TOTAL-BODY NITROGEN AND PROTEIN DETERMINED BY IN VIVO FAST-NEUTRON ACTIVATION ANALYSIS

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Although uses of neutron activation analysis in the biomedical field have been confined to *in vitro* studies until recently, *in vivo* activation analysis is becoming a useful technique for estimating certain elements in the living body. Kellershohn and his associates at the Service Hospitalier Frederic Joliot in France have been irradiating lambs with thermal neutrons from a reactor and measuring the induced ^{128}I in the thyroid gland with a whole-body counter. Their study (*1*) is the first report of radioactivity deliberately induced in the living body by neutrons.

Elements like nitrogen are of considerable interest in biomedical research, but nitrogen is insensitive to thermal neutrons. Since nitrogen can be activated easily by fast neutrons, *in vivo* fast-neutron activation analysis may be of value for determining nitrogen or protein in the body.

In this report, *in vivo* fast-neutron activation analysis for measuring the total-body content of nitrogen and protein in mice is described. Our experiments carried out with 14-MeV fast neutrons, indicate that this technique offers a useful nondestructive method of determining these constituents in the living body.

METHODS

Ten female mice (ddY), weighing 23.1–29.3 gm, were irradiated in a stainless-steel cylindrical container (3.4 × 8.2 cm) with 14-MeV fast neutrons from a Cockcroft-Walton generator (ACTIVAC, Tokyo Shibaura Electronic Co. Ltd.) (Fig. 1). It is well known that this neutron energy is well over the threshold for the n, 2n reaction in ^{14}N but is too low to excite the corresponding reaction in ^{12}C and form ^{11}C.

The neutron generator produced 5 × 10^{10} 14-MeV neutron/sec by the d-t reaction. The neutron output during the 10-min irradiation was measured continuously with a 3 mm^2 silicon surface-barrier diode, and its fluctuation was determined with a 200-channel multiscaler. The neutron output from the tritium target of 10 Ci during each 5-sec period was recorded in each channel of the multiscaler.

After transferring the irradiated mouse into a Lucite container the same size as the stainless-steel one, the whole-body induced gamma radiation was measured with a coaxial Ge(Li) semiconductor detector (ORTEC Model-8102-20) with a 22-cm^3 active volume. The duration of each measurement was 200 sec, and the delay between irradiation and measurement (cooling time) was 60–120 sec. Pulses from the detector were analyzed with an 800-channel pulse-height analyzer. Nitrogen was determined by counting the 0.511-MeV annihilation photons from

Received April 30, 1968; original accepted Sept. 24, 1968.

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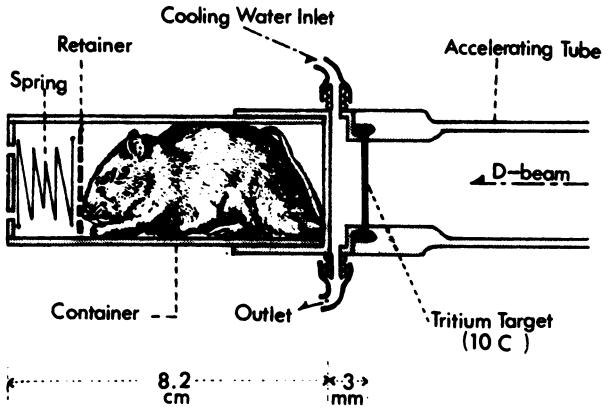

FIG. 1. Diagram of target assembly and irradiation container showing position of mouse for irradiation.

the decay of ^{13}N formed by the n, 2n reaction on ^{14}N in the body. A 10-min irradiation of each mouse gave reasonable counting rates.

After the radioactivity was measured, seven of these animals were sacrificed, placed into a deep freezer and used for chemical analysis.

Polyethylene phantoms (commercial liquid-scintillation-counting vials; 2.8 × 6.5 cm) containing 20 ml of a series of known concentrations of NH_4NO_3, irradiated under exactly the same conditions as the mouse, were used to calibrate the detector.

Analyses of the induced gamma spectra, correction for the physical decay of each gamma emitter and corrections for the influence of fluctuations in neutron output and for the ratio of the neutron doses to each subject were programmed and automatically normalized with a digital computer (GE-635). The computer programs included the following steps: smoothing, background subtraction, peak sorting and normalization (*2,3*).

The activities in the annihilation peaks from the mice and the nitrogen phantoms were compared under standard conditions: a 10-min irradiation with 5×10^{10} n/sec and 200-sec counting after 1-min cooling.

