CCCXXIV. THE CHEMICAL COMPOSITION OF TEETH.

II. THE COMPOSITION OF HUMAN ENAMEL AND DENTINE.

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It is known that the chief inorganic constituent of enamel and dentine is calcium phosphate. Bassett [1917] concluded that the calcium phosphate was in the form of hydroxyapatite. Gassmann [1910; 1921; 1928] found in bones and teeth a constant ratio of $10\text{Ca} : 6\text{P} : 1\text{CO}_2$ indicating that the bone compound was $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaCO}_3$, calcium carbonatophosphate or carbonatoapatite. The best evidence that teeth are composed of apatite was obtained from X-ray analysis by de Jong [1926], Taylor and Sheard [1929], Roseberry *et al.* [1931], Thewlis [1932], Klement and Tromel [1932] and Bredig [1933]. From this method it is difficult to decide which form of apatite is present and no information is gained about substances present in small amounts. The most complete chemical analysis of the inorganic constituents was made by Gabriel [1894] on ox teeth and his figures are those most generally quoted. Since that time determinations have been made of the amounts of certain elements and their possible variations under different conditions. Gassmann [1921] determined Ca, P, CO_2 and Mg of whole teeth, working chiefly with wisdom teeth. Kaushansky [1930; 1932] and Howe [1926] studied the Ca, P and Mg in caries and pyorrhoëa. These and other determinations were made on crowns or whole teeth, that is on a variable mixture of enamel and dentine. Fish [1932] investigated the Ca content of dentine of different ages and under different conditions.

With regard to the organic matter of enamel, Evans [1913] concluded that there was 1–2 % present. Sprawson and Bury [1928] stated that, judged from the combustible carbon and the nitrogen, only a very small amount of protein is present.

The present investigation aimed at obtaining values for the composition of sound human teeth as a basis for comparison. The material was very carefully selected; therein lies the difference between this and much previous work, carried out before it was realised that even sound teeth vary in structure. Sound (non-carious) permanent premolar teeth of children under 14 years of age, removed for orthodonture purposes, were collected from the school clinics. These teeth were examined with a lens and probe in the manner described by Mellanby [1923; 1934] and separated into five classes, comprising perfect teeth and four degrees of hypoplasia. For a tooth to be graded as perfect it had to have a hard, smooth, shining surface devoid of markings, pits or fissures. Such teeth were rarely encountered. This can be understood, since a perfect tooth is not likely to occur in an overcrowded mouth. The next class of tooth, showing a very mild degree of hypoplasia, usually restricted in area, occurred more frequently. The determinations reported in this investigation were all carried out on this class of tooth. In this way we avoided variations in composition due to differences in age and type of tooth [Fish, 1932].

Preparation of material.

After classification the teeth were placed in hot water, brushed and filed to clean the surface and then put into neutral 95 % alcohol till required. Enamel and dentine were analysed separately and were obtained in the following manner. The cleaned tooth was held in a hand clamp and the enamel ground from the outside of the tooth by a rapidly rotating cylindrical file of very hard steel. The file was surrounded by a celluloid guard and the fine enamel powder was directed down a chute into a beaker below by means of a gentle current of air. By careful examination of the tooth every few minutes, it was possible to ensure that only enamel was removed in this way, grinding being stopped before the dentine was reached. To obtain the dentine, the cutting edge of the tooth was then ground down on a carborundum wheel till the dentine was exposed. The file was replaced by a dentist's rose head burr and the dentine bored out and collected as in the case of enamel until only a shell of dentine and enamel remained. The separated powders were freed of iron by means of a magnet and magnetised needle. The material was further examined with a lens for specks of extraneous matter such as cotton fibres *etc.* The enamel and dentine powders were dried at 105–110° and transferred to a vacuum desiccator until used for analysis.

The analyses.

