Activation Analysis in Biological and Medical Sciences

The following are summaries of four of the papers presented at a meeting of the Radiochemical Methods Group held on November 21st, 1974, and reported in the January issue of *Proceedings* (p. 7).

Whole Body *In Vivo* Activation Analysis

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The aim of this paper is to give a brief account of the contribution made by neutron-activation analysis to the determination of the body content of certain elements. Interest in determining body composition has been actively pursued only in the past 30 years and the first method adopted was chemical analysis, which of course was not only extremely laborious and time consuming but also of purely academic interest. At about the same time as these chemical analyses were being performed (the early 1950s), the introduction of radioisotopes permitted the measurement by isotopic dilution of an exchangeable proportion of certain body elements. The introduction 10 years ago of *in vivo* neutron-activation analysis allowed for the first time a measurement of the total body content of certain elements on live volunteers, and this time the information was of potential practical use in the management of diseased states. Since 1964, a number of groups of workers have used the technique to determine total body sodium, chlorine, calcium, phosphorus and nitrogen.

Total Body Neutron-activation Analysis (TBNAA)

The determination of the whole body content of sodium, chlorine and calcium by TBNAA depends on the detection of the radionuclides ^{24}Na, ^{38}Cl and ^{49}Ca induced by thermal (n,γ) reactions, and the determination of the whole body content of nitrogen and phosphorus on the detection of the radionuclides ^{13}N and ^{28}Al induced by fast reactions (n, 2n and n,α, respectively). The radioactivity produced by the subject is compared with that produced in a man-like model similarly irradiated. Since the feasibility of the technique was first demonstrated in 1964 by Anderson *et al.*[1] for the determination of sodium and chlorine (and possibly calcium), other groups have applied the basic method to the determination of body elements. These experiments have shown that measurable activity can be produced by an acceptable dose of radiation. All of the techniques currently employed have achieved uniformity of neutron flow throughout the subject and man-like model to varying extents, and involve bilateral irradiations with fast neutrons moderated in the body tissues and surrounding materials to produce the thermal flux.

The reactions that are of interest in the technique are shown in Table I, but for ease of consideration it is probably best to deal with the TBNAA of each element in turn.

TABLE I

NUCLEAR REACTIONS OF INTEREST IN TOTAL BODY NEUTRON-ACTIVATION ANALYSIS

Element of interest		Reaction*	Expected total body content/ g	Target nuclide relative abundance, per cent	Induced radionuclide		
					$T_{\frac{1}{2}}$	γ-ray energies/MeV	
Sodium	D:	$^{23}Na(n,\gamma)^{24}Na$	Na, 105	100	14·9 h	1·38	2·75
	I:	$^{24}Mg(n,p)^{24}Na$	Mg, 35	78·7	14·9 h	1·38	2·75
Chlorine	D:	$^{37}Cl(n,\gamma)^{38}Cl$	Cl, 105	24·47	37·7 min	1·6	2·15
	I:	$^{39}K(n,2n)^{38m}K$	K, 140	93·10	7·7 min		2·16
	I:	$^{41}K(n,\alpha)^{38}Cl$	K, 140	6·88	37·7 min	1·6	2·15
	I:	$^{35}Cl(n,2n)^{34m}Cl$	Cl, 105	75·53	32·5 min		2·13
	I:	$^{35}Cl(n,\gamma)^{36}Cl$	Cl, 105	75·53	3×10^5 yr	Possible flux depression	
Calcium	D:	$^{48}Ca(n,\gamma)^{49}Ca$	Ca, 1050	0·18	8·75 min		3·07
	I:	$^{37}Cl(n,p)^{37}S$	Cl, 105	24·47	5·0 min		3·10
	D:	$^{40}Ca(n,\alpha)^{37}Ar$	Ca, 1050	96·97	35·1 d	Auger electrons	
	D:	$^{44}Ca(n,\alpha)^{41}Ar$	Ca, 1050	2·06	1·82 h	1·29	
	I:	$^{41}K(n,p)^{41}Ar$	K, 140	6·88	1·82 h	1·29	
Phosphorus	D:	$^{31}P(n,\alpha)^{28}Al$	P, 700	100	2·31 min	1·79	
	I:	$^{28}Si(n,p)^{28}Al$	Si, <5	92·21	2·31 min	1·79	
Nitrogen	D:	$^{14}N(n,2n)^{13}N$	N, 2100	99·63	10·08 min	0·51 from β^+	
	I:	$^{31}P(n,2n)^{30}P$	P, 700	100	2·56 min	0·51 from β^+	
	I:	$^{16}O(p,\alpha)^{13}N$	O, 45 500	99·76	10·08 min	0·51 from β^+	
	D:	$^{14}N(n,\gamma)^{15}N$	N, 2100	99·63			10·8

