NEUTRON ACTIVATION ANALYSIS OF TRACE ELEMENT CONTENTS IN THE CROWNS OF HUMAN PERMANENT TEETH

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Introduction

The apatite phases of teeth can apparently be affected by trace element incorporation into teeth with effects on the physicochemical properties[1-4]. It is the reason why deficiency or excess of F, Sr, Mn, Fe and some other trace elements is one of the factors which determines the degree of susceptibility of caries and other dental diseases.[5,6] So, chemical element analysis of teeth expands the knowledge of etiology of dental diseases and may be apply for diagnostic, therapeutic and preventive purposes. Furthermore, teeth have been suggested as dose monitors for the exposure of human body to elements concentrated in calcified tissues.[7,8] In accordance with it teeth are used in an occupational medicine and environmental health studies.[9-12] Moreover, a chemical element analysis of human teeth are often used in paleoanthropology for dietary and environment reconstruction, assessment of the social and economic status of human groups.[13-18] It developed that for efficient application of teeth analysis in all above-mentioned directions it is necessary to know the normal levels and age-related changes of teeth trace elements in large scale.

There are several reviews and text in regard to trace elements of teeth, using chemical analysis techniques and instrumental methods.[1,2,5,19-22] However, the majority of these data are based upon non intact teeth but separated tissues of teeth such as enamel, dentin or cement. It was shown that samples are contaminated some trace elements from stainless steel tools during the separation.[23,24] In most cases, teeth samples are treated with solvents in order to remove organic matrix, and are then ashed and acid digested. There is evidence that by these methods some chemical elements are lost, their relationship also being affected.[25,26]

In the present study the effect of age on Co, Eu, Fe, Hg, Sb, Sr, and Zn mass fraction in crown of permanent teeth were analyzed with two objectives. The first objective was to use intact teeth. The second objective was to perform measurements on crown – the only part of teeth available for *in vivo* examination.

Experimental

Samples of human permanent teeth were obtained at postmortems, from intact cadavers (24 males, with ages from 16 to 55 years) within 24 hours of death. Each death had resulted from trauma, due to automobile accidents, falls, shootings, knifings, hanging, acute alcohol poisoning, and freezing. Mainly molars and premolars were extracted. The crowns contacted with the stainless steel surgical instruments were clean by the alcohol moist gauze tampons. A tool made of titanium was used to cut and to scrub soft tissue and blood off the

roots. After separating the crown from root with a titanium knife, samples were freeze dried until constant mass was obtained. The crowns were used in the study only.

To determine concentrations of the elements by a relative way, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins in the Institute of Physics, Georgian Academy of Sciences specifically for INAA were used[27]. The BSS were 4 mm diameter tablets weighing about 30 mg. Corrected certified values of BSS element contents were reported by us before[28]. The tooth crown samples, ten CRM IAEA H-5 (Animal Bone) and SRM NIST 1486 (Bone Meal) subsamples weighing about 50-100 mg, together with SSB tablets were wrapped separately in a high-purity aluminum foil washed with rectified alcohol beforehand and put into a nitric acid-washed quartz ampoule. To arrange reference material samples and standards in the quartz ampoule, they were distributed among tooth crown samples along the whole ampoule length. This enabled correcting an influence of neutron flux non-uniformity in the reactor channel. The quartz ampoule was soldered, positioned in a transport aluminum container and exposed to 20-hour neutron irradiation in a vertical channel with neutron flux $1.7 \cdot 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$. Seven days after irradiation samples were reweighed and repacked.

The measurement of each sample was made twice, 7-10 and 40-60 days after irradiation. The second measurements started 40 days after irradiation due to the high intensity of ^{32}P β-particles ($T_{1/2}$=14.3 d). To reduce ^{32}P β-particles background, a combined lead-cadmium-copper-aluminum filter was used. The duration of the first and second measurements was 10 min and 10 hours, respectively. The gamma spectrometer included an HPGe detector (GEM 15180P, ORTEC, relative efficiency 15%) and a MAA combined with a PC (ASPRO-NUC) was used. The spectrometer provided resolution 1.8 keV on the ^{60}Co 1332 keV line. Processing of gamma spectra and computations of chemical element mass fractions in the examined samples were carried out using ASPRO software package[29].

Irradiation, expose and measurement times as well as the sample-detector distance were regarded optimum in terms of providing feasibility of simultaneous measurement for the maximum number of elements with acceptable statistical error for each of them. A dedicated computer program of NAA mode optimization was used for preliminary estimation of these parameters[30].