These included determinations of total ash and nitrogen to give an indication of the organic matter and combined water and carbon dioxide. The nitrogen was estimated by micro-Kjeldahl incineration and distillation followed by nesslerisation. The inorganic analyses included the determination of Ca, Mg, Na, K, P, CO₂, Si, F and Cl. The Ca, P and Mg were determined on the same dilute acid solution of the material, the Ca by titration with $N/50$ KMnO₄ of the calcium oxalate precipitated at p_H 5.6–5.8 and the P by Robison's modification of the Briggs method. The Mg determinations gave considerable trouble. The precipitation as magnesium ammonium phosphate gave very discordant results. The method of Greenberg and Mackay [1932] for the estimation of Mg in blood was modified and successfully applied. For the Na determinations it was necessary to remove the Ca and the phosphate before the zinc-uranyl acetate method of Salit [1932] could be applied. Careful addition of ammonia conveniently removed both as Ca₃(PO₄)₂. In the case of dentine, fat and protein extractions were first carried out. In spite of the fact that we used the purest chemicals available, we found it necessary to perform blanks on the reagents and to account for the sodium arising from the glass in the course of manipulations. For the potassium estimations the material was treated with H₂SO₄ to convert into K₂SO₄, taken up in water and filtered. The determination was made by the method of Shohl and Bennett [1928], which involves precipitation as K₂PtCl₆, treatment with KI and titration of the resulting iodo-compound with thio-sulphate. The combined CO₂ was estimated in a specially adapted Van Slyke apparatus which had at the top a one-way tap with a large bore (7 mm.). Through this tap the substance was introduced as a fine powder by a special device. This consisted of a very small glass funnel with a stem of such a size and length that it would go through the tap. The funnel was fitted with a cork which carried a glass plug for the stem. The plug was inserted into the stem of the funnel, about 0.1 g. powder introduced, the cork placed in position and the whole weighed on a watch glass. The sample was introduced into the apparatus by placing the stem through the tap and pulling out the cork and plug; the powder then dropped into the burette. The funnel was re-weighed immediately. 2*N* HCl was used to liberate

the CO_2 . We found it necessary to standardise the conditions of the determinations on a known mixture of pure $\text{Ca}_3(\text{PO}_4)_2$ and CaCO_3 . The CO_2 determinations were also carried out on samples after they had been heated in a furnace to a temperature and for a time previously determined as sufficient to drive off the CO_2 from CaCO_3 and MgCO_3 . The chlorine was determined by the silver nitrate-thiocyanate procedure in an aqueous extract and on a HNO_3 solution of the same sample. The determination of the F has been dealt with fully elsewhere [Bowes and Murray, 1935]. Recently we have used distillation of the F as H_2SiF_6 by treatment of the material with HClO_4 and then applied the Zr-alizarin colorimetric determination. Silicon was estimated in whole teeth by the method of King and Stantial [1933]; all processes in this determination were carried out either in platinum or "bakelite" vessels.

In almost all the determinations the methods employed had to be adapted to the particular needs, because the high proportion of $\text{Ca}_3(\text{PO}_4)_2$ interfered with most of the estimations. The methods were tested on known samples of $\text{Ca}_3(\text{PO}_4)_2$ to which the particular constituent had been added in suitable proportion. In this way we were able to determine the degree of accuracy to be expected. The determinations on the tooth material were repeated until we could claim an accuracy of 0.2 % for the Ca and P, 2 % for the Mg and less accuracy for the other elements. We are doubtful as to the presence of K, the values obtained being almost within the limits of experimental error.

Consideration of the enamel results.

The first thing to be noticed (Table I) is the very small amounts of Si and F. It is continually stated in textbooks that teeth contain significant quantities of both of these elements; moreover F is probably not present in all teeth. The Cl content is greater than the F content. This Cl is part of the tooth substance

Table I. *Composition of enamel.*

	Dry enamel
Ash	95.38 %
Nitrogen	0.156 %
Combined H_2O (calculated)	1.347 %
Combined CO_2	1.952 % (After heating 0.54%)
Calcium	37.07 %
Magnesium	0.464 %
Sodium	0.25 % (Aqueous extract 0.1 %)
Potassium	0.05 % or less
Phosphorus	17.22 %
Chlorine	0.3 %
Fluorine	0.025 %
Silicon	0.003 % (Whole teeth)
Ca/P	2.153 (Ca/P for apatite is 2.151)
Ca/Mg	79.89
P/ CO_2	8.822

$$\text{Ca} : \text{P} : \text{CO}_2 \text{ (molar proportions)} = 10 : 5.994 : 0.479.$$

and is not present adventitiously as a salt soluble in water, because before the enamel was dissolved in HNO_3 for this estimation it was extracted with hot water. The loss in weight on ashing represents the organic matter, the bound water and part of the CO_2 .

The value of the Ca/P ratio and the relatively small amount of other substances present indicate that a form of apatite $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaX}$ is the chief constituent of enamel, representing approximately 90 % of the dry and 95 % of the

