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The mineral composition of enamel from two South African population groups

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SUMMARY

The mineral composition of pooled bulk enamel from Black and White South Africans respectively, resident in the Johannesburg area, was determined by neutron activation analysis and high resolution gamma spectrometry. The differences between the concentrations of Ca, Cl, Mg, Na, Br and Co in the enamel of the two population groups were apparently not significant. There was a trend for the concentrations of Al, Ag, Au, Fe, Sb, and Zn to be higher in the enamel from the White subjects and for the concentrations of Mn, Se and Sr to be higher in the enamel from the Black subjects.

OPSOMMING

Die minerale samestelling van tandglasuur van wit en swart Suid-Afrikaners onderskeidelik wat in die omgewing van Johannesburg woon, is vasgestel deur neutron-aktiverings-ontleding en hoë gamma spektrometriese ontbinding. Die verskil tussen Ca, Cl, Mg, Br en Co in die glasuur van die twee bevolkingsgroepe, was blykbaar nie betekinsvol nie. Daar was 'n neiging tot hoër konsentrasie van Al, Ag, Au, Fe, Sb en Zn in die glasuur van die Blankes en die konsentrasie van Mn, Se en Sr was weer hoër in die van die Swartes.

INTRODUCTION

Dental caries is one of the most common chronic diseases afflicting mankind and in many areas it assumes rampant proportions. Epidemiological studies have shown significant differences in caries prevalence according to geographic location (Hadjimarkos, 1956; De Jager, 1963; Ludwig and Bibby, 1969; Baume, 1973). It has been suggested that variations in the prevalence of dental caries in various geographical areas might be attributable to the trace-element content of the water supplies (Hadjimarkos, 1966; Ludwig, Adkins and Losee, 1970; Glass *et al.*, 1973) diet (Losee and Adkins, 1968; Curzon, Kubota and Bibby, 1971) and soil (Losee, Cadell and Davies, 1961; Pearce, Ludwig and Darwin, 1974).

The effects of race and socioeconomic status on dental caries prevalence have also been evaluated (Infante and Russell, 1976; Heifetz and Horowitz, 1976; Moreira and Viera, 1977). The results of these studies showed that a high caries prevalence was not always associated with a particular racial group. Marked differences, however, in the caries rate in black and white high-school children in South Africa were recently reported (Retief, Cleaton-Jones and Walker, 1975).

Apart from fluoride, only from one to 20 per cent of the daily micronutrient intake is derived from the drinking water (Schroeder, 1966). Because of the variable intake of micronutrients from the diet, the effect of trace elements on dental caries could be more accurately evaluated by relating the mineral content of enamel to caries prevalence. Sensitive analytical techniques such as neutron activation analysis (Söremark and Samsahl, 1961; Retief *et al.*, 1971), atomic absorption spectro-

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scopy (Nixon and Helsby, 1976; Lokamaa and Rytömaa, 1977) and spark source mass spectrometry (Curzon, Losee and Macalister, 1975) have been used to determine the mineral content of human enamel.

The object of this pilot investigation was to determine by neutron activation and high resolution gamma spectrometry, the mineral composition of pooled bulk enamel from Black and White South Africans respectively.

MATERIALS AND METHODS

(a) Preparation of the enamel samples

Freshly extracted, noncarious molar teeth from adult White and Black subjects resident in Johannesburg were used in this investigation. The sex, age, caries experience and period of residence in the Johannesburg area of the tooth donors were not known. The teeth were extracted because of periodontal disease. The teeth were cleaned with a brush and pumice on a dental lathe and thoroughly washed in deionized water. Although this procedure may remove the superficial ion rich layer of enamel it was carried out on all the extracted teeth to eliminate contamination introduced by the stainless steel forceps during the extraction procedure. The teeth were dried for a few days in a desiccator that contained silica gel. Afterwards the enamel was mechanically separated from the dentine by holding the teeth firmly by their roots and gently tapping the crowns of the teeth on the edge of an agate mortar with an agate pestle. All the enamel obtained from approximately 20 teeth of each of the population groups were pooled separately and ground to a fine powder in the agate mortar to obtain a homogeneous mixture. Approximately 200 mg of this enamel was accurately weighed into clean polyethylene containers and quartz ampoules and sealed.

