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IN VITRO ACTIVATION OF BONE WITH 14 MeV NEUTRONS

P. HOLMBERG, M. HYVÖNEN, M. TARVAINEN

Department of Medical Physics, University of Helsinki
Siltavuorenpenger 10, SF–00170 Helsinki 17 (Finland)

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Samples of compact bone, bone marrow and spongiosa of cow femur have been irradiated in vitro with 14 MeV neutrons. The Ca/P ratio for compact bone was found to be 2.16 ± 0.24. The suitability of using 14 MeV neutrons and the $^{31}P(n, \alpha)^{28}Al$ reaction for studying the bone mineral composition in vitro is discussed.

Introduction

The determination of the bone mineral content, in particular the amount of calcium, is of great medical interest, as a demineralisation process is observed under various physiological conditions such as hormonal imbalance, renal diseases and immobilisation. Much work has therefore been directed to investigating different possibilities for determining the whole-body or partial-body calcium content.

Since it is difficult to observe small changes in the bone mineral content from radiological skeletal surveys alone and since changes up to 30% may still be undetectable by that method, clearly new techniques for more accurate determination of the mineral content are urgently needed.

The technical development has proceeded along two main lines. One technique is based upon the attenuation of photons passing through the area of interest. Although this method is fast and easily applied it has the drawback of not being element selective. The other technique is that of neutron activation analysis, which also provides a method for performing both in vivo and in vitro measurements of the body calcium content.

Since the early work by ANDERSON et al.,[1] there has been a rapid development in the technique for determining the main body elements by neutron activation. ANDERSON et al. made measurements of sodium and chlorine and also pointed out the possibility of determining body calcium by the same technique.

Today several types of neutron sources are available. Very often in this technique the neutron sources produce fast neutrons with a wide range of energies. How-

ever, most investigations use thermal neutrons in the activation process so that the neutrons are deliberately thermalised by a moderator or naturally in the soft tissue surrounding the bone. In the bone, thermal neutrons give rise to the reaction $^{48}Ca(n, \gamma)^{49}Ca$. The abundance of ^{48}Ca in natural calcium is 0.19% and the half-life of the induced ^{49}Ca activity is T = 8.72 min. The β^--decay of ^{49}Ca is followed by a 3084 keV gamma-ray. Although the intensity of this gamma-ray is weak, it can be extracted from NaI-spectra because of its high energy. Neutrons from a cyclotron,[2] 14 MeV neutrons from the D, T reaction[3-5] and neutrons from (α, n) sources[6-8] have been used to determine the calcium content or body elemental composition.

It has also been suggested that the $^{31}P(n, \alpha)^{28}Al$ reaction can be used for determination of the calcium content.[8,10,11] Since the bone mineral hydroxyapatite $Ca_{10}(OH)_2(PO_4)_6$ contains calcium and phosphorus, methods have been developed to measure also the phosphorus content in bone. For example MAZIERE et al.[12] have recently reported a method using neutrons from ^{252}Cf and (α, n) sources for determining the Ca/P ratio by in vivo activation: the amount of calcium was determined using the $^{48}Ca(n_{th}, \gamma)^{49}Ca$ reaction induced by thermal neutrons; phosphorus was determined from the $^{31}P(n, \alpha)^{28}Al$ reaction, achieved with fast neutrons.

Despite the recent developments in neutron activation analysis of biological materials, 14 MeV neutrons are rarely used for activation purposes. Nor are high resolution gamma-ray spectrometers very common in practice. In the present work we therefore report irradiations of bone samples in vitro with 14 MeV neutrons. The resulting gamma-ray spectra were measured with a high energy resolution Ge(Li) detector. In this way we activated several elements. We further discuss the usefulness of the $^{31}P(n, \alpha)^{28}Al$ reaction for determination of the calcium content of bone.

