FAST NEUTRON ACTIVATION ANALYSIS OF THE MAJOR BODY ELEMENTS

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Most of the reported work in the field of in vivo neutron activation analysis has taken advantage of the fact that the bulk elements of the body, namely oxygen, nitrogen, carbon, and hydrogen, do not readily undergo reactions with thermal neutrons (which would lead to unwanted radioactivity), thereby enabling some of the less abundant elements such as calcium, sodium, chlorine, and iodine to be detected. Far less has been published on total-body measurements of the four major elements. Rundo and Bunce (*1*) have reported a method of total-body hydrogen determination by measuring slow neutron capture gamma rays, while Palmer, Jenkins, and McCall (*2*) have shown that a measurable amount of ^{11}C is induced in the body by a relatively low dose of high-energy bremsstrahlung radiation. Palmer, Nelp, Murano, and Rich (*3*) have measured nitrogen in a tissue-equivalent phantom, and Nagai, Fujii, Muto, and Inouye (*4*), using 14-MeV neutrons to produce the positron-emitting ^{13}N in the reaction $^{14}N(n,2n)^{13}N$, have found a very accurate value for the nitrogen content of mice which was confirmed by chemical assay.

The experiments reported here parallel the work of Nagai and his associates in so far as total-body nitrogen in mice has been determined both by counting the annihilation radiation from ^{13}N and by chemical analysis. However, neutrons of energy up to 37 MeV have been used in an attempt to estimate total-body oxygen, nitrogen, and carbon simultaneously by following the decay of the positron emitters ^{15}O, ^{13}N, and ^{11}C. These are formed by n,2n reactions on ^{16}O, ^{14}N, and ^{12}C, respectively, the threshold energies for these reactions being given in Table 1 which also shows three positron emitters which interfere with the nuclides of interest because of similarities in half-lives. After analysis of the complex decay of the annihilation radiation, the amount of each element was determined by comparison with a known standard, and corrections for the interferences were applied.

TABLE 1. THREE MAJOR REACTIONS AND THEIR PRINCIPAL INTERFERENCES

Reaction	Neutron threshold energy (MeV)	Half-life (min)
$^{16}O(n,2n)^{15}O$	16.7	2.1
$^{31}P(n,2n)^{30}P$	12.6	2.5
$^{14}N(n,2n)^{13}N$	11.3	10.0
$^{39}K(n,2n)^{38}K$	13.4	7.7
$^{12}C(n,2n)^{11}C$	20.2	20.3
$^{35}Cl(n,2n)^{34m}Cl$	13.1	32.0

METHODS

Ten CBA mice, freshly sacrificed for ease of handling, and weighing between 4.9 and 32.0 gm, were irradiated in polyethylene vials with fast neutrons produced by the $^{9}Be(^{3}He,n)^{11}C$ reaction on the Nuffield 152-cm cyclotron, University of Birmingham. The bombardment of a thick beryllium target with 30-MeV ^{3}He ions gives neutrons in the energy range 0 to 37 MeV along the beam axis, which allows the production of ^{15}O, ^{13}N, and ^{11}C (Table 1). The neutron yield from this target is 2×10^{9} neutron/sec/μA with an average energy of about 10 MeV (*5*). Assuming an RBE of 10 for the neutron flux, the dose rate as measured by neutron film badges is 100 rem/min/μA at the irradiation distance of 10 cm. The irradiation time was 50 min, which allows saturation of the ^{15}O and ^{13}N activities and more than four-fifths of the activity available in ^{11}C with a beam current of 4 μA. Figure 1 shows the buildup of activities in the three nuclides which follows the $1 - e^{-\lambda t}$ law.

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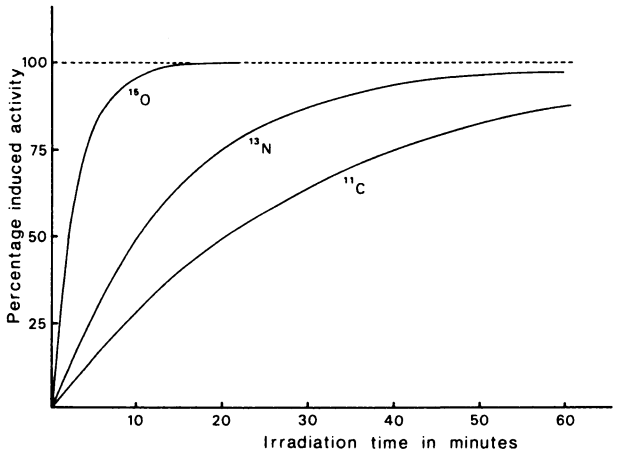

FIG. 1. Buildup of activity in three positron emitters as function of irradiation time.