To evaluate whether this technique could be used to measure protein content, phantoms containing a series of known concentrations of protein solution (human plasma protein; 88%-albumin, 7%-α globulin, 5%-β globulin and also pure bovine albumin; fraction-5) were also irradiated. To determine contributions from other gamma emitters produced by fast-neutron reactions in the body, phantoms containing various elements (H, O, C, Cl, Na, K, Ca, Mg, Fe, Cu and P, etc.) were irradiated and the induced gamma spectra were analyzed.

The nitrogen and protein contents of the irradiated mice, the human plasma protein solution and the pure bovine plasma albumin solution were determined by chemical analysis. These results were compared with the results obtained by activation analysis. The entire mouse except the skin was homogenized and the skin was digested in a Kjeldahl flask. Aliquots were then analyzed chemically (Kjeldahl method for nitrogen and biuret method for protein).

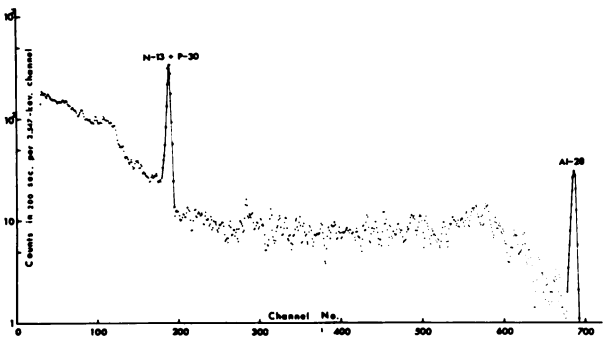

FIG. 2. Smoothed gamma-ray spectrum from irradiated mouse (No. 3).

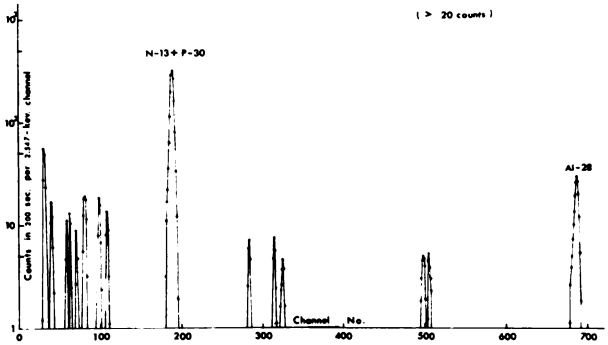

FIG. 3. Automatically sorted background-subtracted gamma peaks of irradiated mouse (No. 3). Peaks more than 20 counts/200 sec are shown.

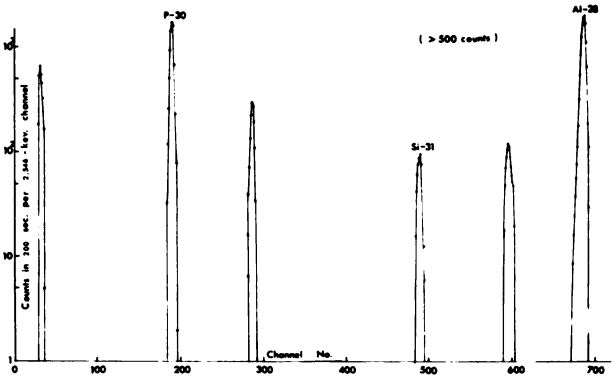

FIG. 4. Automatically sorted background-subtracted gamma peaks of irradiated phosphorus phantom. Peaks more than 500 counts/200 sec are shown.

RESULTS

The gamma spectra from the irradiated mice showed two prominent peaks at 0.511 MeV and 1.78 MeV (Figs. 2 and 3). The decay at 0.511 MeV was followed with a 200-channel multiscaler, and it was confirmed after approximately 10-min cooling that its half-life was identical with that of ^{13}N (10.0 min). The peak at 1.78 MeV was assumed to be due to ^{28}Al (half-life 2.27 min). ^{28}Al can be formed from ^{31}P, ^{27}Al or ^{28}Si, but it was apparent that the induced ^{28}Al was produced by the n, α reaction in ^{31}P which is the only common element of the three in the body constituents.