*D = Desired reaction; I = possible interfering reaction.

Sodium

In the first investigation by Anderson *et al.*[1] using essentially 14-MeV incident neutrons, two subjects were irradiated while lying on a couch approximating to an arc of a circle of 1·1 m radius centred on the target of a Cockcroft - Walton accelerator. The subjects and the man-like model were enclosed by polyethylene sheets and polyethylene bottles filled with water were placed beside the legs so as to reduce the loss of slow neutrons. The subjects were given a bilateral and the man-like model a unilateral irradiation. The phantom contained 105 g of sodium and 105 g of chlorine. The induced radioactivity was measured in a whole body counter, the duration of the count being 35 min. Further measurements were made at about 5 h (when ^{38}Cl had decayed to about 0·3 per cent.) and at 24 h.

The results of this experiment showed an average whole body sodium content in healthy adult males of 73·3 g per 70 kg body weight. In the light of subsequent experiments,[2] this

sodium value could be reduced by 0·102 g for each gram of magnesium present in the subject (on the assumption of 35 g of magnesium per 70 kg body weight) produced by the reaction $^{24}Mg(n,p)^{24}Na$. The revised value for sodium then becomes 69·9 g per 70 kg body weight. The 24-h exchangeable sodium on the same two subjects gave a mean value of 74·8 g per 70 kg body weight. The results further suggested that the then accepted total body content of sodium of 105 g had been over-estimated by chemical analysis and hence there was a much smaller difference between total and exchangeable components (at the time it seemed from these results as if there was no difference).

The smaller value for total body sodium obtained by TBNAA than that previously held was confirmed by the Birmingham group in 1968, who used a cyclotron as a neutron source.[3] The resulting neutron spectrum was continuous from 0·1 to 8·0 MeV with a peak of 3·5 MeV. This choice of neutron energy avoided any serious interfering reactions. They also measured exchangeable sodium and found that in this and further experiments 19 $\pm$ 4 per cent. of total body sodium was non-exchangeable.

Subsequent work by the King's College group[4] on six male volunteers and the Seattle group[5] on five male volunteers, together with various measurements in diseased states by the Brookhaven group, has shown that total body sodium by TBNAA is about 80 g per 70 kg body weight, of which in normal adults up to about 25 per cent. may be exchangeable.

Chlorine

The total body content of chlorine is determined from the reaction $^{37}Cl(n,\gamma)^{38}Cl$. The possible contribution of the 2·16-MeV γ-ray from ^{38m}K from the reaction $^{39}K(n,2n)^{38m}K$ on the 2·15-MeV γ-ray from ^{38}Cl was allowed for by preferential decay, but with a resulting loss of statistical accuracy of counting, when the first experiment was performed in 1964 by Anderson *et al.*[1] As with sodium, however, the estimated chlorine content of 73·5 g per 70 kg body weight was well below the accepted value of 105 g.

The possible interfering reactions were subsequently shown by Battye *et al.*[2] to contribute only a few per cent. to the activity from the desired reaction when using neutrons of 14-MeV energy. The contribution of interfering reactions can be minimised even further by using incident neutrons of energy lower than 14 MeV.

