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ANALYSIS OF SMALL BIOPSY SAMPLES BY NEUTRON ACTIVATION ANALYSIS

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An accurate knowledge of the ratio Ca/P in bone biopsies is very useful in the diagnosis of osteoporosis. Similarly, measurements on muscle biopsies are important in understanding the disturbance of intracellular fluid and electrolyte (Na, Cl, K and P) balance in patients with metabolic myopathies (muscle diseases) and acid-base disturbances including renal failure. The ratio Ca/P in normal subjects was found to be 2.30. Similarly, the ratio K/P was found to be very nearly constant in normal subjects.

Introduction

The object of the present investigation is to present results obtained on small muscle and bone biopsy samples by neutron activation analysis.

Hitherto only large muscle samples, obtained at operation, have been analysed, usually by chemical methods. With the introduction of the technique of needle biopsy for muscle samples (introduced by Bergstrom) it has become possible to obtain small muscle samples (8 – 30 mg wet weight) without a surgical operation and without excessive trauma to the patient.

In the case of bone samples the technique introduced by Van Slooten and Hampe in 1957 is used. The samples obtained by this technique usually weight 8 – 80 mg.

Activation analysis has the distinct advantage over chemical methods of being non-destructive, so that the samples can subsequently be investigated by other methods. For example it is intended to analyse sections for the bone biopsies by the electron probe analyser.

The elements to be analysed are Na, Cl, K and P in muscle biopsies. The information is likely to have diagnostic value.

Measurements on muscle biopsies will further the investigations of disturbance of intracellular fluid and electrolytes balance in patients with metabolic myopathies (muscle diseases) and acid-base disturbances including renal failure. Since chlorine and sodium are essentially extracellular "ions" the chlorine or sodium "space" can be used for the calculation of intracellular concentrations of metabolytes from the total measurements in whole biopsy samples.

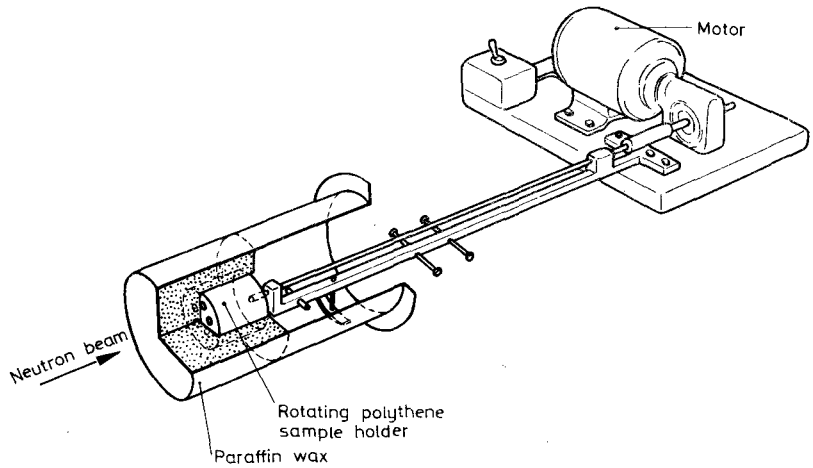

Fig. 1. Scheme of the sample holder

Phosphorus is measured a tissue standard to which other measurements can be related and potassium because of its relation to carbohydrate metabolism and muscle excitability.

In bone biopsies, the ratio Ca/P is thought to be significant in the diagnosis of osteoporosis.

Experimental

Samples and standards

Muscle samples, freeze-dried, dissected to remove connective tissue and weighed, were enclosed in polythene tubes which had been rinsed with deionized water. Anhydrous Na_2HPO_4 and KCl were used as standards.

Similarly, bone samples, dried, fat-extracted and weighed were enclosed in polythene tubes. $CaCO_3$ and Li_3PO_4 were used as standards.

Irradiations

The samples, together with standards were irradiated simultaneously in the neutron beam from the MRC cyclotron. The neutrons, of mean energy 7.5 MeV, are produced by bombarding copper-backed beryllium with 16.7 MeV deuterons. Samples were mounted on a disc of polythene and rotated during irradiation (Fig. 1)

For thermal neutron activation the disc was rotated within a paraffin wax moderator. Fluxes were $5 \cdot 10^{10}$ n · cm^{-2} · sec^{-1} of fast neutrons and 10^9 n · cm^{-2} · sec^{-1} of thermal.

