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DETERMINATION OF THE SODIUM-TO-CALCIUM RATIO IN SECTIONS OF UNDECALCIFIED BONE TISSUE BY NEUTRON ACTIVATION ANALYSIS

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The feasibility has been shown of instrumental determination of the Na/Ca ratio with a standard deviation of 4.7% in sections of undecalcified bone tissue made from standard biopsy or autopsy bone samples (about 0.5 mg in weight) embedded in poly(methyl methacrylate) blocks. The determination was based on short-time activation and counting the ^{24}Na and ^{49}Ca radionuclides. Blank values and detection limits of the elements were evaluated and possibilities to detect changes of the Na/Ca ratio are discussed.

INTRODUCTION

In our recent works[1-3], we have obtained interesting results concerning the state of sodium in bone mineral. A brief outline of the use of neutron activation analysis (NAA) for determining various elements (Ca, P, Sr,

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Ba, rare earths, Na, Cl, K, F, Al, Zn, U, As, Ga, I, Mn, Mo, Ru, Se, W, V, Co, Cr, Fe, Hf, Hg, Sb, Sc, Ta, and Th) can be found in our review[4].

The determination of sodium in bone samples by NAA is easy and it is known already from the classical work by Druyan, Mitchell and King[5]. In the present work, we investigated the feasibility of sodium determination in sections of undecalcified bone tissue obtained with the help of a microtome for cutting hard tissues from standard biopsy or autopsy samples embedded in poly(methyl methacrylate) (PMMA). The determination is connected with the following two problems: what unit should the sodium content be related to (the bone tissue mass in the PMMA section can only be estimated), and what will be the detection limit, accuracy and precision of the method, particularly with respect to the matrix effect to PMMA.

EXPERIMENTAL

Six 10 μm sections with the bone tissue and six 10 μm sections of PMMA containing no biological tissue (serving as blanks) were packaged for irradiation in precleaned snap-cap polyethylene (PE) vials. Sodium and calcium standards (15 and 175 μg of the elements, respectively) were prepared by weighing aliquots of solutions of known element concentrations onto precleaned PE disks of 25 mm in diameter. The solutions were carefully evaporated, the residue covered with another PE disk, and heat-sealed. In the same way, about 5 mg of the reference material IAEA Animal Bone H-5 was prepared for irradiation.

The samples and standards were irradiated separately with neutron fluence monitors (a Cu foil of about 1 mg

in weight) in a nuclear reactor LWR-15 of the NRI, Řež, in a thermal neutron flux density of 6×10^{13} n.cm^{-2} s^{-1} for 2 min with the aid of a pneumatic facility. The PMMA sections were removed from their irradiation containers and transferred into clean PE bags for counting. After a 6 min cooling time, activities of the samples and standards were measured with a HPGe coaxial detector (relative efficiency 11%, resolution FWHM 1.70 keV for the 1332.5 keV photons of ^{60}Co) coupled to a Nuclear Data γ-spectrometry system controlled by a PDP 11/73 computer, which has been described in detail elsewhere[6]. Counting time amounted to 10 min and sample distance from the cap of the detector was 2 cm. Both the 1368.6 and 2754.0 keV γ-lines of ^{24}Na and the 3084.5 keV γ-line of ^{49}Ca were used for the respective element quantification.

RESULTS AND DISCUSSION

Sample data and results for the PMMA blanks are given in Table 1. In addition to ^{24}Na, comparable activities of ^{28}Al and ^{38}Cl, and trace activities of ^{41}Ar, ^{56}Mn, ^{60m}Co were also detected in the blanks in the given experimental conditions. Obviously, the sodium content in the blanks in a crucial factor for sodium detection limit in this type of analysis. Information on the origin of sodium content in the PMMA blanks and on possibilities of the sodium blank reduction can be inferred from a comparison of the relative standard deviations of the absolute sodium amounts, S_a, the blank weights, S_w, and the sodium concentrations, S_c. The relative standard deviations, S_a, S_w, and S_c should follow the equation

$$S_c = (S_a^2 - S_w^2)^{1/2},$$

TABLE 1

Data and results for PMMA sections free of bone tissue (blanks)

Blank No.	Blank mass, mg	Ca_b, μg	Na_b, μg	Na_b, μg g^{-1}
1	4.15	<0.24	0.075	18.1
2	3.61	<0.25	0.135	37.4
3	3.79	<0.25	0.052	13.7
4	3.93	<0.25	0.091	23.2
5	4.06	<0.25	0.123	30.3
6	4.19	<0.25	0.082	19.6
Mean	3.96	<0.25	0.093	23.7
relative S.D. (%)	5.7	-	33.3	36.8

if all sodium originates from endogenous, homogeneously distributed sodium in PMMA. However, somewhat larger S_c than S_a was observed in our experiments. This can be explained by sodium inhomogeneity in the PMMA matrix (sections of about 4 mg in weight) or by the presence of sodium external contamination. The latter would be, however, only a minor part of the whole sodium blank value, because the variances S_c^2 and S_a^2 do not differ significantly at the $(1-\alpha) = 0.95$ confidence level. Thus, the blank value cannot be significantly lowered and the sodium detection limit amounts to 0.186 μg (a $x_o \pm 3\sigma_o$ criterion). The calcium detection limit is given by counting statistics only and equals to 0.250 μg (a $3\sigma_o$ criterion) in the given experimental conditions.