TABLE 2. CHEMICAL AND ELEMENTAL COMPOSITION OF STANDARD PHANTOM

Component	Weight (gm)	Element	% by weight
Water	12.81	Oxygen	70.3
Glycerol	6.40	Carbon	15.1
Urea	1.61	Hydrogen	9.5
Sucrose	1.57	Nitrogen	3.25
Potassium phosphate	0.188	Sodium	0.64
Sodium phosphate	0.458	Phosphorus	0.58
Potassium chloride	0.055	Potassium	0.49
		Chlorine	0.11

A beam current integrator and a standard of each element were required. In a system with half-lives differing by such large amounts, a conventional electronic beam current integrator would have been of limited use because changes in current at different times in the irradiation would have different effects on the ratio of the final activities produced in the three nuclides. As a result, a chemically tissue-equivalent liquid (Table 2) in a polyethylene vial was irradiated simultaneously with each mouse and served as the standard phantom. Since the amount of each element in the mouse was derived from a ratio of mouse counts to standard counts, the result was independent of beam fluctuation. By making the standard tissue equivalent, the best simulation of the neutron flux through the mouse was obtained and the accuracy of the determinations increased.

Another consideration of importance is that of uniformity of activation and detection. Even across the comparatively small width of a mouse there will be absorption of the neutron flux so that the rear side will not be activated to the same extent as that facing the target. If the elements of interest are uniformly distributed through the specimen this is not so important because rotation of the sample during counting provides an average value. In the case of carbon, however, which is not evenly distributed in the body, (ICRP 1959 shows that 60% of total-body carbon is in 14% of the body) uniformity of activation does become important. An experiment was therefore conducted to assess the uniformity of activation of carbon pellets placed at various depths in a stack of 25 1-cm-thick polyethylene sheets. This gave a relaxation length through the phantom of 35 ± 5 cm. In other words, there would be only a 5% drop in carbon activation through the thickness of a typical mouse. It was not felt that this justified rotation of the sample during activation although such a facility would of course be beneficial.

After irradiation, the mouse and standard were transferred to nonactive polyethylene vials, and a 5-in. NaI(Tl) crystal linked to a Northern NS600 512-channel analyzer measured the whole-body induced gamma radiation. A delay of 45 sec between irradiation and counting allowed the annihilation radiation from ^{39}Ca and ^{31}S with half-lives of 0.87 and 2.7 sec, respectively, to die away. Although a Ge(Li) detector would have given better energy resolution, it was felt that detection efficiency was the important consideration. Furthermore, the use of a NaI(Tl) crystal facilitated extrapolation of the results to human patients by comparison with an existing whole-body counting system (*6*). However, a Ge(Li) detector was employed for a section of the experiment as is described later.

As discussed previously, the vials were rotated at 20 rpm about the vertical axis on a small turntable in front of the crystal. The mouse and standard were counted for 30 sec alternately each minute, for a total time of 1 hr. This method allowed several counts of each vial over the initially high decay, while leaving adequate time for printout of the 0.51-MeV channels of interest. The 0.51-MeV decay of a mouse-standard pair is shown in Fig. 2. One-minute counts and complete spectra of mouse and standard were obtained after 5.25 and 10.25 min, respectively.

In addition, four mice with tissue-equivalent standards were irradiated with 14-MeV neutrons produced by the T(d,n)^{4}He reaction. The 2.2-MeV deuteron beam of the Nuffield cyclotron was degraded in a 15.5-micron copper foil located in front of the tritium target. This reduced the deuteron energy sufficiently to take advantage of the (d,n) cross-section resonance which occurs at an energy of 110 keV, and hence the neutron flux was maximized and stabilized in energy. A beam current of 20 μA and an irradiation time of 30 min were used with the counting arrange-

ment as previously described. Nitrogen and potassium phantoms were also irradiated and a correction factor found for the potassium interference.

After measurement, four of the mice were homogenized in distilled water, aliquots being used in subsequent chemical analysis—the Kjeldahl method for nitrogen and the flame photometer method for potassium.

