XIV. THE CHEMICAL COMPOSITION OF TEETH.

I. THE ESTIMATION OF FLUORINE AND THE FLUORINE CONTENT OF NORMAL TEETH.

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THIS investigation is part of a general consideration of the composition of dental enamel. The main object is to determine the composition of teeth of good structure, so that later the question of the relationship between chemical constitution and hypoplasia may be considered. The term hypoplasia is used in the sense intended by Mellanby [1923; 1934], who established a correlation between surface texture and histological structure, and the hypoplasia therein exhibited, with susceptibility to caries.

It is fairly generally agreed that the calcium phosphate of teeth and bones is not present as tertiary calcium phosphate, but as a phosphate of the apatite type $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaX}_2$, in which X can be F_2 , Cl_2 , $(\text{OH})_2$ or CO_3 . Gassmann [1910] suggested carbonato-apatite and Bassett [1927] hydroxyapatite as the bone compound. Robison and Soames's [1925] results on bone support the idea that the chief constituent is an apatite. In a general consideration of the results of various workers for the calcium and phosphorus contents of bones and teeth Robison [1932] came to the same conclusion.

Of recent years bones and teeth have been subjected to X-ray analysis by de Jong [1926], Taylor and Sheard [1929], Roseberry *et al.* [1931], Thewlis [1932], Klement and Tromel [1932] and Bredig [1933]; all have concluded that the chief mineral present is an apatite. The application of this method has so far not been able to settle the question of the particular form of apatite.

Fluoroapatite is geologically common, but other isomorphous forms exist and have been considered in relation to the substance in bone and teeth by Hendricks *et al.* [1932]. The occurrence of fluorine in teeth has been investigated by many workers, but there does not seem to be any generally accepted idea as to the quantity of this element present. The reason is probably the difficulty of estimation. Several textbooks give values of the order of 0.3 % fluorine. The fluorine content of fluoroapatite is theoretically 3.77 %. No recent determinations on teeth have given values of this order of magnitude, so obviously the inorganic substance of teeth is not all fluoroapatite.

Fluorine is not an essential element in the diet. Sharpless and McCollum [1933] proved that fluorine-free diets were better for teeth formation, in rats, than diets containing relatively small amounts, *e.g.* 0.005 %, of fluorine. The occurrence of fluorine in drinking waters, to the extent of more than about two parts per million, is now recognised as the cause of that condition known as "mottled teeth." Such teeth show either a dull opaque chalky appearance or characteristic unsightly brown stains on the enamel. A more detailed description is to be found in a paper by Ainsworth [1933]. Extensive investigations have been carried out in America by McKay, originating in the work of Black and McKay [1916] and reported in the numerous publications of McKay and his collaborators on the

subject of mottled teeth. Independently since 1930 Smith *et al.* [1931] have published the results of investigations which have led to the association of fluorine in drinking water to the extent of more than 1.5 parts per million with the occurrence of mottled teeth. Churchill [1931] proved this connection by determination of the fluorine content of the waters.

The effects of feeding sodium or calcium fluoride to animals had been observed by several earlier investigators, notably Sonntag [1916]. McCollum *et al.* [1925] revived the interest in this subject and reported that the inclusion of sodium fluoride in the diet of rats caused marked changes in the growth and appearance of the incisor teeth.

The danger of feeding rock phosphate, which frequently contains fluoro-apatite, has been well established by Velu [1931]. He found that natural fluoro-phosphates fed to rats caused marked diminution of food intake; the animals did not thrive and many died. This condition of chronic fluorosis he called fluoride cachexia. Bone changes resembling osteomalacia have been observed by Christiani and Gautier [1925] in animals living on vegetation in the neighbourhood of chemical works which liberate fluorine, or certain of its compounds, into the atmosphere.

In this country an investigation of the teeth of persons living in Maldon, Essex, by Ainsworth [1933] revealed that mottled teeth are endemic there. The water of Maldon was found at that time to contain 4.5 to 5.5 parts of fluorine per million.