Results

Radionuclides and some of their characteristics used for INAA of Ag, Ce, Co, Cr, Cs, Eu, Fe, Gd, Hf, Hg, Mo, Rb, Sb, Sc, Se, Sr, Ta, Tb, Th, Yb, Zn, and Zr contents in tooth crown and reference material samples are given in Table 1.

Table 2 shows our results for ten sub-samples of CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal reference material and the certified values of this material.

Table 3 gives some statistical parameters of trace element contents in the intact tooth crown of healthy men such as arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels.

Finally, Table 4 presents the comparison of our and reference data of trace element contents in human teeth.

Table 1. Nuclear reactions and some characteristics of radionuclides used for INAA of crowns of human permanent teeth

Element	Nuclear reaction	Radionuclide	Half-life	γ-energy used keV
Ag	^{109}Ag (n, γ)	^{110m}Ag	250.0 d	658, 1384
As	^{75}As (n, γ)	^{76}As	1.12 d	559
Au	^{197}Au (n, γ)	^{198}Au	2.7 d	412
Ba	^{130}Ba(n, γ)	^{131}Ba	11.52 d	216, 373, 496
Br	^{81}Br(n, γ)	^{82}Br	1.47 d	698, 777, 1044
Cd	^{114}Cd(n, γ)	^{115}Cd	2.2 d	336
Ce	^{140}Ce(n, γ)	^{141}Ce	32.5 d	145
Co	^{59}Co(n, γ)	^{60}Co	5.64 y	1173, 1332
Cr	^{50}Cr(n, γ)	^{51}Cr	27.8 d	320
Cs	^{133}Cs(n, γ)	^{134}Cs	2.05 y	796
Eu	^{151}Eu(n, γ)	^{152}Eu	13.6 y	1408
Fe	^{58}Fe(n, γ)	^{59}Fe	45.6 d	1099, 1292
Gd	^{152}Gd(n, γ)	^{151}Gd	120 d	154
Hf	^{180}Hf(n, γ)	^{181}Hf	42.4 d	482
Hg	^{202}Hg(n, γ)	^{203}Hg	46.91 d	279
La	^{139}La(n, γ)	^{140}La	1.68 d	487, 816, 1595
Lu	^{177}Lu(n, γ)	^{177}Lu	6.74 d	208
Nd	^{146}Nd(n, γ)	^{147}Nd	11.02 d	91
Rb	^{85}Rb(n, γ)	^{86}Rb	18.66 d	1076
Sb	^{121}Sb(n, γ)	^{122}Sb	2.74 d	564
	^{123}Sb(n, γ)	^{124}Sb	60.9 d	1691
Sc	^{45}Sc(n, γ)	^{46}Sc	83.89 d	889, 1121
Se	^{74}Se(n, γ)	^{75}Se	120.4 d	136, 265, 401
Sm	^{152}Sm(n, γ)	^{153}Sm	1.96 d	103
Sr	^{84}Sr(n, γ)	^{85}Sr	64.8 d	514
Ta	^{181}Ta(n, γ)	^{182}Ta	115 d	1221
Tb	^{159}Tb(n, γ)	^{160}Tb	72.3 d	879, 966
Th	^{232}Th(n, γ)	^{233}Pa	27.0 d	312
U	^{238}U(n, γ)	^{239}U(β→^{239}Np)	23.5 m (2.36 d)	228, 278
Yb	^{174}Yb(n, γ)	^{175}Yb	4.19 d	396
Zn	^{64}Zn(n, γ)	^{65}Zn	245.7 d	1115
Zr	^{94}Zr(n, γ)	^{95}Zr	65.5 d	724, 757

Table 2. INAA data of trace elements of CRM IAEA H-5 Animal Bone and SRM NIST 1486 Bone Meal (mg/kg on dry weight basis)