ANALYSIS OF DATA

The percentage by weight of oxygen, nitrogen, and carbon in each mouse was determined by analysis of the decay of the annihilation radiation on an IBM 360 computer. The programming involved a multiexponential least-squares fit to the decay of the 0.51-MeV peak to obtain a comparison between the counting rates at time zero of mouse and standard for the three elements and a test of significance on the result. The basic equation used to obtain the elemental percentage by weight in the mouse is

$$\%A = \frac{A_m}{A_s} \times \frac{W_s^A}{W},$$

in which A_m and A_s are corrected counting rates at time zero of element A in the mouse and standard, respectively, W_s^A is the weight of element A in the standard vial, and W is the weight of the mouse. Because of similarities in half-lives, the computer analysis could not distinguish between counts from oxygen and phosphorus, nitrogen and potassium, nor carbon and chlorine, and so correction factors were derived from other features of the radiations from the interfering nuclides.

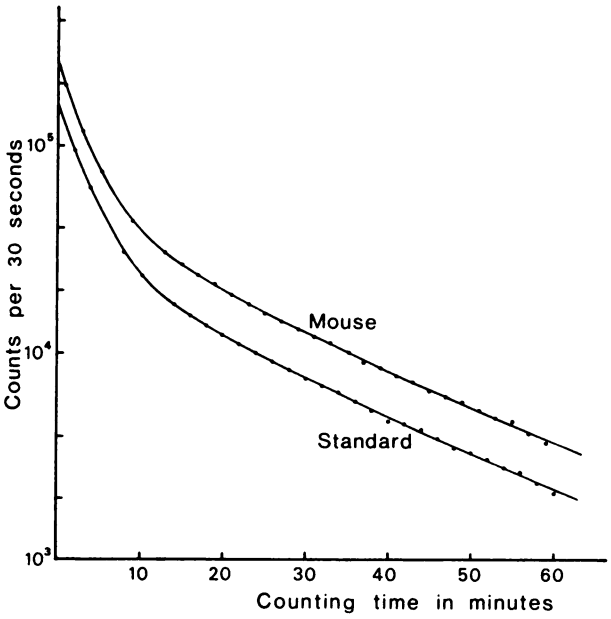

FIG. 2. Decay of annihilation peak in mouse-standard pair.

The gamma spectra from the activated mice showed prominent peaks at 1.78, 2.15, and 2.75 MeV besides the annihilation peak which is mainly the result of n,2n reactions on ^{16}O, ^{14}N, and ^{12}C giving ^{15}O, ^{13}N, and ^{11}C, although the positron decays of ^{30}P, ^{38}K, and ^{34m}Cl also contribute. A typical spectrum is reproduced in Fig. 3. Although the moderation of fast neutrons in animals of this size is a small effect, the 2.75-MeV gamma ray is observed from the $^{23}Na(n,\gamma)^{24}Na$ reaction because the neutron energy spectrum is continuous down to zero energy. The 1.78-MeV line is attributed to ^{28}Al (2.3 min) which is produced in the $^{31}P(n,\alpha)^{28}Al$ reaction. The diffuse peak around 2.15 MeV was expected to contain

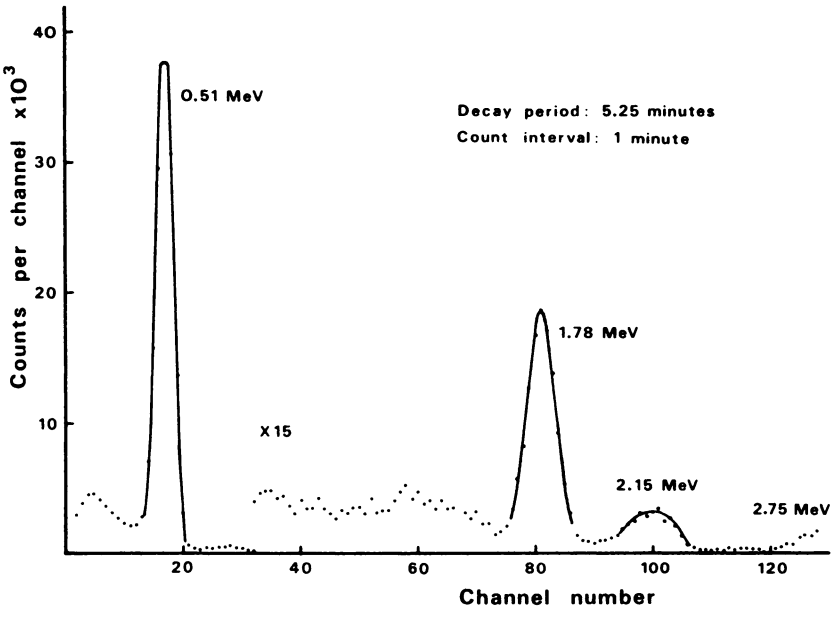

FIG. 3. Gamma-ray spectrum from irradiated mouse showing prominent peaks used in analysis.

