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THE QUANTITATIVE DETERMINATION OF Ca, Na, Al, Mg and Cl IN NORMAL ENAMEL AND DENTINE BY NEUTRON ACTIVATION AND HIGH RESOLUTION GAMMA SPECTROMETRY†

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INTRODUCTION

IT is a well known fact that the ingestion of an optimum amount of fluoride during the developmental period of the crowns of the teeth renders them more resistant to caries. It is possible that other trace elements may also assist in reducing the incidence of the disease: for example, by influencing the solubility of the calcified dental tissues, by producing changes in the morphology of the teeth or by altering the crystallinity of the enamel[1]. Before the function of various elements in teeth can be fully explained or the changes that occur in their distribution in pathological states can be interpreted, their normal distribution must be determined. Thus the methods adopted for the quantitative analysis of the elements in teeth are continually being improved.

Of the recently developed techniques, neutron activation analysis, because of its high degree of sensitivity, enables the presence of the major and minor mineral constituents of teeth to be revealed. The principles involved in activation analysis in general and neutron activation analysis in particular were discussed in a recent paper[2]. The distinctive features of neutron activation analysis may be applied to determine the various elements in biological materials; the inherently high sensitivity of this technique makes it suitable for the detection of mere traces of elements.

Previously NaI (Tl) scintillation counters were used and lengthy radiochemical separations were necessary prior to the determination of one or more components in an irradiated sample[3]. With the development of high resolution Ge(Li) detectors and large, fully transistorised multi-channel analysers, significant progress has been made in activation analysis; this is especially the case in non-destructive multiple-element analysis[4], which has since been applied to a diversity of sample matrices[5,6], including biological materials[7,8].

As most of the quantitative analysis of various elements in human teeth by activation analysis involve radiochemical separation, preliminary investigations were undertaken to ascertain the elements that could be manifested qualitatively by instrumental activation analysis[9]. They numbered eleven.

The object of the present investigation was to determine, by high-resolution γ-spectrometry, the concentration of some of the elements which give rise to short-lived radioisotopes in normal human enamel and dentine: Ca, Na, Al, Mg and Cl.

EXPERIMENTAL METHODS

(a) *Preparation of the enamel and dentine samples.*

Freshly extracted, sound teeth with no restorations were used. Debris and tartar were removed, and the teeth were thoroughly washed in deionised water for a few minutes and dried in an oven at 105°C. The enamel was separated from the dentine mechanically by chipping, and these tissues were then ground to a fine powder in an agate mortar.

(b) *Procedure.*

100 mg of powdered enamel and dentine were accurately weighed and sealed in polyethylene containers. Standards were weighed portions of a mixed solution of Ca, Na, Al, Mg and Cl and other elements present in enamel and dentine. The standard samples contained approximately the same amount of these elements as is present in 100 mg of

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enamel and dentine. The reagents used were of analytical grade, without further purification. The standard liquid mixtures were weighed in polyethylene containers, carefully evaporated to dryness and sealed.

A sample and a standard were irradiated simultaneously for four minutes in the pneumatic facility of SAFARI-I, an ORR-type reactor, in a thermal neutron flux of 1.2×10^{13} n.cm^{-2} sec.$^{-1}$. Westcott's epithermal index, r, for this irradiation position is 0.0087. Gamma spectrometry of the irradiated samples was done 15 seconds after irradiation by placing them 13cm below the Ge(Li) detector. The detector used was a 15 cc Ge(Li) diode (Princeton Gamma Tech., Princeton, N.J., U.S.A.) mounted in a cryostat and cooled with liquid nitrogen. An uncooled TC 135 M Tennelec preamplifier, a TC 200 Tennelec amplifier and a TC 250 Tennelec bias amplifier were used to obtain low noise amplification of the diode signals. The spectrum analysis was done on an Intertechnique 400 channel analyser (Model SA 40B) bypassing the built-in linear amplifier of the analyser. The resolution of the counting system is 4.2 keV (f.w.h.m.) for the 1,333 keV photopeak of ^{60}Co. The standards were counted after a cooling period of approximately three minutes using the same geometry. Magnesium, sodium, chlorine, aluminium and calcium were measured using the 842 keV, 1368 keV, 1643 keV and 1780 keV photopeaks of ^{27}Mg, ^{24}Na, ^{38}Cl and ^{28}Al respectively and the 2080 keV—double escape peak of ^{49}Ca.

Yule's method[10] of photopeak analysis was employed to obtain the true counts under the different photopeaks.

(c) *Nuclear data.*

The application of reactor neutron activation to human enamel and dentine results in the formation of several radioisotopes[11,12]. Table I shows the most important radionuclides of Ca, Na, Al, Mg, Cl, F, and Mn produced by thermal neutron bombardment.

The interference of the ^{24}Mg (n,p) ^{24}Na reaction can also be ignored because the thermal neutron cross-section of sodium is much higher than the fast neutron cross-section of magnesium[14]. In the determination of magnesium interference due to the reaction ^{27}Al (n,p) ^{27}Mg can be neglected because of the low concentration of aluminium compared to that of magnesium in enamel and dentine, and as the fast neutron cross-section of aluminium for (n,p) reactions is low[15]. Besides, the fast neutron flux is less than 1.0 per cent of the thermal neutron flux.