Data on bone samples in the PMMA sections, their calcium and sodium contents, and the Na/Ca ratios are shown in Table 2. A weight of the bone samples was

TABLE 2

Data and results for PMMA sections with bone tissue (samples)

Sample No.	Sample mass, mg	Ca_s, µg	Na_s, µg	$Na_{corr.}$*, µg	$Na_{corr.}/Ca_s$
1	4.72	123.2	2.39	2.297	0.0186
2	4.68	105.4	2.14	2.047	0.0194
3	5.25	139.9	2.63	2.537	0.0181
4	4.98	102.1	1.86	1.767	0.0173
5	5.34	110.9	2.09	1.997	0.0180
6	5.38	119.4	2.14	2.047	0.0171
Mean	5.06	116.8	2.21	2.115	0.0181
relative S.D. (%)	6.1	11.9	-	12.6	4.7

* - Values corrected by subtracting the mean Na_b value from each Na_s value.
Subscripts s and b stand for sample and blank, respectively.

estimated from their geometrical parameters and the bone specific density to amount to about 0.5 mg. Considering this sample weight, the mean calcium and sodium concentrations can be calculated to amount to 23.4% and 0.42%, respectively. The former value compares well with the 10.8-35.6% range of calcium values while the latter is at the lower end of the 0.56-1.41% range of sodium compiled by Iyengar et al.[7] for normal human bone. An attempt was also made to demonstrate the accuracy of our results by control analysis of a 5 mg sample of the reference material IAEA H-5 Animal Bone[8]. Our values of 14.9% and 0.375% of calcium and sodium, respectively, are about 25-30% lower compared to the respective certified values of 21.2±0.81% and 0.500±0.028%. However, the certified values are quaran-

teed for sample weights larger than 100 mg, so that the differences observed can be explained by insufficient homogeneity of the reference material. Apparently, for such small bone samples as analyzed in this work no suitable reference material is available with respect to the matrix composition and/or analyte levels. Thus, a direct proof of accuracy cannot be given, but it can be inferred from the accuracy of our INAA procedure which was demonstrated for more complicated matrices elsewhere[6].

Uncertainty of the Na/Ca ratio was also evaluated (rel. S.D. 4.7%, Cf. Table 2) because its knowledge can help in predicting how large differences of the ratio can significantly be distinguished. Let us consider that the same number of test bone samples (with pathologically changed element concentrations) will be analyzed and the Na/Ca ratio will yield approximately the same standard deviation as for the samples analyzed in this work. Under these circumstances, it follows from the t-test that a 7% difference in the Na/Ca ratio can be detected as significant at the $(1-\alpha) = 0.95$ confidence level.

It can be noted that besides the ^{24}Na and ^{49}Ca activities, the radionuclides ^{27}Mg, ^{28}Al, ^{38}Cl, ^{60m}Co, ^{66}Cu, and sometimes ^{52}V were also detected in the bone samples. However, no attempt was made to determine these particular elements, because Al determination is hampered by the $^{31}P(n,\alpha)^{28}Al$ reaction with fast neutrons, and for the remaining elements, except for Cl and Mg, it is not clear if they are bone endogenous or originate from external contamination on cutting the PMMA sections with the microtome.

CONCLUSIONS

The detection limits of the elements, accuracy and precision of the method used were shown to be sufficient for Ca and Na determination in minute bone samples embedded in the PMMA sections, since the mean sodium blank of the PMMA matrix was only about 5% of the actual sodium bone levels and the variation of the blank was not excessive. The method offers a very good possibility to relate the sodium amount in the bone section to the calcium amount. This method of expressing the sodium content can be considered as quite useful, because it permits to give the sodium concentration in the bone mineral, which can be used in the study of heteroionic exchange of Na^+ ions in the bone hydroxyapatite. The method can be particularly valuable with respect to the fact that bone biopsy (autopsy) samples embedded in PMMA blocks are commonly stored at medical institutions. Sections of this material can be used to study the slowly exchangeable sodium under various pathological conditions.

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