But in spite of this interest in the effects of fluorine, few determinations of the fluorine content of teeth have been made within recent years, so that there have been no definite conclusions as to whether the fluorine affects the deposition of the normal constituents of teeth or whether it enters into the composition of mottled teeth.

As a preliminary to the investigation of this problem it was necessary to find a reliable method for the estimation of fluorine in small amounts of material and suitable for very small percentages of the element. It is with this problem that the present communication deals; it is hoped that later a series of determinations of the fluorine content of the teeth of animals on fluorine-containing diets and of mottled teeth will be possible.

The estimation of fluorine in dental material, with its large proportion of calcium phosphate, is a matter of some difficulty. The earlier workers usually employed volatilisation as hydrofluoric acid or as silicon tetrafluoride, in combination with the glass etching test. Gassmann [1910] using this method found no fluorine in bones or teeth, but Gautier and Clausmann [1913] found 0.02 to 0.056 % in bones and 0.062 % in teeth. Mayrhofer *et al.* [1932] used the silicon tetrafluoride procedure combined with indirect determination of the fluorine by colorimetric estimation of the silicon. This later step is unwise, particularly since the gas was caught in caustic soda in a glass vessel. In the determination of silicon only platinum or "bakelite" vessels should be used. Sharpless and McCollum [1933] used the silicon tetrafluoride method for bones; their determination is referred to later. A method described by Willard and Winter [1933], involving treatment with perchloric acid, in the presence of glass beads, thereby converting the fluorine into hydrofluorosilicic acid, followed by thorium nitrate titration of the fluorine with zirconium alizarinate as an indicator, seems to have much to commend it. Preliminary trials on a known calcium phosphate-sodium fluoride mixture were not satisfactory with regard to the volatilisation. It is to be noted that probably one gram of tooth material contains approximately 0.2 mg. fluorine, so that the determination would be close to the limit of accuracy of the

method. About 30 rats' incisors are required to give 1 g. The success of the volatilisation methods seems to depend very much on the amount present, being less reliable for small quantities.

Other methods which have been used include that of Meyer and Schulz [1925; 1926]. This involves precipitation as a lanthanum fluoride-acetate complex. Trebitsch [1927] used this and found 0.29 to 0.59 % in tooth crowns. When we applied this method to a known fluorine-containing powder it proved quite unreliable; the results were too high, as we believe those of Trebitsch to be.

The next method tried was Mervin's [1909] pertitanate method. When fluorides are added to a solution of a pertitanate, made by addition of hydrogen peroxide to a solution of titanous sulphate in sulphuric acid, bleaching of the orange-yellow colour occurs. The extent of the bleaching is compared with that caused by a standard. Since this work has been in progress, Sharpless and McCollum [1933] have used a modification of the titanium method for the fluorine content of bones.

The pertitanate method is certainly more reliable than the lanthanum method, but it is not very sensitive and so is not ideal when small quantities of material only can be obtained. Estimations by this method were carried out in the following way. Approximately one gram of powdered enamel was fused with sodium carbonate in a platinum crucible. The melt was extracted with successive quantities of hot water, a considerable volume being used. The solution was filtered, concentrated and partially neutralised by the addition of 4*N* sulphuric acid in presence of an aqueous solution of phenolphthalein, care being taken to avoid excess of acid. Excess silver sulphate was then added to remove phosphates and the precipitate filtered off and washed. The excess of silver was removed by addition of sodium chloride, the solution filtered, the precipitate washed and the filtrate brought to a known volume by evaporation. Several 100 ml. graduated flasks were then taken and to each were added 10 ml. 65 % by volume sulphuric acid, 10 ml. of the titanous sulphate solution, made according to the directions of Warren *et al.* [1925], 3 ml. 10 volume hydrogen peroxide and then either a known volume of the unknown fluoride or a known volume of a comparable standard sodium fluoride solution. The solutions were allowed to stand and then were compared in a colorimeter. The values obtained for human enamel by this procedure varied from nothing to 0.04 %, the most usual value being 0.01 %. Ten estimations in all were made¹. We then tested the sensitivity of this method and considered that the low results might be due to its lack of sensitivity. Sharpless and McCollum [1933] obtained less than 0.04 % fluorine in the bones of rats fed on a diet containing very little fluorine, *i.e.* 0.001 % of the ration.

