CHEMICAL CONSTITUTION OF ENAMEL AND DENTIN

I. PRINCIPAL COMPONENTS*

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An understanding of the calcification process requires exact knowledge of the nature and variations of composition of the mineral phase of calcified tissues. Other investigators have continued the study, begun years ago, of the composition of the inorganic salts of bone, but a comprehensive study of the results of analysis of enamel and dentin by modern methods has not been described. Crowell, Hodge, and Line (1934) have reported the results of a very complete survey of the composition of the mineral phase of whole adult teeth. These authors present a comprehensive summary of the literature on the composition of whole teeth, enamel, and dentin. Recently Le Fevre, Bale, and Hodge (1937) described the composition of the mineral phase of whole fetal teeth. The analyses reported in both papers were made on the inorganic residue prepared by boiling the specimen in a solution of potassium hydroxide and ethylene glycol.

The variations in relative amounts of enamel, dentin, and cementum in teeth make the interpretation of analyses of whole teeth difficult and it is, therefore, desirable to determine the composition of enamel and dentin separately. The older studies described the composition of only a few specimens and in many cases the analyses were made on ashed materials. Changes in composition of the mineral phase are known to result during ignition (Bowes and Murray, 1935; Howland, Marriott, and Kra-

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mer, 1931). In recent years only a few attempts have been made to analyze enamel and dentin. Bowes and Murray (1935, 1936) have reported the composition of the pooled enamel and dentin of a number of human teeth. Logan (1935) presented the analyses of the enamel and dentin fractions of two human and two dog teeth and compared the results with those of bone obtained by the same methods. The paucity of information relative to the composition of these fractions of teeth is undoubtedly due to the difficulty experienced in their quantitative separation. Other workers separated enamel and dentin by grinding or other laborious and uncertain mechanical procedures. The method previously described (Brekhus and Armstrong, 1935) by us has permitted a more ready separation of enamel and dentin from each other and from cementum.

We have completed the analyses of a sufficient number of specimens to describe, with statistical significance, the normal variations of composition of enamel and dentin. The possible difference in the composition of enamel with respect to the principal elements of sound and carious teeth has been investigated. The relationship of composition of enamel to the varying susceptibility of the teeth of the human dentition to decay has been studied. Other factors which might be expected to influence the amounts of the constituents of enamel have been considered. The analytical procedures were applied to the unashed protein-containing specimens to eliminate the possibility of chemical alteration of the material in preparation for analysis. Such analyses can be directly compared with those of bone made by Kramer and Shear (1928).

Methods

The teeth, immediately following extraction, were stored in 95 per cent alcohol until preliminary procedures were applied. The lesions of carious teeth were entirely ground away until only sound enamel and dentin remained, since to have left the decayed areas in place would have resulted in the determination of alterations of composition secondary to the carious process. The specimens were cleaned with the aid of dental instruments, broken into bits, and the pulp removed. The fragments were made into a packet in cloth and extracted for 6 hours, in a Sohxlet apparatus, with a mixture of equal parts of alcohol and ether. Each specimen was

dried at 60°, pulverized until all passed a 100 mesh sieve, and the powder submitted to the enamel and dentin separation process (Brekhus and Armstrong, 1935).

With certain exceptions, the analytical procedures[1] are modifications of those used by Kramer and Howland (1926) and Shear and Kramer (1928) for the analysis of bone. Approximately 50 mg. of enamel or 70 mg. of dentin were put in solution in 20 cc. of M HCl by heating the flask in a boiling water bath for 15 minutes. The solution was diluted to 100 cc., that of dentin being first filtered through retentive paper to remove a slight cloudiness, probably caused by undissolved protein.

Calcium was determined in duplicate on 2 cc. aliquots of the solution according to the method of Kramer and Tisdall. The supernatant liquid was siphoned from the precipitate of calcium oxalate which was then washed twice with dilute ammonium hydroxide solution. Potassium permanganate (0.01 N), standardized against sodium oxalate from the Bureau of Standards, was employed. It is important to determine separate blanks for the titration of the standard oxalate solutions and unknowns if the final volumes of the titrated solutions of the standards are considerably greater than those of the unknowns, since the amount of excess permanganate required to color the solutions varies directly as their volumes. Ten analyses of solutions containing 0.446 mg. of calcium per 2 cc. were made. The largest error was + 2 per cent and the error of six results was +0.7 per cent of the calculated amount. Five samples of enamel were analyzed in the routine manner and several months later five determinations were made on each sample. The greatest difference between the first and the average of the three most concordant of the subsequent determinations on any one specimen was 1.4 per cent.