Gamma-ray spectroscopy

After irradiation the samples were counted on a 6" x 4" NaI(Tl) counter couple to a multichannel analyser. Since we had access to the whole body counter we were able to count 5 samples simultaneously. This, indeed, is an asset as one does not have to allow for the decay of the isotopes produced and is particularly valuable in the estimation of phosphorus using 2.3-min ^{28}Al produced by (n, α) reaction on ^{31}P.

Reactions

The following nuclear reactions shown in Table 1 were chosen for the estimation sodium, chlorine, calcium, potassium and phosphorus.

Other interfering nuclear reactions were thoroughly investigated and the interference was found to be negligible.

Fig. 2 shows the gamma-ray spectra taken at 25 min and 4.5 hrs after irradiation for the estimation of sodium and chlorine. As can be seen, the γ-lines at 1.6 and 2.16 MeV corresponding to ^{38}Cl are not present at 4.5 hrs. Thus chlorine can be estimated after a suitable subtraction for sodium from the first spectrum. Sodium can be estimated from the measurement at 4.5 hrs.

Fig. 3 shows the gamma-ray spectrum taken after 2 min decay for the estimation of calcium.

Table 1
Nuclear reactions for the estimation of Na, Cl, Ca, K and P

Reaction	Half-life	E_γ, MeV
^{23}Na(n, γ)^{24}Na	14.96 h	1.37 and 2.76
^{37}Cl(n, γ)^{38}Cl	37.3 m	1.61 and 2.16
^{48}Ca(n, γ)^{49}Ca	8.7 m	3.1
^{41}K(n, p)^{41}Ar	1.83 h	1.293
^{31}P(n, α)^{28}Al	2.31 m	1.78

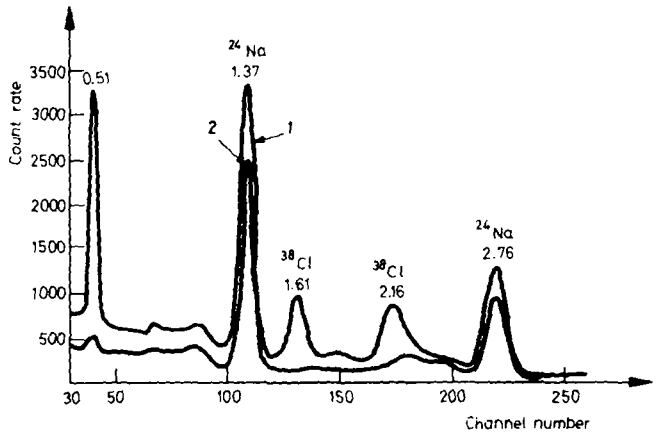

Fig. 2. Gamma-ray spectra taken at 25 min (Curve 1) and 4.5 hrs (Curves 2) after thermal irradiation

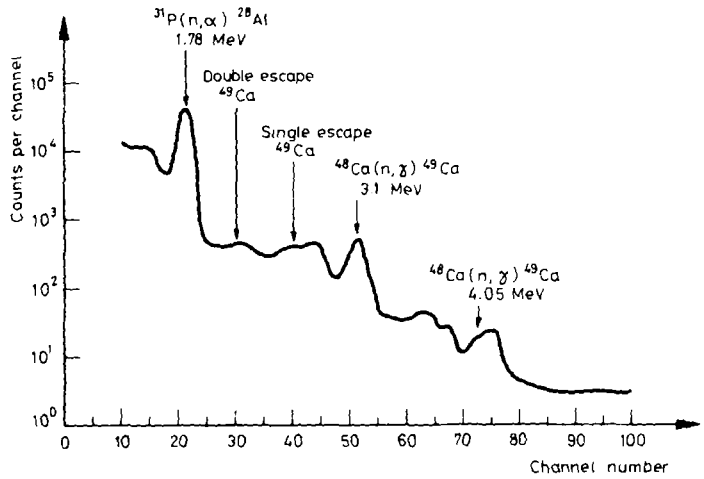

Fig. 3. Gamma-ray spectrum of a bone sample after 2 min decay

In Fig. 4 the upper spectrum was taken after 2 min decay and an irradiation of about 5 min with fast neutrons for the estimation of phosphorus. As is clear from the spectrum, there is no interference in the estimation of phosphorus. The lower spectrum was taken after 25 min decay and an irradiation period of 45 min with fast neutrons for the estimation of potassium in muscle samples. As can be seen from