The estimation of fluorine in sea-water and in the waters of districts where mottled teeth are endemic has of recent years been made by a method originally introduced by de Boer [1924] and latterly used and modified by Casares and Casares [1930].

The principle of the method is that solutions of zirconium salts, whether nitrate or oxychloride, and sodium alizarinsulphonate form a red lake, stable in acid solution. The coloured lake is slowly bleached by fluorine, the bleaching being proportional to the fluorine concentration, hence by colorimetric comparison with a standard fluoride solution, an estimation can be made.

Taylor and Thompson [1933] applied this method to sea-water and Elvove [1933] to drinking water. In our determinations we first used the Taylor and Thompson directions for making the lake, but later we employed the Elvove [1933] solutions.

¹ We are indebted to Miss M. O. Barrie for these titanium method determinations.

The estimation of fluorine in teeth by this method was complicated by the presence of other substances tending to cause precipitation of the lake. Taylor and Thompson, in their report on the estimation of fluorine in sea-water, state that chlorine and sulphate ions have bleaching actions, but that phosphate and calcium ions do not interfere. It was found however that the concentration of calcium and phosphate ions in our solutions of tooth material definitely caused precipitation of the lake. Samples of sea-water and London tap-water also showed precipitation after 18 hours. Various methods for removing interfering substances were tried. Firstly phosphate precipitation was effected with calcium chloride and ammonia. This procedure when checked on fluoroapatite gave a value of 4.733 % fluorine, which was known to be too high. When greater care was taken to remove ammonia the result was nearer the correct value but still there was a tendency to precipitation. We next tried precipitation of interfering substances by excess of zirconium nitrate, but though this removed the source of precipitation of the lake, excess zirconium salt prevented the bleaching action of fluorides.

Finally we used precipitation by silver nitrate and removal of excess silver by sodium chloride. In many of these preliminary tests, a known fused mixture of calcium phosphate and calcium fluoride was employed in place of enamel. We always determined the chlorine content of our solutions and adjusted the chlorine content of the standard fluorine solutions to be the same. Finally we determined the nitrate content by calculation and adjusted this by addition of sodium nitrate to the standards.

In all these methods, the resulting solution was brought to a known volume, and an aliquot part taken, and to it were added 2 ml. 6*N* HCl and 2 ml. of the zirconium lake solution used by Taylor and Thompson. The solution was then brought to the boiling-point and left for several hours. At the same time solutions containing different standard amounts of sodium fluoride and having the same chlorine and nitrate contents were treated similarly.

The values obtained for the fluorine of human enamel by these procedures were as follows:

Method	Fluorine %
Lanthanum method	Unreliable values
Titanium method	0.0 to 0.04
Zirconium-alizarin method with calcium chloride precipitation	0.093
Zirconium-alizarin method with calcium chloride precipitation and removal of ammonia	0.06
Zirconium-alizarin method, silver precipitation, chlorine content adjusted	0.07
Zirconium-alizarin method adjusted for chlorine and nitrate concentrations	0.02

We realised throughout the determinations that the tooth solutions might contain small traces of substances capable of affecting the colour of the lake and we therefore considered it necessary to make certain that we were estimating fluorine by taking a sample of enamel and treating a portion of it with sulphuric acid to drive off the fluorine as hydrofluoric acid.