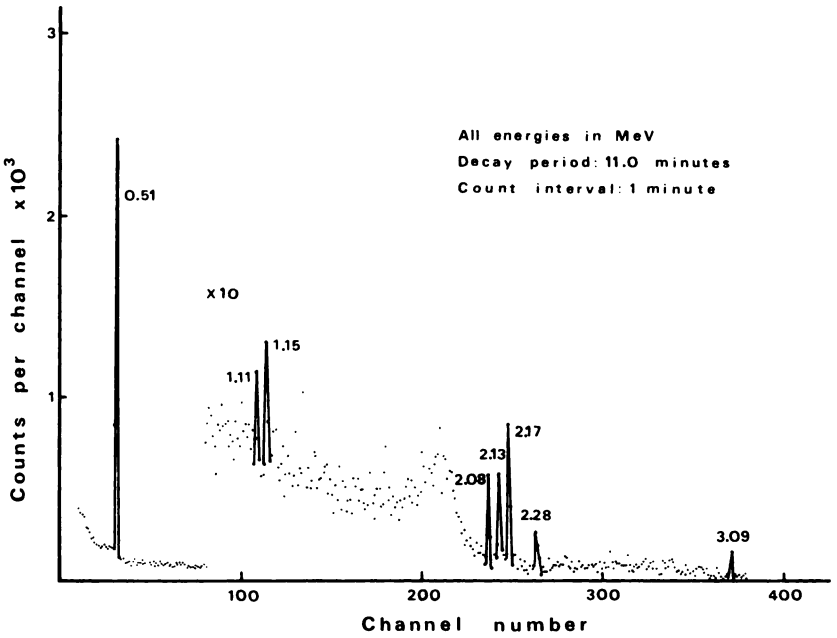

FIG. 4. Gamma-ray spectrum from KCl sample using Ge(Li) detector to investigate high-energy potassium-chlorine complex.

two distinct gamma rays produced by three reactions. ^{38}K (7.7 min) formed by the n,2n reaction on ^{39}K gives a component at 2.17 MeV as does ^{38}Cl (37.3 min) from the $^{37}Cl(n,\gamma)^{38}Cl$ reaction, and a component at 2.13 MeV arises from $^{35}Cl(n,2n)^{34m}Cl$ (32.0 min). A 7.0-cc Ge(Li) detector was used to determine the structure of this high-energy complex, and additional peaks at 2.08 and 2.28 MeV were found to be present besides the two aforementioned. These are the second escape peaks from full-energy gamma rays at 3.09 and 3.30 MeV from $^{37}Cl(n,p)^{37}S$ and $^{35}Cl(n,2n)^{34m}Cl$, respectively. This spectrum is given in Fig. 4.

The 1.78- and 2.17-MeV peaks were used to give correction terms to be applied to the provisional results for oxygen, nitrogen, and carbon, and made possible a determination of the amounts of phosphorus and potassium in the mice. The interferences from phosphorus, potassium, and chlorine were determined in the following way. Separate sensitivity runs were performed with samples of phosphorus and potassium chloride. By recording the decay of the 0.51-MeV and high-energy peaks of each sample, the relationship between 0.51-MeV counts and high-energy counts at any instant was obtained. The counts in each high-energy peak of mouse or standard were compared with this relationship in each sample at the time when the full spectrum of mouse or standard was taken. Knowing the number of 0.51-MeV counts arising from the interfering element in this full spectrum, extrapolation along the 0.51-MeV decay to time zero gave the initial decay rate for that element in mouse or standard. By programming the computer to subtract these three decays from the gross results, correction was made for phosphorus, potassium, and chlorine interferences for each mouse and standard. The problem of the high-energy potassium-chlorine complex was minimized by using the Ge(Li) detector to determine the intensities and decay rates of the 2.08, 2.13, 2.17, and 2.28 MeV lines independently.

