

CHEMICAL ANALYSIS OF HUMAN TEETH

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THE dental literature contains numerous articles concerning the chemical analysis of human teeth.¹⁻⁹ Most of this work was done in the early 1930's. Naturally, in these analyses the common chemical methods and procedures known at the time were used. Recently, a request was received for an analysis of some Greek teeth to compare their chemical compositions with that of domestic teeth. In recent years new methods in analytical chemistry have been developed which make the analysis of calcium, magnesium and phosphorus easier, faster, and more accurate. In this investigation some of these new methods have been applied. It is the purpose of this report to describe these methods and the compositions obtained.

Some investigators^{1, 7} have shown that the composition of enamel and dentine of the teeth of one person may vary as much as that obtained from the teeth of several individuals. Others² have shown that even the difference between the chemical compositions of well-developed and hypoplastic enamel is of the same order as the difference between one individual and another. Thus, the sampling of human teeth for chemical analysis is quite important. In this analysis, 10 domestic and 10 Greek teeth were crushed separately and the results reported represent an average value for each group.

The human tooth is made up largely of inorganic material, and many investigators have analyzed only this portion of enamel and dentin. However, teeth contain organic as well as inorganic components, and some investigators, such as Deakins and Volker,³ have reported the organic content of the enamel of human teeth. This report describes only the determination of the inorganic content of enamel and dentin.

After analyzing the inorganic part of human teeth, different investigators tried to assign a chemical formula to this structure. As a result, 3 rather important formulas have been given to tooth structure.

1. Tricalcium phosphate and calcium carbonate $\text{Ca}_3(\text{PO}_4)_2 + \text{Ca CO}_3$
2. Carbonate apatite $\text{Ca}_{10}(\text{PO}_4)_6 \text{CO}_3$
3. Hydrozyl apatite $\text{Ca}_5(\text{PO}_4)_3\text{OH} + \text{Ca CO}_3$

The first formula is one that is rather old and outdated. The main point about it is the constant ratio between the phosphorus and calcium. Thewlis, Glock, and Murray¹⁵ and Klement and Trömel¹⁰ showed by x-ray methods of study that the phosphate of enamel is of an apatite nature. They

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showed that all lines in apatite appeared on the x-ray pattern of enamel. Hendricks, Hill, Jacob, and Jefferson,¹¹ Bale, Hodge, and Warren,⁵ and Hodge and McKay¹² also gave the apatite configuration to human enamel.

Apatites are found in nature and have a general formula of $\text{Ca}_5(\text{PO}_4)_3\text{X}$, where X can be replaced by Cl, F, or OH. Magnesium, sodium, or potassium may replace calcium in the crystal lattice. The formula for carbonate apatite is based on the assumption that the CO_3 ion has replaced 2 X's from 2 molecules of apatite. However, some crystallographers, such as Trömel and Moller,¹³ consider the existence of carbonate apatite impossible, and as a result this formula has lacked support. A few of the investigators, such as Klement and Trömel,¹⁰ Bredig,¹⁴ Thewlis,¹⁵ and Bale¹⁶ considered the main substance of the dental enamel to be hydroxyl apatite. Today, hydroxyl apatite is the accepted configuration given to the inorganic content of human teeth, and more work¹⁷ using the powder x-ray method also shows this to be the best possible structure.

METHOD

Separation of Dentin From Enamel.—Human teeth after being cleaned and washed with soap and water were dried in an oven for two to three hours at 180° to 185° F. This removed all the water from the surface and made the teeth easy to pulverize. Dry teeth were placed in a mortar, crushed, and screened through 100-mesh screen. Oversize particles were returned to the mortar, recrushed, and rescreened until all the particles passed through 100-mesh screen.

The difference in the density of enamel and dentin makes the separation of these 2 substances rather simple.^{18, 19} A mixture of 92 ml. of bromoform and 8 ml. of acetone (density 2.70) was used. About 1 Gm. of the pulverized teeth was placed in a centrifuge tube and about 7 ml. of the bromoform mixture was added to the powdered teeth sample. The tube was placed in the centrifuge and the rate of the spinning of the centrifuge was slowly increased to the maximum for the machine. After about seven to eight minutes the centrifuge was stopped and the tube was removed. Dentin having a density of 2.14 floats, while enamel with the density 2.89 to 3.00 settles to the bottom of the tube.

