a much stronger radioactive phosphorus sample prepared by him with the aid of his powerful cyclotron. 900

[1] M. Karshan, J. Dental Res. 15, 388, 1936.

mgm. labelled sodium phosphate per os were administred to a patient 25 years old. 4 days later 10 necrotic teeth and 5 days later still, three more, fairly well preserved, living teeth were extracted. Of the $2.5 \cdot 10^6$ relative radioactive units we can estimate that about $1.8 \cdot 10^6$ were absorbed. As is seen in table 21 6 relative units were found in a fairly well preserved tooth on an average, showing that about 1 : 300,000 part of the labelled phosphorus atoms enter a single tooth; in the case of a 16 years old boy about 1 : 200,000 was found. In the latter case an activity of only 0.5 units (kicks per minute) was shown by a single tooth, and the estimate was accordingly only a very rough one. From the above result it follows that about 1 : 300,000

Table 21.

Labelled phosphorus in the teeth of a 25 year old patient.

a) Necrotic Roots.

Nr.	Fresh weigh	Ash weight	Relative labelled P content: In total root	Relative labelled P content: In 100 mgm. root ash
1	223.7	138.1	2.7	1.96
2	284.1	180.1	4.9	2.72
3	199.4	127.1	2.5	1.97
4	230.5	143.0	1.4	0.98
5	124.8	76.5	3.9	5.13
6	435.2	268.5	5.9	2.19
7	205.7	127.1	4.9	3.86
8	169.5	109	1.6	1.47
9	172.5	106.6	3.8	3.57
10	183.5	115.0	2.1	1.83

b) Necrotic Crowns.

	Fresh weight	Ash weight	Relative labelled P content: In total crown	Relative labelled P content: In 100 mgm. crown ash
One single crown	65.8	39.1	3.7	9.4
Fragments of several crowns	241.7	149.8	12.3	8.2

c) Almost normal roots.

Nr.	Fresh weight	Ash weight	Relative labelled P content In total root	Relative labelled P content In 100 mgm. root ash
1	377.4	241.0	2.8	1.16
2	651.1	413.6	3.7	0.9
3	685.9	430.2	6.7	1.56

d) Almost normal crowns.

Nr.	Fresh weight	Ash weight	Relative labelled P content In total crown	Relative labelled P content In 100 mgm. crown ash
1	533.5	338.7	1.9	0.56
2	862.9	670.9	1.8	0.27
3	464.1	429.9	1.8	0.42

part of the phosphorus taken up with the food finds its way into each tooth of an adult. In the course of time these phosphorus atoms get replaced by others taken up with the food or originating from other bodily organs. The normal diet containing about 2 gm. of phosphorus per day and a human tooth containing about 150 mgm. P, the replacement of 1 % of tooth P by that taken up with the food will take 250 days. Simultaneously a further replacement of the phosphorus atoms takes place with phosphorus originating from other bodily organs.

Summary.

It has been shown that an exchange of phosphorus atoms present in the teeth with those present in the blood plasma takes place.

During the growth of the incisors of rats the newly deposited phosphorus atoms are to a large extent found in close vicinity of the dental pulp, but even in the most

remote part of the incisor the presence of newly substituted phosphorus atoms can be established. An exchange of phosphorus atoms thus takes place even in those parts of the incisors which are entirely outside the range of the pulp. The exchange in the molars was found to be less pronounced than that in the incisors, this being presumably due to the fact that these teeth do not grow.

In the teeth of young cats within few hours, besides an exchange of phosphorus atoms, an increase in the labelled phosphorus content due to the growth of the teeth could already be ascertained.

An exchange of phosphorus has also been proved for human teeth, 1 : 300,000 of the phosphorus administered being found in each tooth. The replacement of 1 % of the phosphorus content of a human tooth by phosphorus atoms taken up with the food takes about 250 days.

The labelled (radioactive) phosphorus applied was partly prepared by us from sulphur under the action of neutrons emitted by a radium-beryllium mixture, most kindly put at our disposal by Professor Niels Bohr, and partly formed a generous gift from Professor Lawrence of the University of California. Besides thanking Professor Bohr and Professor Lawrence, we would also like to express our best thanks to Miss Hilde Levi, Miss A. L. Lindberg and Mr. O. Rebbe for their assistance.

(From the Institute of Theoretical Physics, the Dentistry School and the Zoophysiological Laboratory, Copenhagen.)

Færdig fra Trykkeriet den 23. November 1937.

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