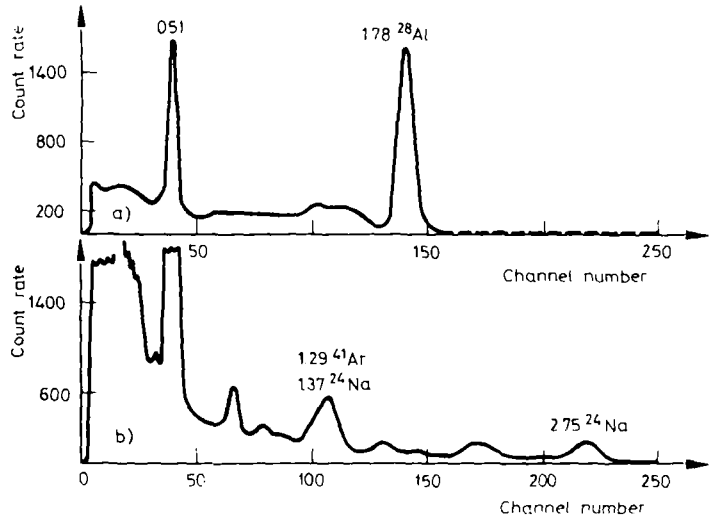

Fig. 4. Gamma-ray spectra of a muscle sample. a – 5 min fast neutron irradiation and prompt counting, b – 45 min fast neutron irradiation and 25 min decay

the spectrum, the 1.293 MeV γ-line of ^{41}Ar produced by (n, p) reaction on ^{41}K cannot be resolved from the 1.37 MeV γ-line of ^{24}Na. But from a previous knowledge of the ratio of the intensities in the photopeaks of 1.37 and 2.76 MeV γ-lines of ^{24}Na, if the number of counts in the photopeak corresponding to 2.76 MeV γ-line are multiplied by a suitable factor and subtracted from the unresolved photopeak of ^{41}Ar and ^{24}Na, one can obtain the total counts corresponding to ^{41}Ar and ^{24}Na, one can obtain the total counts corresponding to ^{41}Ar. This method was found to be quite satisfactory for the estimation of potassium.

Results

Table 2 shows the results obtained for calcium and phosphorus in bone biopsies from five normal subjects obtained at post mortem. As can be seen, the ratio Ca/P is very constant.

Table 3 shows the results obtained for the estimation of sodium, chlorine, potassium and phosphorus in muscle samples. The ratio K/P is very nearly constant.

In conclusion, it can be said that it has been possible to use neutron activation (by neutrons from a cyclotron) for the estimation of Na, Cl, K, P and Ca.

Table 2
Analysis of bone samples

Sample, No.	Weight, mg	Calcium, μg/ mg of bone	Phosphorus μg/mg of bone	Ratio Ca/P
1	36.92	239	103.4	2.31
2	39.61	238	103.5	2.30
2A	20.00	236	103.5	2.28
3	21.52	239	103.0	2.32
3A	30.00	241	103.6	2.32
4	7.54	239	103.50	2.31
4A	46.38	237	103.5	2.29
9	45.24	239	103.5	2.31
9A	53.02	239	103.5	2.31
Mean		238.5	103.44	2.305± 0.030

Table 3
Analysis of muscle samples

No. of samples	Sodium, μg/mg	Chlorine, μg/mg	Potassium, μg/mg	Phosphorus, μg/mg
10	3-4	6-8	16-18	8-10
Mean	3.5	7	17	9

Ratio K/P expressed in milliequivalents: 1.50.