(b) Preparation of the standards

Standard samples were prepared by weighing a known volume of a mixed solution of the elements to be determined into polyethylene containers and quartz ampoules. Analytical grade reagents were used in the preparation of the standard solution. The mass of the elements in the weighed aliquots of the standard solution approximated the mass of these elements present in 200 mg of enamel. After careful evaporation to dryness, the polyethylene containers and quartz ampoules were sealed.

(c) Irradiation procedure

For the determination of Al, Ca, Cl, Mg and Na in the enamel samples, 10 samples from each population group and 20 reference standards, sealed in polyethylene containers, were irradiated separately for precisely 5 min in the pneumatic facility of the SAFARI-1 reactor at Pelindaba. The thermal neutron flux is about 3×10^{13} n.cm^{-2}.sec^{-1}. Westcott's epithermal index, r, for this irradiation position is 0,0087. Relative values of the integrated neutron flux were determined for some enamel samples and reference standards by the simultaneous activation of accurately weighed gold standard samples (~5 μg).

For the determination of Ag, Co, Fe, Sb, and Zn in the enamel samples, 10 samples from each population group with reference standards, all sealed in quartz ampoules, were irradiated for 108 hr in different positions in the poolside facility of SAFARI-1 at a flux of 4×10^{13} n.cm^{-2}.sec^{-1}. Westcott's epithermal index, r, for these positions are between 0,013 and 0,02. Relative values of the integrated neutron flux were determined for each sample and reference standard by the simultaneous activation of accurately weighed iron monitors (~0,03 g of iron) wrapped around each quartz ampoule.

For the determination of Mn and Sr, 10 enamel samples from each population group and reference standards, all sealed in quartz ampoules, were irradiated for 15 hr in 0,5 mm thick cadmium containers. The irradiation was carried out in the poolside facility of the reactor and the integrated neutron flux determined by means of iron monitors.

After two weeks of decay, the same samples and standrards used for the determination of Mn and Sr, were reirradiated for 48 hr in cadmium containers for the determination of gold and bromine. The same irradiation positions in the reactor were used and the integrated neutron flux again determined.

(d) Measurement of gamma activity

Gamma spectrometry of the irradiated samples and standards for the determination of Al, Ca, Cl, Mg and Na was done exactly 2 min after each irradiation by placing them at a fixed distance from the Ge(Li) detector. The detector used in this investigation was a 50 cm^3 coaxial Ge(Li) diode (Princeton Gamma Tech.) connected to an uncooled TC 135 M Tennelec preamplifier. The output pulses were amplified by a TC 200 Tennelec amplifier. Spectrum analysis was performed on an Intertechnique 4000-channel analyzer (Model SA 44). The resolution of this counting system is 3,5 keV (full width at half maxium) for the 1333 keV photopeak of ^{60}Co. Data for peak analysis were recorded on magnetic tapes and processed by computer. Yule's (1968) smoothed first derivative method was applied to obtain the true peak counts under the photopeaks of interest, from which the elemental concentrations were calculated by computer programmes (R. J. N. Brits, 1977, personal communication).

Gamma spectrometry of the samples and reference standards irradiated for 108 hr was carried out after about 30 days of decay using the same counting system and computer programmes. The counting of samples and reference standards irradiated in cadmium containers for 15 hr and 48 hr was done after about 4 hr and 48 hr of decay, respectively. The same counting system and computer programmes mentioned previously were used.

(e) Nuclear data

The radionuclides of the various elements produced by thermal and epithermal neutron activation and the relevant nuclear data are available (Lederer, Hollander and Perlman, 1967).