The spectrum from the phosphorus phantom

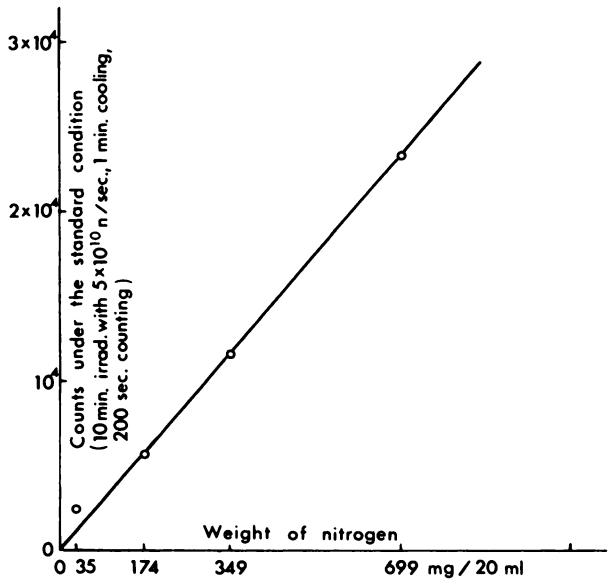

FIG. 5. Calibration curve of nitrogen.

showed two peaks at 1.78 MeV and 0.511 MeV, and several less obvious peaks as shown in Fig. 4. The first peak is attributed to ^{28}Al produced by the n, α reaction, and the peak at 0.511 MeV is attributed to the annihilation radiation due to ^{30}P produced by the n, 2n reaction which can interfere with the measurement of ^{13}N. Therefore to quantify ^{13}N activity in the body, it was necessary to subtract the contribution from ^{30}P. Contributions from other positron emitters such as ^{38m}K, ^{62}Cu, ^{53}Fe or ^{23}Ne produced by fast-neutron reactions in the body were so small that they were practically negligible. Unexpectedly the irradiated polyethylene container itself showed a trace amount of the annihilation peak, and there were no other obvious peaks due to ^{24}Na, ^{38}Cl or ^{49}Ca, etc., in the spectra from the irradiated mice or the standard phantoms.

Figure 5 shows the calibration curve of nitrogen obtained with the NH_4NO_3 standard phantoms. Estimates of total nitrogen and protein contents by fast-neutron activation analysis for protein solution and for mice are shown in Tables 1 and 2 together with the results obtained by chemical analysis. The nitrogen and protein contents of the six mice were found to be 23.00 ± 3.15 (1 s.d.) and 113.49 ± 15.71 mg/gm of gross body weight, respectively. It can be seen that these analytical results obtained by fast-neutron activation analysis are in good agreement with those by the standard chemical analysis.

DISCUSSION

Although the field of 14-MeV fast-neutron activation analysis is not yet as well developed as that with thermal neutrons, progress with the method is very promising and uses of fast neutrons may be of value in analytical research with some elements which are not readily detected by conventional thermal-neutron activation analysis.

Although 14-MeV neutrons have been used for activation analysis by industry to determine oxygen, nitrogen, fluorine or phosphorus, the concentration of nitrogen in biomedical materials has rarely been measured by this technique. Gilmore and his associates (*4*) reported that the concentration of nitrogen in hydrocarbons could be measured by ^{13}N radioactivity produced by irradiation with 14-MeV fast neutrons. Tsuji (*5*) determined nitrogen and phosphorus contents in various compounds with 14-MeV neutron irradiation and Wood (*6*) described fast-neutron activation analysis to determine nitrogen in grain products. Recently, elemental analysis of amino acids and protein by 14-MeV neutron activa-

TABLE 1. RESULTS OF ACTIVATION ANALYSIS AND CHEMICAL ANALYSIS OF NITROGEN IN PROTEIN SOLUTION

	Chemical*		Activation	
Sample	Nitrogen (mg/ml)	Protein† (mg/ml)	Nitrogen (mg/ml)	Protein† (mg/ml)
5% albumin	7.90	49.38	7.33	45.81
10% albumin	15.80	98.76	15.00	93.75
2.5% plasma	3.97	24.78	3.84	24.00
5% plasma	7.93	49.56	8.02	51.12

* Kjeldahl method.
† Factor used for calculating protein content was N × 6.25 for each protein.

TABLE 2. RESULTS OF ACTIVATION ANALYSIS AND CHEMICAL ANALYSIS OF NITROGEN AND PROTEIN IN MICE

		Chemical			Activation	
No.	Gross body weight (gm)	Nitrogen* (mg/gm)	Protein† (mg/gm)	Protein N‡/N (%)	Nitrogen (mg/gm)	Protein‖ (mg/gm)
1	23.7	22.52	94.66	67.27	21.79	107.48
2	23.1	24.99	101.26	64.83	25.08	123.71
3	26.5	23.05	124.97	86.77	26.08	128.64
4	26.1	24.27	136.62	90.03	25.67	126.72
5	27.7	23.72	116.69	78.71	21.42	105.65
6	28.3	21.90	112.58	82.24	18.00	88.78
7	29.3	21.90	113.14	82.65	—	—
Mean		23.19	114.27	78.92	23.00	113.49
		±1.19	±14.01	±9.52	±3.15	±15.71

* Kjeldahl method.
† Biuret method.
‡ Protein N = Protein × 0.16.
‖ N × 0.7892 × 6.25. Figure 0.7892 is mean of ratio of protein-nitrogen (protein × 0.16) to total nitrogen.

tion has been successfully performed by Crambes and his associates (*7*). On the other hand, Anderson and his associates (*8,9*) have measured total-body sodium, chloride and calcium contents in man with *in vivo* activation analysis using partially moderated 14-MeV fast neutrons. They suggested that fast-neutron reactions might be used to estimate nitrogen in the human body.