Methods of measurement

In this investigation 14.7 MeV neutrons were produced in a neutron generator through the D,T reaction. A rotating tritium target assembly was used and the neutron production was approximately 10^{10} n · sec^{-1}. The neutron flux Φ at the position of the sample to be irradiated was $\cong 2 \cdot 10^8$ n · cm^{-2} · sec^{-1}.

The samples were irradiated in polystyrene containers, but for the measuring procedure the sample material was transferred to non-activated containers. The gamma-ray spectra were measured with a 110 cm^3 coaxial Ge(Li) semiconductor detector on line with a PDP 9 computer. In this way it was possible to measure and store up to seven consecutive 4096 channel spectra, which were then analysed with the VIPUNEN program[13] in the Burroughs 6700 computer. For the energy

and intensity calibration of the detector set-up IAEA standard sources were used as well as the well known sources of ^{56}Co and ^{152}Eu.[14-16] After the analysis of the spectra, the gamma-rays were assigned to their reactions on the basis of peak-energies and half-life determinations.

The number of observed counts, N_{obs}, in a spectral peak corresponds to the well known formula for neutron activation analyses

$$N_{obs} = \frac{\sigma_A \Phi N_0 m_A \Theta \epsilon f_\gamma}{M_A \lambda} (1 - e^{-\lambda t_i}) (e^{-\lambda t_o} - e^{-\lambda t_f})$$

where
- σ_A – reaction cross-section for nuclide A;
- Φ – neutron flux at the neutron energy 14 MeV;
- N_0 – Avogadro's number;
- m_A – mass of element A;
- Θ – abundance of the nuclide A in the element;
- M_A – atomic weight of element A;
- λ – decay constant of induced activity;
- ϵ – detector efficiency for detecting the gamma-radiation;
- f_γ – gamma fraction in the decay;
- t_i – irradiation time;
- t_o – waiting time;
- t_f – waiting time plus measuring time.

In Table 1 we present some physical properties for the elements and reactions observed in the activation procedures, including prominent gamma-ray energies, intensities and half-lives.[16,17] The cross-section values for the reactions are taken from the measurements and compilations by HOLMBERG, HYVÖNEN and TARVAINEN.[18]

The in vitro measurements of the bone mineral composition have been carried out using fresh cow femurs. Samples of compact bone, bone marrow and spongiosa have been irradiated. The samples were small, with masses varying from 60 mg to 2 g. During some of the irradiations aluminium foils were used as standards, in which case the samples were sandwiched between two aluminium foils.

The irradiation times varied from 1 min to 30 min and the gamma-ray spectra were recorded after waiting times ranging from 1 min to 24 hrs, depending on the half-life of the activity to be determined. Usually four consecutive spectra were recorded, the time of measurement varying from 5 min to 6 hrs.

Table 1
Some phisical properties of the reactions involved in the present work

Reaction	Cross-section, mb	Half-life	Energy (intensity), keV
$^{44}Ca(n, p)^{44}K$	42	22.15 m	1024.7 (0.06) 1126.2 (0.07) 1157.0 (0.58) 1499.1 (0.08) 2151.3 (0.23) 2519.3 (0.08)
$^{44}Ca(n, \alpha)^{41}Ar$	27	1.83 h	1293.6 (0.99)
$^{42}Ca(n, p)^{42}K$	173	12.36 h	1524.7 (0.18)
$^{43}Ca(n, p)^{43}K$	111	22.2 h	372.9 (1.00) 617.6 (0.92)
$^{48}Ca(n, 2n)^{47}Ca$	616	4.55 d	1297.1 (0.76)
$^{31}P(n, \alpha)^{28}Al$	132	2.24 m	1778.9 (1.00)

Results and discussion

In Fig. 1 we show three spectra measured after 14 MeV neutron irradiation of compact bone and one spectrum from bone marrow, and in Fig. 2 we indicate from which part of the cow femur the samples are taken. The first spectrum (A) of compact bone was obtained by irradiating the sample for 10 min. The measurement was started 21 min after the end of the irradiation and lasted for 20 min. In this spectrum the $^{44}Ca(n, p)^{44}K$ reaction is clearly observed. Even the 1778.9 keV gamma-ray line following the $^{31}P(n, \alpha)^{28}Al$ reaction can still be seen, despite of the relatively long waiting time. As the $^{31}P(n, \alpha)^{28}Al$ reaction is very sensitive, small samples and short waiting times can be used if one is interested in the phosphorus reaction only. Strontium in compact bone gives rise to the reaction $^{88}Sr(n, 2n)^{87m}Sr$, which is manifested by the 388.4 keV gamma-ray peak.