RESULTS

A typical ^{4}-spectrum of an enamel sample which was irradiated for 15 hr in a cadmium shield and recorded after a decay period of 4 hr is shown in Fig. 1. The photopeaks of ^{87m}Sr and ^{56}Mn at 388 keV and 847 keV respectively, were used to measure the concentration of these elements in enamel. Photopeaks similar to those previously described (Retief *et al.*, 1971) were obtained with

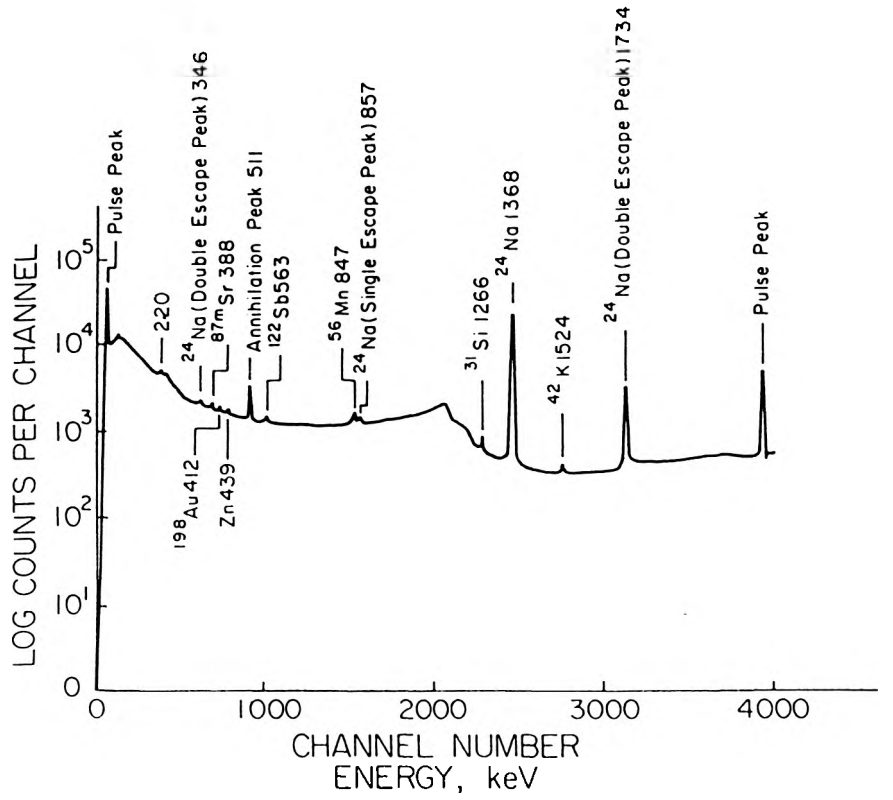

Fig. 1 Gamma spectrum of an enamel sample irradiated for 15 hr, 4 hr after irradiation.

the other irradiation procedures and used to determine the concentrations of the respective elements.

The mean concentrations of the elements determined in the composite enamel samples from the teeth of Black and White South Africans and the standard deviations of the means are given in Table 1. The results for the selenium concentrations, determined in a previous study (Retief *et al.*, 1976), have been included in the table.

The standard deviations of the mean concentration of the elements are a measure of the accuracy of the analytical procedures. A comparison of the concentrations of the elements in the enamel from the two population groups, taking the measurement error in consideration, revealed that the differences between the concentrations of Ca, Cl, Mg, Na, Br and Co were apparently not significant. There was a trend for the concentrations of Al, Ag, Au, Fe, Sb and Zn to be higher in the enamel from the White subjects and for the concentrations of Mn, Se and Sr to be higher in the enamel from Black subjects. It was not possible to determine the significance level of these differences in the mineral composition because of the pooling of the enamel obtained from the extracted teeth from each population group.