The TBNAA of chlorine does not appear to have been so popular as the TBNAA of other elements—an indication not so much of difficulties with this particular element (although some difficulties may exist) but rather the lack of knowledge of the role of chloride in diseased states. Values for total body chlorine that have been reported for various diseased states have ranged from 76·0 to 92·4 g per 70 kg body weight.[6,7]

Calcium

A peak at about 3·1 MeV was seen in the post-irradiation spectra of the two subjects irradiated in 1964 by Anderson *et al.*[1] This peak was attributed to ^{49}Ca from the reaction $^{48}Ca(n,\gamma)^{49}Ca$. Although the irradiated man-like phantom on this occasion contained no calcium to serve as a standard, the calcium content of each subject was derived by comparison with the sodium content and its induced ^{24}Na. The two values obtained for total body calcium were 1330 and 1090 g per 70 kg body weight.

The possibility of using TBNAA for the determination of total body calcium content aroused particular clinical interest because it would provide a direct method of ascertaining the progress of treatment on various bone diseases. The isotope dilution technique, using ^{45}Ca and/or ^{47}Ca, had not found particular favour because of the lengthy course of sampling involved and the controversial compartmental analysis necessary to elucidate the results. Calcium balance procedures are also lengthy and expensive and the analyses are laborious. Radiography is relatively insensitive in detecting changes in calcification, 25–30 per cent. demineralisation being necessary to produce clear changes in the X-ray pictures.

With 14-MeV neutrons there is an interfering reaction, namely the production of ^{37}S with a half-life of 5·0 min and a γ-ray of energy 3·10 MeV, both close to the values for ^{49}Ca of 8·75 min and 3·07 MeV, respectively. The contribution of the ^{37}S activity, however, is only a few per cent. of the calcium count and can easily be allowed for.

Three groups of workers at Birmingham, Seattle and Brookhaven have carried out extensive studies on patients with various bone diseases. The Birmingham group have validated the

relevance of sequential whole body calcium measurements in a number of conditions, and have stated that the method can be applied with confidence to conditions in which the direction of change is unpredictable, such as renal dialysis.[8]

The American groups have also published extensively on calcium determinations by TBNAA, and express their results in absolute terms of calcium. The mean total body calcium of eight normal men was 1093 g per 70 kg body weight,[9] which for this element agrees favourably with the ICRP value of 1050 g per 70 kg.[10]

An interesting recent development in the TBNAA of calcium has arisen from a feasibility study reported by Palmer[11] of measuring ^{37}Ar in expired air. The ^{37}Ar is produced by the reaction $^{40}Ca(n,\alpha)^{37}Ar$, and is assayed in preference to the ^{41}Ar from the reaction $^{44}Ca(n,\alpha)^{41}Ar$ because of the interference from the reaction $^{41}K(n,p)^{41}Ar$. This development is considered in the following paper.[12]

Phosphorus

Fast neutrons will interact with phosphorus to produce ^{28}Al by the reaction $^{31}P(n,\alpha)^{28}Al$. The ^{28}Al has a half-life of 2·30 min and emits a 1·78-MeV γ-ray for each disintegration.

The reaction has been used to measure the total body content of phosphorus in small animals where uniformity of flux can be assured throughout the body thickness, and in humans where, however, the use of the method is more difficult because of the larger body size.

The values that have been published for humans[6,13] were low compared with the values given for standard man, but whether this was due to the method or the diseased state of the patients is not certain. At present, the main interest in total body phosphorus measurements arises from the possibility of its use as a substitute for calcium in determining total body bone mass. However, as only about 66–75 per cent. of the total body phosphorus resides in the skeleton compared with 99 per cent. of the total body calcium, it will never be as good an indicator of bone mass as calcium.

Nitrogen

The first method for measuring nitrogen in humans[6] used 14-MeV neutrons to induce the reaction $^{14}N(n,2n)^{13}N$. The ^{13}N has a half-life of 10·08 min and emits two 0·51-MeV annihilation photons per disintegration.