Dentin and enamel collected this way were not pure and had to be purified. For purifying the enamel, pure bromoform (density 2.85) was used. Again the enamel collected on the bottom. The pure liquid bromoform, having a higher density than the bromoform-acetone mixture, allows the small amount of dentin to float easily. In this way the enamel was purified. For purification of dentin, bromoform and acetone with the density of 2.42 were used (79 ml. bromoform, 21 ml. acetone). Dentin and cementum, having lower density than the liquid, floated on the surface, while enamel was collected on the bottom. Finally, a bromoform and acetone mixture with a density of 2.07 (62 ml. bromoform, 38 ml. acetone) was used for separation of dentin from cementum. Dentin collected on the bottom, while cementum (density 2.03) floated on the surface.

Extraction of Organic Part and Dissolving of Teeth.—Organic parts of enamel and dentin were extracted by the modified Gabriels method which was discussed briefly by Crowell, Hodge, and Line.⁷ By using 3 per cent solution of KOH in ethylene glycol, the organic contents of enamel and dentin were extracted, leaving the inorganic residue in the flask. The inorganic residue of enamel and dentin, after being washed with distilled water for a few times, was dried and weighed.

A known amount of the inorganic part of enamel and dentin was dissolved in nitric acid and was transferred into 100 ml. volumetric flasks. These solutions were used in determining the phosphorus content of enamel and dentin.

Phosphorus Determination.—The prepared solution as mentioned above was used for the determination of phosphorus. Phosphorus was precipitated as ammonium molybdiphosphate, which is usually called a yellow precipitate. This precipitate is pure and theoretical only when it is formed under exact conditions. The facts which must be considered in precipitating phosphorus as ammonium molybdiphosphates are: (1) All phosphorus must be present as orthophosphoric acid— H_3PO_4 , (2) A certain excess of free nitric acid must be present and a large excess of it must be avoided, (3) The presence of a large amount of ammonium nitrate is necessary, and (4) The precipitate should be made from a hot solution at $65^\circ C.$, and should not be allowed to stand at this temperature more than fifteen minutes.

Preparation of Molybdic Acid Solution.—To 50 Gm. of pure molybdic anhydride, MoO_3 , 50 ml. of concentrated ammonia and 150 ml. of water were added. The mixture was heated gently until all of the molybdic acid was dissolved. At this point the solution was checked by smelling for the presence of ammonia. Some ammonia was added until the distinct odor of ammonia was noticeable. About 250 ml. of concentrated nitric acid was diluted to 600 ml. Both of the solutions were cooled and mixed by pouring the molybdate solution into the diluted nitric acid slowly with constant stirring. More water was added until the total volume was 1 liter.

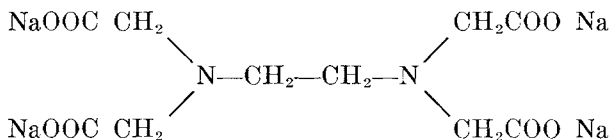
Precipitation of Ammonium Molybdiphosphate.—By means of a pipette, 25 ml. from the solution of teeth dissolved in nitric acid was drawn off. Approximately 50 ml. of fresh molybdic acid solution was heated to nearly $60^\circ C.$ and added slowly with constant stirring to the teeth solution which was previously heated to $60^\circ C.$ The mixture was put aside for two hours at room temperature until the precipitation was completed. After the precipitation was completed, it was filtered through a Gooch crucible. The precipitate was dried at 120° to $130^\circ C.$ and weighed. The residue contained 1.64 per cent of phosphorus. Since the residue is rather hygroscopic, it must be weighed quickly.