The spectrum in part B of Fig. 1 was obtained when the waiting time was 60 min. For this case the gamma-ray peaks from the decays of ^{28}Al and ^{44}K are unobserved or weak, but the long-lived activities due to the $^{42}Ca(n, p)^{42}K$ (T = 12.36 h), the $^{43}Ca(n, p)^{43}K$ (T = 22.2 h) and $^{24}Mg(n, p)^{24}Na$ (T = 15.0 h) reactions are clearly seen. In this spectrum the two strong gamma-transitions following the decay of ^{24}Na appear. The sodium activity originates mainly from the $^{24}Mg(n, p)^{24}Na$ reaction, but the possibility of an interfering $^{23}Na(n, \gamma)^{24}Na$ reaction cannot be rejected even though the sizes of the samples were small.

For still longer waiting times (t_0 = 6 hrs) we obtained the spectrum C, in which the peaks arising from the $^{48}Ca(n, 2n)^{47}Ca$ reaction are observed. The peak at

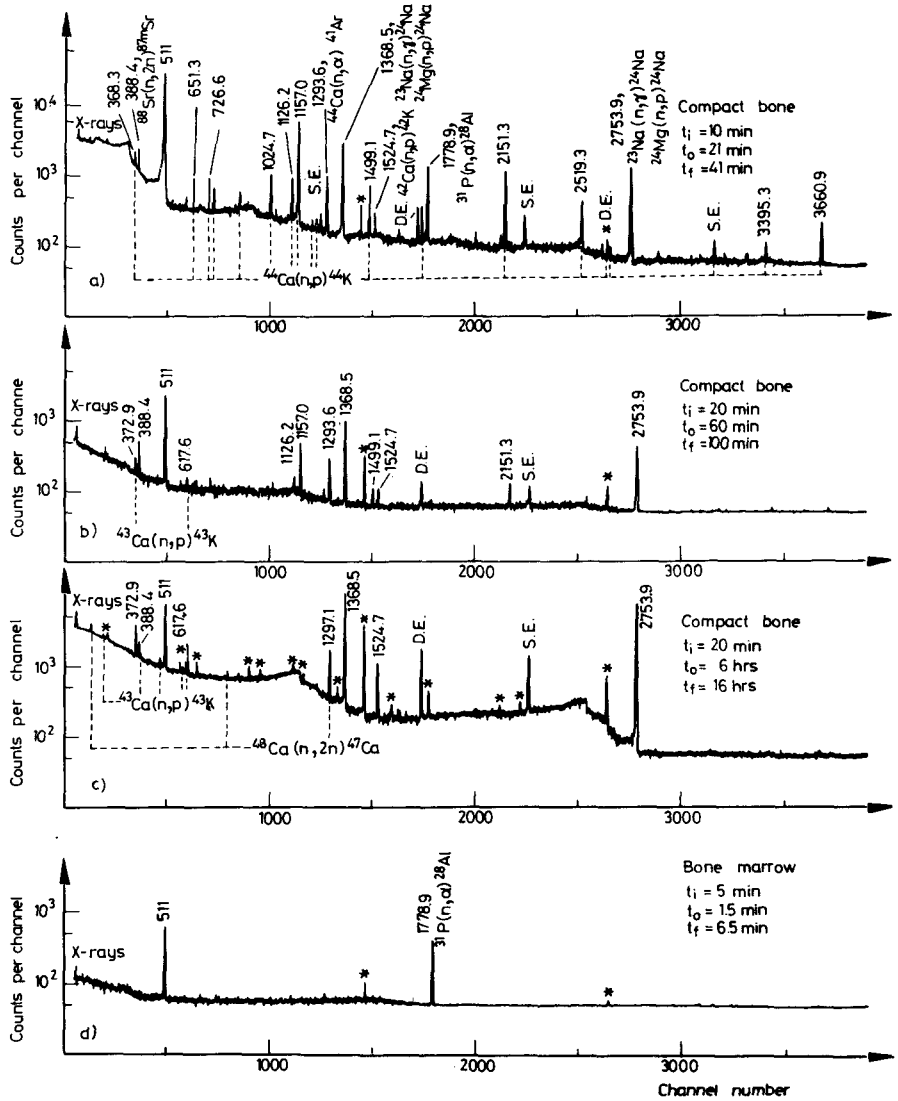

Fig. 1. Gamma-ray spectra measured after 14 MeV neutron irradiation of compact bone and bone marrow of cow femur. Main reactions and gamma-ray energies are indicated in the figures. Asterisks indicate background peaks. A constant of 50 pulses is added to each channel

$E_\gamma = 1297.1$ keV is due totally to the (n, 2n) reaction, as the interfering activity from the $^{44}Ca(n, \alpha)^{41}Ar$ reaction (T = 1.83 h, $E_\gamma = 1293.6$ keV) present in spectra A and B has now fully decayed.

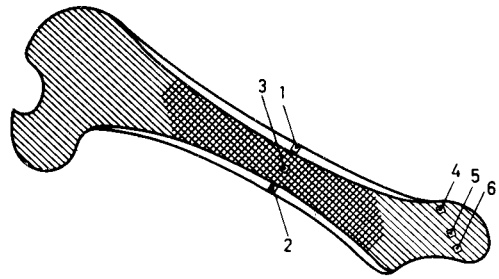

Fig. 2. Cross-section of the cow femur showing the parts from which the samples were taken. Samples: 1, 2 – compact bone, wet mass: 700 mg – 4.5 g; 3 – bone marrow, wet mass: 320 mg – 3.8 g; 4, 5, 6 – spongiosa, wet mass: 67–510 mg

