In Vivo Measurement of Body Nitrogen by Analysis of Prompt Gammas from Neutron Capture

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A method for the in vivo determination of body nitrogen by prompt gamma photons from neutron capture is described. An 85-Ci ^{238}Pu-Be source provides the neutrons. The gamma detection system consists of two 15.24 × 15.24 cm NaI(Tl) detectors placed above the patient. Absolute value of body nitrogen is determined using body hydrogen as an internal standard. The reproducibility of the method is ±3% for a body dose of 26 mrem.

J Nucl Med 20: 1158–1165, 1979

Nitrogen is central to the structure of living matter. It is not only present in all amino acids, which form the fundamental proteins for the body, but is also present in such important biological molecules as the DNA of the cell nucleus. Knowledge of nitrogen balance, or of changes in body nitrogen, finds useful applications in medicine and biochemistry.

While metabolic balance studies have been used to measure changes in body nitrogen, there are several serious limitations to this approach. First, it is very time consuming; second, the errors associated with collection of feces and urine over long periods can be considerable. Wallace (*1*) has noted that intake and collection errors are not necessarily random, and may lead to overestimation and underestimation of positive and negative balance. Forbes (*2*) pointed out another source of error in metabolic balance studies: physiological adjustments to dietary changes may well be much slower than has hitherto been thought. A direct measure of body nitrogen can provide the clinician with a potent tool for clinical investigation of disorders involving nitrogen metabolism and the effects of different therapies and diets. It can also provide norms of reference through study of normal subjects.

Received March 22, 1979; revision accepted May 23, 1979.

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The first nuclear methods for direct determination of total body nitrogen (TBN) in vivo, in mice (*3*), and in humans (*4,5*) used the $^{14}N(n,2n)^{13}N$ reaction. The reaction has a high neutron energy threshold, 11.3 MeV. The product N-13 decays ($T_{1/2}$ = 101 min) by positron emission to C-13. No gamma photon characteristic of N is emitted—only the 511-keV annihilation quanta. This lack of specificity poses problems of interference from positron emitters produced from other body elements (*6,7*). For example, N-13 produced by the knock-on protons in the $^{16}O(p,\alpha)^{13}N$ reaction, contributes as much as 19% to the nitrogen counts (*6*).

Another problem associated with this method is the lack of uniformity of the fast-neutron fluence in the body (*4,8*). Values of ±7 to ±22% RMS deviation from the mean were reported for water-phantom depths ranging from 15 to 30 cm (*9*). At present two groups (*5,10*) are using the (n,2n) method of N determination.

The Birmingham University group (*11*) was the first to use prompt gamma photons from neutron capture for the in vivo measurement of TBN. In slow neutron capture by N-14, the total energy available is 10.83 MeV. Approximately 15% of the de-excitations take place directly to the ground state of N-15. Since no other major body element has a neutron-capture gamma of this energy, it is possible to determine body N by measuring the 10.83-MeV photons.

The Birmingham system has been described in detail

by Ettinger et al. (*12*). A cyclotron is used as the source of neutrons. Because counting is performed during irradiation, this method has encountered serious problems with background levels. These were countered by collimating the neutrons, pulsing the neutron beam, and shielding the detectors. In sequential measurements, an accuracy of ±2% was obtained for an incident dose of 0.1 rem.

Harvey et al. (*13*) reported application of this system for assessing changes in TBN content in normal and diseased humans. Walsh et al. (*14*) studied changes in TBN in newly diagnosed diabetics with this system. Vartsky et al. (*15,23*) described a method for absolute measurement of TBN using body hydrogen as an internal standard. Dabek et al. (*16*) showed correlations between absolute body N and body K in healthy and diseased subjects.

Recently Mernagh et al. (*17*) reported the determination of N by a similar approach using four 5-Ci ^{238}Pu-Be neutron sources. The N in a section of the chest 20 × 20 cm in area was determined. An error of approximately ±3% was reported for a dose of 50 mrem.