Phosphorus was determined in duplicate on 5 cc. aliquots of the solution by the gravimetric method of Embden (1921) which employs the precipitation of strychnine phosphomolybdate. It is important to use not more than 3 drops of brom-phenol blue solution, added after the aliquots are diluted to 30 cc., since an excess of indicator causes high results. The troublesome formation of cracks in the precipitate in the Gooch crucible, which

[1] A detailed description of the analytical methods used in this study has been published (McClendon and Pettibone, 1936).

hinders adequate washing, is prevented if the suction is regulated at 100 mm. of mercury and the precipitate kept wet with wash liquid. Precipitates containing less than 0.5 mg. of phosphorus rarely develop cracks with any form of treatment. Solutions containing 0.5 mg. of phosphorus were analyzed with errors not greater than 1 per cent.

Magnesium was determined in duplicate on 20 cc. aliquots of the solution by an adaptation of the Briggs (1922) method. In the case of enamel and dentin, a quantitative separation of calcium and magnesium oxalates is not obtained at pH 6.2, as described by Kramer and Howland (1926) for bone. In solutions containing a large excess of calcium, the precipitation of calcium oxalate at about pH 4 (just pink to methyl red) permits the most accurate results to be obtained on the analysis for known amounts of magnesium.

To each aliquot 2 drops of methyl red solution and 1 cc. of 0.5 M oxalic acid were added. The solution was heated to boiling and M NH_4OH added until the color was yellow-pink. Very dilute HCl was then added until the color was light pink, following which the mixture was digested on a steam bath for 3 hours. The precipitate of calcium oxalate was removed by filtration through Whatman No. 42 paper, the beaker and filter being washed with four 10 cc. volumes of 0.1 M NH_4OH. The filtrate was evaporated to about 7:5 cc., transferred to a pointed 50 cc. centrifuge tube, and the determination finished in the manner described by Kramer and Howland. The magnesium ammonium phosphate precipitate, which was allowed to collect overnight in a refrigerator, was washed with four 10 cc. volumes of 3 M NH_4OH. The supernatant fluid was removed, after centrifugation, with the aid of a siphon. The standard solutions contained 0.05 and 0.2 mg. of phosphorus for the analysis of enamel and dentin respectively. All solutions were diluted to 50 cc. for colorimetric analysis.

Numerous determinations of the magnesium content of solutions containing 0.15 mg. of magnesium, 10 mg. of phosphorus, 15 mg. of calcium, and 20 cc. of M HCl per 100 cc. gave results which were within 5 per cent of the theoretical content.

Carbonate was determined as carbon dioxide on separate 40 to 50 mg. samples in the Van Slyke volumetric apparatus. The sample was washed into the chamber of the apparatus with 4 cc.

of water divided into small portions, and another 1 cc. was added to overlay the material. The fluid level in the gas burette was raised until it entered the bore of the stop-cock of the filling bulb and the stop-cock was closed. Then 1 cc. of water was placed in the filling bulb and underlaid with 5 cc. of 5 N HCl which was rapidly admitted to contact with the sample by opening the upper stop-cock, after producing a slight vacuum in the chamber. The determination was finished in the manner described by Kramer and Howland (1926). Since 10 cc. of liquid were present in the chamber of the apparatus, the calculation of the result according to the formulas of Van Slyke (1917) (*cf.* Kramer and Howland, 1926) was simplified. Attempts were made to use the solution removed from the Van Slyke apparatus for the determination of calcium, magnesium, and phosphorus but the results did not agree with those made directly on the sample. The quantity of enamel obtainable from a tooth is limited and not all determinations of carbonate on this material were done in duplicate.

Nitrogen was determined in duplicate on separate samples of dentin by the Kjeldahl method according to the technique of Cavett (1931). The determinations of nitrogen in enamel, which required nesslerization of ammonia, are not reported because the sample limitations permitted only a few determinations, whose results were variable.

The mean differences in duplicate analyses of enamel when the results are expressed as percentage of the sample were as follows: calcium 0.189, phosphorus 0.087, magnesium 0.025, and carbon dioxide 0.044. These differences in the analyses of dentin were calcium 0.100, phosphorus 0.039, magnesium 0.022, carbon dioxide 0.034, and nitrogen 0.058. Duplicate analyses which failed to check within the usual range were repeated wherever possible.

Results

Table I is a summary[2] of all results of analysis of sound enamel and dentin. Most of the analyses were made on the enamel and dentin fractions of a single tooth, but in some instances it was

[2] The data of the analyses of the individual specimens, identified as to sex and age of the person and position of the tooth in the mouth, will be supplied to those wishing them. The composition of the enamel and dentin fractions of the same teeth can then be compared.

necessary to pool paired teeth from one individual to increase the amount of enamel to a quantity which would permit all analytical procedures to be applied.