RESULTS AND DISCUSSION

A typical γ-spectrum of an enamel sample under these experimental conditions is shown in Fig. I. The γ-spectrum obtained from a dentine sample is similar except that the chlorine photopeak is much reduced due to its low concentration in dentine. It can be seen that the pronounced 1368 - keV, 1643 keV and 1780 - keV photopeaks of ^{24}Na, ^{38}Cl and ^{28}Al respectively and also the 2080 - keV double escape peak of ^{49}Ca are well separated from other peaks. These photopeaks can therefore be used as a measure of the sodium, chlorine, aluminium and calcium

TABLE I*: Nuclear Data of Ca, Na, Al, Mg, Cl, F and MN.

Stable Isotope	*Abundance* %	*Activation cross section (Barnes)*	*Radio-isotope*	*Half-life of Radio-isotope*	*γ-ray photo peak measured-keV*
^{48}Ca	0.185	1.1	^{49}Ca	8.8 min.	2080
^{23}Na	100	0.13	^{24}Na	15.05 hrs.	1368
^{27}Al	100	0.235	^{28}Al	2.3 min.	1780
^{26}Mg	11.17	0.034	^{27}Mg	9.46 min.	842
^{37}Cl	24.47	0.430	^{38}Cl	37.3 min.	1643
^{19}F	100	0.0098	^{20}F	11.2 sec.	1630
^{55}Mn	100	13.3	^{56}Mn	2.57 hrs.	847, 1810

*In the determination of sodium, interference due to the reaction ^{27}Al (n, α) ^{24}Na can be ignored because of the low concentration of aluminium compared to that of sodium in dentine and enamel, and as the fast neutron cross-section of aluminium is much lower than the thermal neutron cross-section of sodium.[13]

contents of irradiated enamel and dentine samples.

The 842 - keV photopeak of ^{27}Mg was used to measure the magnesium content. Due to the relatively low concentration of manganese in enamel and dentine (<1 p.p.m.), the contribution of the 846 keV photopeak of ^{56}Mn has been ignored. This is further evidenced by the absence of the second prominent photopeak of ^{56}Mn at 1811 - keV.

During this investigation an attempt was made to do a quantitative analysis of fluorine by thermal neutron activation. However, for two reasons this technique failed: first, due to the strong interference of the 1643 - keV photopeak of ^{38}Cl over the 1630 - keV photopeak of ^{20}F which lies on the Compton edge of ^{38}Cl; and secondly, because of the higher content of chlorine in enamel and dentine. In addition it was impossible to commence counting sooner than 15 seconds after irradiation, whereas the half-life of ^{20}F is only 11.2 seconds.

The results obtained appear in Tables II and III. Some of the data shows deviations up to 30 per cent, which cannot be attributed to differences in the weights of the samples or to poor precision of the non-destructive reactor activation analytical technique used. It is the authors' contention that the somewhat erratic behaviour of the experimental data can be ascribed to other factors such as incomplete grinding and mixing of the enamel and dentine respectively after their separation from the teeth.

Separating enamel from dentine by chipping is a slow process and leads to incomplete recovery of enamel. Further, its contamination by dentine is unavoidable especially if large amounts have to be collected for repeated analyses. The low results obtained for the calcium concentration in enamel compared to the accepted one (± 36 per cent), indicate the extent of dentine contamination of the enamel samples used in this investigation. Manly and Hodge[16] developed a separating method which applies a centrifugal technique to the usual flotation