The present paper describes a facility for measurement of TBN based on the $^{14}N(n,\gamma)^{15}N$ reaction. This system uses an 85-Ci ^{238}Pu-Be neutron source, and uses the measurement of neutron capture prompt gamma-rays of body hydrogen as an internal standard. The body N thus determined in conjunction with a determination of other body parameters such as body water, potassium, calcium, fat, and weight, permits a detailed investigation of body composition in health and disease.

MATERIALS AND METHODS

Radiative neutron capture in N-14 and H-1. The following nuclear reaction provides the prompt-gamma radiation.

$$^{14}N + n \rightarrow {}^{15}N^* \; [\sim 10^{-15} \text{ sec}] \; {}^{15}N(\text{stable}) + \gamma$$

This reaction is most probable at thermal neutron energies. The lifetime of the compound nucleus $^{15}N^*$ is of the order of 10^{-15} sec; it de-excites to the ground state by emission of a cascade of gamma photons. The total energy available from the capture, due to the binding energy of a thermal neutron, is 10.83 MeV. Approximately 15% of the de-excitations take place directly to the ground state of N-15. The cross section for the capture by N-14 (0.08 barn, *18*) is relatively small, but the 10.83-MeV energy of the resulting gamma makes it measurable among the more numerous captures by body chlorine and hydrogen.

With the capture of slow neutron in hydrogen, a 2.23-MeV gamma is emitted. The yield of this photon is 100% per neutron capture; the neutron-capture cross section is 0.33 barn.

Interfering reactions. The elements that emit prompt gamma photons in the energy region of N and H are listed in Table 1. Although many of them are found in the body, their low isotopic abundances and low percentages of emission per neutron capture make their contributions to the regions of N and H peaks insignificant (0.03% and 0.09% for N and H, respectively). The elements iron, silicon, and magnesium are found in

TABLE 1. POSSIBLE INTERFERENCES FOR N AND H

			N gamma photon 10.83 MeV			
Element	Cross section (barns)	E (MeV)	I (γs/100 neutrons)	S (σ·I/A)*	Wt in body (g)	S × Wt
^{14}N	0.08	10.83	15	0.08	2000	160
^{40}K	70	10.11	?	—	0.017	—
^{43}Ca	6	11.14	?	—	1.5	—
^{25}Mg	0.18	11.09	0.03	$2.16 \cdot 10^{-4}$	3.5	0.001
^{57}Fe	2.4	10.03	0.1	$4.2 \cdot 10^{-2}$	0.94	0.04
^{10}B†	0.5	11.45	0.8			
^{29}Si†	0.3	10.60	0.2			
			H gamma photon 2.23 MeV			
H	0.33	2.23	100	33	7000	$2.3 \cdot 10^5$
N	0.08	2.16	6.2	0.03	2000	60
Na	0.53	2.19	2.5	0.06	105	6.3
P	0.19	2.15	16.7	0.10	700	70
K	2.1	2.29	3.4	0.18	140	25
Ca	0.34	2.29	1.8	0.02	1050	21

* A = atomic weight.
† Structural materials.

structural materials such as steel and concrete, and therefore may contribute to the background at the energy of interest. For this reason, the use of steel was avoided in the support structure for the detectors. The use of hydrogenous materials was also avoided in the construction wherever possible. However, when a vigorous moderation of neutrons was necessary, as in the collimator or in the detector shielding, resin heavily doped with ^6LiF and $LiCO_3$ was used. The high neutron-capture cross section of Li minimizes radiative neutron capture by H present in the resin. The 2.23-MeV H gamma background due to the H in the concrete and shielding is about 20% of the total H counts measured from the body. Since this background is independent of patient size, as will be shown later, it does not contribute an unknown variable to the determination of body H.