Composition of Mineral Phase of Enamel and Dentin—In Table II are presented certain conventional calculations based on the data of Table I. Practically every publication on the chemical composition of calcified tissues includes such calculations, but it should be realized that the concept of state of combination of the elements implied by these ratios is fictitious. It will be noted that the residual Ca to total P ratio of enamel and dentin does not equal that of the hypothetical $Ca_3(PO_4)_2$, namely 1.94, as is the case with bone (Shear and Kramer, 1928). The high magnesium content of these materials requires that the phosphorus calculated

TABLE I

Analyses of Enamel and Dentin of Sound Teeth

	Enamel			Dentin		
	Mean	Standard deviation	No. of analyses	Mean	Standard deviation	No. of analyses
	per cent	*per cent*		*per cent*	*per cent*	
Ca	35.41	0.963	42	26.18	0.342	20
P	17.45	0.513	42	12.74	0.482	20
Mg	0.30	0.054	34	0.83	0.083	20
CO_2	3.00	0.249	41	3.57	0.103	20
N				3.36	0.145	20

to be in combination as $Mg_3(PO_4)_2$ be considered if the above ratio is to approach 1.94.

With the exception of magnesium, the constituents of dentin vary less than those of enamel, as denoted by the lower values of the standard deviation of the means for dentin. This observation may find an explanation in the fact that dentin retains, after the eruption of the tooth, a mechanism, far superior to that of enamel, for interchange between itself and the circulating fluids. Opportunity is thereby afforded throughout the life of the dental pulp for adjustment of the composition of dentin towards a uniform content of each element.

The magnesium and carbonate contents of dentin are greater than those of enamel in spite of the very much larger protein and

hence smaller mineral content of dentin. The mean protein content of dentin is 22.24 per cent (Armstrong, Brekhus, and Cavett, 1936), while that of enamel is certainly less than 1 per cent. The calcium and phosphorus contents of dentin, while lower than those of enamel, do not stand in direct relationship to the difference in the protein contents of the two materials, as would be the case were the inorganic phases of enamel and dentin identical. For these reasons, it was considered (Armstrong, 1935; Brekhus and Armstrong, 1935, *b*) that the elementary composition of the mineral phase of enamel differs from that of dentin. In order to demonstrate more clearly the differences in the analyt-

TABLE II

Ratios of Elements of Mineral Phases of Enamel and Dentin

	Enamel	Dentin
$\frac{\%Ca}{\%P}$	2.03	2.05
$\frac{\text{Residual Ca}^*}{\text{Total P}}$	1.87	1.80
$\frac{\text{Residual Ca}}{\text{Residual P}^\dagger}$	1.90	1.90
$\frac{\%Ca_3(PO_4)_2}{\%CaCO_3}$	12.60	7.41

* Residual Ca = per cent total Ca minus per cent $CO_2 \times 0.91$ = Ca uncombined as $CaCO_3$.

† Residual P = per cent total P minus per cent Mg $\times$ 0.86 = P uncombined as $Mg_3(PO_4)_2$.

ical composition of the inorganic phases of enamel and dentin, which are partially obscured when the analyses are made on the protein-containing material, the experiment whose results are shown in Table III was performed. The pooled enamel and dentin fractions of four teeth were analyzed directly. The protein-free inorganic residue of each lot of material was prepared according to the modification developed by Crowell, Hodge, and Line (1934) of Gabriel's original method. The analytical composition of the protein-free mineral phase of enamel is obviously different from that of dentin.

The physicochemical conditions required for calcification can

be studied better if the nature of the products of this process are known, and it is customary to derive, from the results of analyses, empirical formulas which purport to describe the character of the inorganic salt of calcified tissues. Morgulis (1931), among others, has derived such a formula for bone, and Crowell, Hodge, and Line (1934) tentatively proposed the formula $Ca(OH)_2 \cdot CaCO_3 \cdot 3\text{-}Ca_3(PO_4)_2$ for the inorganic residue of mixtures of enamel, dentin, and cementum. The *empirical* formulas $MCO_3 \cdot [M_3(PO_4)_2]_{4.1}$ and $MCO_3[M_3(PO_4)_2]_{2.5}$ for enamel and dentin, respectively, almost exactly fit the means of the analytical data. In these formulas M represents calcium and magnesium, the atomic proportions of these elements being 76:1 and 19.1:1 in enamel and dentin, respectively.

