CX. CHEMICAL COMPOSITION OF TEETH

V. SPECTROGRAPHIC ANALYSIS

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THE value of spectrographic analysis applied to the identification and to some extent the estimation of elements present in small amounts or traces in biological material has been emphasized of recent years [Gerlach & Gerlach, 1934; Ramage, 1929; 1933]. Dental tissues with their high proportion of mineral matter are obviously suitable material for spectrum analysis. Howe [1926] stated that such analysis would be profitable. Ramage & Fox [1931] included human teeth in the list of organs studied, but did not report much detail of the results, which were obtained by the use of an oxy-coal gas flame. They found a low Mg, a considerable Na and very much lower K content, a trace of Fe, but more Cu. Bossevain & Drea [1933] made spectrograms of teeth for finding their fluorine content.¹

The results to be recorded here, a preliminary account of which was given to the British Empire Dental Meeting in July 1936 [Lowater & Murray, 1936], form part of a general investigation into the chemical constitution of dental tissues [Bowes & Murray, 1935, 2]. By means of the spectrographic analysis it was hoped among other things to deal with the following points:

- (1) to verify or refute the results of chemical analysis;
- (2) to identify the presence of "trace" elements and to give particular consideration, for reasons to be discussed below, to certain elements, namely, F, Cl, K, Fe, Cu and Zn;
- (3) to make an exhaustive analysis of sound human enamel and dentine, the results of which would act as a basis for comparison with other human teeth and the teeth of animals, in particular of dogs, because of their high immunity to caries;
- (4) to analyse "mottled teeth";
- (5) to show the influence of feeding low concentrations of sodium fluoride on the composition of rats' teeth.

Preparation of the material. The details for the preparation of the enamel and dentine have been described previously [Bowes & Murray, 1935, 1].

Spectrographic method. The spectrographic analyses were carried out by means of a quartz spectrograph (Hilger E 2) and a glass Littrow instrument. Spectrograms from the former cover a range of wave-lengths 2100–5200 Å.; those from the latter the range 3800–6200 Å. Within these ranges characteristic sensitive lines or bands of all known elements and compounds lie. A specimen to be analysed was placed on the positive pole of a carbon arc and its spectrum photographed as the central strip of the spectrogram. Above the central strip was photographed the spectrum of iron, which served as a means of determination of the wave-lengths of the lines in the specimen and hence enabled the elements present to be identified. Below was photographed the spectrum of the carbon arc, for the identification of its lines and bands which

¹ After the completion of this work Drea [1936] published qualitative results on human teeth which agree in most respects with the results reported here.

also appeared in the spectrum of the specimen on the central strip. In order to facilitate the detection of zinc in the specimens, minute pieces of it were placed on the carbon arc used for a comparison spectrum. In these cases very few lines other than those of zinc appeared. The prints in Plate V are enlargements of some of the spectrograms, magnified about four times.

Enamel and dentine are known to contain a high percentage of calcium phosphate; in the carbon arc this substance is decomposed and the products appear in the spectrum as P and PO and as Ca and CaO. Certain specimens contained F and Cl which appear, in the spectroscopist's terminology, as diatomic charged molecules CaF and CaCl.

The results obtained were mainly qualitative. (A full record of the elements and groups identified is given in Table I A.) The comparison of the amounts of the different elements or groups in any specimen can only be made by assigning symbols such as "very strong", "strong", "present", "weak", "very weak", "trace", and "possible trace". The elements listed "present" represent a group with great variations. The terms "weak" and "very weak" are applied to lines of low levels of energy but not limited to the lowest, e.g. K by lines 4044 Å. and 4047 Å. The distinction between "weak" and "very weak" indicates that the lines are fainter in the latter case. The words "trace" and "possible trace" indicate that the substance is represented only by the most sensitive lines, those due to the lowest energy levels, e.g. Zn by the line 2138 Å. Table I A gives the results in this form. Marked differences in the strengths of the lines or bands enabled a rough grading of the relative amounts of any particular element or group in the different specimens to be made. These results are given in Table I B where an attempt has been made to give an idea of the relative abundance of individual elements in the different samples. The figures in a horizontal row correlate the amount of an element in the different samples. The figures for one element bear no relationship to those for any other element. These estimates could not be made satisfactorily for the last five elements in the table, because they were present as impurities in the carbon arc and because the intensities of their lines in the spectrum of the arc alone differed little from those of their lines in the spectrum of the specimen in the carbon arc, except for the Felines in rats' teeth.

With the exception of two short regions where nothing additional is revealed, a complete spectrogram of non-carious human enamel of good structure is shown in Plate V, and should be considered as the standard for comparison. In order to compare the other specimens, differences, qualitative or quantitative, between these and the above standard only are mentioned in the text. Throughout, the possible significance of the spectrographic findings has been briefly indicated.