Irradiation and detection facility. The reactions of interest, $^{14}N(n,\gamma)^{15}N$ and $^1H(n,\gamma)D$, occur predominantly with slow neutrons, but because these have poor penetration into the body (diffusion length about 3 cm in soft tissue), it is necessary to use fast neutrons for the irradiation of such an extended object. Our ^{238}Pu-Be source delivers neutrons with energy ranging from 0 to about 11 MeV and a mean energy of about 4.5 MeV (*19*); the neutron yield is 2.3×10^8 n/sec.

The source is housed in a collimator made of epoxy resin doped with $LiCO_3$ and ^6LiF compounds (Fig. 1). The collimator is designed to provide a rectangular beam measuring 13 × 60 cm at the level of the bed, situated 50 cm above the neutron source. The collimation of the neutrons can be changed to provide smaller beam fields for partial body irradiation of a selected organ e.g., the liver (*20*). The fast-neutron flux at the level of the bed is 7200 n/cm^2· sec.

The neutron shield consists of boxes filled with polyester resin mixed with $LiCO_3$ and ^6LiF. These compounds minimize radiative capture in the resin's hydrogen. The whole system is covered with a 20-cm-thick layer of lead; the layer under the detectors is 30-cm thick. The lead reduces the intensity of gamma photons emitted from the source as well as those produced in the neutron shielding material by neutron capture and in elastic scattering. The neutron shield reduces the radiation dose to 1 mrem/hr at about 1 m from the facility; thus a clinician or nurse may stand near the patient during the measurement. The movable bed consists of a 5-mm-thick aluminum plate driven by a motor so that the patient's body passes continuously through the neutron beam.

A certain degree of premoderation of the neutrons is necessary in order to provide uniformity of composite sensitivity (defined in the "Phantom Studies" section) through the thickness of the body. However, since the 2.23-MeV gamma photons from body hydrogen are measured, no hydrogenous premoderation could be used. The premoderation is provided by a 5-cm-thick box made of aluminum filled with D_2O. The box is positioned in

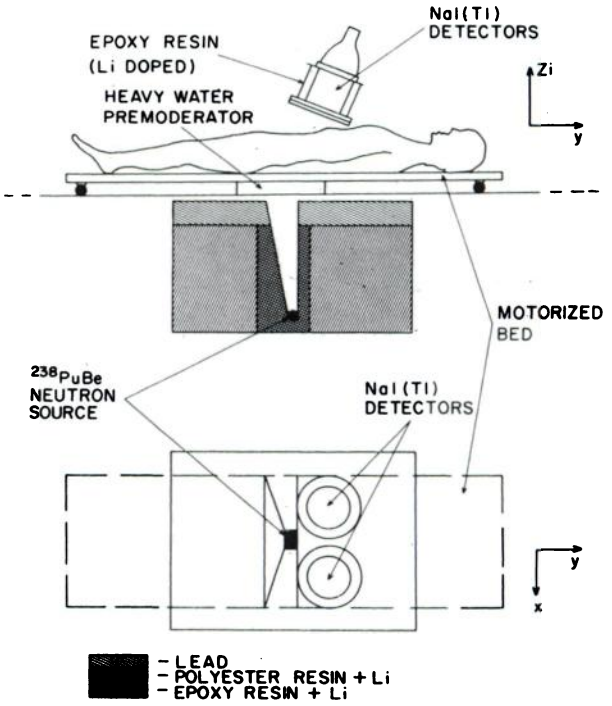

FIG. 1. Irradiation-detection facility.

the neutron beam and pressed against the aluminum bed, with a thin Teflon sheet in between to reduce friction. The deuterium in the neutron field produces tritium by the $D(n,\gamma)T$ reaction, but calculations of the tritium yield showed production of only 5 nCi/yr.

The neutron-capture gammas emitted from the body are detected by two 15.24- × 15.24-cm NaI(Tl) crystals positioned above the body, with aluminum for structural support. The detector geometry is designed to minimize the spatial variation of sensitivity for N and H photons; (see details in the "Phantom Studies" section). To reduce the neutron flux reaching the detectors, they are surrounded by cups made of resin doped with Li compounds.