The last spectrum (D) of Fig. 1 shows the activities that arise in a sample of bone marrow after a 5 min irradiation. This spectrum differs drastically from that from compact bone and shows only the peak at $E_\gamma = 1778.9$ keV due to the $^{31}P(n, \alpha)^{28}Al$ reaction.

From several measurements the Ca/P ratio in compact bone was deduced and was found to be 2.16 ± 0.24, which is consistent with the chemical formula for hydroxyapatite and with earlier measurements.[12,19,20]

Also samples from the spongiosa of the femur were irradiated (Fig. 2). The measurements indicate that the amount of phosphorus and calcium decreases, as well as the amount of other minerals found in the femur, as the central part of the spongiosa is approached. In these samples from the spongiosa part phosphorus is easily activated, although the amount of phosphorus is lower than that of compact bone. The amount of calcium in samples 3–6 was too small for an accurate determination of the ratio Ca/P.

For an accurate determination of phosphorus from the gamma-ray energy peak at 1778.9 keV the interfering gamma-ray peak at 1775.5 keV from the $^{44}Ca(n, p)^{44}K$ reaction should be taken into account. The intensity of this interfering gamma-ray line is weak, and moreover depends on the irradiation and measuring times. For short irradiation and waiting times and with small samples the influence is minimal and may be neglected, but in other cases a correction has to be made. This correction may be made through the 1752.9 keV gamma-ray transition which also follows the decay of ^{44}K, since the intensity ratio for the 1775.5 keV and 1752.9 keV gamma-rays is known to be 0.49.[16] The use of the 1752.9 keV gamma-ray transition as a reference line in favour of other strong lines also present in the decay of ^{44}K is dictated by the very small change in detector efficiency in this narrow energy interval.