Human enamel. The absence of K from the enamel and dentine of the teeth described above is to be noted. The presence of traces of F and Cl identified as CaF_2 and CaCl_2 verified the chemical results. Many of the other elements found to be present have already been recognized in tissues generally [Ramage, 1934]; for example, Fe, Cu, Mn and Ni.

It is believed that the general occurrence of Ti and V has not been recognized before. The latter element is of particular interest since it has been established beyond doubt that the tooth substance belongs to the apatite series and that vanadium can substitute in the apatite molecule [Saupe, 1934-35].

Human dentine. Dentine showed a greater Mg content than the enamel of the same teeth. This confirmed previous chemical determinations [Murray, 1936].

Dogs' dentine. It proved difficult to collect sufficient enamel from the dogs' teeth but comparison of dog's dentine with human dentine showed practically no differences.

Table I A

Constituents	Human enamel non-carious good structure London	Human dentine non-carious London	Human enamel "mottled" Maldon	Human dentine Maldon	Dog dentine London	Rat enamel control	Rat enamel NaF in diet	Maldon water residue
Not present in the carbon arc:								
Ca, Ca II	vS	vS	vS	vS	vS	vS	vS	vS
CaO	vS	vS	vS	vS	vS	vS	vS	vS
Mg, Mg II	P	P	P	P	P	S	S	W
P, PO	S	S	S	S	S	S	S	nF
*CaF	pT	pT	W	W	nF	nF	P	W
*CaCl	pT	pT	T	nF	nF	nF	pT	†
Ag	W	W	P	W	W	P	P	nF
Pb	W	W	P	W	W	P	P	T
Na	P	P	P	P	P	P	P	S
Sr, Sr II	P	P	P	P	P	P	P	S
Ba, Ba II	P	P	P	P	P	P	P	P
K	nF	nF	vW	vW	nF	P	P	P
Cr	T	T	T	T	T	T	T	nF
Sn	T	T	P	T	pT	T	T	T
Zn	T	T	T	T	T	T	T	T
Weak in arc:								
Mn	P	P	P	P	P	P	P	P
Ti, Ti II	P	P	W	P	P	P	P	nF
Ni	vW	P	T	P	P	P	P	T
V	P	P	P	P	P	P	P	nF

Al, Si, B, Cu and Fe were present in all specimens, but were also present in arc.

Table I B

Constituents	Human enamel non-carious good structure London	Human dentine non-carious London	Human enamel "mottled" Maldon	Human dentine Maldon	Dog dentine London	Rat enamel control	Rat enamel NaF in diet	Maldon water residue
Not present in the arc:								
Ca, Ca II	4	3	4	3	2	5	5	1
CaO	vS	vS	vS	vS	vS	vS	vS	vS†
Mg, Mg II	4	5	4	5	3	6	6	1
P, PO	1	2	1	2	2	5	5	T
CaF	pT	pT	1	1	0	0	10	1
CaCl	pT	pT	T	0	0	0	pT	†
Ag	3	1	5	1	2	4	3	0
Pb	1	1	1	1	2	2	4	T
Na	1	1	1	1	2	3	3	15
Sr, Sr II	1	1	1	1	2	2	2	3
Ba, Ba II	1	1	1	1	3	2	2	1
K	0	0	1	1	0	1	8	3
Cr	1	1	1	1	T	1	1	T
Sn	T	T	S	T	pT	T	T	T
Zn	T	T	T	T	T	T	T	T
Weak in arc:								
Mn	1	1	1	1	1	1	1	1
Ti, Ti II	3	2	2	2	2	1	1	pT
Ni	2	1	1	1	1	1	1	T
V	3	2	2	2	1	2	2	0

Al, Si, B, Cu and Fe difficult to grade because also present in carbon arc.

* See Text.

† If present hidden by diffuse Na.

‡ CaO was added to prevent loss of fluorine.

Key. vS=very strong, S=strong, P=present (easily detected), W=weak, vW=very weak, T=trace, pT=possible trace, nF=not found.

Roman numerals indicate charged atoms, e.g. Ca II, Mg II, etc.

Note. Numbers in horizontal rows compare amounts of a particular element in the different specimens. Numbers for one element bear no relationship to those for any other element.

Human mottled teeth. Enamel and dentine of mottled teeth from Maldon, Essex, were analysed with special reference to fluorine. By comparison the Maldon enamel and dentine both showed greater F contents than London enamel and dentine. It must, however, be emphasized how very small is the concentration of this element even in mottled teeth. It is hoped that this observation and other work done recently on the question of F in teeth [Bossevain & Drea, 1933; Bowes & Murray, 1935, 1, 2], will correct the view, still put forward in dental and biochemical literature, that fluorine as CaF_2 is an essential and considerable constituent of dental tissues. It was not found in dog's dentine. The presence of relatively considerable amounts of Ag and Sn in the Maldon enamel may be due to contamination with tooth-filling materials. A limited number only of these Maldon mottled teeth were available and by using sound parts of the teeth we had hoped to avoid such an occurrence. This contamination was ruled out in all the other specimens.