Element	CRM IAEA H-5		Own results	SRM NIST 1486		Own results
	Mean	Type*	Mean±S.D.	Mean	Type*	Mean±S.D.
Ag	-	-	<0.02	-	-	<0.02
As	0.013	N	<0.1	0.006	N	<0.1
Au	-	-	<0.01	-	-	<0.01
Ba	72±28	C	<100	-	-	270
Br	3.6	N	<10	-	-	<10
Cd	0.023	-	<2	0,003	N	<2
Ce	-	-	<0.03	-	-	<0.03
Co	0.25	N	0.56±0.25	-	-	0.11±0.02
Cr	2.56	N	<0.8	-	-	<0.9
Cs	-	-	<0.05	-	-	<0.06
Eu	-	-	<0.015	-	-	<0.02
Fe	79±11	C	85±17	99±8	C	93±11
Gd	-	-	<0.25	-	-	<0.25
Hf	-	-	<0.04	-	-	<0.04
Hg	0.008	N	<0.01	-	-	<0.01
La	-	-	<0.05	-	-	<0.05
Lu	-	-	<0.003	-	-	<0.003
Nd	-	-	<0.1	-	-	<0.1
Rb	1.07	N	<1.0	-	-	<0.9
Sb	0.024	N	<0,02	-	-	<0.02
Sc	-	-	<0.001	-	-	<0.001
Se	0.054	N	<0.05	0.13	N	<0.05
Sm	-	-	<0.01	-	-	<0.01
Sr	96±17	C	103±9	264±7	C	263±10
Ta	-	-	<0.08	-	-	<0.08
Tb	-	-	<0.005	-	-	<0.006
Th	-	-	<0.05	-	-	<0.05
U	-	-	<0.07	-	-	<0.07
Yb	-	-	<0.03	-	-	<0.03
Zn	89±15	C	86±7	147±16	C	153±29
Zr	-	-	<0.2	-	-	<0.2

* "C" - certified values, "N" - non-certified values

Table 3. Some statistical parameters of trace element contents in the intact crowns of human permanent teeth of healthy men (mg/kg on dry weight basis)

Element	Mean	SD	SEM	Min	Max	Med	Per0.025	Per0.975
Ag	<0.02	-	-	-	-	-	-	-
As	<0.1	-	-	-	-	-	-	-
Au	<0.01	-	-	-	-	-	-	-
Ba	<100	-	-	-	-	-	-	-
Br	<10	-	-	-	-	-	-	-
Cd	<2	-	-	-	-	-	-	-
Ce	<0.02	-	-	-	-	-	-	-
Co	0.0030	0.0032	0.0012	0.00045	0.0098	0.0021	0.00053	0.00882
Cr	<0.3	-	-	-	-	-	-	-
Cs	<0.02	-	-	<0.002	0.056	-	-	-
Eu	0.00081	0,00056	0,00023	0,00030	0.00161	0,00057	0,00031	0,00158
Fe	4.67	3.13	1.10	2.43	11.1	3.72	2.43	10.4
Gd	<0.1	-	-	-	-	-	-	-
Hf	<0.03	-	-	-	-	-	-	-
Hg	0.00053	0.00041	0.00020	0.00021	0.00113	0.00040	0.00022	0.00108
La	<0.05	-	-	-	-	-	-	-
Lu	<0.003	-	-	-	-	-	-	-
Nd	<0.1	-	-	-	-	-	-	-
Rb	<0.6	-	-	-	-	-	-	-
Sb	0.022	0.011	0.007	0.012	0.035	0.020	0.012	0.034
Sc	<0.0009	-	-	-	-	-	-	-
Se	<0.04	-	-	-	-	-	-	-
Sm	<0.01	-	-	-	-	-	-	-
Sr	198	200	40	31.7	860	108	38.5	746
Ta	<0.007	-	-	-	-	-	-	-
Tb	<0.01	-	-	-	-	-	-	-
Th	<0.05	-	-	-	-	-	-	-
U	<0.07	-	-	-	-	-	-	-
Yb	<0.03	-	-	-	-	-	-	-
Zn	136	50.2	11	42.6	285	136	60.4	246
Zr	<0.7	-	-	-	-	-	-	-

M –mean
SD – standard deviation
SEM – standard error of mean
min – minimal value
max – maximal value
Med – median
Per0,025 – percentile with 0,025 level
Per0,975 – percentile with 0,975 level

Table 4. Mean, range of means or upper level of chemical element contents (mg/kg on dry weight basis) in the enamel or dentin according to data from the literature in comparison with our data for crowns of human permanent teeth (superscripted number in the column headings designate reference number)