The detectors are coupled to photomultiplier tubes, 12.7 cm in diameter†, connected with the cathode at high negative potential and the anode close to ground potential. In this mode the signal can be taken directly from the anode without the need of a blocking capacitor. Figure 2 shows the electronic system associated with the detectors. The method of pulse-height analysis is that of fractional charge collection (*21*), which is necessary since the total detector count rate is high (73,000 cps) thus providing a high probability of a pulse pile-up. The signals from the two anodes are passed through a pole-zero cancellation filter. This element shortens the duration of the anode-current pulse from approximately 1 μsec to 70 nsec. The anode signal is then amplified and split into two branches: one feeds a linear gate and stretcher through a suitable delay, while the second triggers a fast NIM discriminator that provides a logic

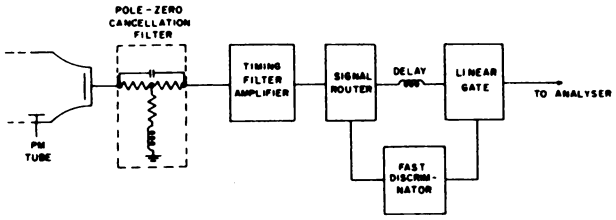

FIG. 2. Electronic system for fractional charge collection.

signal to the linear gate. Only signals that are above the discrimination level are integrated in the linear gate and analyzed.

Figure 3 shows spectra of the 10.83-MeV gamma photons of N obtained from a tissue-equivalent phantom, together with backgrounds obtained from a N-free phantom. Figure 3A used the conventional mode of analysis, and 3B was obtained with the fractional-charge collection method. The net N counts obtained by the latter method are 6% higher and the background is down to about 30%, thus improving the overall accuracy by a factor of 1.7. The signal for analysis of hydrogen is taken from the last dynode of the photomultiplier, fed to a preamplifier, amplifier, and discriminator, and analyzed.

DOSIMETRY

Measurement of the absorbed dose from fast neutrons and gammas was made with tissue-equivalent chambers and LiF TLD dosimeters. The dose rates for neutrons and gammas at the center of the beam spot at the level of the bed were 66.5 and 33 mrad/hr, respectively. The collimator defines the profiles of the neutron and gamma dose rates, as shown in Fig. 4. The distribution of the fast-neutron flux along the width of the collimator (y axis, Fig. 1) was measured with cadmium-shielded indium foils. The gamma-ray distribution was assumed to be similar.

If a body passes above the collimator with constant speed, the y axis in Fig. 4 can be transformed to time (sec) spent in the beam. The actual dose absorbed by a unit volume passing through the beam can then be calculated from the time integral of the dose-rate function—i.e., the area under the dose-rate curve. With a bed speed of 0.23 cm/sec, the absorbed dose from the neutrons is 1.25 mrad/scan, and 0.6 mrad/scan from the photons. For a prone-and-supine irradiation, the total dose is 2.5 mrad for neutrons and 1.2 mrad for the gammas. Using an RBE of 10 for fast neutrons, the dose is approximately 26 mrem. This figure is the skin dose; the actual body dose is smaller due to the attenuation of the fast-neutron flux in the body.