Table 1 Mineral composition of pooled bulk enamel from black and white South Africans

Element	Black South Africans				White South Africans			
	n	$\bar{x}$	±	S.D.	n	$\bar{x}$	±	S.D.
Al ppm	10	72,2	±	5,8	10	93,2	±	17,4
Ag ppm	10	0,11	±	0,07	10	0,23	±	0,23
Au ppm	10	0,015	±	0,004	10	0,025	±	0,002
Br ppm	10	20,9	±	2,3	10	22,2	±	2,0
Ca %	10	36,8	±	1,8	10	36,9	±	1,8
Cl %	10	0,79	±	0,06	10	0,82	±	0,04
Co ppm	10	0,09	±	0,04	10	0,07	±	0,04
Fe ppm	10	34,2	±	10,0	10	86,3	±	16,0
Mg %	10	0,24	±	0,03	10	0,27	±	0,03
Mn ppm	10	1,87	±	0,49	10	0,99	±	0,35
Na %	10	0,69	±	0,02	10	0,70	±	0,01
Sb ppm	10	0,61	±	0,05	10	1,33	±	0,24
Se ppm	10	0,08	±	0,03	10	0,012	±	0,004
Sr ppm	10	177,7	±	28,3	10	103,0	±	23,6
Zn ppm	10	157,8	±	18,0	10	177,3	±	20,4

n =Number of samples.
$\bar{x}$ = Mean.
S.D. = Standard Deviation

DISCUSSION

In this investigation the enamel was mechanically separated from the dentine by chipping. Although this is a slow process and leads to incomplete recovery of enamel, grinding procedures were not used because the concentrations of some trace elements in enamel were markedly increased by such separating techniques (Battistone, Feldman and Reba, 1967). Preliminary studies indicated that approximately 200 mg of enamel is required to determine the concentrations of the minor mineral components with this analytical technique.

The neutron flux in a nuclear reactor is not homogeneous but varies from one irradiation position to another. The gold and iron monitors used in this study enabled us to calculate the relative value of the neutron flux to which the standard and enamel samples were exposed during irradiation.

The concentrations of Au, Br, Mn and Sr in enamel are low when compared with the concentrations of Al, Ca, Cl, K, Mg, Na and P. The elements Au, Br and Sr are very sensitive to epithermal neutron activation,

whereas the activities of the major elements are depressed by irradiation with epithermal neutrons. The sensitivity of Mn is enhanced as a result of the lower activity of epithermally irradiated samples. The use of a cadmium shield reduces the thermal neutron flux with respect to the epithermal neutron flux and makes possible the quantitative analysis of Au, Br, Mn and Sr.

Derise, Ritchey and Furr (1974) determined the concentrations of the macrominerals Ca, P, Cl, K and Mg in enamel and related it to the age, sex and caries experience of the donors. They reported that the correlations between the concentrations of these minerals and caries incidence were low. Armstrong and Brekhus (1937) had previously reported that no significant differences could be detected in the Ca, P and Mg concentrations of the enamel of sound and carious teeth of the same person. This was confirmed by Ockerse (1943) who found no significant differences in the concentrations of Ca, Mg and P in carious and non-carious enamel from people in high- and low-caries areas in South Africa. Efforts to relate the mineral composition of enamel to caries prevalence should therefore be concentrated on the trace element composition of enamel.

Although a trend indicating differences in the concentrations of several trace elements in the enamel from the two population groups was obtained in this pilot study, the association between these elements and dental caries experience could not be established because of the sampling procedure used.

It is possible to obtain approximately 500 mg of enamel from a single molar tooth with the chipping technique used in this investigation to separate enamel from dentine. Approximately 400 mg of enamel is required for the three irradiation procedures used to determine the concentrations of Ag, Co, Fe, Sb, Zn, Mn, Sr, Au and Br.

Analyses of teeth obtained from Black and White subjects of similar age groups and with known caries experience and who had lived permanently in a well defined geographical area supplied with drinking water from a common source, may reveal an association between the trace element composition of enamel and dental caries. The material for such a study is available in Johannesburg and neutron activation analysis and high resolution gamma spectrometry is an elegant analytical tool for an investigation of this nature.

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