The counting of ^{13}N annihilation photons has interference from ^{30}P and some ^{13}N is produced from oxygen by the reaction $^{16}O(p,\alpha)^{13}N$. This reaction with oxygen is produced by protons formed from high-energy neutron collisions with hydrogen. From studies by Palmer and Nelp[14] on beef tissue, less than 10 per cent. of the ^{13}N will be produced from oxygen in the body but a correction for this interference must be made.

A more recent method developed by the Birmingham group[8] measures the prompt 10·8-MeV γ-ray emitted when ^{14}N captures a thermal neutron. Upon thermal neutron capture by ^{14}N in the reaction $^{14}N(n,\gamma)^{15}N$, about 15 per cent. of the excited ^{15}N nuclei decay directly, emitting 10·8-MeV γ-rays. As the γ-rays resulting from thermal neutron capture in the major body elements are mostly of energies below 7 MeV, it is possible to measure the body nitrogen content by monitoring the 10·8-MeV γ-rays with a large sodium iodide crystal spectrometer. The irradiation and counting are carried out during alternating short periods while the patient remains in a fixed position. The neutrons from the cyclotron are pulsed in repetitive cycles consisting of irradiation for 10 μs followed by an interval of 140 μs. After the neutrons are turned off, the counting is performed during the next few hundred microseconds as the neutrons are thermalised and captured within the body.

Sequential studies of patients to determine the magnitude and rate of change of body nitrogen have been undertaken using this reaction, although determination of the absolute value is not yet possible.

The only published results for total body nitrogen in humans are those of Cohn and Dombrowski and, like the phosphorus results, were obtained in conjunction with total body calcium, sodium and chlorine measurements. The uniformity for nitrogen activation was poor as irradiation conditions were optimised for thermal neutron activation. The total body nitrogen results[6,13] were high when compared with ICRP values for standard man.

To summarise, a number of centres are now actively pursuing the technique of TBNAA since it was introduced 10 years ago. It has found application in clinical investigations of many

diseased states, where it has been used for the determination of total body content of sodium, chlorine, calcium, phosphorus and nitrogen. Modifications and improvements in the technique are still being implemented and may reduce the irradiation dose necessary for the determination of the major body elements.

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Argonaut: An (n,α) Reaction Used to Measure Calcium *In Vivo*

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Ten years ago, Anderson *et al.*[1] reported on the use of *in vivo* neutron-activation analysis (IVNAA) as a means of determining elemental concentrations in living subjects. Calcium was one of the elements which they suggested might be measured in this way. Following this suggestion, several laboratories developed systems capable of monitoring human calcium levels.[2–4] These systems made use of the thermal neutron reaction $^{48}Ca(n,\gamma)^{49}Ca$, and detected the 3·07-MeV γ-ray emitted from the decay of the ^{49}Ca with a half-life of 8·7 min.

More recently, Palmer and co-workers[5,6] investigated the feasibility of using the reaction $^{40}Ca(n,\alpha)^{37}Ar$ to measure calcium. This paper describes an IVNAA system that has been established at Birmingham to use this reaction for calcium measurements. The ^{37}Ar decays with a half-life of 34·3 d, emitting 2·62-keV Auger electrons and X-rays. As the energy is low, the ^{37}Ar cannot be counted inside the body but, being chemically inert, it is easily released from the body and exhaled.

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The method employed can be conveniently divided into four phases. Firstly, the subject is prepared for irradiation by being given pure oxygen to breathe, which has the effect of depleting the nitrogen concentration in the subject's lungs. The irradiation is carried out with neutrons produced by the reaction $^{9}Be(^{3}He,n)^{11}C$. The ^{3}He particles are accelerated to 30 MeV in the 60-in Nuffield cyclotron.

The second aspect of the method is the collection of the exhaled gases from the subject. This starts at the beginning of the irradiation and continues for some time. The subject, whether animal or human, is in a closed breathing circuit that can be sampled periodically.