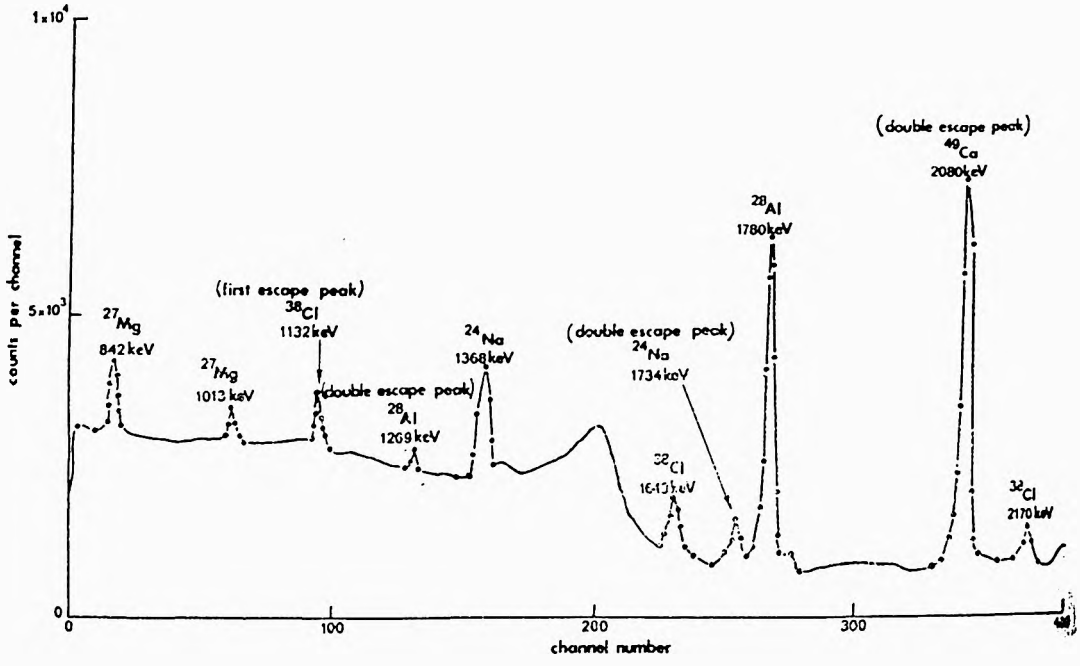


FIGURE 1. Gamma spectrum of an irradiated enamel sample 15 seconds after irradiation.

TABLE II: The Concentration of Ca, Na, Al, Mg and Cl in Normal Human Enamel.

Element	*Number of Samples Analysed*	*Mean Conc.*	*Standard Error of Mean*	*Standard Deviation*	*95% Confidence Interval for the True Mean*
Ca	7	31.69%	0.33	0.88	30.94 - 32.45
Na	6	0.52%	0.02	0.04	0.47 - 0.56
Al	6	236.3 ppm	9.4	22.9	214.7 - 257.8
Mg	5	0.37%	0.01	0.22	0.34 - 0.39
Cl	5	0.33%	0.01	0.22	0.31 - 0.35

TABLE III: The Concentration of Ca, Na, Al, Mg and Cl in Normal Human Dentine.

Element	*Number of Samples Analysed*	*Mean Conc.*	*Standard Error of Mean*	*Standard Deviation*	*95% Confidence Interval for the True Mean*
Ca	7	26.24%	0.56	1.49	24.97 - 27.51
Na	6	0.55%	0.01	0.03	0.53 - 0.57
Al	7	68.6 ppm	8.5	22.5	49.4 - 87.8
Mg	6	0.87%	0.01	0.03	0.85 - 0.90
Cl	8	0.035%	0.003	0.01	0.029 - 0.041

procedure. It enabled them to prepare 99.4 per cent pure enamel and 99.7 per cent pure dentine with approximately 10 per cent loss of material. The bromoform acetone mixture used in the differential flotation technique cannot be obtained sufficiently pure to avoid contamination of the enamel and dentine prior to irradiation, which unfortunately excludes this method of separation when employing activation analysis. To avoid its contamination with enamel, the dentine was collected from the root portion of the tooth and no attempt was made to keep it free from cementum. The removal of enamel by grinding with a separating disc was considered, but the idea was abandoned because neutron activation analysis of the disc showed the presence of Mn, Cu and Al.

Phosphorus, the other major constituent of enamel and dentine, cannot be determined with neutron activation and gamma-ray spectrometry. Although phosphorus is readily activated by reactor neutrons, the resulting radionuclide, ^{32}P, is a pure β-emitter. The β-activity can be measured with a Geiger-Müller or proportional counter.

The authors' findings for Ca, Mg and Cl are similar to those published by Hawk *et al*[1]. In the case of aluminium, however, whereas Harwick and Martin[16], using mass spectrometry, found its concentration in both tissues to range from 10 - 100 p.p.m., in this investigation its mean concentration in dentine was 68.6 p.p.m. and 236 p.p.m. in enamel.

It is the authors' intention to extend this present work to determine the concentrations of the other elements in enamel and dentine

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BIOLOGIC CONSIDERATIONS IN THE SELECTION AND USE OF RESTORATIVE MATERIALS

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UNTIL recently, relatively little attention had been given to the biologic effects of restorative materials upon the pulpo-dentinal organ and on the gingivae. For a long time, the major emphasis was upon the physical and chemical properties of filling materials, and dental research concentrated upon improvement of these properties, such as hardness, resistance to wear, solubility in saliva, setting rate, etc. The National Bureau of Standards under Paffenbarger, and dental materials research laboratories in a number of universities (Skinner at Northwestern, Phillips at Indiana, Peyton at Michigan and Ryge at Marquette) did a great deal to improve the quality of the filling materials for the profession and set standards which all the world now follows.

With the appearance of reports from early workers such as Pallazzi (1923), Fasoli (1924), Hellner (1927-28) in Germany, Manel of London (1936), Gurley and Van Huysen (1937) in the United States, Shroff of New Zealand (1947), and many others, the importance of the effects of filling materials upon the pulp began to be better appreciated and the emphasis shifted from purely physicochemical research to include the biologic aspects of restorative procedures. One of the reasons for the relatively late appearance of biologic research in the area of restorative dentistry and dental materials was the lack of fundamental studies in pulpal biology and dentinal responses to injury. The classic studies of Sir Wilfred Fish of London and Beust in the United States in 1931-33 did much to give impetus in this direction. The reparative potential of the dentin has since that time become better clarified so that the effects of medicaments and filling materials upon the dentinal defenses and pulpal repair are now becoming the object of serious research.

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