RESULTS

Table 3 contains our results for the mean percentages by weight of oxygen, carbon, nitrogen, phosphorus, and potassium in the group of ten mice activated by 37-MeV neutrons. The results of the Kjeldahl chemical assay and the 14-MeV neutron determination for nitrogen together with the flame photometer method for potassium are also presented in Table 4.

We quote the standard deviation on the elemental content of a single mouse which is calculated from the range of values obtained from the experimental mouse population. Repeated measurements on individual mice showed much higher precision. As Table 5 indicates, the reproducibility obtained using the continuous neutron spectrum was $\pm 3\%$ for oxygen, $\pm 5\%$ for nitrogen, and $\pm 2\%$ for carbon, the corresponding figure for nitrogen being $\pm 2\%$ for the 14-MeV neutron irradiation. Thus the standard deviation shown arises from the natural variation in elemental composition of the mice used, the extent of which is due to the large variation in weight and age.

TABLE 3. RESULTS OF ACTIVATION ANALYSIS USING NEUTRONS OF ENERGY UP TO 37 MeV

No.	Mouse weight (gm)	Oxygen	Carbon	Nitrogen	Phosphorus	Potassium
1	4.9	64.0	13.1	3.19	0.862	0.241
2	5.6	69.3	13.6	2.85	0.536	0.166
3	6.7	72.0	16.2	3.60	0.815	0.265
4	7.1	72.2	16.8	3.06	0.761	0.215
5	12.9	65.8	13.8	2.56	0.647	0.213
6	15.8	66.3	14.9	3.09	0.674	0.257
7	18.1	63.4	15.1	3.39	0.702	0.216
8	23.4	67.0	16.5	3.13	0.733	0.281
9	24.0	70.1	17.6	2.92	0.754	0.311
10	32.0	72.2	16.4	3.24	0.698	0.213
Mean		68.2	15.4	3.10	0.72	0.24
		±3.5	±1.5	±0.3	±0.08	±0.04

All results are expressed as percentage by weight of animal.

TABLE 4. COMPARISON OF TECHNIQUES FOR NITROGEN AND POTASSIUM DETERMINATIONS

		Nitrogen			Potassium	
No.	Mouse weight (gm)	14 MeV	37 MeV	Kjeldahl	Activation	Flame photometry
11	30.5	3.26	—	—	—	—
12	29.1	3.45	—	3.37	—	0.247
13	28.3	3.40	—	3.51	—	0.273
14	27.7	3.37	—	—	—	—
6	15.8	—	3.09	3.24	0.257	0.240
7	18.1	—	3.39	3.32	0.216	0.223

TABLE 5. REPRODUCIBILITY OF ACTIVATION MEASUREMENTS

				Nitrogen	
No.	Mouse weight (gm)	Oxygen	Carbon	37 MeV	14 MeV
		71.0	18.6	3.34	
15	30.8	73.2	18.5	3.20	
		71.6	18.2	3.24	
14	27.7				3.37
					3.35
					3.47

DISCUSSION

Analysis of the complex decay of the annihilation peak gives expected values for the amounts of oxygen, nitrogen, and carbon in mice after correction for the contributions of phosphorus, potassium, and chlorine. Unfortunately there are no comparisons to be made with previous work for oxygen and carbon, and so detailed discussion of the validity of our measurements is necessarily limited to nitrogen and a comparison with the work of Nagai and his associates, who measured nitrogen levels in mice of 2.30 ± 0.31% by neutron activation analysis and 2.32 ± 0.12% by the Kjeldahl chemical assay. Whereas the simultaneous evaluation of the three bulk elements does not allow the high accuracy for an individual element inherent in nitrogen measurement with 14-MeV neutrons, the results of our own 14-MeV neutron experiment and Kjeldahl determination give confidence in our value. The range of weights of the irradiated mice was larger than that used by Nagai, and it may be that younger mice have relatively more nitrogen than older mice. Because of the spread in individual values and the small number of measurements, we could not show correlations between weight, strain, and nitrogen content. To find percentage nitrogen content as a function of weight, the accuracy of the 14-MeV system is required.

Although the primary function of this work was to quantify the bulk elements, useful values of total-body phosphorus and potassium in mice have been obtained. Our value for phosphorus content is in agreement with that obtained by Williams, Cargol, Pailthorp, and Nelp (7) using 14-MeV neutrons. The counts in the 1.78-MeV peak are assumed to

be from the $^{31}P(n,\alpha)^{28}Al$ reaction, the contribution of the n,γ reaction on ^{27}Al being negligible in comparison because of the relative amounts of the two elements in the body. For total-body potassium, our activation value was confirmed by chemical assay, but the intricacy of the high-energy potassium-chlorine complex puts this method at a disadvantage to the relatively more simple ^{40}K measurement at present employed (*8*).