TABLE I

REPRODUCIBILITY FOR HAMSTER

Run No.	Flux monitor counts	^{37}Ar counts	$\frac{^{37}Ar \text{ counts}}{\text{monitor counts}}$
1	121 158	16 409	0·1354
2	145 575	19 064	0·1309
3	119 102	16 019	0·1344
4	131 584	16 769	0·1271
5	133 683	17 488	0·1308
	Mean		0·132 ± 0·003
	Reproducibility		2·25%

Thirdly, the exhaled gases must be purified so as to leave only the various isotopes of argon and possibly trace amounts of other inert gases. This is necessary because any electronegative gas impurities will impair the counting of the ^{37}Ar. A sample is stored on a cooled activated charcoal trap, and is slowly released through a furnace containing 7·5 kg of copper turnings heated to 540 °C, a molecular sieve (type 3A) is used as a drying agent and any residual carbon dioxide or water is removed in the cold trap and the phosphorus pentoxide trap. A second furnace containing copper removes trace amounts of oxygen. Finally, the nitrogen, already depleted by the use of pure oxygen as a breathing gas, is removed in a furnace containing about 150 g of calcium turnings heated to 600 °C.

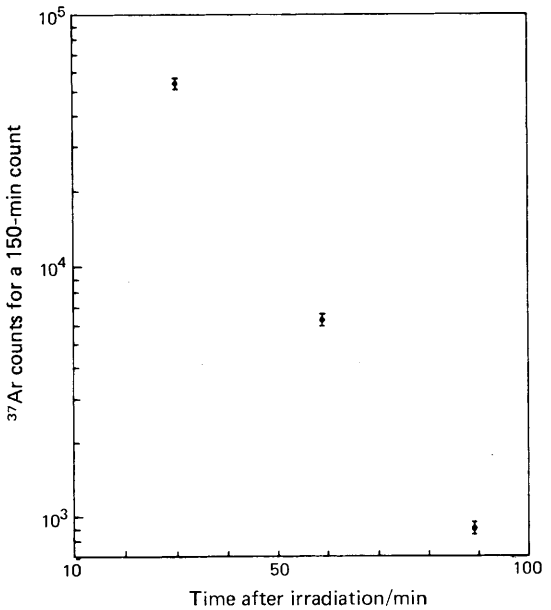

Fig. 1. Exhalation graph for rabbit.

The final part of the method is the counting of the ^{37}Ar. The now purified sample is counted internally in a gas proportional counter. A 9+1 argon - methane mixture is used as a filling gas in the counter, which is, typically, filled to a pressure of 560 torr, and carries a potential of 2500 V on its anode wire.

The reproducibility of the system has been investigated by performing measurements on the same mature hamster five times during a period of 10 d. The results of these measurements, which had a coefficient of variation of 2·25 per cent. are summarised in Table I. The rate at which the ^{37}Ar is exhaled from the body has been measured for a rabbit and a man. The rabbit's exhalation graph is shown in Fig. 1. These data are consistent with an exponential exhalation with a half-life of about 20 min. Fig. 2(*a*) shows the over-all human exhalation graph and Fig. 2(*b*) shows the same data plotted on an expanded time scale. An exponential fitted to the first five data points has a half-life of 21 min, while the longer lived component could represent either a second exponential with a half-life of the order of 20 h or a nearly constant level with respect to the 36 h covered by the experiment.