In the present work we have demonstrated that 14 MeV neutrons can be used for the determination of calcium and phosphorus in bone with the aid of the $^{44}Ca(n, p)^{44}K$ and $^{31}P(n, \alpha)^{28}Al$ reactions, respectively. As the Ca/P ratio for compact bone is known, the calcium content can be deduced indirectly from a determination of the phosphorus. This method has the advantage of not requiring moderator materials, as in the case when the $^{48}Ca(n, \gamma)^{49}Ca$ reaction is employed. Also the half-life of the ^{28}Al activity is short (T = 2.243 m) so that only short irradiation times are necessary.

A further advantage is that since the phosphorus reaction is easily observed, even with small samples, the 14 MeV neutron activation method may be of value when only small biopsy samples are available.

References

1. J. ANDERSON, S. B. OSBORN, R. W. S. TOMLINSON, D. NEWTON, J. RUNDO, J. SALMON, J. W. SMITH, Lancet II (1964) 1201.
2. M. J. CHAMBERLAIN, J. H. FREMLIN, D. K. PETERS, H. PHILIPS, Brit. Med. J., 2 (1968) 581.
3. S. H. COHN, C. S. DOMBROWSKI, R. G. FAIRCHILD, Intern. J. Appl. Radiation Isotopes, 21 (1970) 127.
4. S. H. COHN, C. S. DOMBROWSKI, J. Nucl. Med., 12 (1971) 499.
5. S. H. COHN, C. S. DOMBROWSKI, IEEE Trans. Nucl. Sci., 18 (1971) 73.
6. S. H. COHN, K. K.SHUKLA, C. S. DOMBROWSKI, R. G. FAIRCHILD, J. Nucl.Med., 13 (1972) 487.
7. K. G. McNEILL, B. J. THOMAS, W. C. STURTRIDGE, J. E. HARRISON, J. Nucl. Med., 14 (1973) 501.
8. K. BODDY, D. GLAROS, Intern. J. Appl. Radiation Isotopes, 24 (1973) 179.
9. H. C. BIGGINS, W. D. MORGAN, J. Nucl. Med., 12 (1971) 508.
10. T. NAGAI, I. FUJII, H. MUTO, T. INOUYE, J. Nucl. Med., 10 (1969) 192.
11. J. L. WILLIAMS, L. H. CARGOL, K. G. PAILTHORP, W. B. NELP, J. Nucl. Med., 11 (1970) 576.
12. B. MAZIERE, M. HYVÖNEN, D. COMAR, Proc. Symp. Radioaktive Isotope in Klinik und Forschung, Bad Gastein, Egermann, Vienna, Vol. 12, 1976, p. 767.
13. P. PAATERO, E. TUURA, A. SIIVOLA, Dept. Physics, Univ. Helsinki, Rep. Ser. Phys., No A17 (1973).
14. P. HOLMBERG, Dept. Physics, Univ. Oulu, Rep. No. 34, 1973.
15. A. KIURU, P. HOLMBERG, A. SIIVOLA, Comment. Phys. -Math., 40 (1970) 113.
16. W. W. BOWMANN, K. W. MACMURDO. Atomic Data and Nuclear Data Tables, 13 (1974) 89.
17. G. ERDTMANN, W. SOYKA, Die γ-Linien der Radionuklide, Kernforschungsanlage Jülich, Jul–1003–AC, 1974.
18. P. HOLMBERG, M. HYVÖNEN, M. TARVAINEN, Cross-sections of 14 MeV neutron reactions on phosphorus and calcium. J. Radioanal. Chem., (in the press)
19. G. J. BATRA, D. K. BEWLEY, J. Radioanal. Chem., 16 (1973) 275.
20. ICRP No. 23, Report of the Task Group on Reference Man, W. S. SNYDER, M. J. COOK, E. S. NASSET, L. R. KARHAUSEN, G. PARRY HOWELLS, I. H. TIPTON (Eds), Pergamon, Oxford, 1975.