The use of neutrons of this energy (up to 37 MeV) for in vivo activation analysis may prove as valuable in clinical medicine as thermal activation is at the present time. It may be feasible to measure inulin clearance by neutron activation of carbon more conveniently than by the method at present being used by Zagzebski and Kelsey (*9*) with high-energy bremsstrahlung radiation. Nitrogen is of interest in such diseases as gout or uremia and in patients suffering from malnutrition. The combination of carbon and nitrogen determinations to obtain a parameter representing the fat-to-muscle ratio may well be of clinical significance. Mazess, Cameron, and Sorenson (*10*) have recently reported a method of obtaining this ratio in the middle upper arm by gamma-ray absorption, using the different absorption characteristics of fat and lean components of tissue. Whether the upper arm is representative of the whole body in this respect is not conclusive, and as total-body gamma-ray absorption measurements do not appear feasible, it may be that a total-body fat-to-muscle ratio is more favorably obtained from a carbon-to-nitrogen ratio.

The probable errors in the counts comprising the elemental ratios at time zero between mouse and standard were 2% for nitrogen and carbon and 1% for oxygen. Assuming this statistical accuracy to be adequate, we may discuss the feasibility of patient irradiation by extrapolation from our system. The dose given to each mouse during irradiation was 20 krem, and the photopeak detection efficiency for the annihilation radiation was estimated to be 10%. The dose required by a typical patient to give the same counting statistics would be about 3 rem, but with a whole-body counter detection efficiency of a few percent roughly 8 rem would be needed. This figure may be halved by an equivalent reduction in the irradiation time, which would only cause a 30% reduction in carbon counts. Moreover, by reducing the dose rate (beam current) by a further factor of two, the standard deviations on the three individual ratios would each be less than 4%. Thus information might be obtained on oxygen, nitrogen, and carbon levels in patients at a dose of less than 2 rem.

Extrapolation from mouse to man is, however, more complicated than this simple argument about the probable dose requirements. The problem of self-absorption in the body leading to nonuniformity may require a bilateral irradiation of the patient. Threshold-detector measurements using carbon pellets and nitrides indicate a nonuniformity of carbon activation of $\pm 4\%$ and of nitrogen activation of $\pm 9\%$ under conditions of bilateral irradiation. This latter figure is in agreement with the work of Palmer et al (*3*). The progressive modification of the neutron spectrum through a patient causing extensive thermalization of neutrons would increase the occurrence of thermal relative to fast reactions. The unwanted annihilation radiation from energetic gamma rays of isotopes such as ^{24}Na, ^{38}Cl, and ^{49}Ca may be as important in patient irradiation as the phosphorus, potassium, and chlorine interferences.

It would be impracticable to implement the concept of a tissue-equivalent standard irradiated simultaneously with the patient to serve as beam-current integrator and standard phantom. Beam fluctuations might be compensated for by registering the time variation of the output of a suitable neutron detector during patient irradiation, and modifying the results appropriately. Continuous monitoring of the induced annihilation radiation by multichannel scaling would increase precision of the results.

Without the facility of a simultaneously irradiated standard, another interference arises. Additional ^{13}N may be produced by a p,α reaction on ^{16}O, a fast neutron giving energy to a hydrogen atom to initiate the reaction. This leads to an increase in nitrogen counts which is a function of oxygen concentration. In the work reported here, the use of the comparator technique with a standard solution of very nearly the same major elemental proportions as mouse tissue (Table 2) ensured the effect was minimized since the same percentage interference occurred in both mouse and standard. Irradiation of distilled water indicated that this interference gave a 10–15% contribution to the nitrogen counts in a mouse. Hence, in a patient irradiation with no standard, this interference would also require correction.

Further work is in progress using human phantoms to verify the proposals put forward in this paper.

CONCLUSION

The accuracy of the oxygen, nitrogen, and carbon determination is such that the method described could be employed in serial measurements where changes in elemental percentages are of interest rather than absolute levels. This system is the first reported for total-body oxygen and carbon estimation by neutron activation analysis, but if nitrogen estima-

tion is also of interest, it would appear better to use a separate irradiation with 14-MeV neutrons, when neither carbon nor oxygen would be activated.

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