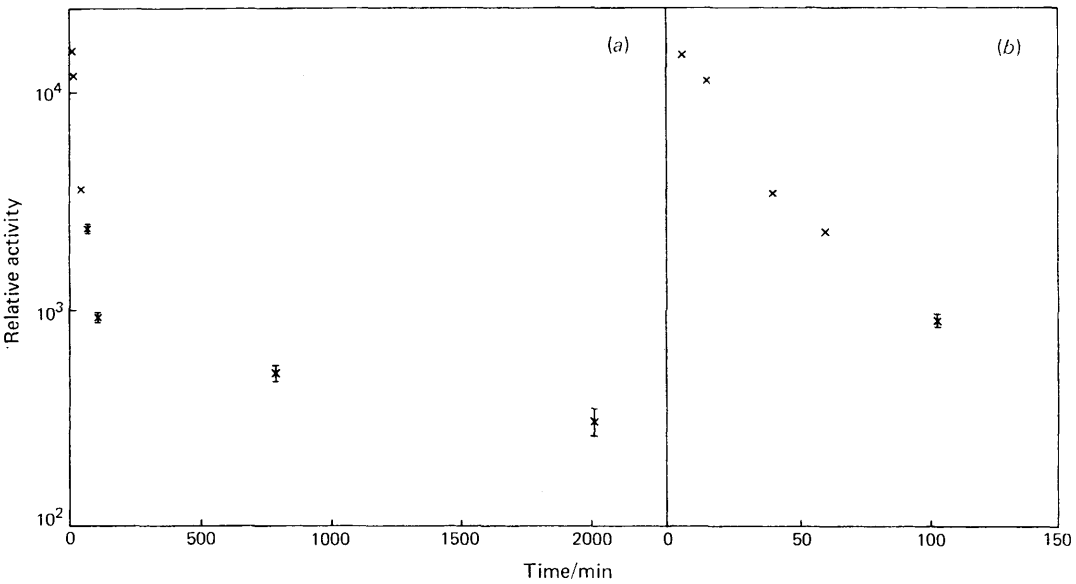

Fig. 2. Human exhalation graph. (*a*) J.H.F., 2 d; and (*b*) J.H.F., 2 h.

This technique can usefully be compared with the already established $^{48}Ca(n,\gamma)^{49}Ca$ technique. Fig. 3 summarises the nuclear properties associated with the two techniques. It can be seen that ^{40}Ca is much more abundant than ^{48}Ca. The cross-sections for the two reactions are not directly comparable. The 0·1-b cross-section quoted for the $^{40}Ca(n,\alpha)^{37}Ar$ reaction is an estimate of the effective cross-section for the incident neutron spectrum, taking into account the variation of cross-section with energy. The thermal neutron capture cross-section is quoted for the $^{48}Ca(n,\gamma)^{49}Ca$ reaction but the effective cross-section for any non-

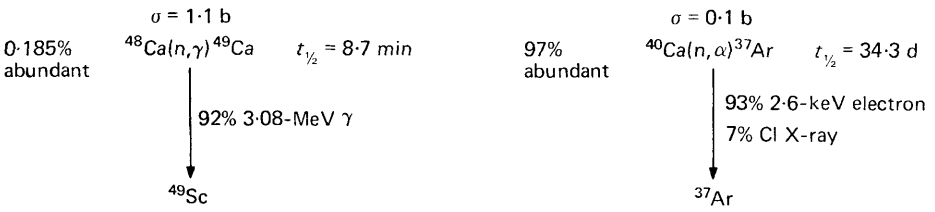

Fig. 3. Nuclear properties associated with the two techniques.

thermal incident neutron beam must be less than 1·1 b. The long half-life of ^{37}Ar means that it is impracticable to count more than 2 per cent. of the induced activity, while, allowing for transfer of the subject from irradiation area to counting area, about 40 per cent. of the induced activity of ^{49}Ca can be monitored. The over-all detection efficiency for ^{37}Ar depends only on the efficiency of transfer of the ^{37}Ar from the body to the counter, because once in the counter it will be detected with effectively 100 per cent. efficiency. The efficiency for the detection of ^{49}Ca depends on the intrinsic photopeak efficiency of the sodium iodide scintillator and on the geometrical arrangement. These two factors give a combined efficiency of about 1·5 per cent. for an array of 54 6- $\times$ 2-in crystals.[7] Taking all the factors together and assuming that 50 per cent. of the ^{37}Ar is collected and that it is counted for 12 h, the ^{37}Ar method should require a dose 40 times less than the ^{48}Ca method in order to obtain statistically similar information.

Another aspect worthy of comment is the shape of the exhalation curve. This contains information on the transport of the ^{37}Ar out of the body and it is possible that this information could be usefully interpreted. If the long-lived component represents a substantial proportion of the total induced activity, absolute measurements of total body calcium may be difficult to obtain, but this should not impair the usefulness of sequential measurements of calcium on the same patient.

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