Maldon water residue. This was obtained by evaporation of the water to dryness after the addition of CaO to prevent loss of fluorine. The strength of the Ca lines is due in part to this added Ca. The notable points are the presence of fluorine (actually the water contained 5 parts per million fluorine), the strength of the Na lines (the water was brackish), and the considerable intensity of the Sr lines.

Rats' teeth. Control and fluorine-fed rats. In dealing with the rats' teeth there was a different object in view. Only the incisor teeth were used. These are teeth of persistent growth. The pulp cavity contents were removed as well as possible. The teeth were heated, in which process the enamel with pigmented surface layer separated itself in small pieces from the rest of the tooth. The two parts, the enamel with the pigment and the dentine, were separately examined. Spectra were taken of the teeth of control rats and of rats given NaF for 6 weeks, incorporated into the diet by moistening it with 0.05% NaF in the proportion of 1 ml. to 1 g. of dry ingredients. The daily amount of NaF was 7 mg. per rat per day. Probably not all of this was absorbed. The increase in the intensity of the CaF band was the most noticeable difference between the two groups of rats' teeth (see Plate V). It is of interest to note that the values for the fluorine contents of the various specimens determined chemically were:

London E and D	Maldon E and D	Control rats. Whole incisor	NaF-fed rats Whole incisor
0.0025 %	0.032-0.073 %	0.002 %	0.2 %

Table I gives the corresponding spectrograph record. The loss of the normal pigmentation of the teeth is the first sign of chronic fluorosis. The normal pigment is inorganic and by qualitative tests appeared to be an iron derivative. Loss of pigment also occurs on iron-free diets and under other conditions,

Table II

Wave-length of Fe line examined	Enamel and pigment layer only		Dentine	
	Normal rat	Fluoride- fed rats	Normal rat	Fluoride- fed rats
2343 and 2348 Å.	4	3	2	1
2406 and 2410 Å.	4	3	2	1
2625 Å. group	4	3	2	1
2912 Å.	4	3	2	1
3059 Å.	4	3	2	1

Nos. 1, 2, 3, 4 indicate the order of intensity of the lines, 1 the weakest, 4 the strongest. These results emphasize the greater iron content of the pigmented enamel of rats' teeth as compared with the dentine, and the loss of iron from both enamel and dentine in chronic fluorosis.

Human enamel, non-carious, good structure, London

Human enamel (Maldon) "mottled"

Enamel of fluoride-fed rat

Spectra of teeth

Spectrograms of enamel enlarged about four times. Strips 1-8. Spectrum of non-carious human enamel of good structure (London). Strip 9. That of human enamel (Maldon) with a faint CaF band. Strip 10. That of fluoride-fed rats' enamel with a strong CaF band. In Strips 1-4 the Fe comparison spectrum is at the upper edge, but in 5-10 it is at the lower edge of the spectrograms.

including lack of vitamin A. The spectrograms showed the different iron contents of the enamel and dentine of the teeth from the control and NaF-fed rats. The results are listed in Table II.

Zinc in teeth. Recently the physiological significance of zinc has been considered from different aspects [Hart & Elvehjem, 1936]. Bones contain a higher concentration of zinc than other tissues, the amounts determined chemically were [Lutz, 1926] 0.0126% and [Drinker *et al.* 1927] 0.0227%. This led Cruickshank [1936] to consider the Zn content of teeth. He found that enamel contained 0.025% Zn.

It was with some difficulty that Zn was identified spectrographically in teeth. Using the line 2138.61 Å. Zn was found as a trace in all human specimens. By comparison with the spectra given by $\text{Ca}_3(\text{PO}_4)_2$ to which small measured graded amounts of Zn had been added, the teeth specimens were found to contain between 0.01 and 0.005% of Zn.

SUMMARY

1. Spectrograms of human enamel and dentine, dog dentine and rats' teeth, covering the ranges 2100–6200 Å. were obtained and analysed to identify "trace elements". Special consideration was given to the identification of fluorine, and the specimens analysed included "mottled teeth" from Maldon, Essex, and teeth of fluoride-fed rats.

2. In addition to the major constituents known to exist in dental tissues the following elements were found in all specimens; Na, Ag, Pb, Sr, Ba, Cr, Sn, Zn, Mn, Ti, Ni, V, Al, Si, B, Cu and Fe.

3. Fluorine was not found in the dog or rat specimens; there was a "possible trace" in the London human specimens, a definite trace in the human "mottled teeth" and a considerable amount in the teeth of fluoride-fed rats.

4. Potassium was not found generally but was present in enamel and dentine of "mottled teeth" and in the fluoride-fed rats' teeth.

5. Diminution of the Fe content was noted in the enamel and dentine of the fluoride-fed rats.

6. An attempt has been made to grade roughly the amount of different elements in the specimens and further to assess relatively the amount of a particular element in the different specimens.

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