PHANTOM STUDIES

Uniformity of activation and detection. Measurement

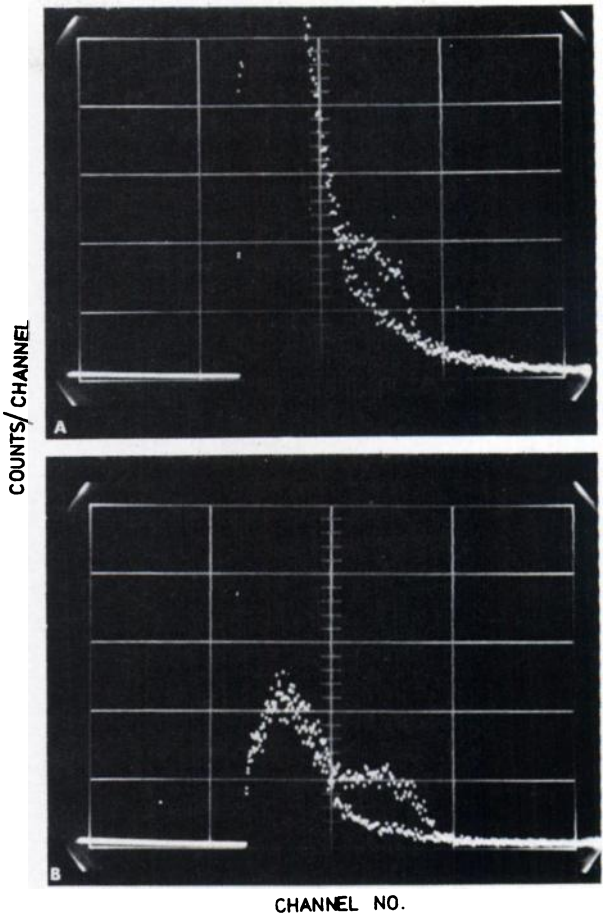

FIG. 3. Nitrogen 10.38-MeV gamma and background spectra: (A) conventional mode of analysis, (B) fractional charge collection method. Vertical axis = counts/channel, horizontal axis = channel number.

of body nitrogen with hydrogen as an internal standard requires a reasonably uniform composite sensitivity in the body (*17*). Composite sensitivity is defined as the number of counts detected from the body per unit mass of element under investigation per unit dose delivered to the body. The factors that influence the composite sensitivity are: (a) the activating neutron fluence, (b) the self-absorption of the escaping photons, and (c) the detection efficiency for these photons. All of these factors are space-dependent, whereas ideally their product should be spatially independent.

The slow-neutron flux that produces the $^{14}N(n,\gamma)^{15}N$ and $H(n,\gamma)D$ reactions was measured with Cu foils. The cross section for neutron capture in Cu-63 producing Cu-64 ($T_{1/2}$ = 12.7 hr) has a 1/v behavior that is characteristic of the neutron-capture cross sections in nitrogen and hydrogen. The composite sensitivity was measured using nickel samples placed in various positions in the phantom. Nickel is used rather than nitrogen because of the difficulty of producing a concentrated small nitrogen sample. The prompt gamma photons of nickel have an energy of 8.99 MeV, which is close to the

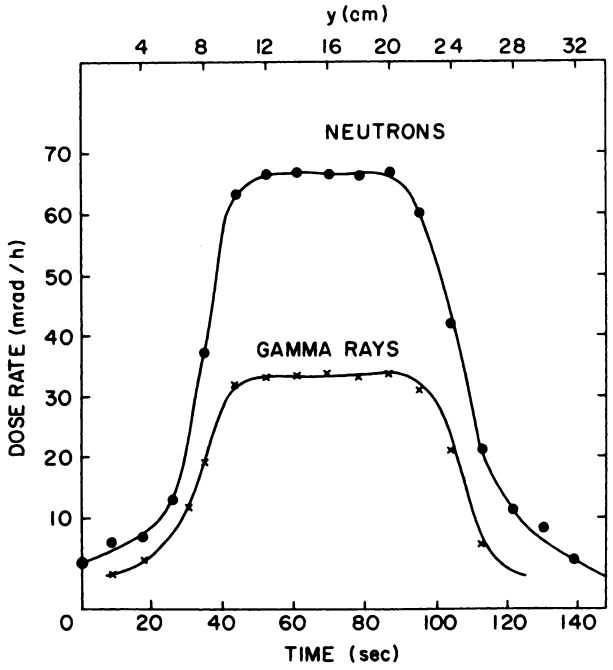

FIG. 4. Distribution of neutron and photon dose rates as defined by collimator at level of bed.