The results of 2 runs made by this method were as follows:

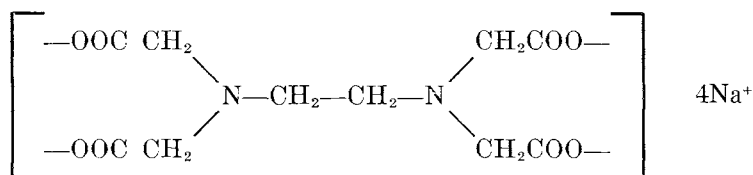
PERCENTAGE OF PHOSPHORUS

	DOMESTIC		GREEK	
	DENTIN (PER CENT)	ENAMEL (PER CENT)	DENTIN (PER CENT)	ENAMEL (PER CENT)
Sample I	13.15	16.38	12.56	14.47
Sample II	13.36	16.37	12.46	14.69

Determination of Calcium and Magnesium.—Calcium and magnesium in dentin and enamel were determined by titration with Versene.* The chemical structure of this compound is usually presented as



Since it is a salt of a strong base, it is completely ionized in solution. Consequently, its structure may also be presented by the formula



which could be contracted to Na_4Ve . The sodium and other alkali metal salts of ethylenediaminetetraacetic acid are quite soluble in water. The acid itself is insoluble. Thus, the addition of strong acid to sodium versenate results in the formation of a crystalline precipitate of the acid.

Versene reacts with alkaline earth and heavy metals to form an unusually stable soluble metal chelate compound. The chemical reactions which take place can be shown as follows:



In titration of calcium with Versene, the Versene indicator was used. The indicator is blue in color but forms a red color with the magnesium ion and stays blue in the presence of calcium ion. The calcium ion reacts with Versene completely before the magnesium ion is being attacked. With this method, therefore, calcium and magnesium ions will be titrated as a total. Having the magnesium ion in the solution, the indicator will have a red color which will be converted to a bluish purple at the end point. In order to separate the amount of magnesium from that of calcium, calcium was precipitated, filtered, redissolved, and titrated separately. The amount of magnesium was found by the difference. When calcium is titrated separately, in order to have a sharp end point, a known concentration of the magnesium ion was added.

For the best results in these titrations, the pH of the solution should be about 10.²¹ With the presence of the phosphorus ion, however, at pH of 10, calcium will form calcium phosphate in which case the end point may not be sharp. Therefore, an ion exchange technique was applied to separate the phosphorus ion from that of calcium.

Principle of Separation.—After dissolving the sample in hydrochloric acid, the solution was passed through a column containing a cation exchange resin.²²⁻²⁴ The resin was in the hydrogen form on which the metal cations in the solution

*Versene is the aqueous solution of the tetra sodium salt of ethylenediaminetetraacetic acid made by the Bersworth Chemical Company of Framingham, Mass.²⁰

were retained, being thus separated from the phosphoric acid which passes quantitatively through the column. After the column was washed with water, approximately 4 normal hydrochloric acid solution was passed through the column. The acid solution carries down all the metal cations from which calcium and magnesium were determined.

Preparation of Standard Versenate Solution.—About 10 Gm. of disodium-dihydrogenethylenediaminetetraacetate dihydrate* and 2 Gm. of sodium hydroxide pellets were dissolved in water and made up to one liter. This solution was standardized with standard calcium chloride solution which was made by dissolving a known amount of calcium carbonate in hydrochloric acid.

The actual values obtained by this method are:

PERCENTAGE OF CALCIUM AND MAGNESIUM

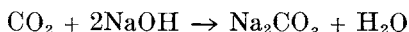
	DOMESTIC		GREEK	
	DENTIN (PER CENT)	ENAMEL (PER CENT)	DENTIN (PER CENT)	ENAMEL (PER CENT)
Sample I (Ca + Mg)	30.97	37.20	27.25	33.47
Sample II (Ca + Mg)	31.02	37.05	27.22	33.25
Average (Ca + Mg)	31.00	37.12	27.24	33.37
Sample I Ca	30.36	36.16	26.73	32.87
Sample II Ca	30.14	36.15	26.89	32.79
Average Ca	30.25	36.16	26.81	32.83
Average Mg	0.75	0.96	0.43	0.54