Element	From review of Cutress[7]		Our review		Our data
	Mean or range of means		Mean or range of means		Mean contents in crown
	enamel	dentin	enamel	dentin	
Ag, mg/kg	0.005-1.11	0.005-17	0.0049-10.9	0.004-2.18	<0.02
As, mg/kg	<0.02-0.07	-	<0.007->10	11-111	<0.1
Au, μg/kg	0.0001-0.11	0.03-0.07	0.0001-0.11	<0.0004-0.07	<0.01
Ba, mg/kg	2.1-125	129	2.1-125	129	<100
Br, mg/kg	1.1-33.8	4-114	0.45->100	4-114	<10
Cd, mg/kg	0.04-14.9	-	0.026-14.9	0.086-0.097	<2
Ce, mg/kg	-	-	0.07	-	<0.02
Co, mg/kg	0.0002-34.3	0.0003-32	0.0002-37.3	0.00034-33	0.0030
Cr, mg/kg	0.004-6.4	0.005-2.0	0.003-28	0.005-6.6	<0.3
Cs, mg/kg	0.04	-	0.04	-	<0.02
Eu, μg/kg	-	-	<0.04	-	0.00081
Fe, mg/kg	2.77-338	2.0-110	2.77-876	2.0-110	4,67
Gd, mg/kg	-	-	<0.08	-	<0.1
Hf, mg/kg	-	-	<0.08	-	<0.03
Hg, mg/kg	<0.11-2.6	-	0.02-86	0.18-1.03	0.00053
La, mg/kg	0.004-0.005	-	0.004-0.005	-	<0.05
Lu, mg/kg	-	-	<0.02	-	<0.003
Nd, mg/kg	0.045-0.050	-	0.045-0.050	-	<0.1
Rb, mg/kg	0.41-73	6-69	0.39-73	5.6-69	<0.6
Sb, mg/kg	0.001-0.96	0.7	0.012-1.0	0.1-2.7	0.022
Sc, μg/kg	0.0007	-	0.0007-53	20-71	<0.0009
Se, mg/kg	0.01-1.47	0.3-0.5	0.004-14.5	0.07-4.9	<0.04
Sm, mg/kg	-	-	<0.08	-	<0.01
Sr, mg/kg	76.2-286	64-570	14.5-610	64-256	198
Ta, mg/kg	-	-	<0.1	-	<0.007
Tb, mg/kg	<0.005	-	<0.005	-	<0.01
Th, mg/kg	-	-	-	-	<0.05
U, μg/kg	-	-	<0.02-0.03	-	<0.07
Yb, mg/kg	<0.01	-	<0.01	-	<0.03
Zn, mg/kg	126-740	160-2200	64-1670	148-1860	136
Zr, mg/kg	0.08-1.8	-	0.07-175	-	<0.7

Discussion

Of 6 (Ba, Br, Fe, Pb, Sr, Zn) and of 3 (Fe, Sr, Zn) trace elements with certified values for the CRM IAEA H-5 and the SRM NIST 1486 reference materials respectively, we determined contents of Fe, Sr, and Zn (Table 2). Mean values for these elements were in the range of 95% confidence interval. Mean values and 95% confidence intervals of Co, Cr, Hg, Rb, Sb, and Se were reported for CRM IAEA H-5, although those were not certified[31] (Table 2). Of these elements, a good agreement was obtained for Sb and Se. Mean contents obtained for Co, Cr, and Hg appeared to be considerably lower than the appropriate values of Parr's report[31]. Good agreement with the certified data of CRM IAEA H-5 and SRM NIST 1486 reference materials indicate an acceptable accuracy of the results obtained in the study of trace elements of the teeth as shown in Table 3.

The mean values and all selected statistical parameters were calculated for 7 (Co, Eu, Fe, Hg, Sb, Sr, and Zn) trace elements (Table 3). The contents of these trace elements were measured in all or majority part of teeth samples. The contents of Cs were determined in a few samples of collection only. The upper limit of mean for the elements was found as the normalized sum of all individual contents and detection limits. The contents of Ag, As, Au, Ba, Br, Cd, Ce, Cr, Gd, Hf, La, Lu, Nd, Rb, Sc, Se, Sm, Ta, Tb, Th, U, Yb, and Zr were lover of detection limits in all samples.

The obtained means for Co, Fe, Sb, Sr and Zn, as shown in Table 4, agree well with ranges of the mean values cited by other researches for teeth. [1,2,5,19-22] The Eu and Hg means are two orders of magnitude lower than range of previously reported data. The upper limit of mean for Ce, Cs, Hf, Lu, Sm, and Ta are some lower than of previously reported results (Table 4).

Statistically significant age difference is shown for none of the studied trace elements. It conforms well to the data by Little et al.[32] (Fe, Sr, Zn), Derise and Ritchey[5] (Fe), Nixon et al.[33,34] (Hg, Sb), Brudevold et al.[35] (Zn), and Steadman et al.[36] (Sr). On the contrary the negative Sr-, Zn- and positive Co-, Se-age correlation in teeth have been observed by Derise and Ritchey.[5]

Our data for Co, Eu, Fe, Hg, Sb, Sr, and Zn mass fractions in intact crown of permanent teeth may serve as indicative normal values for male residents of the Russian Central European region.

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