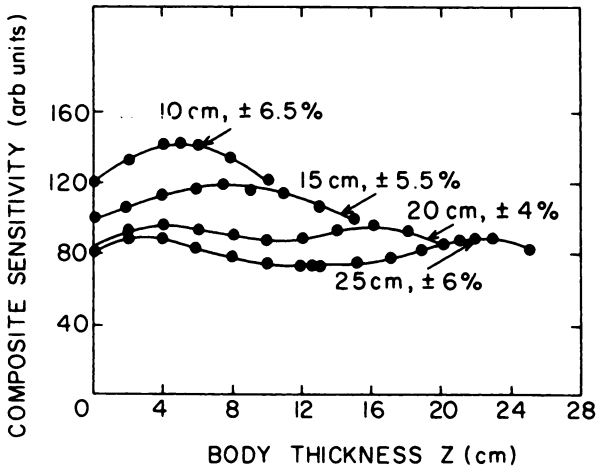

FIG. 6. Composite sensitivity distribution for front-and-back irradiation of different body thicknesses. Numbers above or below curves are body thicknesses in cm and uniformity of composite sensitivity in RMS percentages.

10.83-MeV gamma of nitrogen.

Figure 5 shows the slow-neutron flux distribution in a 25-cm-thick phantom. As can be observed, there is an initial buildup of slow neutrons, reaching a maximum at about 4 cm, and then a slow decline of the flux. The buildup can be eliminated by introducing a suitable premoderator. Placement of the detectors above the body, opposite the irradiated side, will partially compensate for the drop in the slow-neutron flux, since both the detection efficiency and the self-absorption of the escaping photons act in opposite directions to the flux depression. Figure 6 shows the composite sensitivity for a bilaterial irradiation, measured with a 100-g Ni sample along different body thicknesses. With the use of the heavy-water premoderator described in the previous section, the uniformities obtained here have acceptable RMS values, (4-6.5%), for thicknesses ranging from 10-25 cm.

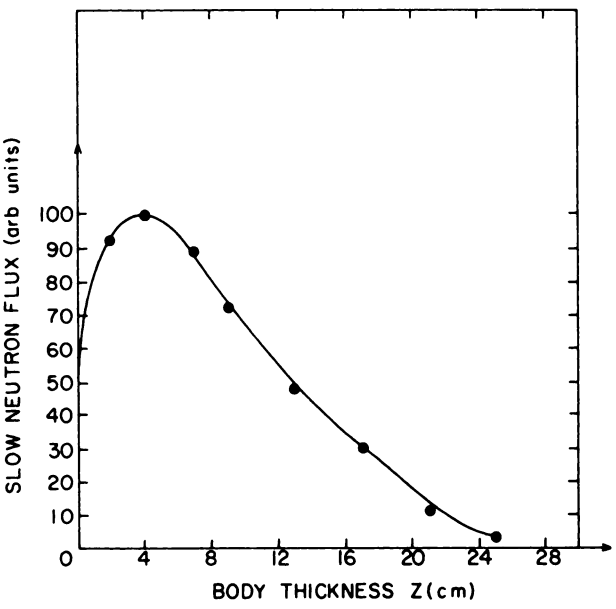

FIG. 5. Slow-neutron flux distribution in a phantom 25 cm thick.

In order to check the effect of body shape on the nitrogen to hydrogen count ratio, the Alderson anthropomorphic phantom was filled with a tissue-equivalent solution and the N/H ratio was measured at different sections of the phantom. With appropriate correction for different thicknesses of body sections (*15,23*), the coefficient of variation of N/H from shoulders to knees was ±3%. The N/H count ratios are shown in Table 2. We conclude that the shape of the body has only a small influence on the N/H count ratio; only the thickness of the patient has to be considered when correction for a differing geometry is made.

Background radiation. Since the background under the nitrogen peak contributes about half of the gross counts obtained with a healthy volunteer, and might contribute an even larger proportion for a wasted patient, determination of the nitrogen background for the individual patient plays an important role on the overall accuracy of a nitrogen determination. The relatively high background in the nitrogen region is thought to be due to fast neutrons interacting in the NaI(Tl) detectors. These neutrons produce (n,p) and (n,α) reactions and radiative neutron capture in the crystals.