ashed substance. This is what would have been expected from the results of X-ray analysis. This latter method, however, is not able to distinguish definitely between the various forms of apatite, and opinions differ as to which form represents the bone and tooth compound. The possible forms are chloro-, fluoro-, carbonato-, hydroxy- and oxy-, where the appropriate element or radical represents the X in the general formula stated previously. Bredig [1933] has compared the X-ray patterns of the teeth and bones with the artificially prepared apatites and is of the opinion that the compounds of bone and teeth are essentially the same and are hydroxyapatite. Klement and Tromel [1932] are of the same opinion. Hendricks *et al.* [1932] favour the presence of carbonatoapatite in bone, whilst de Jong states that he is unable to distinguish between the various apatite forms. Dealing with bone, on which much work has been done, opinion also varies as to whether the complex $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaX}$ is the only compound deposited and whether it is necessary to consider the separate deposition of salts such as CaHPO_4 and CaCO_3 . Robison [1932] considers that there is no reason to postulate the separate formation of CaCO_3 . Roseberry *et al.* [1931] state that there is no evidence for the separate deposition. On the other hand, Marek *et al.* [1934], as well as discussing the previous evidence for and against the question of the existence in bone of one single compound, concluded from their own analyses of young pig bone that the $\text{Ca}_3(\text{PO}_4)_2$ and the CaCO_3 were deposited separately, because the relative amounts of these two substances were found to vary in different parts of the bone. A similar conclusion was drawn by Burns and Henderson [1934], working on young bone. Gassmann [1921] considered that the ratio $\text{Ca} : \text{P} : \text{CO}_2$, found by him in bones and whole teeth, was so near to that required by carbonatoapatite that he deduced that this was the chief constituent of both of these. The values obtained in this investigation did not agree entirely with those of Gassmann who, it must be remembered, worked on whole teeth. Enamel, though probably a form of apatite, is not exclusively carbonatoapatite. It is probably not justifiable to consider the bone and teeth to be of the same chemical composition; dentine but not enamel is comparable with bone. The results reported here show that the enamel and dentine are not identical in their inorganic constituents. In normal enamel the P is probably only present as apatite whereas dentine possibly contains CaHPO_4 in addition; there are also distinct differences in the CO_2 contents. Dentine is in all ways more similar to bone; the CO_2 contents are approximately equal; enamel contains much less CO_2 . It is significant that enamel, on prolonged heating, does not lose all its carbon dioxide whereas dentine and bone, heated under the same conditions, lose all. CaCO_3 and MgCO_3 heated for the same time and to the same temperature also lose all their CO_2 . It has not been possible to test the effect of heat on carbonatoapatite for no sufficiently pure specimens can be made. Since it is probable that the CO_2 combined as apatite would be more firmly held, it was assumed that the CO_2 remaining in the enamel after heating was present as apatite, whilst that driven off was present as CaCO_3 or MgCO_3 . Gassmann [1921] emphasised the stability of the complex calcium carbonatophosphate as compared with $\text{Ca}_3(\text{PO}_4)_2$ and CaCO_3 . Working on this assumption it is possible, by calculation and deduction on a percentage basis, to form an idea of the probable combination of the elements composing the enamel. The CO_2 was considered to be distributed in the following way: that remaining in the heated enamel was taken to be present as carbonatoapatite; that driven off by heating to be combined with the Mg and the excess with Ca as carbonate. The amounts of fluoro- and chloro-apatite were calculated from the F and Cl values. The remainder of the Ca was taken to be present as hydroxyapatite. The values obtained by

these calculations are given in Table II. The corresponding figures for P found in this way by calculation agreed within experimental limits with those determined practically. It is possible that a very small amount of organically bound P occurs; this is indicated by the slight rise in Ca/P ratio on ashing.

Table II. *The composition of human enamel found by calculation, using the figures given in Table I.*

	%		Ca % in enamel	P % in enamel
MgCO ₃	1.624		—	—
CaCO ₃	1.331	≡	0.532	—
Carbonatoapatite	12.06	≡	4.684	2.178
Fluoroapatite	0.663	≡	0.26	0.122
Chloroapatite	4.397	≡	1.69	0.786
Hydroxyapatite	75.04	≡	29.90	13.90
Sodium	0.25		—	—
Loss on heating (other than CO ₂ and H ₂ O)	1.837		—	—
Total	97.202		37.066	16.986
Determined values (see Table I)	—		37.07	17.22

The loss in weight on ashing (4.62 %) represents the bound water, the greater part of the CO₂ and the organic matter. Assuming that the water is driven off from the hydroxyapatite to form oxyapatite a value (1.837 %) for the organic content can be obtained; this is probably not the true value. The protein content calculated from the N would be 0.98 %. The N value obtained by us was greater than that found by Sprawson and Bury [1928]. The utmost care was taken in the collection of the enamel. Blank determinations were made on the reagents and the recovery tested by distillation of standard (NH₄)₂SO₄ solutions.

Dentine.