This experiment was a crucial one and will be described in detail. Two portions of ashed human enamel, 1.1684 g. each, were taken and fused with 6 g. Na₂CO₃. The melts were extracted very thoroughly with boiling water. One sample B was evaporated to dryness, H₂SO₄ added and the whole heated cautiously to drive off the HF. The residue was extracted with water and solid Na₂CO₃ added to neutralise; the resulting solution was the "blank." This solution and the

solution of the other melt, solution A or the "unknown," were each brought to a volume of about 100 ml. and both were treated in the following way. 60 ml. of a strong solution of AgNO_3 were added, the precipitates filtered off in the dark and washed. The excess silver was removed by addition of a solution of NaCl and the precipitates filtered off and washed. The resulting filtrates were brought to a known volume and the chlorine concentration of each determined by titration with standard AgNO_3 , so that later, the chlorine contents of the solutions could be equalised and that of the standard fluorine made the same.

Sulphate ions interfere with the determination; sulphate was therefore removed from B by the addition of the calculated quantity of $\text{Ba}(\text{NO}_3)_2$, after determination of sulphate. After filtration and concentration, the solution was tested for sulphate and barium; a very small amount of sulphate was present. A trace of Na_2SO_4 was added to A and to the fluoride standards to equalise the sulphate throughout.

The two solutions A and B were then adjusted to the same chlorine content, so that they finally contained equal amounts of their different constituents except fluorine. A solution C was made having the same concentration of NaCl , NaNO_3 and Na_2SO_4 as A and B; this served for the making of the standard fluoride solutions. 25 ml. portions of solutions A and B were taken and to them were added HCl and the zirconium-alizarin lake; the solutions were brought to the boil. At the same time, portions of solution C to which were added varying amounts of NaF standard solution were similarly treated. All the volumes were made equal and the solutions matched in Nessler tubes. We found it better to find an exact match for the unknown solutions rather than to compare them in a colorimeter because there is a change of tint as well as intensity.

The unknown solution was definitely bleached as compared with the acid-treated blank. The amount of fluorine in the unknown was determined by finding a fluorine standard which compared with it. It was found that 2 ml. of the standard sodium fluoride (1 ml. = 0.01 mg.) gave slightly less bleaching, while 3 ml. of the standard gave definitely more bleaching.

$$\begin{aligned}\text{Hence 25 ml. A} &\equiv 0.025 \text{ mg. F} \\ \therefore 250 \text{ ml. A} &\equiv 0.25 \text{ ,,} \\ \% \text{ Fluorine} &\equiv \frac{100 \times 0.25}{1168.4} \% \\ &\equiv 0.0214 \text{ \%}.\end{aligned}$$

Another comparison was made by taking the acid blank B and adding fluoride to it. When 2 ml. were added there was too little bleaching and when 3 ml. were added too much bleaching, as compared with the unknown. Hence this procedure gave the same answer as before. (It is of interest to note that this fluorine value indicated that the teeth contained 0.51 % fluoroapatite.)

The process is a lengthy one because of the precautions necessary to eliminate precipitation effects and alteration of dye colour by substances other than fluorine.

Recently we have carried out determinations by the titanium method and the zirconium-alizarin method on the same sample of rats' teeth, the results were:

Titanium method	0.034 %,
Zirconium method	0.030 %.

That the latter is more sensitive is shown by the fact that for this method only 20 ml. of the tooth solution were used and matched against a standard solution containing 0.06 mg. fluorine per 100 ml., whereas for the titanium

method, 75 ml. solution were taken and matched against a standard containing 2 mg. per 100 ml.

We are at present applying the zirconium method to the estimation of the fluorine content of teeth of rats under various experimental conditions and to mottled teeth.

It has become clear during this investigation that normal teeth, that is non-mottled, may vary in their fluorine content within certain limits.

SUMMARY AND CONCLUSIONS.

1. Methods used in recent years for the estimation of small quantities of fluorine have been reviewed.

2. The zirconium-alizarin method has been applied to solutions of tooth material.

3. The quantity of fluorine in human enamel of the London area was 0.02 %.

4. The teeth of rats fed on diets, not designed to be fluorine-free, contained 0.02 % to 0.03 % fluorine.

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