Phantom studies have shown that the background levels depend on thickness and shape of the patient, but

TABLE 2. CORRECTED N/H RATIO IN ALDERSON PHANTOM

Section	Thickness (cm)	N/H count ratio $\times 10^{-3}$
Shoulders	16	2.36
Thorax	20.4	2.85
Abdomen	19.3	3.01
Hips	19.4	2.87
Upper thigh	16.1	2.80
Lower thigh	13.4	2.85
Knees	12.0	2.91
		$\overline{N/H} = 2.89 \pm 0.07$

the shape of the background spectrum remains invariant.

Figure 7 shows a spectrum obtained from an Alderson phantom containing tissue-equivalent liquid, and a background obtained from nitrogen-free phantom. It can be seen that on a semilogarithmic plot the background can be approximated by two straight lines. The patient's background is found, therefore, by fitting a line on each side of the nitrogen peaks.

The contribution of the surroundings to the hydrogen peak was determined with a phantom filled with heavy water. Different shapes and thicknesses of phantoms were used, and it was found that this hydrogen background is independent of the presence of the phantom. It contributes about 20% of the counts in the patient's hydrogen peak. Since the count rate of this contribution is constant, this background does not introduce an unknown variable to the net count rate for hydrogen.

Reproducibility. The reproducibility of the method has been tested by multiple measurements of the Alderson phantom filled with tissue-equivalent liquid. In eight measurements, the N/H count ratio had a coefficient of variation of ±3%. With an error due to counting statistics of 2.6%, the reproducibility of the ratio was found to be 1.5%.

IN VIVO NITROGEN STUDIES

Measurement routine. The following patient irradiation routine was adopted.

1. Patient thickness was measured in eight places between shoulder and knees.
2. The shoulder-to-knee region was scanned with the patient in supine and prone positions to provide front-and-back irradiation.
3. The measured N/H ratio was corrected for the differences between patient thickness and the Alderson phantom.
4. Total body nitrogen was determined with the formula:

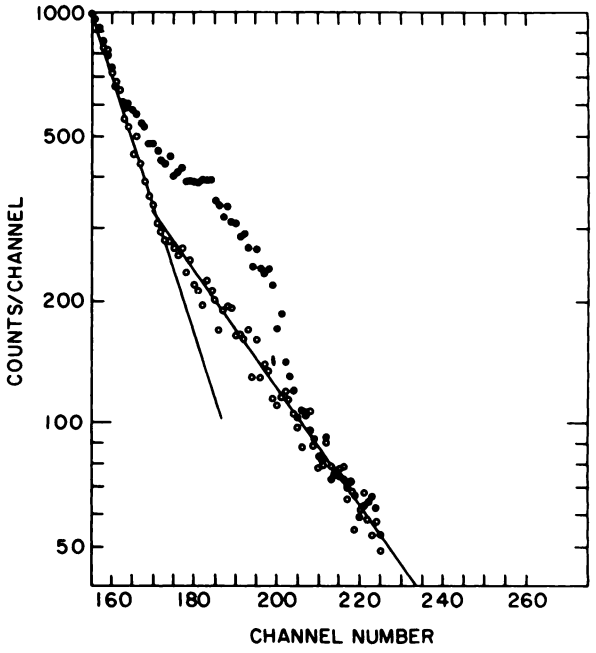

FIG. 7. Nitrogen 10.83-MeV gamma spectrum from Alderson tissue-equivalent phantom, and background from nitrogen-free Alderson phantom.

$$TBN = k \cdot N/H \cdot TBH$$

where k is a constant determined from irradiation of an Alderson phantom containing known amounts of nitrogen and hydrogen. TBN is total body nitrogen and TBH is total body hydrogen; N/H is the corrected ratio. Total body hydrogen (TBH), is calculated from body water, fat, and weight (determined apart from the nitrogen measurements) according to the relation:

$$TBH = 0.11 \text{ water} + 0.12 \text{ fat} + 0.052 \text{ (body weight-water-fat)}$$

The formula is based on the known fraction of hydrogen by weight in each body compartment, namely, 0.11, 0.12, and 0.07 for water, fat, and protein, respectively (*22*). The hydrogen content of ash is assumed to be negligible.