The capability for measuring total-body nitrogen is important in clinical medicine because of the direct relationship between nitrogen and protein content. In this paper the possibility of estimating total-body nitrogen and protein in the living body of mice with 14-MeV fast neutrons is described. Our results suggested that *in vivo* fast-neutron activation analysis can be used for selective activation of nitrogen and protein in the body.

The only positron emitter that might be confused with ^{13}N is ^{30}P which can be formed from phosphorus in the body. Fortunately, the induced ^{30}P is accompanied by ^{28}Al from phosphorus. The contribution from ^{30}P in the body can be calculated on the basis of the ratio of ^{30}P-to-^{28}Al normalized radioactivity of the phosphorus phantom and subtracted from the annihilation photons of the irradiated mouse. The contribution from ^{30}P can also be eliminated by counting the annihilation protons after 20-min cooling which results in the complete decay of ^{30}P (half-life 2.55 min). Standard reference spectra of the potassium, copper, iron and sodium phantoms excluded the possibility that ^{38m}K, ^{62}Cu, ^{53}Fe or ^{23}Ne formed in the living body could be mistaken for ^{13}N. The hydrogen-carbon reaction by recoil from collision with fast neutrons may be possible, but this reaction only gives rise to ^{13}N. The hydrogen-carbon reaction by recoil from collision with fast neutrons may be possible, but this reaction might only give rise to ^{13}N activity corresponding to a hundred parts per million of nitrogen.

It is well known that thermal-neutron activation analysis of almost any biomedical material is hampered by the great excess of ^{24}Na over the more desired trace-element activity. In this study, however, the gamma spectra were not distorted by ^{24}Na activity, showing that the fast neutrons did not slow down to thermal energies within the body of the mouse. Approximately uniform activation could be achieved for nitrogen because the animals were small. The 1- or 2-min cooling time is long enough to allow decay of ^{16}N (half-life 7.3 sec) formed from oxygen in the body.

Phosphorus content in the whole body can be determined from the ^{28}Al activity, and further studies measuring total-body phosphorus will be reported in a later paper.

Further development of *in vivo* 14-MeV fast-neutron activation analysis may result in many applications in clinical medicine. For example, assessment of the muscle wasting process, malnutrition, hypoproteinemia, gout or uremia may be possible with whole-body irradiation. Detection of abnormal contents of nitrogen or phosphorus in malignant tumors, in calf muscles of patients with progressive muscular dystrophy or in the hand of patients with gout may also be possible with partial irradiation.

A major concern is always the total-body radiation dose to the subject. Anderson's group reported that the radiation dose from neutron irradiation and from the decay of all the induced radioactivity is no greater than that of a barium x-ray examination. In our study three of the irradiated mice were alive months after irradiation with only slight weight loss, but further investigation is needed in both physical and biological aspects including not only simple radiation effects but also hot-atom effects before this technique can be introduced into clinical research.

Moreover, ^{13}N or ^{30}P-labeled organic compounds produced with fast neutrons may also be used as tracers to follow the metabolism of amino acids, proteins, nucleic acids or other organic compounds in biomedical research. For example, with a stationary imaging device like Anger's positron camera ^{13}N-labeled methionine or tryptophane could be used for pancreas imaging, and ^{30}P might be useful for malignant-tumor scanning. Crambes' group (*7*) reported that the infrared spectra of cystine after irradiation with fast neutrons showed no detectable bond alterations.

SUMMARY

In vivo 14-MeV fast-neutron activation analysis for total-body nitrogen and protein in mice has been successfully performed with a high degree of accuracy. The results obtained with this technique agree to a high confidence level with the analyses performed by standard chemical methods. An appraisal of this study indicates that *in vivo* 14-MeV fast-neutron activation analysis is a simple, useful and nondestructive technique for measuring nitrogen and protein in the living body without serious radiation effects. Automated data processing with a digital computer gave highly accurate results.

ACKNOWLEDGMENT

The authors are indebted to Miss Harumi Ohyama and Miss Aiko Endo of the National Institute of Radiological Sciences for assisting with the chemical analysis.

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