Determination of Carbonate Ion in Teeth.—Enamel and dentin samples, after being weighed, were placed on a special gas-tight apparatus. The success of determination of carbonate ion largely depends upon construction of a compact gas-tight train. The apparatus used for this purpose was formed of a wide mouth decomposition flask which was supported on a wire gauze on a ring stand. The flask was closed with a 2-hole rubber stopper containing a calcium chloride tube filled with "ascarite" (sodium hydroxide asbestos absorbent). By means of a 6 mm. diameter piece of glass tubing, the condenser was connected to a U-form tube. Two of these U-form tubes were employed and were supported with a copper wire from an iron rod which was held by two ring stands. The first U-tube served to absorb water vapor that escapes from the condenser as well as to remove any hydrochloric acid or hydrogen sulfide from the gas. The first third of the first U-tube was filled with anhydrous copper sulfate which served to remove any hydrogen sulfide, and the last two thirds with dehydrite $Mg(ClO_4)_2$, which is an effective absorbent of water. The second U-tube was filled with the drying agent, dehydrite. From the second U-tube a suitable shaped glass tube led to the special absorption bulb which was filled with fresh ascarite on top of which was placed a layer of drying agent, about 1 cm. thick. From the absorption bulb a tube led to another bulb containing concentrated sulfuric acid. This bulb served both to indicate the rapidity of the gas flow through the train and to prevent any water vapor from entering the train from the suction end. The last bulb in

*From Bersworth Chemical Company.

the train was an empty one which acted as a trap to catch any water that might be drawn back from the suction. This bulb was connected to the suction by heavy walled rubber tubing.

Before running the actual determination, the train was tested for any possible leakage. A known amount of calcium carbonate was placed in the decomposition flask, and through a separatory funnel hydrochloric acid was added very slowly. The hydrochloric acid decomposed the calcium carbonate, and suction pulled the formed gases through different tubes and bulbs as described. Eventually, carbon dioxide was absorbed in the absorption bulb and the amount of it was found by weighing the bulb before and after each experiment. In the absorption bulb the following chemical reaction takes place:



The water vapor formed from this reaction will be absorbed by the drying agent which was placed on the top of the ascarite in the same bulb. Therefore, the change of weight of this bulb is due to 2 factors: (1) Conversion of 2NaOH to NaCO₃, and (2) for every mole of CO₂, one mole of water will be formed which would be absorbed in the same bulb also.

The results of runs made by this method are:

PERCENTAGE OF CO₂

	DOMESTIC		GREEK	
	DENTIN (PER CENT)	ENAMEL (PER CENT)	DENTIN (PER CENT)	ENAMEL (PER CENT)
CO ₂ in Sample I	3.33	4.95	3.47	2.43
CO ₂ in Sample II	3.74	4.65	3.56	2.11

Other Elements Present in the Teeth.—Human teeth contain besides calcium, phosphorus, magnesium, and carbonate ions, which are the major ingredients, other elements in a very low concentration. With usual chemical analysis, these elements cannot be detected. Therefore, spectroscopic analysis was applied in order to identify these minor elements. It was found that domestic dentin and enamel have the same elements as Greek dentin and enamel, but with different concentrations. At the moment, the quantitative spectroscopic analysis for these elements has not been completed. By this method the qualitative presence of the following elements with a low concentration were identified: Pb, Zn, Si, Ag, Cu, Fe, Al, Sn, Na, Cl, F.

RESULTS OF THE CHEMICAL ANALYSIS OF TEETH

PER CENT OF ELEMENTS	DOMESTIC TEETH		GREEK TEETH	
	DENTIN	ENAMEL	DENTIN	ENAMEL
Ca	30.25	36.16	26.81	32.83
Mg	0.75	0.96	0.43	0.54
P	13.25	16.37	12.51	14.58
CO ₂	3.54	4.80	3.51	2.27
Ca/P	2.28	2.19	2.14	2.26

SUMMARY

Greek and domestic teeth were analyzed and the results are reported. In this analysis dentin was separated from enamel by the Manly and Hodge

method.¹⁹ Phosphorus was determined by precipitating it as ammonium molybdophosphate which is usually called the yellow precipitation method. Calcium and magnesium were determined by titrating with Versene^{20, 21} at a pH of about 10 from phosphorus free solution. In this solution phosphorus was removed by means of ion exchange column.²²⁻²⁴ The carbonate ion is reported as CO₂. By adding hydrochloric acid, CO₂ was released and absorbed by sodium hydroxide. A special apparatus was used for this determination. For the qualitative determination of other elements present in teeth at very low concentrations, spectroscopic analysis was used.

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