Results. Nitrogen was measured in 14 healthy male volunteers within the age range of 20–31 yr. All were athletic individuals. Table 3 shows the nitrogen results obtained in this study together with body potassium as determined by whole-body counting. TBN represented (3.38 ± 0.15) % of lean body mass and (2.7 ± 0.2) % of body weight. The correlation coefficient between nitrogen and potassium was 0.93 and the standard error of the estimate was ±4%.

SUMMARY AND CONCLUSIONS

A method has been developed for absolute measurement of whole-body nitrogen by the use of a technique involving body H as an internal standard. A wide rec-

TABLE 3. N AND K IN NORMAL MALE VOLUNTEERS

Subject No.	Age (yr)	Weight (kg)	Nitrogen (kg)	Potassium (g)
1	31	85.4	2.11	156.9
2	30	65.0	1.88	138.7
3	22	70.0	1.97	139.3
4	22	77.3	2.40	183.2
5	27	88.2	2.28	178.5
6	27	83.6	1.98	142.9
7	23	78.6	2.08	152.1
8	24	77.3	2.20	179.4
9	21	75.0	2.16	150.2
10	26	60.9	1.68	124.8
11	23	73.6	1.89	147.3
12	25	68.2	1.78	141.0
13	23	87.3	2.39	174.8
14	20	62.7	1.77	125.2

tangular neutron beam, 60 × 13 cm, permits the entire width of the patient to be irradiated as the bed passes over the neutron beam. The gamma detection system comprises two 15.24 × 15.24 cm NaI(Tl) detectors placed above the patient. This detector geometry yields a more uniform composite sensitivity in the patient, as compared with other detector geometries (*8*). The usual requirement of a very uniform composite sensitivity for absolute measurements is not essential when an internal standard is used.

The problem of pulse pile-up due to high count rate was minimized by the method of fractional charge collection and selective pulse integration.

The advantage of the internal standard method over the conventional method of analysis is that errors due to different irradiation and detection conditions, as well as different shape and position of the patient, are greatly reduced. This makes sequential nitrogen measurements much more reliable, especially when the patient's weight has changed significantly. In addition, positioning errors are reduced. The present method of nitrogen measurement is reasonably comfortable for the patient; it requires only that he change from a supine to a prone position between the scans. The patient does not have to be surrounded by premoderating material, as is common practice with other methods of neutron-activation analysis. With the restricted beam and good collimation, the presence of a clinician or nurse near the patient is possible during the irradiation. The total measurement time is about 20 min; the dose delivered to the irradiated region is 26 mrem.

Based on the limited number of measurements of healthy young volunteers, the average nitrogen mass was (2.7 ± 0.2)% of body weight and (3.38 ± 0.15)% of lean body mass. Correlation between body nitrogen and potassium was high (r = 0.93) and standard error of the estimate was ±4%.

The precision of the method (±3%) as determined from multiple irradiations of the Alderson phantom, could be improved by adding two more NaI(Tl) detectors positioned symmetrically to the two used at present. Further improvement could probably be achieved by reducing the background in the nitrogen region through use of neutron/gamma event discrimination by a pulse-shape discrimination technique.

Since the facility uses ^{238}Pu-Be sources with a constant neutron output, no continuous monitoring of the patient's radiation dose is necessary. Furthermore, the portability of these sources makes the whole system mobile.

FOOTNOTE

† RCA 4522.

ACKNOWLEDGMENT

Research was carried out under the auspices of the U.S. Dept. of Energy under NIH-NCI Contract No. YO-1-CP-80207.

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