TABLE III

Comparison of Composition of Enamel and Dentin and Their Mineral Phases

	Dentin	Enamel	Mineral phase of dentin	Mineral phase of enamel
	per cent	*per cent*	*per cent*	*per cent*
Ca	25.86	36.10	33.69	36.41
P	12.53	17.31	15.78	17.39
Mg	0.73	0.23	0.89	0.24
CO_2	3.64	3.05	4.76	3.07
N	3.43	0.098	0.00	0.00

These formulas, in spite of their agreement with the analytical results, cannot represent the true state of combination of the elements in enamel and dentin. If the formula of the mineral phase of any calcified tissue is to be correctly represented, it is necessary to account for the fact that the x-ray spectrograms of the mineral phases of all calcifications are practically identical with each other and with pure apatites (Taylor and Sheard, 1929). On the other hand, in view of the analytical differences between enamel and dentin and the other calcifications, the data of x-ray analysis cannot be interpreted to indicate an exactly identical composition of the mineral phases of these materials.

An explanation is needed of the fact that several substances may exhibit almost identical x-ray diffraction patterns and yet their compositions be so diverse as to appear irreconcilable by the

substitution of equivalent atoms in the apatite crystal lattice, as it is now conceived. Until such explanation is forthcoming, attempts to depict the composition of the inorganic fraction of bone, enamel, and dentin as a single molecular species are futile. These results emphasize that the composition of the mineral deposited in calcified tissues is not absolutely fixed.

Relation of Composition of Enamel to Decay—Table IV is a summary of the analyses of fifteen specimens of enamel of carious teeth. The means of the results and the standard deviations of the means are not significantly different from those of enamel of sound teeth. No significant difference could be detected in the composition of the enamel of sound and carious teeth of the same person. The age of the persons from whom the carious teeth were obtained varied from 20 to 49 years. Specimens of each type of

TABLE IV

Composition of Enamel of Carious Teeth

	Mean	Standard deviation	No. of analyses
	per cent	*per cent*	
Ca	35.64	0.598	15
P	17.21	0.398	15
Mg	0.32	0.053	15
CO_2	3.01	0.145	14

tooth were studied and in some instances it was necessary to pool the enamel of homologous teeth from one person to obtain sufficient material for analysis. These results indicate that no significant alteration is produced in enamel, with respect to the content of the principal elements, beyond the site of the carious lesion.

Brekhus (1931) has determined the incidence of caries in the several teeth of the human dentition, and it is possible that there are differences in composition of the enamel of sound teeth which could be correlated with the known individual susceptibility of the tooth types of decay. A rigid test of this hypothesis would require the analysis of the enamel fractions of a number of sound, newly erupted permanent teeth. It has been possible to obtain only a few specimens of sound teeth from youths. The data of Table I were rearranged in three groups according to the resistance of the tooth types to decay. The mean calcium contents of the

groups, in order of susceptibility to decay, were 35.14, 35.85, and 35.07 per cent. These means were compared for significant differences by "Student's" method. The calculated probability of the high calcium content of the enamel of the moderately susceptible group being a result of random sampling is less than 3 chances in 100. Except for calcium, there was no possibility of real differences in composition of the three groups. Because of the small and irregular differences in calcium content, and because of the few specimens in each group, it is not possible to conclude that the caries resistance of the tooth types constituting each group is related to the calcium content of the enamel.

A summary of the results of separate analysis of fourteen speci-

TABLE V

Composition of Enamel of Teeth of One Person Compared with That of Several Persons

	Enamel of teeth of patient A			Enamel of all other teeth		
	Mean	Standard deviation	No. of analyses	Mean	Standard deviation	No. of analyses
	per cent	*per cent*		*per cent*	*per cent*	
Ca	35.86	0.540	14	35.19	0.903	28
P	17.60	0.566	14	17.38	0.210	28
Mg	0.28	0.028	13	0.31	0.073	21
CO_2	3.01	0.230	14	3.00	0.190	27

mens of enamel prepared from the teeth of patient A is shown in Table V. Since the enamel fractions of homologous teeth were pooled in many instances, the composition of the enamel of twenty teeth is represented in the means. As indicated by the values of the standard deviation of the means, the composition of the enamel of this person varied about as much as that of all other specimens considered together. Patient A was 48 years old and his teeth were unusually free of caries. There were no certain differences of composition of the enamel of these teeth in comparison with that of the sound teeth of other persons or with that of carious teeth which could account for their resistance to decay.

No definite relationship could be discovered between the composition of enamel and the age of eruption of the teeth.

SUMMARY

1. Variations of composition of enamel and dentin have been described.

2. Since the magnesium and carbonate contents of dentin are higher than those of enamel, and because of other differences in composition, it is concluded that the mineral phases of enamel and dentin are not identical.

3. The composition of the enamel of carious teeth does not differ with respect to the elements determined from that of sound teeth.

4. There has been discovered no correlation of composition of enamel with susceptibility to decay or with the age of eruption of the teeth.

5. The composition of the enamel of the teeth of one person varies as much as that obtained from the teeth of several individuals.

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