The analysis of the dentine presented a difficulty because of its high organic (protein and fat) content. It is easy to remove the organic part by extraction but it is difficult to get quantitative recovery and so calculate back to the dry material. Calculating from the P content of the dry dentine and the loss in weight on ashing, it appeared that 0.4 % of the P is lost in the ashing process. It can be deduced from the N values that not all the organic matter is protein; fat extraction raises the Ca/P ratio; these two facts indicate that this loss of P is due to the presence of lipid P and that no inorganic P is lost. Hence it was considered possible to use the values obtained on ashed dentine for the calculations dealing with the consideration of the composition.

Consideration of the dentine results.

Dentine differs from enamel in several respects (Table III). The former is devoid of chlorine; if the values for the ashed dentine are compared with those of the enamel it can be seen that dentine contains more Mg; the P content of dentine is also greater; this gives a lower Ca/P ratio, which does not correspond so closely to the ratio for apatite as does that of enamel. All the CO₂ was lost on heating, which was taken to mean that all was present as simple carbonate. We considered that all the Mg was present as MgCO₃ and assumed the rest of the CO₂ to be combined as CaCO₃. In this way the following figures were obtained:

0.835 g. Mg (found)	2.922 g. MgCO ₃ (dry dentine)	1.958 g. MgO (ashed).
1.645 g. CO ₂	3.738 g. CaCO ₃ (dry dentine)	2.945 g. CaO (ashed).

Table III. *The composition of dentine.*

	Dry dentine	Ash of dentine
Ash %	71.09	—
Nitrogen %	3.43	—
Calcium %	27.79	39.83
Magnesium %	0.835	1.175
Sodium %	0.19	0.267
Potassium %	0.07 or less	0.1013
Phosphorus %	13.81	19.04
CO ₂ %	3.176	Nil
Chlorine %	Nil	Nil
Fluorine %	0.0246	0.0346
Ca/P	2.012	2.091
Ca/Mg	33.29	33.90
P/CO ₂	4.349	—
Ca : P : CO ₂ (molar prop.)	10 : 6.412 : 1.036	10 : 6.17 : 0

The Ca left in the ashed dentine was then 37.727 % and this with a P value of 19.04 % (Ca/P=1.981) could not be present as apatite only. From the ratio of Ca/P, the possibilities are that the Ca and P are present as (1) Ca₃(PO₄)₂ or (2) Ca₃(PO₄)₂+CaHPO₄ or (3) 3Ca₃(PO₄)₂.CaX+CaHPO₄. The X-ray analyses have shown that dentine contains considerable quantities of apatite and hence the third possibility stated above seemed the most probable. Working on this assumption, the following figures were obtained: 11.67 % CaHPO₄ and 58.03 % of hydroxyapatite in dry dentine, due consideration having been given to the fluoroapatite. Table IV gives a complete account of the results of these calculations based on the analyses given in Table III above.

Table IV.

	Dry dentine %		Ashed dentine %	Ca in ashed dentine %	P in ashed dentine %
MgCO ₃	2.922	MgO	1.958	—	—
CaCO ₃	3.738	CaO	2.945	≡ 2.103	—
CaHPO ₄	11.67	CaHPO ₄	16.42	≡ 4.829	3.742
Hydroxyapatite	58.03	Oxyapatite	80.19	≡ 32.528	15.12
Fluoroapatite	0.663	Fluoroapatite	0.933	≡ 0.370	0.172
Sodium	0.19	Sodium	0.267	—	—
Organic matter	24.693	—	—	—	—
Total: (Calc.)	101.906		102.713	39.83	19.034
(Found)				39.83	19.04

It is realised that this treatment of the results is not the only possible one but after due consideration it seemed justifiable. Hydroxyapatite is the most abundant constituent of the dentine as it is of enamel. It is to be noted that the quantity of Mg in human teeth is considerably less than in the teeth of some other mammals and that the actual quantity is so small that it cannot replace Ca to any extent in normal teeth. Mg is now considered important for the activity of phosphatases and it may be that the significance of Mg in relation to teeth is in connection with the proper laying down of the Ca₃(PO₄)₂ complex rather than with the formation of part of the inorganic complex. No doubt when Ca is lacking Mg may be laid down in its place, but this is abnormal.

It is hoped that similar analyses of sound teeth showing hypoplastic structure and of carious teeth will throw some light on the problem of dental disease.

SUMMARY.

1. Analyses of enamel and dentine of sound human teeth of good structure and of one type from a defined age group were made. The total ash, N, Ca, Mg, Na, K, P, CO_2 , Cl, F and Si were determined.

2. The compositions of the inorganic parts of enamel and dentine are not identical. Enamel consists mainly of apatite, the greater part of which is in the hydroxy form.

3. Dentine contains less apatite but more carbonate than the enamel. Not all the inorganic P of the dentine is in the form of apatite; CaHPO_4 is probably a constituent.

4. The CO_2 of the enamel is not all driven off by heating; that of the dentine is.

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